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The Journal of American Science

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Cover Page, Introduction, Contents, Call for Papers, All papers in one file

The following manuscripts are presented as online first for peer-review, starting from March 7, 2010.

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	concrete with improved strength. However, the ultimate strength of concrete was gained at 1.0 wt% of cement replacement. The workability of fresh concrete was decreased by increasing the content of Al_2O_3 nanoparticles. It is concluded that partial replacement of cement with nanophase Al_2O_3 particles improves the compressive strength of concrete but decreases its workability. [Journal of American Science 2010;6(5):6-9]. (ISSN: 1545-1003). **Key words:** Al_2O_3 nanoparticles; concrete; compressive strength; workability.	
3	**Study the effect of *Diphenyl Dimethyl Bicarboxylate* and *Dexamethasone* on Immunological and parasitological parameters in murine *Schistosomiasis mansoni*** Ibrahim RB Aly*, Mohamed A Hendawy*, Eman Ali, Mona MF Nosseir**. Departments of Parasitology* and Pathology** Theodor Bilharz Research Institute, Giza, Egypt dr_mona_zaki@yahoo.co.uk **Abstract:** This work aimed to evaluate the effect of Diphenyl Dimethyl Bicarboxylate (DDB) or dexamethasone either alone or combined with praziquantel (PZQ) on different parasitological, immunological, and pathological parameters that reflect disease severity and morbidity in murine schistosomiasis. Diphenyl Dimethyl Bicarboxylate (DDB) or dexamethasone had no effect on worm burden but altered tissue egg distribution. This indicates that under the schedule used, both drugs did not interfere with the development of adult worms or oviposition but it can modulate liver pathology. Meanwhile, dexamethsone showed a marked reduction of granuloma size more than DDB. Dexamethasone-treated mice, also, showed lower levels of serum gamma interferon (IFN-g), interleukin-12 (IL-12), and IL-4 together with higher IL-10 level compared to infected untreated control animals. These data suggested that dexamethasone is a convenient and promising co adjuvant agent causing decreased morbidity in murine schistosomiasis. [Journal of American Science 2010;6(5):10-18]. (ISSN: 1545-1003). **Keywords:** Schistosomiasis – Morbidity – Cytokines – Treatment.	Full Text
4	**Anti-*S. mansoni* MAb-based Latex Agglutination: A reliable field applicable immunodiagnostic test for screening of active Human Schistosomiasis** Ibrahim RB Mohamed SH*, ,Demerdash ZA*.,Diab TM**.,Maher K**., SafwatW***., Shaker ZA*. *Immunology, **Parasitology and***Clinical Departments, Theodor Bilharz Research Institute. dr_mona_zaki@yahoo.co.uk **Abstract:** Schistosomiasis is a major public health problem with a worldwide distribution. Diagnosis of this disease by simple and rapid immunoassays is a priority. The objective of the present study was to standardize and evaluate the latex agglutination test (LAT) as a simple test for the detection of circulating schistosomal antigen (CSA) in serum and urine samples of *S. mansoni* patients and compare it with ELISA. According to stool examination this study included 70 *S. mansoni* infected patients, 32 other parasites infected patients and 30 negative control samples. Characterization of MAb 12D/10F was done using several techniques including: ELISA, immunoelectrophoresis, polyacrylamide gel electrophoresis and immunoblotting, as well as periodate and trichloroacetic acid treatment of target antigen for identification of its chemical nature. A polystyrene latex (0.81 µm) suspension was used as a carrier particle for anti *S. mansoni* adult worm tegumental antigen monoclonal antibody (12D/10F) in the test. The Latex particles sensitized with MAb were used for the detection of CSA in urine and serum samples. The sensitivity of LAT assay was 90% in urine and 87.1% in sera versus 92.9% and 95.7% for	Full Text

	ELISA. The specificity of LAT assay was 88.7% and 93.5% for urine and sera versus 87.1% and 93.6% for ELISA. The diagnostic efficacy of LAT was 89.1% and 90.2% for urine and serum samples, respectively versus 90.2% and 94.7% for ELISA. Moreover, a positive correlation was found between ova count in stool of *S. mansoni* infected patients and both the intensity of LAT and OD readings of ELISA in urine ($r= 0.922$; $p< 0.001$ and $r= 0.865$; $p< 0.001$, respectively) and in serum ($r=0.847$; $p< 0.001$ and $r= 0.781$; $p< 0.001$, respectively). In conclusion, LAT is a suitable applicable diagnostic method in field survey especially when followed by ELISA as a confirmatory test in query false negative results. In the same time, more trials are required to increase the sensitivity and specificity of LAT to allow its use on a large scale in field surveys and as diagnostic kits for multiple parasitic infections. [Journal of American Science 2010;6(5):19-27]. (ISSN: 1545-1003). **Keywords:** Schistosomiasis – Agglutination – immunodiagnostic – Human	
5	**The Impacts of Urbanization on Kaduna River Flooding** Alayande Adegoke Waheed[1], Agunwamba, Jonah Chukwuemeka[2] [1]National Water Resources Institute, P.M.B. 2309, Kaduna. Nigeria. [2]Department of Civil Engineering, University of Nigeria, Nsukka. Nigeria. walayande@yahoo.co.uk, jcagunwamba@yahoo.com **Abstract:** Population growth, urbanization and expansion of structural developments into traditional flood prone areas of urban settlements of Nigeria are challenges requiring dynamic predictions of inundation areas; development of models for the propagation of flood waves on the floodplain; and the development of a rapid response and flood warning systems. In this study the impact of urbanization on geomorphic parameters of the Kaduna River along the City of Kaduna were investigated. The results obtained indicated that increasing urbanization along the Kaduna River floodplain is responsible for the problem of flooding experienced in recent times along the river floodplain and that encroachment into the traditional flood prone areas of the Kaduna River as a result of urbanization has attained 85.31%, 68.47% and 67.54% respectively in Reach 2, Reach 3 and Reach 1 respectively over the period 1962 and 2009. Because the Kaduna River usually attained its bankfull flow capacities in all its sections along the City of Kaduna early August each year, the result further indicated that the 2yr, 5yr, 10yr, 25yr, 50yr, and 100yr floods when occur can cause maximum inundation of between 82.53% to 94.48% of the floodplain area between the Eastern Byepass bridge and the Kaduna South Waterworks with Ungwan Rimi, Kabala Doki and Kigo road extension as the most critical areas where the right banks are lower than the left banks and developments are almost to the right bank of the river. [Journal of American Science 2010;6(5):28-35]. (ISSN: 1545-1003). **Keywords:** Urbanization, River Flooding, Geomorphology, Urbanization, Floodplain Development.	Full Text
6	**Extraction Conditions of Inulin from Jerusalem Artichoke Tubers and its Effects on Blood Glucose and Lipid Profile in Diabetic Rats** [1]A.M. Gaafar; [2]M.F. Serag El-Din; [2]E.A. Boudy and [3]H.H. El-Gazar [1] Food Technology Research Institute, Agricultural Research Center, Giza [2]Nutrition and Food Science, Faculty of Home Economics, Minufiya University [3] National Nutrition Institute, Cairo, Egypt. dr_mona_zaki@yahoo.co.uk **Abstract:** This study aimed to analyze Jerusalem artichoke tubers to identify its contents and to optimize conventional extraction of inulin, various time extract, temperature, and solvent ratio were used. 30 male albino rats divided into 5 groups (6 rats) were used to evaluated the extricated inulin as	Full Text

	Hypoglycemic agents. It could be concluded that, the highest yield of inulin was recovered from Jerusalem artichoke tuber by using the following condition, sample to solvent ratio was 1:5 w/v at 80°C for 90 minutes. The crude inulin extracted from Jerusalem artichoke tubers were used for production of orange juice and chocolate and estimated by aid of 10 panelists. The reduction of glucose was observed after one week of feeding till the end of experimental period, also, high level of inulin 15% led to a more reduction of blood glucose level comparing with the low level especially in the end of experimental period. The crude inulin extracted from Jerusalem artichoke tubers were used in diet for diabetic rats on different levels of inulin (10 and 15%) had significantly lower in total cholesterol, triglyceride and total lipids in comparing to positive diabetic rats fed on control diet. Meanwhile, HDL level was increased significantly after fed on 10 and 15% inulin. On the other hand, LDL and VLDL levels were decreased significantly after fed on (10 and 15%) inulin in comparing to positive group rats fed on control diet. [Journal of American Science 2010;6(5):36-43]. (ISSN: 1545-1003). **Keywords:** Jerusalem artichoke, Extraction Conditions, inulin, blood glucose and lipid profile	
7	**Potential impact of bee pollen administration during pregnancy in rats** **Eman I. AbdEl-Gawad** Radioisotopes Department-Atomic Energy Authority-Cairo – Egypt dr_mona_zaki@yahoo.co.uk **Abstract:** Although bee pollen was recommended as a supplemental diet for its nutritionally beneficial components, it is warning for its usage during pregnancy. In this study bee pollen (PB) water extract with different doses (2.5 & 5 and 10 g/kg b.w./day) was delivered to pregnant rats orally from day 1 to day 21 of gestation to address the physiological relevance of bee pollen rich in proteins and phytoestrogen during pregnancy in rats and to examine whether bee pollen administration modifies the serum steroid hormones involved in fetal outcome. The results revealed that bee pollen administration at high doses (5 & 10 g/kg/day) during pregnancy has an adverse effect on mothers and fetal outcome manifested by dams death, failure in implantation processes, resorbtion of fetuses, reduction in fetal numbers, retardation in fetal and placental weights. Lipid oxidation markers such as MAD and GSH levels were changed on day 21 of gestation in bee pollen treated rats referring to incidence of imbalance of oxidant/antioxidant system. Circulating profile of estradiol (E_2), testosterone and progesterone were changed at selected time intervals (7,12,17 and 21) of gestation. Bee pollen had no apparently effect on cholesterol value and decreasing effect on triglyceride, HDL-cholesterol and LDL-cholesterol values through gestational period, it produced hypercholesterimia and hyperlipidemia on day 21 of gestation especially at high doses. On determining the concentration of total protein and albumin, it was showed a significant increase particularly, in the second half of pregnancy pertaining to the groups administered bee pollen at a dose of 5 & 10 mg/kg b.w./day. The present results revealed that supplemental of pregnant rats with bee pollen throughout gestational period had harmful effect to a great extent on mothers and fetuses life. [Journal of American Science 2010;6(5):44-53]. (ISSN: 1545-1003). **Key Words:** pollen - pregnancy - rats	Full Text
8	**Neurobehavioral toxicity produced by sodium fluoride in drinking water of laboratory rats** H. El-lethey[1], M. Kamel[1,*], I.B. Shaheed[2] *[1]Department of Animal Hygiene and Management, [2]Department of Pathology, Faculty of veterinary Medicine, Cairo University, Cairo, Egypt*	Full Text

	Correspondence: E-mail: mevy58@yahoo.com* **Abstract: The effect of exposure to different concentrations of sodium fluoride (Na-F) for different durations on learning and memory tasks in rats (non-associative and associative learning) was assessed in our study. Three groups of fifteen pregnant Wistar female rats each, were administered Na-F in drinking water at one of three concentrations; 0, 50 and 100 ppm from second trimester of pregnancy till weaning of their pups at 30 days of age. Pups were then allocated into 5 groups of 20 animals each, where Na-F was administered in three different concentrations with different exposure periods throughout the study. Brain tissue specimens, representing all treatment groups, were taken for histopathological examination. The average body weight gain was significantly lower in group of rats exposed to high Na-F doses for long duration, with distinct hair loss. Open field revealed a significant influence of dose of Na-F on exploratory motor activities (EMA) and emotionality with marked impairment in habituation in rats exposed to high Na-F. Moreover, learning and memory assessed during maze test showed reduced memory retention in rats exposed to high Na-F for long periods. In novelty acquisition test, despite evidence of occurrence of habituation in all groups, a noticeable reduced degree was demonstrated in rats continued to administer high Na-F for long duration. Furthermore, histopathological evaluation revealed distinct neurodegenerative changes of nerve cells especially in hippocampus. Our results suggest that exposure of rats to Na-F in high doses for long duration has detrimental effects on the brain as reflected in diminished learning and memory. [Journal of American Science 2010;6(5):54-63]. (ISSN : 1545-1003). **Keywords:** Neurobehavioral-toxicity-sodium fluoride-drinking water - laboratory rats	
9	**Indirect Boundary Element Method for Calculation of Compressible Flow past a Symmetric Aerofoil with Constant Element Approach** Muhammad Mushtaq* & Nawazish Ali Shah Department of Mathematics, University of Engineering & Technology Lahore – 54890, Pakistan Corresponding Author, e-mail: mushtaqmalik2004@yahoo.co.uk **Abstract:** In this paper, an indirect boundary element method is applied to calculate the compressible flow past a symmetric aerofoil. The velocity distribution for the flow over the surface of the symmetric aerofoil has been calculated using constant boundary element approach. To check the accuracy of the method, the computed flow velocity is compared with the exact velocity. It is found that the computed results are in good agreement with the analytical results. [Journal of American Science 2010;6(5):64-71]. (ISSN : 1545-1003). **Keywords:** Indirect boundary element method, Compressible flow, Velocity distribution, Symmetric aerofoil, Constant element.	Full Text
10	**Phytoplankton Dynamics of River Oli in Kainji Lake National Park, Nigeria during Dry Season.** Adesalu, Taofikat. Abosede. University of Lagos, Department of Botany and Microbiology, Lagos, Nigeria boseadesalu@yahoo.com **Abstract:** This paper examined the phytoplankton of River Oli (Borgu sector) of kainji Lake National Park for the first time. It recorded total of fifty five taxa, belonging to fourma jor divisions; bacillariophyta, chlorophyta, euglenophyta and cyanophyta. The taxa were dominated qualitatively by green algae and quantitatively by euglenoids in particular *Euglena acus*. [The Journal of American Science. 2010;6(5):72-76]. (ISSN 1545-1003). **Keywords:** River Oli, phytoplankton, Nigeria, diversity.	Full Text
11	**Global Food Crisis and its Implications in Nigeria**	Full

	[1]Oparaeke, A.M. and [2]Ofor, M.O., [2]Ibeawuchi I.I [1]Department of Crop Protection, Ahmadu Bello University, Zaria [2] Department of Crop Science and Technology, Federal University of Technology, Owerri mariofor2002@yahoo.com ; ii_ibeawuchi@yahoo.co.uk **Abstract:** The increasing world population is putting pressure on the productive lands, resulting to decline in yield and hence food to feed the ever teeming world population, thus causing food crisis globally. The food crisis has resulted in problems leading to riots in Bangladesh, India, Pakistan, Burma, Egypt, Morocco Ethiopia, France, Spain, Brazil, Venezuela, Kenya and most recently, Madagascar. These problems could have far reaching effect on the fertility i.e. reproductive capacity of the population. Therefore, Food crisis has become a global issue since it occurs in virtually all parts of the world. Some constraints to food production in the world include land policies, poverty, rural-urban migration, bad governance, disease (especially the AIDS scourge). Execution of Research findings from Research institutes, deliberate government policies to alleviate poverty and disease are some of the ways of tackling the crisis. [Journal of American Science 2010;6(5):77-79]. (ISSN : 1545-1003). **Keywords:** food crisis, constraints, world population	Text
12	**A Reliable 3D Laser Triangulation-based Scanner with a New Simple but Accurate Procedure for Finding Scanner Parameters** Ali Peiravi[1], Behrad Taabbodi[2] Ferdowsi University of Mashhad, Department of Electrical Engineering, School of Engineering, Mashhad IRAN Telephone number: (0098) 511-881-5100; Fax number: (0098) 511-8763302 [1] Ali_peiravi@yahoo.com, [2]behrad@yahoo.com **Abstract:** In this paper, a low occlusion laser triangulation 3D scanner based on two different color lasers and one color CCD camera is proposed. By placing a laser source in each side of the camera, occlusion problems are decreased to a minimum. Finding scanner parameters is one of the critical issues in 3D scanner accuracy. A new simple procedure is proposed to accurately find scanner parameters. [Journal of American Science 2010;6(5):80-85]. (ISSN : 1545-1003). **Key words:** 3D scanner, laser triangulation, low occlusion, single camera	Full Text
13	**Intelligence as a predictor of creativity among undergraduate students** Habibollah. Naderi[1], Rohani. Abdullah[2] 1. Department of Educational Studies, University of Mazandaran, Street of Pasdaran, Babolsar, Iran 2. Department of Human Development & Family Studies, University Putra Malaysia, Serdang 43400, Malaysia naderihabibollah@yahoo.com **Abstract:** This research examined how intelligence predicts level of creativity and different constituent of creativity; Something about myself, Environmental sensitivity, Initiative, Intellectuality, Self-strength, Individuality and Artistry among undergraduate students. One hundred and fifty three Iranian students were selected from six Malaysian universities to participate in the research. Data was analyzed using multiple regression analysis. The total variance accounted for by the intelligence factor is 13.5% (multiple R2 = 0.135), F (7, 145) = 3.222, p= .003<0,01). This implies that intelligence is important when considering the factors that influence creativity of students. [Journal of American Science 2010;6(5):86-90]. (ISSN : 1545-1003). **Keywords:** Intelligence, Creativity, Something about myself, Environmental sensitivity, Initiative, Intellectuality, Self-strength, Individuality and Artistry	Full Text

14	**Proximate and Nutrient Analysis of the Locally Manufactured Herbal Medicines and its Raw Material** Javid Hussain [1] , Ali Bahader[1] , Farman Ullah[2] , Najeeb Ur Rehman[1] , Abdul Latif Khan[1] ,[4] Wasi Ullah[1] and Zabta Khan Shinwari[3] [1]Department of Chemistry, Kohat University of Science & Technology, Kohat [2]Department of Biotechnology, Kohat University of Science & Technology, Kohat [3]Department of Biotechnology, Plant Sciences, Quaid-i-Azam University, Islamabad [4]School of Applied Biosciences, College of Agriculture & Life Sciences, Kyungpook National University, Daegu, Republic of Korea. javidhej@yahoo.com **Abstract:** Herbal medicines have unique therapeutic properties and therefore, used in rural areas to cure different diseases. Proximate analysis and elemental composition of the locally manufactured formulations from *Hypericum perforatum, Allium sativum, Zingiber officinalis and Valeriana officinalis* were carried out. The heavy metals including Cu, Ni, Zn, Pb, Co, Cd, Fe, and Cr were determined using Atomic Absorption Spectroscopic standard method. Na and Ca was estimated using flame emission spectrophotometer. *Z. officinalis* has highest percentage of carbohydrate, fats, fiber and energy values while in herbal formulations St. John Wort has the highest. In case of micro analysis, St. John Wort Capsules has highest concentrations of Zn while Valerian has highest concentration of Cu, Co, Cd and Fe compared to others, while in medicinal plant species, the content of Cu, Zn, Co and Fe was highest in *V. officinalis*. The level of macronutrients (Ca and Na) was highest in St. John Wort Capsule, *H. perforatum* and *V. officinalis* . However, the concentration of these nutrients in both the medicinal plants and herbal formations were in the optimum level of WHO standards. . [Journal of American Science 2010;6(5):91-96]. (ISSN : 1545-1003). **Keywords:** Proximate analysis, herbal formulations, nutrient analysis, Pakistan.	Full Text
15	**Effect of stratification on seed germination and seedling performance of wild pomegranate** J.M .S. Rawat*, Y .K . Tomar and Vidyawati Rawat Department of Horticulture, Chauras Campus, HNB Garhwal University, Srinagar (Garhwal), 246174, Uttarakhand, India jms_rawat99@yahoo.co.in **Abstract:** The germination response of *Punica granatum* seeds to different stratification periods was studied. The germination of *P. granatum* significantly improved with increasing stratification periods. Stratification for 30 days at 5^{0}C showed highest germination percentage, longest radicle, maximum root and shoot length, number of leaves and highest survival of seedlings. The longest plumule, maximum collar diameter, highest shoot and root dry weight were recorded with 25 days of stratification. Thus the results of present investigation clearly reveal that 25 to 30 days stratification of *P. granatum* seeds was more suitable for uniform and faster germination as well as best growth in early stage of seedlings as compared to the control. [Journal of American Science 2010;6(5):97-99]. (ISSN : 1545-1003). **Keywords:** wild pomegranate, Stratification, germination, survival per cent, radical and plumule length	Full Text
16	**Application of Different Levels of Zinc and Boron on Concentration and Uptake of Zinc and Boron in the Corn Grain** Farshid Aref Department of Soil Science, Faculty of Agriculture Islamic Azad University of Firouz Abad, Iran	Full Text

	Tel: +989173383896 farshidared@yahoo.com **Abstract:** For the purpose of studying the effect of Zn and B application on the concentration and total uptake of Zn and B in corn grain, a field experiment was conducted in Fars Province, Iran. Treatments including five levels of Zn (0, 15, 30 and 45 kg ha^{-1} and Zn foliar spray) and four levels of B (0, 4, and 8 kg ha^{-1} and B foliar spray) in a completely randomized block design were set up. The findings showed that the presence of Zn prevented from the increase in Zn concentration in the grain, by B application; while B applied in the presence of Zn had no effect on the amount of Zn uptake by the grain. At the level that lacked B, Zn use increased Zn concentration and uptake in the grain but at the levels where B was used, the presence of B prevented from the effect of Zn application on the Zn concentration and uptake in the grain. The minimum concentration and uptake of Zn in the grain was observed by lack of Zn and B use or the control treatment. Therefore, an antagonism between Zn and B was observed as regards concentration and uptake of Zn in the grain. At the highest Zn level, the B use caused an increase in concentration and uptake of B in the grain. Also, at the high B level, application of Zn caused an increase in the B concentration in the grain. Boron use at low levels and Zn solution spray, had no effect on the uptake of B in the grain, but at high B levels, it increased the B uptake in the grain. Therefore, the presence of a high amount of Zn or B in the soil, assisted in the effect of B or Zn on increasing concentration and uptake of B in the plant. That is, a synergism was seen between the Zn and B as effecting the concentration and uptake of B in the grain. [Journal of American Science 2010;6(5):100-106]. (ISSN: 1545-1003). **Keywords:** Interaction, Zinc, Boron, Concentration and Corn grain	
17	**Air quality depreciation index in a coal mining area- a case study from eastern India** Papaya Roy[1], Gurdeep Singh[2], Asim Kumar Pal[3] [1] JRF, Dept. of Environmental Sc.& Engg., Indian School of Mines, Dhanbad, Jharkhand, India [2]Professor& Head, Dept. of Environmental Sc.& Engg., Indian School of Mines, Dhanbad, Jharkhand, India [3]Associate Professor, Dept. of Environmental Sc.& Engg., Indian School of Mines, Dhanbad, Jharkhand, India papiyaroy_ism@yahoo.co.in **Abstract:** The comparison with National Ambient Air Quality Standards does not always depict a true picture of the Air Quality Status of a study area. As an alternative an index that measures depreciation in Air Quality on more realistic terms has been proposed and applied to the ambient air monitoring data collected from Talcher Coalfields in India. Results have been discussed in detail to illustrate the application of the proposed index and utility in bringing out more realistic air quality assessment [Journal of American Science 2010;6(5):107-114]. (ISSN: 1545-1003). **Keywords:** National Ambient Air Quality Standards, value function curves, air quality depreciation index	Full Text
18	**Enhancing the rate of ferulic acid bioconversion utilizing glucose as carbon source** Prakash Kumar Sarangi↑ and Hara Prasad Sahoo PG Department of Botany and Biotechnology, Ravenshaw University, Cuttack, India, 753003 ↑*Author for correspondence: Telephone:* 00 91-674-2471284, 00 91-9437305796; sarangi77@yahoo.co.in Qtr. No-2RB/115, Road No-1, Unit-9, Bhubaneswar, Orissa, 751022 **Abstract:** Work has been carried out to study the effect of glucose addition	Full Text

	into the medium during the biotransformation of ferulic acid into vanillin using *Staphylococcus aureus* . Study showed that microorganism consumed ferulic acid very quickly nearly 5-fold accumulation of vanillin (45.7 mg/l) on 2nd day as compared to 9.8 mg/ml of vanillin accumulation on 7th day without addition of glucose. [Journal of American Science 2010;6(5):115-117]. (ISSN : 1545-1003). **Keywords:** biotransformation, ferulic acid, *Staphylococcus aureus*, glucose, vanillin	
19	**Investigation Of The Influence Of Systematic Errors In Least Squares Estimation** Eltahir Mohamed Elhadi[1,2] and Ehadi E. Ibrahim[2] 1-China University of Geosciences Faculty of Resources, Wuhan, 430074, China, 2-Sudan University of Science and Technology Faculty of Engineering, Khartoum, Sudan, tahirco2006@yahoo.com, hadeena2005@hotmail.com **Abstract:** The least squares method is widely accepted as a computational method, that covers different branches of Surveying and Photogrammetry. Basically, it is applied when the observations contain random errors only. This paper is directed towards the investigation of the effects of systematic errors on the least squares estimates. The main conclusions are: (1) The use of observations containing systematic errors beside the random ones, gives different values for the parameters and the residuals. (2) The value of the standard error of unit weight will increase in the presence of systematic errors. (3) Modeling of systematic errors will enable the evaluation of systematic errors and their effects. [Journal of American Science 2010;6(5):118-123]. (ISSN : 1545-1003). **Keywords:** systematic errors, dimensional adjustment, parameters, residuals.	Full Text
20	**A Situational Analysis of Waste Management in Freetown, Sierra Leone.** Alhaji Brima Gogra[a], Jun Yao[a,*], Victor Tamba Simbay Kabba[b], Edward Hinga Sandy[a], Gyula Zaray[c], Solomon Peter Gbanie[a], Tamba Samuel Bandagba[d] [a] State Key Laboratory of Biogeology and Environmental Geology of Chinese Ministry of Education, School of Environmental Studies and Sino-Hungarian Joint Laboratory of Environmental Science and Health, China University of Geosciences, 430074 Wuhan, PR China. [b] State Key Laboratory of Geological Processes and Mineral Resources of Chinese Ministry of Education, Department of Land Resources Management, School of Management, China University of Geosciences, 430074 Wuhan, PR China. [c] Department of Chemical Technology and Environmental Chemistry, Eötvös University, H-1518 Budapest, P.O. Box 32, Hungary. [d] Department of Hydrology and Water Resources, School of Environmental Science, China University of Geosciences, 430074 Wuhan, PR China. * Corresponding author. *E-mail address:* yaojun@cug.edu.cn (J. Yao) or abgogra@yahoo.co.uk (A. B. Gogra) **Abstract:** Freetown served as save haven for thousands of people from the provinces during the war and suffered a corresponding increase in the rate of generation of waste with very little waste management facility as such facilities were vandalized or completely destroyed. Solid waste management in Freetown has been under variable organizations, with each change further deteriorating the system, bringing it on the verge of collapse. Freetown Waste Management Company (FWMC) is struggling to manage the wastes, hence, the need for the intervention of potential investors/donors to ameliorate this waste	Full Text

	management problem by helping address this problem sustainably for the betterment of the lives of all Freetown residents. Streams of waste are characterized by their sources, the types of waste produced, and the composition and generation rates; therefore, knowledge of these characteristics is required in order to design and operate appropriate waste management systems, hence, the need for the Sierra Leone Government or FWMC to set limits on certain physical characteristics and properties for waste classifications; having significant implications for the collection and disposal of various waste streams, since any material deemed hazardous must be handled with specific protocols. The total quantities and characteristics of waste streams generated are yet unknown, with uncategorized refuse, poorly collected, dumped at the two city's insanitary landfills, hence exposing FWMC workers, scavengers, etc., to the dangers of hazardous waste. This appalling garbage situation needs efficient corrective measures or serious rehabilitation; otherwise it will adversely impact the living conditions of the people, further endangering their environment and health. [Journal of American Science 2010;6(5):124-135]. (ISSN : 1545-1003). **Keywords:** Sierra Leone, Hazardous waste, health care waste, landfills, Freetown Waste Management Company.	
21	**Determination of oil life for crane Liebherr Model D9408 diesel engine by Oil Condition Monitoring** Hojat Ahmadi, and Payman Salami * Department of Agricultural Machinery Engineering, Faculty of Biosystems Engineering, University of Tehran, P.O. Box 4111, Karaj 31587-77871, Iran; salamip@ut.ac.ir **Abstract:** The aim of this study is to choose and investigate the best oil replacement time by oil condition monitoring for crane Liebherr model D9408 diesel engine. This is achieved by investigating different oil sample analyses of crane Liebherr model D9408 diesel engine. According to the majority indices results of the oil analysis, But not for all of them, they had an acceptable function after 160 running hours. The variation percent of plumb in wear debris analysis was above 50 percent. According to the Total Base Number (TBN) analysis, the oil had an acceptable function until 150 running hours. Additive depletion results showed that the oil had an acceptable function after 150 running hours, and absolute variation percent of each additive material after 160 running hours were not more 50 percent. Pollutant materials results of this table showed that the variation percent of each pollutant material after 160 running hours was not more than 50 percent. Also the Particle Quantifier (PQ) results showed that the variation percent of PQ after 160 running hours was not more than 50 percent. Results of oils analysis for viscosity didn't give us a reliable consequence. Right now, the oil of the diesel engine is replacing every 125 hours, but overall the best time for replacing the oil for this engine has been calculated as 150 running hours. [Journal of American Science 2010;6(5):136-141]. (ISSN : 1545-1003). **Keywords:** Oil analysis; Oil Condition Monitoring; Oil time replacement; Machine Condition Monitoring; Wear debris materials	Full Text
22	**Cold Laser as a Complementary Drug in the Treatment of Osteoarthritis** [1]Samir W. Aziz; [1]Bassem M. Raafat; [1]Nahed S. Hasan and [2]Ahmed Hanafy [1]Genetic Engineering and Biotechnology Division, Biochemistry Department, Biophysics Group, National Research Center; [2]Head of Rheumatology Department, Air Force Hospital	Full Text

	Samir_Wassef@yahoo.com ; bassemraafat@hotmail.com ; nahedsha33@yahoo.com ; [2]hanafy2003@hotmail.com **Abstract:** Osteoarthritis is a common cartilage condition and a major cause of pain and disability in older adults. Osteoarthritis most often occurs at the ends of the fingers, thumbs, neck, lower back, knees, and hips. Osteoarthritis hurts people in more than their joints: their finances and lifestyles also are affected. Magnetic susceptibility, dielectric relaxation in the frequency range 100 KHz up to 10 MHz of Hb molecule of osteoarthritic patients receiving anti inflammatory drugs were compared to those received drugs and subjected to soft laser emitted from He-Ne laser with two IR diodes. In addition SOD and whole blood ATP concentration enzyme were measured. The dielectric results indicated that the molecular shape tends to deviate from the non spherical form in patients treated with non steroidal anti inflammatory drugs, to spherical one in those receiving soft laser as an additive drug. Low power laser has significant ability to decrease the pain and suffering of arthritis as well as reducing the disease symptoms. Side effects of medications were reduced in patients received cold laser as a complementary drug. [Journal of American Science 2010;6(5):142-152]. (ISSN : 1545-1003). **Keywords** : Osteoarthritis, dielectric properties, soft laser, hemoglobin, magnetic susceptibility.	
23	**Association of serum Leptin and Adiponectin with Atherosclerosis in obese and non-obese Type 2 Diabetes Mellitus patients** Mohga S Abdalla[1], Hayat M Sharada[1], Ashraf I Amin[2], Nervana Samy[3], Magda Sayed[3], Esmat Ashour[3] and Elham M Youssef-Elabd[3]. [1]Biochemistry Dept, Faculty of Science, Helwan University, Egypt; [2]Clinical Pathology Dept, National Institute of Diabetes & Endocrinology, Cairo, Egypt. [3]Biochemistry Dept, National Research Centre, Dokki, Giza, Egypt Nervana91@hotmail.com **Abstract** : Obesity is a major risk factor for insulin resistance, type 2 diabetes, heart disease, and many other chronic diseases The current study was designed to investigate the endogenous mechanism by which obesity may increase the risk of CVD by examining whether serum adiponectin, Leptin or insulin mediate the association of obesity and type2 diabetes and cardiovascular risk factors in Egyptian adult patients. **Patients and Methods:** This study included 82 subjects, 30 patients suffering from type 2 diabetes and 52 patients suffering from type 2 diabetes together with coronary artery disease (CAD) together with another group having CAD without diabetes. They were classified according to their body mass index (BMI) into obese and non-obese groups, also 25 healthy volunteers were considered as controls. All patients were subjected to anthropometric assessment and laboratory determination of serum Adiponectin, Leptin, insulin and glucose. Insulin resistance was established by homeostasis model assessment (HOMA-IR) Differences in clinical or laboratory parameters among groups were compared by using one-way ANOVA test. **Results** revealed highly significant decrease in Adiponectin levels and highly significant increase in serum Leptin in non obese groups (G1 (T2D), G2 (CAD) and G3 (T2D+ CAD) as compared to controls. However, there were no statistical variations between non obese groups when compared to each others. HOMA-IR showed highly significant increase in non obese groups as compared to both controls and each other. Also, the results showed highly significant decrease in Adiponectin and highly significant increase in Leptin in obese groups (G4 (T2D), G5 (CAD) and G6 (T2D+CAD) when compared to	Full Text

	controls. However, there were no statistical variations between obese groups when compared to each others as regard Adiponectin, while Leptin showed statistical increase between (G4) and (G5) groups when compared to each others, HOMA-IR showed highly significant increase in the two obese groups only (G4 and G6) as compared to controls, while there was no significant variation in (G5) when compared to controls. Moreover, there was a significant increase in all obese groups when compared to each other. Also, there was significant correlation between serum Adiponectin and Leptin in obese DM patients. **Conclusion:** The coexistence of correlation between serum leptin and Adiponectin levels in addition to increase of serum leptin and decrease serum Adiponectin levels in obese DM patients in the current study; support the hypothesis of their susceptibility to atherosclerosis. [Journal of American Science 2010;6(5):153-164]. (ISSN: 1545-1003). **Keywords** : Type2 Diabetes, Cardiovascular disease, Adiponectin, Leptin, HOMA-IR	
24	**Effects of L-carnitine on growth performance of Nile tilapia (*Oreochromis niloticus)* fingerlings fed basal diet or diets containing decreasing protein levels** Abdel-Fattah M. El-Sayed[1*], Nabil F. Abdel-Hakim[2], Hanan A. Abo-State[3], Khaled F. El-Kholy[4], Dosoky A. Al-Azab[1] [1]Oceanography Department, Faculty of Science, Alexandria University, Alexandria, Egypt [2]Animal Production Department, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt. [3]Animal Production Department, National Research Center, Dokki, Giza, Egypt. [4]Animal Production Research Institute, Utilization of by–Products Department, Ministry of Agriculture and Land Reclamation, Dokki, Giza, Egypt hanan_abostate@yahoo.com **Abstract:** The effects of L-carnitine on growth rate, feed utilization efficiency and protein sparing of Nile tilapia (*Oreochromis niloticus* L.) fingerlings were investigated in two consecutive experiments. In experiment 1, triplicate groups of 10 fingerlings (4.16 ±0.07) each were stocked in 85 L glass aquaria, filled with 70 L dechlorinated tap water. Five levels of L-carnitine (0, 75, 150, 300, 450 mg/kg) were separately added to the basal diet (30% crude protein and 18.74 MJGE/kg). The fish were fed the diets, at a daily rate of 5% BW, twice a day for 70 days. The results revealed that fish growth rates, feed utilization and whole body protein and lipid levels were increased with increasing L-carnitine levels. In experiment 2, Nile tilapia fingerlings (4.3±0.1 g) were fed diets containing decreasing levels of protein (30, 25, and 20%) and supplemented with 450 mg L-carnitine/kg diet, for 84 days. Fish performance was not significantly affected with decreasing dietary protein levels up to 20%. These results suggest that dietary inclusion of L-carnitine in Nile tilapia diets may significantly reduce dietary protein requirements and may facilitate the use of fatty acids for obtaining energy and consequently, can spare dietary protein for somatic growth. [Journal of American Science 2010;6(5):165-172]. (ISSN: 1545-1003).	Full Text

	Keywords: L-carnitine, Nile tilapia, performance, protein sparing.	
25	**ESTIMATION OF TOXIC METALS IN CANNED MILK PRODUCTS FROM UNLAQUERED TIN PLATE CANS.** [1] Itodo U. Adams and [2] Itodo U. Happiness [1]Department of Applied Chemistry, Kebbi State University of Science and Technology, Aliero, Nigeria. [2]Department of Chemistry, Benue State University, Makurdi, Nigeria itodoson2002@yahoo.com **Abstract:** Branded canned milk (B1, B2, B3 and B4) were selected in triplicate, using market basket approach. The samples were pre-treated and analysed for heavy metals. Their physicochemical variables were estimated. The metal concentration (in μgg^{-1}, using AAS) of some toxic metals compared to those of uncanned dairy products include: 0.02±0.008 (006±0.003); 1.61±0.21 (0.01±0.01); 1.47±0.73 (0.01±0.01); 1.64±0.66 (0.05±0.03) and 1.75±0.29 (1.54±1.2) for Cd, Co, Cr, Ni, and Pb found in canned and (uncanned) milk products respectively. Further analysis revealed that Nickel contents in milk is less, compared to those of canned fish products. Unlike Cd contents, Cr and Pb concentration were above the threshold limit values (TLV) of $2.0 \mu gg^{-1}$. . [Journal of American Science 2010;6(5):173-178]. (ISSN: 1545-1003). **Key words:** Toxic metals, canned milk, Corrosion, Health	Full Text
26	**Green Tea Extract Ameliorate Liver Toxicity and Immune System Dysfunction Induced by Cyproterone Acetate in Female Rats** Heba Barakat Department of Biochemistry and Nutrition, Women`s College, Ain Shams University, **dr_hebabarakat@yahoo.com** **Abstract:** Green tea, consumed worldwide since ancient times, is considered beneficial to human health. The present study aimed to evaluate the effect of green tea extract (GTE) on liver damage and immune system function in female rats treated with cyproterone acetate (CPA). Forty healthy female adult albino rats were randomly assigned to four groups. Group (1) was fed on a standard diet as a control. Group (2) was fed on a standard diet and received an intraperitoneally injection of 25mg/Kg/day. Group (3) was fed on a standard diet supplemented with 1 g GTE% and received a daily injection. Group (4) was fed on the supplemented diet for 7 days prior to receiving the daily injection. The experimental duration lasted for 3 weeks initiated from the first injection. The results showed CPA alone led to diminish liver function, hepatic antioxidant enzyme activities and elevated hepatic oxidative stress and serum IgG and IgM levels comparing with the control group of rats. However, the injection of GTE either along with or prior to the CPA treatment could significantly improve the function of liver, hepatic oxidative stress and hepatic antioxidant status and elevate the IgG and IgM levels. These data suggested that, GTE possesses a protective effect on the liver against the inducer CPA toxicity by increasing auto immunity and countering the hepatic oxidative stress. [Journal of American Science 2010;6(5):179-185]. (ISSN: 1545-1003). **Key words:** Cyproterone acetate, green tea extract, liver toxicity, oxidative stress, immunity	Full Text

27	**Screening of some Biopesticides for the control of *Callosobruchus chinesis* in Stored Black Beans (*Vigna mungo*) in Imo State.** [1] Dialoke Sunday Ani [1] Department of Crop Science and Technology, Federal University of Technology, P.M.B 1526, Owerri Imo State, Nigeria. akuamka@yahoo.com **Abstract:** The influence of some oil extracts comprising Cashew nut oil (CNO), Coconut oil (CONO), Udara nut oil (UDNO), and Neem leaf oil in controlling stored black beans weevil (*Callosobruchus chinesis*) was investigated. The experiment was laid down in the laboratory using Completely Randomized Design (CRD). The results showed that the number of eggs and exit holes of C. chinesis were not significant at 5% probability level before treatment with the extracts. Then after two months in storage the black beans were treated with the extracts and there was significant reduction of rate of oviposition and number of exit holes. The plots treated with coconut oil extract proved more effective than other oils and was therefore recommended for use by farmers for black beans storage under our agro-ecological zone. [Journal of American Science 2010; 6(5):186-188]. (ISSN: 1545-1003). **Keywords:** Sceening, biopesticides, black beans, *callosobruchus chinesis*, extracts	Full Text
28	**Neurobehavioural, neurochemical and neuromorphological effects of cadmium in male rats** Hussein A. Kaoud[1]*, Mervat M. Kamel[1], Abeer H. Abdel-Razek[1], Gehan M. Kamel[2] and Kawkb A. Ahmad[3] [1] Departments of Animal, Hygiene and Management. [2] Department of Pharmacology. [3] Department of Pathology. Faculty of Veterinary Medicine, Cairo University, Cairo, Egypt. *ka-oud@link.net **Abstract:** AS Cadmium is a widespread toxic environmental and industrial pollutant. The present study was carried out to investigate the possible effect of cadmium chloride ($CdCl_2$) on memory, exploratory motor activity (EMA) and motor balance in male rats. Forty five male Wistar rats weighing (100-120 gm) were administered $CdCl_2$ in drinking water at one of three concentrations; 0, 5 and 50 mg/L dissolved in water for a period of 60 days. Memory retention was evaluated through open-field habituation test (non associative learning), classic maze test (associative learning) as well as working spatial memory in a Y-maze. Moreover, exploratory motor activity and motor coordination were evaluated. Brain tissue specimens, representing all treatment groups, were taken for histopathological and biochemical examination. The average body weigh	Full Text

	significantly lower in group of rats exposed to high $CdCl_2$ doses. Open field revealed marked impairment in habituation with noticed influence on both anxiety and fear in rats exposed to high $CdCl_2$. Moreover, learning and memory assessed during classic maze test and Y-maze test showed reduced memory retention in cadmium exposed animals as compared to control group .In novelty acquisition test ,a reduced exploratory motor activity in rats exposed to high $CdCl_2$ was noticed Additionally ,complex motor behaviour (motor coordination)was significantly impaired due to cadmium intoxication. Furthermore, ,histopathological and biochemical evaluation revealed distinct neurodegenerative changes of nerve cells especially in hippocampus , inhibition of cholinesterase activity ,aswell as decrease in the antioxidant enzymes activity (GST and SOD). Overall, these results suggest that intoxication with cadmium chloride has potentially deleterious effects on brain as reflected in impairment learning and memory. Also exploratory motor activity and motor coordination were reduced. [Journal of American Science 2010; 6(5):189-202-]. (ISSN 1545-1003). **Keywords:** Cadmium intoxication; learning and memory; motor activity; hippocampus, AChE; SOD ;GST ;Rats	
29	**Antioxidative properties of flavonoids from *Cheilanthes anceps* Swartz.** Sanyukta Chowdhary [a] ,D .L .Verma [b] ,Rachana Pande [b] and Harish Kumar [c*] [a] Department of Botany, Kumaun University, S. S. J. Campus, Almora-263601, India. [b] Department of Chemistry, Kumaun University, S. S. J. Campus Almora-263601, India [c*] Department of Botany and Microbiology, Gurukul Kangri University, Haridwar-249404, India **Email*:** harish2129@gmail.com ,hellosanyukta28@gmail.com **Abstract:** Antioxidative guided chromatographic fractionation of BuOH fraction from aqueous-ethanolic extract of fern fronds of *Cheilanthus anceps* gave flavonol glycosides, Quercetin-3-O-δ–L-rhamnopyranosyl(1→ 2)-β-D-glucopyranoside-7-O-β-D-glucopyranoside, Kaempferol-3-O-δ-L-rhamnopyranosyl (1→ 2)-β-D-glucopyranoside-7 O-β-D-glucopyranoside, Quercetin-3-O-β-D-glucosyl (1→ 2)-β-D-galactoside-7-O-β-D-glucoside, Quercetin-3-methyl ether-5-O-glucoside, Quercetin-3-O-glucoside and Kaempferol-3-O-glucoside. Of these flavonol-glycosides, the glycosides of Quercetin showed prominent antioxidative activity compared to Kaempferol glycosides. [Journal of American Science 2010; 6(5):203-207]. (ISSN : 1545-1003). **Keywords:** *Cheilanthes anceps* ,Flavonol glycosides, Antioxidative activity	Full Text
30	**Effect of Treatment with Antifibrotic Drugs in Combination with PZQ in Immunized *Schistosoma mansoni* Infected Murine Model** Ibrahim Rabia[1] ,Faten Nagy[2] 2,Eman Aly[1] ,Amina Mohamed[3] Fayza EL-Assal[3] and Azaa El-Amir[3] 1 Parasitiology, 2 Immunology Departments, Theodor Bilharz Research Institute, (TBRI) Giza, Egypt and 3 Faculty of Science, Cairo Univeresity.	Full Text

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Abstract: The main problem in schistosomal hepatic morbidity is fibrosis and extensive scarring induced by living eggs. In this study, we tried to study the effect of treatment using antihelminthic drug (PZQ) and/or antifibrotic drugs (PTX and silymarin) in combination with immunization. The parasitological parameters, the dynamics of serum-specific immunoglobulins and splenic cytokines associated with changes in granuloma diameter were assessed. Naïve mice were immunized intravenously with 10 ug of SEA in three doses at 2 days intervals 6 weeks before infection. Animals were infected by tail immersion with 100 cercariae and divided into several groups. Three groups were treated with PZQ, PTX or silymarin administered alone. Another two groups were treated with PZQ combined with PTX or silymarin. All treated animals and respective controls were sacrificed 12 weeks post infection. Immunization did not affect worm reduction, but slight decrease in granuloma diameter, increase in immunoglobulins and cytokines was observed. Reduction in worm burden was associated with reduction in ova count and changes in oogram pattern which were mainly due to PZQ treatment. Increasing reduction in granuloma diameter, elevation of immunoglobulins and cytokines levels were observed in the groups treated with PZQ alone or combined with PTX or silymarin. In conclusion, in this study, treatment with PZQ complemented with immunization resulted in significant reduction of parasitological parameters and rise of specific Igs. Addition of antifibrotic drugs PTX or silymarin to PZQ, potentiated an antipathology effect which minimized and ameliorated liver fibrosis by inhibition of HSC activation and accentuation of the effect of suppressor Treg cells. [Journal of American Science 2010;6(5):208-216]. (ISSN: 1545-1003).

Key word: *Schistosoma mansoni,* Praziquantel, Pentoxifyllin, silymarin

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FMSIND: A Framework of Multi-Agent Systems Interaction during Natural Disaster

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Abstract: Multi-agent systems have a potential to collaborate with each other using their language but the challenge is to make them work intelligently during the situation of catastrophic disaster. In such situations, it is extremely viable to diagnose and dispose resources like ambulances, volunteers, etc. timely, in order to help out people and reduce casualties. We studied the existing frameworks and methodologies in this area but none of them satisfy the requirements on the whole. If one lacks the coordination between agents then other has deficiency of decision support system. This was a motivation for us to propose a framework that covers all aspects of the problem. In this paper, we propose an algorithm to find out the plans of other collaborative agents for coordination and a complete architecture of the framework. The decision support system has been incorporated in the framework for taking optimized decisions. We take a scenario as a case study to verify and validate the

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proposed framework. .We also show the implementation of interaction among the agents. [Journal of American Science 2010; 6(5):217-224]. (ISSN: 1545-1003).

Keywords: Agents,Multi-agent Systems, JADE,Decision Support Systems

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Postpartum Performance Of Buffaloes Treated With Gnrh To Overcome The Impact Of Placenta Retention

El-Malky,O.M.;Youssef*,M.M.;Abdel-Aziz,N.A.and Abd El-Salaam,A.M.
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Abstract: This study aimed to investigate impacts of GnRh treatment on post-partum productive and reproductive performance of buffaloes subjected to placenta retention. A number of 30 female buffaloes were used in the study among them 20 buffaloes were detected with retained placenta (RP), while 10 buffaloes were normally calved (NRP). Buffaloes with RP were divided into two groups (10 buffaloes each) where group (RPT) were injected with 10 ml GnRH at the 7th day postpartum and group (RPC) served as control group. Blood samples were collected twice weekly from each buffalo cow during late pregnancy and postpartum period for determination of progesterone (P4), estradiol 17β (EST) as well as some blood metabolites. Placental tissue samples were taken from four animals with normal and retained placenta for histological examination. Postpartum loss in live body weight was greater (P <0.01) in NRP buffaloes than animals with RP. Differences between groups in calf birth weight (CBW) were insignificant while differences between newborn males and females were highly significant (P < 0.01). Volume of fetal fluids was greater in NRP group comparing with the other groups (P < 0.01) whereas no significant differences were detected in weight of fetal membranes between groups. Time elapsed for placenta expulsion in was 4.23, 17.26 and 18.7 hr. in NRP, RPT and RPC groups, respectively. Sex of newly born calf had only a significant effect (P < 0.01) on CBW and CBW/DAM. The normal group of buffaloes (NRP) achieved the least (P < 0.01) calving interval (CI) and days open (DO) as compared with buffalo groups with RP. However, GnRH treatment had significantly (P < 0.05) reduced CI and DO for group RPT than that for group RPC by 10.41% and 28.33%, respectively. No. of services per conception declined in response to GnRH treatment (2.6) when compared with RPC group (3.5). Differences between the studied groups in milk traits (total milk yield, days in milk and daily milk yield) were highly significant (P < 0.01) not only in the current milking season but also in the previous and next milking season. Buffaloes treated with GnRH (RPT group) achieved greater milk productivity (13.27%) than RPC group. Postpartum concentrations of P4 were significantly (P <0.05) greater in NRP animals than that in buffaloes with RP throughout the experimental months. GnRH treatment increased significantly (P < 0.05) postpartum EST concentrations during 5th to 8th week as compared with non-treated animals. Concentrations of all studied metabolic parameters were relatively lees in RP groups than that in non retained group (NRP). GnRH treatment had relatively ameliorated the metabolic function in treated buffaloes via increasing concentrations of blood total protein, glucose, creatine, creatinine, clacium and inorganic phosphorus. The histological

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	sections revealed dismaturation of the RP denoted by limited number of trophblastic giant cells, decomposition and fragmentation of the placental tissue and chorionic villi concomitant with hyperplasia in the chorionic epithelial cell of the villi. [Journal of American Science 2010; 6(5):225-233]. (ISSN: 1545-1003). **Keywords:** Buffaloes, retained placenta, GnRH, productive and reproductive traits	
33	**Physico-Chemical Characteristics, Microbial Assessment And Antibiotic Susceptibility Of Pathogenic Bacteria Of Ismailia Canal Water, River Nile, Egypt** [1]Abdo, M. H.; [1]Sabae, S. Z.; [2]Haroon, B. M.; [2]Refaat, B M.; [2]Mohammed, A. S. 1: National Institute of Oceanography and Fisheries, Inland Waters and Aquaculture Branch. 2: Faculty of Science, Al-Azhar University mh_omr@yahoo.com **Abstract:** Thirty two water samples were collected during four successive seasons during the period between August, & April 2006 along the area extending from El-Mazallat square to Mostorud area (River Nile, Egypt). These samples represented the effect of the factories effluent discharge onto the canal on water quality. Physical characteristics (air and water temperature, transparency and electrical conductivity) and chemical characteristics (pH, DO, BOD, COD, CO_3^-, HCO_3^-, SO_4^{--}, NO_2^-, NO_3^-, NH_3 and PO_4^{3-}) were measured to identify the Ismailia Canal water quality. These measurements showed slight variations during different seasons at different stations. Additionally, the bacteriological analysis for water samples was done and included the total viable bacterial counts at 22 and 37 ^{0}C and the bacterial indicators of faecal pollution (*total coliformas, faecal coliforms* and *faecal streptococci*). The pathogenic bacteria were identified as ***E.coli, Salmonella, Choleraesuis,*** ***Streptococcus faecium and Pseuedomones aeruginosa.*** Antibiotics susceptibility testing was selected, the families Beta-lactams (ampicillin & cefeprime), Aminology cosides (gentamycin & Kanamycin), Macrolides (erythromycin, spiramycin, tylosin and spectinomycin), Tetracyclines (oxytetracycline base, doxycycline HCl and chlorotetracycline HCl) and Amino acids (neomycin & streptomycin). Pathogenic bacterial isolates revealed resistance against most applied antibiotics pathogenic bacterial isolates were also subjected to fifteen herbal extracts. The test herbal extract extended an antimicrobial activity, ***P. aeruginosa*** was sensitive to coriander and ***E. coli*** was sensitive to Cinnamon. [Journal of American Science 2010; 6(5):234-250]. (ISSN: 1545-1003). **Key words:** Physico-chemical characteristics, microbial diversity, antibiotic susceptibility, Ismailia Canal, Egypt	Full Text
34	**New Synthesis of Furochromenyl Imidazo [2a-**	Full Text

1b] Thiazole Derivatives, Studies on Their Antitumor Activities.

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Abstract: 4, 9-Dimethoxy-5-oxo-5H-furo [3, 2-g] benzopyran-6-carboxaldehyde 1 was condensed with 2-thiox-4-imidazolinone 2 to form 3. Treatment of 3 with α-chloroacetyl chloride gave 4. Cyclization of 4 with acetic anhydride took place by heating to give 5. Condensation of 5 with aromatic aldehydes gave the arylidene derivatives 6a-c. Coupling of 5 with diazonium salts gave azo derivatives 7a-c. The work was further extended to investigate the behavior of 3 with 1, 2-dichloroethane to give (4Z)-2-(2-chloroethylthio)-4-((4, 9-dimethoxy-5-oxo-5H-furo [3, 2-g] chromen-6-yl) methylene)-1H-imidazol-5(4H)-one 8. Then 8 was cyclized with acetic anhydride to give (6Z)-2, 3-dihydro-6-[(4, 9-dimethoxy-5-oxo-5H-furo-[3, 2-g] chromen-6-yl) methylene] imidazo [2, 1-b] thiazol-5-(6H)-one 9. [Journal of American Science 2010; 6(5):251-256]. (ISSN: 1545-1003).

Key words: Furochromon; arylidene derivatives; azo; antitumor activity

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The effect of *Botrytis Cinerea* and *Rhizopus Stolonifer* on pre-harvest energy losses of strawberry production in Iran

Full Text

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Abstract: It is well accepted that agricultural production must be increased considerably in the foreseeable future to meet the food and feed demands of a rising human population and increasing livestock production. Crop protection plays a key role in safeguarding crop productivity against competition from weeds, animal pests, pathogens and viruses. The aim of this study was to evaluate the amount of energy losses caused by pre-harvest strawberry losses in the Kurdistan province of Iran. These losses were caused by *Botrytis cinerea* (Gray Mold) and *Rhizopus stolonifer*. The average pre-harvest losses of strawberry production were found to be 6% in this study, thus the average losses were found to be about 544.3 kg ha^{-1}. The total energy losses of strawberry production in the study area are estimated to be 2.585 TJ. This amount of losses is equal to 422.5 BOE (Barrel of Oil Equivalent), also the total pre-harvest strawberry losses are equal to 1,673,412.3 $. Tools and techniques are needed to assist in developing strategies that can lead to higher food production, prevent crop production losses, and ensure minimal greenhouse gas emissions while maintaining soil fertility. [Journal of American Science 2010;6(5):257-260]. (ISSN: 1545-1003).

	Key words :*Botrytis* ; energy losses; Kurdistan; Iran;*Rhizopus* ; strawberry	
36	**Acid Washing Of "Zeolite A": Performance Assessment And Optimization** Heba A. Hanfi[(1)], Shadia R. Tewfik[(1)], Mohamed H. Sorour[(1)], Nabil Abdel Moneem[(2)] [(1)]Chemical Engineering and Pilot Plant Department, National Research Center, Dokki, Cairo, Egypt [(2)]Chemical Engineering Department, Faculty of Engineering, Cairo University, Giza, Egypt Email:hi_heba@yahoo.com **Abstract** Zeolite A has been developed, characterized and tested for the removal of chromium (III) from solutions. To facilitate the industrial manufacture of zeolite A, numerous experimental trials and testing procedures have been undertaken to develop prediction methodology for the synthesis of zeolite A with specified characteristics. However, conditioning of the prepared zeolite to the required pH and purity necessitated extensive washing cycles and time. In this paper an approach has been developed, through modeling and optimization techniques, to predict the range of operating parameters governing the washing of zeolite A using oxalic acid and elucidate the mechanism governing the acid washing process in a batch stirred tank reactor. Several washing parameters have been addressed comprising acid gram equivalent, liquid to solid ratios, temperature and stirring speed. It is thus possible through this prediction methodology to define the conditions required for minimum washing costs and high chromium (III) uptake. Determination of the effectiveness factor indicates that the chemical reaction controls the washing rate. The results of this paper indicate that the amount of water required for washing decreased from 310 m^3 to 20 m^3 per ton zeolite A using oxalic acid, consequently the washing cost decreased by about 22%. The results of optimization indicate that the acid washed zeolite A was able to adsorb 179 mg Cr^{3+}/g as compared to 184 mg Cr^{3+}/g for the conventionally washed zeolite A. [Journal of American Science 2010; 6(5):261-271]. (ISSN: 1545-1003). **Key words:** zeolite A, washing, prediction, optimization, chromium	Full Text
37	**Effect of Spearmint Essential Oil on Chemical Composition and Sensory Properties of White Cheese** Mervat I. Foda[2]*; M A. El-Sayed[2]; Amal A. Hassan[2]; Nagwa M. Rasmy[2] and Marwa M. El-Moghazy[1] [1*]Dairy Dept., National Research Center, Cairo, Egypt. [2] Food Science Dept., Fac. of Agric. Ain Shams Univ., Cairo, Egypt. * Corresponding author: mervatlm@yahoo.com, **Abstract:** Spearmint (*Mentha spicata*) the most common herb in the Mediterranean region, widely used as a source of essential oil for flavoring, spearmint essential oil contains about 24 constituents representing 98.45% of the total essential oil; the main compounds are carvone (68.58%) and limonene (16.42 %). Two lipid model systems (DPPH scavenging activity and β-carotene bleaching test) were used to determine the antioxidant activity of	Full Text

	spearmint essential oil. White cheeses with different concentrations of spearmint essential oil (0.5 to 2.5 ml/kg retentate) were prepared and stored at (7 ^{0}C ± 2) for 5 weeks. The chemical composition and ripening index of spearmint white cheese were determined. Obtained results showed that lower concentrations of spearmint essential oil increased titratable acidity values significantly, while ripening index was increased significantly by increasing the concentration of essential oil. Prolonging the cold storage period for five weeks increased these values significantly. Panel study showed that lower concentration of essential oil got the highest total acceptability scores. [Journal of American Science 2010; 6(5):272-279]. (ISSN : 1545-1003). **Key words:** white cheese, spearmint, essential oil, sensory evaluation, antioxidant activity, ripening index	
38	**Alginate/ Polyvinyl Alcohol - Kaolin Composite for Removal of Methylene Blue from Aqueous Solution in a Batch Stirred Tank Reactor** M.M. Abd El-Latif[1], M.F. El-Kady[1], Amal M. Ibrahim[2] Mona E. Ossman[3] Mubarak City for Scientific Research and Technology Applications, Institute of New Materials & advanced technologies, Fabrication Technology Department, Alexandria, Egypt.[1] Surface Chemistry and Catalysis Laboratory, National Research Center, Cairo, Egypt.[2] Mubarak City for Scientific Research and Technology Applications, Informatics Research Institute, IRI, Alexandria, Egypt.[3] **Abstract:** The investigation of possible use of Alginate/ polyvinyl alcohol - kaolin composite instead of free kaolin in the removal of methylene blue from aqueous solutions was studied. Various experiments have been carried out using batch adsorption technique to study the effects of the process variables, which include contact time, beads diameter, beads swelling, organic-kaolin composite dosage, initial dye concentration, pH, agitation speed and solution temperature on the adsorption process. In the batch kinetic study of methylene blue, the order of the reaction, the half-life and the rate constant were determined. Numerical correlations using regression analysis for maximum percentage removal of dye with operating condition of the process were presented. The result showed that the adsorption attained to equilibrium in 360 min and the kinetics followed first order in nature. [Journal of American Science 2010; 6(5):280-292]. (ISSN : 1545-1003). **Keywords:** Adsorption, Cationic dye, Isotherms, Kaolin composite, binding polymers	Full Text
39	**Assessment Of Cell Kinetics In The Tissues Of Brownbanded Bamboosharks (*Chiloscyllium Punctatum*) By Using Bromodeoxyuridine (Brdu) And Anti-Brdu Monoclonal Antibody**	

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Abstract: The 5-bromo-2'-deoxyuridine (BrdU) labeling method has been used to assess the quantity of proliferative potential in organs and tissues in various mammals. For application of this method in fish, it was necessary to determine conditions that optimize the detection of the BrdU epitope. In the present investigation, we investigated the localization of proliferative cells as well as various conditions for detection of S-phase cells in the tissues of adult brownbanded bamboosharks by means of the BrdU immunohistochemical method. Our results demonstrated that BrdU-positive cells were satisfactorily demonstrated in the tissues of brownbanded bamboosharks treated with BrdU at a dose of 6 mg/kg or higher. However, there was no difference in BrdU reactivity between routes of administration, including intravenous, subcutaneous and intraperitoneal injections. BrdU-incorporated cells were detected both in formalin-fixed and 70% ethanol-fixed tissues with enzymatic treatment and acid hydrolysis in the shark tissues, while formalin-and ethanol-fixed brownbanded bamboosharks tissues that did not undergo the enzymatic procedure showed no BrdU reactive cells. Importantly, samples were quickly fixed in heated formalin solution and treated with 5N HCL and 0.01% Nagarase at 37 C for 30 seconds to one minute. In conclusion, the BrdU labeling method was useful in a cell kinetic study detecting S-shaped cells in sharks, as in other mammals. [Journal of American Science 2010;6(5):293-299]. (ISSN: 1545-1003).

Keywords: BrdU, IHC, Labeling method, Brownbanded bamboosharks (*Chiloscyllium punctatum*)

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Creative Perception Inventory as a predictor of I.Q

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Abstract: This research examines the extent to which the level of creativity and different components of creativity: What Kind of Person Are You , Acceptance of authority, Self confidence, Inquisitiveness, Awareness of others, Disciplined Imagination among undergraduate students predict intelligence. Respondents in the research comprises of 153 from six Malaysian universities. Multiple regression analysis reveals that a total variance in intelligences accounted for by the creativity factors is 16.4 % (multiple R2 = 0.164, (6, 146) = 4.761, p = .000). This implies that creativity is significant when considering the factors that influence the intelligence of students. [Journal of American Science 2010;6(5):1-5]. (ISSN: 1545-1003).

Keywords: Intelligence, Creativity, What Kind of Person Are You, Acceptance of authority, Self confidence, Inquisitiveness, Awareness of others, Disciplined Imagination.

1. Introduction:

We have several definitions from theoreticians and researchers for intelligence. Sternberg (1985a, 1985b) views the conceptions and definitions on the nature of intelligence and determines an underlying theme beginning at the research that mentions that intelligence is a capacity to learn from experience and to adapt to one's environment. Researcher's (Sternberg et al., 1981) refined their thoughts on intelligence to include verbal intelligence, problem solving and practical intelligence (Sternberg, 1985a). (Sternberg, 1985a) explained his first ideas by stating; "Intelligence is a mental activity directed toward purposeful adaptation to and selection and shaping of real-world environments relevant to one's life". Sternberg & Lumbart (1991) cited, "the two main aspects of intelligence (the ability to define and redefine problems and the ability to think insightfully) are relevant to creativity".

Cattell (1971) suggested that intelligence comprises general ability at the top of a hierarchy, followed by fluid and crystallized abilities. Crystallized intelligence is the ability to bring previously acquired often culturally defined, problem-solving methods to bear on the current problem (Woodcock, McGrew, & Mather, 2001). Note that this implies the problem solver both knows the methods and recognizes that they are relevant in the current situation. Fluid intelligence is the ability to develop techniques for solving problems that are new and unusual, from the perspective of the problem solver (Woodcock et al., 2001). To conclude, intelligence may mean, it seems to involve the ability to learn, to solve problems, and to behave in a way that allows a person to achieve goals effectively. Intelligence in this study is a fluid intelligence.

Furnham & Bachtiar (2008) stated there are more than 60 definitions of creativity with no single authoritative and agreed upon definition, or operational measure. An easy meaning of creativity view it as generating something novel, original, an expected (Sternberg & Lubart, 1999). According to Palaniappan (2007b) creativity is some of the many intellectual constructs that has been defined as many different ways as the number of researchers investigating them. For the purpose of this study, creativity is investigated as a personality (KTCPI as the measure), because it is new measure for assessment of creativity by this instrument. Creativity Perception refers to the perception of oneself as being creative and capable of creative productions. It is one of the most important personality traits related to creativity (Biondi, 1976; Davis, 1983).

The conception of creativity is regularly related with intelligence (Furnham & Bachtiar, 2008), but according to note's (Furnham & Bachtiar, 2008) several early researchers (Andrews, 1930; Getzels & Jackson, 1962; McCloy. W and N.C. Meier, 1931) have been shown the relation between creativity and intelligence has only modest correlations (r= .07, .22, .26, respectively). In a study conducted by (Olatoye & Oyundoyin, 2007) on the creativity and intelligence among 460 students were randomly selected from 20 secondary schools, it was found that intelligence quotient (as measured by Slosson's Intelligence Test) was significantly related to creativity (Ibadan Creative Assessment Scale). Their finding has been shown intelligence quotient accounted for 8% of variance in creativity (R2 = 0.80). This percentage is statistically significant. According to this result also intelligence quotient significantly predicts each of the four components of creativity (fluency, originality, flexibility and creativity motivation).

Furnham & Bachtiar (2008) intelligence (as measured by the Wonderlic Personnel Test) was not correlated with any of the creativity (as measured by the Divergent Thinking , Biographical Inventory of Creative Behaviours , Self-Rating of creativity , Barron–Welsh Art Scale). Funchs & Karen (1993) studied on the creativity and intelligence in which four hundred and ninety six preschoolers of children looking admission to a special program for gifted preschoolers participated, it was found that creativity (as assessed by the Thinking Creativity in Action and Movement Scales) was significantly related to intelligence . According to Naderi, H.& Abdullah, R. (2009) studies creativity predicts intelligence, however the fact is that the value is low i.e. 13.5% (multiple R2 = 0.135), (F7, 145=3.222,

p<0.05). They found no significant-relation between each of the creativity components (Environmental Sensitivity, Initiative, Intellectuality, Self-strength, Individuality and Artistry) except Environmental Sensitivity).

This research was hence designed to examine the influence of creative perception inventory and the different component of creativity; What Kind of Person Are You, Acceptance of authority, Self confidence, Inquisitiveness, Awareness of others, Disciplined Imagination on intelligence among Iranian undergraduate students in Malaysian Universities. This study look for investigate the following hypotheses; creative perception inventory will not significantly predict the intelligence among students. The components of creativity will not significantly predict intelligence among the students.

2. Methodology

2.1Sample

One hundred and fifty three Iranian undergraduate students in Malaysian Universities (31.4% females and 68.6% males) were recruited as respondents in this study. Their ages ranged from 18-27 years for females and 19-27 years for males.

2.2 Measures

Catell Culture Fair Intelligence Test

To evaluate the intelligence, every student was administered by a Scale 3 of the Catell Culture fair Intelligence Test (CFIT-3a & b). Roberto Colom, Botella, & Santacreu (2002) reported that this test is a well-known test on fluid intelligence (GF). Participants completed Cattell's culture fair intelligence test battery to assess individual differences in fluid intelligence. Cattell's Culture Fair Intelligence Test (1971), which is a nonverbal test of fluid intelligence or Spearman's general of intelligence. This test contained four individually timed subsections a) Series, b) Classification, c) Matrices, d) Typology, each with multiple-choice problems progressing in difficulty and incorporating a particular aspect of visuospatial reasoning. Raw scores on each subtest are summed together to form a composite score, which may also be converted into a standardized IQ.

Khatena-Torrance Creative Perception Inventory (KTCPI)

Every student was examined using a Khatena-Torrance Creative Perception Inventory (KTCPI) to measure the creative perception of the undergraduate students (A. K. Palaniappan, 2005). The KTCPI instrument was comprised of two subscales, namely, "Something About Myself" (SAM) and "What Kind of Person Are You" (WKOPAY)?

Creativity in this study is a What Kind Of Person Are You?. The (WKOPAY), which is a creative personality measure based on the rational that an individual has a psychological self whose structures have incorporated both creative and noncreative ways of behaving Khatena & Torrance (1990) .

The WKOPAY measure of creative perception is based on the rationale that an individual has a psychological self whose structures have incorporated both creative and noncreative ways of behaving. It covers five factors: Acceptance of Authority, Self-confidence, Inquisitiveness, Awareness of Others, and Disciplined Imagination. The Creative Perception score is the total score obtained on the 'What Kind of Person Are You?' inventory (A. K. Palaniappan, 2005; A. K Palaniappan, 2007).

According to (A. K. Palaniappan, 2005; A. K Palaniappan, 2007) Acceptance of Authority relates to being obedient, courteous, conforming, and accepting of the judgments of authorities; Self-confidence relates to being socially well adjusted, self-confident, energetic, curious, thorough and remembering well; Inquisitiveness relates to always asking questions, being self-assertive, feeling strong emotions, being talkative and obedient; Awareness of Others relates to being courteous, socially well-adjusted, popular or well-liked, considerate of others, and preferring to work in a group; Disciplined Imagination relates to being energetic, persistent, thorough, industrious, imaginative, adventurous, never bored, attempting difficult tasks and preferring complex tasks.

Cumulative Grade Point Average (CGPA)

For the purposes of this study, Cumulative Grade Point Average (CGPA) was used as a proxy of academic achievement. The CGPA was calculated by dividing the total number of grade points earned by the total number of credit hours attempted. A student's academic achievement was based on their mid-year examination results. Academic achievement was the aggregate or the total number of grade points in the mid-year examinations. In these examinations, each university subject was graded along a one hundred (or four) point scale, the best grade point being one hundred (or four) and the lowest being zero. Hence the aggregate would range from 75 to 100 (3 to 4); notably the lower the aggregate, the better the academic achievement. This approach was used because other researchers have used the measure and found it an acceptable one for measuring academic achievement Palaniappan (2007a) cited several researchers (Nuss, 1961; Parker, 1979; Taylor, 1958; Wilson, 1968).

2.3 Procedure

The students who participated in this study were all undergraduates. The research questions posed for the study required the students to identify and analyze the distributions and correlations of certain creativity perception were best addressed in the form of a descriptive study. Creativity levels were assessed by self- report instruments and were confirmed by consideration of the results from the administration offices of the universities (described below). They were then divided by gender, with the total scores and subscales calculated for each male and female. The participant sample, women (18-27 years) and men (19-27years), was asked to respond during the regular course time. Both written and oral instructions were given for all participants, and the subjects were ready to answer upcoming questions in the class. Multiple significance tests were conducted, and the data were analyzed by Regression analysis. Participants answered the tests either using their name or anonymously (whichever they preferred). They received no rewards for participating but were advised they would be given information of their results in the form of a self-referenced level of abilities at a later date. Scores for the intelligence, the creativity scale and its factors, were entered into the SPSS statistical program.

3. Result

3.1 Descriptive Statistics

Table.1 shows descriptive statistics on intelligence. The finding of this result shows that the mean score for intelligence was 104.55, standard deviation (15.70), while the mean scores for creativity and its components were as follows: What kind of person are you? (M=28.2745, SD= 5.03571), Disciplined Imagination (M= 4.5882, SD= 1.80471), Awareness of others (M= 5.7059, SD=1.98309), Inquisitiveness (M=2.7190, SD= 1.17237), Self confidence (M=6.0654, SD=1.88021), and Acceptance of authority (M=2.2876, SD=1.46302). However, the mean score and standard deviation for cumulative grade point average were (M= 2.96, SD.53).

3.2 Data Analysis

Hypothesis One

It states that the creativity of the subjects will not significantly predict intelligence. In Table 2, creativity significantly predicts intelligence among subjects. The total variance accounted for by the creativity factor is 16.4 % (multiple R2 = 0.16.4), F (6, 146) = 4.761, p = .000). This implies that creativity is important when considering the factors that influence intelligence of Iranian undergraduate students in Malaysian universities.

Hypothesis Two

It states that each of the constituents of creativity of the subjects will not significantly predict intelligence. In Table 3, the multiple R2 columns reveal the total variance in intelligence accounted for by each of the creativity components of students. The highest contributing component to intelligence is Environmental Sensitivity (R2 = 0.165). This is closely followed by Intellectuality (R2 = 0.134), then, followed by Initiative (R2 =0.122), artistry (R2 = 0 .114), Individuality (R2 = 0.113) and lastly, by Self Strength (R2 = 0.090). The contribution of each of the component is different. The difference between the highest and lowest contributors is 0.156 (15.6%). each component of creativity except Environmental Sensitivity (Sig= .041). Each component of creativity except Environmental Sensitivity (Sig= .041) does not significantly predict intelligence. However, Normal P-P Plot graphs (Expected Cumulative Probability by Observed Cumulative Probability) were obtained for intelligence scores is shown in Figure 1.

Table 1. Descriptive Statistics (N=153)

	Min	Max	Mean	SD
What kind of person are you?	15	39	28.2745	5.03571
IQ	69	141	1.0455	15.70113
CGPA	1.21	4	2.9677	.53684
Disciplined Imagination	.00	8	4.5882	1.80471
Awareness of others	.00	10	5.7059	1.98309
Inquisitiveness	.00	5	2.7190	1.17237
Self confidence	1	9	6.0654	1.88021
Acceptance of authority	.00	7	2.2876	1.46302
Valid N (listwise)	153			

4. Discussion

The creativity factors predict I.Q in this research. In numerous review articles and background studies the opposite is reported greatly, however is the value is low i.e. 16.4% (multiple R2 = 0.164), F (7, 145) =3.222, p<0.01). The result of this research is not in the right place. It supports the relation between intelligence and creativity found in studies conducted by (Funchs & Karen, 1993; Olatoye & Oyundoyin, 2007). They found a

significant relationship between the creativity and intelligence. Creativity is a positive predictor of Intelligence. It is advised and suggested that employers of universities and teachers would that include assignments have need for creative skills for high I.Q.

Table 2. Regression summary table showing the effect of intelligence on creativity b

	Sum of Squares	Df	Mean Square	F	Sig*
Regression	6131.7	6	1021.9	.761	.000[a]
Residual	31340.2	146	214.659		
Total	37471.9	152			

a. Predictors: (Constant), Creativity (What kind of person are you?), Acceptance of authority, Inquisitiveness, Self confidence, Disciplined Imagination, Awareness of others
b. Dependent Variable: intelligence
* = Significant at 0.01
Multiple R= .405
Multiple R2 = .164
Adjusted R2 = .129
Standard Error of the Estimate= 14.65124

Table 3. Regression summary table showing relative effect of intelligence on each of the creativity constituents

Creativity components	R	Multiple R Square	F	Sig
Acceptance of authority	.013	.000	.026	.871
Self confidence	.048	.002	.005	.942
Inquisitiveness	.006	.000	1.228	.270
Disciplined Imagination	.317	.101	16.903	.000*
Awareness of others	.232	.054	8.628	.004*

- Significant at 0.01 level of confidence

Creativity as used in this research has five components, namely; Acceptance of authority, Self confidence, Inquisitiveness, Awareness of others, Disciplined Imagination. The relative effect of each of the creativity component considered in this investigation on intelligence indicates that their contributions are each unique. On its own, each of the creativity components (except Disciplined Imagination & Awareness of others) is not sufficient to measure the creativity of the students. This means that if a counsellor or teacher wishes to measure creativity, using any of the components separately (except Disciplined Imagination & Awareness of others) will not be sufficient to measure a student's creativity.

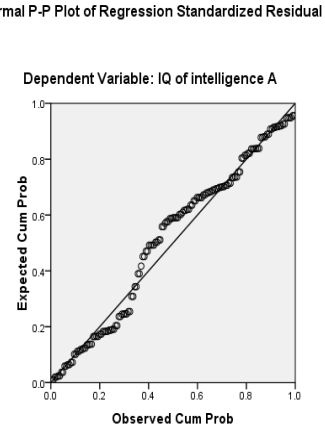

Figure 1. Normal P-P plot of Regression Standardized Residual

It supports the creativity as a predictor of intelligence among undergraduate students in study conducted by Naderi, H.& Abdullah, R. (2009) they found no significant-related between each of the creativity components (Environmental Sensitivity, Initiative, Intellectuality, Self-strength, Individuality and Artistry) except Environmental Sensitivity). However, Olatoye and Oyundoyin, (2007) found I.Q significantly predicts each of the four components of creativity (fluency, originality, flexibility and creativity motivation). Therefore, the conclusion in this study needs to be verified by conducting similar studies in other nations (Naderi et. al. 2009).

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Influence of Al_2O_3 nanoparticles on the compressive strength and workability of blended concrete

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Abstract: The compressive strength and workability of concrete by partial replacement of cement with nano-phase Al_2O_3 particles. Al_2O_3 nanoparticles with the average diameter of 15 nm were used with four different contents of 0.5%, 0.1%, 1.5% and 2.0% by weight. The results showed that the use of nano-Al_2O_3 particles up to maximum replacement level of 2.0% produces concrete with improved strength. However, the ultimate strength of concrete was gained at 1.0 wt% of cement replacement. The workability of fresh concrete was decreased by increasing the content of Al_2O_3 nanoparticles. It is concluded that partial replacement of cement with nanophase Al_2O_3 particles improves the compressive strength of concrete but decreases its workability. [Journal of American Science 2010;6(5):6-9]. (ISSN: 1545-1003).

Key words: Al_2O_3 nanoparticles; concrete; compressive strength; workability.

1. Introduction

Portland cement-based binders are the primary active components of concretes used in most modern construction. The other components are water and both fine and coarse aggregate. Binders are made from Portland 'clinker' ground together with a little calcium sulfate, and frequently also contain fine mineral powders such as limestone, pozzolan (typically volcanic ash), fly ash (usually from coal-burning power plants) and granulated blast furnace slag. Such powders are referred to as supplementary cementitious materials (SCMs) since they are used to replace some of the more expensive clinker. Chemical admixtures such as superplasticisers and air-entraining agents can be added in small amounts to modify the properties of a concrete for specific applications.

Another type of admixtures recently used are nanoparticles. However, there are few reports on incorporation of nanoparticles in cement-based concrete. Hui et al. (2003) [1] investigated the properties of cement mortars blended with nanoparticles to explore their super mechanical and smart (temperature and strain sensing) potentials. Also useful applications of nano-SiO_2 are addressed by the Fuji Chimera Research Institute (2002). However, until now, research performed over the years has been mainly aimed at achieving high mechanical performance with cement replacement materials in micro level. Recently, the effect of nano-SiO_2 particles by adding to blended concrete has been reviewed by Nazari et al. (2010) [2]. Several researchers have demonstrated that the finer the SiO_2 particle sizes in micron level, the higher the compressive strength. But there is a lack of knowledge on effects of ultra fine and nano-size particles on concrete's properties. Lu and Young [3] achieved high strengths on compressed samples and Richard and Cheyrezy [4] developed Reactive Power Concretes (RPCs) with high compressive strength and appropriate fracture energy. The development of an ultrahigh strength concrete was made possible by the application of DSP (Densified System containing homogeneously arranged ultra-fine Particles) with super plasticizer and silica fume content [5].

In this work, the influences of nano-Al_2O_3 on workability and compressive strength of binary blended concrete has been investigated. The reason for using Al_2O_3 as a partial replacement by cement is the C-A-H (lime-alumina-calcium sulfate) gel formation in concrete. The major constituent of a pozzolan is the alumina that can be amorphous or glassy. This component reacts with calcium hydroxide produced from the hydration of calcium aluminates. The rate of the pozzolanic reaction is proportional to the amount of surface area available for reaction. Therefore, it is possible to add nano-Al_2O_3 of a high purity (99.9%) and a high Blaine fineness value (60 m^2/g) in order to improve the characteristics of cement mortars [5]. In this study an attempt has been made to prove that using new materials, it is possible to obtain HPC or HSC with slight increase in cost.

HPC and HSC are very useful in constructions and multistory buildings because they can decrease the cross-

sectional area of the structural fundamentals.

2. Materials and Methods

2.1. Materials and mixtures

2.1.1. Cement

Ordinary Portland Cement (OPC) obtained from Holcim Cement Manufacturing Company of Malaysia conforming to ASTM C150 standard was used as received. The chemical and physical properties of the cement are shown in Table 1.

Table 1. Chemical and physical properties of Portland cement (*Wt. %)* Chemical properties

Material	SiO_2	Al_2O_3	Fe_2O_3	CaO	MgO
Cement	21.89	5.3	3.34	53.27	6.45
Material	SO_3	Na_2O	K_2O	Loss on ignition	
Cement	3.67	0.18	0.98	3.21	

Specific gravity: 1.7 g/cm^3

2.1.2. Nano- Al_2O_3 particles

Nano– Al_2O_3 with average particle size of 15 nm was used as received. The properties of nano- Al_2O_3 particles are shown in Table 2.

Table 2. The properties of nano-Al_2O_3

Diameter (nm)	Surface Volume ratio (m^2/g)	Density (g/cm^3)	Purity (%)
15 ± 3	165 ± 12	< 0.1	>99.9

2.1.3. Aggregates

Locally available natural sand with particles smaller than 0.5mm and fineness modulus of 2.25 and specific gravity of 2.58g/cm^3 was used as fine aggregate. Crushed basalt stored in the laboratory with maximum size of 15mm and specific gravity of 2.96 g/cm³ was used as coarse aggregate.

2.1.4. Mixture proportioning

A total of two series of mixtures were prepared in the laboratory trials. Series C0 mixtures were prepared as control specimens. The control mixtures were made of natural aggregates, cement and water. Series N were prepared with different contents of nano- Al_2O_3 particles with average particle size of 15 nm. The mixtures were prepared with the cement replacement of 0.5%, 1.0%, 1.5% and 2.0% by weight. The water to binder ratio for all mixtures was set at 0.40 [9]. The aggregates for the mixtures consisted of a combination of crushed basalt and of fine sand, with the sand percentage of 30% by weight. The binder content of all mixtures was 550 kg/m^3. The proportions of the mixtures are presented in Table 3.

2.2. Preparation of test specimens

Series N mixtures were prepared by mixing the course aggregates, fine aggregates and powder materials (cement and nano- Al_2O_3 particles) in a laboratory concrete drum mixer. The powder material in the series C0 mixtures was only cement. They were mixed in dry condition for two minutes, and for another three minutes after adding the water. Slumps of the fresh concrete were determined immediately to evaluate the workability following the mixing procedure. Cubes of 100 mm edge were cast and compacted in two layers on a vibrating table, where each layer was vibrated for 10 s [10]. The moulds were covered with polyethylene sheets and moistened for 24 h. Then the specimens were demoulded and cured in water at a temperature of 20^o C prior to test days. The compressive strengths tests of the concrete samples were determined at 7, 28 and 90 days. The reported results are the average of three trials.

Table 3. Mixture proportion of nano-Al_2O_3 particles blended concretes

Sample designation	nano-Al_2O_3 particles	Quantities (kg/m^3)	
		Cement	nano- Al_2O_3 particles
C0 (control)	0	550	0
N1	0.5	547.25	2.75
N2	1.0	544.50	5.50
N3	1.5	541.75	8.25
N4	2.0	539.00	11.00

Water to binder [cement + nano-Al_2O_3] ratio of 0.40, sand 492 kg/m^3, and aggregate 1148 kg/m^3

2.3. Compressive strength of nano-Al_2O_3 particles blended concrete

Compressive strength of nano-Al_2O_3 particles blended cement concrete cubes was determined as per ASTM C 39 after 7, 28 and 90 days of moisture curing.

2.4. Workability

Standard slump tests conforming to ASTM C143 were used to determine the workability of the concrete.

3. Experimental results and discussion

The compressive strength results obtained from the experimental investigations are showed in tables and the comparison between the results of workability test is presented in form of bar chart. All the values are the average of the three trails in each case in the testing program of this study. The results are discussed as follows.

3.1. Compressive strength

The compressive strength results of series C0 and N mixtures are shown in Table 4. Comparison of the results from the 7, 28 and 90 days samples shows that the

compressive strength increases with nano-Al_2O_3 particles up to 1.0% replacement (N2) and then it decreases, although the results of 2.0% replacement (N4) is still higher than those of the plain cement concrete (C0). It was shown that the use of 2.0% nano-Al_2O_3 particles decreases the compressive strength to a value which is near to the control concrete. This may be due to the fact that the quantity of nano-Al_2O_3 particles (pozzolan) present in the mix is higher than the amount required to combine with the liberated lime during the process of hydration thus leading to excess silica leaching out and causing a deficiency in strength as it replaces part of the cementitious material but does not contribute to strength [11]. Also, it may be due to the defects generated in dispersion of nanoparticles that causes weak zones.

Table 4. Compressive strength of nano-Al_2O_3 particle blended cement mortars

		Compressive strength (MPa)		
Sample designation	nano- Al_2O_3 particle (%)	7 days	28 days	90 days
C0 (control)	0	27.3	36.8	42.3
N1	0.5	30.4	41.1	44.1
N2	1.0	31.7	42.3	46.1
N3	1.5	31.9	42.8	45.3
N4	2.0	27.5	37.7	42.6

Water to binder [cement + nano-Al_2O_3] ratio of 0.40

The high enhancement of compressive strength in the N series blended concrete are due to the rapid consuming of $Ca(OH)_2$ which was formed during hydration of Portland cement specially at early ages related to the high reactivity of nano-Al_2O_3 particles. As a consequence, the hydration of cement is accelerated and larger volumes of reaction products are formed. Also nano-Al_2O_3 particles recover the particle packing density of the blended cement, directing to a reduced volume of larger pores in the cement paste.

3.2. Workability

A high-quality concrete is one which has acceptable workability (around 6.5 cm slump height) in the fresh condition and develops sufficient strength. Basically, the bigger the measured height of slump, the better the workability will be, indicating that the concrete flows easily but at the same time is free from segregation [12, 13]. Maximum strength of concrete is related to the workability and can only be obtained if the concrete has adequate degree of workability because of self compacting ability. Self-compacting repair mortars, as new technology products, are especially preferred for the rehabilitation and repair of reinforced concrete structures [14]. The water/powder (cement, fly ash, limestone filler, silica fume, nano-particles, etc.) ratio of mortar and the type of chemical admixtures should be determined, in order to place the fresh mortar without any external compaction and at the same time without causing any segregation [15]. In other words, the rheology of paste phase of self-repairing mortar should have suitable properties from flowability and segregation point of view [16–19].

The workability of C0 and N series concrete are presented in Figure 1. The figure shows the influence of nano- Al_2O_3 particles content on the workability of mixtures at constant water to binder ratio of 0.40. The results show that unlike the C0 series, all investigated nano-Al_2O_3 particles blended mixtures had low slump values and non-acceptable workability. This may be due to the increasing in the surface area of powder after adding nanoparticles that needs more water to wetting the cement particles.

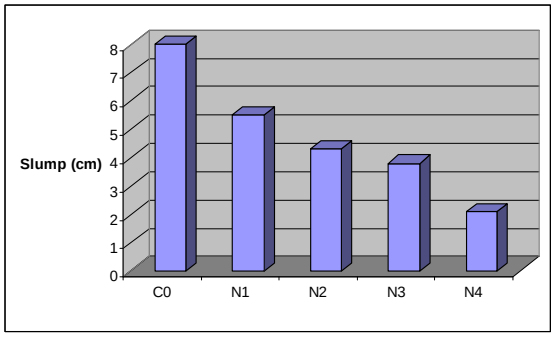

Figure 1. Particle size effects of nano-Al_2O_3 on workability of concrete. N1, N2, N3 and N4 are the series N blended concrete with 0.5, 1.0, 1.5 and 2.0 percent of nano-Al_2O_3 particles, respectively.

With the improvement of novel plasticizers, to obtain high filling rates is possible even for compound molding systems. The fresh characteristics of concrete, strength and durability of mortars can be improved by the addition of inert or pozzolanic [20]. The selection of the amount and the type of cementitious or inert powders depends on the physical and physico-chemical properties of these powders which are affecting the performance of fresh paste such as particle shape, surface texture, surface porosity and rate of superplasticizer adsorption, surface energy (zeta potential), finest fraction content, Blaine fineness and particle size distribution.

There is no universally accepted agreement on the effect of these factors due to the complex influence of the combination of these factors [21].

Usually, increasing the fine particles content in cements changes the rheological properties of pastes and

consequently influences the workability of mortars and fresh concrete mixtures. The observed changes can be advantageous or not as a result of many factors influencing the rheology of cement pastes [22]. It is usually expected that, if the volume concentration of a solid is held constant, for a specific workability, the replacement of cement with a fine powder will increase the water demand due to the increase in surface area. This is more observed for nanoparticles blended concrete. However, in some cases, the above-mentioned conclusion is not appropriate. Lange et al. [23] obtained same results with fly ash blended concrete. But In this study, the addition of nano-Al_2O_3 particles decreased the fluidity and increased the water demand for normal consistency

Conclusions

The results show that the nano-Al_2O_3 particles blended concrete had significantly higher compressive strength compare to that of the concrete without nano-Al_2O_3 particles. It is found that the cement could be advantageously replaced with nano-Al_2O_3 particles up to maximum limit of 2.0% with average particle sizes of 15 nm. Although, the optimal level of nano-Al_2O_3 particles content was achieved with 1.0% replacement. Partial replacement of cement by nano-Al_2O_3 particles decreased workability of fresh concrete; therefore use of super plasticizer is substantial.

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Effect of *Diphenyl Dimethyl Bicarboxylate* and *Dexamethasone* on Immunological and parasitological parameters in murine *Schistosomiasis mansoni*

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Abstract: This work aimed to evaluate the effect of Diphenyl Dimethyl Bicarboxylate (DDB) or dexamethasone either alone or combined with praziquantel (PZQ) on different parasitological, immunological, and pathological parameters that reflect disease severity and morbidity in murine schistosomiasis. Diphenyl Dimethyl Bicarboxylate (DDB) or dexamethasone had no effect on worm burden but altered tissue egg distribution. This indicates that under the schedule used, both drugs did not interfere with the development of adult worms or oviposition but it can modulate liver pathology. Meanwhile, dexamethsone showed a marked reduction of granuloma size more than DDB. Dexamethasone-treated mice, also, showed lower levels of serum gamma interferon (IFN-γ), interleukin-12 (IL-12), and IL-4 together with higher IL-10 level compared to infected untreated control animals. These data suggested that dexamethasone is a convenient and promising co adjuvant agent causing decreased morbidity in murine schistosomiasis. [Journal of American Science 2010;6(5):10-18]. (ISSN: 1545-1003).

Keywords: Schistosomiasis – Morbidity – Cytokines – Treatment.

1. Introduction

Schistosomiasis is a chronic and debilitating disease that remains one of the most prevalent parasitic infections in the humid tropics, with an estimated 650 million people at risk of infection and 200 million actually infected in 74 countries (WHO, 2002). It is encouraging that significant progress in the control of schistosomiasis has been achieved over the last several years in Brazil, China and Egypt. However, because of environmental changes linked to water resources development and the rapidly increasing sizes and movements of population, the disease has spread to previously non-endemic or low endemic areas (Engels *et al.* 2002).

The main cause of morbidity and mortality in human schistosomiasis is hepatic fibrosis, which essentially involves portal spaces, without severe lesions in the hepatic parenchyma. Management of schistosomiasis has focused primarily on treating and preventing the complications of portal hypertension. Unfortunately, no therapy has been proved to prevent progressive hepatic fibrosis which is associated with granulomatous hypersensitivity to parasite eggs. A proportion of patients with chronic schistosomiasis retain the hepatic fibrous scarring of the liver, following antihelminthic treatment. This problem leads to the suggestion that addition of anti-fibrotic agents as an adjuvant to anti-schistosomal chemotheraby may be useful in the treatment of *Schistosoma mansoni (S. mansoni)* infection (Mohamed *et al.* 1991).

Praziquantel (PZQ) remains the only antibilharzial drug effective against the four main schistosomes pathogenic to man (Gönnert & Andrews, 1977; WHO, 2002). Although it has been reported that PZQ has minimal side effects, control of schistosomiasis using PZQ at a population level faces some problems. Resistance to PZQ has been recently induced in schistosomes by laboratory selection (Fallon & Doenhoff, 1994). Reduced cure rates and failure of treatment after PZQ have been reported in Senegalese, Kenyan and Egyptian patients (Ismail *et al.* 1999; Fallon *et al.* 2000, Gryseels *et al.* 2001).

DDB (dimethyl – 4 , 4 '- dimethoxy – 5 , 6 , 5 ', 6' -dimethylene dioxybiphenyl- 2,2'-dicarboxylate), a component derived from *Shizandrae,* is a curative agent for the treatment of hepatitis used clinically in East Asia (e.g. China & Korea). It protects liver tissue against carbon tetrachloride-, galactosamine-, thioacetamide- or prednisolone-induced injuries, and enhances antibody production (Liu, 1987; Salama *et al.* 2004). A long term randomized controlled human study has shown DDB to substantially improve the liver function of patients with the hepatitis B virus (Liu, 1987). We have reported that the pharmacological effect of DDB was associated with the inhibition of NF-KB activation and TNFβ (Salama *et al.* 2004).Previous studies on the effect of corticoids in murine schistosomiasis showed variations according to the dose, the type and the schedule of treatment used (Harrison & Doenhoff , 1983, Morrison *et al. 1986,* Hermeto *et al* . 1990). It was proposed that the decrease in worm burden was due to impairment in the initial phase of parasite penetration into host tissues (Hermeto *et al . 1994*). Dexamethasone also decreased the level of collagen synthesis and the level of post-translational enzymes associated with

collagen synthesis (Newman & Cutroneo, 1978; James *et al.* 1983).

This study aimed to explore the effects of DDB or dexamethasone either alone or in combination with PZQ on several parasitological, immunological, and pathological parameters in murine schistosomiasis.

2. Material and Methods

Animals:

Male, Swiss albino Laboratory-bred mice, each weighing 18-20 grams were used in this study. They were maintained, in conditioned rooms at 21ºC, on sterile water adlibitum and balanced dry food containing 24% protein. The animal experiment was carried out according to the internationally valid guidelines (Nessim *et al.* 2000) at Schistosome Biological Supply Program Unit of Theodor Bilharz Research Institute (SBSP/TBRI, Giza, Egypt).

Cercariae:

Schistosoma mansoni cercariae suspension (0.2 ml) was obtained from SBSP/TBRI and placed drop by drop on a glass plate. The cercariae on the plate were killed by the addition of one drop of 1% iodine. With the aid of a dissecting microscope, the number of cercariae was determined. Generally five counts were made to calculate the number of cercariae per ml of the suspension and the average number per 0.1 ml was used. Infection was performed by the subcutaneous injection of 100 *S. mansoni* cercariae to each mouse (Stirewalt & Dorsey, 1974).

Drug regimen:

Praziquantel

Tablets (600mg) were grinded as white powder and suspended in 13 ml of 2% cremophore-EL, as it is insoluble in water. The drug was freshly prepared before oral administeration to mice using a stainless steel oral canula. The dose given was 500 mg/kg body weight for two consecutive days.

Diphenyl Dimethyl Bicarboxylate (DDB)

Bimethyl-4,4′-dimethoxy-5,6,5′,6′-dimethylene-dioxybiphenyl-2,2′ dicarboxylate was supplied by Dongkwang Pharmaceutical Co. DDB was administered orally to mice using a stainless steel oral canula. The dose given was 25mg/kg body weight, three times a week until the end of the experiment.

Dexamethasone disodium phosphate

Decadron (Prodome, Brazil) was injected by the intramuscular route at 1 mg/kg body weight three times a week until the end of the experiment.

Experimental design:

Mice (70) were divided into seven groups (each composed of ten mice) as follows:

Group 1: Normal control group.

Group 2: Infected untreated control group, in which mice were infected with 100 *S. mansoni* cercariae.

Group 3: Infected-treated group, with 25 mg/kg DDB orally, three times/ week from the first day of infection to the end of the experiment.

Group 4: Infected-treated group, with 25 mg/kg DDB orally, three times/ week from the first day of infection to the end of the experiment and 500 mg/kg body weight of PZQ orally six weeks post-infection for two consecutive days.

Group 5: Infected-treated group, with intramuscular injection of 1 mg/kg dexamethasone, three times/week from the first day of infection to the end of the experiment.

Group 6: Infected-treated group, with intramuscular injection of 1 mg/kg dexamethasone, three times/week from the first day of infection to the end of the experiment and 500 mg/kg body weight of PZQ orally six weeks post-infection for two consecutive days.

Group 7: Infected with *S. mansoni* cercariae and treated with 500 mg/kg body weight of PZQ orally six weeks post-infection for two consecutive days.

Animals of all groups were killed under anesthesia, 8 weeks post infection.

Parasitological Parameters

Worm burden: Hepatic and portomesenteric vessels were perfused to recover worms for subsequent counting (Duvall & DeWitt, 1967).

Tissue egg load: The number of ova/gm intestinal or hepatic tissue was counted after digestion overnight in 5% KOH (Cheever, 1968; Kamel *et al.* 1977).

Percentage egg developmental stages "Oogram pattern": The percentage of eggs at different developmental stages were examined in three samples/mouse and the mean of each stage/animal was obtained (Pellegrino *et al.* 1962).

Immunological study

Serum enzyme assessment

Animals of all groups were weighed, then killed and blood was collected. The serum was separated by centrifugation at 3000×*g* for 10 minutes and stored at −20°C for the assay of ALT [EC 2.6.1.2] (Reitman & Frankel, 1957), GGT [EC 2.3.2.2] using Boehringer reagent kit (Mannheim, Germany), AP [EC 3.1.3.1] (Kind & King, 1954), total protein (Weichselbaum, 1946) and albumin (Doumas et al. 1971).

Cytokine assay

Serum IFN-γ, IL-12, IL-4, and IL-10 levels were measured 8 weeks post-infection by a sandwich enzyme-linked immunosorbent assay technique with capture and detection antibodies according to the instructions of the manufacturer (PharMingen, San Diego, Calif.). Recombinant cytokines were used as standards. Briefly, plates (Nunc, Roskilde, Denmark) were coated with capture antibodies with 100 ul of serum sample or recombinant cytokine. Following addition of the biotinylated detection antibody and streptavidin-alkaline phosphatase conjugate, the reaction was developed with *para*-nitrophenyl phosphate (Sigma). Absorbance at 405 nm was measured with a Benchmark reader (Bio-Rad Laboratories Inc., Hercules, Calif.). Assays were performed in duplicate. The cytokine concentration was obtained from a regression curve prepared with the help of Microplate Manager software (Bio-Rad).

Histopathological study

Liver specimens were fixed in 10% buffered formalin and embedded in paraffin blocks. The prepared 4µm thick sections were examined by light microscopy using Hematoxylin and eosin and Masson trichrome stains.

Measurement of mean granuloma diameter per group was calculated at a microscopic magnification of X100 using an occular micrometer. Only lobular granulomas containing eggs in their centers and non-confluent ones were measured (Lichtenberg, 1962).

Statistical analysis

The data were presented as mean ± standard error of the mean (X±SE). The means of the different groups were compared globally using the analysis of variance ANOVA. Data were considered significant if p values were less than 0.05.different groups were compared globally using the analysis of variance ANOVA. Data were considered significant if p values were less than 0.05.

3. Results

The Worm burden and tissue egg load in the intestine and liver of each studied group were calculated as the mean ± SE. In the infected control group, the total number of worms counted was 29.6±0.26, divided between liver (43%) and portomesenteric vein (57%). Oral administration of DDB to infected mice with *S. mansoni* reduced the total number of worm burden to 23.3±0.29 (21.37% reduction); treatment of mice with dexamethasone alone reduced the total number of worm burden to 25.6 ± 0.20 (13.5 % reduction) especially those in the liver (Table 1). On the other hand, PZQ caused a marked reduction in worm burden reaching 95.6%, with 60% of the worms shifted to the liver; this inhibition was slightly improved when PZQ was given in combination with DDB (95.9%). The oogram pattern after PZQ treatment showed a complete disappearance of all immature ova from the wall of the intestine, a reduction in the number of mature ova and a four fold increase in dead ova. dexamethsone alone affected the number of dead ova significantly, reduced the number of mature ova (19–20%) while hardly affecting the immature ova. Combination of PZQ with DDB or dexamethasone augmented its effect on the mature ova to reach 85% 95% respectively (Table 2). In principle, the same observation was noted in egg load, where PZQ reduced it in both intestine (95.7%) and liver (96.4%), slightly expanded upon combination with DDB. DDB or dexamethsone alone showed a decrease also in the egg load mounted to 76.8%, 72.7% in the intestine, 76.1% and 66.5% in the liver.

Hepatic granuloma in each studied group was measured as the mean of granuloma diameter± SE and it was 318.8 µm ± 26.3 for the infected control group. Oral administration of DDB to infected mice with *S. mansoni* decreased the granuloma diameter to 194.1 µm ± 21.2 (39.1 % reduction) while administration of DDB in combination with PZQ decreased the mean of granuloma diameter to 168.1µm ± 32.11 (47.3% reduction). Intramuscular administration of dexamethsone to infected mice with *S. mansoni* decreased the granuloma diameter to 142.2 µm ± 25.1 (55.4 % reduction) that reached 119.2 µm ± 29.5 (62.6 % reduction) (Table 3). Accordingly, a significant reduction ($p<0.001$) was observed in granuloma diameter in the two treated groups either alone or combined with PZQ relative to infected control.

Table 1: Effect of oral administration of DDB and intramuscular administration of Dexamethasone on Worm burden and tissue egg load in different studied group**s**

Animal group	Mean No. of worms ± SE	% Reduction	Mean no. of ova count ± SE			
			Intestine	% reduction	Liver	% reduction
Infected Control	29.6 ± 0.26	-	14199±1342	-	2877±411	-
Dexamethson	25.6 ± 0.31***	13.5 %	3877± 211***	72.7%	965±255***	66.5 %
Dexamethson +PZQ	1.6 ± 0.35***	94.6 %	715 ± 121***	95 %	178± 40***	93.8 %
DDB	23.3 ± 0.29***	21.3 %	3292 ± 233***	76.8 %	689 ± 98***	76.1 %
DDB + PZQ	1.2 ± 0.29***	95.9 %	545 ± 133***	96.2 %	94±13***	96.7 %
PZQ	1.3 ± 0.35***	95.6 %	612 ± 156***	95.7 %	101±13***	96.4 %

***** Statistically significant difference at p< 0.001 compared to infected control group.**
***** Statistically significant difference at p< 0.001 compared to infected control group.**

Table 2: Effect of oral administration of DDB and intramuscular administration of Dexamethasone oogram pattern of mice infected with 80 *S. mansoni* cercariae and sacrificed 8 weeks postinfection.

Group Name	**Oogram pattern (% ova)**		
	Immature	**Mature**	**Dead**
Infected Control	65.3 ± 5.4	31.1 ± 2.6	3.6 ± 0.7*
Dexamethson	50.2± 4.2***	30.7 ± 3.4***	19.1 ± 1.5***
Dexamethson +PZQ	5.3 ± 5.1**	4.3 ± 1.4	90.4 ± 8.3**
DDB	20.2± 5.4***	43.2± 6.1***	36.6± 2.4***
DDB + PZQ	10.3 ± 1.9***	6.5 ± 1.4***	83.2 ± 5.4***
PZQ	2.3 ± 0.4	1.9 ± 0.2	95.8 ± 4.9

* Statistically significant difference at p< 0.05 compared to infected control group
** Statistically significant difference at p< 0.01 compared to infected control group
*** Statistically significant difference at p< 0.001 compared to infected control group

Table 3:- Effect of oral administration of DDB and intramuscular administration of Dexamethasone on hepatic granuloma diameter of mice infected with *S. mansoni*.

Group Name	**Hepatic granuloma diameter** – X GD ± SE	% Reduction
Infected Control	318.8 ± 26.3	-
Dexamethson	142.2 ± 25.1	55.4 % ***
Dexamethson +PZQ	119.2 ± 29.5	62.6% ***
DDB	194.1 ± 21.2	39.1% **
DDB + PZQ	168.1 ± 32.11	47.3 % **
PZQ	201.1 ± 25.3	24.1 %

GD :- Granuloma diameter SE:- Standard Error.
** Statistically significant difference at p< 0.01 compared to infected control group
*** Statistically significant difference at p< 0.001 compared to infected control group

Cytokines assy.

Cytokines are believed to modulate the amount of fibrosis and granuloma size and play a fundamental role in the pathology of schistosomal infection. In order to investigate if the modulatory effects of both DDB and dexamethasone on granulomas were mediated through alteration of cytokine production, the levels of these mediators in serum were measured. Intramuscular administration of dexamethasone to infected mice induced significant decrease in the levels of IL-4, IFN-γ or IL-12 when compared their levels on infected untreated control group, while treatment of mice with DDB induced insignificant decreases in the levels of IL-4, IFN-γ, and IL-12 (Table 4). On the other hand, significant increases in serum IL-10 levels were detected in groups treated with DDB or dexamethasone comparing to the infected untreated control group.

Table 4:- Effect of oral administration of DDB or intramuscular injection of dexamethasone either alone or combined with PZQ on cytokine production of mice infected with 100 *S. mansoni* cercariae and sacrificed 8 weeks post-infection.

Group Name	IL-4 pg/ml X ± SE	IL-10 pg/ml X ± SE	IL-12 pg/ml X ± SE	IFN-γ pg/ml X ± SE
Infected Control	770±160.1	512±19.1	150±10.1	672±74.0
DDB	687±144.1	612±46.5***	131±9.6	607±63.0
DDB + PZQ	712±155.2	668±50.3	138±12.5	613±72.0
Dexamethasone	272±57.1***	711±23.1***	52±16.1***	127±54.0***
Dexamethasone+PZQ	288±100.3	698±33.0	45±14.4	192±94.0
PZQ	711±133.0	588±45.5	163±11.7	633±53.0

X = Mean SE= Standard Error.
**** Significant difference at p< 0.01 compared to infected control group.**
*** significant difference at p< 0.001 compared to infected control group.

Liver Enzymes Assay.
Treatment of mice with DDB or dexamethasone was found to reduce serum enzyme levels characteristic of hepatic damage induced by infection, as indicated by a lowering in the raised levels of serum ALT (78%, 85% respectively), GGT (73%, 86% respectively) and AP (76%, 93% respectively). Treatment of mice with DDB or dexamethasone, also tended to normalize the lowered levels of serum albumin. Untreated infected mice showed a two fold elevation of liver enzymes as compared with normal control animals. Treatment with PZQ alone reduced liver enzymes insignificantly compared to untreated infected mice. The highest significant reduction ($p<0.100$) in liver enzymes was observed in combination of PZQ with DDB or dexamethason.

4. Discussions

Schistosomiasis is a major public health problem in tropics, with tens of millions infected and many more at risk (Boros, 1999). It has been estimated that greater than 250,000 deaths per year are directly attributable to this disease (Botros et al. 2000), and the subtle morbidities associated with chronic infection have a more serious impact. Treatment relies on a single drug, praziquantel, to eliminate the adult worms but this has no prophylactic properties and is ineffective against resistant strains (Botros et al. 2000).

Previous studies have been reported using non-steroidal anti-inflammatory drugs (NSAIDs) e.g tiaprofenic acid and piroxicam either alone or as adjuvant to praziquantel in treating hepatic granuloma in *S.mansoni*-infected mice (Hegazy et al. 1997). The possibility of using another NSAIDs namely, ibuprofen (CAS 15687-27-1) and naproxen (CAS 22204-53-1), either alone or in combination with praziquantel (CAS 55268-74-1) has been studied to induce regression of hepatic morbidity or to ameliorate the biochemical and histopathological consequences and intensity of infection (Mahmoud et al. 2002). However, in the current study, we aimed to investigate the possible role of DDB or dexamethasone alone or as co adjuvant therapy in the treatment of murine schistosomiasis.

Oral administration of DDB to *S. mansoni*-infected mice showed insignificant decrease in the worm burden, and alteration of egg load in intestinal and liver tissue (76.8% and 76.1% reduction, respectively). However, combination with PZQ decreased the egg load in the intestinal and hepatic tissue giving 96.2% and 96.7% reduction, respectively. Moreover, reduction in granuloma diameter and cytokine production, indicated that administration of DDB to *S. mansoni*-infected mice may improve disease morbidity.

The protective effects of DDB on chemically induced damage of isolated suspended rat hepatocytes were studied by Fu and Liu (1992) and its reversing effect on the phenotyes of human hepatocarcinoma cell line has been evaluated (Liu et al. 1996). Recently, administration of DDB on tamoxifen-induced liver injury in rats showed that prolonged treatment revealed a potent anti-fibrogenic role (El-Beshbishy, 2005). Moreover, a pharmaceutical composition of garlic oil and DDB, as active ingredients for enzyme induction and liver protection has been used as a curative preparation for patients with acute or chronic viral hepatitis (Park et al. 2005).

On the other hand, dexamethasone was previously investigated as a co adjuvant immunomodulator in treatment of chronic schistosomiasis (Pyrrho *et al.* 2002).

Our results showed insignificant effect of dexamethasone on parasite number which agreed with Lambertucci et al. (1989). However, investigators who used hydrocortisone or a dose of dexamethasone 50 times higher than ours reported decrease in the parasite burden (Coker, 1957, Hermeto *et al. 1993*). Following oviposition, the eggs are carried mainly to intestinal and hepatic veins then to the lungs and other tissues. Although a lack of *S. mansoni* fecundity using dexamethasone in vitro has been described (Morrison, 1986), another decrease in the amount of oviposition was reported after oral administration of dexamethasone in vivo (Lambertucci et al. 1989). Our results demonstrated that with the therapeutic schedule used, neither worm development nor oviposition was significantly modified, but the treatment altered egg distribution in tissue, favoring a more intense deposition in the intestine. The mechanism by which dexamethasone alters the egg distribution in tissue is unknown. Also, little is known about the effect of this glucocorticoid on the migration of female parasites and on the intravascular sites of oviposition. A reduction in the rate of egg excretion following treatment of infected mice with corticosteroids or hydrocortisone acetate was observed (Newsome, 1963; Doenhoff et al. 1978) with consequent changes in the places where the eggs are trapped in the tissues. Since granulomas are composed of several cell types and extracellular matrix components, the action of dexamethasone on these elements is pleiotropic and difficult to evaluate in vivo. However, granuloma size in animals treated with dexamethasone showed significant decrease, probably due to the high levels of IL-10 induced by treatment. This observation is in accordance with Franchimont et al. (1999) who showed that administration of exogenous IL-10 resulted in reduction of granuloma size. Furthermore, an

opposite effect was seen in IL-10-deficient mice (Wynn et al. 1998). Rezende et al. (1997) suggested that immunocomplexes from patients with chronic intestinal schistosomiasis are able to modulate granulomatous hypersensitivity to *S. mansoni* eggs by inducing prostaglandin E production that augments IL-10 level.

Cytokines are believed to modulate the amount of fibrosis and granuloma size and play a fundamental role in the pathology of schistosoma infection. In order to investigate if the modulatory effects of both DDB and dexamethasone on granulomas were mediated through alteration of cytokine production, the levels of these mediators in serum were measured. Administration of exogenous IL-4 increases the amount of fibrosis (Yamashita & Boros. 1992), while administration of anti-IL-4 or exogenous IFN-γ decreases the level of collagen deposition (Cheever et al. 1994, Czaja et al. 1989). In murine models, IL-12 was also involved in reduction of the amount of fibrosis and granuloma size (Wynn et al. 1995, Hoffmann et al. 1998). However, compared to wild-type mice, mice lacking IL-4 (IL-4 knockout mice) showed diminution in catalase levels, increased hepatotoxicity that resulted in early mortality (Fallon et al. 2000, La Flamme et al. 2001). In our study, administration of DDB or dexamethasone to *S. mansoni*-infected mice decreased serum IL-12 and IFN-γ levels and induced a pronounced reduction of IL-4 levels, but it is possible that despite the decrease in the level of IL-4 production, the circulating levels of this cytokine are enough to exert a protective effect and has been suggested that it plays an important role in the severity of *S. mansoni* infection and may influence the course of disease (Brunet et al. 1998, Fallon et al. 2000). Since dexamethasone also increased serum IL-10 levels, our data are in agreement with those from previous reports (Hoffmann et al. 1999; 2000; Wynn et al. 1997), indicating that production of IL-10 is the key factor in preventing the polarization toward a Th1 or Th2 profile and therefore avoiding an increase in rates of disease morbidity.

In conclusion, the use of DDB or dexamethasone as a coadjuvant treatment with praziquantel in murine schistosomiasis, in addition to minimizing the morbidity of infection, may give an insight into the mechanisms involved in its pathogenesis.

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Anti-*S. mansoni* MAb-based Latex Agglutination: A reliable field applicable immunodiagnostic test for screening of active Human Schistosomiasis

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Abstract: Schistosomiasis is a major public health problem with a worldwide distribution. Diagnosis of this disease by simple and rapid immunoassays is a priority. The objective of the present study was to standardize and evaluate the latex agglutination test (LAT) as a simple test for the detection of circulating schistosomal antigen (CSA) in serum and urine samples of *S. mansoni* patients and compare it with ELISA. According to stool examination this study included 70 *S. mansoni* infected patients, 32 other parasites infected patients and 30 negative control samples. Characterization of MAb 12D/10F was done using several techniques including: ELISA, immunoelectrophoresis, polyacrylamide gel electrophoresis and immunoblotting as well as periodate and trichloroacetic acid treatment of target antigen for identification of its chemical nature. A polystyrene latex (0.81 µm) suspension was used as a carrier particle for anti-*S. mansoni* adult worm tegumental antigen monoclonal antibody (12D/10F) in the test. The Latex particles sensitized with MAb were used for the detection of CSA in urine and serum samples. The sensitivity of LAT assay was 90% in urine and 87.1% in sera versus 92.9% and 95.7% for ELISA. The specificity of LAT assay was 88.7% and 93.5% for urine and sera versus 87.1% and 93.6% for ELISA. The diagnostic efficacy of LAT was 89.1% and 90.2% for urine and serum samples, respectively versus 90.2% and 94.7% for ELISA. Moreover, a positive correlation was found between ova count in stool of *S. mansoni* infected patients and both the intensity of LAT and OD readings of ELISA in urine ($r= 0.922$; $p< 0.001$ and $r= 0.865$; $p< 0.001$, respectively) and in serum ($r=0.847$; $p< 0.001$ and $r= 0.781$; $p< 0.001$, respectively). In conclusion, LAT is a suitable applicable diagnostic method in field survey especially when followed by ELISA as a confirmatory test in query false negative results. In the same time, more trials are required to increase the sensitivity and specificity of LAT to allow its use on a large scale in field surveys and as diagnostic kits for multiple parasitic infections. [Journal of American Science 2010;6(5):19-27]. (ISSN: 1545-1003).

Keywords: Schistosomiasis – Agglutination – immunodiagnostic – Human

1. Introduction

Diagnosis of schistosomiasis is usually based on the microscopic detection of eggs in stool and urine samples. These methods showed high specificity and low sensitivity especially in light infection with the presence of daily variation phenomenon (De Vlas and Gryseels, 1992). Immunodiagnostic assays have been developed and used for the detection of specific antibodies in serum. However these assays cannot differentiate between recent and past infection and has the problem of cross reactivity among different helminthic parasitic infections (Mott and Dixon, 1982).

Antigen detection assays represent a useful alternative diagnostic tool in two respects. Firstly, the high sensitivity of the assays allowing diagnosis of active infection (Deelder *et al.*, 1989; 1994; Gryseels *et al.*, 1994; Demerdash *et al.*, 1995; Hanallah *et al.*, 2003); secondly, antigen assays allow direct measurement of worm burden than quantitative parasitological techniques that would be extremely valuable for immuno-epidemiological studies, ranging from transmission dynamics over morbidity to immune responses and vaccine trials. Moreover, circulating antigen assays could be modified to be easy performed and field applicable. They are widely used for diagnosis of *Schistosoma* infection (WHO, 2000). However, up till now it is not introduced for community diagnosis of schistosomiasis in Egypt.

The use of monoclonal antibodies (MAbs) has greatly increased the sensitivity and specificity of assays for detection of circulating schistosomal antigen (CSA) (Demerdash *et al.*, 1995). Tegumental antigens develop within 3 hours of host penetration by cercariae and thus their detection would diagnose active *S. mansoni* infection very early and reflect worm burden (Davis, 1986) and proved to be an efficient immunodiagnostic tool for schistosomiasis (Hanallah *et al.*, 2003).

The development of simple, rapid and sensitive methods for detecting CSA are needed as most available assays require several laboratory equipment and highly skilled persons. Being simple and rapid; latex-based diagnostic tests have been

used for detecting specific antigens or antibodies in several diseases (Bangs, 1990).

This study aimed at the development of MAb-based LAT agglutination test as a simple, rapid and field applicable screening test for CSA in urine and serum samples of human schistosomiasis.

2. Material and Methods

Parasitological Examination

This work was conducted on 70 *S. mansoni* infected patients, 32 patients harboring other parasites than *Schisosoma* [*Fasciola* (n= 15), *Echinoccocus granulosus* (n= 10) and *H. nana* (n= 7)] and 30 healthy individuals. All patients were subjected to the following investigations: stool examination using merthiolate iodine formaldehyde-concentration (MIFC) method for detection of all helminth eggs and protozoal cysts. Three slides were prepared for egg count of schistosome eggs in stool using Kato-thick smear technique (Siongok *et al.*, 1976). Blood samples were collected from all cases and sera were separated, aliquoted and kept at -70° C until used. According to the egg count per gram faeces, schistosomiasis group was classified into high (> 300 eggs/g faeces), moderate (100-299 eggs/g faeces) and light (<100 eggs/g faeces) subgroups according to their intensity of infection.

Collection and processing of urine

Patients and healthy subjects (negative controls) were asked to provide freshly voided urine in 20 ml test tubes, and 1 ml of urine was transferred to an eppendorf (Hamburg, Germany) tube. The tubes were put into a tube holder and placed into a boiling water bath for five minutes, and then allowed to cool to ambient temperature before conducting the test.

Preparation of adult worm tegumental antigen

Viable *S. mansoni* adult worms were purchased from the Schistosome Biological Supply Program at Theodor Bilharz Research Institute, Giza, Egypt. The *S. mansoni* adult worm tegumental antigen (Sm AWTA) was prepared from living worms according to Oaks *et al.* (1981).

Monoclonal antibody production

Spleen cells from BALB/c mice immunized with Sm AWTA were fused with non-secreting murine myeloma cells (P3 X 63 Ag. 8). Immunization was performed according to Cianfriglia *et al.* (1983). Fusion was performed in the presence of 43% polyethylene glycol (Sigma) as modified from Galfre and Milstein (1981).

Hybridomas were screened for anti-Sm AWTA antibodies by ELISA. Hybrids that were highly reactive to Sm AWTA and not reactive to *Fasciola* or *Echinococcus granulosus* were cloned by limiting dilution method, using splenocyte feeder layer according to Galfre and Milstein (1981). Isotypic analysis of MAb 12D/10F was done and proved to be of IgM class using a mouse hybridoma subtyping kit (Boehringer). Hybridoma cells were injected intraperitoneally into BALB/c mice for ascites production. Monoclonal antibody 12D/10F (IgM) was purified from ascitic fluid by euglobulin precipitation in distilled water according to Garcia-Gonzalez *et al.* (1988).

Characterization of target antigen recognized by monoclonal antibody

Target antigen recognized by monoclonal antibody 12D/10F was identified using the following techniques; immunoelecrophoresis (IEP) for determination of ability of MAb to recognize a repetitive epitope according to Capron *et al.* (1965). For identification of chemical nature of target antigen, the reactivity of MAb 12D/10F against the antigen before and after treatment with 20 mM sodium periodate and 4% trichloroacetic acid was tested for by indirect ELISA according to Woodward *et al.* (1985). For determination of molecular weight range of recognized antigen, polyacrylamide gel electrophoresis (PAGE) of antigen followed by enzyme linked immunoelectrotransfer blot technique (EITB) was performed according to Tsang *et al.* (1983).

Sandwich ELISA

Quantification of the target antigen of the MAb was achieved by sandwich ELISA using MAb 12D/10F both as antigen capture and detection antibody, being found to recognize an antigen with repetitive epitope. Labeling of MAb with horseradish peroxidase was performed by periodate method according to Nakane and Kawaoi (Nakane and Kawaoi, 1974).

After several optimization trials, the following sandwich ELISA originally described by Engvall and Perlmann (1971), was performed. Microtitration plates (Dynatech) were coated with 10 µg/ml of purified MAb in 0.1 M carbonate buffer, pH 9.6 dispensed as 100 µl/well and left overnight at room temperature. Plates were blocked by adding 200ul/well of 3% fetal calf serum/PBS/Tween for 1 hour at 37°C (3% FCS/PBS/T was used as diluting buffer and PBS/T as washing buffer). Undiluted sera were added (100 µl/well) and incubated for 2 hours at 37°C. Plates were washed with washing buffer. One hundred µl/well of 1:1000 dilution of peroxidase-conjugated MAb (5µg/ml) were added and incubated for 2 hours at room temperature, and then plates were washed as before. The reaction was

visualized by the addition of 100 µl/well of O-phenylene diamine (OPD) substrate solution for 30 minutes in the dark at room temperature. The reaction was stopped by adding 50 µl/well of 8 N H_2SO_4 and plates were read at 492 nm using ELISA microplate reader (Bio Rad).

Detection of circulating schistosomal antigen in serum by Latex agglutination test (LAT)

A polystyrene latex suspension (0.81 µm; Sigma, St. Louis, MO) was used in this test. 1% standardized polysterene latex suspension was prepared by mixing 0.1 ml of latex suspension with 9.9 ml of 0.02 M glycine-buffered saline (GBS), pH 8.4. This was stored at 4 °C until used. One ml of 1% latex suspension was mixed with 1 ml of purified MAb (1.0 mg/ml). The mixture was incubated at 37°C for two hours in a water bath. After incubation, antibody-sensitized latex particles were washed two times with GBS, pH 8.4, and centrifuged at 3000 x g for five minutes. The pellet of MAb-sensitized latex particles was emulsified with 1% bovine serum albumin/GBS, pH 8.4 to make a suspension of 2%. The particles were stored at 4°C until used. Latex particles coated with normal rabbit serum were used as negative control.

The test was performed on a clean slide divided with a glass marking pen into two halves. A drop of test serum or urine (50 µl) was placed on each half of the slide. An equal volume of sensitized latex reagent was added to the serum or urine on one half. The same volume of control latex suspension was added to the serum or urine on the other half as a negative control. The slide was then manually rotated for two minutes then inspected. Agglutination with sensitized latex reagent and not with the control latex reagent was considered a positive result. Appropriate controls were examined in parallel in each test.

Interpretation of results: According to the intensity of agglutination accumulated around the edge of the reaction zone, the positivity was classified into high (+++), moderate (++), low (+). When no agglutination was seen, the result was considered negative (-).

Statistical analysis

Data were expressed as mean ± standard deviation (SD) or number (%). Correlations between different parameters were performed using Spearman's rank correlation coefficient. SPSS computer program (version 13 windows) was used for data analysis

3. Results

Characterization of target antigen recognized by monoclonal antibody

Immunoelectropheresis of MAb versus AWTA in agarose gel showed a high density precipitation arc, proving the ability of MAb to recognize a repetitive epitope on AWTA (Fig. 1). The EITB technique revealed that MAb 12D/10F recognized *S. mansoni* AWTA antigen at 50, 60 and 65 kDa bands (Fig. 2). Binding of MAb 12D/10F to *S. mansoni* AWTA coated microtitration plates was strongly inhibited by treatment with 20 mM sodium periodate (39.0%) and slightly inhibited (9.0%) by treatment with 4% trichloroacetic acid denoting that target antigen for MAb 12D/10F was a proteoglycan.

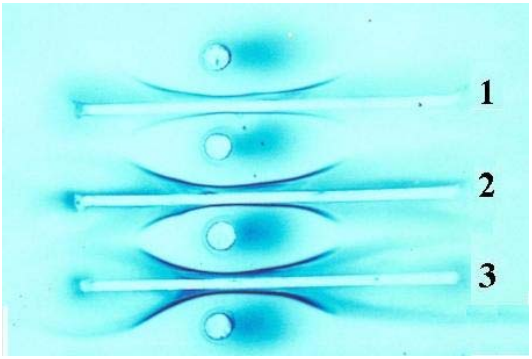

Fig. 1: Immunoelectrophoresis pattern of *S. mansoni* adult worm tegumental antigen (in wells) against 12D/10F MAb (in trough; 1: tissue culture supernatant, 2 and 3: ascitic fluid).

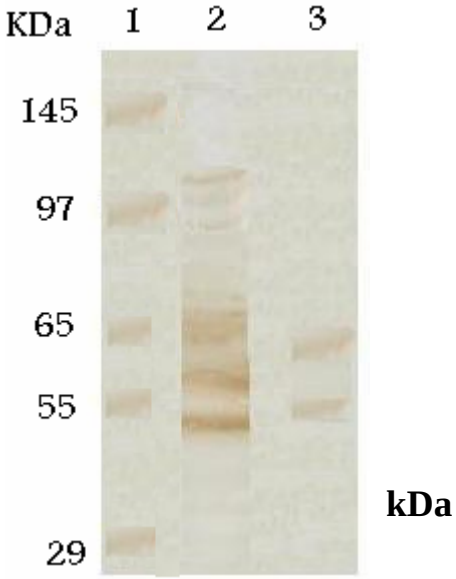

Fig. 2: Enzyme linked immunoelectrotransfer blotting pattern of *S. mansoni* tegumental worm antigen and *S. mansoni* SEA recognized by anti-*S. mansoni* AWTA MAb (12D/10F).
Lane 1: Molecular weight standard proteins.

Lane 2: Pattern of *S. mansoni* adult worm tegumental antigen (AWTA).
Lane 3: Pattern of *S. mansoni* soluble egg antigen (SEA).

In urine, LAT was positive in 63 out of 70 *Schistosoma* infected patients and the sensitivity of the assay was 90%, while in serum LAT was positive in 61 out of 70 *Schistosoma* infected patients and the sensitivity of the assay was 87.1%. The seven and nine patients that showed false negative results in urine and serum respectively were among the light infection subgroup and the mean number of ova in their stool/g was 25.78 ± 9.31 and 28.49 ± 7.78, respectively. The specificity of the assay was determined as the sum of results of negative control group and other parasites group. All the 30 negative controls were LAT negative in both urine and serum while 7 patients out of 32 other parasites group showed positive LAT in urine and 4 patients in serum and were considered as false positives. The specificity of the assay in urine and serum, therefore, was determined to be 88.7% and 93.5%, respectively. The diagnostic efficacy of the assay was 89.4% and 90.2%, respectively (Tables 1 & 2).

Levels of circulating schistosomal antigens in urine and serum of different studied groups were also measured by ELISA at OD readings equal to 492 nm. The cut off value was calculated as the mean OD reading of negative controls + 2 standard deviation of the mean. The OD readings equal to or less than cut off value were considered negative while those readings greater than the cut off value were considered positive.

In urine, the cutoff was 0.245 and ELISA was positive in 65 out of 70 schistosomiasis patients and the sensitivity of the assay was 92.9% while in serum the cutoff was 0.390 and ELISA was positive in 67 out of 70 schistosomiasis patients and the sensitivity of the assay was 95.7%. Also, the 5 and 3 patients that showed false negative results in urine and serum were among the light infection subgroup. All the 30 negative controls were ELISA negative while 8 and 4 patients in other parasites group showed positive ELISA in urine and sera respectively and were considered as false positives. The specificity of the assay was 87.1% and 93.6%, respectively. The diagnostic efficacy of the assay was 90.2% and 94.7%, respectively (Tables 1 & 2).

In *S. mansoni* infected group, there was a significant positive correlation between schistosome egg count in stool and both the intensity of LAT and OD reading of ELISA in urine ($r= 0.847$; $p< 0.001$ and $r= 0.915$; $p< 0.001$, respectively) and in serum ($r=0.863$; $p< 0.001$ and $r= 0.801$; $p< 0.001$, respectively) (Figs. 1 & 2).

Table 1: Mean ELISA OD readings at 492 nm in urine and serum samples classified according to intensity of LAT positivity in schistosomal group (n= 70).

Intensity of LAT	**Urine**		**Serum**	
	Positivity [n (%)]	***ELISA (cutoff= 0.245)***	***Positivity [n (%)]***	***ELISA (cutoff=0.390)***
High (+++)	19 (27.1%)	1.25±0.22	20 (28.6%)	1.59± 0.29
Moderate (++)	33 (47.1%)	0.85±0.12	28 (40%)	0.97 ± 0.14
Low (+)	11(15.7%)	0.52±0.11	13 (18.6%)	0.76 ± 0.13
No agglut.	7 (10.1%)	0.211±0.09	9 (12.8%)	0.299 ± 0.07

Table 2: Sensitivity, specificity and diagnostic efficacy of LAT and ELISA.

	LAT		***ELISA***	
	Urine	***Serum***	***Urine***	***Serum***
Sensitivity	90%	87.1%	92.9%	95.7%
Specificity	88.7%	93.5%	87.1%	93.6%
Diagnostic efficacy	89.4%	90.2%	90.2%	94.7%

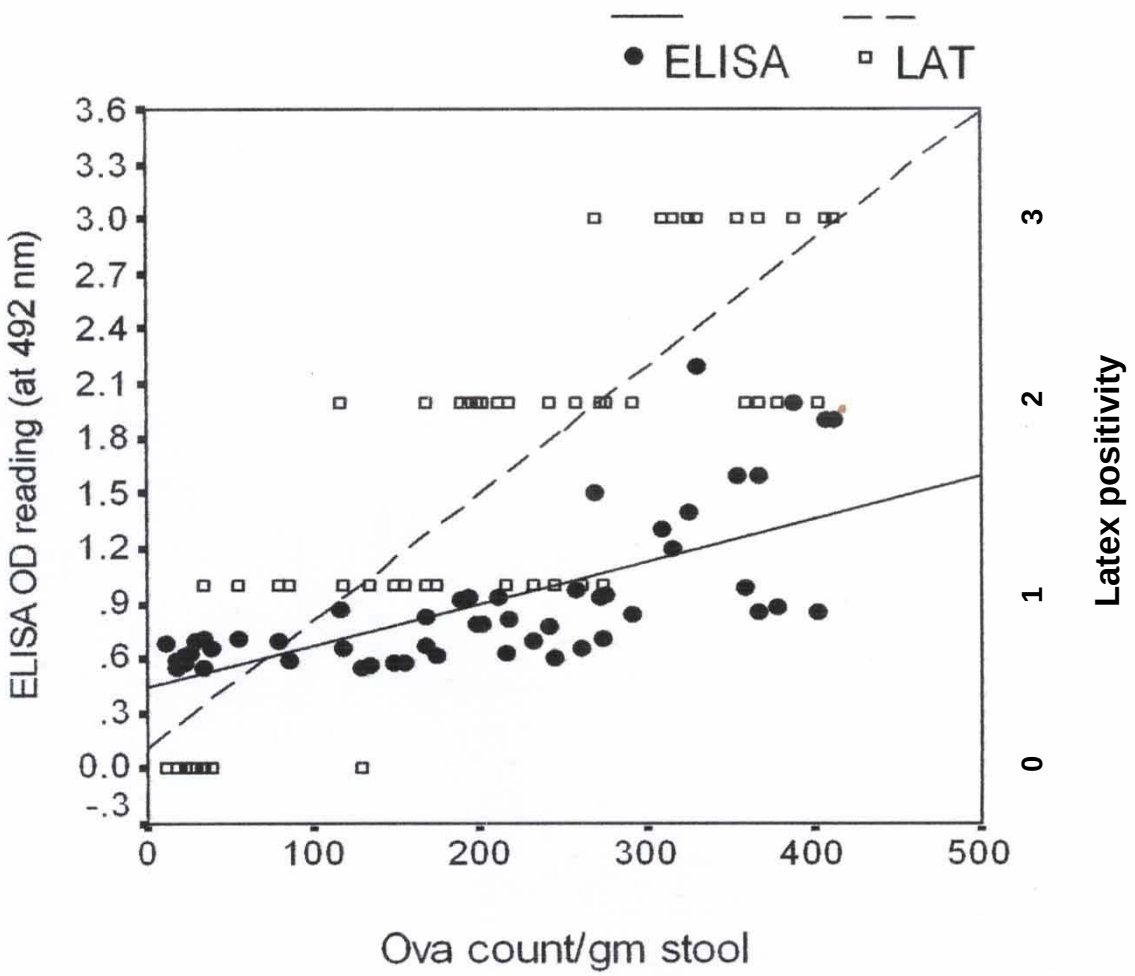

Fig. 3: Correlation between schistosome egg count in stool and both intensity of LAT positivity (r=0.863; p< 0.001) and OD reading of ELISA (r= 0.801; p< 0.001) in serum samples of *S. mansoni* infected group.

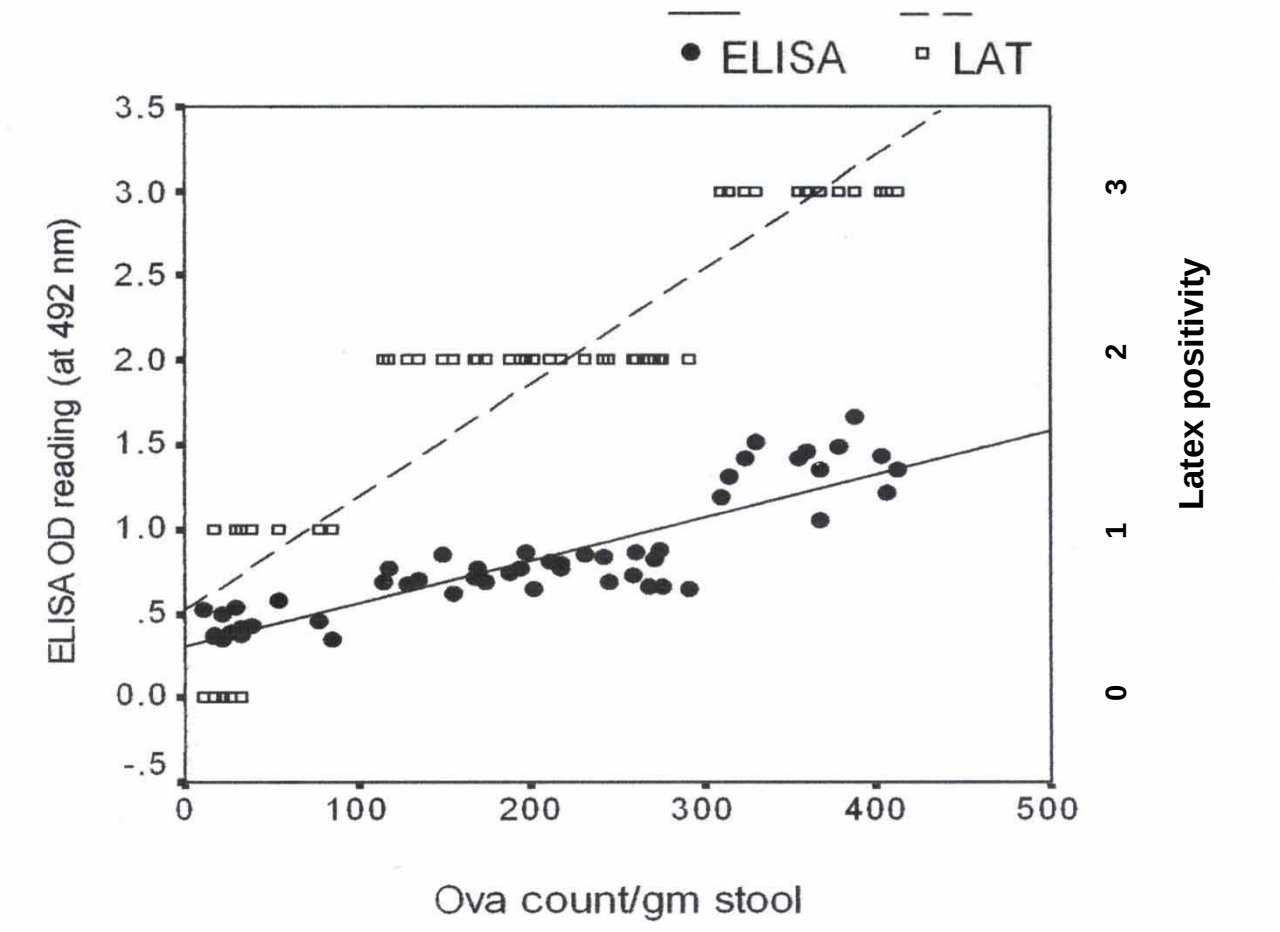

Fig. 4: Correlation between schistosome egg count in stool and both intensity of LAT positivity (r= 0.847; p< 0.001) and OD reading of ELISA (r= 0.915; p< 0.001) in urine samples of *S. mansoni* infected group .

4. Discussions

The development of simple, rapid and sensitive methods for detecting CSA are needed as most available assays require several laboratory equipment and highly skilled persons.

Being able to recognize an antigen with repeating epitope on *S. mansoni* AWTA by IEP, MAb (12D/10F) was selected from a panel of MAbs and employed as both antigen capturing and detecting antibody in sandwich ELISA for detection of CSA in diagnostic extracts of *S. mansoni* and Pardo *et al.* (2004) denoted the 65 kDa as the major recognized band.

The latex agglutination test (LAT) is one of the simplest slide agglutination tests available in a diagnostic parasitology laboratory. The test has been used in the diagnosis of meningococcal meningitis (Gray and Fedorko, 1992). Since then, latex agglutination has been used to detect antibodies in a variety of parasitic diseases such as visceral leishmaniasis (Arya, 1997; Bagchi *et al.*, 1998), toxoplasmosis (Mazumder *et al.*, 1988), *Schistosoma japonicum* (Wang *et al.*, 2006) and *Echinococcosus granulosus* (Barbieri *et al.*, 1993). Devi and Parija (2003) used LAT in detecting circulating *Echinococcus granulosus* antigens in serum, the sensitivity and specificity of the assay was 72% and 98%, respectively.

Although LAT has been used to detect antibodies to schistosomal antigens in serum, the test has schistosomiasis patients' sera. MAb (12D/10F) is an IgM that recognized a proteoglycan antigen of *S. mansoni* AWTA in the molecular weight regions of 50-65 kDa. This is similar to anti-*S. mansoni* IgG MAb of Attallah *et al.* (1999b) which recognized a common band at 63 kDa in three stages of the parasite life cycle; cercariae, soluble egg antigen and adult worms. Moreover, using schistosomiasis patients' sera, Sulahian *et al.* (2005) detected three bands in the range of 65 to 120kDa as

yet to be evaluated for the detection of CSA in urine and serum. The present study was carried out for detection of CSA in both urine and serum samples of a group of *S. mansoni* infected patients using a simple MAb based-latex agglutination test comparing its results with MAb-based sandwich ELISA as a well established reference test for CSA assay. A group of patients infected with parasites other than *Schistosoma* and healthy individuals group were also included in the study. The sensitivity of CSA assay in urine and serum samples by LAT was 90% and 87.1% respectively compared to 92.9% and 95.7% by sandwich ELISA. The specificity of LAT was 88.7% and 93.5% for CSA assay in urine and sera respectively versus 87.1% and 93.6% by sandwich ELISA. This means that sensitivity for CSA assay in urine samples by LAT was comparable to sandwich ELISA but specificity was higher. Therefore, the diagnostic efficacy for CSA assay in urine samples was slightly lower 89.4% using LAT compared to 90.2% by sandwich ELISA. However, sandwich ELISA gave a higher diagnostic efficacy for CSA assay in serum samples (94.7%) compared to 90.2% by LAT.

These results agree with those of Demerdash *et al.* (1995) and Hanallah *et al.* (1995) who used different MAbs-based sandwich ELISA for detection of CSA in both urine and serum samples of *S. mansoni* infected patients and reported a sensitivity of 90.0% and 94.8% in urine respectively, while in serum it was 97.0% and 98.4%, respectively. Also, El-Bassiouny *et al.* (2005) used a pair of MAbs in sandwich ELISA for detection CSA in serum samples of *S. mansoni* infected patients and found 96.7% sensitivity and 92% specificity.

Detection of CSA in urine has a potential for development of non-invasive screening test (Van Etten *et al.*, 1994; 1997; Polman *et al.*, 1995), while serum antigen detection may provide a more direct measure of worm burden for epidemiological research (Van Etten *et al.*, 1994; 1997; Polman *et al.*, 1995; Van Lieshout *et al.*, 1998).

In this study, a significant correlation was observed between the level of CSA detected by ELISA and LAT in both serum and urine and the number of eggs excreted in stool of schistosomiasis patients denoting the reliability of CSA detection as an indication for intensity of infection. These results were in parallel with those of Hendawy *et al.* (2006).

The false negative results observed in LAT and ELISA were found in patients with low number of ova/g stool and this could be due to the possibility that the intact ova of *S. mansoni* may release only small undetectable amounts of antigen into the circulation. Another possibility is that the antigen released from the parasite form immune complexes with circulating antibodies (Carlier *et al.*, 1983; Nash, 1984). Moreover, the disappearance of CSA could be due to the effect of successful chemotherapy denoting the reliability of CSA assay as a cure monitor (Van Lieshout *et al.*, 1993; Demerdash *et al.*, 1995).

Finally, detection of CSA by LAT in urine is very simple, portable, non-invasive especially for children, sensitive and easy to perform a slide agglutination test. The test offers many advantages for its use in the diagnosis of *S. mansoni* infection in poorly equipped laboratories, does not require any special equipment or reagents, and can be performed under difficult field conditions. First and foremost, paramedical health personnel in a rural health center can perform this test on a microscopic glass slide. The test does not require any training or specific skills. Second, this test is cost-effective. Third, LAT is a rapid test, in which results can be obtained within minutes of performing the test. Finally, it can be used to monitor the efficacy of chemotherapy.

In conclusion, the use of LAT for CSA assay could be a valuable applicable screening diagnostic technique in field survey especially for urine samples. A confirmatory sandwich ELISA for CSA assessment in sera is recommended for query false negative results. At the same time, more studies have to be performed to improve the sensitivity and specificity of LAT and hence encourage its use on a large scale for diagnosis of multiple parasitic infections in field surveys.

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The Impacts of Urbanization on Kaduna River Flooding

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Abstract: Population growth, urbanization and expansion of structural developments into traditional flood prone areas of urban settlements of Nigeria are challenges requiring dynamic predictions of inundation areas; development of models for the propagation of flood waves on the floodplain; and the development of a rapid response and flood warning systems. In this study the impact of urbanization on geomorphic parameters of the Kaduna River along the City of Kaduna were investigated. The results obtained indicated that increasing urbanization along the Kaduna River floodplain is responsible for the problem of flooding experienced in recent times along the river floodplain and that encroachment into the traditional flood prone areas of the Kaduna River as a result of urbanization has attained 85.31%, 68.47% and 67.54% respectively in Reach 2, Reach 3 and Reach 1 respectively over the period 1962 and 2009. Because the Kaduna River usually attained its bankfull flow capacities in all its sections along the City of Kaduna early August each year, the result further indicated that the 2yr, 5yr, 10yr, 25yr, 50yr, and 100yr floods when occur can cause maximum inundation of between 82.53% to 94.48% of the floodplain area between the Eastern Byepass bridge and the Kaduna South Waterworks with Ungwan Rimi, Kabala Doki and Kigo road extension as the most critical areas where the right banks are lower than the left banks and developments are almost to the right bank of the river. [Journal of American Science 2010;6(5):28-35]. (ISSN: 1545-1003).

Keywords: Urbanization, River Flooding, Geomorphology, Urbanization, Floodplain Development.

1. Introduction.

The geomorphology of a river system is directly influenced by major variables including channel width, depth, velocity, discharge, channel slope, roughness of channel materials, sediment load and sediment size (Leopold et al. 1964). A change in one variable causes a series of channel adjustments which lead to changes in the other variables, resulting in channel pattern alterations and the manner the channel respond to flood flows flowing through it. Increasing urbanization along the Kaduna River floodplain has led to the problem of flooding which have highlighted the need to understand the consequences of urban developmental activities on the geomorphology of the Kaduna River and the propagation of the flood wave along the river channel.

There have been several cases of floods in Nigeria mostly resulting from heavy downpour and excess releases from dams whose operational capacities could not cope with excessive inflows into their reservoir areas. In most cases these releases are made mainly to safe the dams whose failure could be more catastrophic than the consequences of the releases. Managing flood and other disasters focuses on palate measures and reducing the socio-economic impacts of these disasters through mobilizing relief materials with little investments onto research efforts aiming at understanding the dynamics of these natural events and reducing the impacts of future flood events. In fact a standing National Emergency Management Agency (NEMA) was established by government at the federal level and State Emergency Management Agency (SEMA) at State levels to rapidly respond to the plight of the people in the cases of disasters including flooding. Flood simulations are rarely used in disaster preparations and management either at policy making or implementation levels.

Cases of these floods affects urban centres and rural settlements along the floodplain (Etiosa, 2006; NWRI, 2008), and in all cases houses, property, farm produce and animals were destroyed running into billions of naira each year (Vanguard, 2005 and 2007; The Punch, 2003). Of particular attention and the main focus of this paper is the Kaduna 2003 flood disaster which occurred on Friday 6th September 2003, when Kaduna River overflew its banks spilling flood waters into the adjoin properties along its flood plain across the city of Kaduna. The water stages in the channel and damages to properties along the floodplain were unprecedented, lives were lost,

properties worth about N500 million were destroyed while thousands of people were rendered homeless in the City by the ravaging flood which brought the socio-economic activities of the city to standstill for three consecutive days before the flood waters recedes. In this study, the impact of urbanization on the channel geometry variables of the Kaduna River including channel width, depth, velocity, discharge, channel slope, and roughness of channel materials were investigated in relation to the 2003 flood event.
The Kaduna river took its source on the Jos Plateau, flows northwest across the Kaduna plains cutting several gorges through rugged terrain between Kaduna and Zungeru. Finally, the river flows southwards through the broad, level Niger valley, and enters the Niger River near Wuya in Niger State having drained about 70,200 square kilometers of land area in a 550km long main river course (MNS Encarta 2007) covering Kaduna, Niger, FCT, parts of Plateau, Nasarawa, and Kano States. Major tributaries joining the Kaduna along its course include rivers Karami, Galma,Tubo, Sarkin Pawa and Mariga in that order from source. Kaduna is the only state capital the main channel passes through and Shiroro hydropower reservoir is the only major dam across the main.

2. Existing Development with Kaduna River Floodplain.

The project area covers the reaches of the Kaduna River extending between the confluence with the Kangimi River located upstream and the Western Byepass at Nasarawa downstream Kaduna city respectively. For the purpose of this study, the part of the Kaduna city adjoining the Kaduna River as it flows past the city was divided into three distinct reaches namely Upper, the middle and lower reaches. The Upper reach extending between the confluence of the Kaduna River with the Kangimi to just upstream the Kaduna Eastern Bye Pass Bridge at Malali. Important settlements along this reach include Raafin Gusa, Angwan Dosa, part of Malali and the Makarfi new town. In this reach we have the Kaduna North Waterworks, Federal Government College and substantial parts of the Malali Government Reserve Areas developed for residential accommodation were located within the floodplain. The Kaduna basin especially upstream this reach has a large concentration of small to medium scale dams for water supply and irrigation and which regulates the flows into this reach and with the potential to generate flash floods during raining season. The Galma River, one of the major tributary discharges into the Kaduna River some 30km upstream this reach has two major dams on its main channel. The Kangimi reservoir is about one kilometer upstream this reach and releases its flow into the Kaduna River to augment the flow in the main channel during the low flow period. The middle reach extends from the Eastern Bye Pass Bridge at Malali to the main Kaduna Bridge by the Stadium. The reach is the most developed of the three reaches in terms of physical developments within the floodplain and host to the Ahmadu Bello Stadium, Angwan Rimi GRA, Kigo, Living Faith Church, Kabala Doki and Barnawa. The 2003 flood has its devastating impacts concentrated in this reach. The lower reach extends downstream the main Kaduna Bridge to the Eastern Byepass Bridge This reach adjoins the Zango, Kudenda Industrial layout, Kakuri, Nasarawa, Abattoir, and Moslem burial ground. Hydraulic structures along this reach include, the railway Bridge, the Kaduna South Waterworks, three intake pumping stations belonging to the Nigerian Breweries Plc, the United Nigerian Textile and Arewa Textiles while the Western Bypass Expressway Bridge crosses the Kaduna River within this reach. This reach is usually characterized by very low flow and almost dry situation at the peak of the dry season and many industrial effluents are discharged into this segment of the river. Physical development activities are fast emerging in the floodplain within this reach especially around Zango, Angwan Muazu, Kakuri, and Kudenda industrial layout. The reach profile is characterized by visible rock rapids causing braiding and flow bifurcations at various segments of the reach.

3. Materials and Methods.

3.1 Hydrological Analysis.

3.1.1 Analysis of Rainfall and Streamflow Data.

A comprehensive hydrological investigation aimed at determining the causes, level and the probability of occurrences of flooding in the Kaduna River valley along the City of Kaduna was carried out. Statistical analyses of the rainfall data for the period 1955 to 2004; daily Streamflow data for the period 1967 to 1992 and daily water stages record for the period 1993 to 2004 available for Kaduna River at Kaduna South Waterworks were carried on Microsoft EXCEL to create four extreme rainfall and streamflow databases maximum daily, maximum annual, five days and seven days moving averages. Both data sets were characterized by several months of missing records due to gauge not operational or wash away by flood. No discharge

measurements were conducted at the station for the period 1993 to 2007 because of obsolete equipment and the data for these periods were converted to discharge using the 1994 rating table. Available data were examined for "spurious peak" and suspicious record verified.

The Kaduna River was completely ungauged during the 2003 flood and in order to reconstruct the 2003 flood level, eye witness account by the author and interview made during the field survey indicated that stage record data corresponding to 2003 flood level marks at the Kaduna Railway Bridge is 0.61m below the top of the central pier of the bridge. The top level of the bridge is at 574.55m amsl and the top of the pier is 0.65m to the top level of the bridge. Therefore the 2003 flood level measured at the railway bridge is (574.55-0.65-0.61)m or 573.29m amsl. With a right bank valley slope of 0.042% and distance of 905m to the cross section at the Kaduna South Waterworks, the corresponding level at this cross section is 573.29-0.042%*905 or 572.91m amsl. Extending the rating curve at Kaduna South Water Works to 572.91m gives the corresponding discharge for the 2003 flood as 3,485.31m^3/sec.

3.1.2 Flood Frequency Analysis

The extreme rainfall and streamflow databases analysis indicated that flooding along the Kaduna City adjoining the Kaduna River are rainfall induced and the river channel are expected to be on higher risks of flooding when the channel is flowing bankfull capacity coincides with high rainfall. Consequently the flood frequency analysis was carried out separately on the extreme flow and rainfall databases by fitting the Log Pearson Type III distribution to the database to determine floods levels corresponding to 200, 100, 50, 20, 10, 5 and 2 years annual recurrence intervals.

The Log-Pearson Type III distribution is a statistical technique for fitting frequency distribution data to predict the design flood for a river at some site. The Log-Pearson Type III distribution is calculated using equation (1).

$$\log x = \overline{\log x} + K\sigma_{\log x} \quad (1)$$

where x is the flood discharge value of some specified probability, $\overline{\log x}$ is the average of the *log x* discharge values, *K* is a frequency factor, and σ is the standard deviation of the *log x* values. The frequency factor *K* is a function of the skewness coefficient and return period and can be found using the frequency table. The flood magnitudes for the various return periods were found by solving the equation (1) on Microsoft EXCEL. The analysis results are presented in Tables 1 and 2.

Table 1 Flood Frequency Analysis for Kaduna River at Kaduna South Waterworks
Log Pearson Type III Estimated Flood Flows (m3/sec)

	Return Period (years)						
	2	5	10	25	50	100	200
Max Daily Q	1,578.60	2,181.72	2,607.43	3,175.96	3,621.59	4,086.07	4,573.47
Max 5days Q	1,218.57	1,535.55	1,607.96	1,641.22	1,649.64	1,652.41	1,654.22
Max 7 days Q	1,108.94	1,343.03	1,350.97	1,403.08	1,406.81	1,407.59	1,408.20

Table 2 Flood Frequency Analysis of Rainfall at Kaduna Airport
Log Pearson Type III Estimated Rainfall (mm)

	Return Period (years)						
	2	5	10	25	50	100	200
Max Daily	67.73	75.20	79.27	83.75	86.70	89.40	91.91
Max 5-day Total	124.78	134.11	138.97	144.14	147.47	150.43	153.14
Max 7-day Total	150.20	160.53	165.49	170.41	173.38	175.91	178.10
Annual Rainfall	1,235.79	1,317.84	1,346.89	1,368.80	1,378.77	1,385.40	1,389.93

3.2 Geomorphological Characterization and Channel Planform Classification.

The field investigation and topographic surveys were organized in three distinct reaches of the Kaduna River principally to collect project related data on geomorphology, River Mechanics, and channel hydraulic geometry. Instrumentation mobilized for these activities includes **eTrex Garmin GPS** for positioning and distance measurements; **Total Station Instrument** for spot heights and positions**,** and **digital camera** for picture documentation on existing conditions. The entire

activities were carried out by traversing the river course while assessing the river and its floodplains for changes in river geomorphology. Survey of the river cross sections were carried out using the **Total Station** instrument and canoe was used to carry the reflector across the sections of the river where water was flowing at the time of survey. A total of fifty nine cross sections were surveyed consisting of 21 in reach 1, 25 in Reach 2 and 13 in Reach 3 and the cross sections were spaced along the longitudinal direction in a manner to capture the changes along the channel and extending across the width of the floodplain at the section.

All the field generated data were analysed using a combination of software including Microsoft Excel 2007, Surface Mapping System Software (Surfer Version 8.01) and AUTOCAD 2007 that facilitated the management of the information collected for the determination of the following Channel Morphology classification parameters (Rosgen, 1996).

- The channel sinuosity which is an index of channel pattern, determined from a ratio of stream length divided by valley length; or estimated from a ratio of valley slope divided by channel slope.
- The entrenchment ratio (ER) is the ratio of the flood-prone area width divided by bankfull channel width.
- The width to depth ratio is the ratio of the bankfull width to the mean depth of the stream channel at bankfull stage elevation.

The bankfull width is the width of the stream channel at the bankfull stage elevation in a riffle section. The mean depth is the depth of the stream channel at the bankfull stage elevation in a riffle section. The maximum depth is the depth of the bankfull channel cross-section, or vertical distance between the bankfull stage and thalweg elevations, in a riffle section. The flood-prone area width is measured at an elevation that is twice the maximum depth at the location that the maximum depth was determined. Table 3 presents the summary of the geomorphological parameters for the three reaches while the channel geometry parameters related to bankfull and flood dimensions for selected cross sections, presented in Table 4 for Reach 2, were calculated at each cross section and averaged over each of the three reaches provides the baseline data upon which the changes in geomorphology of Kaduna River arising from anthropological changes were evaluated.

3.2.1 Planform Description

The Google Earth images of reaches of Kaduna River under investigation were downloaded and employed for the channel pattern description. In Reach 1 which is the uppermost portions under investigation the river channel exhibits a regular sinuous meanders at its downstream portion while at its uppermost portion, the channel exhibits braiding and mild meanders with several aggregation and degradation points. Commercial mining of good quality aggregates for infrastructural development in Kaduna City has been going on for years in this reach and still a daily activity.

Table 3 Summary of Gemorphological Parameters.

Parameter		Reach 1	Reach 2	Reach 3
Channel Plan View		Single Threaded	Multi Threaded	Single Threaded
Average water surface slope (S) m/m		0.000109671	8.45557E-05	0.000306698
Stream or channel length (SL) m		21,097.14	24,032.93	4,650.63
Stream or Channel Slope		0.00038	0.00051	0.00157
Valley length (VL) m	Left Bank	20,898.43	24,708.42	4,561.13
	Right Bank	21,097.14	22,483.18	4,802.10
Valley slope (VS) m/m	Left Bank	0.000306281	0.00064	0.00140
	Right Bank	0.000375634	0.000515	0.001142501
Sinuosity (VS/SL)		0.907686209	1.13997	0.809256879
Sinuosity (SL/VL)		1.004731857	1.018525	0.993382274
Entrenchment ratio (W_{fpa}/W_{bfl})		2.057847328	1.795573	1.936598257
Width / Depth Ratio		103.074	221.600	142.171
Stream Power (N/m/s)		2.41	1.49	2.67

Table 4 Channel Geometry Parameters Related to Bankfull and Flood Dimensions for Selected Cross Sections, for Reach 2

PARAMETER	X-14	X-21	X- 22	X- 15	X- 16	X- 23	X--32	X-38	Eastern Bye Pass
Bankfull Dimensions									
X-section area (m.sq.)	484.29	162.42	1,130.9	637.66	404.27	345.14	367.75	345.21	877.78
Width (m)	145.89	167.36	341.85	277.70	204.88	223.82	240.81	169.37	276.70
Mean depth (m)	1.30	1.15	2.97	2.10	0.84	1.20	1.29	2.86	1.99
Max depth (m)	2.74	2.13	5.18	3.35	3.96	2.13	2.44	5.18	4.27
Flood Dimensions									
Flood prone area Width (W_{fpa}) m	262.22	283.43	364.80	379.19	247.98	238.47	294.24	275.25	137.39
Width Left Floodplain	209.75	158.19	106.40	106.40	232.04	483.35	470.00	604.69	589.98
Width Left Floodplain Encroached (m)	144.10	124.08	106.40	106.40	206.18	457.19	0.00	0.00	0.00
Width Right Floodplain	499.95	492.86	439.41	437.02	571.30	494.42	488.50	415.81	638.44
Width Right Floodplain Encroached (m)	480.68	409.96	386.89	395.87	544.63	479.89	432.50	343.50	599.85
Low bank height	571.50	570.89	572.41	571.80	573.02	574.55	583.39	579.12	584.00
Max riffle depth	568.76	570.59	569.98	569.98	570.59	573.02	580.95	579.12	579.73
Bank height ratio (LBH/max riffle depth)	1.0048	1.0005	1.0043	1.0032	1.0043	1.0027	1.0042	1.0000	1.0074
Flood prone area Elevation (EL_{fpa}) m	574.24	574.85	580.34	576.68	578.51	577.29	585.83	589.48	588.26
Maximum Level in the Cross Section	573.94	573.94	575.16	575.46	574.85	575.46	585.22	588.87	591.62

Reach 2 is the most urbanized of the three reaches and the river channel is multi channeled characterized by heavy braiding and heavy anastomosing occasioned by heavy concentration of rock outcrops all across the river length and cross section. The river width and its flood plain is greatest in this reach most especially between Malali and Kigo road extension where the 2003 flood unleashed the most devastating effect on the city. Two other left side tributaries confluence with the Kaduna main channel within this reach which makes this reach very critical for this study. The river and its floodplain narrowed to just 269.13m at its exit into Reach 3 due to construction of the Kaduna River main bridge and fences by properties owner around.

The Kaduna river flow into Reach 3 with a very sharp U shaped meanders around the Moslem burial ground and characterized by heavy braiding and major flow bifurcations occasioned by occurrences of two vegetated bars just downstream the Kaduna South Waterworks Intake.

3.2.2 Flood Plain Encroachments and Flood Risk Zone Delineation

Urban expansion in all the communities located near the main stream channel of the Kaduna River has caused floodplain areas to be developed. High rise hollow block wall fences had been placed to allow the use and development of areas that originally provided zones for natural floodwater storage and conveyance. As a result, channel floodway zones have become constrained most especially in the middle reach where the 2003 has caused severe damages. Consequences of these developments are many for instance flood passage through these areas may results in higher stages and low velocities and shortage of flood attenuation potential. In other reaches, encroachment may impede the downstream progression of the floodwave such that backwater effects may cause high local flood levels.

To determine the extent of encroachments into the floodplain consequent to urbanization development, the surveyed cross section data was overlaid with the topographical map of the area taken in 1962. The two extremities of the cross sections are limits of floodplain development as at the time of surveys March 2009. The limit of the floodplain as delineated by contour elevation 1900feet was compared with the width of the cross section surveyed to quantitatively give the extent of floodplain encroachment between 1962 and 2009. Table 5 presents the extent of floodplain development between 1962 and 2009 while Figure 1 shows typical cross sections and floodwall at section in Reach 2.

Table 5 Extent of Kaduna River Floodplain Development

	Reach 3		Reach 2		Reach 1*	
	Left	Right	Left	Right	Left	Right
Area of floodplain (ha) as at 1962	124.41	130.48	388.55	455.48	453.46	406.20
Developed Area (ha) as at 2009	85.19	36.04	68.06	388.55	0.00	274.35
Developed Area as % of 1962 Coverage	68.47	27.62	17.52	85.31	0	67.54
Undeveloped Area (ha)	39.23	94.44	320.50	66.93	453.46	131.85
Undeveloped Area as % of 1962 Coverage	31.53	72.38	82.48	14.69	100	32.46

*Measured to Rafin Gusa (Limit of Kaduna City)

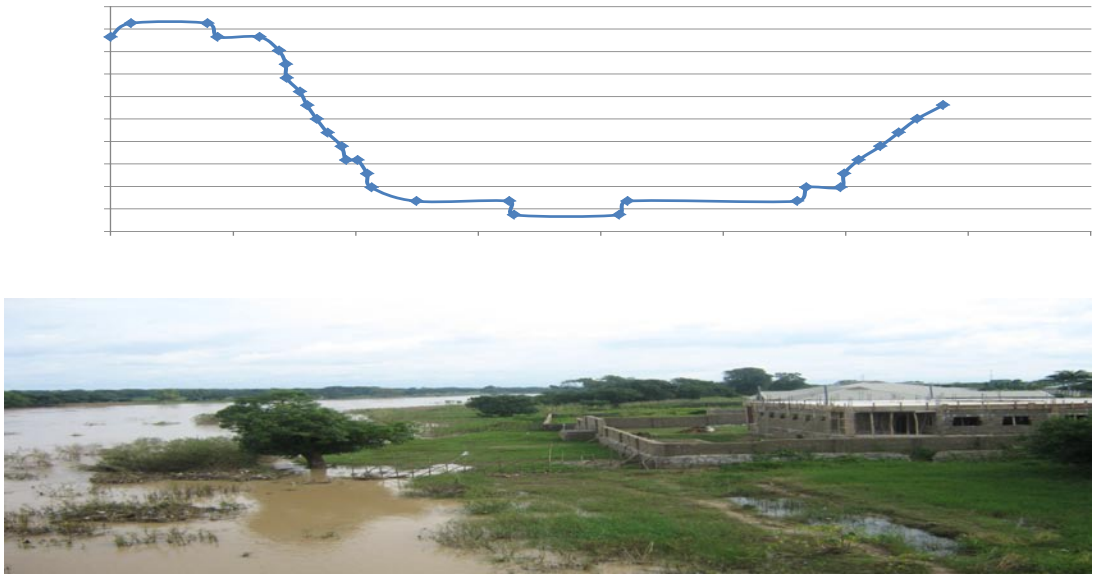

Figure 1 Typical Cross Section in Reach II and Floodwall Protection Structure
(Source: Alayande, 2010)

The extent of flood risk zones corresponding to the 2yr, 5yr, 10yr, 25yr, 50yr, 100yr and 200yr flood levels were determined by reading off the water stages corresponding to these flood levels discharges on the rating curves established for each of the 59 cross sections. These levels were compared with the floodprone area elevation and the maximum elevation in the cross section. Where maximum elevation in the cross section is less than the flood level elevation, or the flood level elevation is greater than the floodprone area elevation then there is the risk of flooding.

4.0 Results and Discussions

Hydrological analysis of rainfall data 1955 to 2004 revealed that the year 2003 annual rainfall of 1459.4mm was the third historical maximum annual rainfall coming after 1691.34mm and 1674.88mm of 1955 and 1957 respectively. Also the month of August 2003 was fifth wettest month during the period. Available record did not indicated flooding in the basin in 1955 and 1957 but what was certain was that the level of urbanization and structural developments was higher in 2003 than in 1955 and 1957. The analyses of streamflow data for the period 1967 and 2004 indicated that the month of August and September are the wettest months each year producing the maximum daily flow annually for ten and fifteen months respectively during the period under investigation. The historical maximum daily flow of 2,926.31m^3/sec was recorded on the 18th September 1994 followed by 2,871.75m^3/sec and 2,579.50m^3/sec for 1986 and 1992 respectively.

Analysis of 5-days and 7-days consecutive rainfall and average daily flows did not show and significant pointer to the occurrence of flooding in the basin. The flood frequency analysis shows that the basin's 2yr, 5yr, 10yr, 25yr, 50yr, 100yr and 200yr floods are respectively 1,578.6m^3/sec, 2,181.72m^3/sec, 2,607.43 m^3/sec, 3,175.86m^3/sec, 3,621.59m^3/sec, 4,086.07m^3/sec and 4,573.47m^3/sec and what we experienced in 2003 could be as much as 3,485.31m^3/sec . From the hydrological point of view, the Kaduna 2003 was rainfall-induced or as a result of high rainfall aggravated by indiscriminate structural developments in the floodplain that progressively reduces the width of the floodplain.

The geomorphological characterization classify the reaches investigated as class B stream segment defined as moderately entrenched, moderate width-to-depth ratio, moderate gradient, riffle dominated channel with gently sloping valleys; rapids predominates with scour pools infrequently spaced; very stable plan and profile. The average main channel slope is 0.0416% while the longitudinal slope for both banks is 0.042%. The Kaduna River channel develops into several low, medium and high flows braided reaches and five bifurcated reaches characteristically overgrown with forested vegetation, or shrubs while the soils in the bars are consolidated eroded materials from the catchments. Nineteen tributaries flow into the main Kaduna channel with a tributary density of 1.93 tributaries per kilometer.

Impact analysis of urbanization shows that the Kaduna River floodplain is increasing urbanized at a maximum encroachment rate of 85.31%, 68.47% and 67.54% respectively in Reach 2, Reach 3 and Reach 1 over the period 1962 and 2009. The flood risk zones were determined by comparing the floodprone area elevation with the maximum elevation in each of the cross sections. Where maximum elevation in the cross section is less than the floodprone area elevation, there is the risk of flooding. The analysis indicated that 39 out of 59 cross sections are under the risk of overbank spills of flood waters into the adjoin properties with Reach 2 the most vulnerable having 21 cross sections susceptible to overbank spills out of 21 cross sections. Existing floodwalls made of hollow bricks are quite inadequate in capacities for flood control as they can be pulled down under severe flooding. Also these walls are not continuous a situation that can lead to flood water flowing through unprotected sections to inundates properties.

In view of the fact that the Kaduna River usually attained its bankfull flow capacities in all its sections along the City of Kaduna in early August each year, when this situation coincides with the occurrences of the 2yr, 5yr, 10yr, 25yr, 50yr, and 100yr floods, the level of flood plain inundation could be as much as 82.53% to 94.48% of the floodplain area between the Eastern Byepass bridge and the Kaduna South Waterworks with Ungwan Rimi, Kabala Doki and Kigo road extension as the most critical areas where the right banks are lower than the left banks and developments are almost to the right bank of the river.

5. Conclusion

The results from this study indicated that urbanization is progressively modifying the Kaduna River floodplain and its flow. This situation if persisted without proper flood protection works will endanger both lives and properties in the floodplain. Existing flood protection measures cannot and will never put to check the menace of flooding along Kaduna River. A concerted effort in the form holistic approach towards controlling the flood is urgently required. It is therefore recommended that the Kaduna State Government should immediately put in place a policy to regulate infrastructural development along the Kaduna floodplain as a short term measure and construct dyke along the banks to shield already developed area from flood water as a long term measure.

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Extraction Conditions of Inulin from Jerusalem Artichoke Tubers and its Effects on Blood Glucose and Lipid Profile in Diabetic Rats

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Abstract: This study aimed to analyze Jerusalem artichoke tubers to identify its contents and to optimize conventional extraction of inulin, various time extract, temperature, and solvent ratio were used. 30 male albino rats divided into 5 groups (6 rats) were used to evaluated the extricated inulin as Hypoglycemic agents. It could be concluded that, the highest yield of inulin was recovered from Jerusalem artichoke tuber by using the following condition, sample to solvent ratio was 1: 5 w/v at 80°C for 90 minutes. The crude inulin extracted from Jerusalem artichoke tubers were used for production of orange juice and chocolate and estimated by aid of 10 panelists. The reduction of glucose was observed after one week of feeding till the end of experimental period, also, high level of inulin 15% led to amore reduction of blood glucose level comparing with the low level especially in the end of experimental period. The crude inulin extracted from Jerusalem artichoke tubers were used in diet for diabetic rats on different levels of inulin (10 and 15%) had significantly lower in total cholesterol, triglyceride and total lipids in comparing to positive diabetic rats fed on control diet. Meanwhile, HDL level was increased significantly after fed on 10 and 15% inulin. On the other hand, LDL and VLDL levels were decreased significantly after fed on (10 and 15%) inulin in comparing to positive group rats fed on control diet. [Journal of American Science 2010;6(5):36-43]. (ISSN: 1545-1003).

Keywords: Jerusalem artichoke, Extraction Conditions, inulin, blood glucose and lipid profile

1. Introduction

Today, the industrialized countries are facing, among others, three major challenges. Firstly, to control the cost of health care, secondly, to offer to their aging population a real opportunity to live, not only longer, but also better and thirdly, to provide to more and more. Jobs, consumers, a choice of healthy processed or ready to eat foods **(Roberfroid, 1999)**. Busy life styles and the increasing demand from consumers for meals and snacks that are quick sources of good nutrition have prompted the food industry to develop foods like nutrition bars that combine convenience and nutrition **(Izzo and Niness, 2001)**.

Inulin is a storage polysaccharide consisting of a chain of fructose molecules with a terminal glucose molecule. **Silva (1996)**, inulin is water soluble, the solubility being temperature dependent. At 10°C its solubility is about 6% whereas at 90°C it is about 35%. Inulin type fructans, are the best-documented oligosaccharides for their effect on intestinal bifidobacteria and are considered important prebiotic substrates **(Vos *et al.*, 2006)**. Jerusalem artichoke tubers with 14–19% inulin can be a valuable source of inulin **(Vanloo, et al., 1995)**.
Several methods for inulin extraction from Jerusalem artichoke have been developed. A pretreatment step involving boiling water-extracting for 10. 15 min of the ground tubers had been used **(Laurenzo *et al.*, 1999)**.

Initially, the application of inulin in the food industry was restricted to the production of a drink similar to coffee, due to its bitter taste. However, it was found that inulin could substitute sugar and fat with the advantage of exhibiting low caloric value **(Applied Technology, 1993)**. The application of inulin as a fat substitute is associated with its capacity of producing a cream-like substance, similar to fat dissolved in water, which can act as a rheological modifier **(Cândido and Campos, 1995 and Silva, 1996)**. Inulin shows interesting technological properties, as a low-calorie sweetener, as a fat substitute, or it can be used to modify texture **(Tungland and Meyer, 2002)**. These properties are linked to the degree of chain polymerisation. Inulin are added during cheese processing to decrease its fat percentage without losing its organoleptic characteristics, such as texture and flavour. One of the interesting functions of inulin and oligofructose in human nutrition is related to their prebiotic effect, i.e. the specific stimulation of growth and/or activity of a limited number of colonic bacteria beneficial to the host, as well as the growth inhibition of pathogens and harmful microorganisms **(Roberfroid, 2007**).

The combination of prebiotics and probiotics has given rise to the so-called 'synbiotics', with promising healthy properties (**Buritiet al., 2007; Pool- Zobel and Sauer, 2007**).

The functional effects of inulin on humans and experimental animals include relief of constipation lower blood glucose levels, improved absorption of calcium, reduced fasting triglycerides, LDL cholesterol, and inhibition of the growth of various kinds of tumors **(Kaur *and Gupta* 2002)**. **Marchetti (1993)** reported that, inulin is a natural polymer that not hydrolysable by the intestinal enzymes, because it has β (2-1) link which is not be hydrolyzed. So it could be considered a calorie free fiber, although some calories may occur due to the digestible fermentation of these by products in the colon. Inulin is such carbohydrates have a high potential nutritional advantage as low energy dietary supplements. It can be used as a source of carbohydrates for diabetic patients and more generally as dietary fiber. During 4 to 6 weeks improves glucose tolerance, decreases glycemia, and partially restores insulin secretion **(Cani *et al.*, 2005)**. Moreover, an improvement of glucose/insulin ratio has also been observed in rats receiving Oligofructose added in a high fructose diet (inulin) **(Busserolles *et al.*, 2003)**. In the case of low-calorie chocolates and derivatives, fibre compounds such as inulin and oligofructose are used as sugar substitutes **(Gonze and Van der Schueren, 1997)**. This study was carried out to investigate studying the best conditions to obtain inulin from Jerusalem artichoke for production of new foods and beverages with high biological value and studying the health benefits of inulin as functional food on diabetic and hyperlipidemic rats.

2. Material and Methods

Jerusalem artichoke tubers (*Helianthus tuberosus*) were purchased from the experimental station, Agriculture Research Center, Cairo, Egypt. inulin was isolated from the tubers of Jerusalem artichoke. Cholesterol, casein, bile acids, kits and cholin cholerid were purchased from El-Gomhoreria Co.

Technological methods:-

Preparation of Jerusalem artichoke tubers:

The samples of Jerusalem artichoke tubers were cleaned with tap water to remove dust and other undesirable materials. The cleaned tubers were cut into small pieces and used immediately.

Extraction of inulin from Jerusalem artichoke by different methods:

Jerusalem artichoke tubers were mixed with different volumes of hot water according to **Yamazaki (1994)** (sample to water ratio were 1 : 1, 1 : 3, 1 : 5 and 1 : 7 w/v) tuber : water. The effect of using different temperature (70, 80, 90 and 100°C) as will as different times (60, 70, 80 and 90 minutes) were also done to choose the best condition of extraction of inulin.

Preparation of juice and chocolate: Orange juice and Chocolate were prepared according to the method of **El-Gendy (1970).**

Sensory evaluation:

Juice and chocolate:

Orange juice, chocolate, juice and chocolate supplemented by different levels of inulin (10, 15, 20 and 25%) estimated by aid of 10 panelists. The following hedonic scale was used **Kramer and Twigg (1970)** for juice **and Kramer and Twigg (1970)** for chocolate.

Analytical methods:

The prepared samples were subjected to the chemical analysis moisture, protein, fat and ash by the methods described by **A.O.A.C. (1990)**. Crude fiber was measured using the method described by **Kirk and Sawyer (1991)**. Total carbohydrates were determined using the method described by **James (1995)**. Determination of inulin was recommended by **Prosky and Hoebregs (1999).**

Biological experiments:

Animals:

30 adult male albino rats weighting between 115 to 120 g were divided to 5 groups and (each group consisted of 6 rats), each rat housed individually in wire cages with wire bottoms.

Preparation of diabetic rats:

Pure fine was used for induction diabetes in normal healthy adult male albino rats by intraperitoneal injection of alloxan (150mg/kg body weight) according to the method described by **(Desia and Bhide, 1985)**, fasting blood samples obtained from retro orbital plexus (Superficial blood sample) to determined serum glucose. Diabetic rates were divided into 4 groups, 6 rats in each group fed on certain diets for 28 days as follows: First group was fed on basal diet only and used as control negative group. Second group diabetic rats were fed on basal diet and used as the control positive group. Third and fourth group diabetic rats were fed on basal diet containing 10 and 15% inulin.

Biological Evaluation:

At the end of trials, the animals were sacrificed, under ether anaesthetized and blood

samples were collected in clean dry centrifuge tube from hepatic portal vein. Serum was separated by centrifugation at 4000 r.p.m. for 10 minutes at room temperature then kept in plastic vials at-20c until analysis.

Biochemical Analysis:

Glucose was determined by enzymatic methods using kits according to **Trinder (1969)**. Determination of triglycerides in serum was determined calorimetrically according to **(Fossatip and Prancipel, 1982)**. Total cholesterol was determined by colorimetric method according to **Allain(1974)**. HDL Cholesterol was determined according to **Lopez (1977)**. Total lipid was determined by colorimetric method according to **Schimit (1964)**. Calculation of LDL and VLDL in mg/dl according to **Lee and Nieman (1996)**.

LDL cholesterol = Total Cholesterol - (HDL + T.G / 5) mg / dl.

VLDL cholesterol = Triglycerides / 5

Statistical analysis:

The results expressed as mean ± SD, arid performing using student (t) test. The oplained results will be analyzed to determine the degree of significances between different groups ($p \leq 0.05$) using one way analyzing of various (ANOVA) **(SAS, 1988)**.

3. Results and Discussion

Chemical composition of Jerusalem artichoke and extracted inulin:

Chemical composition of Jerusalem artichoke tubers percentages were calculated as dry weight (Table 1). Data obtained from this table showed that, Jerusalem artichoke had a low level of moisture content, their was 6.36% apparent also, from the same table that Jerusalem artichoke tubers seems to have inulin content (72.99%). Also, total carbohydrate content of Jerusalem artichoke were 78.03%, our results are in line with those of **Sahar (2003)** who reported that chemical composition of Jerusalem artichoke, Moisture, total carbohydrate, inulin, crude protein, crude fiber and ash were 6.50, 86.21, 71.78, 7.40, 7.52 and 5.30 g / 100 g, respectively. Also, these results are slightly with those of **Fleming and Groot-Wassink (1979); Guiraud *et al.* (1981) and Rashwan (1996)**, who reported that, Jerusalem artichoke tubers contained 85. 95% carbohydrates that were recovered mainly in the form of inulin. From the previous results, it could be concluded that, Jerusalem artichoke tubers have level of inulin high enough to be utilized commercially.

Meanwhile, Data in Table (1) showed chemical composition of extracted inulin from Jerusalem artichoke tuber. As shown the mean value of moisture, ether extract, crude protein, ash, inulin and crude fiber after chemical analysis of extracted inulin their were 4.57, 0.35, 0.49, 0.75, 96.87 and 1.54, respectively. our findings are in harmony with those of **Shalaby (2000)** who found that, inulin isolated from Jerusalem artichoke tubers was characterized by high value of inulin 96.25%.

Effect of solvent ratio, temperatures and extraction time on extractable inulin (%) from Jerusalem artichoke tuber:

Data obtained from Table (2) showed the effect of solvent ratio to samples, different temperatures and extraction time on extractable inulin (%) from Jerusalem artichoke. The optimum ratio to recover the highest yield of inulin was 1: 5 sample to solvent. This may be due to the presence of enough amounts of water required to dissolve and separate the maximum amounts of inulin found in the cell. From the same data it colud be noticed that increasing sample to solvent ratio from (1 : 5) to (1 : 7) had no significant effect. Data obtained from the same Table showed the influence of using different temperatures on the extractable inulin (%), as shown the mean values of extractable inulin after using different temperatures (70, 80, 90 and 100°C), their were 88.55, 92.46, 92.81 and 93.36, respectively, and these results were in agreement with the results recorded by **Margaritis and Bajpai (1982)** who extracted inulin from artichoke chips with water at temperatures ranged from 70 to 100°C.

Data recorded in Table (2) indicated that extractable inulin was increased with increasing the extraction time. Finally, it could be concluded that, the highest yield of inulin was recovered from Jerusalem artichoke tuber by using the following condition, sample to solvent ratio was 1 : 5 w/v at 80°C for 90 minutes. Our results are in agreement with the data obtained from **Sahar (2003)** who reported nearly the same condition.

Organoleptic evaluation:

Juice:

Organoleptic evaluation of the different manufactured orange juice is presented in Table (3). The data indicated that, orange juice prepared without inulin (control) had the highest values of taste, odor, color, texture and overall acceptability comparing to those prepared by adding different levels of inulin as judged by a group of panelists from nutrition and food science department, in addition, it should be noted from obtained data that, there were no significant differences between control and some levels of inulin (10 and 15%) on values of taste, odor, color, texture and overall acceptability.

On the other hand, using (20 and 25%) of inulin as a percent of substitution had a significant differences in comparingto control and those prepared with 10 and 15% inulin.

Chocolate:

Sensorial results of the chocolate studied did not indicate any significant differences in preference between control sample and different levels of inulin 10 and 15%, even though control sample was considered the most preferred trial of chocolate studied (Table 4). On the other hand, different levels of inulin 20 and 25% had a significant difference in comparing to control sample and samples prepared with 10 and 15% inulin. The appearance of the chocolates with partial fat replacement was similar to the appearance of conventional chocolate which is important because appearance in one of the four main sensory characteristics that plays a role in sensory acceptability **(Jones, 1996)**. **Thompson *et al.* (2004)** reported that, *Cocoa aroma* is a major driver influencing acceptability of chocolate milks.

Biological experiments:

Effect of different levels of inulin (10 and 15%) on glucose levels of diabetic rats:

Data presented in Table (5) showed the effect of using different levels of inulin 10 and 15% on serum glucose levels of diabetic rats. As shown the mean values of serum glucose levels for control negative group after first week, second week and fourth week were 78.63, 81.96 and 84.46, respectively. With regard to the mean values of glucose levels for control positive group after first, second and fourth week was recorded 231.00, 244.30 and 220.00, respectively. Apparent, also from the same table that the mean values of serum glucose levels of diabetic rats after receiving different levels of inulin (10 and 15%) first, second and fourth weeks were (224.70, 195.30 and 173.90) for positive group rats receiving 10% inulin and (226.40, 183.73 and 136.86) for positive group of rats receiving 15% inulin. It could be concluded that feeding on different, levels of inulin (10 and 15%) led to significant decrease in serum glucose level of positive group rats when compared with diabetic rats receiving standard diet. The reduction of serum glucose was observed after one week of feeding till the end of experimental period, also, high level of inulin 15% led to a more reduction of blood glucose level comparing with the 10% inulin level especially at the end of experimental period.

Our results are in agreement with those of **Alles *et al.* (1999) and Niness (1999)** who recorded that, inulin and oligofructose play an active role in reducing the caloric value and they do not lead to a rise in serum glucose or stimulate insulin secretion. However, **Molis *et al.* (2002)** reported that aforementioned action to the possible beneficial effects of inulin on blood glucose. However, there are some evidences that, inulin may decreased fasting blood sugar in type 2 diabetic that, can be explained as follow: inulin may delay gastric emptying and shorten small intestinal transit time, propionate may inhibit gluconeogensis. This can be done by the metabolic conversion to methyl malonyl-Co. A and succinyl-Co. **Roberfroid and Delzenne (1998)** reported that, supplementation with Jerusalem artichoke significantly decreased blood glucose levels in non-insulin dependent diabetes. Also, **Giacco *et al.* (2002)** found that, dietary fiber in particular the soluble fiber fraction plays the important role in controlling glucose concentration in serum and other risk factors associated with diabetes.

Effect of different levels of inulin (10 and 15%) on TC, TG and T. lipids of diabetic rats:

Data in Table (6) show the mean value of total cholesterol TC after receiving 10 and 15% inulin their were 148.40 and 123.06 while the mean value of TC for positive and negative control groups were 160.06 and 111.60, respectively. Apparent, also from this table the level of triglyceride TG of rats received different levels of inulin (10 and 15%) ranged from 195.63 for 10% and 165.83 for 15% inulin and ranged from 134.26 to 215.70 for negative and positive control groups. Data in this table indicated that total lipids for diabetic rates received 10 and 15% inulin ranged from 545.16 for 10% and 495.00 for 15% inulin, in comparing to positive and negative control groups which ranged from 650.96 for control positive and 421.86 for control negative. Finally, it could be concluded that, diabetic rats received different levels of inulin (10 and 15%) had a significant decreased in total cholesterol, triglyceride and total lipids when compared to positive diabetic rats received standard diet. Our findings are in agreement with those of **Levrat *et al.* (1991)** who found that, dietary inulin played active role in reducing serum cholesterol concentration in rats fed on diet contains inulin for 3 weeks. **Pushparaj *et al.* (2007)** reported that, administration of inulin extract of *Cichorium intybus* produced a significant reduction in serum glucose, triglycerides and total cholesterol in diabetic rats.

Effect of different levels of inulin (10 and 15%) on HDL, LDL and VLDL of diabetic rats:

Data in Table (7) show the effect of different levels of inulin (10 and 15%) on HDL, LDL and VLDL in serum of diabetic rats. It is noticed that HDL level was increased significantly after received

10 and 15% inulin by the means of 57.90 and 57.63 in comparing to control positive group rats which was 50.13. Data from the same table showed no significant difference between 10 and 15% inulin on HDL level of diabetic rats. On the other hand, LDL level was decreased significantly after fed a diet supplemented with 10 and 15% inulin by the means of 51.36 and 32.26 inulin in comparing to positive rats group which received the basal diet their was (66.83). In the same table, VLDL level was decreased significantly after received different levels of inulin by the means of 39.13 and 33.16 in comparing to positive rats group which fed the basal diet (43.10). In conclusion, data presented in this table indicated that, addition of inulin in different levels (10 and 15%) had significantly higher serum HDL in comparing to rats (control positive group) fed the basal diets, also, significantly lower in serum LDL and VLDL. Propionate, a product that yields from inulin fermentation in colon may inhibit hydroxymethylglutaryl-Co. A reductase which is considered the ratlimiting step in cholesterol biosynthesis **(Deleznne and Kok, 2001)**.

Table (1). Chemical composition of Jerusalem artichoke tuber and extracted inulin (as dry weight).

Constituents	Jerusalem artichoke tuber	Inulin
Moisture	6.36±0.97	4.57±0.56
Ether extract	1.40± 0.10	0.35± 0.16
Crude protein	7.55± 0.34	0.49± 0.11
Ash	5.72± 0.21	0.75± 0.15
Crude fiber	6.51± 0.17	1.45± 0.12
*Inulin	72.99± 2.34	95.36± 2.96
Total carbohydrates	78.03± 1.35	95.51± 2.45

* Inulin from total carbohydrates

Table 2: Effect of extraction time, solvent ratio and temperatures on extractable inulin (%) from Jerusalem artichoke tuber.

Time of extraction (min)	Extractable inulin (%)	Sample to solvent ratio	Extractable inulin (%)	Temperate of extraction (°C)	Extractable inulin (%)
60	$85.37^{b} + 2.12$	1:1	$77.61^{c} \pm 0.66$	70	$88.55^{b} \pm 0.80$
70	$87.92^{b} + 1.38$	1:3	$86.64^{b} \pm 0.71$	80	$92.46^{a} \pm 0.79$
80	$92.53^{a} + 1.21$	1:5	$92.26^{a} \pm 0.93$	90	$92.81^{a} \pm 0.57$
90	$93.12^{a} + 1.42$	1:7	$92.64^{a} \pm 0.83$	100	$93.36^{a} \pm 0.89$

Mean[1] in the same column with different letters are significantly different ($p \leq 0.05$).

Table (3). Organoleptic evaluation of orange juices substituted their sucrose with different levels of extracted inulin.

Type of juices	Quality index				
	Taste	Odor	Color	Texture	Overall acceptability
Control	$8.92^{a} \pm 0.13$	$8.82^{a} \pm 0.17$	$8.94^{a} \pm 0.13$	$8.84^{a} \pm 0.20$	$8.94^{a} \pm 0.02$
10% inulin	$8.88^{a} \pm 0.13$	$8.84^{a} \pm 0.15$	$8.74^{a} \pm 0.26$	$8.80^{a} \pm 0.21$	$8.74^{a} \pm 0.20$
15% inulin	$8.80^{a} \pm 0.15$	$8.74^{a} \pm 0.20$	$8.74^{a} \pm 0.25$	$8.68^{a} \pm 0.28$	$8.68^{a} \pm 0.34$
20% inulin	$7.66^{b} \pm 0.34$	$7.56^{b} \pm 0.38$	$7.52^{b} \pm 0.31$	$7.64^{b} \pm 0.30$	$7.54^{b} \pm 0.25$
25% inulin	$6.74^{c} \pm 0.45$	$6.80^{c} \pm 0.43$	$6.84^{c} \pm 0.45$	$6.74^{c} \pm 0.36$	$6.68^{c} \pm 0.27$

Means in the same row with different superscript letters are significantly different ($p \leq 0.05$).

Table (4). Organoleptic evaluation of chocolate substituted their sucrose with different levels of extracted inulin.

Type of chocolate	Quality index				
	Appearance	Texture	Flavour	Aroma	Overall acceptability
Control	8.42^{a}±0.10	8.42^{a}±0.07	8.46^{a}±0.08	8.36^{a}±0.05	8.44^{a}±0.05
10% inulin	8.36^{a}±0.05	8.40^{a}±0.04	8.38^{a}±0.05	8.32^{a}±0.04	8.36^{a}±0.05
15% inulin	8.24^{a}±0.05	8.40^{a}±0.12	8.36^{a}±0.13	8.28^{a}±0.04	8.34^{a}±0.05
20% inulin	7.62^{b}±0.13	7.48^{b}±0.08	7.46^{b}±0.05	7.48^{b}±0.08	7.34^{b}±0.11
25% inulin	6.78^{c}±0.27	6.42^{c}±0.08	6.52^{c}±0.19	6.40^{c}±0.15	6.38^{c}±0.13

Mean[1] in the same column with different letters are significantly different ($p \leq 0.05$).

Table (5). Effect of 10 and 15% inulin on (glucose level in blood) of diabetic rats.

Period	Groups				Mean [1]
	Control (-)	Control (+)	10 % Inulin	15 % Inulin	
0	78.63±1.11	231.6±2.31	224.7± 2.60	226.4± 4.84	**190.3^{c}± 67.4**
7 days	81.96±1.64	224.3±3.45	195.3± 2.35	183.7± 2.44	**171.31^{b}± 56.0**
14 days	84.46±2.10	220.0±1.62	173.9± 1.91	136.8± 4.76	**153.79^{a}± 82.0**
Mean [2]	**81.68^{a}±2.9**	**225.3^{d}± 5.5**	**197.9^{c}±22.16**	**182.3^{b}± 38.95**	

Mean[1] in the same column with different letters are significantly different ($p \leq 0.05$), LSD = 2.230
Mean[2] in the same row with different letters are significantly different ($p \leq 0.05$), LSD = 3.841

Table (6). Effect of different levels of inulin (10 and 15%) on TC, TG and TL of diabetic rats.

Groups / Variables	Control (-)	Control (+)	10 % Inulin	15 % Inulin	LSD
Total cholesterol (mg/dl)	111.6^{a} ± 1.56	160.06^{d} ± 2.11	148.4^{c}± 2.75	123.06^{b} ± 1.94	4.02
Triglyceride (g/dl)	134.26^{a}± 1.44	215.7^{d}± 5.55	195.63^{c}± 3.7	165.83^{b}± 1.89	6.67
Total lipids	421.86^{a}± 15.8	650.96^{d}± 16.3	545.16^{c}± 22.06	495.0^{b}± 6.84	30.52

Means in the same row with different superscript letters are significantly different ($p \leq 0.05$).
Each value in the table is the average of six replicates.

Table (7). Effect of different levels of inulin (10 and 15%) on HDL, LDL and VLDL of diabetic rats.

Groups / Variables	Control (-)	Control (+)	10 % Inulin	15 % Inulin	LSD
HDL (mg/dl)	63.96^{a}±1.75	50.13^{c}±1.61	57.9^{b}±1.66	57.63^{b}±1.05	2.91
LDL (mg/dl)	20.80^{a}± 0.88	66.83^{d}±1.33	51.36^{c}±2.40	32.26^{b}±1.4	3.04
VLDL (mg/dl)	26.83^{a}± 0.28	43.10^{d}±1.1	39.13^{c}±0.76	33.16^{b}±0.37	1.33

Means in the same row with different superscript letters are significantly different ($p \leq 0.05$).
Each value in the table is the average of six replicates.

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Potential impact of bee pollen administration during pregnancy in rats

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Abstract: Although bee pollen was recommended as a supplemental diet for its nutritionally beneficial components, it is warning for its usage during pregnancy. In this study bee pollen (PB) water extract with different doses (2.5 & 5 and 10 g/kg b.w./day) was delivered to pregnant rats orally from day 1 to day 21 of gestation to address the physiological relevance of bee pollen rich in proteins and phytoestrogen during pregnancy in rats and to examine whether bee pollen administration modifies the serum steroid hormones involved in fetal outcome. The results revealed that bee pollen administration at high doses (5 &10 g/kg/day) during pregnancy has an adverse effect on mothers and fetal outcome manifested by dams death, failure in implantation processes, resorbtion of fetuses, reduction in fetal numbers, retardation in fetal and placental weights. Lipid oxidation markers such as MAD and GSH levels were changed on day 21 of gestation in bee pollen treated rats referring to incidence of imbalance of oxidant/antioxidant system. Circulating profile of estradiol (E_2), testosterone and progesterone were changed at selected time intervals (7,12,17 and 21) of gestation. Bee pollen had no apparently effect on cholesterol value and decreasing effect on triglyceride, HDL-cholesterol and LDL-cholesterol values through gestational period, it produced hypercholestermia and hyperlipidemia on day 21 of gestation especially at high doses. On determining the concentration of total protein and albumin, it was showed a significant increase particularly, in the second half of pregnancy pertaining to the groups administered bee pollen at a dose of 5 & 10 mg/kg b.w./day. The present results revealed that supplemental of pregnant rats with bee pollen throughout gestational period had harmful effect to a great extent on mothers and fetuses life. [Journal of American Science 2010;6(5):44-53]. (ISSN: 1545-1003).

Key Words: pollen- pregnancy - rats

1- Introduction

In modern times perception-that bee pollen as apicultural product is focused for human diet because of its nutritionally beneficial compounds has increasingly come under attack. Bee pollen (BP) is flower pollen collected from selected flower species by the honey bee, is known to be of particular essentiality for the reproduction and survival of these creatures. It is well documented that bee pollens are rich in essential amino acids, protein, unsaturated fatty acids and also contains many vitamins, minerals and trace elements which contribute to the health effects **(Campos et al., 2003)**. Because of its nutrient-rich components, it has been used as a folk medicine for centuries, to alleviate or cure conditions such as cold, flu, ulcer, premature aging, anemia, colitis, enteritis, and allergic reactions **(Šarić et al., 2009)**.The main active ingredients of bee pollen are primarily phytoestrogens including isoflavones, flavonols and lignans, otherwise known as plant hormones, compounds with well-documented hormonal benefits for both men and women **(Kristoffersen et al., 1997 and Moon et al., 2006).** Also, the phenolic components of bee pollen were reported to exhibit high levels of antioxidant and radical scavenging activity **(Carpes et al., 2007 and Eraslan et al., 2009).** Although its precise mode of action is still obscure, evidence gained from experiments suggested that the extract's lipid soluble fraction contains the material responsible for inhibiting the biosynthesis of prostaglandins and leukotrienes **(Šarić et al., 2009)**. Accordingly, BP is one alternative therapy utilizing plant-derived products (phytotherapy) as the treatment of choice for many chronic conditions and can be for developing preventive and therapeutic agents for various estrogen-mediated diseases **(Šarić et al., 2009).**

Though nutritional benefit has been noted with this dietary supplement, one study only, up to author knowledge, suggested that bee pollen could improvement nutrition without affecting normal fetal development, noting pollen to be a practical and effective nutrient during pregnancy but they warned from its usage **(Xie et al., 1994).** To asses the manifestations effect of bee pollen on pregnancy, oral injection to pregnant rats was performed to study the adverse effects resulting from bee pollen administration. Furthermore and more important, the aim of the present study was to demonstrate the effect of bee pollen on fetal outcome associated with

maternal hormonal pattern changes. The present study bears originality with regard to the inexistence of any previous study in which bee pollen was used as nutritional diet during pregnancy.

2. Material and Methods

Bee Pollen Extracts:

The pollen was collected from clover (*Trefoil Alexandrinum*) using special pollen traps from bee hives located at Sharkia Governate, Egypt. Firstly, the bee pollen was ground and this ground was stored continuously at 2–8 °C in desiccators. Microscopic examination demonstrated a purity of greater than 97%, with less than 0.5% foreign bee pollen and less than 0.9% plant parts. The powder of bee pollen (5g) was suspended in distilled water (20 ml) and mixed vigorously for 2 h. Following filtration and centrifugation (10,000×g, 20 min), the combined solution was concentrated and finally freeze dried. For use in experiments, the bee pollen of extract was dissolved in distilled water **(Masayoshi et al., 2007).**

Animals and Treatments:

Rats were kept under an environmentally controlled room (19 C, range 18-21°C, relative humidity 64%). Food pellets and tap water were freely available. Adult rats were housed in groups of two females and one male in a plastic cage measuring 36 × 23 × 20 cm during a 4-day period. Vaginal smears were collected each day, with the first day of detectable sperm designated as embryonic day 1. On the first day of gestation, females were isolated in individual cages and randomly divided into four groups, each consisted of 10 rats.

The water-solubilized extract of bee pollen was orally administered at doses of 2.5,5 and10 gm/kg b.w./day to pregnant rat groups through a stomach tube from day 1 to day 21 of gestation. Control rats were received distilled water orally.

Pregnancy assessment and blood samples collection:

Maternal survival and mortality were recorded throughout gestational period and the dead animals were compensated by others to obtain the desired numbers of animals (n=10) in each group. All animals were fasted over night prior to scarifying. Blood samples were collected from orbital venous plexus of pregnant rats at time intervals of 2, 7, 12, 17 and 21 days of gestation and centrifuged at 3000 rpm for 10 min to separate plasma and serum. Subsequently, the animals were killed on day 21 of gestation with an overdose of ether and the uteri were removed with their contents to determine the number of live, dead and resorbed fetuses. The uterus of apparently non pregnant rats was stained with a 10% solution of sodium sulphide and evaluated for evidence of early resorption or implantation sites **(Chahoud and Paumgartten, 2005)**. Live fetuses and their placentas were weighed to the nearest milligram.

Hormonal analysis:

Serum estradiol (E_2), progesterone and testosterone concentrations were quantified by radioimmunoassay according to the method of **Burtis and Ashwood (1994), Smith (1985)** and **Wilson and Foster (1992),** respectively.

Measurement of oxidative stress markers and biochemical parameters:

Plasma malondialdehyde (MDA) levels and blood reduced glutathione concentration were determined as lipid oxidation markers according to the methods described by **Yoshioka et al. (1979)** and **Beutler et al., 1963**, respectively. Serum total cholesterol, triglycerides and HDL-cholesterol levels were measured by kinetic method using commercial kits according to the method of **Stein (1986), Walleyed (1974)** and **Wieland and Seidel (1981),** respectively and LDL was calculated. Also, serum protein and albumin contents were measured according to the method **of Dumas and Biggs (1975).**

Statistical analysis:

All results are presented as mean ± standard error of the mean. Statistical significances of the differences between the mean of the two groups of samples were assessed using Student's *t* test. Differences were considered to be statistically significant at $p < 0.05$ and 0.01.

3. Results

The adverse effect of bee pollen supplementation to pregnant rats firstly appeared on the inability of dams for continuity to survive. Since, there are 30% dams died on day 11 and 30% on day 13 of gestation in groups which received 5 &10 g/kg/day bee pollen, respectively.

Dissection of the uteri revealed that, in the females with uniform-sized uterine swellings, pregnancy appeared normal and general appearance of the fetuses. Opening of the uterine horns of the females with several sizes of uterine swellings revealed that normal viable fetuses were associated with the largest swellings, whereas the smaller one contained disintegrating or no fetal tissue. The uterine swellings that appeared abnormally small contained no fetal

tissue at the sites of implantation or placentation, and resorptions were considered to be completed at these sites. Resorbing fetuses within the closed uterine compartments showed different degrees of disintegration. The uterine lumen was open between viable fetuses, whereas uterine compartments isolated the sites with disintegrating or no fetal tissue. The endometrium within these compartments was extensively folded, greatly enlarging the luminal surface. Placentas were smaller at sites where no fetal tissue remained than that of viable fetuses, but the general appearance of it was similar in two cases.

As shown in Table 1, bee pollen administration to rats over a period of gestation reduced fetal number at full term and this effect was dependent on the administered dose. Fetal weights were lighter in all groups administered bee pollen than controls, and this was accompanied with similar reductions in placental weight. Among 30 pregnant rats treated with bee pollen, 11 cases showed red spots in the uterine segment as a signs of implantation with no fetal tissues and 11 cases showed sign of resorptions.

Table 1: Uterine implantation, fetal resorbtion, live fetuses, fetal and placental weights of control and bee pollen groups on day 21 of gestation in rats

Groups / Parameters	Control group	Bee pollen administered groups		
		2.5 g/kg	5 g/kg	10 g/kg
Live fetal numbers	7.1± 1.44	6.5 ±1.16	4.4 ± 0.44**	2.4 ± 0.14**
Live fetal weights	4.1 ±0.40	3.8 ± 0.28	3.1 ± 0.14*	3.1 ± 0.20*
Placental weights	0.54 ±0.03	0.49 ±0.07	0.45 ± 0.23*	0.39 ±0.04**
Implanted sites Recorded cases No. of implanted sites No. of live fetuses	Zero	2 7 6	4 13 5	5 24 Zero
Resorbed fetuses Recorded cases No. of resoebed fetuses No. of live fetuses	Zero	4 8 10	4 8 12	3 8 10

Values are represented as means ±S E for the recorded cases; *P< 0.05, **P<0.01

In table (2), administration of bee pollen extract with high doses (5 &10 g/kg b.w.) through gestational period to pregnant rats appeared to increase significantly plasma MDA associated by significant decrease in blood GSH value on day 21 of gestation as compared to controls. It, however, showed non significant change in either MAD or GSH values in animals received the lower dose (2.5 g/kg b.w.) of bee pollen.

Table 2: plasma MDA and blood GSH of control and bee pollen groups on day 21 of gestation in rats.

Groups / Parameters	Control group	Bee pollen administered groups		
		2.5 g/kg	5 g/kg	10 g/kg
MDA (nmol/ml)	7. 45±0.34	9.0±0.76	10.5**±0.66	12.0** ± 1.01
GSH (mg/dl)	61.47±2.0	58.8± 1.44	53. 6*± 2.61	50.0*±2.11

Values are represented as means ±S E for the recorded cases. * P< 0.05, ** P<0.01

Among all treated animals, bee pollen extract appeared modifying effect in E_2, testosterone and progesterone patterns at selected time intervals through gestational period and this effect was dose dependent. Since, bee pollen administration caused a significant decrease in circulating E_2 level on days 7 (fig 1) concomitant with non significant change in testosterone (fig 2) and progesterone (fig 3) levels followed by a sharp increase in E2 and testosterone levels only on day 12 of gestation. The three hormonal levels showed sharp decrease on day 17 of gestation than control remaining to day 21 except progesterone level, increase significantly at term.

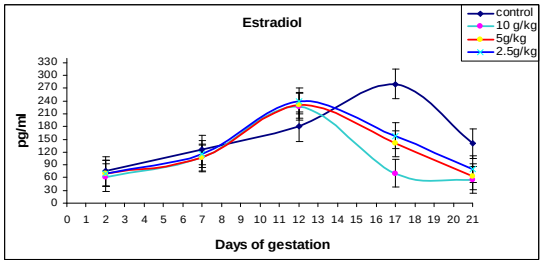

Fig 1: Serum estradiol pattern in control and bee pollen groups

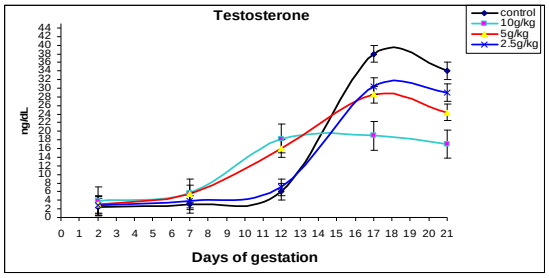

Fig 2: Serum testosterone pattern in control and bee pollen groups.

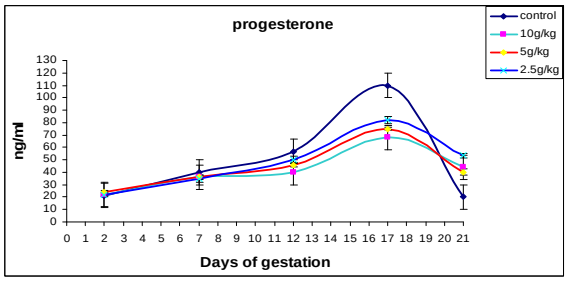

Fig 3: Serum progesterone pattern in control and bee pollen groups.

Neither serum cholesterol nor triglyceride concentrations differed among the four groups (control and treated groups) on day 2 of gestation. Thereafter, levels of cholesterol were maintained within control range among all BP treated animals until day 21 of gestation where it increased only in animals receiving 10g/kg b.w/day. BP. But triglyceride levels decreased significantly in animals receiving 5 and 10 g/kg BP on days 7 and 12 of gestation returning to approach control value on day 17 and increased sharply on day 21 of gestation. Whereas, the animals that received 2.5 g/kg BP, the triglyceride level recorded significant decrease on day 12 and 17 of gestation and returned to control level on day 21 (Fig 4 &5).

Animal group receiving 10g/kg of BP, showed significant decrease in HDL-cholesterol and LDL-cholesterol levels from day 7 till day 21 of gestation. Whereas, the animals received 5g/kg b.w. of bee pollen showed significant decrease in HDL-cholesterol and LDL-cholesterol levels from day 12 to day 21 of gestation. HDL-cholesterol and LDL-cholesterol in animals administered 2.5 g/kg of BP were slightly variable but not statistically differ than control (Fig 6 &7).

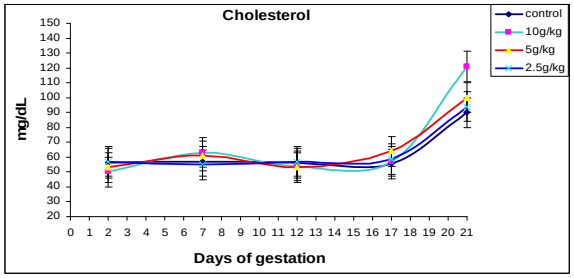

Fig 4: Serum cholesterol pattern in control and bee pollen group

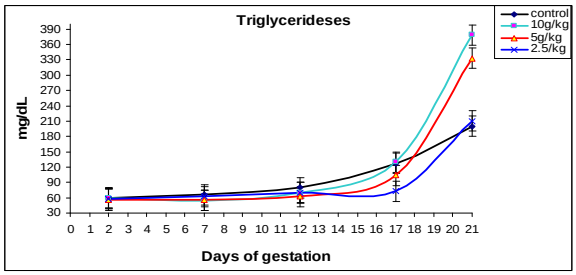

Fig 5: Serum triglyceride pattern in control and bee pollen groups.

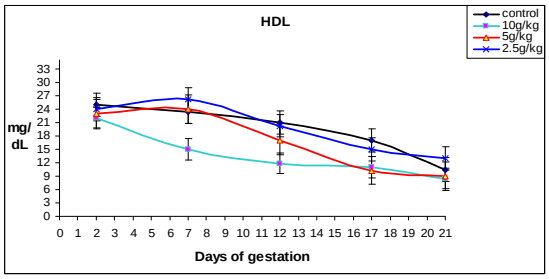

Fig 6: Serum HDL-cholesterol concentration in control and bee pollen groups.

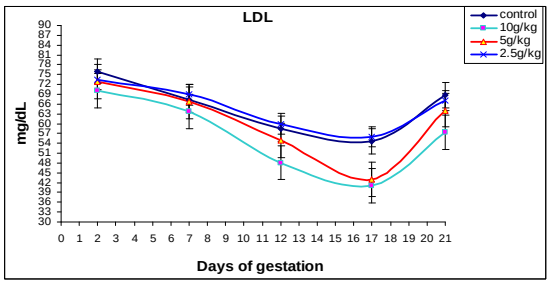

Fig7: Serum LDL-cholesterol concentration in control and bee pollen groups.

Administration of BP extract (10g/kg b.w./day) to pregnant rats caused significant increase in total protein concentration from day 7 till day 21 of gestation and this increase was dose dependent. Relative to control, little gradual increase was observed in protein concentration of animals that received 2.5 and 5 g/kg BP on days 7 and 12

followed by significant increase on days 17 and 21 of gestation. These changes were contaminant with noticeable variable and non significant increase, especially on the second half of gestation, in albumin concentration among all animals administered BP.

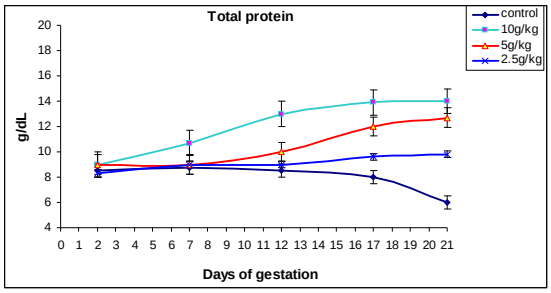

Fig 8: Serum total protein pattern in control and bee pollen groups.

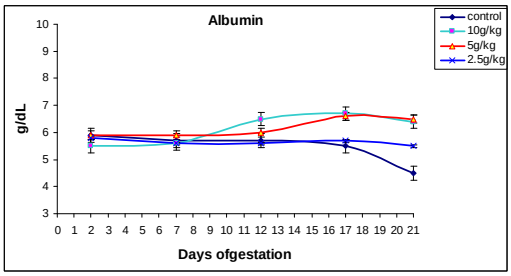

Fig 9: Serum albumin pattern in control and bee pollen groups.

4. Discussions

The quality of diet has long been known to affect the fetal condition, a better maternal diet being associated with better condition of the offspring. Extremes of diet are well recognized as adversely affecting the outcome of pregnancy (Law et al., 2000). However, inadequate nutrition during fetal development can alter aspects of morphologic and physiologic development increasing the predisposition in adult life to metabolic diseases such as diabetes mellitus and cardiovascular disease (Gillman, 2002). Fetal nutrition depends on the concentration of nutrients in the maternal bloodstream, placental perfusion and the transfer of nutrients through the placenta (Pisani et al., 2008).

In the current study, about 30% of dams administered the high doses of bee pollen (5 & 10g/kg b.w.) died in mid-pregnency. The mortaliry of dams may incident as a consequent to ingestion of certain toxins intake of bee pollen into the body. Bee pollen may constitute *ochratoxin* A **(Medina et al., 2004)** and bacterial spores of *Clostridium botulinum* **(Compas et al., 2008).** The analysis of ready-to-eat bee pollen samples have revealed contamination with potential mycotoxin producing species, including *Penicillium verrucosum*, *Aspergillus niger* aggregate, *Aspergillus carbonarius*, *Aspergillus ochraceus*, *Aspergillus flavus*, *Aspergillus parasiticus* and *Alternaria* spp. **(Gonzalez *et al*., 2005)**, heavy metals **(Jablonski *et al*., 1995; Leita, 1996; Conti and Botre, 2001)** and pesticides (**Fleche *et al*., 1997; Kubik *et al*., 1999**) originating from the environment and from agricultural practices are also considered the main contaminants of bee pollen but the bacterial contamination is a greater problem than pesticide or heavy metal contamination **(Bogdanov, 2006).**

Based upon the previous evidence, it could be suggested that the contamination of bee pollen which was provided into the pregnant rats was the main reason of alteration observed in MDA and GSH concentrations. Another important factor may be involved is, that the administration of bee pollen with high concentration of flavones constitute could be harmful because in condition in which a state of oxidation is not induced, flavonols can itself behave as an oxidant agent, inducing liver damage and a high production of NDA **(Okuda, 1999**). Also, after extensive flavonoid intake, flavonoid concentration in plasma may be insufficient to exert systemic antioxidant effects *in vivo,* probably as a result of flavonoid metabolites (methylated, sulphated or glucuronidated forms) which tend to have decreased antioxidant activity **(Dudov and Starodub, 1994).**
In bee pollen treated rats, the embryonic uterine implantation did not correspond to the numbers of fetuses at term, since a high degree of uterine reabsorption occurred. By examination of dam uteri, it was revealed that decreased litter size was due to increased prenatal mortaliry, predominantly at the post-implantation stage, and that the ovulation rate had not changed associated with placental growth retardation, particularly within groups with highest embryonic mortality. Accordingly, the number and weight of viable fetuses and their placentas recovered in the treated pregnant rats at term were less than in control. Such fecundity defects is usually considered to result from a toxic substance-induced prenatal growth retardation **(Eriksson et al., 2002).** It appeared that the consumption of bee pollen may constitute an important risk factor not only for mothers but also for the fetuses concerning the presence of mycotoxins **(Compas et al., 2008)** associated with oxidative stress which reduced the placenta retained **(Mandang et al., 2007)**. On the other hand, consumption of bee pollen rich in phytoestrogens **(Janeczko and Skoczowski, 2005**

and Moon et al., 2006) may lead to changes in hormonal levels and/or ovarian sensitivity to hormones with subsequent changes in maternal estrogen/progesterone ratio required for pregnancy maintenance **(Ruhlen et al., 2008)**. Because estrogens administration at any stage of gestation can affect various aspects of fetal development **(Ruhlen et al., 2008)**, phytoestrogens consumption during pregnancy represent a potential risk factor for abnormal development **(Janeczko and Skoczowski, 2005).** Phytoestrogen administration during rat pregnancy decreased placental blood flow **(Mahendroo et al., 1997)**, lowered fetal weights **(Becker et al., 2005 and Ruhlen et al., 2008)** and is also lethal to the embryo. Depending upon the modern-day analyses, many of the plants contain phytoestrogens were historically noted for their ability to prevent pregnancies or cause miscarriages **(Ruhlen et al., 2008)**. Although there are a wide variety of effects reported for rats that were exposed to phytoestrogens during gestation, there is inconsistency within the literature which suggests that the amount and administration route may be critical and effective **(Nagao et al., 2001, Dlclos et al., 2001, Lewis, et al., 2003, Kouki et al 2003 and Nikaido et al., 2004)**.

In the current study, the adverse effect of bee pollen on fetal outcome was associated with a change in circulating maternal hormonal pattern through gestational period. Estrogen is essential for ovarian progesterone production throughout pregnancy **(Rothchild, 1983)** and the placenta may be the source of testosterone substrate for ovarian estrogen formation during the second falf of rat pregnancy **(Bartholomeusz et al., 1999)**. It is pertinent the serum estradiol level accumulated in all pregnant rats administered bee pollen with different doses was lower in the first half of pregnancy (days 2&7 of gestation) than control, reaching its peak on day 12 and returned back to sharp decrease on days 17 and 21 of gestation and this change was dose dependent. However, estradiol is among the powerful of estradiol hormones in mammals, and even slight changes in the estrogen response system during gestation can affect fetal development **(Collins, 1996)** or fetal death because the endogenous control of E2 secretion must be carefully regulated to maintain optimum conditions for fetal development **(Bartholomeusz et al., 1999)**. Stimulation of estrogen production by phytoestrogens in trophoblast cells is probably due to estrogen receptor blocking effects of phytoestrogens. Trophoblast cells seem to compensate blocking of its estrogen receptors by higher estrogen production **(Richter et al., 2009)**.

The alteration in estradiol pattern in bee pollen administered dams was concomitant with a similar pattern change in the testosterone level. Since, the significant changes in testosterone value starting from day 12 till day 21 of gestation. Such changes may be related to specific growth-retarding effect of estradiol on the placenta which secondarily limits fetal growth and the ability of estradiol to inhibit placental production of testosterone **(Legrand et al., 1984)**, essential precursor for its own synthesis by the ovary **(Jackson and Albrecht, 1986).** Maternal circulating testosterone in bee pollen treated rats was also responsible for pregnancy disruption because testosterone can affect fetal growth and size through modifying maternal energy homeostasis and decreasing the nutrient supplies to the placenta and fetus **(Steckler et al., 2005)**. Alternatively, testosterone may modify placental function and reduce the capacity for transport of nutrients to the fetus or cross the placenta and exert a direct effect on fetal growth and/or energy homeostasis **(Carlsen et al., 2006).** On the other hand, the decrease in estrogen related to testosterone concentration in rats receiving bee pollen may be due to inhibitory effect of phytoestrogen content on aromatase activity, the enzyme that converts androgen to estrogen whether as a result of competition for its active site or by reducing enzyme expression regulating aromatase activity **(Oh et al., 2000 and Burton and wells, 2002).** There is, however, increasing evidence that phytoestrogens may bind to aromatase and/or 17β-hydroxysteroid dehydrogenase (HSD) and thereby reduce the availability of these enzymes for the production of estrogen from androgen precursors and/or the production of estradiol from weak estrogens **(Le Bail *et al*., 2000).**

With regard to progesterone, growth and development of the embryo and fetus are unaffected over a wide range of progesterone concentrations in the maternal plasma. The present results revealed a marked change in progesterone concentration till term, which implies that bee pollen affects the ovarian function and the uterine environment resulted again on the very vunreable hormonal cycle. However, bee pollen may disturb the production of 20α hydroxysteroid dehydrogenase (20αHSD), the enzyme that converts intraluteal progesterone to its inactive metabolite 20α-dihydroprogesterone **(Arosh *et al.* 2004)**, in which circulating progesterone level elevated at term **(Piekorz *et al.* 2005)** referring to delay of parturition**.** On the other hand, the plant estrogens inhibit enzymes involved in dteroidogenesis because these compounds are estrogenic per se and may thus replace endogenous estrogens. Also, phytoestrogens sufficiently reduce progesterone production in term trophoblast cells **(Jefferson et al., 2006).** Because blockade of

progesterone is a possible mechanism involved in initiation of labor, thus the high doses of phytoestrogens at the feto-maternal interphase could play a negative role in maintenance of pregnancy. There is some concern that bee pollen when used orally might have uterine stimulant effects; avoid using **(Leung and Foster, 1996).**

The present findings are consistent to great extent with the reports of many previously workers such as, **Jefferson et al., (2006)**, who suggested that the plant estrogens are inhibit enzymes involved in steroidogenesis because these compounds are estrogenic per se, and may thus replace endogenous estrogens. Also, phytoestrogens sufficiently reduce progesterone production in term trophoblast cells. Because blockade of progesterone is a possible mechanism involved in initiation of labor, thus the high doses of phytoestrogens intake through bee pollen consumption at the feto-maternal interphase could play a negative role in maintenance of pregnancy **(Richter et al., 2009)**. In another study, **Kao and P;Eng (1995)** revealed the ability of phytoestrogens to interfere in estrogen negative feedback by binding to estrogen receptors in interior pituitary or hypothalamus and indirectly alter ovarian steroidogenesis.

Although the administration of bee pollen had no apparently effect on cholesterol value and decreasing effect on triglyceride, HDL-chlesterol and LDL-cholesterol values through gestational period, it produced hypercholestermia and hyperlipidemia on day 21 of gestation especially at high doses. Perhaps the best approach explanation is the definition of the functional capacity of the placenta to perform metabolic alterations of certain lipids. In most studies the degree of transport across the placenta and the degree of fetal or placental synthesis of lipids varies with gestational age **(Herrera et al., 2006).** Thus, placental size, architecture, developmental and pathological processes, and interaction with the fetus cooperate with transport and metabolic mechanisms to affect placental-fetal nutrient exchange **(William and Hay 1994).** Apparently, earlier in pregnancy, there is more dependence on maternal lipids to provide placental and fetal lipids. The placenta has the capacity of altering those lipids presented to it by selective transport and inter conversions mediated transfer of lipid uptake from lipoproteins, metabolic alteration in the placenta, and release into the fetal plasma **(Hussain et al., 2003).** Also, most lipid classes are synthesized *de novo* in the placenta **(Herrera et al., 2006).** In this study, phytoestrogen content in bee pollen could exert damage on placental cells **(Janeczko and Skoczowski, 2005)** leading to restriction in placental blood supply to carry lipids to meet the high demand of the developing fetus **(Wadsack et al., 2003)** resulting in accumulation of lipid fractions in maternal circulation.

The concentration of total proteins and albumin values showed significant increase particularly, in the second half of pregnancy pertaining to the groups administered bee pollen at a dose of 5 & 10 mg/kg b.w./day. The elevation in protein and albumin concentrations may be due to the high contents of protein and amino acids supplemented in bee pollen (**Xie et al., 1994** and **El-Missiry, 1999),** that provided amino acids greatly in excess of the requirements of the fetuses and the transfer of these amino acids might have been limited by the functional capacity of the placenta **(William 1994).** Interestingly, combustion of pregnant rats to dietary protein caused elevation of estradiol level earlier in pregnancy and a significant decrease in E2 concentrations near term **(Wilson et al., 2000).** It was suggested that a protein rich diet was associated with an increased incidence of premature deliveries, babies with low birth weights and neonatal death **(Malhorta and Sawers 1986).**

One must consider that the present results, although consistent and significant, are not sufficient to a further discussion. The data obtained are suggestive to inspire future research, one can speculate that the bee pollen at a high doses acted mainly during implantation stage, a susceptible period when toxic action of any substance leads to embryonic death. Bee pollen did not acted during organogenesis, because in doing so, it could lead to fetal malformations.

The current work provides evidence that bee pollen toxicity was high enough to affect both mother and embryo. Decline in size and death of litters, death of mothers, as well as pregnancy failure were all observed. The results obtained also show that bee pollen extract toxicity affects embryonic implantation resulting in significant reduction in implantation process.

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Neurobehavioral toxicity produced by sodium fluoride in drinking water of laboratory rats

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Abstract: The effect of exposure to different concentrations of sodium fluoride (Na-F) for different durations on learning and memory tasks in rats (non-associative and associative learning) was assessed in our study. Three groups of fifteen pregnant Wistar female rats each, were administered Na-F in drinking water at one of three concentrations; 0, 50 and 100 ppm from second trimester of pregnancy till weaning of their pups at 30 days of age. Pups were then allocated into 5 groups of 20 animals each, where Na-F was administered in three different concentrations with different exposure periods throughout the study. Brain tissue specimens, representing all treatment groups, were taken for histopathological examination. The average body weight gain was significantly lower in group of rats exposed to high Na-F doses for long duration, with distinct hair loss. Open field revealed a significant influence of dose of Na-F on exploratory motor activities (EMA) and emotionality with marked impairment in habituation in rats exposed to high Na-F. Moreover, learning and memory assessed during maze test showed reduced memory retention in rats exposed to high Na-F for long periods. In novelty acquisition test, despite evidence of occurrence of habituation in all groups, a noticeable reduced degree was demonstrated in rats continued to administer high Na-F for long duration. Furthermore, histopathological evaluation revealed distinct neurodegenerative changes of nerve cells especially in hippocampus. Our results suggest that exposure of rats to Na-F in high doses for long duration has detrimental effects on the brain as reflected in diminished learning and memory. [Journal of American Science 2010;6(5):54-63]. (ISSN: 1545-1003).

Keywords: Neurobehavioral-toxicity-sodium fluoride-drinking water - laboratory rats

1. Introduction

Chronic fluoride toxicity represents a hazard to human health. The fluoride administered during gestation can cross both human and rat placenta and is also present in mother's milk (Drinkard et al., 1985; Fassman, 1993; Hassunuma, 2007). Excessive exposure to fluoride has been reported to be associated with central nervous system dysfunction manifested in lethargy, insomnia and deterioration of learning and memory (Spittle, 1994, 2000, Wu et al., 2006; Sharma et al., 2009). Moreover, chronic fluorosis reduced mental work capacity of adults as well as Intelligence Quotient (IQ) of children (Zhao et al., 1996; Lu et al., 2000; Xiang et al., 2003). Comparable effects are also reported with animal studies in rats (Niu et al., 2008; Gao et al., 2009).

Moreover, chronic exposure to high concentrations of sodium fluoride has been reported to induce disturbances in the development of brain in offspring rats (Liu et al., 1989). Rats exposed to flurosis showed a number of histopathological changes in the brain, including demyelinization, a decrease in the number of Purkinje cells, thickening and disappearance of dendrites, swelling of mitochondria, and dilation of endoplasmic reticulum in neurons (Guan et al., 1986, 1998).

Non-associative behavioural habituation affords one of the most essential forms of learning, both in animals and in humans. Open field is a potentially useful model for simultaneous assessment of anxiety and memory (Weiss and Greenberg, 1996; Weisstaub et al., 2006). Rats submitted for the first time to an open field display higher spatial exploration, a form of information storage (Eichenbaum, 1996), than in successive exposures. Thus, the decrement in response to successive exposures is taken as an index of memory of habituation (Izquierdo et al., 2001; Winograd and Viola, 2004).

Exposure to a novel environment also generates novelty recognition process that involves increased awareness with matching the stored memories of formerly explored places with the new spatial information to judge its novelty (Montag-Sallaz et al., 1999).

Formation of association between choice alternatives and their dependant outcomes is an important aspect of learning that may be sensitive to hippocamal dysfunction (Mahut et al., 1982; Reilly and Good, 1989; Johnson et al., 2008). Many studies in mammals have confirmed the involvement of hippocampus in some forms of associative and non-associative memories (Izquierdo and Medina, 1997;

Zhu et al., 1997; Thiel et al., 1998; Eichenbaum, 1999; McGaugh, 2000).

A deteriorating effect has been reported for sodium fluoride on performance in some memory tasks such as open field habituation in rats (Pereira and Dombrowski, 2009).

To our knowledge, no literatures are available to address the effect of Na-F, regarding the association between different doses and exposure periods, on learning and memory in rats. Furthermore, the involvement of various arrays of measurements to properly evaluate both associative and non-associative learning abilities in rats is not well implemented.

Thus, the objectives of the current study were to investigate the influence of exposure to different concentrations of sodium fluoride during gestation and postnatal period in rats on learning and memory tasks. Moreover, since the hippocampus is greatly involved in the process of learning and memory, histopathological examination was also carried out in order to detect alterations in brain tissues.

2. Material and Methods

2.1. Animals and housing

Forty five mature Wistar female rats weighing about 200-220g were obtained from the Unit for Laboratory Animals at Faculty of Veterinary Medicine, Cairo University and used in our study. They were housed in polypropylene cages with stainless steel wire lids (bedded with wood shavings) and maintained on a standard laboratory feed diet throughout the course of the study. Animals had free access to feed and water and housed at a room temperature of 20-22°c, 60% humidity on a 12h light:dark cycle. All females were mated with males of the same strain. Animal care was in compliance with applicable guidelines from Cairo University Policy on Animal Care and Use.

2.2. Administration of sodium fluoride:

Pregnant females were divided at random into three groups of 15 animals each and received Na-F at one of three different concentrations; 0 (control), 50 and 100 ppm on a mg/kg/day basis of 0, 5.15 and 10.77 Na-F, respectively). Sodium fluoride (Na-F, Sigma Chemical Company) was incorporated in drinking distilled water and administered to pregnant rats for a 44 days period (from day 8 of gestation till termination of lactation and weaning of pups at 30 days of age). After weaning, all pups were then collected and distributed into five groups of 20 animals each, divided on 2 replicates, as following: Group (1) control, n=20: weanling pups were derived from control dams receiving no Na-F. These pups served as a control group, where Na-F-free water was administered throughout the study till completing all assessments of learning and memory behaviours at 105 days of age.

Group (2) low-discontinued (LD), n=20: weanling pups were derived from dams receiving low dose of Na-F. Pups were then exposed to *ad libitum* supply of low dose of Na-F in drinking water, only till weaning at 30 days of age. Group (3) low-continued (LC), n=20: weanling pups were derived from dams receiving low dose of Na-F. Pups were then continually exposed to *ad libitum* supply of low dose of Na-F in drinking water till completing all assessments of learning and memory behaviours at 105 days of age. Group (4) high-discontinued (HD), n=20: weanling pups were derived from dams receiving high dose of Na-F. Pups were then exposed to *ad libitum* supply of high dose of Na-F in drinking water, only till weaning at 30 days of age. Group (5) high-continued (HC), n=20: weanling pups were derived from mothers receiving high dose of Na-F. Pups were then continually exposed to *ad libitum* supply of high dose of Na-F in drinking water till completing all assessments of learning and memory behaviours at 105 days of age.

2.3. Body weight gain

All pups were weighed at the onset of treatment, on day 30, and then individual body weight of all rats per group was recorded weekly throughout the study and body weight gain was calculated as the difference between final and initial weight. Mortalities were recorded as it occurred.

2.4. Behavioural testing

Behavioural testing started at 60 days and ended at 105 days of animals age.

2.4.1. Open field habituation test

The locomotor activity and habituation, a form of non-associative learning, were measured in the open field test (Kelly, 1993; Mello e Souza et al., 2000; Chioca et al., 2008). The open field used was a square wooden arena measured (90 x 90 x 25cm. The wood of the apparatus is covered with a plastic laminate (formica), which prevents absorption of fluids (urine of rats). The floor was divided by black lines into 36 small squares (15 x 15cm). All testing was conducted between 09:00 and 15:00 h. All treatments groups were tested at the same day in a random order. Rats were gently placed into a corner of the arena and allowed to explore the apparatus for 3 minutes. During the three minutes of exploration, the time spent freezing (no movement) was quantified. Exploratory measures as well as non-exploratory behaviours were recorded by the observer (Kalueff et al., 2006). Exploratory motor activity (EMA) measures included horizontal locomotion (the number of squares crossed) as well as vertical activity (rearing). A crossed square was defined as the rat placing its two forepaws in the next square and moving forward (Chioca et al., 2008), while

vertical activity was defined as the number of times an animal stood erect on its hind legs with its fore legs in the air or leaning against the wall of the open field (Brown et al., 1999). Non-exploratory measurements comprised only the vegetative behaviours (numbers of urination episodes and defecation boli). After the 3 minutes test session, the rat was returned to its home cage and the open field was cleaned using 70% ethyl alcohol (to avoid odour cues) and permitted to dry between tests. To assess the process of habituation to the novelty of arena, rats were exposed to the apparatus for a 3 minutes test session, on three consecutive days.

2.4.2. Classic maze test

Associative learning was assessed using classic maze test. The base of the maze measured (100 x 100cm) with walls height of 25cm. The entire maze was made of plywood with a glass cover in order to prevent escape of animals and allow observation. Testing was carried out between 09:00 and 15:00 h, where all groups were randomly allowed for testing at the same day. Rats were deprived from feed for a 23 hours period before start of testing. Rats were given their daily feed amount as a reward at the end of the maze. Animals were given one trial per day for five consecutive days. Time elapsed to locate the feed at the end and numbers of entries of blind alleys were recorded according to Staddon (1983).

2.4.3. "Novelty acquisition for exploration" test

We designed to investigate the exploratory activity, where a "mini-holeboard" consisted of a dark platform (40 x 40cm) containing a hole (5.5cm diameter x 5cm depth) in each quadrant was used. This mini-holeboard was inserted into the base of the recording chamber (40 x 40 x 40cm). A small object, which differed in scent and texture was placed in each hole (stimulus rich). Exploratory behaviour of rats including numbers of rears as well as head dips (to examine the interior of, or the objects within the four holeboard holes) were counted during a 15-min exposure period of the rats to the holeboard. To assess whether learning has occurred in our rats, re-exposure of rats to the same environment was conducted, 14 days later and the same parameters were recorded (Manahan-Vaughan and Braunewell, 1999).

2.5. Histopathological examination

After completing all behavioural assessments, tissue specimens were taken from the brain for histopathological examination. The specimens were fixed in 10% neutral buffer formalin, processed by paraffin embedding method, sectioned at 4-5 um and stained with Hematoxylin and Eosin stain (Bancroft et al., 1996). Axons were examined by using Bielschowsky's stain (Louis and Williams, 1995).

For Immunohistochemical examination, Paraffin sections of brain tissues were immunostained according to Ultra vision detection system (Anti-Polyvalent, HRP/ DAB), using monoclonal antibodies of Glial fibillary Acidic Protein (GFAP Ab-1, NeoMarkers, Fremont, CA) for determining astrocytes (Overmyer et al., 1999).

2.6. Statistical analysis

Data for all variables were subjected to analyses of variance (ANOVA) to assess the effect of dose of Na-F administered to rats, duration of administration as well as session factor for behavioural tests using the general linear models procedure in SPSS® statistical software (SPSS, 2006). When ANOVA was significant, post hoc Tukey HSD test was conducted for individual comparisons. A probability of $p<0.05$ was considered significant for all evaluations. All data are expressed as mean ± SEM.

3. Results

3.1. Body weight gain

The averages of rats' body weight gain allover the study was shown in (Fig. 1). Both dose of Na-F administered to rats ($F_{(1, 45)} = 4.86$; $p = 0.03$) and duration of exposure ($F_{(1, 45)} = 6.04$; $p = 0.02$) had a significant effect on average body weight gain of rats. Lower body weight gain was significantly seen in groups of animals exposed to high concentrations of Na-F for long period (59.91±4.33 g) compared to those exposed to low doses of Na-F for short period (81.76±5.74 g) and rats in control group (85.59±5.45 g).

3.2. Open field test

Only dose of Na-F administered to animals, but not duration of exposure, had a significant influence on exploratory motor activity assessed on first occurrence in the open field test. Although no significant dose difference was found for horizontal activity (numbers of crossed squares; $F_{(1, 45)} = 2.45$; $p = 0.13$), a significant dose effect was noted for the vertical one (numbers of rearing; $F_{(1, 45)} = 7.88$; $p = 0.01$). Correspondingly, regardless of Na-F exposure time, rats exposed to different Na-F concentrations demonstrated a significant different profile of novelty-induced anxiety related behaviours, as measured by time spent freezing and vegetative behaviours. There was a marginally significant dose effect in duration of freezing, with rats administered high Na-F doses spent more time freezing than their counterparts in control group ($F_{(1, 45)} = 3.82$; $p = 0.05$). For vegetative behaviours, rats received high Na-F doses defecated more frequently than rats of other treatments ($F_{(1, 45)} = 10.32$; $p = 0.002$), while no differences were recorded

for numbers of urination episodes ($F_{(1, 45)} = 1.33$; $p = 0.25$).

Habituation over the course of three successive test sessions of open field revealed a significant session factor diminishing effect on the following parameters; numbers of crossed squares ($F_{(2, 135)} = 7.65$; $p = 0.00$), numbers of rearing ($F_{(2, 135)} = 11.54$; $p = 0.00$), time spent freezing ($F_{(2, 135)} = 8.29$; $p = 0.00$), and defecation boli ($F_{(2, 135)} = 7.92$; $p = 0.00$) but not for urination ($F_{(2, 135)} = 2.58$; $p = 0.08$).

As in first occurrence of open field, statistical analysis revealed a significant dose influence for locomotor activity, where high Na-F group displayed more horizontal locomotion (Fig. 2a), ($F_{(1, 135)} = 4.51$; $p = 0.04$) and higher vertical activity (Fig. 2b), ($F_{(1, 135)} = 13.43$; $p = 0.00$) compared to control group. In addition, high Na-F groups were the most anxious group as indicated by duration of freezing (Fig. 2c), ($F_{(1, 135)} = 5.72$; $p = 0.02$), defecation ($F_{(1, 135)} = 9.69$; $p = 0.00$) and urination scores ($F_{(1, 135)} = 5.08$; $p = 0.03$). Again, non of the measured parameters were affected by duration of exposure to Na-F. Hence, over the three test sessions, impairment in habituation was markedly seen in high Na-F group compared to other treatments.

3.3. Maze test

During acquisition on the first day of testing, significant differences among treatments were recorded regarding time elapsed and numbers of entries of blind alleys. Animals received high Na-F doses required more time to locate the feed at the end of the maze ($F_{(1, 45)} = 19.37$; $p = 0.00$) with higher frequency of entering blind alleys ($F_{(1, 45)} = 8.25$; $p = 0.01$), compared to control group. On the other hand, duration of exposure to Na-F had only a significant influence on time spent in maze test ($F_{(1, 45)} = 6.45$; $p = 0.02$).

Over the five days of maze test, all treatments required progressively less time to locate feed ($F_{(4, 225)} = 24.45$; $p = 0.00$). Similar decline trend was also noted for numbers of entries of blind alleys ($F_{(4, 225)} = 34.25$; $p = 0.00$). Learning and memory assessed over five days of maze test showed that groups of animals exposed to high concentrations of Na-F for long period took longer time to locate feed (Fig. 3a), (dose effect, $F_{(1, 225)} = 16.13$; $p = 0.00$), exposure time effect ($F_{(1, 225)} = 15.21$; $p = 0.00$) with higher frequency for entering blind alleys (Fig. 3b), (dose effect, $F_{(1, 225)} = 29.54$; $p = 0.00$), exposure time effect ($F_{(1, 225)} = 26.73$; $p = 0.00$), demonstrating poorer memory retention relative to all other treatments.

3.4. Novelty exploration test

After re-exposure to the novel environment, habituation occurred as evidenced by decreased exploratory activities in all treatments compared to their values during initial exposure. Numbers of both rearing and head dipping were significantly lower during re-exposure ($F_{(1, 90)} = 34.71$; $p = 0.00$) and ($F_{(1, 90)} = 13.40$; $p = 0.00$), respectively.

A significant discrepancy in exploratory activities was found between treatments during both novel exposure and re-exposure. Group of rats administered high Na-F concentrations for long period reared more compared to other groups (Fig. 4a), (dose effect, $F_{(1, 90)} = 17.53$; $p = 0.00$), nevertheless, time of exposure to Na-F had only a marginal significance ($F_{(1, 90)} = 45.12$; $p = 0.05$). The same tendency was also shown for head dips with higher numbers observed in group of animals exposed to high Na-F for long duration (Fig. 4b), (dose effect, $F_{(1, 90)} = 27.09$; $p = 0.00$), exposure time, $F_{(1, 90)} = 6.59$; $p = 0.01$). Thus, a less degree of habituation was noticeably shown in rats with long exposure to high doses of Na-F compared to other treatments.

3.5 Histopathological examination

No pathological changes were detected in the brain of rats in the control group.

The histopathological changes in both treated groups were more or less the same but differ only in their degree of severity. It was obvious and severe in group of rats administered high doses for long duration. The main histopathological changes observed in the brain of rats in both treated groups were congestion of the meningeal, cerebral, cerebellum blood capillaries and choroid plexus (Fig. 5). Areas of hemorrhage in cerebral cortex, cerebellum white matter and in ventricles around choroid plexuses were constant finding in the brain of rats exposed to high Na-F for long duration (Fig. 6). Neurodegenerative changes were detected in nerve cells especially in pyramidal cells of Ammon's horn of hippocampus. The pyramidal cells showed atrophy and necrosis (Fig. 7). Large nerve cells of cerebral cortex showed neurofilaments accumulation in the cytoplasm and the axons. Also, nerve cells of cerebral cortex revealed central chromatolysis, edema, atrophy, necrosis and neuronophagia (Fig. 8), whereas gliosis either focal or diffuse were observed (Fig. 9). Demylination of the nerve fibers in the neuropil was detected in cerebrum accompanied with axonal swelling (Figs. 10 & 11). Signs of encephalitis were observed in the cortex of cerebrum represented by necrotic areas with mononuclear cells aggregations mainly microglial and macrophages cells with perivascular cuffing only in rats exposed to high Na-F for long period (Fig. 12). Glial fibers was detected under the ependymal cells lined the ventricles. The cerebellum showed necrosis of Purkinje cells and edema with necrosis in the granular cell layer in group treated with high dose (Fig. 13). There was an increase in Glial Fibrillary Acidic Protein

(GFAP) expression in astrocytes represented by astrogliosis and astrocytosis in the cerebrum, especially hippocampus. Some astrocytes showed shrinkage of cell bodies and retraction of their processes (Fig. 14).

4. Discussions

Contrary to previous studies that showed no effect of fluoride on body weight (Collins et al., 1995; Chioca et al. 2008; Pereira et al., 2009), lower body weight gain was observed in our study in rats exposed to high daily doses of sodioum fluoride. Similar results derived from other studies with fluoride treated animals (Paul et al., 1998; Ekambaram and Paul, 2001, 2003; Wang et al., 2004). A concomitant reduction in fluid and water consumption was recorded in these studies that might account for body weight reduction (Ross and Daston, 1995). In the study of Das et al. (1994), the atrophic gastritis produced by chronic oral administration of sodium fluoride might be attributed to the decrement in feed intake and consequently lower body weights in rats. Further explanation derived from Shupe et al. (1984) and Ekambaram and Paul (2003) who observed white and chalk-like incisors with broken tips in rats treated with sodium fluoride. These may impair both mastication and swallowing process, resulting in decreased feed intake and body weight.

In the present study, the open field test provides simultaneous measures of both exploratory motor activity (EMA) and anxiety during first occurrence training session. In order to investigate the novel environment, rats were engaged in horizontal as well as vertical activities. Significant enhancement in EMA was noted in high Na-F treated animals, however, the actual increase was related to vertical activity (rearing). This can be interpreted on the basis of increased emotionality in high concentration group. Supportive evidence derived from greater time spent freezing in open field in high Na-F treated group with increased number of faecal boli. The latter was considered the most credible criteria for judging anxious animals. In addition, Davies and Redfern (1973) reported that rearing is not simply a manifestation of exploratory behaviour but might refer to emotionality state of animals. Therefore, it might be concluded that individuals treated with high Na-F were more fearful and highly anxious. However, Chioca et al (2008) reported no impairment in locomotor activity in Na-F treated rats during their first exposure to open field, the impairment in EMA stated by Paul et al (1998) and Ekambaram and Paul (2001, 2003) might be contributed to the high daily doses of Na-F (500 ppm) administered to rats.

Long term habituation to a novel environment is one of the most elementary forms of non-associative learning. In this study, where reduction in spatial exploration during test session was taken as an index for memory habituation (Montag-Sallaz et al., 1999), an impairment in the open field habituation was noticed in Na-F treated group, a result similar to Chioca et al. (2008) using the same concentrations of 50 and 100 ppm Na-F. A proof that learning has taken place is evidenced by habituation on re-exposure to novel environment (File and Wardill, 1975; Platel & Porsolt, 1982). Therefore, sodium fluoride intoxication may impair learning and memory in the present study.

Novelty acquisition during exploration, a hippocampus–dependent phenomenon, has been described as representing a form of information storage (Eichenbaum, 1996; Manahan-Vaughan and Braunewell, 1999). Confirming the data of open field, novelty acquisition test revealed a reduced degree of habituation in rats exposed to high concentrations of Na-F for long duration. Similar results reported by Bera et al. (2007), where impairment of learning was evidenced by reduced habituation on re-exposure to a novel environment in Na-F intoxicated rats.

Concerning the associative learning, memories are based on the acquisition of a predictive link between a specific event and a stimulus. Corresponding to the results of Bhatnagar et al. (2002), where fluoride intoxicated rats performed poorly in maze test, rats with long exposure to high Na-F in the present study demonstrated higher latency with increased numbers of errors in the maze reflecting a poorer memory retention relative to other treatments.

In rats, the hippocampus is involved in learning and memory (Riedel et al. 1999; Wolfman et al., 1999; Vianna et al., 2000; Pittenger et al., 2002, Vianna et al., 2008). Since fluoride is classified as neurotoxic substance, our histopathological examination of the brain confirmed that hippocampus is the most affected region due to fluoride intoxication. This could be attributed to the accumulation of fluoride in different parts of brain in rats especially in hippocampus (Burgstahler and Colquhoun, 1996, Varner et al., 1998). Where fluoride is a chemically active ionized element, it may affect oxygen metabolism and induce oxygen free radicals which appears to play a role in diminishing cognitive ability processes such as learning and memeory (Chirumari and Reddy, 2007). Moreover, fluoride binds antioxidants in the body such as N-acetyl cysteine (NAC) and glutathione (GSH)) and other free-radical destroying enzymes, triggering oxidative stress that leads to cell damage and even cell apoptosis (Rzeuski et al., 1998 and Anuradha et al., 2000). Absence of the compensatory antioxidant system with the presence of oxidative stress due to increased free radicals plays a great role in initiation of damage of nerve cells membrane especially via increased lipid peroxidation (Guan et al., 1998, Ghiselli et al., 2000, and Gao et al., 2009).

The presence of neurofilament in nerve cells as well as the detected increase in expression of Glial fibrillary Acidic Protein (GFAP) in astrocytes may be as a result of interference of fluoride in various steps of protein synthesis inside nerve cells (Miu et al., 2003). Enhanced expression of GFAP has been evidenced to be associated with gliosis and genetic response of CNS to neural injury (Roberts et al., 1989). In addition, Phyllis et al. (1995) has accounted that fluoride may accumulate in both neurons and astrocytes resulting in strong morphological changes, clustering, degeneration and finally death. In addition, learning and memory has been associated with hippocampal activity and cholinergic neurotransmission (Izquierdo et al., 1992; Thiel et al., 1998; Leussis and Bolivar, 2006). Thiel et al. (1999) related the increased release of acetylcholine in the hippocampus to Na-F intoxication in rats, where fluoride may get through the blood brain barrier and accumulate in rat hippocampus resulting in inhibition of cholinesterase activity. Nicotinic acetylcholine receptors (nAChRs) have been also established to play a major role in cognitive processes such as learning and memory. In a fluoride toxicity study for Long et al. (2002), a decreased number of nAChRs was noticed resulting in brain dysfunction. All previous justifications might be responsible for the suppressive effect induced by Na-F intake on learning abilities monitored in the present study.

In conclusion, our study indicated that long exposure for high concentrations of Na-F resulted in clear deleterious effects on brain of rats as reflected in impaired learning and memory.

Since, maternal fluoride ingestion constitutes a great threat to progeny, caution should be exercised when products containing fluoride are administered to nursing mothers.

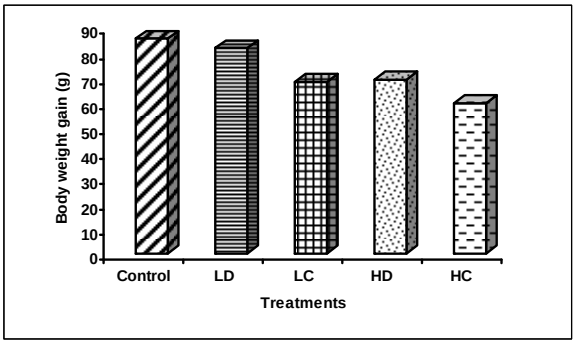
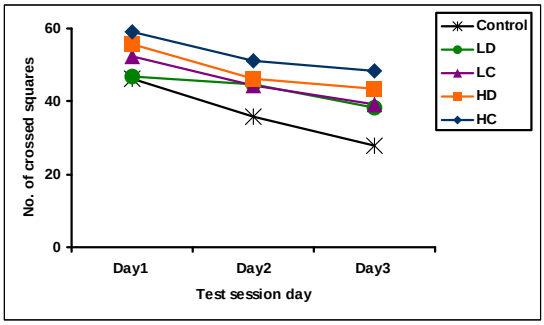
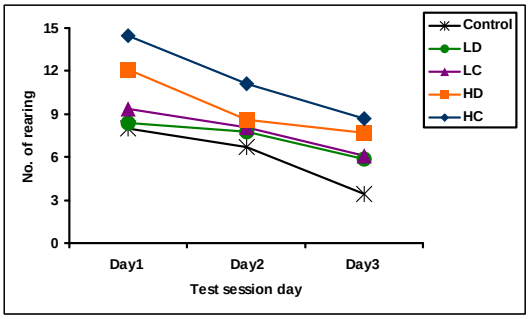


Figure 1: Effects of exposure to different doses and durations of Na-F on body weight gain of rats. Data are presented as mean of 10 animals per treatment.

a) Horizontal activity:



b) Vertical activity:



c) Freezing time:

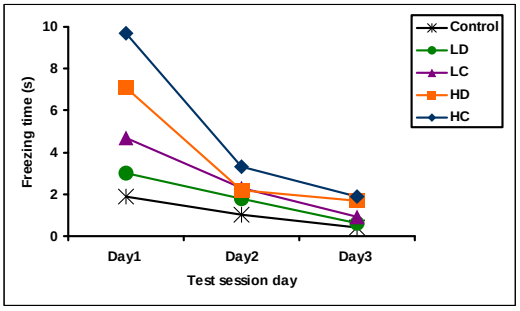
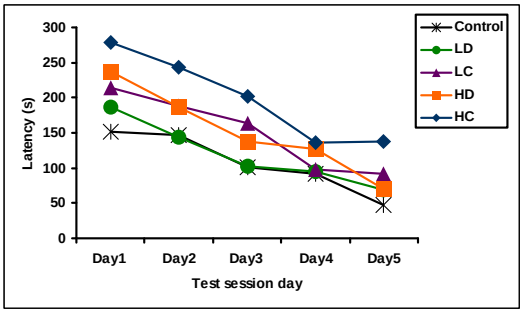


Figure 2: Effects of exposure to different doses and durations of Na-F on measurements of open field test. Data are presented as mean of 10 animals per treatment.

a) Latencies:



b) Numbers of entries of blind alleys:

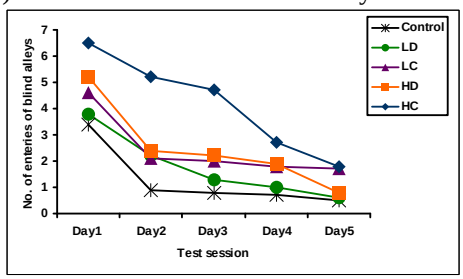
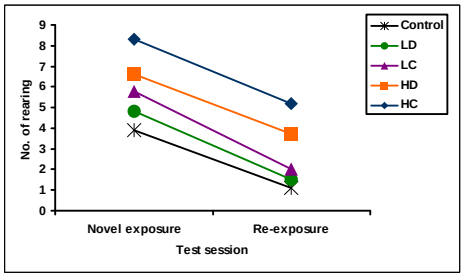


Figure 3: Effects of exposure to different doses and durations of Na-F on measurements of maze test over the course of five days in rats. Data are presented as mean of 10 animals per treatment.

a) Numbers of rearing:



b) Numbers of head dipping:

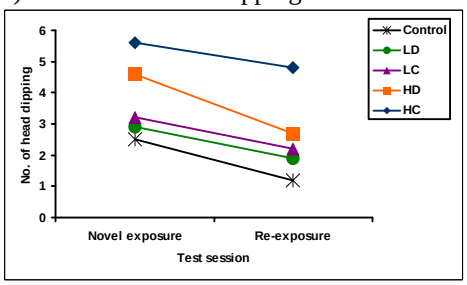


Figure 4: Effects of exposure to different doses and durations of Na-F on measurements of novelty acquisition test in rats. Data are presented as mean of 10 animals per treatment.

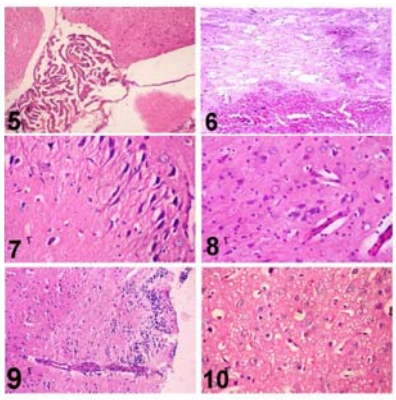


Figure 5: Brain of rat administered high dose of Na-F for long duration showing congestion of choroid plexus, H&E X 100.

Figure 6: Brain of rat administered high dose of Na-F for long duration showing hemorrhagic area in cerebrum, H&E X 100.

Figure 7: Hippocampus of rat administered high dose of Na-F for long duration showing atrophy and necrosis of pyramidal cells H&E X 100.

Figure 8: Brain of rat administered high dose of Na-F for long duration showing necrosis of nerve cells with neuronophagia, H&E X 400.

Figure 9: Brain of rat administered low dose of Na-F for short duration showing focal gliosis, H&E X 100.

Figure 10: Brain of rat administered high dose of Na-F for long duration showing demylination. Notice the cellular edema in the nerve cells, H&E X 400.

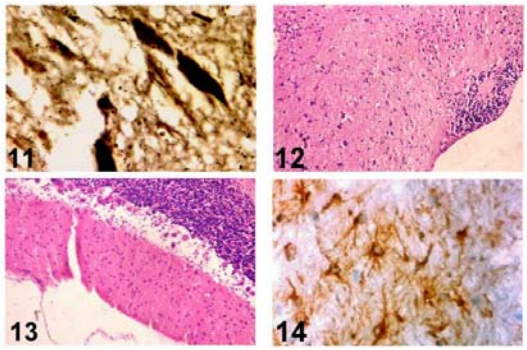


Figure 11: Brain of rat administered high dose of Na-F for long duration showing axonal swelling, Bielschowsky's stain X 400.

Figure 12: Brain of rat administered high dose of Na-F for long duration showing signs of encephalitis, H&E X 100.

Figure 13: Brain of rat administered high dose of Na-F for long duration showing necrosis of Purkinje cells of cerebellum, H&E X 200.

Figure 14: Hippocampus of rat administered with high dose of fluoride showing astrogliosis and astrocytosis stained with Anti-GFAP using DAB chromogen X 400.

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2/1/2010

Indirect Boundary Element Method for Calculation of Compressible Flow past a Symmetric Aerofoil with Constant Element Approach

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Abstract:
In this paper, an indirect boundary element method is applied to calculate the compressible flow past a symmetric aerofoil. The velocity distribution for the flow over the surface of the symmetric aerofoil has been calculated using constant boundary element approach. To check the accuracy of the method, the computed flow velocity is compared with the exact velocity. It is found that the computed results are in good agreement with the analytical results. [Journal of American Science 2010;6(5):64-71]. (ISSN: 1545-1003).

Keywords: Indirect boundary element method, Compressible flow, Velocity distribution, Symmetric aerofoil, Constant element.

1. Introduction

In the past, many numerical techniques such as finite difference method, finite element method, and boundary element method etc. came into being making possible to solve various practical fluid flow problems. Boundary element method has received much attention from the researchers due to its various advantages over the other domain methods. One of the advantages is that with boundary elements one has to discretize only the surface of the body, whereas with domain methods it is essential to discretize the entire region of the flow field. Moreover, this method is well–suited to problems with an infinite domain. The boundary element method can be classified into two categories i.e. direct and indirect. The direct method takes the form of a statement which provides the values of the unknown variables at any field point in terms of the complete set of all the boundary data. On the other hand, the indirect method utilizes a distribution of singularities over the boundary of the body and computes this distribution as the solution of integral equation. The equation of indirect method can be derived from that of direct method. (Lamb, 1932; Milne-Thomson, 1968, Kellogge, 1929 and Brebbia and Walker, 1980). The indirect method has been used in the past for flow field calculations around arbitrary bodies (Hess and Smith, 1967; Muhammad, 2008, Luminita, 2008, Mushtaq, 2009). Most of the work on fluid flow calculations using boundary element methods has been done in the field of incompressible flow. Very few attempts have been made on flow field calculations using boundary element methods in the field of compressible flow. In this paper, the indirect boundary element method has been used for the solution of compressible flows around a symmetric aerofoil.

2. Mathematical Formulation

We know that equation of motion for two – dimensional, steady, irrotational, and isentropic flow is

$$(1 - Ma^2)\frac{\partial^2 \Phi}{\partial X^2} + \frac{\partial^2 \Phi}{\partial Y^2} = 0 \qquad (1)$$

where Ma is the Mach number and Φ is the total velocity potential of the flow. Here X and Y are the space coordinates.

Using the dimensionless variables, $x = X$, $y = \beta Y$, where $\beta = \sqrt{1 - M a^2}$, equation (1) becomes

$$\frac{\partial^2 \Phi}{\partial x^2} + \frac{\partial^2 \Phi}{\partial y^2} = 0$$

or $$\nabla^2 \Phi = 0 \qquad (2)$$

which is Laplace's equation.

3. Symmetric Aerofoil

The Joukowski transformation

$$z = \zeta + \frac{a^2}{\zeta} \qquad (3)$$

transforms the circle shown in figure (1) in the ζ – plane on to symmetric aerofoil in the z-plane.

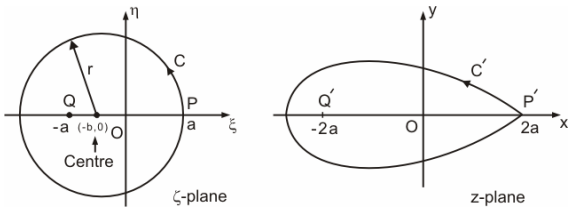

Figure 1

4. Flow Past a Symmetric Aerofoil

Consider the flow past a symmetrical aerofoil and let the onset flow be the uniform stream with velocity U in the positive direction of the x – axis as shown in figure (2) .

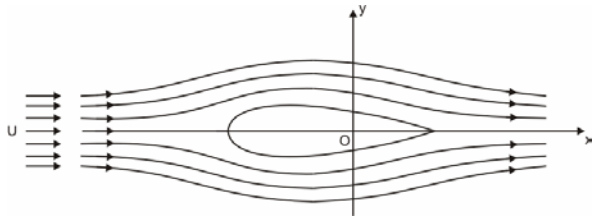

Figure 2: Flow past a symmetric aerofoil.

Exact Velocity

The magnitude of the exact velocity distribution over the boundary of a symmetric aerofoil is given by Chow[3] as

$$V = U \left| \frac{1 - \left(\frac{r}{z-b}\right)^2}{1 - \left(\frac{a}{z}\right)^2} \right|$$

where r = radius of the circular cylinder,
a = Joukowski transformation constant
and b = a – r = x-coordinates of the centre of the circular cylinder

In Cartesian coordinates, we have

$$V = U \frac{\sqrt{\left[\left\{(x-b)^2+y^2\right\}^2 - r^2\left\{(x-b)^2-y^2\right\}\right]^2 + 4r^4y^2(x-b)^2}}{\left[(x-b)^2+y^2\right]^2} \times \frac{\sqrt{\left[(x^2+y^2)^2 - a^2(x^2-y^2)\right]^2 + 4a^4x^2y^2}}{(x^2+y^2)^2 - 2a^2(x^2-y^2) + a^4}$$

Boundary Conditions

Now the condition to be satisfied on the boundary of a symmetric aerofoil is

$$\vec{V} \cdot \hat{n} = 0 \qquad (4)$$

where $\hat{n}$ is the unit normal vector to the boundary of the aerofoil .

Since the motion is irrotational

$$\vec{V} = -\nabla \Phi$$

where Φ is the total velocity potential . Thus equation (4) becomes

$$(-\nabla \Phi) \cdot \hat{n} = 0$$

$$\text{or} \quad \frac{\partial \Phi}{\partial n} = 0 \qquad (5)$$

Now the total velocity potential Φ is the sum of the perturbation velocity potential $\phi_{s.a}$ where the subscript s . a stands for symmetric aerofoil and the velocity potential of the uniform stream $\phi_{u.s}$.

$$\text{i.e.} \quad \Phi = \phi_{u.s} + \phi_{s.a} \qquad (6)$$

$$\text{or} \quad \frac{\partial \Phi}{\partial n} = \frac{\partial \phi_{u.s}}{\partial n} + \frac{\partial \phi_{s.a}}{\partial n} \qquad (7)$$

From equations (5) and (7) , we get

$$\frac{\partial \phi_{s.a}}{\partial n} + \frac{\partial \phi_{u.s}}{\partial n} = 0$$

$$\text{or} \quad \frac{\partial \phi_{s.a}}{\partial n} = -\frac{\partial \phi_{u.s}}{\partial n} \qquad (8)$$

But the velocity potential of the uniform stream , given in Milne – Thomson [6], Shah [7], is

$$\phi_{u.s} = -Ux \qquad (9)$$

$$= -U\frac{\partial x}{\partial n}$$

$$= -U(\hat{n} \cdot \hat{i}) \qquad (10)$$

Thus from equations (8) and (10), we get

$$\frac{\partial u_{s.a}}{\partial n} = U(\hat{n} \cdot \hat{i}) \qquad (11)$$

Now from the figure (3)

$$\vec{A} = (x_2 - x_1)\hat{i} + (y_2 - y_1)\hat{j}$$

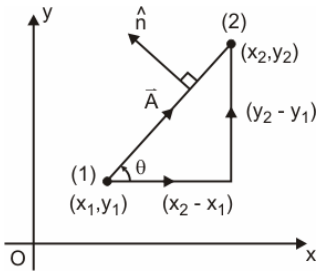

Figure 3

Therefore the unit vector in the direction of the vector $\vec{A}$ is given by

$$\vec{A} = \frac{(x_2 - x_1)\hat{i} + (y_2 - y_1)\hat{j}}{\sqrt{(x_2 - x_1)^2 + (y_2 - y_1)^2}}$$

The outward unit normal vector $\hat{n}$ to the vector $\vec{A}$ is given by

$$= \frac{-(y_2 - y_1)\hat{n} + (x_2 - x_1)\hat{j}}{\sqrt{(x_2 - x_1)^2 + (y_2 - y_1)^2}}$$

Thus $$\hat{n} \cdot \hat{i} = \frac{(y_1 - y_2)}{\sqrt{(x_2 - x_1)^2 + (y_2 - y_1)^2}} \qquad (12)$$

From equations (11) and (12) , we get

$$\frac{\partial \phi_{s.a}}{\partial n} = U \frac{(y_1 - y_2)}{\sqrt{(x_2 - x_1)^2 + (y_2 - y_1)^2}} \qquad (13)$$

Equation (13) is the boundary condition which must be satisfied over the boundary of a symmetric aerofoil.

Equation of Indirect Boundary Element Method

The equation of indirect boundary element method for two–dimensional flow in the case of doublet alone [Muhammad,2008 & Mushtaq, 2008&2009] is :

$$-c_i \Phi_i + \frac{1}{2\pi} \int_{\Gamma - i} \Phi \frac{\partial}{\partial n}\left(\log \frac{1}{r}\right) d\Gamma + \phi_\infty = -(\phi_{u.s})_i \qquad (14)$$

where c_i = 0 when 'i' is within R'

= 1 when 'i' is within R

= $\frac{1}{2}$ when 'i' is on S and S is smooth

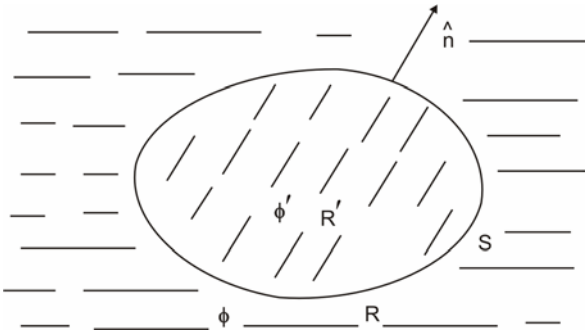

Figure 4

Matrix Formulation

Let the boundary of the region be discretized into m elements , then equation (14) can be written as

$$-c_i \Phi_i + \sum_{j=1}^{m} \left[\frac{1}{2\pi} \int_{\Gamma_j - i} \Phi \frac{\partial}{\partial n}\left(\log \frac{1}{r}\right) d\Gamma \right] + \phi_\infty = -(\phi_{u.s})_i \qquad (15)$$

where $\Gamma_j - i$ is the length of the element 'j' excluding the point 'i' .

For the constant boundary element approach, the value of Φ is assumed to be constant on each element and equal to the values at the mid–node of the element . The number of nodes in this case will be the same as the number of elements m . On each element the variable Φ is specified as a boundary condition . As Φ is constant over each element it can be taken out of the integral . This gives

$$-c_i \Phi_i + \sum_{j=1}^{m} \left[\frac{1}{2\pi} \int_{\Gamma_j - i} \frac{\partial}{\partial n}\left(\log \frac{1}{r}\right) d\Gamma \right] \Phi_j + \phi_\infty = -(\phi_{u.s})_i \qquad (16)$$

Equation (16) applies for a particular node 'i' and the integrals $\frac{1}{2} \int_{\Gamma_j - i} \frac{\partial}{\partial n}\left(\log \frac{1}{r}\right) d\Gamma$ relate the node 'i' with the element 'j' over which integrals are evaluated . These integrals will be denoted by $\hat{H}_{ij}$. Hence equation (16) can be written as

$$-c_i \Phi_i + \sum_{j=1}^{m} \hat{H}_{ij} \Phi_j + \phi_\infty = -(\phi_{u.s})_i \quad (17)$$

or $$\sum_{j=1}^{m} H_{ij} + \phi_\infty = -(\phi_{u.s})_i \quad (18)$$

where $H_{ij} = \begin{cases} \hat{H}_{ij} & \text{when } i \neq j \\ \hat{H}_{ij} - c_i & \text{when } i = j \end{cases}$

When all nodes are taken into consideration, equation (18) is M x (M + 1) system of equations . Which can put in the matrix form in case of constant element as

$$[H]\{\underline{U}\} = \{\underline{R}\} \quad (19)$$

where as usual $[H]$ is a matrix of influence coefficients , $\{\underline{U}\}$ is a vector of unknown total potentials Φ_i and $\{\underline{R}\}$ on the R.H.S. is a known vector whose elements are the negative of the values of the velocity potential of the uniform stream at the nodes on the region of the body . Note that $\{U\}$ in equation (19) has (M + 1) unknowns $\Phi_1, \Phi_2, \ldots, \Phi_m, \phi_\infty$. To solve precisely this system of equations , the value of Φ at some position must be specified . For convenience ϕ_∞ is chosen as zero . Thus M x (M + 1) system reduces to an M x M system of equations which can be solved as before but now the diagonal coefficients of [H] will be found by

$$H_{ii} = -\sum_{\substack{j=1 \\ j \neq i}} H_{ij} - 1 \quad (20)$$

Process of Discretization

Now for the discretization of the boundary of the symmetric aerofoil, the coordinates of the extreme points of the boundary elements can be generated within computer programme using Fortran language as follows:

Divide the boundary of the circular cylinder into m elements in the clockwise direction by using the formula.

$$\theta_k = \left[(m+3) - 2k \right] \frac{\pi}{m}, \quad k = 1, 2, \ldots\ldots, m \quad (21)$$

Then the extreme points of these m elements of circular cylinder are found by

$$\xi_k = -b + r \cos\theta_k$$

$$\eta_k = r \sin\theta_k$$

Now by using Joukowski transformation in equation (3), the extreme points of the symmetric aerofoil are

$$x_k = \xi_k \left(1 + \frac{a^2}{\xi_k^2 + \eta_k^2} \right)$$

$$y_k = \eta_k \left(1 - \frac{a^2}{\xi_k^2 + \eta_k^2} \right)$$

where $k = 1, 2, \ldots\ldots, m$.

The coordinates of the middle node of each boundary element are given by

$$\left.\begin{aligned} x_m &= \frac{x_k + x_{k+1}}{2} \\ y_m &= \frac{y_k + y_{k+1}}{2} \end{aligned}\right\}$$

$$k, m = 1, 2, \ldots\ldots, n \quad (22)$$

and therefore the boundary condition (13) in this case takes the form

$$\frac{\partial \phi_{s.a}}{\partial n} = U \frac{(y_1)_m - (y_2)_m}{\sqrt{\left[(x_2)_m - (x_1)_m\right]^2 + \left[(y_2)_m - (y_1)_m\right]^2}} \quad (23)$$

The following tables show the comparison of computed and analytical velocity distribution over the boundary of a symmetric aerofoil for 8, 16, 32, and 64 constant boundary elements.

Table 1: The comparison of the computed velocity with exact velocity over the boundary of a symmetric aerofoil using 8 constant boundary elements.

ELEMENT	X	Y	$R = \sqrt{X^2 + Y^2}$	VELOCITY	EXACT VELOCITY
1	-1.87	.36	1.91	.83110E+00	.75969E+00
2	-1.36	.86	1.61	.20042E+01	.18480E+01
3	-.64	.86	1.07	.19913E+01	.18561E+01
4	-.13	.35	.38	.82093E+00	.68955E+00
5	-.13	-.35	.38	.82093E+00	.68955E+00
6	-.64	-.86	1.07	.19913E+01	.18561E+01
7	-1.36	-.86	1.61	.20042E+01	.18480E+01
8	-1.87	-.36	1.91	.83109E+00	.75969E+00

Table 2: The comparison of the computed velocity with exact velocity over the boundary of a symmetric aerofoil using 16 constant boundary elements.

ELEMENT	X	Y	$R = \sqrt{X^2 + Y^2}$	VELOCITY	EXACT VELOCITY
1	-2.04	.21	2.05	.39880E+00	.38702E+00
2	-1.88	.59	1.97	.11354E+01	.11044E+01
3	-1.59	.88	1.82	.16984E+01	.16594E+01
4	-1.21	1.03	1.59	.20013E+01	.19661E+01
5	-.80	1.03	1.30	.19967E+01	.19716E+01
6	-.42	.87	.96	.16825E+01	.16645E+01
7	-.12	.57	.58	.10924E+01	.10750E+01
8	.05	.19	.20	.39734E+00	.26843E+00
9	.05	-.19	.20	.39734E+00	.26843E+00
10	-.12	-.57	.58	.10924E+01	.10750E+01
11	-.42	-.87	.96	.16825E+01	.16645E+01
12	-.80	-1.03	1.30	.19967E+01	.19716E+01
13	-1.21	-1.03	1.59	.20013E+01	.19661E+01
14	-1.59	-.88	1.82	.16984E+01	.16594E+01
15	-1.88	-.59	1.97	.11354E+01	.11044E+01
16	-2.04	-.21	2.05	.39880E+00	.38702E+00

Table 3: The comparison of the computed velocity with exact velocity over the boundary of a symmetric aerofoil using 32 constant boundary elements.

ELEMENT	X	Y	$R = \sqrt{X^2 + Y^2}$	VELOCITY	EXACT VELOCITY
1	-2.09	.11	2.09	.19744E+00	.19455E+00
2	-2.05	.32	2.07	.58470E+00	.57600E+00
3	-1.97	.51	2.03	.94941E+00	.93637E+00
4	-1.85	.69	1.97	.12775E+01	.12622E+01
5	-1.70	.84	1.89	.15562E+01	.15410E+01
6	-1.52	.96	1.80	.17748E+01	.17620E+01
7	-1.32	1.04	1.68	.19247E+01	.19163E+01
8	-1.11	1.08	1.55	.20002E+01	.19969E+01
9	-.90	1.08	1.40	.19981E+01	.19999E+01
10	-.69	1.04	1.24	.19182E+01	.19236E+01
11	-.49	.95	1.07	.17632E+01	.17695E+01
12	-.31	.83	.89	.15384E+01	.15417E+01
13	-.16	.68	.70	.12514E+01	.12461E+01
14	-.04	.49	.49	.91024E+00	.88934E+00
15	.06	.28	.29	.52632E+00	.47740E+00
16	.12	.09	.15	.23223E+00	.15912E+00
17	.12	-.09	.15	.23223E+00	.15912E+00
18	.06	-.28	.29	.52632E+00	.47740E+00

19	-.04	-.49	.49	.91024E+00	.88934E+00
20	-.16	-.68	.70	.12514E+01	.12461E+01
21	-.31	-.83	.89	.15384E+01	.15417E+01
22	-.49	-.95	1.07	.17632E+01	.17695E+01
23	-.69	-1.04	1.24	.19182E+01	.19236E+01
24	-.90	-1.08	1.40	.19981E+01	.19999E+01
25	-1.11	-1.08	1.55	.20002E+01	.19969E+01
26	-1.32	-1.04	1.68	.19248E+01	.19163E+01
27	-1.52	-.96	1.80	.17748E+01	.17620E+01
28	-1.70	-.84	1.89	.15562E+01	.15410E+01
29	-1.85	-.69	1.97	.12774E+01	.12622E+01
30	-1.97	-.51	2.03	.94941E+00	.93637E+00
31	-2.05	-.32	2.07	.58471E+00	.57600E+00
32	-2.09	-.11	2.09	.19744E+00	.19455E+00

Table 4: The comparison of the computed velocity with exact velocity over the boundary of a symmetric aerofoil using 64 constant boundary elements.

ELEMENT	X	Y	$R = \sqrt{X^2 + Y^2}$	VELOCITY	EXACT VELOCITY
1	-2.10	.05	2.10	.98527E-01	.97672E-01
2	-2.09	.16	2.10	.29450E+00	.29110E+00
3	-2.07	.27	2.09	.48756E+00	.48207E+00
4	-2.04	.37	2.07	.67617E+00	.66862E+00
5	-2.00	.47	2.05	.85796E+00	.84901E+00
6	-1.95	.56	2.03	.10316E+01	.10216E+01
7	-1.89	.65	2.00	.11952E+01	.11847E+01
8	-1.82	.74	1.96	.13473E+01	.13367E+01
9	-1.74	.81	1.92	.14863E+01	.14763E+01
10	-1.66	.88	1.88	.16109E+01	.16021E+01
11	-1.57	.94	1.83	.17200E+01	.17127E+01
12	-1.47	.99	1.77	.18123E+01	.18072E+01
13	-1.37	1.03	1.72	.18871E+01	.18844E+01
14	-1.27	1.06	1.66	.19437E+01	.19435E+01
15	-1.17	1.08	1.59	.19813E+01	.19839E+01
16	-1.06	1.09	1.52	.19997E+01	.20051E+01
17	-.95	1.09	1.45	.19986E+01	.20065E+01
18	-.84	1.08	1.37	.19781E+01	.19882E+01
19	-.74	1.06	1.29	.19382E+01	.19501E+01
20	-.63	1.03	1.21	.18794E+01	.18922E+01
21	-.53	.98	1.12	.18022E+01	.18151E+01
22	-.44	.93	1.03	.17072E+01	.17193E+01
23	-.35	.87	.94	.15952E+01	.16052E+01
24	-.27	.80	.85	.14672E+01	.14739E+01
25	-.19	.72	.75	.13243E+01	.13260E+01
26	-.12	.64	.65	.11677E+01	.11624E+01
27	-.06	.55	.55	.99845E+00	.98398E+00
28	-.00	.45	.45	.81795E+00	.79117E+00
29	.04	.34	.34	.62778E+00	.58472E+00
30	.08	.23	.24	.43184E+00	.36984E+00
31	.12	.12	.17	.24912E+00	.18920E+00
32	.16	.03	.16	.50082E+00	.18610E+00
33	.16	-.03	.16	.50082E+00	.18610E+00
34	.12	-.12	.17	.24911E+00	.18920E+00
35	.08	-.23	.24	.43183E+00	.36984E+00
36	.04	-.34	.34	.62779E+00	.58472E+00

37	-.00	-.45	.45	.81795E+00	.79117E+00
38	-.06	-.55	.55	.99845E+00	.98398E+00
39	-.12	-.64	.65	.11677E+01	.11624E+01
40	-.19	-.72	.75	.13243E+01	.13260E+01
41	-.27	-.80	.85	.14672E+01	.14739E+01
42	-.35	-.87	.94	.15952E+01	.16052E+01
43	-.44	-.93	1.03	.17072E+01	.17193E+01
44	-.53	-.98	1.12	.18022E+01	.18151E+01
45	-.63	-1.03	1.21	.18794E+01	.18922E+01
46	-.74	-1.06	1.29	.19382E+01	.19501E+01
47	-.84	-1.08	1.37	.19781E+01	.19882E+01
48	-.95	-1.09	1.45	.19986E+01	.20065E+01
49	-1.06	-1.09	1.52	.19997E+01	.20051E+01
50	-1.17	-1.08	1.59	.19813E+01	.19839E+01
51	-1.27	-1.06	1.66	.19437E+01	.19435E+01
52	-1.37	-1.03	1.72	.18871E+01	.18844E+01
53	-1.47	-.99	1.77	.18123E+01	.18072E+01
54	-1.57	-.94	1.83	.17200E+01	.17127E+01
55	-1.66	-.88	1.88	.16109E+01	.16021E+01
56	-1.74	-.81	1.92	.14863E+01	.14763E+01
57	-1.82	-.74	1.96	.13472E+01	.13367E+01
58	-1.89	-.65	2.00	.11953E+01	.11847E+01
59	-1.95	-.56	2.03	.10316E+01	.10216E+01
60	-2.00	-.47	2.05	.85797E+00	.84901E+00
61	-2.04	-.37	2.07	.67615E+00	.66862E+00
62	-2.07	-.27	2.09	.48757E+00	.48207E+00
63	-2.09	-.16	2.10	.29455E+00	.29110E+00
64	-2.10	-.05	2.10	.98468E-01	.97670E-01

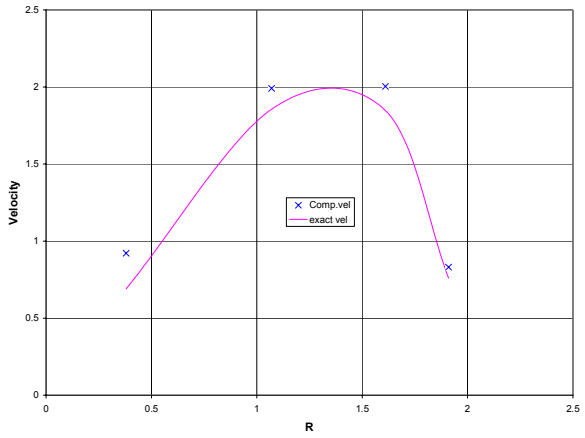

Figure 5: Comparison of computed and analytical velocity distributions over the boundary of a symmetric aerofoil using 8 boundary elements with constant element approach for r = 1.1, a = 0.1 and Ma = 0.7.

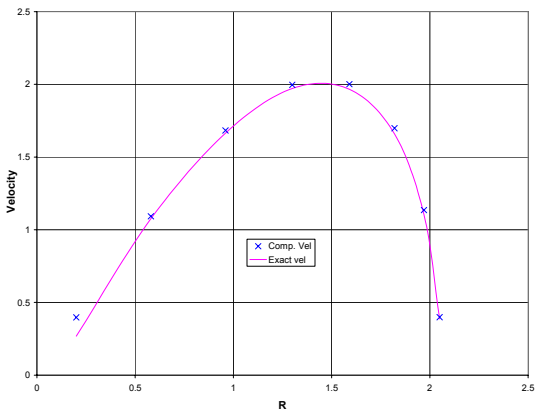

Figure 6: Comparison of computed and analytical velocity distributions over the boundary of a symmetric aerofoil using 16 boundary elements with constant element approach for r = 1.1, a = 0.1 and Ma = 0.7.

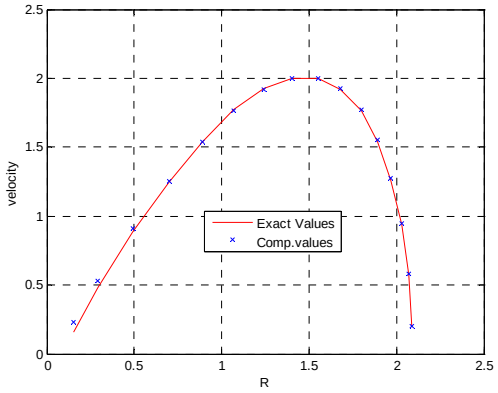

Figure 7: Comparison of computed and analytical velocity distributions over the boundary of a symmetric aerofoil using 32 boundary elements with constant element approach for r = 1.1, a = 0.1 and Ma = 0.7.

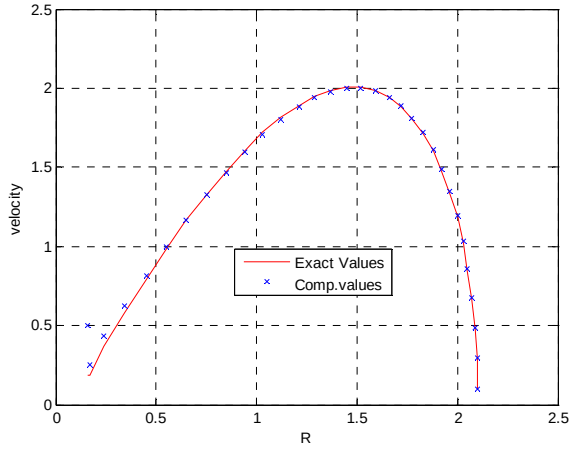

Figure 8: Comparison of computed and analytical velocity distributions over the boundary of a symmetric aerofoil using 64 boundary elements with constant element approach for r = 1.1, a = 0.1 and Ma = 0.7.

5. Conclusion

An indirect boundary element method has been applied for the calculation of compressible flow past a symmetric aerofoil with constant element approach. The calculated flow velocities obtained using this method is compared with the analytical solutions for flow over the boundary of a symmetric aerofoil. It is found that the computed results obtained by this method are good in agreement with the analytical ones for the body under consideration and the accuracy of the result increases due to increase of number of boundary elements.

6. Acknowledgement

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Phytoplankton Dynamics of River Oli in Kainji Lake National Park, Nigeria during Dry Season.

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Abstract: This paper examined the phytoplankton of River Oli (Borgu sector) of kainji Lake National Park for the first time. It recorded total of fifty five taxa, belonging to four major divisions; bacillariophyta, chlorophyta, euglenophyta and cyanophyta. The taxa were dominated qualitatively by green algae and quantitatively by euglenoids in particular *Euglena acus.* [The Journal of American Science. 2010;6(5):72-76]. (ISSN 1545-1003).

Keywords: River Oli, phytoplankton, Nigeria, diversity.

1. Introduction

The Nigerian climate is tropical, characterized by high temperatures and humidity as well as marked wet and dry seasons. The coastal areas have an annual rainfall ranging between 1, 500 and 4,000 mm (Kuruk, 2004). The surface water of the Nigerian coast is basically warm with temperature generally greater than 24^0C. Kuruk (2004) reported that the hydrology of Nigeria is dominated by two great river systems, the Niger-Benue and the Chad systems.

With the exception of a few rivers that empty directly into the Atlantic Ocean, all other flowing waters ultimately find their way into the Chad basin or down the lower Niger to the sea. It was also stated that the two river systems are separated by a primary watershed extending northeast and north-west from the Bauchi Plateau which is the main source of their principal tributaries. Algological studies on lotic system in Nigeria are few and these include that of Egborge (1973, 1974, 1979) who reported the phytoplankton of Oshun river, Egborge and Sagay (1979) on freshwater ecosystem in Ibadan, Holden and Green (1960) on the River Sokoto , Nwadiaro and Ezefili (1986) and Erondu and Chindah (1991) reported the phytoplankton of new Calabar River, while Kadiri (1999) studied the lower River Niger phytoplankton, Kadiri and Azomani (2000) studied the effect of brewery effluent on the growth of two chlorophytes in Ikpoba River, Kadiri and Omozusi (2002) reported the phytoplankton of River Okhuahe in Benin and most recently Kadiri (2007) reported the phytoplankton of River Ethiope.

Of all the mentioned rivers above, River Oli in Kainji Lake National Park and several other water bodies remain without phycological information hence such study remain important because majority of the riverine inhabitant most of the time depend on their surrounding water (River) apart from rain water for their water needs. This study is a pioneer phycological investigation of River Oli in Nigeria.

Description of study area

Kainji Lake National Park was established by Decree 46 of 1979 and is located in Niger and Kwara states of Nigeria, is 560 km north of Lagos, close to the border with the Republic of Benin. It covers an area of about 5340 km^2 and the most important landmark of the park is Kainji Lake. Adjoining the western side of the lake is the 3972 km^2 Borgu sector of the park which harbours River Oli, the major river found in this park. The river is perennial as it breaks into pools during the dry season. At this period its surface rate of flow reduces but the pool remains and is often quite large and provides a source of water to the wildlife population while the wet season is characterized by a period of maximum volume. River Oli takes its source from the River Niger which is the third longest river in Africa and the longest river in West Africa with watershed area covers of about 1,250,000km^2 (John 1986) and finally crossing Nigeria from north-west to south (Iloeje 1991). On the eastern side is the Zugurma sector, both sectors are not connected by land. The larger of the two distinct sectors of the park, Borgu is an ecosystem in Northern Guinea vegetation zone characterized by tall grasses and savannah woodland.

The vegetation of the park is typical of the Sudan-Guinea Savanna, although in some areas it appears more Sahelian. Riparian forests occur on the banks of the larger watercourses and some of the vegetation identified around this river are *Cola laurifolia, Terminalia aficiodis, Xylopia sp., Irvingia smithii, Bambus vulgaris, Burkea Africana, Diaspyrus mesfiformis, Symchnos spinosa, Grewia cubicens, Nauclea latifolia, Maytanus senegalensis* and *Mallotus oppositifolis*. There is a distinct raining season from May to October with maximum rains in

August and September. The park retains a robust animal population including antelope, lion, hippopotamus, buffalo, roan antelope, jackal, baboon, monkey and crocodile. The park is usually open from December to June, with the best time to visit towards the end of the dry season, when the grass has dried out and the animals move closer to the water.

2. Materials and Methods

Collection of samples

The study was based on a single sampling strategy during reconnaissance field trips in the Kainji Lake National Park, Borgu sector. Samples were collected on 16 April, 2009 from two locations due to accessibility as the river has separated into pools which harbors animals like Hippopotamus and crocodile. Station A (Latitude: 09^0 53^1 53.6N, Longitude: 003^0 59^1 07E) was called Hippopotamus pool by the workers in the Park while station B (Latitude: 09^0 54 43.4, Longitude: 003^0 57^1 13.5E) was very close to the Park hostel. Few of the physical parameters analysed include surface water temperature which was measured by mercury in glass thermometer, surface water conductivities and salinity values were recorded using conductivity meter and hand refractometer for stations A and B.

Biological samples were stored in 5 L, concentrated, fixed with 4% unbuffered formalin and analysed with the aid of Olympus XSZ-N107 photomicroscope. Taxanomic keys employed in the identification included Hustedt (1930, 1937, 1942 and 1971); Patrick & Reimer (1966, 1975); Prescott (1964, 1973 and 1982; Whittford and Shumacher 1973). Community structure analyses used in this study have been described elsewhere (Adesalu et al., 2008).

3. Results

Surface water temperature recorded 31^0C for both stations, the pH values of (7.08 and 7.20), surface water conductivities (70.0µScm^{-1} and 71.0µScm^{-1}) and salinity values recorded 0.01‰ and 0.02‰ for stations A and B respectively.

Phytoplankton composition

A total of 55 taxa classified into four major divisions namely, Bacillariophyta, Chlorophyta, Euglenophyta and Cyanophyta were observed in this study. The green algae dominated the phytoplankton spectrum of both stations, it recorded 32.50% and 54.99% of total phytoplankton for stations A and B (Figure 1) respectively with *Scenedesmus quadricauda* and *Pediastrum boryanum var longicorne* accounted for 7.50% and 6.67% of station A and *P. simplex (*13.78%) with *S. quadricauda (*13.63%) for station B. For station A, the diatoms, euglenoids and blue green algae recorded 30.42%; 19.38%; and 17.71% of total phytoplankton in that sequence making the blue-green algae the least represented with only three genera *Oscillatoria, Merismopedia* and *Chroococcus*. Interestingly, Station B did not follow that sequence rather the least represented division was bacillariophyta (9.50%) with *Navicula decusis* accounted for 2.30% (Table 1).

The overall observation showed that the green algae were ably represented by chlorococcales particularly Scenedesmaceae (mostly *Scenedesmus* spp) and the hydrodictyaceae mainly *Pediastrum* spp. *Euastrum sinuosum* and *Tetraedron* sp were among the rare species encountered during investigation. Although the euglenoids recorded lower percentage values for both stations, the taxon had a wide distribution with *Euglena acus* as dominant species. Shannon-Weaver information (H^l) value (3.23) was higher in station A while low equitability value (0.81) was recorded for station B. Species richness d recorded its highest value (5.67) in station A (Figure 2).

Dominance of phytoplankton samples by a few species was reflected by low equitability 'j' value recorded and since Margalef's 'd' value is influenced by the number of species and individuals, high 'd' values recorded in station A reflected high species number and relatively low numbers of individuals. The Shannon-Weaver diversity index (H^1) is influenced by both number of species and equitability. In River Oli, higher H^1 values observed could be attributed to high 'j' value recorded (Table1).

Table 1: Phytoplankton abundance and percentage composition (cells mL^{-1}) of Oli River, Kainji Lake National Park (17/4/09) (%A=Percentage composition of species in station A; %B=Percentage composition of species in station B).

Taxa	**Station A**	% A	**Station B**	% B
Division: Bacillariophyta				
Class: Bacillariophyceae				
Order 1: Aulacoseirales				
Family 1: Aulacoseiraceae				
Aulacoseira granulata (Ehrenb) Ralfs.	14	2.92	4	0.30
Aulacoseira granulata var angustissima O.Muller	37	7.71	26	1.93
Order 2: Achnanthales				

Family: Achnanthaceae				
Achnanthes sp.	4	0.83		
Order 3: Fragilariales				
Family: Fragilariaceae				
Synedra sp.	4	0.83		
Ulnaria ulna (Nitzsc) P Compère	18	3.75	2	0.15
Order 4 : Tabellariales				
Family :Tabellariaceae				
Tabellaria fenestrata (Lyng.)Kutzing			2	0.15
Order 5: Bacillariales				
Nitzschia sp.			2	0.15
Order 6: Naviculales				
Family 1: Naviculaceae				
Frustulia rhokmboides var saxonica (Rabh) de Toni			4	0.30
Gyrosigma scalproides (Raph.) Cleve			4	0.30
Luticola mutica Kutzing.	20	4.17	15	1.11
Mastogloia sp.			14	1.04
Navicula cryptocephala Kutzing	8	1.67	1	0.07
N. decusis Ostrup	4	0.83	31	2.30
N. exigua (Greg.) O. Muller	14	2.92		
N. rhyncocephala Kutzing			12	0.89
Navicula sp.	4	0.83	12	0.89
Pinnularia biceps Gregory	6	1.25	4	0.30
Pinnularia sp.	4	0.83		
Family 2: Cymbellaceae				
Cymbella ventricosa Kutzing			2	0.15
Cymbella sp	3	0.63		
Family 3: Gomphonemataceae				
Gomphonema angustatum var producta Grunow	3	0.63		
*G. parvulum (*Kutzing) Kutzing			2	0.15
Gomphonema sp.	3	0.63		
Division: Chlorophyta				
Class: Chlorophyceae				
Order 1: Chlorococcales				
Euastrum sinuosum Lenor	3	0.63		
Pediastrum boryanum var longicorne Raciboski	32	6.67	128	9.48
P. duplex Meyen			96	7.11
P. simplex (Meyen) Lemm	17	3.54	186	13.78
P. simplex var echinulatum (Wittr)	11	2.29		
P. tetras (Ehr.) Ralfs			16	1.19
Scenedesmus acuminatus (Lag.) Chodat	8	1.67		
S. armatus			24	1.78
S. armatus var bicaudatus (Gugl.-Printz)Chodat	12	2.50		
S. bicaudatus Dedus			104	7.70
S. denticulatus Lagerh.	8	1.67		
S. dimorphus (Turp.) Kutz			16	1.19
S. quadricauda (Turp) Breb	36	7.50	184	13.63
S. quadricauda var maxima W & G.S West	4	0.83	20	1.48
Tetraedron sp.	1	0.21		
Order 2: Desmidiales				
Family: Closteriaceae				
Closterium sp.			4	0.30
Order 3: Volvocales				
Family: Volvocaceae				
Volvox sp	24	5.00	7	0.52
Division: Euglenophyta				
Class: Euglenophyceae				
Order: Euglenales				
Euglena acus Ehr.	34	7.08	234	17.33
E. deses Ehr.			4	0.30
Euglena ehrenbergii Klebs			14	1.04
E. limnophila var limnophila Lemm			14	1.04
Euglena viridis	18	3.75	42	3.11
Euglena sp.	13	2.71		
Phacus longicauda				
Phacus orbicularis Hubner	19	3.96	40	2.96
Phacus sp.	1	0.21		
Strombbonas ovalis	4	0.83		
Strombbonas sp			8	0.59
Trachelomonas sp.	4	0.83		
Division: Cyanophyta				
Class: Cyanophyceae				
Order 1: Chroococcales				
Family 1: Chroococcaceae				
Chroococcus sp.	21	4.38	80	5.93
Family 2:Merismopediaceae				
Merismopedia glauca (Ehr.) Nag	48	10.00	48	3.56
Order				

2:Oscillatoriales				
Family 1:Oscillatoriaceae *Oscillatoria formosa* Bory	16	3.33	28	2.07
Number of Species	36		38	
Total Number of Individuals	480		1442	
Margalef species diversity (d)	5.67		5.09	
Shannon-Weaver (H^1)	3.23		2.96	
Species Evenness (j)	0.90		0.81	

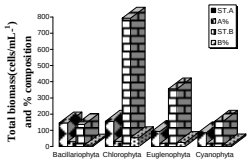

Figure 1: Percentatage composition of different divisions in Oli River

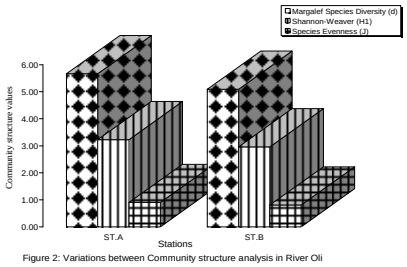

Figure 2: Variations between Community structure analysis in River Oli

4. Discussion

The paucity of phytoplankton population in the River Oli may be partly due to the poor light penetration into highly turbid water, which reduced photosynthetic depth as a result of natural habitat that the river created for hippopotamus and crocodiles which most often mixes up the water for their own body temperature regulation especially during dry season when this study was undertaken. In this study, the Euglenophyceae had a wider distribution and two of the organic pollution indicators species observed were *Euglena acus* Ehr. and *Phacus orbicularis* Hubner.

The observation of more chlorophytes than diatoms and very few cyanophytes in this study conformed to typical trend in tropical water bodies (Kadiri 1999; Kadiri & Omozusi 2002; Coute & Rousselin 1975; Kebede & Belay 1994). Wetzel (1983) reported that chlorococcales inhabit waters of differing salinity and alkalinity. The low desmids recorded could be a pointer that the river is poor in its ionic composition (Kadiri 1993; Nwankwo 1996) because high diversity of desmids is an indication that the water body is largely unpolluted (Egborge and Sagay 1979) and this is supported with *Euglena acus* recording the highest percentage composition (17.33%) for the overall phytoplankton spectrum. According to Caljon (1987) and Conforti (1991) this group is characteristic of eutrophic or nutrient rich water bodies and there abundant is a pointer that probably the study area is organically polluted which could be due to animal faecation. So far, no work has been done on the algal flora of River Oli; hence all these forms constitute new records and this study has provided baseline data for the River Oli.

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Global Food Crisis and its Implications in Nigeria

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Abstract: The increasing world population is putting pressure on the productive lands, resulting to decline in yield and hence food to feed the ever teeming world population, thus causing food crisis globally. The food crisis has resulted in problems leading to riots in Bangladesh, India, Pakistan, Burma, Egypt, Morocco Ethiopia, France, Spain, Brazil, Venezuela, Kenya and most recently, Madagascar. These problems could have far reaching effect on the fertility i.e. reproductive capacity of the population. Therefore, Food crisis has become a global issue since it occurs in virtually all parts of the world. Some constraints to food production in the world include land policies, poverty, rural-urban migration, bad governance, disease (especially the AIDS scourge). Execution of Research findings from Research institutes, deliberate government policies to alleviate poverty and disease are some of the ways of tackling the crisis. [Journal of American Science 2010;6(5):77-79]. (ISSN: 1545-1003).

Keywords: food crisis, constraints, world population

Introduction

Global food crisis is not a new phenomenon. It has been there since the creation of the world, but certainly the current food crisis being experienced in many parts of the world is a big embarrassment to the national governments. Since the Second World War ended in 1945, many Countries have placed emphasis on massive food production to feed the ever-increasing national populations and provide raw materials for industry with positive results. However in some Countries especially in Africa, South-East Asia and Latin America where there are heavy demographic figures, the situation is different. These countries experienced and would continue to experience food deficit as a result of uncontrolled birth rates (which continue to rise), poor agricultural development and "Bad" governance.

The total world population is put at 6.78 billion people of who half is in the Asian continent. South America and Africa constitute a sizeable proportion of the world's demography. Unfortunately, these regions are prone to natural disasters such as earthquakes, volcanic eruptions, floods, desertification and man-made conflicts (wars). With the world population increasing at 3.5% annually and food production at between 1.5% and 2.0%, it becomes difficult to feed the ever-increasing hungry mouths in the world. This scenario aggravated by the current global climatic change (Global warming) which has devastated many countries in the world by destroying farm lands, infrastructures and even human population (in hundreds and thousands) is the main reason for the current global food crisis. The politico-economic crisis in many parts of the world (especially in the third- world countries due to instability) has disrupted the supply of oil to the world market and created influx of refugees which further contributed immensely to the current global food crisis.

The results of these problems cited above include some food related riots in Bangladesh, India, Pakistan, Burma, Egypt, Morocco, Ethiopia, France, Spain, Brazil, Venezuela, Kenya and recently Madagascar. The cost of living in many countries is rising while standard of living is declining. Millions of people world-wide are suffering from hunger and hunger related diseases like Kwashiorkor and Marasmus; squalor, deprivation and are grossly malnourished. This could have a far reaching effect in the fertility (or reproductive capacity) of the population – may be natural birth control measure. The adage 'a hungry man is an angry man' is apt to describe the dilemma the world is facing if urgent steps are not taken to mitigate the suffering of the vulnerable segment of the world active populations.

The Nigerian Perspectives, Constraints, Prospects and Challenges

Nigeria belongs to the community of nations of the world. Whatever is happening at the world scene trickles down into the country. The current global food crisis is also affecting the populace. Cost of living is soaring daily and many families cannot afford one square meal daily let alone three square meals. There is hunger and anger in the land. Net pay of the working class can no longer carry them through-out the month. Even the "rich also cry" as they are not comfortable with the trend where many hungry mouths are looking up to them for succour on a daily basis. With an estimated population of 140

million people (NPC, 2007) growing at the rate of 3.65% per year, it means that by the year 2020, the nation's population will be about 201,320,000 million (an addition of about 61.32 million people in 12 years). If the current food production growth trend of 1.35% annually is not increased to tally with or surpass the population growth rate, then the country is in for a turbulent future.

Constraints to food production in Nigeria

The constraints to increasing food production in Nigeria include land use policy (where the Federal Government claims ownership of all land); farm fragmentation (where farmers cultivate small parcels of land located here and there); use of archaic farm tools (due to poverty and illiteracy); poor farming techniques; rural-urban migration of the active segment of the population in search of 'white-collar jobs'; 'bad' governance (due to crooked, obnoxious and uncoordinated policies or lack of it) by the government in power and hydra-headed monster called corruption and ineptitude. Also, farm land encroachment due to expansion of urban centres; siting of heavy industries; construction of roads, dams and game reserves have all contributed to diminishing arable land available for massive and sustained food production.

The findings of Research conducted in Universities and Research Institutes (albeit poorly funded Research) across the country cannot reach the end users (resource-poor farmers) and primary processors. Banks willingly lend money to traders but are reluctant granting loans to farmers because of the high risk involved. Can anyone explain to Nigerians why every year, the supply of fertilizers to farmers has become a recurrent decimal in the polity? This commodity is neither been imported early enough nor local sources developed and maintained (sustained) so that farmers can have access to the product as at when needed (evidence of planlessness). Even when fertilizers eventually arrive, alas belatedly, it becomes a service tool to politicians, middlemen and cannot get to the real farmers.

Another major constraint (and perhaps most important) is the AIDS pandemic which is fast decimating the active working population of the society with its resultant effect on the loss of manpower (labour force) in all sectors (education, agriculture, etc). Since the first reported case of HIV infection in Nigeria, there has been a systematic effort (by past governments) to respond to the potential epidemic, in view of the large population of the country. There has also been a steady decline in sero-prevalence over the years, from 1.8% in 1991, peaking at 5.8% in 2001, 5.0% in 2003 and lowering to 4.4% in 2005. However in 2008, the national HIV prevalence rate in Nigeria was 4.6%. Sero-prevalence surveys in Nigeria have always shown that the population with the most HIV infection is in the 15-29 years age range. Infection rates among the population segment below 15 years have also grown very rapidly in the last decade (UNICEF, 2009).

This increase may be attributed to the global economic recession which has seen previous donor-nations looking inwards to their own economic down-turn, thereby shifting their attention from the hitherto massive funding on HIV/AIDS control Programmes. If Nigeria, is to avert an ugly food crisis situation (now or in the near future), this is the time all heads must adorn our thinking caps to call a spade a spade.

Prospects and Challenges

Fortunately, Mother Nature has been so kind to the country by giving us benevolent weather, fertile soil and large expanses of arable land. The country is also endowed with abundant manpower and other resources which can be harnessed and ploughed into food production and processing. This raises the question of food security. Recently, the Federal Government established the 'National Programme for Food Security', a deliberate policy to assuage and free the people from hunger, malnutrition and deprivation through actions that would guarantee consistent and adequate food supply at affordable prices. These actions include ecological security (protecting the nations forests from desertification, erosion degradation); technological security (encouraging commercial food production and processing of produce/commodities into durable and nutrient stable products); building of strategic grains reserves across the country and maintaining a buffer stock to ensure price stability, nutrition education (where special lessons or messages are designed to target the "home-makers" on how to prepare high quality nutritious food for the family and "street feeders".

Surveys carried out by FAO (1985) have shown that postharvest losses due to poor storage vary from 30 to 50% in Nigeria. These surveys have also shown that the widely used traditional sun-drying techniques are the most appropriate and economical means of preservation of most of these commodities for low-income consumers. The adoption of modern but small scale solar drying technologies can be a means towards increased food preservation whereby not only excess produce are dried but a deliberate protection of our agricultural produce in good condition to save for the off-season. The establishment of collection centres which would undertake the processing of fresh produce as well as

packaging, storage and marketing should also be encouraged.

The construction of dams to provide potable drinking water and for irrigation would no-doubt encourage dry season farming but they must be maintained for effectiveness. Research Institutes and Universities (as agents of change) should be well funded and their findings made available to the resource-poor farmers and processors for increased yield per hectare and conversion of raw materials into finished products that are durable and nutrient fortified. Banks should be encouraged to assist agro-allied business through soft-loans guaranteed by the government.

The vision of NEEDS (National Economic Empowerment and Development Strategy) which focuses on key strategies like wealth creation, employment generation, poverty reduction and value reorientation must be thoroughly exploited. Among other things, offering farmers improved irrigation, machinery, and crop varieties will help boost agricultural productivity and tackle poverty head on, since 70% of Nigeria's rural people work in farms. Since half of Nigeria's population are children (NPC, 2004), recognizing the importance of improvement of their education would become a viable bridge to a prosperous future where we are self sufficient all round. The plan to improve the system of health care delivery with emphasis on HIV/AIDS and other preventable diseases, such as malaria, tuberculosis, and reproductive health-related illnesses will assuage this major social and health problem which is threatening the country's productivity and economy.

Conclusion

Global food crisis is an aberration. It smacks of lack of fore-sight by the various national rulers (albeit leaders). Both the developed and developing countries must pledge to work together to solve the food crisis through measures that have been outlined above including good governance; zero tolerance on corruption; organizing and encouraging other stake-holders towards increased food production to avert imminent "Armageddon". Execution of the 'Kuru' Declaration of 2001 which is to build a truly great African democratic country, politically united, integrated and stable, economically prosperous, socially organized, with equal opportunity for all, and responsibility from all, to become the catalyst of (African) Renaissance, and making adequate all-embracing contributions, sub-regionally, regionally and globally will help us achieve our goal of averting a global food crisis.

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A Reliable 3D Laser Triangulation-based Scanner with a New Simple but Accurate Procedure for Finding Scanner Parameters

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Abstract: In this paper, a low occlusion laser triangulation 3D scanner based on two different color lasers and one color CCD camera is proposed. By placing a laser source in each side of the camera, occlusion problems are decreased to a minimum. Finding scanner parameters is one of the critical issues in 3D scanner accuracy. A new simple procedure is proposed to accurately find scanner parameters. [Journal of American Science 2010;6(5):80-85]. (ISSN: 1545-1003).

Key words: 3D scanner, laser triangulation, low occlusion, single camera

1. Introduction

Excellent properties of laser triangulation such as being a non-contact method, possessing a simple structure, having high precision and fast measurement speed have made it the best method to obtain three dimensional images. Laser triangulation-based 3D scanners are widely used in quality inspection, surface profile measurement, 3D modeling and reverse engineering applications.

The design of laser triangulation-based 3D scanners presents challenges in the design of optical routing, calculation of the laser spot, and system calibration. Moreover, issues such as laser reflective properties of the work-piece surface, speckle noise, occlusion and detecting the peak of the laser stripe using subpixel precision may affect the precision of the measurement.

A lot of work has been done by researchers to overcome these challenges, and several structures have been proposed as solutions for solving these problems [1]. A FIR filter approach for detecting the peak position of the laser stripe was presented in [2] and its performance was compared with other methods.

The occlusion problem can be somewhat reduced if either two lasers and one camera are used or two cameras and one laser are employed in the scanner. In this study, we use a red and a green laser plus a high resolution color camera to produce a low occlusion, high resolution scanner. If we compare this system with a scanner which uses two cameras and one laser, the two laser scanner has benefits such as a lower price, less complexity, and lower computing to prepare a 3D image. A new simple procedure for accurate calculation of scanner parameters is also presented.

The paper is organized as follows. In the following section, the basic principles of laser triangulation are reviewed. In the third section, our system structure for low occlusion is presented and its advantages are introduced. In the fourth section, camera calibration and the procedure for finding the parameters are introduced. In the fifth section, the main parts making up the proposed system are introduced. In the sixth section, results of scanning an object and scanner parameters are presented. The last section is conclusions.

2. The basic principles of laser triangulation

Laser triangulation is based on the projection of a laser over an object and the image is captured by a digital camera. The 3d position of the laser beam over the object can be calculated by trigonometry, if we know the distance between the laser source and the camera (called baseline) and the angle between the baseline and the laser beam. An example of the triangulation system configuration is shown in Figure 1.

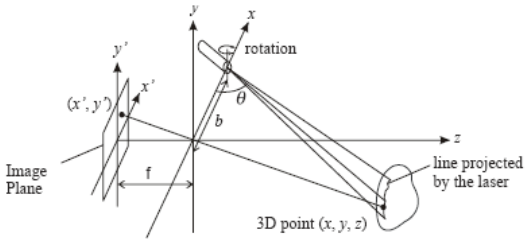

Figure 1. Example of triangulation system adopted from [3]

The coordinate (x,y,z) of a 3D point in real space which is projected onto the image pixel (x', y') can be found from equations (1), (2) and (3):

$$x = \frac{bx'}{f\cot\theta - x'} \quad (1)$$

$$y = \frac{by'}{f\cot\theta - x'} \quad (2)$$

$$z = \frac{bf}{f\cot\theta - x'} \quad (3)$$

where b is baseline, θ is laser beam angle and f is the focal length [3].

3. System structure and its advantages

Our scanner structure is designed with the aim of high reliability, high resolution measurement, minimum occlusion problem, medium scan size, simple architecture and a low price.

Usually in many projects two cameras with two different fields of view are used to decrease the occlusion problem as shown in Figure 2. However, this increase the complexity and price of the scanner because we need two cameras and more computational expense for processing the data for the duplicate volume of picture data. Moreover, this additional hardware complexity would reduce the overall reliability of the system.

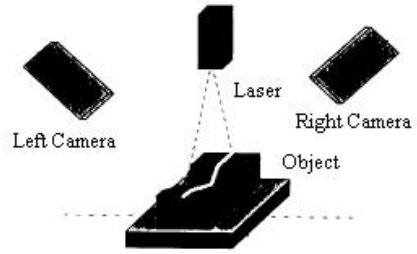

Figure 2. Laser triangulation 3d scanner with two cameras and one laser adopted from [4].

The use of two lasers with red and green color and one color camera is proposed in this paper to increase the overall system reliability. The lasers are placed on different sides of the camera. This architecture minimizes the occlusion problem. The reason for using different laser colors is to employ full size of image sensor for each lasers beam completely. Park et al. [5] proposed a 3D scanner with dual same-color lasers on the left and the right sides of the camera. To avoid the ambiguity in the detection of the two lasers beams, they assigned lasers beams to certain angles so that they never intersect each other in the scanner's field of view. This means that nearly one half of the image sensor is used by each laser, and the resolution of the scanner is drastically reduced and the scanner's measurable depth is decreased. In our proposed design, since we use two lasers with red and green colors, we can distinguish their beams in the RGB color camera.

One may calculate the distance in the Z- axis using equation (3). The measurement error and the parameters which affect it are given by (4). Range error in triangulation-based laser scanner come from the error in estimate of position of the laser on the image sensor. Equation (4) gives the uncertainly approximation in Z [6]

$$\delta z \approx \frac{z^2}{fb}\delta x' \quad (4)$$

where f is focal length, b is the baseline, $\delta x'$ is uncertainty in the laser position and z is the distance of the scanner to the object.

From equation (4), it can be seen that the error measurement in Z decreases with an increase in the camera baseline and the focal length of the lens, but dramatically increases by any increase of the distance.

In order to reduce the $\delta x'$ to a minimum, a 7.2 mega pixel camera was used in this study and the center of mass algorithm was employed to accurately find laser position.

Unfortunately, there is a trade off between range error minimization and other specifications of the scanner such as reduction of occlusion problem and maximization of the camera's field of view. Thus, in the scanner f and b cannot be made as large as desired. The baseline is limited by the occlusion problem since occlusion increases with increasing b, and f is limited by the camera's field of view since the camera's field of view decreases with increasing f. In the proposed scanner design, we set the camera as near as possible to the object that we wish to scan.

Setting the camera very close to the object will lead to a small field of view for the scanner. However, we have solved this problem by moving the camera and the lasers together using an accurate linear motion system and preserved the scanner's field of view to maintain a high precision measurement.

4. Camera calibration and finding scanner parameters

Camera calibration and setting scanner parameters directly affect the scanner's precision. We need to calibrate the camera for its intrinsic parameters such as lens distortion. Also scanner parameters such as camera and laser baseline, laser beam angle, camera focal length, laser tilt versus camera Y axis and the angle between camera X axis and scanner linear motion system should exactly be set to accurately find 3D position of laser stripe on the object.

There are a lot of published papers about camera calibration [7]. However, our new procedure to find scanner parameters is very simple and there is no need for any mechanical calibration gages or any calibration points with exactly known position in three dimensions. The laser beam illumination on the flat table of the scanner with good reflectance properties is used for calculation of major scanner parameters, and sub-pixel resolution can be employed to find parameters with

high precision results. Also in our procedure we consider all of scanner details which cause accurate result in scanning.

In the proposed procedure, we use photogrametery to find some of the parameters in order to calculate laser beam angle. Accurate lithography film is used for reference of measurements. The film is shown in Figure 3.

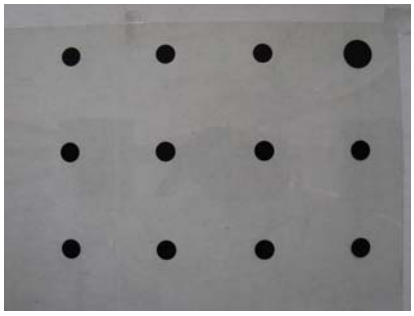

Figure 3. Picture of the lithography film. (Distances between the centers of the circles are 50mm)

Since the camera and the lasers are placed on the linear motion system with the capability of moving in the X and Z axes, we can use the Z axis to calculate the laser beam angle by taking two pictures with the camera. We first turn on the lasers and paste the film on the scanner table. Then we take two pictures. We take one picture when the Z position of the motion system is at z_1 and take the second picture when it is at z_2. Then we process the two pictures and find the distance between the centers of the lasers stripes in the two pictures with consideration of the fixed film in the pictures and the known center positions of the circles on it. Thus, we can calculate the laser beam angle using equation (5):

$$\theta = \cot^{-1}(\frac{\Delta x}{\Delta z}) \qquad (5)$$

where θ is the laser beam angle, Δx is the distance between the centers of the two laser stripes in two pictures and $\Delta z = z_2 - z_1$.

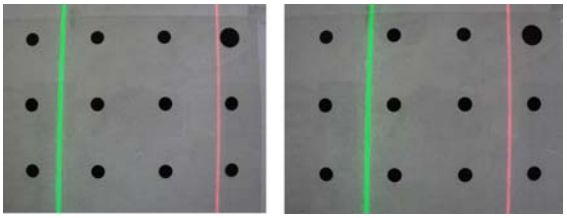

Figure 4. Two pictures which are used to calculate the laser beam angle.

Laser tilt versus camera Y axis is one of the other parameters of the scanner which should be considered. For accurate scanning, it is necessary that the laser stripe be parallel with the Y axis of the camera. This situation needs very precise assembling of scanner parts. However, we can compensate the laser tilt instead of having precision assembly of the system. The laser tilt can be calculated with our accurate lithography film and photogrametery method, as shown in Fig. 5. Equations (6) and (7) show the laser stripe tilt compensation.

$$Tilt = \frac{x_2 - x_1}{y_2 - y_1} \qquad (6)$$

$$x' = x'_{WithLaserTilt} - Tilt \times y' \qquad (7)$$

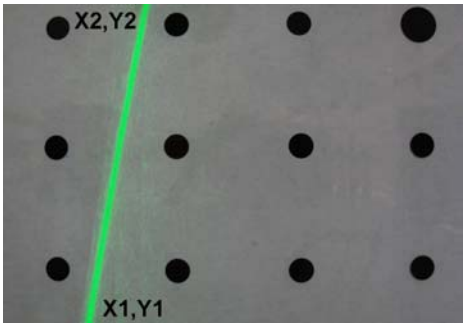

Figure 5. Compensation of the laser stripe tilt.

The angle between the x axis of the camera and the x axis of the scanner linear motion system is the third parameter which we calculate by using photogrametery. If the angle is not equal to zero, we should compensate the results of the process for each stripe with equations (8) and (9):

$$X = y\sin\alpha + x\cos\alpha \qquad (8)$$

$$Y = y\cos\alpha - x\sin\alpha \qquad (9)$$

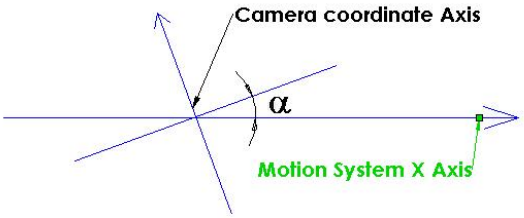

Figure 6. The angle between the x axis of the camera and the x axis of the scanner linear motion system.

To calculate the angle α, we first calibrate the camera with the lithography film as shown in Figure 3. Then we change the film with a new one shown in Figure 7. Then we take two pictures with displacement of Δl in the x axis of the linear motion system. The calculation of the angle α is given in (10):

$$\alpha = \sin^{-1}(\frac{\Delta y}{\Delta l}) \quad (10)$$

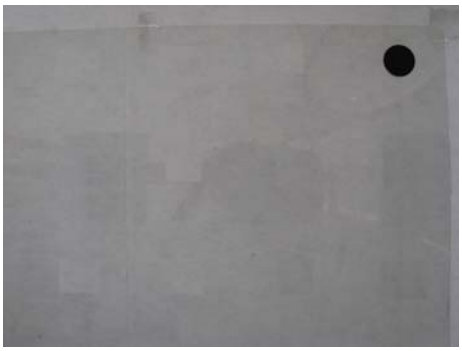

Figure 7. Lithography film used for calculating the α angle.

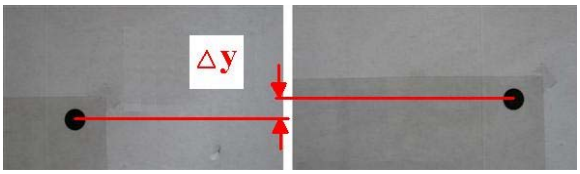

Figure 8. Two camera pictures are used to calculate the α angle.

Finally, to calculate b (baseline) and f (focal length), we turn on the lasers and take 4 pictures with the camera in 4 different movements along the Z axis of the linear motion system from a position where the distance between the origin of coordinates of scanner and table of the scanner on the Z axis is z_0. These 4 movements take us to z_1, z_2, z_3, and z_4 so the distances between the origin of the scanner and the table of the scanner on the Z axis are $z_0 - z_1$, $z_0 - z_2$, $z_0 - z_3$, and $z_0 - z_4$ respectively. Note that we consider the sign of the 4 movements positive when they are in the direction of the scanner table.

So we write equation (3) for $z_0 - z_1$, $z_0 - z_2$, $z_0 - z_3$ and $z_0 - z_4$ as follows:

$$z_0 - z_1 = \frac{bf}{f\cot\theta - x'_A} \quad (11)$$

$$z_0 - z_2 = \frac{bf}{f\cot\theta - x'_B} \quad (12)$$

$$z_0 - z_3 = \frac{bf}{f\cot\theta - x'_C} \quad (13)$$

$$z_0 - z_4 = \frac{bf}{f\cot\theta - x'_D} \quad (14)$$

In the above equations, we know $z_1, z_2, z_3, z_4, x'_A, x'_B, x'_C, x'_D, \theta$ and z_0, f, b are unknown. Then we can calculate f and b by using equations (15) and (16):

$$f = \frac{z_2 x'_B - z_1 x'_A + \frac{x'_B - x'_A}{x'_D - x'_C}(z_3 x'_C - z_4 x'_D)}{\cot\theta(z_2 - z_1 - \frac{x'_B - x'_A}{x'_D - x'_C}(z_4 - z_3))} \quad (15)$$

$$b = \frac{(z_2 - z_1)(f\cot\theta - x'_A)(f\cot\theta - x'_B)}{f(x'_B - x'_A)} \quad (16)$$

With the procedure explained above, all of the scanner parameters may be calculated and used for scanning.

5. Description of the system

A picture of the prototype of the system built is shown in Figure 9. The camera and the laser diodes are placed on a high accuracy Cartesian 3-Axis ball screw drive motion system with 0.05 mm resolution. Here, only the X and Z axes are used in the scanner. Two 5 mw linear red and green laser diodes with 650 nm and 532 nm wave length are placed on the right and left sides of the camera and one Sony DSC-H5 7.2 Mega pixels (3072 pixels x 2304 pixels x 24 bits color) camera is used.

6. A scanned case

To present an example, a computer mouse was scanned. Its photograph is shown in Figure 10. The point cloud models resulting from the green laser scan is shown in Figure 11, and the point cloud models resulting from the red laser scan is shown in Figure 12. The right side of the object did not scan well in the green laser scan because the occlusion problem happened. On the other hand, the left side of the scanned mouse did not scan well in the red laser scan due to the occlusion problem. However, a better point cloud model is produced by combining these two scans together as shown in Figure 13. Results of calculated scanner parameters are shown in Table 1.

7. Conclusions

In this paper, the design and implementation of a new 3D scanner is presented based on laser triangulation which has several advantages over existing systems. In this approach, two laser with different colors and one color CCD camera were used to minimize the occlusion problem and increase the overall system reliability. The proposed scanner is much cheaper and is less complex compared with the scanners which include two cameras and one laser. Also because two different color lasers are used, there

is no ambiguity in distinguishing between the two lasers and the full CCD has been used for each of the lasers.

Another advantage of our scanner is an appropriate field of view with a low range error. This was achieved by placing the scanner near the object to decrease the range error and by moving the camera and lasers together on the linear motion system to increase the field of view.

A new yet simple procedure is presented in this paper to accurately find the scanner parameters with sub-pixel resolution without any need for accurate mechanical gage or calibration points with known precise 3D position.

Table 1: Calculated scanner parameters

Red Laser Beam Angle	51.19
Green Laser Beam Angle	46.63
Red laser Tilt	0.0125
Green laser Tilt	0.0303
Red laser baseline	128.61 mm
Green laser baseline	180.95 mm
Focal length	6.62 mm
α angle	0.587

Acknowledgement

We would like to thank the Office of Vice Chancellor of Research and Technology of Ferdowsi University of Mashhad for their support of this research via the Grant Project 8146 (1387/5/17) that has assisted us in carrying out the research that led to the preparation of this manuscript.

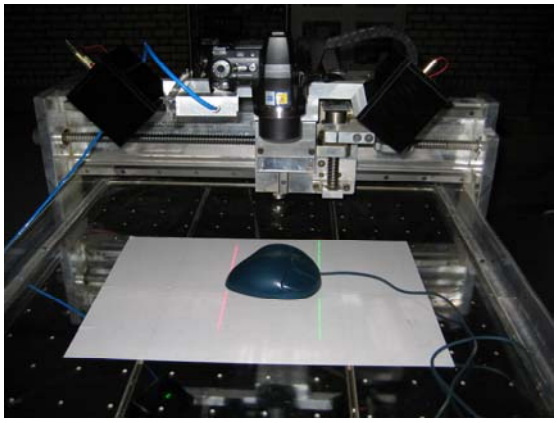

Figure 9. The prototype of the built system showing its architecture

Figure 10. Photograph of computer mouse which was scanned

Figure 11. Result of green laser scan.

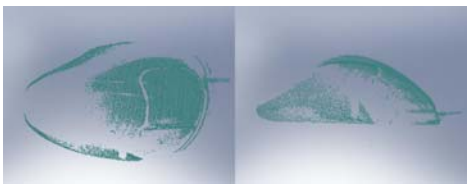

Figure 12. Result of red laser scan.

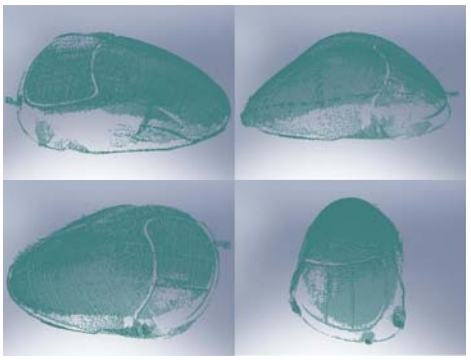

Figure 13. Result of combination of the red and green laser scans.

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9/14/2009

Intelligence as a predictor of creativity among undergraduate students

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Abstract: This research examined how intelligence predicts level of creativity and different constituent of creativity; Something about myself, Environmental sensitivity, Initiative, Intellectuality, Self-strength, Individuality and Artistry among undergraduate students. One hundred and fifty three Iranian students were selected from six Malaysian universities to participate in the research. Data was analyzed using multiple regression analysis. The total variance accounted for by the intelligence factor is 13.5% (multiple R2 = 0.135), F (7, 145) =3.222, p= .003<0/01). This implies that intelligence is important when considering the factors that influence creativity of students. [Journal of American Science 2010;6(5):86-90]. (ISSN: 1545-1003).

Keywords: Intelligence, Creativity, Something about myself, Environmental sensitivity, Initiative, Intellectuality, Self-strength, Individuality and Artistry

Introduction

Furnham & Bachtiar (2008) stated there are more than 60 definitions of creativity with no single authoritative and agreed upon definition, or operational measure. An easy meaning of creativity view is as generating something novel, original, and unexpected(Sternberg & Lubart, 1999). According to Palaniappan (2007b) creativity is some of the many intellectual constructs that has been defined as many different ways as the number of researchers investigating them. Creativity has been defined as a product, process, person as well as the press (environment) that impacts on the individual (Rhodes, 1961). For purpose of this study, creativity is investigated as a personality (KTCPI as the measure), because it is a new measure for assessment of creativity by this instrument. Creativity Perception refers to the perception of oneself as being creative and capable of creative productions. It is one of the most important personality traits related to creativity (Biondi, 1976; Davis, 1983). This is further confirmed by (Khatena, 1977) when he said that " an individual who perceives himself as creative and with accuracy, is a person who can be expected to behave in creative ways".

The conception of creativity is regularly related to intelligence(Furnham & Bachtiar, 2008), but according note's (Furnham & Bachtiar, 2008) several early researchers (Andrews, 1930; Getzels & Jackson, 1962; McCloy. W and N.C. Meier, 1931) have been shown the relation between creativity and intelligence has only modest correlations (r= .07, .22, .26, respectively). In another study (Furnham & Bachtiar, 2008) intelligence [as measured by the Wonderlic Personnel Test (WPT)] was not correlated with any of the creativity [as measured by the Divergent Thinking (DT), Biographical Inventory of Creative Behaviours (BICB), Self-Rating of creativity (SR), Barron–Welsh Art Scale (BWAS)].

In a study conducted by (Olatoye & Oyundoyin, 2007) on the creativity and intelligence among 460 students were randomly selected from 20 secondary schools, it was found that intelligence quotient (I.Q) [as measured by Slosson's Intelligence Test (SIT)] was significantly related to creativity [Ibadan Creative Assessment Scale (ICAS)]. Their finding has been shown intelligence quotient (I.Q) accounted for 8% of variance in creativity (R2 = 0.80). This percentage is statistically significant. According to this result also intelligence quotient (I.Q) significantly predicts each of the four components of creativity (fluency, originality, flexibility and creativity motivation). (Funchs & Karen, 1993) studied on the creativity and intelligence in which four hundred and ninety six preschoolers of children looking admission to a special program for gifted preschoolers participated, it was found that creativity (as assessed by the Thinking Creativity in Action and Movement Scales) was significantly related to intelligence (as assessed by the standard I.Q tests).

This research was hence designed to examine the influence of intelligence on both level of creative perception inventory and the different component of creativity; something about myself, Environmental Sensitivity, Initiative, Intellectuality, Self-strength, Individuality and Artistry among Iranian undergraduate students in Malaysian Universities. This study look for investigate the following hypotheses; intelligence will not

significantly predict the level of creative perception inventory among students. Intelligence will not significantly predict each of the components of creativity among the students.

Material and Methodology

2.1 Sample

One hundred and fifty three Iranian undergraduate students in Malaysian Universities (31.4% females and 68.6% males) were recruited as respondents in this study. Their ages ranged from 18-27 years for females and 19-27 years for males.

2.2 Measures

Catell Culture Fair Intelligence Test

To evaluate the intelligence, every student was administered by a Scale 3 of the Catell Culture fair Intelligence Test (CFIT-3a & b). Roberto Colom, Botella, & Santacreu (2002) reported that this test is a well-known test on fluid intelligence (GF). Participants completed Cattell's culture fair intelligence test battery to assess individual differences in fluid intelligence. Cattell's Culture Fair Intelligence Test (1971), which is a nonverbal test of fluid intelligence or Spearman's general of intelligence. This test contained four individually timed subsections a) Series, b) Classification, c) Matrices, d) Typology, each with multiple-choice problems progressing in difficulty and incorporating a particular aspect of visuospatial reasoning. Raw scores on each subtest are summed together to form a composite score, which may also be converted into a standardized IQ.

Khatena-Torrance Creative Perception Inventory (KTCPI)

Every student was examined using a Khatena-Torrance Creative Perception Inventory (KTCPI) to measure the creative perception of the undergraduate students (A. K. Palaniappan, 2005). The KTCPI instrument was comprised of two subscales, namely, "Something About Myself" (SAM) and "What Kind of Person Are You" (WKOPAY)? The SAM measure of creative perception which is based on the rationale that creative behavior is reflected in an individual's personal creative characteristics, characteristics possessed and in use in creative thinking and creative productions (Palaniappan, 2005; A. K Palaniappan, 2007). It tests six factors, namely, Environmental Sensitivity, Initiative, Intellectuality, Self-strength, Individuality and Artistry (p.125).

According to Palaniappan (2005; 2007) definitions, Environmental Sensitivity relates to being open to ideas of others, relating ideas to what can be seen, touched, or heard, interest in beautiful and humorous aspects of experiences, and sensitivity to meaningful relations; Initiative relates to directing, producing, and /or playing leads in dramatic and musical productions; producing new formulas or new products; and bringing about changes in procedures or organization; Self-strength relates to self-confidence in matching talents against others, resourcefulness, versatility, willingness to take risks, desire to excel and organizational ability; Intellectuality relates to intellectual curiosity, enjoyment of challenging tasks, imagination, preference or adventure over routine, liking for reconstruction of things and ideas to form something different, and dislike for doing things in a prescribed routine; Individuality relates to preference for working by oneself rather than in a group, seeing oneself as a self-starter and somewhat eccentric, critical of others' work, thinking for oneself and working for long periods without getting tired; Artistry relates to production of objects, models, paintings, carvings, musical composition, receiving awards or prizes or holding exhibitions, production of stories, plays, poems and other literary pieces. The SAM consisted of 50 items that required 'yes' or 'no' answers. The scoring of responses to this measure presented little difficulty; it was done by simple frequency counts of the positive responses on the total scale. The reliability was established in a pilot study. The pilot study had good reliability in the assessment of creativity [the SAM (alpha =0.779)].

Cumulative Grade Point Average (CGPA)

For the purposes of this study, Cumulative Grade Point Average (CGPA) was used as a proxy of academic achievement. The CGPA was calculated by dividing the total number of grade points earned by the total number of credit hours attempted. A student's academic achievement was based on their mid-year examination results. Academic achievement was the aggregate or the total number of grade points in the mid-year examinations. In these examinations, each university subject was graded along a one hundred (or four) point scale, the best grade point being one hundred (or four) and the lowest being zero. Hence the aggregate would range from 75 to 100 (3 to 4); notably the lower the aggregate, the better the academic achievement. This approach was used because other researchers have used the measure and found it an acceptable one for measuring academic achievement Palaniappan (2007a) cited several researchers (Nuss, 1961; Parker, 1979; Taylor, 1958; Wilson, 1968).

2.3 Procedure

The students who participated in this study were all undergraduates. The research questions posed for

the study required the students to identify and analyze the distributions and correlations of certain creativity perception were best addressed in the form of a descriptive study. Creativity levels were assessed by self- report instruments and were confirmed by consideration of the results from the administration offices of the universities (described below). They were then divided by gender, with the total scores and subscales calculated for each male and female. The participant sample, women (18-27 years) and men (19-27years), was asked to respond during the regular course time. Both written and oral instructions were given for all participants, and the subjects were ready to answer upcoming questions in the class. Multiple significance tests were conducted, and the data were analyzed by Regression analysis. Participants answered the tests either using their name or anonymously (whichever they preferred). They received no rewards for participating but were advised they would be given information of their results in the form of a self-referenced level of abilities at a later date. Scores for the intelligence, the creativity scale and its factors, were entered into the SPSS statistical program.

3. Result

3.1 Descriptive Statistics

Table.1 shows descriptive statistics of intelligence. The finding of this result has been shown that the intelligences' mean scores were 104.55, standard deviation (15.70), creativity (the SAM) (M=32.30, SD= 4.44), Environmental Sensitivity (M= 4.83, SD= 1.15), Initiative (M= 2.74, SD=1.48), Self Strength (M=7.24, SD= 1.62), Intellectuality (M=6.69, SD=1.70), Individuality (M=3.54, SD=1.39) and Artistry (M= 2.50, SD=1.51).

Table.1 Descriptive Statistics (N=153)

Variables	Mean	Std. Deviation
Intelligence (The A Form)	104.55	15.70
Creativity (The SAM)	32.30	4.44
Environmental Sensitivity	4.83	1.15
Initiative	2.74	1.48
Self Strength	7.24	1.62
Intellectuality	6.69	1.70
Individuality	3.54	1.39
Artistry	2.50	1.51

3.2 Data Analysis

Hypothesis One

It states that the intelligence will not significantly predict creativity of the subjects. In table 1, intelligence significantly predicts creativity among subjects. The total variance accounted for by the intelligence factor is 13.5% (multiple R2 = 0.135), F (7, 145) =3.222, p= .003<0/01). This implies that intelligence is important when considering the factors that influence creativity of Iranian undergraduate students in Malaysian universities.

Table.2. Regression summary table showing the effect of intelligence on creativity b

	Sum of Squares	Df	Mean Square	F	Sig*
Regression	5043.436	7	720.491	3.222	.003a
Residual	32428.446		145	223.644	
Total	37471.882		152		

a. Predictors: (Constant), Artistry, Individuality, Environmental Sensitivity, Self Strength, Intellectuality, Initiative, Creativity(Something About Myself)
b. Dependent Variable: intelligence
* = Significant at 0.01
Multiple R= .367
Multiple R2 = .135
Adjusted R2 = .093
Standard Error of the Estimate= 14.95475

Hypothesis Two

It states that the intelligence will not significantly predict each of the constituents of creativity of the subjects. In table 3, the multiple R2 columns reveals the total variance accounted for by each of the creativity constituents in the total performance of students in creativity. The highest contributory constituents to creativity is environmental sensitivity (R2 = 0.165). This is closely followed by intellectuality (R2 = 0.134), than, followed by initiative (R2 =0.122), artistry (R2 = 0 .114), individuality (R2 = 0.113) and lastly by self strength (R2 = 0.090). The contribution of each of the constituents is almost the different. The difference between the highest and lowest contributors is 0.156 (15.6%). Intelligence was not significantly predicts each constituents of creativity except environmental sensitivity (Sig= .041). However, Normal P-P Plot graphs (Expected Cumulative Probability by Observed Cumulative Probability) were obtained for intelligence scores is shown in Figure 1.

4. Discussion and Conclusion

Intelligence variable predicted creativity in this research, but the fact is that the value is low i.e. 13.5% (multiple R2 = 0.135), (F7, 145=3.222, p<0/05). The findings of researchers like as (Andrews, 1930; Getzels & Jackson, 1962; McCloy. W and N.C. Meier, 1931) has been show low

correlation between intelligence and creativity scores in various instruments. However, the finding of this study is not out of place. Because some researchers, they found relationship between intelligence and creativity. For example, researcher (Funchs & Karen, 1993; Olatoye & Oyundoyin, 2007) found a significant relationship between the intelligence and creativity. Intelligence is a good predictor of creativity. It is recommended and suggested that employers of schools, universities and teachers must search assignments including creativity for high intelligence students. If student reveals lack of creativity on assignments, the teacher must be administering intelligence test in order to know whether the low creativity may be caused by the students' level of intelligence.

Creativity as used in this research has six constituents namely; environmental sensitivity, initiative, intellectuality, self-strength, individuality and artistry. The relative effect of all variables considered in this investigate on each of the creativity constituents indicated that the contribution of them is almost different. Each of the creativity constituents (except environmental sensitivity) is not enough to measure creativity between students, meaning that, if the counselor or teacher, who would like to measure creativity, any of separately these constituents (except environmental sensitivity) can not be taken as the act on creativity. This study was conducted in Kuala Lumpur (capital city) and metropolitan area (Selangor) at Malaysian universities. Thus the extent to which results apply to other cities universities is not known. Therefore, conclusions need to be verified by conducting similar studies across other universities in Malaysia (Naderi et. al. 2009).

Table.3. Regression summary table showing relative effect of intelligence on each of the creativity constituents

Creativity components	R	Multiple R Square	F	Sig
Artistry	.114	.013	1.972	.162
Environmental Sensitivity	.165	.027	4.232	.041*
Self Strength	.090	.008	1.228	.270
Individuality	.113	.013	1.941	.166
Intellectuality	.134	.018	2.751	.099
Initiative	.122	.015	2.279	.132

* Significant at 0.05 level of confidence

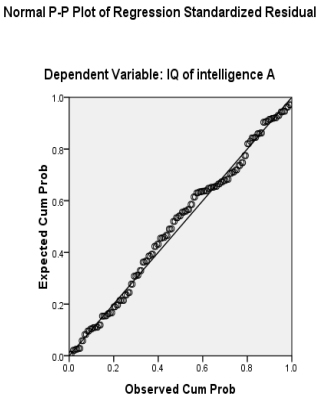

Figure 1. Normal P-P plot of Regression Standardized Residual

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Proximate and Nutrient Analysis of the Locally Manufactured Herbal Medicines and its Raw Material

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Abstract: Herbal medicines have unique therapeutic properties and therefore, used in rural areas to cure different diseases. Proximate analysis and elemental composition of the locally manufactured formulations from *Hypericum perforatum, Allium sativum, Zingiber officinalis and Valeriana officinalis* were carried out. The heavy metals including Cu, Ni, Zn, Pb, Co, Cd, Fe, and Cr were determined using Atomic Absorption Spectroscopic standard method. Na and Ca was estimated using flame emission spectrophotometer. *Z. officinalis* has highest percentage of carbohydrate, fats, fiber and energy values while in herbal formulations St. John Wort has the highest. In case of micro analysis, St. John Wort Capsules has highest concentrations of Zn while Valerian has highest concentration of Cu, Co, Cd and Fe compared to others, while in medicinal plant species, the content of Cu, Zn, Co and Fe was highest in *V. officinalis.* The level of macronutrients (Ca and Na) was highest in St. John Wort Capsule, *H. perforatum* and *V. officinalis*. However, the concentration of these nutrients in both the medicinal plants and herbal formations were in the optimum level of WHO standards. . [Journal of American Science 2010;6(5):91-96]. (ISSN: 1545-1003).

Keywords: Proximate analysis, herbal formulations, nutrient analysis, Pakistan

1. Introduction

The use of traditional medicines is increasing and getting popularity throughout the developed and developing world (Jia and Zhang, 2005). Herbal medicines as finished labeled medicinal product that contain active ingredients, aerial or underground parts of the plant or other plant material or combinations (Chaudhari, 1996; and Ritch, 2000). About 80% of the people in developing countries relay on traditional medicine for their primary health care (Latif et al., 2004). The worth of herbal product industry is approximately US$ 300 million compared to modern drugs that is US$ 2.5 billions while in recent year it has been gained considerable momentum (Shinwari and Shoukat., 2003; Shinwari et al., 2003; and Shinwari et al., 2006).

Proximate and nutrient analysis of edible fruit and vegetables plays a crucial role in assessing their nutritional significance (Pandey et al., 2006). As various medicinal plant species are also used as food along with their medicinal benefits, evaluating their nutritional significance can help to understand the worth of these plant species (Pandey et al., 2006). As far herbal drug's standardization is concerned, WHO also emphasize on the need and importance of determining proximate and micronutrients analysis. Such herbal formulations must pass through standardization processes (Niranjan and Kanaki, 2008).

Hypericum Perforatum, Valaneria officinalis, Zinigiber officinalis and *Allium sativum,* are the important plant species used in preparation of herbal formulations (st. john wort, garlicare tablet, ginger

capsule, and valerian capsule). The extract of aerial parts of *H. perforatum* is usually gathered during the flowering season or shortly before and has been used in herbal medicines (Barnes et al., 2001). *Hypericum* extracts are marketed not only as herbal medicine but also in the form of dietary supplements (Shinwari et al., 2006). Garlic or *Allium sativum* is medicinally important plant species i.e. helpful in the treatment and prevention of a number of diseases e.g. cancer, coronary heart diseases, obesity, hypercholesterolemia, hypertension and gastrointestinal (Capasso et al., 2003). *Valeriana officinalis* have a broad range of applications such as a tranquillizer for people with hyper-excitability and as a smooth-muscle relaxing agent to treat stomach and intestine cramp (Leoeniewicz et al., 2006). Valerian is also a component of many herbal mixtures, which are widely used to treat sleeping disorders (Bent et al., 2006). Ginger (*Zinigiber officinale*), an important constituent of many herbal formulations, is carminative, pungent, stimulant, used widely for indigestion, stomach ache, malaria and fevers. It is said to be used for abdominal pain, chest congestion, chronic bronchitis, colic and vomiting (Jatoi, et al., 2007). Although, these formulations are providing beneficial effects but without any knowledge of their inorganic constituents.

In the present study, the herbal formulations manufactured by Qarshi Ind. Pvt. Ltd. Pakistan and their raw materials were taken for investigation from the north western part of the NWFP, Pakistan. All these selected plant species based formulations have well documented for their phytochemicals and biological significance but no informations the proximate and elemental data. Keeping in view the importance of the inorganic constituents of the herbal medicines their proximate and elemental analysis were undertaken.

2 Material and Methods

2.1 Sampling

The medicinal plant species were collected from various areas of NWFP Pakistan. The collected plants were packed in the Kraft paper and herbarium sheets were prepared. These plants were identified by a plant taxonomist of Botany Department, Kohat University of Science and Technology, Kohat. The herbal formulations were provided by Qarshi Industries, Pakistan.

2.2 Proximate Analysis

Proximate analysis including moisture, ash, crude fiber, fats, carbohydrates and proteins were determined of both formulations and their respective raw material using AOAC (1990). The moisture contents was determined by oven dehydration method at 105 ^{0}C for 5hr using MC (%) = $\frac{W_0}{W_i}\times 100$ formula. Total ash was determined by weighing the furnace incinerated residue at 550 ^{0}C for 12hrs. The formula for calculating the ash in percent is $\frac{M_a}{M_s}\times 100$. Crude fats were determined using petroleum ether as extracting solvent in soxhlet apparatus. The percentage crude fats were calculated by $CF\ (\%)=\frac{M_{ex}}{M_s}\times 100$. The crude fibers of the samples were estimated by treating moisture and fats free material with dilute acidic solution followed by dilute base particularly NaOH. After base treatment the residue was filtered and washed with hot water and then ignited. The loss in weight was calculated from the ash left after incineration in the furnace by $\frac{W_2-W_3}{W_1}\times 100$. The crude protein was determined using micro Kjeldahl method. Percentage carbohydrate was calculated by 100 – (percentage of ash + percentage of moisture + percentage of fat + percentage of protein). All these methods were adopted, with little modification, from AOAC (1990) and Awan & Salim (1997).

2.3 Elemental Analysis

The samples were digested by a mixture of concentrated nitric acid and perchloric acid mixed in 1:1 v/v ratio. The heavy metals including Cu, Ni, Zn, Pb, Co, Cd, Fe, and Cr were determined using Atomic Absorption Spectroscopic standard method. Na and Ca was estimated using flame emission spectrophotometer.

3. Results and Discussions

3.1 Weights of the products

The weight variation among 10 tablets of the selected four herbal formulations is presented in Table 1. The data reveals that there was large difference from tablet to tablet as indicated by their corresponding standard deviation but comparatively ginger tablets shows less variation.

Table 1. Variation in the average weight of a single

S.No.	Product name	Weight
1	St. John Wort	424.89 ± 1.93
2	GarliCare Tablet	573.84 ±1.98
3	Ginger Capsule	625.85 ± 1.74
4	Valerian Capsule	426.13±1.23

± = standard deviation; Means of ten Tables

3.2 Analysis of herbal product

The proximate data for all formulations is tabulated in Table 2 and their respective raw material in the Table 3. The moisture contents was noted highest in Ginger and Valerian capsules i.e. 7.26±0.09 and 7.14±0.05% respectively. The fats contents were found highest in St. John Wort and Valerian tablets i.e. 4.98±0.01 and 4.0±0.03 respectively (Table 2). Looking at the results of carbohydrates, it was highest in St. John Wort Capsule, and (79.37±0.09 and 76.41±0.25). Comparing the crud fiber, it was higher in St. John Wort Capsule and Valerian Tablets (14.33±0.24 and 18.60±0.22 respectively). The protein contents of St. John Wort Capsule and Ginger Tablets was 6.66±0.04 and 8.60±1.0 respectively. The details of other proximate parameter are given Table 2.

The moisture, fats, crude protein, carbohydrates and ash concentrations of the ginger tablets are comparable (6.9%, 8.6%, 6.4%, 72.40% and 5.7%) to the value reported in the Encyclopedia of Chemical Technology (1980).

The data reveals that the formulations based on the selected raw materials are not the rich sources of lipid and can be administered to patients in whom high fats contents is a risk factor. The level of dietary fibre is low in garlicare tablets and high in valerian capsule. This trend is persistent in its respective raw materials, generally the fiber content are high as compared to other plant leaves and seeds in the cited literature (Elegbede, 1998). Although high fiber contents increases digestibility, but on the other hand high level of fibers in the diet can produce intestinal irritation, which ultimately decrease nutrients utilization (Oyenuga and Fetuga, 1975). It can be deduced that the fiber contents of these formulation are mild in concentration and have more beneficial effects rather than hazardous. The ash contents of St. John Wort capsule and Garlicare tablets are comparatively low. It is evident that these formulations besides its targeted objectives offer good source of carbohydrates, and can produce energy, thus meeting the increased demand of energy especially in aged population

3.3 Medicinal plant species

The moisture contents of the *Allium sativum* was found 67.66±0.18 and *Valeriana officinalis* was 6.82±0.09. The fats contents of the *Allium sativum* and *Valeriana officinalis* was found 2.43±0.11 and 4.14±0.09% respectively. The carbohydrate contents of the *Allium sativum* and *Valeriana officinalis* was found 14.98±0.06 and 67.52±0.07 respectively. The fibre contents of the *Allium sativum* was found 2.43±0.07 and *Valeriana officinalis* was 16.78±0.09. The ash contents of the *Allium sativum* and *Valeriana officinalis* was 1.73±0.16 and 17.10±0.08 respectively. The calorific value of the *Allium sativum* and *Valeriana officinalis* was estimated 134.60±0.65 and 324.95±0.52 respectively.

It is essential to quantify the level of toxic trace elements in medicines directly derived from the herbal source and used as it is, due to its deleterious effects upon human health. All the four formulations were analyzed for heavy metal and essential mineral contents. The concentration of Cu, Ni, Zn, Pb, Co, Cd, Fe, and Cr in St. John Wort was 25.4, <0.006, 78.2, <0.015, 2.6, <0.0008, 1020.4 and <0.003 ppm respectively. The Ca and Na in St. John Wort was 192 and 14.84 ppm respectively.

3.4 Nutrient Analysis

3.4.1 Micro-Nutrient in Herbal Products

The amount of Cu, Ni, Zn, Pb, Co, Cd, Fe, and Cr in Garlicare tablets were 12.2, 5.8, 38.2, <0.015, <0.009, <0.0008, 142 and <0.003 ppm respectively. The amount of mineral including Ca and Na in the Garlicare tablets is 64.77 and 7.78 ppm respectively. The quantity of Cu, Ni, Zn, Pb, Co, Cd, Fe, and Cr in Ginger Capsules were 19, 9.4, 52.6, 13.6, <0.009, 0.0008, 226.8 and <0.003 ppm respectively. The quantity of Na and Ca in the Ginger capsule are 5.88 and 74.62 ppm respectively. The concentration of Cu, Ni, Zn, Pb, Co, Cd, Fe, and Cr in Valerian Capsule were 58, <0.006, 55, <0.015, 9.8, 0.2, 1681.8, and <0.003 ppm respectively.

In all four samples the chromium (Cr) concentration was very low, while the Cd concentration is also low except in valerian capsule with value of 0.2 ppm. Cadmium concentration of 0.2 ppm is also not in the limits set by WHO because the daily intake of 0.06–0.07 mg/day is permissible (FAO, 1993). The level of lead (Pb) is also below the permissible level in St. John wort capsule, garlicare and valerian tablets but higher in the ginger tablets with recorded value of 13.6 ppm. This is exceptionally high because the WHO acceptable daily intake of Pb

for adults was 0.21-0.25 mg/day (FAO, 1993). It is well reported in the literature that Pb has pronounced ill effects on the central nervous system especially in children (FAO, 1993).

The range of Ni obtained in this study was lower than 0.05–5 mg/kg reported for plant foods by the National Academy of Sciences in case St. John wort and valerian tablet where it is <0.006; in case of

Table 2. Proximate Analytical Data of the Selected Herbal formulations (in percentage)

Product Name	Moisture	Crude Fat	Carbohydrate	Fibre	Protein	Ash	Energy Value
St. John Wort	4.11±0.09	4.98±0.01	79.37±0.09	14.33±0.24	6.66±0.04	4.89±0.05	389.30±0.68
GarliCare Tablet	2.75±0.12	3.53±0.23	70.24±0.17	1.07±0.03	4.34±0.09	19.15±0.18	330.10±2.2
Ginger Capsule	7.26±0.09	3.60±0.13	76.41±0.25	4.24±0.12	8.60±1.0	4.13±0.06	372.40±0.84
Valerian Capsule	7.14±0.05	4.00±0.03	69.46±0.13	18.60±0.22	5.14±0.02	14.27±0.15	334.39±0.76

Table 3. Proximate Analytical Data of the Raw material used in the selected Medicines

Species Name	Moisture	Fats	Carbohydrate	Fibre	Proteins	Ash	Energy Value
H. perforatum	8.31±0.06	5.06±0.08	72.2±0.09	13.0±0.00	9.54±0.16	4.54±0.014	374.09±0.06
A. sativum	67.66±0.18	2.43±0.11	14.98±0.06	2.43±0.07	13.2±0.04	1.73±0.16	134.60±0.65
Z. officinalis	9.21±0.07	7.30±0.07	72.36±0.04	16.36±0.03	7.27±0.07	4.83±0.07	380.3±0.06
V. officinalis	6.82±0.09	4.14±0.09	67.52±0.07	16.78±0.09	4.39±0.09	17.10±0.08	324.95±0.52

Table 4. Nutrient concentration (ppm) of the preparations

Product Name	Cu	Ni	Zn	Pb	Co	Cd	Fe	Cr
St. John Wort Capsules	25.4	<0.006	78.2	<0.015	2.6	<0.0008	1020.4	<0.003
GarliCare Tablet	12.2	5.8	38.2	<0.015	<0.009	<0.0008	142	<0.003
Ginger Tablet	19	9.4	52.6	13.6	<0.009	<0.0008	226.8	<0.003
Valerian Tablet	58	<0.006	55	<0.015	9.8	0.2	1681.8	<0.003

garlicare it not very high but the value in terms of ginger tablets is very alarming of 9.4 ppm (WHO, 1998; and Pizzaro et al., 1999). Globally the dietary intake of copper (Cu) in healthy non occupational exposed population vary between 0.9-2.2 mg/day (Pizzaro et al., 1999).

Table 5. Mineral contents (ppm) of the Preparation

Specie Name	Cu	Ni	Zn	Pb	Co	Cd	Fe	Cr
H. perforatum	25.4	<0.006	78.2	<0.015	2.6	<0.0008	1020.4	<0.003
A. sativum	14.4	<0.006	52.8	<0.015	<0.009	1.4	165.8	<0.003
Z. officinalis	36.8	<0.006	54.4	5.6	2	<0.0008	518.2	<0.003
V. officinalis	57.4	48.4	177.2	13.6	19	<0.0008	26690	<0.003

The level of copper in all formulations is very high than the acceptable range set by WHO of 2-5 mg intake per day (WHO, 1998; Cantilli, et al., 1994). It has been reported that Cu consumption in excess of 3 ppm of drinking water result in nausea and other adverse effects on the gastrointestinal tract (GIT) (Pizzaro, et al., 1999). The levels of Zn found in the formulations are also not in line with the WHO values of 2-5 mg intake per day. There is no documented evidence of adverse health effects from the intake of Zn normally found in various diets consumed world wide. But chronic zinc ingestion i.e. 300mg/day for six weeks cause suppression of the immune system and decrease in high density lipoproteins (Cantilli et al., 1994).

3.4.2 Macro-Nutrients in Herbal Drugs

The mineral contents comprising sodium and Calcium found in the valerian capsule was 8.942 and 67.1ppm respectively. The recommended daily intake level of iron (Fe) outlined by WHO is about 10-30 mg/day (Cantilli et al., 1994). Keeping in view the recommended level, all of the samples shows high iron contents but comparatively in garlicare and ginger tablets is low, although not very low to be recommended for consumption. The acute chronic dose of iron in infants has been estimated to approximately 20mg/kg and the lethal dose of about 200-300 mg/kg body weight. The chronic iron overloads result in hepatomegaly, cardiac disease and liver chirrosis (Weber, 1988). The level of Ca lay down by WHO is 450-1200 mg/day, which in agreement to the one found in formulations as indicated in the Table 5.

Table 6. Micro Nutrients in Medicinal Plants (ppm)

Product Name	Ca	Na
St. John Wort Capsule	192	14.84
GarliCare Tablet	64.77	7.78
Ginger Tablet	74.62	5.88
Valerian Tablet	67.1	8.942

The highest concentration of micronutrients e.g Cu, Ni, Zn, Pb, Co and Fe were found in *V. officinalis* followed by *H. perforatum* as shown in the Table. 6.

Table 7. Macro Nutrients in medicinal plants (ppm)

Specie Name	Ca	Na
H. perforatum	192	14.84
A. sativum	40.31	8.44
Z. officinalis	11.96	9.33
V. officinalis	176.9	16.32

The Ca concentration in *H. perforatum* was found the highest among all the medicinal plants having 192 ppm concentration followed by *V. officinalis*, *A. sativum*, and *Z. officinalis* has the concentration of 176.9, 40.31 and 11.96 ppm (Table 7). In case of Na, *V. officinalis* has the highest concentration of 16.32 ppm followed by *H. perforatum*, which has a concentration of 14.84 ppm (Table 7).

The findings of the proximate contents of most of the species analyzed in present study were almost complying with the previous reports (Odhav et al., 2007; and Odebunmi et al., 2009). The difference in the analysis might be attributed to the conditions on which the plant species is harvested along with environmental parameters (Nordeide et al., 1996).

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Effect of stratification on seed germination and seedling performance of wild pomegranate

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Abstract: The germination response of *Punica granatum* seeds to different stratification periods was studied. The germination of *P. granatum* significantly improved with increasing stratification periods. Stratification for 30 days at 5^0C showed highest germination percentage, longest radicle, maximum root and shoot length, number of leaves and highest survival of seedlings. The longest plumule, maximum collar diameter, highest shoot and root dry weight were recorded with 25 days of stratification. Thus the results of present investigation clearly reveal that 25 to 30 days stratification of *P. granatum* seeds was more suitable for uniform and faster germination as well as best growth in early stage of seedlings as compared to the control. [Journal of American Science 2010;6(5):97-99]. (ISSN: 1545-1003).

Keywords: wild pomegranate, Stratification, germination, survival per cent, radical and plumule length

1. Introduction

Wild pomegranate (*Punica granatum*), a large deciduous shrub is member of punicaceae growing wild in the sub tropical tracts of north western India, in the foot hills up to 1800 m in the western Himalaya. Pomegranate is a good source of carbohydrates, minerals and moderate source of pectin. The commercial source of *Anardana* is said to be the wild trees of pomegranate (Pujari, 1983). *Anardana* is used as an acidulant in curies, chutneys and *pakora*. Thus, it can play an important role in pushing up the economy of rural poor farmers, where the cultivation of other fruit crops is arduous or less profitable. Regular harvesting of fruits from forests for anardana with negligible planting makes the condition critical for its existence. Research on the germination and growth of selected edible species would provide a basis for their inclusion in afforestation/agroforestry programs and also contribute circa *situ* conservation for certain threatened species (Hebert and Jack, 1998).

Seedling rootstocks are still the most commonly used as they provides deep root system resulted in better outputs. It is therefore necessary to define the physical requirements of seed germination of this species. So this study was under taken to examine the effect of various stratification periods on germination of seeds and seedling performance which facilitates its supply from nursery.

2. Material and Methods

Ripe fruits of *Punica granatum* were collected in the month of August from its natural habitats. After collection, seeds were depulped by washing with water, dried in the shade and stored in polythene bags till the experiment starts. The imbibed seeds were placed between layers of moist peat moss and exposed to chilling temperature (5^0C) in refrigerators for 5 days interval upto 30 days. Germination was carried out under laboratory conditions. Three replicates of 20 seeds for each treatment were allowed to germinate in 9 cm petri dishes on 2-layer filter paper (Whatman No. 1), kept moistened with distilled water. The untreated seeds were used as control. The germination count was made daily for 21 days after sowing and emergence of radical was adopted as a criterion of germination. The plumule and radical length were measured. After 21 days seedlings were planted in previously filled perforated white polythene bags of 2 kg capacity with soil mixture (1:2:1). Survival percentage, shoot and root length, and collar diameter were measured after three month in field conditions. Shoot and root dry weights of the seedlings were estimated after drying at 103 $\pm$ 3^0C as per ISTA (1999) rules.

3. Results and Discussion

Increased time of stratification resulted in higher total seed germination (Table 1). Seeds of *P. granatum* showed best germination (91.66%) when stratification period was 30 days as compared to the control and other treatments. Total seed germination did not differ significantly between 30 days and that of 25 & 20 days but with 10 and 15 days stratification had significantly the lowest germination. For successful germination a population of a cool

climate needed a long stratification (Seneca and Cooper, 1971). Longest radicle (3.96 cm) and maximum survival (83.33%) were also found with 30 days of stratification. Results of the present investigation clearly indicated that the germination per cent of *P. granatum* significantly increased with the increased duration of stratification (Table 1). The maximum germination with longest stratification period is attributed to the reduction of germination inhibitors present in seeds during stratification. It is also showed by the data of mean germination time and germination index (Fig. 1).

Table 1. Effect of different stratification periods on germination and physiological parameters of seedling in wild pomegranate in under laboratory condition

Stratification periods	At the end of test under laboratory condition			
	G %	P L (cm)	R L (cm)	SVI
10 days	51.66^{bc}	1.28^{b}	2.71^{b}	139.99^{cd}
15 days	56.66^{bc}	1.74^{ab}	2.19^{b}	124.08^{cd}
20 days	77.5^{ab}	2.27a	3.09^{ab}	239.47^{bc}
25 days	81.66^{a}	**2.31^{a}**	3.79^{a}	309.49^{ab}
30 days	**91.66^{b}**	2.18^{a}	**3.96^{a}**	**362.97^{a}**
Control	39.99^{b}	2.11^{a}	2.60^{b}	103.97^{d}
SEM ±	**5.94**	**0.26**	**0.38**	**44.8**
CD at 5%	**18.72**	**0.83**	**1.21**	**117.80**

Means followed by same letter within each column are not significant (P<0.05)
Highest value of a parameter in bold

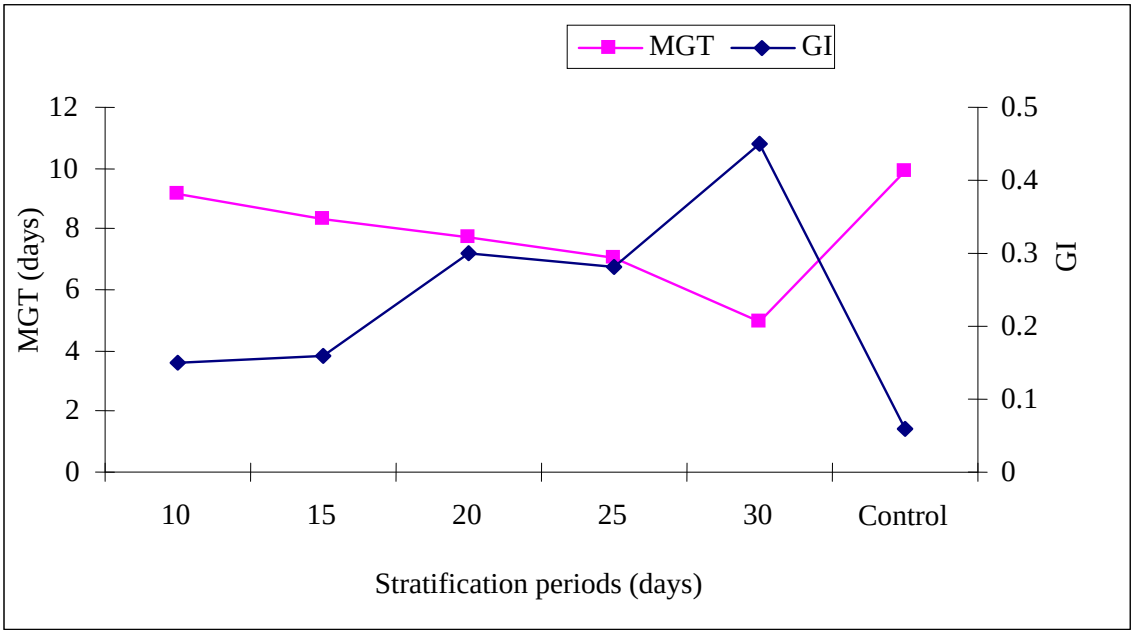

Fig. 1. Effect of stratification periods on MGT and GI of wild pomegranate seeds

The mean germination time was also decreased with increasing stratification and the germination index was increased with increasing stratification periods. Early germination may results into longest radicle, which helps in early establishment of new seedling to produce maximum food material with the help of photosynthesis that resulted into the maximum survival of seedlings. The results are in close in conformity with the results of Bose and Mitra (1991) in apricot and Bhatt *et al.* (2000) in *Myrica esculenta*. Stratification for 25 days gave the maximum plumule length (2.31cm), followed by 20 and 30 days of stratification. The maximum shoot length (8.49 cm), root length (35.14 cm) and number of leaves (32.21) were also produced by 30 days of stratification. The maximum shoot dry weight (0.24 g), root dry weight (0.25 g) and collar diameter (0.24 cm) were produced by 25 days of stratification (Table 2).

Table 2. Effect of different stratification periods on germination and physiological parameters of seedling in wild pomegranate in under field condition

Stratification periods	After three months in the						
	Survival %	S L (cm)	R L (cm)	NOL	SDW (g)	RDW (g)	CD (cm)
10 days	68.33[a]	5.54[c]	26.23[bc]	18.93[b]	0.12[d]	0.11[d]	0.20[b]
15 days	68.33[a]	5.68[c]	27.14[b]	20.00[b]	0.08[e]	0.05[e]	0.20[b]
20 days	79.99[ab]	5.86[bc]	29.07[b]	26.23[ab]	0.15[c]	0.14[c]	0.20[b]
25 days	81.66[a]	6.82[b]	31.97[ab]	27.36[a]	**0.24[a]**	**0.25[a]**	**0.24[a]**
30 days	**83.33[a]**	**8.49[a]**	**35.14[a]**	**32.21[a]**	0.20[b]	0.21[b]	**0.24[a]**
Control	69.99[a]	5.04[c]	22.26[c]	18.33[b]	0.11[d]	0.09	0.13[c]
SEM ±	**7.90**	**0.33**	**1.91**	**2.63**	**0.01**	**0.01**	**0.06**
CD at 5%	**24.91**	**1.03**	**6.02**	**8.31**	**0.03**	**0.03**	**0.03**

Means followed by same letter within each column are not significant (P<0.05)
Highest value of a parameter in bold

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Application of Different Levels of Zinc and Boron on Concentration and Uptake of Zinc and Boron in the Corn Grain

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Abstract: For the purpose of studying the effect of Zn and B application on the concentration and total uptake of Zn and B in corn grain, a field experiment was conducted in Fars Province, Iran. Treatments including five levels of Zn (0, 15, 30 and 45 kg ha^{-1} and Zn foliar spray) and four levels of B (0, 4, and 8 kg ha^{-1} and B foliar spray) in a completely randomized block design were set up. The findings showed that the presence of Zn prevented from the increase in Zn concentration in the grain, by B application; while B applied in the presence of Zn had no effect on the amount of Zn uptake by the grain. At the level that lacked B, Zn use increased Zn concentration and uptake in the grain but at the levels where B was used, the presence of B prevented from the effect of Zn application on the Zn concentration and uptake in the grain. The minimum concentration and uptake of Zn in the grain was observed by lack of Zn and B use or the control treatment. Therefore, an antagonism between Zn and B was observed as regards concentration and uptake of Zn in the grain. At the highest Zn level, the B use caused an increase in concentration and uptake of B in the grain. Also, at the high B level, application of Zn caused an increase in the B concentration in the grain. Boron use at low levels and Zn solution spray, had no effect on the uptake of B in the grain, but at high B levels, it increased the B uptake in the grain. Therefore, the presence of a high amount of Zn or B in the soil, assisted in the effect of B or Zn on increasing concentration and uptake of B in the plant. That is, a synergism was seen between the Zn and B as effecting the concentration and uptake of B in the grain. [Journal of American Science 2010;6(5):100-106]. (ISSN: 1545-1003).

Keywords: Interaction, Zinc, Boron, Concentration and Corngrain

1. Introduction

Zinc is involved in a large number of enzymes as a cofactor. For example, it is involved in activation of different enzymes such as dihydrogenase, aldolase, isomerase, transphosphorase and DNA polymerase (Marschner, 1995). In spite of the fact that the total amount of Zn in the soil is relatively high, but a small fraction of it is available to the plant. Numerous factors affect Zn availability, including the soil calcium carbonate content, which reduces the Zn availability in the soil (Dutta et al., 1989; Mandal et al., 1992). Among the micronutrients, Zn deficiency is perhaps most extensive in the world. Zinc deficiency is most common in low- and high pH soils, low- and high organic matter, sandy, sodic, calcareous soils and waterlogged without ventilation (Takkar and Walker, 1993). Corn is among the plants most sensitive to Zn deficiency. The amount of Zn uptake from the soil is 130 gr for each metric ton of corn grain (Tandon, 1995). If the harvest of the corn grain is 9.5 metric tons per ha, the Zn uptake by the plant would be 10 kg ha^{-1} (Marschner, 1995). Boron plays a very important role in vital functions of the plant, including meristem tissue cell division, petal and leaf bud formation, vascular tissue repair, sugar and hydrocarbon metabolism and their transfer, RNA and indoleacetic acid metabolism, membrane stability, cytokinin production and transfer, pollen budding and seed formation (Shelp, 1993; Marschner, 1995). Boron and Zn deficiency upset the order of grains on the corns and make them deformed so that some parts of the corn ear are free from grains (Marschner, 1995). Boron deficiency is common in sandy and highly calcareous rich soils since there is an interaction between the Ca ion and the available B and high Ca levels at high pH reduces B uptake (Marschner, 1995).

A negative Zn-B interaction has been seen in the plant (Harsharn et al., 1998). Singh et al. (1990) reported that B application increased its concentration and uptake in wheat and the same increased when Zn was not used. Moreover, when B was not used, application of Zn reduced B concentration and uptake in the plant. Adding B to the soil caused a low effect by Zn application on B concentration and uptake. A large number of authors have reported that using Zn increased its concentration in the plant (Gupta and Singh, 1985; Darajeh et al, 1991; Karimian and Yasrebi, 1995). Mozafar (1987), by studying the effects of 12 nutrients on the corn graining, observed that concentrations of Zn, B, Cu, Mn, Ca, Mn, K, P, N and Na in corn ear and concentrations of elements Zn, B, Fe, Cu, Mn, Mo, Ca and P in the unripe corn ear stem were similar and no significant difference was seen between them. Massey et al. (1996) while studying the Zn concentration in the grain observed

that the least and the highest Zn concentration in the grain were 15.5 and 38.4 mg kg^{-1}, respectively. The increase of B concentration in the plant using B is reported by some authors (Grant and Baily, 1990; Wei et al., 1996; Hu and Brown, 1997).

Considering numerous cases of corn use in human, animal and poultry nutrition and extraction of about 500 different products, from it, we see that the grain being rich in Zn and B elements plays an effective role in human health. Also, richness of the grain in these elements reflects quantitative and qualitative increase in the grain harvest. Therefore, by studying Zn-B interaction in the grain, we can enrich the grain while understanding its indirect effects which are quantitative and qualitative improvement in the grain harvest. Moreover, it is an established fact that if grains rich in these elements are used as seeds, the harvest will improve quantitatively and qualitatively.

2. Materials and Methods

A field experiment was conducted at the farms of Firuz Abad, in Fars province, Iran, on the corn (*Zea mays L.*), cultivar "Single Cross 401" during 2008 cropping season. This experiment included 20 treatments and 3 replications in the form of completely randomized block design and factorial that combinations of five levels Zn (0, 15, 30 and 45 kg ha^{-1} Zn, and Zn foliar spray) and four levels of B (0, 4, and 8 kg ha^{-1} and B, and B foliar spray). Nitrogen: P: K used at 400, 160 and 160 kg ha^{-1} according to the recommendation, from sources of urea, triple super phosphate and potassium sulfate, respectively, were added to all treatments (plots). Moreover, 50% of the urea was used when planting and the remainder two times: At vegetative growth (35 days after planting) and when the corn ears were formed. Potassium and P used before planting. Zinc and B, from zinc sulfate and boric acid sources, respectively, were used by two methods, adding to the soil and spraying. Addition to the soil was made at the time of plantation and the sprayings were made at 5 per thousand (0.5%) Zn sulfate and 3 per thousand (0.3%) B two times: one at vegetative growth stage and the other after corn ears formation. The Zn and B were both applied to the leaves with uniform coverage at a volume solution of 2500 L/ha using a knapsack sprayer. Each experimental plot was 8m length and 3m width, had 5 beds and 4 rows, equally spaced, and seeds 20cm apart on the rows. At the end of the growth stage (4.5 months after planting) the grain yield, dry matter and the residual available phosphorus and Zn in the soil after corn harvest were measured.

Analysis of the grain and the soil was carried out using common lab procedures (Soil and Plant Analysis Council, 2004). Phosphorous was measured by Olsen method, available potassium by Ammonium Acetate extraction method and potassium assessment in the extract by flame photometer, organic carbon by the Walkley and Black method. Available Fe, Zn, Mn and Cu in the soil were first extracted by DTPA and then were read by atomic absorption (Shimatzu Model AA-670). The soil's available B was extracted by hot water and then was measured by spectrophotometer by curcamin method, considering the intensity of the color produced. Digestion method by dry burning was used to measure Zn and then was measured by atomic absorption setup. Statistical analysis of data was made using MSTATC and SAS software with Duncan test and regression equations via the SPSS program.

3. Result and Discussion

The results of soil analysis before plantation are presented in table 1. The soil P and K content in the soil is lower than the critical level suggested in scientific sources (Rashid et al., 1994; Karimian and Yasrebi, 1995). Karimian and Ghanbari (1990) have reported the critical P level by the Olsen method in calcareous soils as 18 mg kg^{-1}. The soil Zn content was lower than the critical level but the

B content was at medium level down to deficiency. The soil Mn, Cu and Fe content was above the critical level. Sims and Johnson (1991) have reported the critical levels of Fe, Zn, Mn and Cu by the DTPA extraction method and B by the hot water in the soil method to be 2.5-5, 0.2-2, 1-5, 0.1-2.5 and 0.1-2 mg kg^{-1}, respectively. Agrawala (1992), reports the critical levels of Fe, Zn, Mn and Cu in soil with DTPA extractor as 2.5, 0.8, 5.5 and 0.78 mg kg^{-1} soil, respectively.

Table 1.The result of soil analysis

Depth of soil (cm)	Soil texture	pH	EC (ds m^{-1})	Organic matter (%)	T.N.V (%)	P	K	Fe	Mn	Zn	Cu	B
									mg kg^{1}			
0-30	Loam	8.1	2.33	0.62	31	11.6	234	1.8	7.76	0.28	0.5	0.81

3.1. The grain Zn content

The main effect of Zn on the grain Zn content (mg kg^{-1}) was insignificant at the 5% level (table 2). Boron application in spray form led into a significant reduction in grain Zn content from 28.47 to 24.27 mg kg^{-1} (14.75 percent reduction as compared with zero B level). However, adding B to the soil had no significant effect on the grain Zn content. The highest and the lowest mean grain Zn content at 28.47 and 24.27 mg kg^{-1} levels were seen at zero B level and B spraying level. Therefore, a negative interaction was seen between B application in spray form and grain Zn content.

In analysis of Zn and B interaction affecting the grain Zn contents it was seen that B application at 30 kg ha^{-1} Zn reduced grain Zn content but at other Zn levels, it had no significant effect on Zn content. Application of 4 kg ha^{-1} and spraying B at 30 kg ha^{-1} Zn reduced grain Zn content from 35.67 to 24.67 and 23.76 mg kg^{-1}, respectively (30.83 and 33.64 percent reduction) but application of 8 kg ha^{-1} B had no significant effect. At zero B level, only the use of 30 kg ha^{-1} Zn significantly increased grain Zn content from 20.67 to 35.67 mg kg^{-1} (72.56% increase). But at high levels (4 and 8 kg ha^{-1} B) and B spraying, Zn application had no effect on grain Zn content. In fact, B use as added to soil or sprayed, prevented from application of B to affect grain Zn content. The least grain Zn content at 2.67 mg kg^{-1}, was observed due to nonuse of Zn and B (the control treatment). The highest grain Zn content, at 35.67 mg kg^{-1} was obtained by applying 30 kg ha^{-1} Zn, showing an increase of 72.56% as compared with the control. Except the treatment with the highest grain Zn content, the treatments showed no significant difference from the control.

Increase in corn grain Zn content by Zn application has been reported by different authors (Victor et al., 1990; Grewal et al., 1997; Sugreeve et al., 1998; Singh and Verma, 1999). According to Massey and Loeffel (1996), grain Zn content was at least 15.5 and at most 38.4 mg kg^{-1}. In this study, the grain Zn content in the control treatment was 20.67 mg kg^{-1}, which is higher than the minimum suggested.

Table 2. The effect of Zn and B on Zn concentration in the grain (mg kg^{-1})

B (kg ha^{-1})	Zn (kg ha-1)					
	0	15	30	45	Foliar Spray	Mean
0	20.67 b	30 ab	35.67 a	28 ab	28 ab	28.47 a
4	24.67 b	27.67 ab	24.67 b	28.67 ab	26.67 ab	26.57 ab
8	23.33 b	25.67 ab	27.33 ab	26 ab	27 ab	25.87 ab
Foliar Spray	25 b	23 b	23.67 b	22.33 b	27.33 ab	24.27 b
Mean	23.42 a	26.58 a	27.83 a	26.25 a	27.25 a	

3.2. Zinc uptake by the grain

The effect of application of different B levels on Zn uptake by the grain (gr ha^{-1}), was insignificant at 5% level, but the main effect on Zn uptake was significant at 1% level (table 3). The least mean Zn uptake by the grain, 174.77 gr ha^{-1}, was seen at zero Zn level and Zn application at all levels (to the soil and spraying), significantly increased Zn uptake by the grain. Application of 15, 30 and 45 kg ha^{-1} Zn, increased Zn uptake by the grain from 174.77 gr ha^{-1} at no Zn level to 233.73, 246.36 and 230.12 gr ha^{-1}, respectively (33.73, 40.96 and 31.67 percent increase, in that order). But there was no significant difference between levels of Zn added to the soil. Zinc spraying significantly increased Zn uptake by the grain to 238.47 gr ha^{-1} (36.44 percent increase as compared with the no Zn level), while there was no significant difference from that of Zn being added to the soil. The effect of Zn-B interaction on the Zn uptake by the grain showed that B application in any Zn level had no significant effect on Zn uptake by the grain. Zinc use at no B level increased Zn uptake by the grain, but at other levels of B it had no significant effect on the Zn uptake. Application of Zn at 15 and 30 kg ha^{-1} Zn levels at no B level, significantly increased Zn uptake by the grain from 139.4 gr ha^{-1}, to 258.9 and 269.73 gr ha^{-1}, respectively (85.72 and 93.49 percent increase relative to no Zn use at the level of B). Zinc spraying at no B level, increased Zn uptake by the grain from 39.4 to 247.8 gr ha^{-1} (77.76% increase), but it had no significant difference from when Zn was applied to the soil. Probably the antagonism between Zn and B, applying B to the soil or spraying, prevented from an increase in Zn uptake by the grain, by the Zn application. The least and the most Zn uptake by the grain, 139.4 and 269.73 (93.49% increase relative to the control) (gr ha^{-1}), were observed in the control treatment (no Zn and B use) and 30 kg ha^{-1} Zn, respectively.

Table 3. The effect of Zn and B on Zn uptake by the grain (gr ha^{-1})

B (kg ha^{-1})	Zn (kg ha-1)					
	0	15	30	45	Foliar Spray	Mean
0	139.4 c	30 ab	258.9 ab	216.1 abc	247.8 ab	226.39 a
4	202.63 abc	254.23 ab	222.33 abc	263.83 ab	234.63 abc	235.53 a
8	168.47 bc	222.27 abc	263.2 ab	211.17 abc	244.1 ab	221.84 a
Foliar Spray	188.57 abc	199.5 abc	230.17 abc	229.37 abc	227.33 abc	214.99 a
Mean	174.77 B	233.73 a	246.36 a	230.12 a	238.47 a	

3.3. The grain B content

The main effect of Zn on Grain B content (mg kg^{-1}) was not significant at 5% level, but application of different levels of B was significant at 5% level (table 4). The least and the most mean grain B content, 7.94 and 10.49 mg kg^{-1}, were observed at no B level and 8 kg ha^{-1} B. The use of 4 and 8 kg ha^{-1} B, significantly increased B concentration in the grain from 7.94 mg kg^{-1} at no B level, to 9.38 and 40.49 mg kg^{-1}, respectively (33.8 and 32.11 percent increase, in that order), but there was no significant difference between these two B levels. Boron spraying showed no significant difference with no B level and adding B to the soil. Examination of the Zn-B interaction showed that at high Zn level (45 kg ha^{-1} Zn), only the increase of 4 kg ha^{-1} in B increased the grain B content from 6.04 to 12.12 mg kg^{-1} (100.66% increase). However, at other Zn levels, B use showed no significant effect on grain B content. Zinc application at 30 kg ha^{-1} Zn at a high B level (8 kg ha^{-1} B), increased grain B content from 9.13 to 13.93 mg kg^{-1} (52.57% increase), while other levels of Zn had no significant effect. At low levels (zero and 4 kg ha^{-1} B) and B spraying, Zn use had no significant effect on the grain B content. Except for the treatment with the highest grain B content, all treatments showed no significant difference from the control. Joint use of 30 kg ha^{-1} Zn and 8 kg ha^{-1} B, made the maximum grain content: 13.93 mg kg^{-1}, a 68.44% increase relative to the control 8.27 mg kg^{-1}. Increase in wheat grain boron content by application of B has been reported by Singh et al. (1990), Singh and Singh (1980) and Shen et al (1998).

Table 4. The effect of Zn and B on B concentration in the grain (mg kg^{-1})

B (kg ha^{-1})	Zn (kg ha-1)					
	0	15	30	45	Foliar Spray	Mean
0	8.27 bcd	8.88 bcd	9.43 abcd	6.04 d	7.1 cd	7.94 b
4	8.53 bcd	9.13 bcd	8.4 bcd	12.12 ab	10.93 abc	9.83 a
8	9.13 bcd	10.54 abcd	13.93 a	9.33 abcd	9.5 abcd	10.49 a
Foliar Spray	9.37 abcd	9.07 bcd	9 bcd	9.83 abcd	9.77 abcd	9.41 ab
Mean	8.83 a	9.41 a	10.19 a	9.33 a	9.33 a	

3.4. Boron uptake by the grain

Among different levels of Zn, application of 30 kg ha^{-1} Zn significantly increase B uptake by the grain (gr ha^{-1}) relative to no Zn level (positive Zn-B interaction), while other levels of Zn had no significant effect on B uptake (table 5). The least mean B uptake by the grain, 65.47 gr ha^{-1} was seen at no Zn level. The highest mean B uptake by the grain, 92.37 gr ha^{-1}, was seen at 30 kg ha^{-1} Zn, a 40.72% increase as compared with no Zn level.

The main effect of B on B uptake by the grain was significant at 5% level. The lowest and the highest mean B uptake by the grain, 63.13 and 90.49 gr ha^{-1}, were seen at no B level and 8 kg ha^{-1} B. Boron application at all levels, significantly increased B uptake by the grain relative to the no B level. The use of 4 and 8 kg ha^{-1} B, significantly

increased B uptake by the grain from 63.13 at no B level, to 86.95 and 90.49 gr ha^{-1}, respectively (37.73 and 43.34 percent increase) but there was no significant difference between those two B levels. Boron spraying significantly increased B uptake by the grain, to 83.53 gr ha^{-1}, showing a 32.31 percent increase relative to the no B level, but showed no significant difference from when B was applied to the soil.

Table 5. The effect of Zn and B on B uptake by the grain (gr ha^{-1})

B (kg ha^{-1})	Zn (kg ha-1)					
	0	15	30	45	Foliar Spray	Mean
0	55.87 cd	263 bcd	71.67 bcd	46.7 d	65.1 bcd	63.13 b
4	70 bcd	82.13 bcd	75.23 bcd	112.5 ab	94.87 abcd	86.95 a
8	65.9 bcd	91.53 abcd	134.67 a	76.1 bcd	84.27 bcd	90.49 a
Foliar Spray	70.8 bcd	76.77 bcd	78.9 bcd	100.97 abc	81.23 bcd	83.53 a
Mean	65.64 b	81.86 ab	92.37 a	84.07 ab	81.37 ab	

The effect of Zn-B interaction on B uptake by the grain showed B application at low levels (zero and 15 kg ha^{-1} Zn), and Zn spraying had no effect on B uptake by the grain, while at high Zn levels (30 and 45 kg ha^{-1} Zn), increased B uptake by the grain. At 30 kg ha^{-1} Zn, only application of 8 kg ha^{-1} B increased B uptake by the grain from 71.67 to 34.67 gr ha^{-1} (87.9% increase). Application of 4 kg ha^{-1} and spraying of B at high Zn level (45 kg ha^{-1} sulfate) increased B uptake by the grain from 46.7 to 112.5 and 100.97 gr ha^{-1}, respectively (140.9 and 116.2% increase), while using 8 kg ha^{-1} B had no significant effect. At 8 kg ha^{-1} B, only the use of 30 kg ha^{-1} Zn significantly increased B uptake by the grain from 65.9 to 134.67 gr ha^{-1} (104.35% increase). But at other B levels, Zn application had no significant effect on B uptake by the grain. No treatment, except the treatment with the highest B uptake by the grain, showed a significant difference from the control. The highest B uptake by the grain, 134.67 gr ha^{-1}, was obtained by the joint use of 8 kg ha^{-1} B and 30 kg ha^{-1} Zn, a 141% increase relative to the control, with an uptake of 55.87 gr ha^{-1}.

Singh et al. (1990) observed that by B application, its concentration and uptake in the grain increased, and that increase was higher when a Zn deficiency prevailed. With increasing the B level, Zn application decreased B concentration and total B uptake.

3.5. Concentration and uptake of Zn and B in grain with other variables

Concentration and uptake and other variables, correlation coefficients (R) and (R^2) between different variables were computed using the Pearson method and equations relating to each variable were derived using the step-by-step method. The symbols * and ** in equations denote significance at 5 percent level ($\alpha = 0.05$) and 1 percent level ($\alpha = 0.01$) respectively.

3.6. The grain Zn content

The grain Zn content showed a positive correlation with leaf P content (R= 0.5*), Mn content (R= 0.57*), and Zn content (R= 0.42), grain Mn content (R= 0.49*), grain's Mn (R= 0.35), and Zn (R= 0.76*) uptake and the number of grain along the corn ear (R= 0.35) and a negative correlation with leaf B content (R= -0.4), % of grains in the ear (R= -0.33) and the dry matter (R= -0.36). The equation of which was: ZnG = 27.624 + 0.182 ZnUG - 0.00323 TGY - 0.0223 FeG + 0.141 ZnS + 0.0769 PS + 0.0000985 DM $R^2 = 0.998$*

ZnG, ZnUG, TGY, FeS, ZnS, PS and DM denote grain Zn content (mg kg^{-1}), Zn uptake by the grain (gr ha^{-1}), total grain harvest (kg ha^{-1}), grain Fe content (mg kg^{-1}), soil Fe content after harvest (mg kg^{-1}), soil Zn content after harvest (mg kg^{-1}), soil P content after harvest (mg kg^{-1}) and dry matter (mg ha^{-1}).

3.7. The Zn uptake by the grain

There was a positive correlation between Zn uptake by the grain and the leaf P content (R= 0.57**), Zn content (R= 0.56**), the grain Mn content (R= 0.71**), Zn content (R= 0.76**)and B content (R= 0.73), the grain's uptake of N (R= 0.56**), P (R= 0.56**), K (R= 0.63**), Fe (R= 0.35), Mn (R= 0.76**), Cu (R= 0.39) and B (R= 0.54*), ear's weight (R= 0.36), grain weight in the ear (R= 0.32), total grain harvest (R= 0.63**), number of grains along the ear (R= 0.45*), number of grains across the ear diameter (R= 0.5*) and a negative correlation with leaf B content (R= -0.4) and the ear diameter (R= -0.3). The relevant equation was ZnUG = -214.814 + 8.155 ZnG + 0.0255 TGY +

0.0197 FeUG - 1.14 ZnS -0.66PS + 0.000781 DM R^2 = 0.999*.

3.8. The grain B content

The grain B content showed a positive correlation with the grain N content (R= 0.43), P content (R= 0.58**) and Mn content (R= 0.61**), the grains' uptake of N (R= 0.53*), P (R= 0.6**), K (R= 0.47*), Fe (R= 0.45*), Mn (R= 0.66**), Zn (R= 0.37) and B (R= 0.92**), ear weight (R= 0.48*), grain weight in the ear (R= 0.48*), total grain harvest (R= 0.41), the number of grains in the ear length (R= 0.42), the number of grains across the ear diameter (R= 0.32) and grain protein content (R= 0.43) and a negative correlation with leaf N content (R= -0.41), Mn content (R= -0.43), and Cu content (R= -0.38).

BG = 0.114 + 0.11 BUG - 0.00101 TGY R^2 = 0.989**

BG, BUG and TGY are grain B content (mg kg^{-1}), B uptake by the grain (gr ha^{-1}) and total grain harvest (kg ha^{-1}), respectively.

3.9. The B uptake by the grain

There was a positive correlation between B uptake by the grain and the grain N content (R= 0.42), P content (R= 0.58*), Mn content (R= 0.66**) and B content (R= 0.92**), the grain N uptake (R= 0.76**), P uptake (R= 0.82**), K uptake (R= 0.68**), Fe uptake (R= 0.49*), Mn uptake (R= 0.8**) and Zn uptake (R= 0.54*), ear weight (R= 0.64**), grain weight in the ear (R= 0.65**), total grain harvest (R= 0.72**), the number of grain along the ear (R= 0.62**), the number of grains across the ear diameter (R= 0.43), and the grain protein content (R= 0.42) and a negative correlation with the leaf Mn content (R= -0.45*) and Cu content (R= -0.4).

BUG = -0.82.261 + 8.843 BG + 0.00924 TGY R^2 = 0.994**

4. Conclusion

In conclusion, Zn application had no effect on the grain Zn and B contents, but application of Zn to the soil as well as Zn spraying increases Zn uptake by the grain. Boron spraying decreased the grain Zn content but B application to the soil had no effect on it. Boron application had no effect on the Zn uptake by the grain. Boron application to the soil increased grain B content, but B spraying had no effect on it. Also, B application to the soil and its spraying increased B uptake by the grain.

The presence of Zn prevents from grain Zn content increase by B application so that B use at 30 kg/ ha Zn, reduced grain Zn content. Also, application of B in the Zn presence had no effect on Zn uptake by the grain. At no B level, Zn application increased grain Zn content and uptake while at B use levels, the presence of B prevented from the effect of Zn application on the grain Zn content and uptake. The least grain Zn content and uptake was seen by no Zn and B application or in the control treatment. Therefore, an antagonism existed between Zn and B affecting grain Zn content and uptake.

At high Zn level (45 kg ha^{-1} Zn), B use increased grain B content; also at high B (8 kg ha^{-1} B) level, Zn application increased grain B content. Such an effect was seen for B uptake by the grain as well. Application of B at low levels (zero and 15 kg ha^{-1} Zn) and at Zn spraying level, had no effect on the B uptake by the grain but at high Zn levels (30 and 45 kg /ha Zn) it increased B uptake by the grain. Zinc use at high B level, increased B uptake by the grain but had no effect on it at other B levels. Therefore, a high soil B or Zn content, helped with the B or Zn effect on the increase in plant B content and uptake. That is, a synergism existed between B and Zn affecting grain B content and uptake. The Zn uptake by the grain was positively related with the leaf P and Zn contents, and negatively related with the leaf B content. The grain B content had a positive correlation with the grain N, P and Mn content, total grain harvest and grain protein content while it had a negative correlation with the leaf N, Mn and Cu content. Boron uptake by the grain was positively related with grain N, P, Mn and B contents and N, K, phosphorus, iron, Mn and Zn uptake by the grain, total grain harvest and grain protein and negatively related to the leaf Mn and Cu contents.

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Air quality depreciation index in a coal mining area- a case study from eastern India

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Abstract: The comparison with National Ambient Air Quality Standards does not always depict a true picture of the Air Quality Status of a study area. As an alternative an index that measures depreciation in Air Quality on more realistic terms has been proposed and applied to the ambient air monitoring data collected from Talcher Coalfields in India. Results have been discussed in detail to illustrate the application of the proposed index and utility in bringing out more realistic air quality assessment [Journal of American Science 2010;6(5):107-114]. (ISSN: 1545-1003).

Key words: National Ambient Air Quality Standards, value function curves, air quality depreciation index

1.0 Introduction

Coal mining and coal based thermal generation activities result in serious pollution problems due to release of particulates and noxious gases in the atmosphere. Over the past few years, with the introduction of mechanized mining techniques and heavy earth moving equipments, this problem has been further aggravated (Singh and Sharma, 1991; Sharma and Singh, 1992). Air quality assessment in Korba Coalfield also revealed an unsatisfactory air quality there. The concentration of respirable particulate matter (RPM) was found to be at an alarming level there (Singh and Puri, 2004). In opencast mines all the major mining activities directly or indirectly contribute to air pollution. Sharma & Singh (1990) found that unloading and loading, transportation of coal, poor condition of roads and huge quantities of open air coal burning were responsible for air pollution in coal mines.

Mining operations share a number of common stages or activities each of which has potentially adverse impacts on the natural environment. The health and safety status of the occupational and the communities in the environs of the mine may also be affected as a result of mining. Like other industries, social and cultural conditions of the concerned environment may accordingly be modified. In absence of appropriate control measures mining operations may lead to environmental disturbances. The impact of a mining operation commences with exploration activities, extends through extraction and processing of minerals and may continue even after closure of the operation. One of the important environmental impact of mining is the degradation in the air quality.

The monitoring and evaluation of ambient air quality is first important step in controlling air pollution. Current approaches to the evaluation of air quality in India are based entirely on the comparison of measured concentration of pollutants with National Ambient Air Quality Standards (NAAQS). A comparison of data with NAAQS serves the purpose to some extent, but this cannot map the periodical degradation in the air quality, particularly if the measured values remain below NAAQS. A number of air quality indices have been formulated (Babcock, 1970 and Ricci, 1979). Most of the indices take NAAQS standards as the base for devising the scale. There are other systems, which are independent of the NAAQS and based on the measurement of air quality (with due weightage to the potential of pollutants to affect biophysical, health and aesthetic attributes) on an absolutely environmental quality scale and not in relation to NAAQS.

Although the use of this approach to some extent helps to maintain a 'desired' environmental quality, it does a little to 'map' periodic degradation in air quality, particularly if the measured values remain below NAAQS. The reason behind this drawback arises from the fact that by providing an upper threshold concentration value in the form of a standard, air quality tends to get categorized either as 'good' or 'bad' depending on whether the standards have been exceeded on not. In reality, however, there are instances where concentration of pollutants become sufficiently high to pose environmental and

health problems, but owing to the fact that may not falsely interpreted to represent 'acceptable' air quality.

Viewed in this backdrop, the present paper attempts to propose an air quality depreciation index that measures deterioration in air quality (with due weightage to the potential capacity of pollutants to affect bio-physical, health and aesthetic attributes) on an 'absolute' environmental quality scale independent of NAAQS.

1.1 Air Quality Depreciation Index

The air quality depreciation index, as proposed here, attempts to measure deterioration in air quality on an arbitrary scale that ranges between 0 and –10. An index value of '0'represents most desirable air quality having no depreciation from the best possible air quality with respect to the pollutants under consideration while an index value of –10 represents maximum depreciation or worst air quality. Index values differing from 0 towards –10 represent successive depreciation in air quality from the most desirable. The air quality depreciation index is defined as follows:

$$AQ_{dep} = \sum_{i=1}^{n}(AQ_i \times CW_i) - \sum_{i=1}^{n} CW_i \quad \ldots(1)$$

where,

AQ_i = Air quality index value for i^{th} parameter

CW_i = Composite weight for i^{th} parameter

n = Total no. of pollutants considered

The values of the AQ_i are obtained from the value function curves. In the value function curves the value of 0 signifies worst air quality and value of 1 represents the best air quality for corresponding pollutant concentration. Typical value function curves for SPM, SO_2, NO_x and (TSP x SO_2) are given in Figures 1, 2, 3 and 4, respectively.

Value of CW_i in equation (1) is computed using the following expression:

$$CWi = \frac{TW_i}{\sum_{i=1}^{n} TW_i} \times 10 \quad \ldots\ldots\ldots\ldots(2)$$

where,

TW_i = Total weight of i^{th} parameter

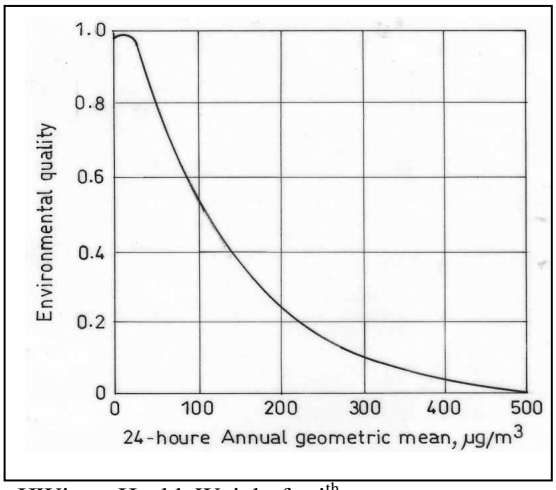
$$= AW_i + BPIW_i + HW_i$$

where,

AWi = Aesthetic weight for i^{th} parameter

BPIWi = Bio- Physical Impact Weight for i^{th} parameter

be high enough to cross the threshold value, they are



HWi = Health Weight for i^{th} parameter

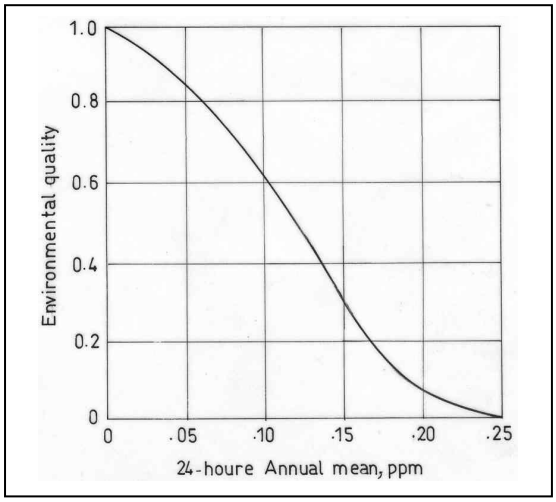


Figure 1. Value function curve for suspended particulate matter (Jain et al. 1977)

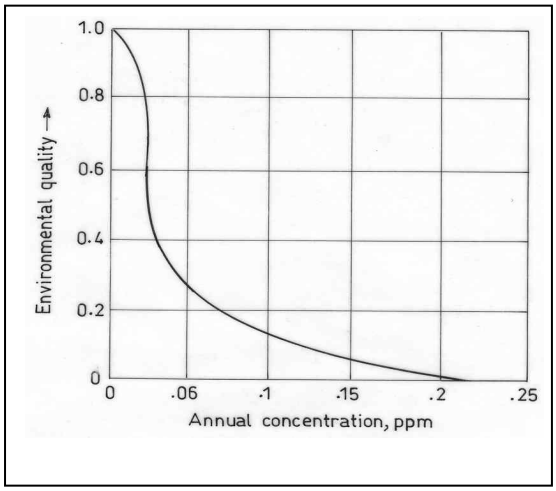
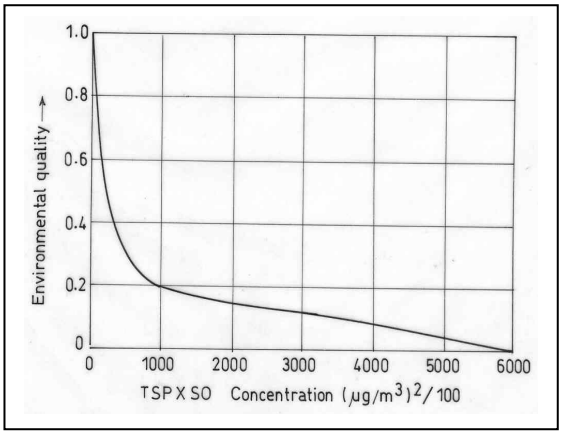


Figure 2. Value function curve for sulphur dioxide (Jain et al., 1977)
Figure 3. Value function curve for nitrogen oxides



(Jain et al., 1977)

Figure 4. Value function curve for TSP × SO_2 (Luhar & Khanna, 1988)

In computing TW_i, an importance weight between 1 to 5 is subjectively assigned to AWi, BPIWi and HWi (i.e. for the i^{th} pollutant) by a team of assessors or experts. Least important assignment is 1 and most important marking is 5. The weights are then aggregated in accordance with equations (2) and (3).

2.0 Materials and methods

The air quality depreciation index was applied to the set of data obtained from monitoring ambient air quality of some coal mining areas of Talcher coal field (Fig. 5) of MCL (Orissa). The Talcher Coalfields constitutes mostly the south-eastern part of the Lower Gondwana Mahanadi Master Basin and occupies an area of over 1813 sq km. The coalfield is bounded by latitudes 20°50' N and 21°15' N and longitudes 84°09' E and 85°33' E. This basin mainly occupies the Brahmani River Valley. It covers parts of Dhenkanal and Angu1 districts along with a small portion of the adjoining Sambalpur District of Orissa. The main sources of air pollutants located in this region are Talcher Thermal Power Station (TTPS) and the extensive mining industries of MCL and other allied industries.

Systematic air quality monitoring was carried out at twenty one sampling stations (Fig. 6) in the study area using Respirable Dust Samplers (Envirotech-make Model APM 460) with thermoelectrically cooled impinger attachment for gaseous sampling. 24-hourly ambient air samples were collected for SPM, PM_{10}, SO_2 and NO_X. The impinger samples (containing SO_2, NO_X in specific absorbing solutions) were analyzed spectrophotometrically using Scanning Visible Spectrophotometer (VIS-7200). Improved West-Gaeke method and Jacob & Hocheiser modified methods were used for analysis of SO_2 and NO_X, respectively as per standard methods prescribed by Central Pollution Control Board (CPCB, July 2003), India.

3.0 Results and discussions

Assignment and computation of Composite Weight for different pollutants is given in Table 1. Air Quality monitoring results for Talcher mining Belt is summarised in Table 2. Values for AQ_i and AQ_{dep} calculated as per equation (1) are given in Table 3. For MCL Coalfield the air quality depreciation values are depicted in Table 3.

Comparison with NAAQS cannot forcefully categorize air quality as 'objectionable' or 'unacceptable'. Till standards are exceeded, there is no indication of deterioration in air quality from what can be considered 'truly acceptable air quality'. Results of air quality monitoring in the study areas show that the concentration levels of SPM and PM_{10} exceeds the NAAQS while concentration levels of NOx and SO_2 are found to be below the NAAQS at most of the sampling stations of both the study area. So the overall results do not provide a clear picture about the Ambient Air Quality status of the study areas. If all the pollutants exceeded the NAAQS then the air quality of the station could be referred to as 'objectionable' or 'unacceptable'. But this situation does not prevail in the study areas. Application of the air quality depreciation index to the observed data, however, clearly 'maps' this deterioration in the quality of air around these mining sites. Depreciation in air quality from the desired value of 0 is clearly apparent, as AQ_{dep} values at all the locations of the study area are less than -1.0.

The deterioration in the Air Quality in the coal mining areas of Talcher Coalfields can be undoubtedly visualised in the Table 3. Depreciation in air quality from the most desired value of '0' is clearly apparent, as AQ_{dep} values at all the locations are less than -1.0. After assigning rank to the resulted depreciation values it was found that Ananta OCP (A3) had the highest depreciation value (-4.59) followed by Jagannath OCP (A4), Lingraj OCP(A1) and Bhubaneswari mines (A2) with depreciation values -4.50, -3.21 and -3.20, respectively. Among residential and other areas Dera chowk (A15) had higher depreciation value (-3.10) followed by Jagannathpur village (A13) with depreciation value -2.85.

This pointed out that mining premise was the most polluted area followed by traffic junction. The results also suggest that the Raghunathpur Village has the best air quality followed by TTPS Guest House Colony.

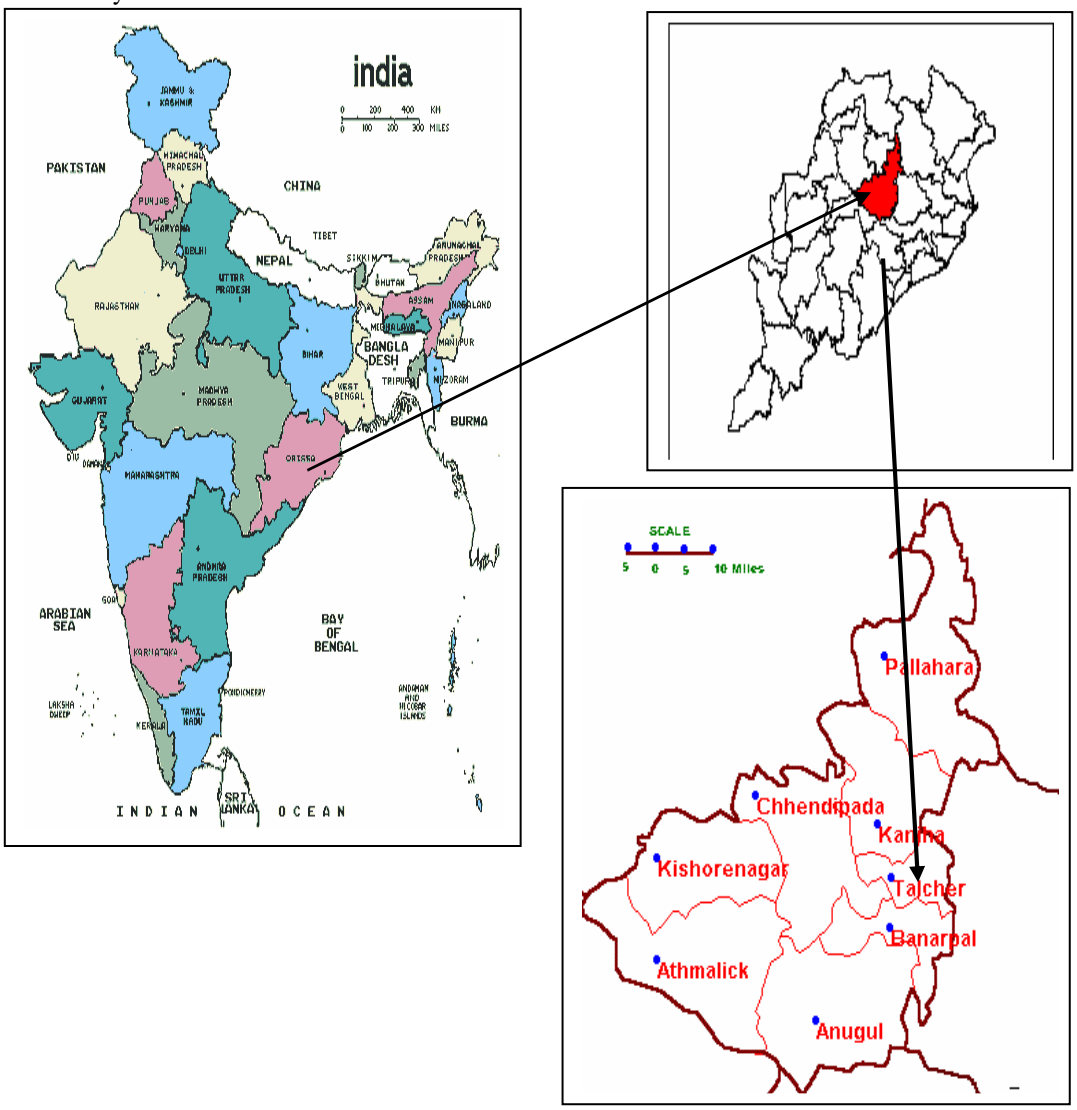
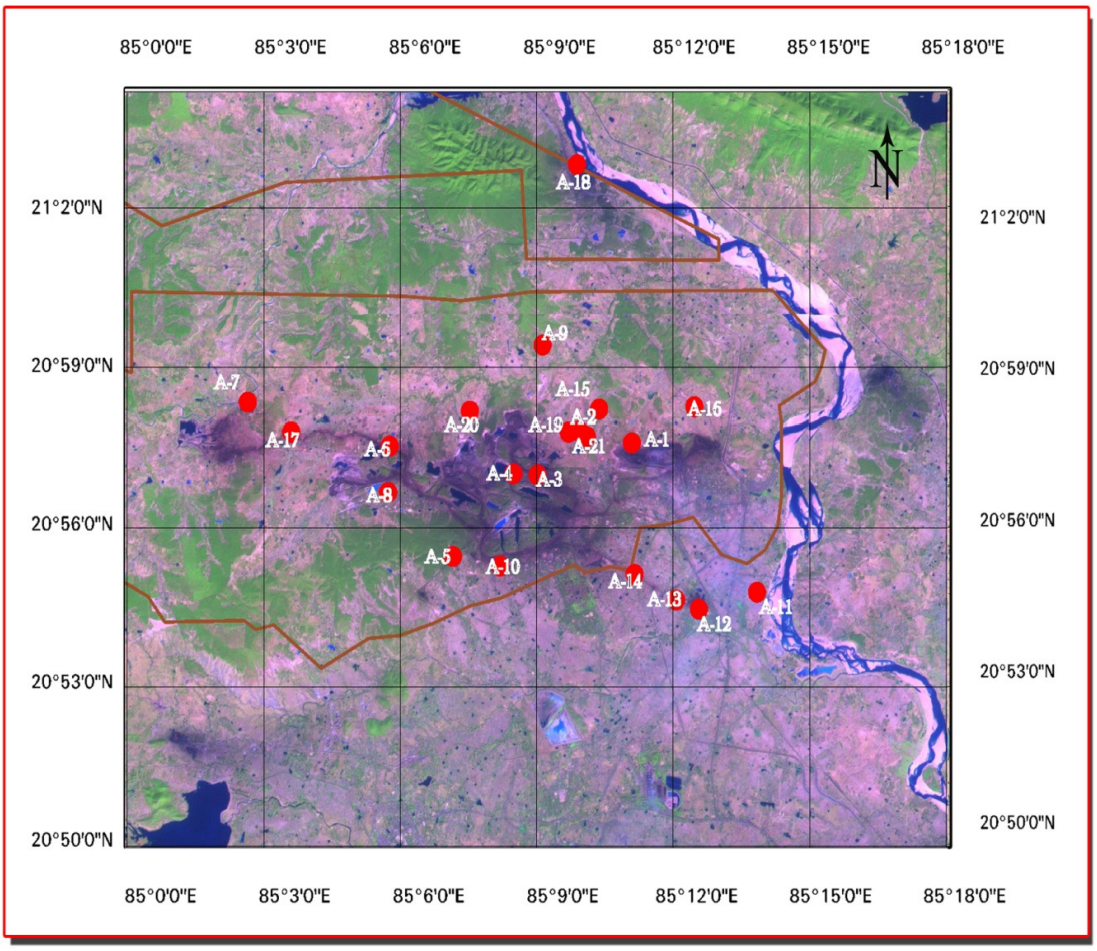


Figure 5. Location map of the study area.

Table 1. Assignments and computation of composite weight for different pollutants

Pollutants	AWi (Range 1-5)	BPIWi (Range 1-5)	HWi (Range 1-5)	TW_i	CW_i
SPM	4	4	3	11	3.1
SO_2	1	4	4	9	2.5
NO_x	2	3	3	8	2.2
SPM X SO_2	1	2	5	8	2.2
			$\sum_{i=1}^{n} TW_i = 36$		



A1-Lingraj OCP[a]	A2- Bhubaneswari Mines	A3-Ananta OCP	A4-Jagannath OCP
A5-Bharatpur Colony	A6-kalinga Township	A7-Gopal Prasad village	A8-Donnara village
A9-Raghunathpur village	A10-Barasinghra village	A11-Talcher guest house	A12-TTPS Residential area
A13-Jagannathpur village	A14-Sharma chowk	A15-Dera chowk	A16-Talbera village
A17-Kalamchui village	A18-Ekgharia village	A19-Ananta Guest House	A20-Rakash village
A21-Mukundnali village	[a]Open cast project		

Air sampling stations

Coalfield boundary

Scale

Kilometers

Figure 6. Air quality sampling stations

Table 2. Ambient air quality monitoring results of MCL coal mines of Talcher coalfield

Locations	Code	SPM[1]	TSP[2]	SO_2	NOx	SPM[3]	TSP*SO_2/ 100
Lingraj OCP	A1	278.92	422.87	27.04	34.75	274.11	1.16
Bhubaneswari Mines	A2	233.58	324.87	28.29	35.71	227.93	0.93
Ananta OCP	A3	500.71	773.79	29.33	31.83	490.73	2.31
Jagannath OCP	A4	440.79	639.29	31.0	36.0	434.29	2.03
Bharatpur Colony	A5	190.83	280.71	25.5	30.92	188.39	0.73
Kalinga village	A6	196.67	286.29	25.96	28.25	193.80	0.76
Gopalprasad village	A7	121.37	181	23.58	27.12	119.85	0.43
Utkal village	A8	118.62	176.21	21.21	23.46	117.21	0.38
Donnara	A9	177.25	268.13	19.91	26.62	166.86	0.55
Raghunathpur village	A10	109.42	165.08	24.33	27.79	105.20	0.41
Barasingra village	A11	193.92	286	22.45	31.75	192.97	0.65
TTPS Guest House	A12	117.58	178.96	25.37	27.79	116.08	0.45
TTPS residential	A13	129.37	195.46	26.95	29.37	125.27	0.53
Jagannathpur village	A14	224.75	330.12	25.83	31.71	212.01	0.86
Sharma Chawk	A15	152.46	237.29	28.08	31.17	144.56	0.67
Dera Chawk	A16	287.83	437.58	37.58	38.08	280.92	1.68
Talbera village	A17	126.75	183.67	20.66	24.83	126.00	0.38
Kalamchui village	A18	141.12	206.37	23.63	25.92	136.35	0.48
Ekghari village	A19	149.5	212.83	23.54	26.42	144.9	0.50
Ananta Guest House	A20	148.46	213.12	26.75	28.37	140.74	0.57
Rakash village	A21	153.29	221.25	25.33	25.17	139.95	0.60
Mukundnali village	A22	120.21	170.62	24.66	29.25	117.16	0.42
[4]NAAQS		500	800	120	120		

1 - Arithmetic Mean Value of annual air quality monitoring results
2 - Total Suspended Particulate (Sum of preceding two columns)
3 - Geometric Mean Value of annual air quality monitoring results
4 National Ambient Air Quality Standards.

Table 3. Value functions and AQ_{dep} values for different sampling locations of Talcher coalfield

	SPM	SO_2	NOx	TSP×SO_2	Weighted AQi	AQ_{dep}	Rank
A1	0.15	0.98	0.9	0.699	6.787	-3.21	3
A2	0.19	0.981	0.899	0.56	6.977	-3.02	5
A3	0.01	0.98	0.93	1.386	5.407	-4.59	1
A4	0.04	0.972	0.898	1.22	5.498	-4.50	2
A5	0.23	0.992	0.91	0.436	7.175	-2.83	7
A6	0.24	0.99	0.921	0.456	7.397	-2.60	9
A7	0.47	0.998	0.948	0.259	8.106	-1.89	16
A8	0.29	0.99	0.932	0.332	7.448	-2.55	10
A9	0.52	0.99	0.95	0.249	8.223	-1.78	19
A10	0.26	0.992	0.928	0.391	7.308	-2.70	8
A11	0.5	0.999	0.945	0.273	8.063	-1.94	15
A12	0.44	0.98	0.933	0.319	7.789	-2.21	11
A13	0.24	0.99	0.918	0.518	7.153	-2.85	6
A14	0.38	0.998	0.941	0.406	7.957	-2.04	13
A15	0.14	0.991	0.928	1.01	6.905	-3.10	4
A16	0.44	0.99	0.938	0.228	8.339	-1.66	21
A17	0.42	0.99	0.93	0.286	8.241	-1.76	20
A18	0.38	0.992	0.927	0.3	8.124	-1.88	17
A19	0.4	0.99	0.93	0.342	7.975	-2.03	14
A20	0.4	0.992	0.939	0.362	7.941	-2.06	12
A21	0.48	0.99	0.921	0.25	8.189	-1.81	18

The air quality of Raghunathpur Village may be considered as the background status of Air Quality Depreciation as this location is free from any major air pollution sources.

While comparing with the Air Quality Depreciation Index data of other mining sites reported in other studies, it was found that index calculated was quite high as compared to other mining areas. In a study, Jharia was found to have the most degraded air quality status having index value upto -7.8 followed by Korba coalfield with a index value upto -5.94 (Singh, 2006). The highest index was calculated for the Raniganj coalfield with the index upto -3.31. So it can be inferred that the Talcher coal mine areas has a better air quality as compared to other mining areas.

4.0 Conclusions

The application of the proposed Air Quality Depreciation Index has shown that the index allows for more realistic air quality assessment as compared to interpretive evaluations that revolve around comparing observed concentrations to national ambient air quality standards. The Air Quality Depreciation index can be an invaluable tool to map periodic deterioration in air quality with respect to its potential for environmental damages. We believe that adoption of such an index to monitor air quality at all the mining locations in India will help mutual comparisons in a much more realistic and meaningful manner. This work is just a step in this direction. Since the air quality depreciation index is neither geographically specific nor constrained for the type or number of pollutants, it can be easily used for different situations and applications.

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Enhancing the rate of ferulic acid bioconversion utilizing glucose as carbon source

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Abstract: Work has been carried out to study the effect of glucose addition into the medium during the biotransformation of ferulic acid into vanillin using *Staphylococcus aureus*. Study showed that microorganism consumed ferulic acid very quickly having more than 4-fold accumulation of vanillin (45.7 mg/l) on 2nd day as compared to 9.8 mg/ml of vanillin accumulation on 7th day without addition of glucose. [Journal of American Science 2010; 6(5):115-117]. (ISSN: 1545-1003).

Key words: biotransformation, ferulic acid, *Staphylococcus aureus,* glucose, vanillin

1. Introduction

Hydroxycinnamic acids such as ferulic acid and *p*-coumaric acid occur widely in the cell walls of graminaceous plants (Grabber et al 1995, Harris and Hartley 1980). Ferulic acid is a very important component for the structure and the biology of cell wall as it can cross link polysaccharide chains through dimerisation reaction as described by Ishii (1997). Microbes transform hydroxycinnamic acids to their corresponding hydroxybenzoates upon the production of ferulic acid esterase (FAE) enzyme. FAE has the greater industrial importance like lipases having microbial origin (Jooyandeh et al 2009). These benzoates are important components of natural flavours and fragrances. Like the antioxidant activities of some plants extracts as described by Jayaprakasha et al (2008), hydroxycinnamates can also act as precursors for a variety of antioxidant compounds, signaling molecules and phytoalexins that play an important role in plant defense responses (Dixon et al 1995). A number of industrial and food applications were reported for ferulic acid, especially based on its microbial degradation to vanillin. Vanillin is the world's most highly prized natural flavour. It is one of the most important aromatic flavour compounds used in foods, beverages, perfumes and pharmaceuticals (Clark 1990)**.** Thus, considering the increasing interest in 'natural' products, the production of flavours via biotechnological processes offers a viable alternative to natural and chemical sources (Walton et al 2003). This work reports the capability of *Staphylococcus aureus* to degrade ferulic acid into vanillin. Effect of carbon sources on the production of metabolites was a study of interest (Vijayendra et al 2008). In this case study the effect of supplementation of glucose into the medium was analyzed during the biotransformation of ferulic acid into vanillin.

2. Materials and Methods

Microorganism:

Staphylococcus aureus was isolated from soil on the basis of its ability to grow in ferulic acid containing medium. Pure cultures of these strains were maintained on a mixed medium containing both beef extract and peptone.

Medium and Culture conditions:

After growth on a mixed broth medium containing both beef extract and peptone for 5 days, 1 ml cell suspension was transferred into the 100 ml flask each containing 25 ml of minimal medium (Muheim and Lerch, 1999) along with ferulic acid as a sole carbon source. The pH of the media was adjusted to 7.2. The cultures were incubated at 35^0C and analyses were carried out on day-to-day basis upto 8 days of incubation to detect the degradation product of ferulic acid. Each experiment was carried out in triplicate.

Extraction and detection of metabolites from the culture media:

For the extraction of ferulic acid and its degradation product from the culture media, culture supernatants were prepared by centrifugation. These were acidified (pH 1-2) and extracted with equal volume of ethyl acetate. The ethyl acetate was evaporated in vacuum and residue was re-dissolved in 50% methanol. This processed culture filtrate was subjected to HPLC. Quantification of ferulic acid and its degradation products were performed at 254 nm and 310 nm. (Ghosh *et al* 2005, Sachan *et al* 2004). Compound in sample was identified by comparison with authentic standard.

Supplementation of glucose in addition to ferulic acid: In order to make high density culture of *Staphylococcus aureus***,** microorganism was allowed to grow in minimal media supplemented with glucose (0.1% w/v) as a sole carbon sources. When glucose was completely consumed by the

microorganism, ferulic acid (5.0 mM) was added into minimal medium.

3. Results and Discussion

Day basis analyses were performed to detect the product of ferulic acid bioconversion by *Staphylococcus aureus*. Vanillin was detected as the major degradation product (9.8 mg/l) after 6 days of incubation period in absence of glucose (Table 1 & Figure 1). Amount of vanillin was enhanced (45.7 mg/L) only on 48 hours of incubation period when glucose was added into the medium (Fig.2). Quantification of ferulic acid and its degradation product such as vanillin and was analyzed through HPLC. The use of additional carbon source helped in the formation of high density cultures (Oddou et al 1999) which helps in the formation of product in a shorter period of incubation period. Microorganism was pregrown on minimal media containing glucose as a carbon source. Ferulic acid utilized more rapidly when microorganism was pregrown on minimal media supplemented with glucose. Maximum accumulation of vanillin (45.7 mg/L) was observed in 48 hours of incubation period. Hence, use of the high density culture of this *Staphylococcus aureus* resulted more than four fold enhancement of vanillin formation in a shorter incubation period (48 hrs).

Table 1. Vanillin formation from ferulic acid by *Staphylococcus aureus* on a time-course basis (Without glucose).

Period of Incubation (days)	Amount of Ferulic acid (mg/l)	Amount of vanillin (mg/l)
0	520	0
1	375	0
2	165	1.2
3	18	3.4
4	11	5.7
5	5	6.9
6	1.2	8.1
7	0.65	9.8
8	0.22	5.2

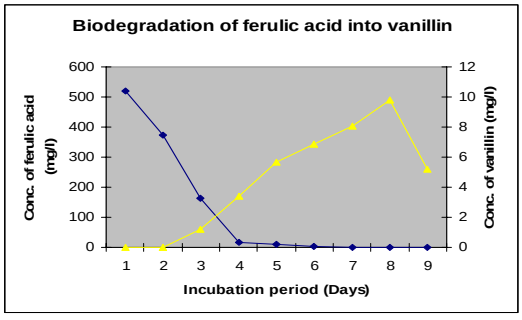

Figure 1.Time course degradation of ferulic acid and subsequent formation of vanillin by *Staphylococcus aureus* (Without glucose)

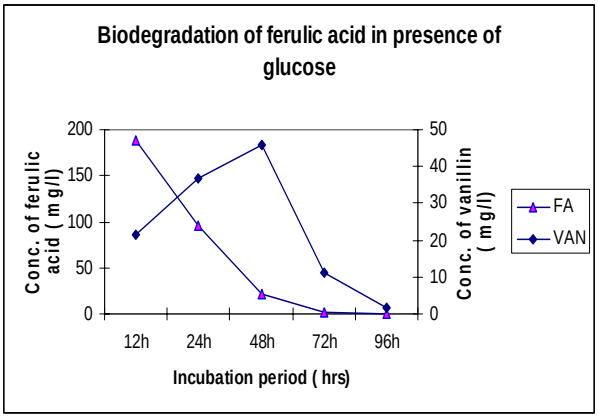

Figure 2. Time course degradation of ferulic acid and detection of vanillin in the culture media of *Staphylococcus aureus***.** (presence of glucose).

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Investigation Of The Influence Of Systematic Errors In Least Squares Estimation

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Abstract: The least squares method is widely accepted as a computational method, that covers different branches of Surveying and Photogrammetry.Basically, it is applied when the observations contain random errors only. This paper is directed towards the investigation of the effects of systematic errors on the least squares estimates. The main conclusions are: (1) The use of observations containing systematic errors beside the random ones, gives different values for the parameters and the residuals. (2)The value of the standard error of unit weight will increase in the presence of systematic errors.(3)Modeling of systematic errors will enable the evaluation of systematic errors and their effects. [Journal of American Science 2010; 6(5):118-123]. (ISSN: 1545-1003).

Keywords: systematic errors, dimensional adjustment, parameters, residuals.

1. Introduction

The main Surveyor's task is to design the survey systems; plan for field operations, carry out the measurements; and thereafter adjust and analyze his results (Edward, M.andGordon, G., 1981). The analysis of the survey results depends on considering the three types of errors and the cost of the total survey operations (Rainsford, H.F., 1957).In general, any successful survey work has to meet certain requirements. Firstly, the number of observations must be sufficiently enough for the purpose; redundant observations are recommended for computational reasons. Secondly, these observations must have a good quality as far as possible. In addition, the minimum cost of operations and processing is preferable (Cross, 1983).

One of the factors that directly affect the above requirements is the presence of systematic errors. Therefore they have a considerable effect on the analysis of survey networks (Cooper, M.A.R., 1947 and Cross, P.A., 1983). However, with the traditional methods of computation, the existence of errors will cause a misclosure in the final results. This misclosure must be examined to decide whether it is due to systematic, gross or random effects. If it is a systematic effect, it will be compensated for, either graphically or analytically (Wolf, 1985).Recently, the method of least squares appear as an effective computational method, that replaces the traditional methods, because of it's computational advantages.

The observation equation method of least squares is the most easiest to apply. The application of the observation equations method of least squares gives estimated parameters and residuals that statistically possess certain properties, provided that the observation contains only random errors. (Cross, 1983). However, the case with those observations which contains systematic errors is the aim of this paper. It is directed towards:

- The investigation of the effect of systematic errors on the estimated parameters, residuals and any other related quantities.
- Modeling of systematic effects in the least squares model.
- Comparison between systematic and gross errors.

To satisfy these objectives, a network consisting of six points (Figure 1.1) is established (as in Cooper, M.A.R., 1987) and different types of observations were taken by means of a theodolite and an E.D.M. (Tables (1.1),(1.2),(1.3),(1.4),(1.5),(1.6),(1.7) and (1.8)).

Methodology

In connection with any survey project many tasks must be performed, from planning stage, up to the final presentation. These tasks are based on the so called observations or "measurements". Before any observations can take place certain preparations are

necessary. For example centering, levelling, pointing, matching, setting and reading (Allan, A.L.Hollwy, J.R.and Maynes, J.H.B., 1968). The end product is a single numerical value which represents the measurement of a certain quantity. Any measurement taken by the surveyor, by means of a particular instrument, in a certain physical or environmental conditions is subject to variation, due to the above mentioned factors. This variation is known as the ***error*** in the measured value. According to the behavior of the observational errors, they have been classically classified to:

1- Gross errors.
2- Systematic errors, and
3- Random errors.

In this paper we used observations containing systematic errors beside the random ones, to investigate the effect of these errors on the estimated parameters, residuals and any other related quantities. Also we made comparison between systematic and random errors. And finally, we discussed the analysis of the least squares results in terms of precision and reliability.

Precision:

Measures of precision are most conveniently done by the use of the variance covariance matrix because it contains all elements of precision. The construction of the variance covariance matrix depends on the weight matrix, Which is equal to the inverse of the variance covariance matrix of the observations,

i.e.

$W=c^{-1}_{l}$

Where:

W is the weight matrix

c^{-1}_{l} is the inverse of the variance covariance matrix of the observations.

Reliability:

The word reliable is defined as "consistently good in quality or performance, and so deserving trust". In estimation problems, reliability is meant to be the ability of the system to detect gross error in observation or, as (Cross,P.A.,1983) defined it, a measure of the ease with which gross errors may be detected. The detectable error is generally given the term Marginally Detectable Error (MDE) which, in case of a diagonal weight matrix

Generally, we can differentiate between two aspects of reliability; internal and external.

Internal Reliability:

Internal reliability is the one which considers the size of the gross error (assuming normal distribution and the presence of one gross error). If we consider type one error then the simple test can be carried out as follows:

(i) Specify a level of significance (for type one error).

(ii) Determine *w*-statistic (w_t) from tables (using a two tailed test).

(iii) Compute w_i using the equation

$$w_i = \hat{v}_i / \hat{\sigma}_{vi}$$

(iv) Compare with w_t

If w_t is larger, then no gross error is present.

The test is applied separately to each observation. This is known as data snooping. If we specify the probability of type two error, we can use the equation developed by Barada(1968).

$$\Delta_i^u = \delta_i^u \sigma_i^{\,2} / \sigma_{vi}$$

Where Δ_i^u is the MDE

δ_i^u is the value computed from specified probabilities of type one and two errors.

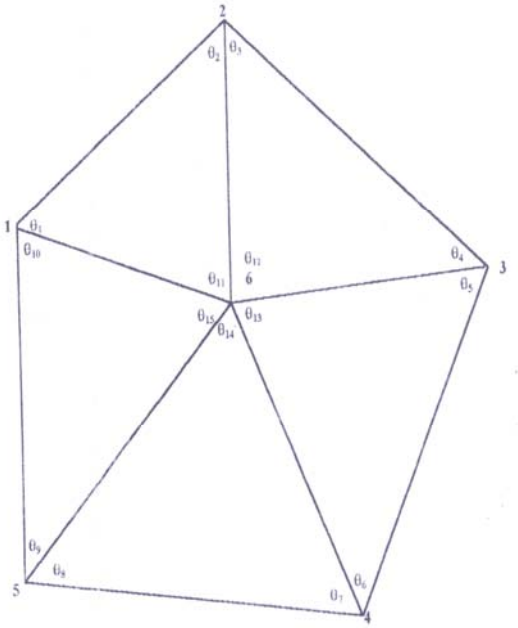

Figure 1. A network consisted of six points

External reliability:

It is the effect of an undetected gross errors on the parameters and on the quantities computed from them, Barada (1968) .In a sense, therefore, external reliability is more important than internal reliability as we don not care too much about the size of an undetected gross error as long as it has no effect on the determined parameters.

After least squares estimation of the parameters, the effect of (MDE) (for each observations) on the parameters is given by:

$$\Delta \hat{x}_i = (A^T wA)^{-1} A^T w \, \Delta b_i$$

Where Δb_i is a null vector except for the ith position which is equal to Δ_i^u

The largest elements of will be considered as the a measure of the effect of an undetected gross error of the size of a marginally detectable error on the estimated parameters.

Consider (q) as a quantity estimated from parameters and to be the effect of (MDE) on the derived quantities. It can be shown (see Cross, P.A., 1983) when W is diagonal would be given by

$$\Delta \hat{q}_i \leq \delta_i^u \gamma_i \hat{\sigma}_{qi}$$

Where:

$\gamma_i = \hat{\sigma}_i / \hat{\sigma}_{v_i}$

$\hat{\sigma}_{qi}$ Is the standard deviation of q

σ_i Is standard error of the ith observation

$\hat{\sigma}_{v_i}$ Is the square root of the ith diagonal element of C_v

for un correlated observations, the following could be established**:**

$\rho_i = \sigma_i / \hat{\sigma v}_i$

$\rho_i^2 = \sigma_i^2 / \hat{\sigma}^2_{vi}$

$\gamma_i^2 = \sigma_i^2 / \sigma^2_{vi} = \sigma_i^2 / \sigma^2_{vi} - 1$

$\therefore \gamma_i^2 = \rho^2_i - 1$

It follows that if an observation has high internal reliability if must also have high external reliability, and conversely low internal reliability reflects low external reliability.

Testing and results

Before testing the effects of the systematic errors, the coordinates of the unknown points (fig(1-1)) were computed Using the observed data in. (Tables (1.1),(1.2),(1.3),(1.4),(1.5),(1.6),(1.7) and (1.8)).

The following approximate values were obtained:

Table 3.1: The coordinates of unknown points in fig (1.1).

Point	X (easting)	Y (northing)	Z
1(*fixed*)	1000.00	1000.00	382.035
2	1918.46	1150.044	382.943
3	2023.341	669.079	384.181
4	1455.81	153.494	383.903
5	982.863	509.239	382.875
6	1580.526	656.280	382.933

One dimensional adjustment

Using the slant distances shown in Table(1.6) and the zenith angles in Table(1.5), ten observations were sued for the one dimensional adjustment (as in Methley, B.D.F., 1986). After applying the above corrections, the adjusted heights of the unknown points together with their standard errors were obtained as in Table(3.2) below:

Table 3.2: The adjusted heights and their standard errors of the unknown points

Point	Z (m)	Standard Error
1	382.035	-
2	382.944	±0.01
3	384.183	±0.03
4	383.902	±0.02
5	382.872	±0.01
6	382.937	±0.01

With a standard error of unit weight:

$\sigma_0^{\wedge} = 1.161$

Two dimensional adjustments

A two dimensional adjustment was carried out from observed horizontal angles (1.1), azimuth (1.5) and horizontal distances (1.3). The least squares estimates of the coordinates were obtained as follows:

Table 3.3: The least squares estimates of the coordinates

Point	X(m)	S.E	Y(m)	S.E
1(*fixed*)	1000.000	-	1000.00	-
2	1718.455	± 0.01	1149.984	± 0.03
3	2023.318	± 0.01	669.000	± 0.04
4	1455.766	± 0.03	153.441	± 0.02
5	982.862	± 0.02	509.241	± 0.01
6	1580.502	± 0.01	656.236	± 0.03

And the standard error of unit weight:

$\sigma_0^{\wedge} = 1.114$

Three dimensional adjustments

Slant distances and zenith angles in Table (1.6), and Table (1.5) were used for the three dimensional adjustment with the following results (assuming points 1 and 2 as fixed stations):

The value of a standard error of unit weight equals to :

$\sigma_0^{\wedge} = 0.721$

Note that the values of adjusted coordinates in Tables (3.2),(3.3)and (3.4)agree.

Table 3.4: The least squares estimates of the coordinates

Point	E(m)	S.E	N(m)	S.E	Z(m)	S.E
1(*fixed*)	1000.000	-	1000.00	-	382.035	-
2(*fixed*)	1718.465	-	1150.044	-	382.943	-
3	2023.348	±. 006	669.083	±. 006	374.183	±.01
4	1455.832	±. 01	153.480	±. 005	383.906	±.02
5	982.899	±.02	509.238	±. 004	382.876	±.01
6	1580.532	±.005	656.286	±. 004	382.934	±.02

Testing of the effects of systematic errors

To test the effect of systematic errors on the estimated parameters, a scale error of (0.996) was introduced on the horizontal observed distances Table (1.7).Thereafter the two dimensional coordinates of different points in the network was estimated. The following results were obtained by using ten of the observations:

The least squares estimates of the coordinates and their standard errors respectively are:

The following table shows the adjusted values of the coordinates of the stations after applying two iterations.

Table 3.5: The adjusted values of the coordinates of the stations

Point	X(m)	S.E	Y(m)	S.E
1(*fixed*)	1000.000	-	1000.00	-
2	1715.601	±0.01	1149.384	±0.03
3	2019.224	±0.01	670.324	±0.04
4	1453.943	±0.03	156.829	±0.02
5	982.931	±0.02	511.204	±0.01
6	1578.180	±0.01	657.611	±0.03

And the standard error of unit weight:

$\sigma_0^{\wedge} = 2.543$

Comparing the values in table (3.3) and table (3.5), it can be easily noticed that, the presence of systematic errors will change the values of the estimated parameters, the estimated residuals and the value of the standard error of unit weight will change as well, which gives the sense as if a gross error exists, while the variance covariance matrix remain the same.

Adjustment with additional parameters

As mentioned above, we can account for the systematic errors in the least squares model, and then

estimate the coordinates of the points. Taking into account the scale error inserted in the observed distances as an unknown parameter (assuming that it's approximate value is one). The following results were obtained.

Table 3.6: The least squares estimates of the coordinates

Point	X(m)	S.E	Y(m)	S.E
1(*fixed*)	1000.000	-	1000.00	-
2(*fixed*)	1718.467	–	1150.040	–
3	2023.379	±0.01	669.071	±0.04
4	1455.846	±0.03	153.435	±0.02
5	982.891	±0.02	509.216	±0.01
6	1580.528	±0.01	656.278	±0.03

With the standard error of unit weight:

$\hat{\sigma_0} = 1.477$

Noting that the same values of corrections are obtained, therefore the identical value of the adjusted coordinates will be obtained and the value of is again maintained. And the estimated value for the scale error can be computed by applying the related correction to it's initial value.i.e:

=1-0.00406=0.996

Which agrees with the introduced value.

Table 1.1: Horizontal Angles

Angle	Observed value		
θ_1	42 °	25 ´	30 ″
θ_2	62	35	44
θ_3	47	49	46
θ_4	59	26	04
θ_5	40	35	55
θ_6	61	40	36
θ_7	39	07	08
θ_8	50	46	19
θ_9	74	10	41
θ_{10}	61	22	14
θ_{11}	74	58	48
θ_{12}	72	44	07
θ_{13}	77	43	30
θ_{14}	90	06	30
θ_{15}	44	27	01

Table 1.2: Magnetic Bearing

From	To	F.Bearing	B. Bearing
1	5	180° 00 ´ 00 ″	02° 00 ´ 00 ″

Table 1.3: Horizontal Distances

From	To	Observed distances(m)
1	2	733.965
2	3	569.453
3	4	766.759
4	5	591.805
1	5	591.060
1	6	674.651
2	6	512.660
3	6	442.996
4	6	518.033
5	6	615.450

Table 1.4: Difference in Height

From	To	Observed difference(m)	Remarks
1	2	0.943	RISE
2	3	1.238	RISE
4	3	0.278	RISE
5	4	0.028	RISE
1	5	0.875	RISE
1	6	0.933	RISE
6	2	0.010	RISE
6	3	1.248	RISE
6	4	0.970	RISE
5	6	0.058	RISE

Table1.5: Zenith Angles

From	To	Observed zenith angle		
1	2	89°	55 ´	34 ″
2	3	89	52	31
4	3	89	58	45
5	4	89	59	50
1	5	89	59	23
1	6	89	55	14
6	2	89	59	51
6	3	89	50	20
6	4	89	53	30
5	6	89	59	45

Table 1.6: Slant Distances

From	To	Observed distances (m)
1	2	733.965
2	3	569.456
3	4	766.762
4	5	591.805
1	5	491.062
1	6	674.652
2	6	512.660
3	6	443.000
4	6	518.038
5	6	615.458

Table 1.7: Horizontal Distances with Scale Error

From	To	Observed distances (m)
1	2	731.029
2	3	567.175
3	4	763.691
4	5	589.437
1	5	489.095
1	6	671.952
2	6	510.609
3	6	441.224
4	6	515.960
5	6	612.988

Table1.8 Standard Errors for Observed Quantities:

Observed quantity	S.E.
Horizontal Distance	±0.003 m
Horizontal Angle	±3″
Bearing	±10″
slant distance	±0.007 m
zenith Angle	±7″

Conclusion

1. The estimated values for heights obtained from a one dimensional estimation and a three dimensional one are identical, on the other hand the estimated values for eastings & northings obtained from the two dimensional and three dimensional adjustments are identical.

2. When a systematic error is introduced to measured distances the following is noticed:

i) The estimated parameters and residuals will take different values from those with no systematic error.

ii) The value of the standard error of unit weight will also increase.

iiii) Application of least squares model containing additional parameters will enable the evaluation of the systematic errors and their effect.

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A Situational Analysis of Waste Management in Freetown, Sierra Leone.

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Abstract: Freetown served as safe haven for thousands of people from the provinces during the war and suffered a corresponding increase in the rate of generation of waste with very little waste management facility as such facilities were vandalized or completely destroyed. Solid waste management in Freetown has been under variable organizations, with each change further deteriorating the system, bringing it on the verge of collapse. Freetown Waste Management Company (FWMC) is struggling to manage the wastes, hence, the need for the intervention of potential investors/donors to ameliorate this waste management problem by helping address this problem sustainably for the betterment of the lives of all Freetown residents. Streams of waste are characterized by their sources, the types of waste produced, and the composition and generation rates; therefore, knowledge of these characteristics is required in order to design and operate appropriate waste management systems, hence, the need for the Sierra Leone Government or FWMC to set limits on certain physical characteristics and properties for waste classifications; having significant implications for the collection and disposal of various waste streams, since any material deemed hazardous must be handled with specific protocols. The total quantities and characteristics of waste streams generated are yet unknown, with uncategorized refuse, poorly collected, dumped at the two city's insanitary landfills, hence exposing FWMC workers, scavengers, etc., to the dangers of hazardous waste. This appalling garbage situation needs efficient corrective measures or serious rehabilitation; otherwise it will adversely impact the living conditions of the people, further endangering their environment and health. [Journal of American Science 2010;6(5):124-135]. (**ISSN**: 1545-1003).

Keywords: Sierra Leone, Hazardous waste, health care waste, landfills, Freetown Waste Management Company.

I. Introduction

Management of waste in Freetown poses costly and annoying problems (including low service coverage – averaging 40%, insufficient budgets, highly inadequate equipment, substantial inefficiencies such as high costs, low quality service, low labour productivity, poor public attitudes, and widespread illegal dumping). With respect to waste management, a direct relationship exists between a city's population size and both the percentage of waste removed and rate of household enjoying regular waste collection. If solid wastes are not managed properly, they can pose many environmental and human health risks for many Freetown's inhabitants; for instance, refuse blocking storm drains can cause malaria and other diseases and fires set at disposal sites can cause major air pollution, causing illness (mostly respiratory) and reducing visibility, making disposal sites dangerously unstable and possibly spreading contaminants to adjacent property. Unfortunately, Freetown's poor bear an uneven burden of the impact of externalities resulting from poor management of municipal solid (and/or liquid) wastes.

In a study carried out by Sood (2004) for the Government of Sierra Leone, it was estimated that over 745 tons day^{-1} (averaging 0.45 kg $person^{-1}$ day^{-1}) of garbage is generated in the Freetown municipality, of which, biodegradable organic waste, mostly from residential areas and vegetable markets, accounts for over 84%. Construction, demolition debris and yard wastes are not included in this estimate as these are highly variable and skew quantity assessments.

However, medical, toxic, and hazardous wastes are included, as these are currently disposed off with regular wastes. Additionally, the few Freetown industries contribute approximately 20 tons day^{-1} of wastes, mainly, broken bottles and glasses, waste cans, rags and plastics, and small amounts of hazardous wastes.

II. History of Waste Management in Freetown and other parts of Sierra Leone.

Waste management in Freetown has been under variable organizations, both public and private. Unfortunately, each change further deteriorated the system, bringing it on the verge of collapse. The Freetown Waste Management Company (FWMC), the current authority, is struggling to manage the wastes under tight budgets, limited trained but inexperienced manpower, and little or no legislative authority and experience in waste management. Given the lack of education and awareness, and coupled with the very weak penalties (if any) for non-compliance, the public at large is also generally non-cooperative.

The main issues noticeable in the system are highly inadequate and malfunctioning equipment; inefficient collection practices with quite a variable levels of service, poor and unhygienic operating practices; including no environmental control systems; open burning of garbage; indiscriminate illegal dumping and littering; and a public with seemingly little sensitivity to the garbage around them or any awareness of what represents responsible waste management. Coupled with changing waste management authority, the appalling garbage situation, with its present state of management in Freetown, which borders collapse, needs efficient corrective measures. A collapse of the system will adversely impact the living conditions of the city dwellers, further endangering their environment and health. Freetown's solid waste management system needs serious rehabilitation, first on an emergency basis, followed by development and implementation of long-term, sustainable measures. It also needs a change in behavior of individuals and the society. A successful solid waste management depends on an efficient operational system from the outset. It is commonly recognized that four technical pillars of any SWM system are: (i) storage at or near the point of generation, (ii) collection of waste, (iii) street cleansing, and (iv) transport and disposal of wastes. Each of these precepts for sustainable SWM also requires careful planning and implementation by a financially sound, well-footed institute that has executive authorities and appropriate policy and legislative support. In addition, the participation, organization and management of relationship between all key stakeholders must also include consensus building throughout the planning process, which also requires regular revisions and updating. A sound solid waste management system is also essential for sustained economic growth, which in turn can also help generate better revenues and potentially better waste management resources and services (World Bank, 1999). Unfortunately, a sustainable solid waste management system is beyond the ability of any municipal government alone, as is the case of the Freetown City Council (FCC). To meet this need, SWM authorities in many countries are increasing involving private sector and communities as key participants.

In terms of solid waste management, in Freetown, there is too much to do, and at present, there is too little to do it with. Waste management in Freetown, under shifting authorities, has been treated as a political football. Table 1 shows the Record of our Solid Waste Management Responsibility.

The Sierra Leone Department of Health and Human Services (DoHSS) was assigned the responsibility in the 60s which nominated FCC in 1971, an urban Health Authority to manage Freetown's solid wastes. However, the FCC had difficulty in providing the services, and in late seventies, given the hosting of the 1980 Organization of African Unity (OAU) conference in Freetown, the Health minister, while launching a "Keep the City Clean" campaign, also transferred the waste management to the Ministry of Health, DoHS's (Department of Health and Sanitation) new name. In early 80's sanitation was added, and a new name - the Ministry of Health and Sanitation (MoHS) emerged. In 1987, the MoHS assigned the waste management responsibility to it's (then newly created) public health units under its Environmental Health Division (EHD).

The *Kreditanstalt fur Wiederaufbau* (KFW) of the Federal Republic of Germany helped the EHD's Public Health Units with technical and financial assistance during the 1980-1990 periods, which assistance included provision of waste management vehicles, equipment and consultancy services. The equipment provided included ten (10) skip trucks, two (2) tippers, two (2) front-end loaders, three (3) monitoring vehicles and one (1) one-track bulldozer. The assistance, however, was abruptly halted in 1994, because of the Sierra Leone's government's political misunderstanding, and declaration of the German Ambassador as *persona nongrata*.

The World Bank, in 1995, under its Freetown Infrastructure Rehabilitation Program (FIRP), provided two (2) skip trucks, two (2) monitoring vehicles and thirty (30) skip containers to the city. Additionally, the project also provided one (1) truck in 1997. Unfortunately, by this time, most of the skip trucks provided earlier by Germany had ceased to operate, creating an acute shortage of skip trucks

needed to cope with the city's growing requirement. Coupled with the domestic insurgency, by this time, most of the equipment was damaged or destroyed. In 1999, based on government's request the British government provided used waste management equipment including four (4) skip trucks, three (3) cesspit emptier, two (2) waste bowzer, and two (2) tippers. Almost all of these vehicles have since been grounded due to lack of maintenance.

Table 1: History of waste management in Freetown, Sierra Leone.

Date	Name of Authority
Before 1961	Department of Health and Human Services (DoHSS)
1971	Freetown City Council (FCC
1980	Ministry of Health (MoH)
1982	Ministry of Health, German Assistance and Ajibu Jalloh – Private Contractor
1987	Environmental Health Division (MoH), German Assistance and Ajibu Jalloh-Private Contractor
1993	Environmental Health Division (MoH) with assistance from Freetown Infrastructure Rehabilitation Project (FIRP)
1995	Environmental Health Division (MoH) with assistance from Freetown Infrastructure Rehabilitation Project (FIRP)
May 2003	National Youth Multi-purpose Cooperative Society (NYMCOS) under Ministry of Youths and Sports (MoYS)
March 2005	Freetown City Council (FCC)
February 2008	Freetown Waste Management Company (FWMC

The waste management situation further deteriorated significantly under the EHD's resumption of Freetown's waste management responsibilities. As before, key contributing factors were essentially the same and included high bureaucratic inefficiency, corruption allegations especially in the procurement of spare parts and existence of "ghost" workers, poor management, lack of accountability, and lack of funds. The continued restructuring also placed junior, inexperienced, and incompetent staff over the qualified and the experienced ones, adversely impacting staff morale and performance.

At this time, EHD's key solid waste personnel included its chief, one Senior Sanitary Engineer (SSE), one Sanitary Engineer (SE) and three Public Health Inspectors. In addition, EHD also had 57 junior staff and 328 labourers. In terms of equipment, the EHD's main operating equipment (until 1994) included one (1) bulldozer, nine (9) skip trucks (average availability 80%), two (2) dump trucks (90% availability), two (2) loaders, ten (10) other vehicles, and three (3) cesspit emptier and 2295 m^3 vehicles. Waste containers were emptied according to the appraised requirements, daily or less frequently.

Following several field missions consisting of the MoHS and UNDP, assessing Environmental Health and Waste Management situations in five major towns and Freetown city in 2005, a project concept on Sustainable Waste Management was designed by the MoHS, in cooperation with the UNDP Governance Unit. At the same time, the World Bank, as a key partner, agreed to fund the provision and preparation of landfill sites. To formally launch the project in the different towns, a 2-day Validation Workshop (called "Write-Shops") was organised in each of the six locations: Koidu, Bonthe, Bo, Kenema, Makeni and Freetown. Detailed implementation plans were put up at these Write-Shops, and the local community; from paramount chiefs to religious leaders, to representatives from schools and local police, was at the forefront of their development.

As an emergency measure, also the IDA Transport Sector Project (TSP) financed a solid waste collection program for Freetown, Bo and Kenema designed mainly to generate employment, through local contracts; and was implemented by the Sierra Leone Roads Authority (SLRA) under the overall supervision of the Coordinating and Monitoring Unit (CMU) of the Ministry of Transport and Communications (MoTC). Makeni, the headquarter town of the Northern Province, was not included at that time due to the problem of inaccessibility caused by the war. Although relatively successful, these service contracts ended in March 2002, and the MoHS continues to be responsible for the management and sustenance of refuse collection and disposal in the country.

The Ministry of Youth and Sports (MoYS) was responsible for managing the city's wastes in May 2003. The transfer of solid waste management to the MoYS also created an ideal enabling environment to partially tackle unemployment, drug abuse, and the homelessness of city's vast numbers of unemployed youth. For collection, the MoYS has assigned the responsibility to one of its (non-professional) branches, called "National Youth Multi-purpose Cooperative Society", (NYMCOS). Earlier, the NYMCOS youths were engaged in mostly voluntary services in the cleaning of strategic public places, streets, drainages, and sidewalks.

However, in March 2005 the responsibility of the management of Freetown's waste was transferred to the FCC, which used to receive between 35 and 40 million Leones per year from government for garbage

collection by paying staff monthly salaries, hiring and fuelling vehicles and machines, providing protective gears and medical care for the workers. To compound the problems, there were very few official garbage dumping sites, so the overcrowded-city residents use gutters and other unofficial sites resulting in choked waterways/streams that flow down to the sea, depositing waste into the waters that only wash up again on the beaches, destroying the environment and beautiful tourism sceneries. The reality on the ground was that there was an inadequate number of trucks to clear garbage as they were generated. The vehicles and few trucks the council was using were donated by the Libyan President and some trucks were out of service by then. This waste disposal situation would have brought about many health hazards in the city. For instance, residents of Fort Street and Lucas Street among others trapped in heaps of filth and unbearable stench have complained about dirt-related sicknesses. One of the residents remarked that, mosquitoes and flies continue to increase, respectively, malarial- and diarrhoeal-related deaths in the communities and that the transit points are now garbage fortresses sometimes blocking human and vehicular traffic (Concord Times-Freetown, 2008a).

Based on the recommendations of a study by Sood (2004), a World Bank project aimed to help Freetown manage its waste in an effective and sustainable manner, will fund equipment for much-needed emergency and the short-term, two to four year cleanups, as well as helping establish an independent organization, Freetown Solid Waste Management Company (FSWMC, named proposed by Sood 2004 report), capable of implementing these activities. Equally important, for Freetown, the outputs can provide long-term sustainable solid waste management (SWM) services.

To implement the recommendations of the report by Sood, the Sierra Leone Government, in 2008, decided to take garbage collection from the authority of FCC, and called for its privatization to ensure Freetown from continuing been filthy and to avert huge capital investment spent in solving the traditional structural problems in waste management. The council no longer had the logistical capacity to dispose of the city's mounting garbage as all the vehicles used by FCC were not able to collect the volume of garbage at dumpsites and it was not pleased with government's decision as garbage collection has traditionally been the responsibility of the local council. The FWMC (a name almost the same as that proposed by Sood) was given that mandate backed by a three (3) million US dollar World Bank loan and it started operations on February 1, 2008. This company inherited 520 cleaners from the GTZ/Klin Salone (GTZ – Germany's agency for overseas development/German technical cooperation in collaboration with Klin Salone - a youth-based enterprise) programme; whereas the real running cost they inherited was one hundred and twenty one (121) million Leones a month from the government and GTZ, together with its, providing the balance money needed for the cost of providing safety gears and salaries of 520 cleaners and the running and maintenance of 11 trucks and other equipment, 2 tippers and 9 compactors which had to be fueled on a daily basis and repaired and other administrative costs. The Project Manager of GTZ in Sierra Leone said they came up with the Klin Salone project to promote health, a cleaner environment, and create jobs for some hundreds of marginalized youths (the most vulnerable in the country) through the private sector after years of war and political instability. For the past one year and half, GTZ worked with 42 youth groups in Freetown who have been actively involved in both the public and door-to-door collection of wastes.

III. Study Area

Freetown is the capital and largest city of Sierra Leone. A major port city on the Atlantic Ocean located in the western region of the West African country. The climate of Sierra Leone is tropical (hot, humid); with the Rainy Season lasting from May to December and the Dry Season from December to April, and rainfall along the coast can reach 495 cm a year with Freetown having the highest amount of rainfall, greater than 3500 mm, hence one of the wettest places along coastal western Africa. The other main towns in Sierra Leone include Bo, Kenema, Makeni and Koidu.

The ten-year (1991-2001) old rebel war severely impacted Freetown's economic and infrastructural developments, including the vandalization or complete destruction of waste management equipment such as skip trucks, skips/large containers, etc, coupled with swelling its population from 1.2 million in 1994 to an estimated high of 1.4 million in 2006 (Rosenberg 2006). Recent UN and World Bank estimates indicate a projected annual population increase of 4.0 for Freetown (World Factbook 2008), which would proportionately increase the amount of solid waste generated with resultant inadequate sanitation, etc. The result of serious population migration has been squalor, poor housing, inadequate sanitation, congestion, pollution, poor public services, and chronic unemployment, particularly among the youth, most of who are without any employable skills. The ubiquitous pile-up of garbage, can be seen everywhere in Freetown. Also, most city drains are clogged with garbage and even a number of manhole covers have been removed to dump garbage. Many existing skips/containers that also act as transfer stations for the solid waste are broken. Often, garbage is strewn around, where scavengers (mostly

children and wandering dogs, birds, pigs, and other stray animals) forage amongst the rubbish, spreading it around. City's coastal area residents dump their wastes into the sea, whilst, in poor neighborhoods, collected waste is often set on fire. The situation is a major contributor to the city's significant rise of the incidence of vector-propagated diseases.

Besides increasing population, in general, problems with solid waste management particularly in Freetown and Sierra Leone in general, are a lack of continuity in implementation of government policies (which are sometimes inadequately formulated), financial and operational constraints, and unfortunate attitude of citizens towards waste management. Poorly collected waste is subjected to much quicker putrefaction, stronger stinks, and more flies (vectors of diseases) and during the long rainy season of Freetown the waste, being uncovered, becomes soggy, smelly and difficult to handle (collect and transport).

IV. Analysis of the Existing Situation

As indicated earlier, the ten-year rebel war severely impacted the infrastructure, the agriculture, and the economy of Sierra Leone. During and by the end of the war, thousands of refugees, mostly rural poor migrated to Freetown, swelling its population.

The ubiquitous pile-up of garbage is a significant contributor to the city's significant rise of the incidence of vector-propagated diseases. Currently, most of the city's drains are choked with rubbish. A number of manhole covers have been removed so that garbage can be dumped there. Where special dumps or public "dustbins" or containers (skips included) are provided, garbage is often dumped outside due to lack of capacity, poor collection, and/or public insensitivity. Open Dumps allow free access to waste pickers or scavengers, animals, and flies; and often produce unpleasant and hazardous smoke from slow-burning fires. Garbage can be seen strewn everywhere, scattered, or in small or large piles, many of which are regularly set on fire, used as a waste disposal option. Waste generation in Freetown far outstrips its collection and transport.

From media reports, it seems as if the company presently in charge of waste management in the city, FWMC, is struggling to cope with the present situation (Concord Times-Freetown, 2008a, 2008b and 2009) as it suffered so many strike action threats from workers in 2008 and 2009 because of reasons of poor conditions of service and unfair treatments, including but not limited to, late or none payments of salaries, non-provision of workers protective gears, no medical care for the workers, the attitude (molestations) of some bosses to their workers; inadequate funding; lack of heavy equipment and other working tools to do the job; lack of trained and experienced workers to efficiently do the job; insufficient availability of official garbage dumpsites; etc.

Kroo Bay, one of the largest and poorest slums in Freetown, is located at the mouth of one of the rivers which crosses Freetown, so all waste dumped in the streets and in the drainage systems all over the city will all end up there causing a health risk and a serious environmental disaster (for instance, massive flooding during the rains) to the inhabitants. And also drains along the streets of Freetown, meant to collect rainwater in the rainy season, become clogged with waste and during heavy rainstorms entire areas of the city are flooded as a result of bad management of the waste.

At the two dumpsites (i.e., Granville Brooke Landfill in the East and Kingtom Landfill in the West of the city) in Freetown, thousands of scavengers make their living from the collection of waste. They collect cans and other metallic objects, plastics, and other products in order to sell them for few Leones (the local currency). Healthcare waste is also dumped at the dumpsites, mixed with domestic waste, increasing the risk of infection with Hepatitis B and HIV and other diseases (World Bank, 2000).

Silvia Garcia, a researcher, Caledonian Environmental Centre/PhD student and 2009 Gordon Masterton/Magnusson Award winner, went on a successful working visit to Freetown in April 2009 as a part of twelve (12) professionals working in the waste management sector in the United Kingdom (UK); on which visit, this group was able to review the city's waste problem from top to bottom and held meetings with a number of key stakeholders including the FCC Mayor, FWMC, GTZ, the British Council, Klin Salone, MoH, the World Bank, hospitals and universities. These experts delivered training in relevant waste management approaches to a mix of waste practitioners and universities and organized environmental awareness sessions with a large group of very enthusiastic school children; and also delivered a session with the aim of launching an ecoschools programme in few schools selected by the British Council. This opportunity was used by them to raise awareness of waste and environmental issues and assist in setting up environmental clubs. They gave to the School of Environmental Sciences (Njala University, NU) some waste management books donated by Dr. Gholam Jamnejad of the Built and Natural Environment Department at Glasgow University; and are currently working on potential partnerships between Sierra Leone and UK universities. Before their departure, the group also undertook a waste and environmental audit for the Freetown's British Council offices; and later presented the main findings of their visit to the group of stakeholders.

Upon their arrival to the UK, Silvia, on behalf of the group, expressed her appreciation, "Thanks to the Magnus Magnusson Award and my employer, Caledonian Environment Centre, I am part of this project. It has had a great impact on me, both professionally and personally. After being in direct contact with the severe poverty my perception of life has changed; my "problems" are not problems anymore and I have realized how well we live in the UK. Professionally, waste in developing countries is a new area to develop which is much more challenging than my everyday job in the UK. I believe that nobody can remain indifferent after such an experience. We are therefore very keen to continue our project, in order to work towards a sustainable transformation of waste management in Freetown. We are currently working in a document that summarizes our findings, intended activities and future actions, which will be presented to potential investors/donors in order to address the waste management issue in Freetown".

In a recent media report (Sierra Express Media, 2009), contrary to Concord Times-Freetown (2008a, 2008b and 2009), the General Manager and Operations Manager of FWMC claimed that, the company was embarked on recruiting more manpower to help clean the city and that they were engaged constantly in efforts to sensitize the residents of Freetown about the need for respect for sanity and cleanliness; there's an ongoing construction of garbage disposal points all over the city; and the company has procured more vehicles, motor cycles and push carts to make sure that the city is clean on a twenty-four hour basis.

In August 2009, the Government of the Republic of Sierra Leone (through the Ministry of Finance and Economic Planning), on behalf of the FWMC, released an "Invitation for Bids" notice for the construction of Transit Points (including perimeter fence walls) in Eastern and Western Freetown and Access Roads within the Kissy Grandville Brooke Landfill and the Kingtom Landfill, Freetown, and Rehabilitation of Offices and Garages at Works Yard, Blackhall Road, Freetown. The government recently received this financing from the International Development Association (IDA) toward the cost of the Sierra Leone Water and Power Project (Water and Sanitation Component), of which funds IDA intended applying a portion, through the FWMC, to eligible payments under the contract for IFB №: FWMC/NCB/08/01.

V. Waste Collection Practices, Categorization and Disposal Methods

5.1 Collection Practices

The snags to an efficient or rather house-to-house waste collection in Freetown include, the unwillingness and/or inability of the residents to pay for such services; coupled with large areas of the city been highly congested, making up more than two-thirds of all city neighbourhoods, mostly inhabited by low-income communities. Additionally, waste storage practices at homes are rather poor, adding to the insurmountable collection difficulties. Unsorted waste is often stored in old leaky buckets, and used paper/plastic bags instead of a bin lined with plastic bags. Given the small-scale house-to-house collection, pre-collection from homes to the public or communal skips placed at strategic spots in the city, has to be organized by households or some informal private groups; thus, household waste is thrown by a family member, usually either a child or a family servant and since 2005 this has being done on a very limited basis by an arm of National Youth Multi-purpose Cooperative Society (NYMCOS), doing the service for a negotiated payment from the households concerned. To add to the waste collection problem, there has never been any transfer station, a common situation to most Africa countries. Rubbish picked up by collection workers (not provided with safety gears, including gloves, etc.) from communal skips is moved straight for the city's two disposal sites.

Table 2: Garbage Skips Distribution and their Average Monthly Collection Rates (Adopted from Sood, 2004)

Zone #	Zone Range	# of Skips	Collection Frequency	Estimated Population
1	Calaba Town to Ferry Junction	11	30	185,000–200,000
2	Ferry Junction to East End Police Station	9	20	185,000–210,000
3	East End Police Station to St. John	8	25	250,000-285,000
4	St. John to Juba Bridge (7th Battalion)	26	50	275,000-410,000
	Total No. of Operational Skips	54	31.2 (Avg.)	

The then Ministry of Youth and Sports (MoYS) in early 2000 divided the city into four zones for waste collection (as shown in Table 2); each zonal team consisted of ten members who had access to tipper trucks, 5-7 ton capacity wheelbarrows, and related equipment including shovels, long and short brooms, rakes, shovels, etc.

Household waste in Freetown is collected using 6 m^3 skips, which are strategically located along various streets and given their (skips) highly inadequate number, wastes are often illegally deposited in small dumps along city streets, and market and business districts, making collection inefficient and expensive. Furthermore, often immobilization rate of waste collection vehicle reaches about 70% in Freetown, thereby seriously impacting the rate of collection. The volume of waste to be collected in areas, where manual collection is performed, often far exceeds the capacity of the collection system. To salvage the situation, a few community groups collect their own waste; which, however, often end up as garbage mounts elsewhere. In economically better neighborhoods, such as Signal Hill, and Wilkinson Road, etc., waste collection is performed at least three times a week, on the average, considered a desirable collection frequency, but poor neighborhoods, like Calaba Town, Wellington, etc., are serviced less frequently, once a week, on the average; the reasons cited for the variation been better roads, little or no congestion, etc., in economically better neighborhoods making vehicular waste collection easy.

Streams of waste, broadly categorized into "controlled" and "non-controlled", are characterized by their sources, the types of waste produced, and the composition and generation rates; therefore, knowledge of these characteristics is required in order to design and operate appropriate waste management systems. The single most important part of waste classification is accuracy because all other waste management requirements (including monitoring and controlling the existing waste management systems, and making regulatory, financial, and institutional decisions) hinge on this one assessment. It's also proper to determine the volume, density and weight of solid waste produced to estimate the storage requirements and collection frequencies and devise suitable collection methods.

5.2 Waste Categorization

Streams of waste, broadly categorized into "controlled" and "non-controlled", are characterized by their sources, the types of waste produced, and the composition and generation rates; therefore, knowledge of these characteristics is required in order to design and operate appropriate waste management systems. The single most important part of waste classification is accuracy because all other waste management requirements (including monitoring and controlling the existing waste management systems, and making regulatory, financial, and institutional decisions) hinge on this one assessment. It's also proper to determine the volume, density and weight of solid waste produced to estimate the storage requirements and collection frequencies and devise suitable collection methods.

Table 3: The eight major categories of Solid Wastes (modified from Sood, 2004)

Source	Typical waste generators	Types of solid wastes
[#] Residential	Single and multifamily dwellings	Food wastes, paper, cardboard, plastics, Textiles, leather, wood, glass, bulky items, and household hazardous wastes
[#] Industrial	Light and heavy manufacturing	Housekeeping wastes, e-waste, packaging, food wastes, demolition materials, wastes from mining industries (mine tailings), etc.
[#] Commercial	Stores, hotels, restaurants, markets	Paper, cardboard, plastics, wood, food wastes, hazardous wastes, e-waste, etc
[#] Institutional	Schools, hospitals, prisons	Same as commercial, government centers, new construction sites, road repair, Wood, steel, concrete wastes, e-waste, etc.
[#] Municipal Services	Street cleaning, etc.	Street sweepings; landscape and tree Trimmings, general wastes from parks, sludge water, e-waste, etc
[#] Process wastes	Heavy and light manufacturing	Slag, mineral tailings, etc
Agriculture	Crops, orchards, vineyards, dairies	Spoiled food wastes, agricultural wastes, etc.

[#] All should be included as "municipal solid waste"

Principally, the three main classifications of urban solid wastes are municipal, industrial and hazardous. But, the designation of a material as 'municipal waste' depends upon the individual city's definition of

municipal solid waste. Nonetheless, the current waste authority, the Freetown Waste Management Company (FWMC) handles solid waste, known as "controlled waste", from households, markets and institutions, street and public open spaces, dead animals; "uncontrolled waste" from agriculture, mines and quarries; and non-hazardous waste from processing and industries. The eight major categorizations of solid waste generators are as shown in Table 3.

5.3 Disposal Technologies

Freetown's wastes are disposed of at the city's two landfills, which are essentially open dumps; which approach can be classified as the primitive stage of landfill development and is the predominant waste disposal option in Freetown. These uncontrolled or insanitary open dumps have no environmental safeguards, hence, can pose major public health threats, and affect the landscape of Freetown.

5.3.1 Municipal Wastes

The two landfills, Kingtom and Granville Brooke, located at the western and eastern ends of the city, respectively, were initially designed as controlled dumps. In addition to the disposal at these landfills, there is also significant illegal dumping of wastes at vacant lots, street corners, roadside, the city's drains (mostly clogged with garbage), and the few streams from the mountainside that empty into the sea.

Bulk of the refuse deposited at these landfills is mainly domestic refuse and market-refuse, mainly from the public markets; with organic, biodegradable waste accounting for the largest component with lesser amounts of industrial and street-refuse, in addition to the city's medical, hazardous, and toxic wastes. Uncategorized refuse are dumped at these landfills as all refuse is mixed and piled at available or accessible areas at each dumpsite. Some commercial and other institutions, which pay little or no fee to the waste management authorities, do their own dumping using their own refuse carts or vehicles. Waste is tipped in heaps at each of the landfills, and leveling of these occurs in a several-day rhythm depending on the availability of a bulldozer [given the high daily hire costs of \$600 day^{-1} in late nineties and early 2000 (Bartone, 2001)], which works diagonal to slope. With infrequent bulldozing, smaller fraction of all collected medical waste disposed with regular waste, come up to the surface of the dumpsite. Uncertain bulldozer availability often results in garbage heaps that are intermittently burnt to decrease volume, and to make space for incoming garbage, thereby polluting the environment and posing some health risks to the residents. There exists the potential for open as well as controlled dumps to significantly pollute an area's groundwater; as water percolates through the solid waste in landfills, it absorbs chemicals and microorganisms present in the rotting materials. The uncontrolled discharge of liquid formed in solid waste dumps or landfills, known as leachate, contaminates ground and surface waters, and thus, pose environmental and public health risks to the local area. Additionally, the emission of harmful gases such as methane (highly flammable gas having the risk of explosion and affects global warming), given its high calorific value, need to be controlled and economically utilized. Each of the two landfills has at least one (1) rudimentary office and no weigh station or formal tipping area. The staff at each landfill is skeletal and it's composed of five laborers, two supervisors, one clerk, at least one health inspector and two security guards.

The 2-3 skip trucks, used to transport skips to the city's wastes to the nearest dump, are supported by two front-end loaders dump trucks to haul garbage. When in good conditions, these trucks work right around the clock, sometimes, driving over scattered waste dumps; as use of bulldozer for waste leveling is highly irregular, given the high daily hire costs, when available for renting from a private company. Because the city's environment is congested, a huge number of skips are hauled at night usually by a crew of four, including a driver and during the day, pushcarts, both small and large, transport wastes from neighborhood to the nearest skip/container or illegal dump, many of which seem to have never been cleared. In many of these containers, garbage is regularly set on fire to dispose of wastes. Sood (2004) estimated that over 40-50 percent of the total garbage in Freetown is disposed of illegally, including large quantities been dumped in open drains, sewers, street corners and so on. Furthermore, each of the landfills, particularly the Kingtom's, is also reaching its designed capacity, which situation is exacerbated by the lack of appropriate equipment, in particular to level the refuse, preventing "refuse hills." The two landfills have already failed, having been pushed beyond their engineered limits; and due to poor operational practices, each landfill has almost degraded into potentially hazardous and toxic dump. At the fringes of each of the landfills, some vegetable gardening is done by squatters living in makeshift huts and they are also engaged in various small-scale industrial activities. The leachates from these open dumps entering the adjacent surface and ground waters will expose downstream residents to disease organisms in their bathing, irrigation, and drinking water supplies, and through eating contaminated fish and other foods. Consequently, proper management of the two landfills can effectively remedy this situation.

5.3.2 Industrial Wastes

Commercial and industrial wastes are privately collected and transported to nearest dump site. For instance, the Sierra Leone Brewery Limited (SLBL) collects and deposes its waste at the nearest dumpsite, at no cost. Forms of wastes from Freetown's industries, including the SLBL, Freetown Cold Storage Company Limited (FCSC), range from solid (broken bottles, plastics, spent grains and yeast), liquid (including detergents used for cleaning bottles and other equipments) to gas (basically CO_2 which is a product of fermentation). The non-biodegradable ones include bottles and plastics and the spent yeast extracts from Brewery are believed to be biodegradable, thus, releasing dangerous gases. The SLBL's liquid waste, mostly of unknown composition, is discharged untreated into a nearby Rogers Stream as the SLBL has no wastewater treatment plant. However, it must also be noted, that an effort is been made in the factory to minimize the amount of CO_2 going into the atmosphere as some percentage is trapped and used in the gasification or carbonation of the final products. Also used in the manufacturing process is caustic soda and its wasted excess, being a base, will cause alkalinity (increase in pH) of the surrounding streams to which it is released, hence aquatic life will be threatened.

Surface mining methods to extract ore are employed by most of the mining industries in most rural areas of Sierra Leone. By its very nature, surface mining causes disturbance to the surface of the earth and its associated activities is certainly detrimental to humans, animals and plant lives in the short term. The mining industry, however, differs from the other production industries in generating an extremely large quantity of waste materials in the form of overburden tailings heaps, slags, sludge and mineralized deleterious wastes; hence, causing adverse environmental effects on the landscape in its broadest sense and on the community depending very much on the particular mining company. Some 700,000 tonnes of slimy, red (mainly $\leq$2.5mm tailings consisting mainly of alumina, silica, kaoline and iron oxide) wastes from the Sierra Leone Ore and Metal Company (SIEROMCO) process plant are disposed of into impoundment areas in valley adjacent to the plant, ending up into the Jong River. Sierra Rutile Limited (SRL), mining and processing mineral sands (including rutile, TiO_2; ilmenite, $FeO.TiO_2$ or $FeTiO_3$; and zircon, $ZrSiO_4$ or ZrO_2SiO_2), generates tailings and high concentrates of acidic pyrites (FeS_2) and marcasites (FeS_2) that are pumped back into the pond and sand tailings pumped to the back of the dredge; ending into Nitti harbour and the other bodies in this mining area. The other mining industries including, Gold Mining, Marampa Iron Ore Mining Company, and lot of diamond mining industries, also deposit their wastes into their immediate surrounding adjacent water bodies. The main resultant effects from such operations are traffic, noise, visibility, dust, water pollution, vibration, displacement of residents in the affected areas, the destruction of current land use, and so on.

5.3.3 Hazardous, Toxic and Medical Wastes

Hazardous wastes, which can be in the form of solid, liquid, sludge or even gas, contain highly persistent inorganic or organic chemicals and compounds with acute and chronic (immediate, short-term, as well as long-term) impacts on human/public health and on environment; with direct contact (such as during handling of waste) been the most common exposure route. They also vary in the degree of hazard posed.

5.3.3.1 Industrial and Hazardous Wastes

Key industries in Freetown are plastics, soap manufacturing, tanneries, Freetown Cold Storage Company Limited (FCSC), National Confectionary Company Limited (NATCO), Aureol Tobacco Company (ATC, non-functional at present), Sierra Leone Brewery Limited (SLBL), R. K. Distilleries, G. Shankerdas and Sons Limited (GSS), and others; none of which has any effluent controls. Waste lubricating oil, motor and gearbox oils, and some cutting oil; small amounts of organic solvents; flesh and hide cuttings contaminated with sulfide and chromium salts; waste batteries; and textile dyeing wastes which contain toxic metals like cyanide, are the main hazardous and toxic wastes arising from these facilities. Additionally, there is rubbish from production processes, including, floor sweepings, rags, discarded cardboard and wooden packaging materials, broken glass, metal offcuts, and swarf, whilst the office waste is mainly paper and cardboard. In Freetown, there is no heavy industry, large production or processing of chemicals, oil refining or other similar industrial operations that can generate significant quantities of hazardous wastes. Moreover, inhalation of dust from waste storage or dumpsites may also constitute a hazard at the facilities.

Generally, however, the industrial units are small, with the exceptions of SLBL and FCSC; and all dispose of their wastes, mostly by private arrangements at the nearest landfill. SLBL also gives waste malt to area farmers who use it as cattle or pig feed at no cost. Smoke from burning tires, often used to provide heat to small manufacturing operations, can be seen in a number of places around the city.

5.3.3.2 Health Care/Medical Wastes

Another category of waste that requires special care in handling and disposal is HCW, defined as the total waste stream from a healthcare establishment, research facilities, laboratories, and emergency relief donations. HCW is broadly classified

into communal and special wastes; with communal waste usually having the characteristics of regular municipal waste, such as food waste, packaging materials, waste plastic, cardboards, and office supplies. It can be safely disposed of with regular municipal waste. The remaining HCWs, called special waste, require special attention.

Medical waste is generated by Freetown's health care facilities, including veterinary hospitals. The government medical hospitals in Freetown include Connaught Hospital, PCM Hospital, Under Fives Hospital, Kingharman Road Hospital, Rokupa Hospital, Macauley Street Hospital, and Children's Hospital. The Ministry of Health and Sanitation's (MoHS) 2004 estimates of the total number of beds, including those at the city's major private clinics and health centers (including The Good Shepherd Clinic, Yearima Memorial Clinic and Lumley Health Center, Curney Barnes Hospital, etc.) is 1,455. It is unfortunate that, the overall health care delivery has significantly deteriorated in terms of quality and patient care, coupled with an inefficient waste handling and disposal system in the city's limited number of hospitals; hence, no current estimates of total quantity of medical wastes generated in Freetown are available. Average rates have been projected at 0.55 kg bed^{-1} day^{-1} (Sood 2004), to an estimated total of 727 kg day^{-1} depending upon the number of beds occupied and based on similar city data. The numerous ways used for safe handling and disposal of medical waste (of which the infectious waste can vary from 3%-30% of the total medical wastes) include incineration, non-burn technologies such as use of microwave (radiation) systems, shredding and sterilizing, shredding and chlorination, autoclave, electric arc systems and mechanical systems.

5.3.4 Sludge/Sewage Disposal

With Freetown having no central sewage treatment plant, and at household level, about 60 percent of the city's total population uses pit latrines, and over 30% have septic tanks coupled with the given improper maintenance and servicing, each of these systems represents serious health and environmental hazards to the public. The emptying of cesspits at household and industrial levels has been the duty of the MoHS and now FWMC's and other cesspit emptying private companies. Slurry trucks or "cesspit bowzers are used to collect and transport faeces to one of the city's two landfills, the Kingtom landfill, where the faeces are spread in a polder with alternating pits (each currently overflowing) for dewatering and drying up. Upon drying, a polder's contents are covered with soil and after few months the product, "night soil", is used as fertilizer. There must be some risk concerns as inappropriate treatment and disposal methods are used; the existing polder/slurry pond has run out of capacity and its overflowing sewage is led, in its vicinity, through a 6-8 feet-connecting pipe to an unlined pit, which is further connected to a source of tidal water which takes the untreated sewage out to the sea. Inadvertently, a number of families have set up homes close to the tidal pipe and often, these families use waste plastic to prevent the sewage pass their front doors. There is no water supply in the area, and the situation presents an environmental and health nightmare.

VI. Discussions

Freetown waste management has been under various authorities, both public and private, with each change associated with further deterioration, and bringing the system on the verge of collapse. The current authority, FWMC, is struggling to manage the wastes under the aforementioned prevailing conditions and given the illiteracy rate and awareness, coupled with the very weak penalties (if any) for non-compliance, the society at large is also generally uncooperative with seemingly little sensitivity to the garbage around them or any awareness of what represents responsible waste management.

There are no reliable estimates of the quantities of hazardous wastes produced by Freetown's approximately more than 30 manufacturing companies but a German study gives an estimate of 7,500 tons $year^{-1}$ (GOPA 1995). Also a study carried out by Sood (2004) estimated that an average of 0.45 kg $person^{-1}$ day^{-1} of garbage is generated in the Freetown municipality, of which, biodegradable organic waste accounts for over 84%, excluding construction, demolition debris and yard wastes; but including medical, toxic, and hazardous wastes, as these are currently disposed off with regular wastes. The few Freetown industries account for over 20 tons day^{-1} of solid wastes, and small amounts of hazardous wastes and the key industries that have the potential to generate hazardous wastes include, soap, paint manufacturing, the large Germany's Heineken-owned brewery (SLBL), chemical, kernel oil and other products. It is likely that given poor economic growth, past domestic insurgency and other factors, these quantities may not have changed. There is also no separation or pretreatment of wastes or polluted effluents at any of these facilities and no existing environmental monitoring, either voluntarily or by authorities of industrial wastes in Sierra Leone. Most industrial wastes are disposed off at the city's landfills by private arrangements. In a few cases, such as, during the operations of ATC, wastes such as tobacco dust and cigarette wrappings were disposed at the facility. In some cases, the effluents are illegally discharged into city drains. Unfortunately, this is also

the case at the SLBL. A used oil recycling facility (recycling used oil from the Sierra Leone Ports Authority and National Power Authority) located at Rokel in the eastern outskirts of Freetown also engages in illegal waste disposal, disposing the potentially dangerous residues in an unlined earthen pit at the facility. Sierra Leone also lacks industry-specific environmental regulations and has an overall weak institutional capacity, which aspect needs to be reviewed through establishment and strengthening of institutional framework.

Generally, frequency of waste collection in Freetown is very low as its estimates range between 35 and 55 percent of the total waste generated and given such low collection rates, the uncollected waste is sometimes burnt, buried, or illegally deposited in open spaces, water bodies, and storm-drainage channels, along the streets or roadsides; with particular days set up for removal of bulky items such as furniture, tree stumps and tree cuttings. The key issues apparent in the system are highly inadequate equipment; poor, unhygienic operating and inefficient collection practices with quite variable levels of service; littering, widespread illegitimate dumping and open burning of garbage; inefficient or no environmental control systems; and a public with apparently little or no sensitivity to the garbage around them or any awareness of what characterizes reliable waste management.

VII. Conclusion

As per above discussions, a sound institution is essential to sustainable solid waste management (SWM) operation. Experience in developing countries indicates that an efficient waste management institute should be autonomous, and has executive authority to design, monitor and implement sustainable SWM strategies; and given the needs for its multi-sectoral role, such an institution must also possess authority, visibility, adequate budgets, legislative and policy support, administrative capacity, and a strong constituency to advocate its plans and their potential implementation.

The FWMC, the current authority, seems to have many shortcomings, particularly, on the areas of management and implementation. Additionally, coupled with equipment shortage, inadequate budgets, lack of authority, the company is struggling with very weak staff capacities at all levels. Lack of adequate records and information related to the SWM costs; lack of internal controls; lack of institutional and regulatory frameworks for procurement, and legislative enforcement; etc.; are some of the snags on the operational side of FWMC. Minimum standards will have to be set and implemented for all World Bank projects with FWMC.

One of the major weaknesses of SWM in Freetown is administration, though tight or limited budgets, inability to raise revenues through user fees, municipal bonds, or other means, as well as poor organizational set up are also serious limitations to effectively implement and run the solid waste management projects. The service ultimately depends on effective administrative and organizational systems and hence, they are very crucial to a sustainable SWM system. It's proper to make provisions for both public feedback and input from related public organizations in planning, evaluation and upgrading of the system. As a role of a private sector, cost-recovery contributes to sustainability. After the setting up of sound institutional structures, it's possible to adopt sustained improvements through labour-intensive, low-capital alternatives, and enabling administrative changes, when necessary.

And on this note, one can really tell the severity of waste management problems in Freetown, despite the invaluable joint efforts of the new company, the government and its partners (World Bank, IDA, GTZ/Klin Salone, etc.) to clean the city of its heaps of waste. There is the need for the intervention of potential investors/donors to ameliorate or lay to rest this waste management problem by helping address this problem sustainably, once and for all for the betterment of the lives of all Freetown inhabitants.

Thus, in the context of Freetown, there is a dire need of a sound institute, if a sound and proper waste management is to be realized.

VIII. Recommendations

Based on this study, this report is proposing that this appalling garbage situation needs efficient corrective measures/serious rehabilitation, first on an emergency basis, followed by development and implementation of long-term sustainable measures; otherwise it will adversely impact the living conditions of the city dwellers, further endangering their environment and health. It also needs a change in behavior of individuals and the society. In addition, the participation, organization and management of relationship between or/and among all key stakeholders must also include consensus building throughout the planning process, which also requires regular revisions and updating. A sound solid waste management system is also essential for sustained economic growth, which in turn can also help generate better revenues and potentially better waste management resources and services. Unfortunately, a sustainable solid waste management system is beyond the ability of any municipal government alone, as it's the case of the FWMC. To meet this need, waste management authorities in many countries are increasingly

involving private sector and communities as key participants.

Regulatory requirements making it easier to classify waste in Freetown has either not commenced or are dormant. To assist the waste industry in meeting the changed requirements for waste classification, the FWMC needs to replace its environmental guidelines (if any), which will outline a clear and easy-to-follow step-by-step process for classifying waste. There should be regulations on special waste, which will provide effective system of control for wastes that are difficult to handle. The regulations will ensure that dangerous wastes are soundly managed from their production to their final destination or recovery. Any would-be transfrontier shipment of hazardous wastes is to be controlled by a national legislation as they can pose threat to both human health and the environment. For instance, the UK legislation on this is governed by the EC Directive, which is based on international multilateral and environmental agreements.

There should be proper management of HCW, both within and outside healthcare facilities, to lessen risks, the first priority been the segregation of wastes, preferably, at the point of generation, into reusable and non-reusable, hazardous and non-hazardous components; and the other important steps been the instituting of a sharps (i.e., sharp instruments) management system, waste reduction, avoidance of hazardous substances such as the PVC-containing products, mercury thermometers and others, wherever possible, ensuring workers' safety, providing secure methods of waste collection and transportation, and installing safe waste treatment and disposal mechanisms.

It is envisioned that successful implementation of the measures recommended in the study can help establish a long- term, 10-year and beyond, self-sustainable waste management system in Freetown.

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Determination of oil life for crane Liebherr Model D9408 diesel engine by Oil Condition Monitoring

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Abstract: The aim of this study is to choose and investigate the best oil replacement time by oil condition monitoring for crane Liebherr model D9408 diesel engine. This is achieved by investigating different oil sample analyses of crane Liebherr model D9408 diesel engine. According to the majority indices results of the oil analysis, But not for all of them, they had an acceptable function after 160 running hours. The variation percent of plumb in wear debris analysis was above 50 percent. According to the Total Base Number (TBN) analysis, the oil had an acceptable function until 150 running hours. Additive depletion results showed that the oil had an acceptable function after 150 running hours, and absolute variation percent of each additive material after 160 running hours were not more 50 percent. Pollutant materials results of this table showed that the variation percent of each pollutant material after 160 running hours was not more than 50 percent. Also the Particle Quantifier (PQ) results showed that the variation percent of PQ after 160 running hours was not more than 50 percent. Results of oils analysis for viscosity didn't give us a reliable consequence. Right now, the oil of the diesel engine is replacing every 125 hours, but overall the best time for replacing the oil for this engine has been calculated as 150 running hours. [Journal of American Science 2010;6(5):136-141]. (ISSN: 1545-1003).

Keywords: Oil analysis; Oil Condition Monitoring; Oil time replacement; Machine Condition Monitoring; Wear debris materials

1. Introduction

Machine condition monitoring has long been accepted as one of the most effective and cost efficient approaches to avoid catastrophic failures of machines (Toms, 1998; Barron, 1997). There are several methods to monitor the condition of a machine, such as acoustic condition monitoring, thermal condition monitoring, vibration condition monitoring, and others. However, practical experience has shown that oil condition monitoring technique is a suitable method for reciprocating machinery (Barron, 1997).

Oil condition instruments are suitable for use in all major industrial applications, including iron and steel, agriculture, military, mines and quarries, power generation transport, and used oil analysis. Recent evidence showed that oil analysis technique provided greater and more reliable information, resulting in a more effective maintenance program with large cost benefits to industry (Troyer, 1999; Byington,1999).

Preventative maintenance program is essential for optimizing operational efficiency and performance of machinery and lubricant oil. The main limitation is that it is comparatively expensive to operate and can also be a time-consuming activity. The employment of this technique can be used as both predictive and proactive tools in order to identify machine wear and diagnose faults occurring inside machinery against different kinds of oil s. However, recent evidence shows that oil analysis technique provides greater and more reliable in formation, thereby resulting in a more effective maintenance program with large cost benefits to industry (Byington, 1999; Mathew, 1987; Maxwell, 1997; Troyer, 1999).

Lubricating oil in internal combustion engines is exposed to various strains depending on the operating conditions, the fuel quality, the ambient conditions and operating parameters. The rate of deterioration strongly depends on these influences. In order to avoid an engine failure, the oil must be changed before it looses its protective properties. At the same time, an unnecessary oil change should be avoided for environmental and economical reasons. In order to determine the optimum oil change interval reliably, it is necessary to monitor the actual physical and chemical condition of the oil. The oil's ageing process is very much influenced among other things, by the fuel quality, due to the blow-by gases of the combustion process. Therefore, especially for gas engines fueled with biogas of a priori unknown and fluctuating fuel quality, the direct monitoring of the oil condition is essential (Agoston, 2005).

The monitoring of oil and oil-based liquids (including emulsions) is an important task in a number of application areas ranging from the food industry to automotive applications. In the latter field, there has recently been increased interest in monitoring the condition of lubricants facilitating proper engine operation. Monitoring the engine oil condition at first instance allows the implementation of increased oil drain intervals. Moreover, it provides increased insight into the actual state of the engine, which enables the detection of possibly approaching engine failures but also the monitoring of the performance of engine oils of varying quality. Similar considerations hold for other applications where oils are used as lubricants (Jakoby, 2003).

As a general rule, machines do not break down or fail without some form of warning, which is indicated by increased wear debris materials. By measuring and analyzing the oil of a machine, it is possible to determine both the nature and severity of the defect, and hence predict the machine's failure (Davies, 1997; Williams, 1994) and also choose the best oil for the machine and investigate optimum operating time for that oil. Oil analysis may have two purposes: safeguarding the oil quality (contamination by parts, moist) and safeguarding the components involved (characterization of parts). Oil analysis is mostly executed off-line by taking samples. However, for safeguarding the oil quality, application of on-line sensors is increasing. Sensors are nowadays available, at an acceptable price level, for count the part and measure the moisture of oil. Besides this, Safe-guarding the state of the oil filter (pressure loss over the filter) is mostly applied nowadays for hydraulic as well as for lubrication oil.

Engine lubrication oil degrades at varying rates depending on the lubricant, engine type and application. Traditional maintenance programs are designed to change oil on predetermined intervals (such as run time/mileage), with more advanced algorithms taking into account load and operating temperature of the engine, or lab analysis. Conservative interval based maintenance programs spend too many resources changing oil and longer intervals may result in engine damage. Lab based oil condition approaches also have significant time lag and other logistical difficulties (Bennett, 2005).

One of the most important aspects of oil analysis that requires improvement is the fact that the wear debris quantification does not always correlate with the real wear that one means to measure. Measurements are affected by different factors that should be compensated or accounted for if a proper wear analysis is to be achieved. Also, for practical operating equipment, there are many factors which affect the wear of parts such as engine age, type of service, environmental conditions of work, engine metallurgy, etc. These are difficult to evaluate (Macian, 2003).

2. Materials and methods

The experimental and testing was conducted on crane Liebherr model D9408 diesel engine. Details of engine components are given in table 1. Six running hours were conducted, they were 110, 120, 130, 140, 150, and 160 hours. Right now, the oil of the diesel engine is replacing every 125 hours. The objective of this research is to choose and investigate the best oil replacement time by oil condition monitoring for crane Liebherr model D9408 diesel engine. This is achieved by investigating different oil sample analyses of crane Liebherr model D9408 diesel engine.

Table 1: Details of crane Liebherr model D9408 diesel engine

Engine component	Description
Model of engine	LIEBHERR D9408, diesel engine
Number of cylinder	8 cylinders
Maximum power (kW)	400 KW at 1900 rpm
Cooling system	Water

Wear means the loss of solid material due to the effects of friction of contacting surfaces. According the results of researchers if the concentration wear debris materials were between 50 to 100 ppm, fifty percent change in wear debris materials could show the fault in engine (Poley, 2000). An important factor in any monitoring program is the ability to obtain reliable trend information or details of gradual changes with time or running hours. A careful observation of these trends can be very revealing. Any significant variation from the trends such as rapid increase or decrease in a measured value, gives early warning of an impending problem, well before the limit value is reached. Oil condition monitoring involves sampling lubricants from critical rotating plant and equipment and then analyzing the lubricant for clues as to the operational condition of the machinery under inspection (Ahmadi et al., 2009b).

3. Results and discussion

3.1. Wear debris analysis

According to ASTM D-6595 standard and by using the Atomic Emission Spectroscopy (AES) tests, the amount of the wear debris materials of oil samples has been investigated. The wear debris materials of oil samples between 110 and 160 hours and the results were shown in figure 1. It has been shown that there weren't any significant difference between values.

It has been shown in table 2 that the variation percent of the majority of wear debris materials were below than 50 percent. Only the variation percent of plumb was above 50 percent. Also the results showed that variation percent of each wear debris material until 160 running hours was below than 50 percent.

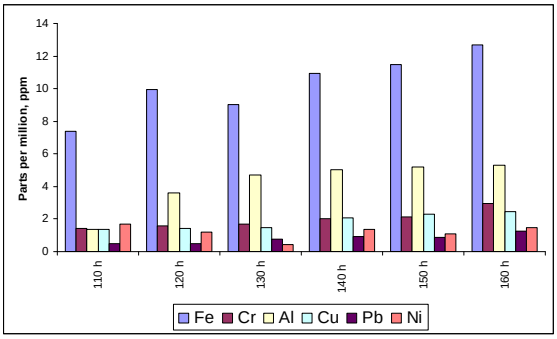

Fig 1: Wear debris analysis results during different running hours

Ahmadi et al. explained that according to the results of researchers if the concentration of wear debris materials were between 50 to 100 ppm, fifty percent change in wear debris materials could show the fault in engine. An important factor in any monitoring program is the ability to obtain reliable trend information or details of gradual changes with time or running hours. A careful observation of these trends can be very revealing. Any significant variation from the trends such as rapid increase or decrease in a measured value, gives early warning of an impending problem, well before the limit value is reached (Ahmadi et al., 2009b).

Table 2: Value, warning zone and variation percent of wear debris materials of oil in 160 running hours

Wear debris material	Value (ppm)	Average + 1* standard deviation (ppm)	Average + 2* standard deviation (ppm)	Variation percent
Fe	12.67	12.12	14.00	23.7
Cr	2.93	2.51	3.05	49.2
Al	5.33	5.73	7.25	27.0
Cu	2.47	2.34	2.82	32.6
Pb	1.24	1.09	1.37	55.0

3.2. Additive depletion

Once dispersion becomes "loaded" any added sludge, resin or soot will cause the oil to dump whatever it has collected and refuse to collect anymore. This results within a rapid period build-up engine deposits. The value of each additive material between 110 and 160 running hours has shown in figure 2. Results showed that the oil had an acceptable function after 150 running hours, and absolute variation percent of each additive material after 160 running hours were not more 50 percent.

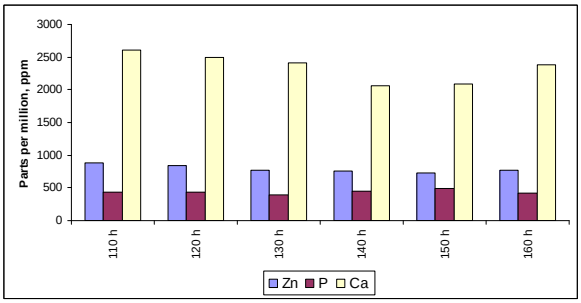

Fig 2: Result of additive materials analysis during different running hours

Ahmadi et al. illustrated that the building blocks of lube oil are known as base oil. Generally speaking, base oil is a mixture of various fractions from the crude oil refining process. Additives are then mixed within this base oil to impart additional desirable properties to the base oil. Base oil is refined by solvent extraction (usually with propane at a pressure high enough to keep it in liquid form) and hydrotreatment (reaction with hydrogen) (Ahmadi et al., 2009b).

3.3. Pollutant Materials

Silicon and sodium usually enter the oil from environment and they are known as pollutant materials. Figure 3 shows the amount of pollutant materials, silicon and sodium, in different oil analysis. The existence of silicon in oil analysis would be through entering dust into the engine.

Figure 3 and table 3 shows that the value of each pollutant material after 160 running hours was between the average of pollutant material plus two times of standard deviation of data those gotten during different running hours. Also the results of this table showed that the variation percent of each pollutant material after 160 running hours was not more than 50

percent. These results showed that the oil after 160 running hours could be used at more time and this shows that it is not commodious to change the oil at 125 running hour. There was no significant difference of pollutant materials among different running hours.

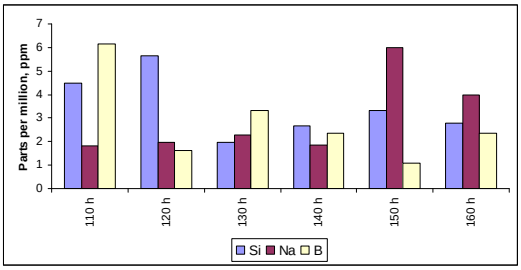

Fig 3: Pollutant materials analysis results during different running hours

pollutant material	Value (ppm)	Average + 1* standard deviation (ppm)	Average + 2* standard deviation (ppm)	Variation percent
Si	2.78	4.83	6.18	20.0
Na	3.98	4.66	6.34	33.8

Table 3: Value, warning zone and variation percent of pollutant materials of oil in 160 running hours

3.4. Wear Indices

Wear indices are belonged to important indices for showing the increasing of wear in engine parts. There are different wear indices, such as Particle Quantifier (PQ). This index shows the amount of particles that are bigger than 10 µm. Figure 4 shows the amount of PQ in the investigation between 110 and 160 running hours.

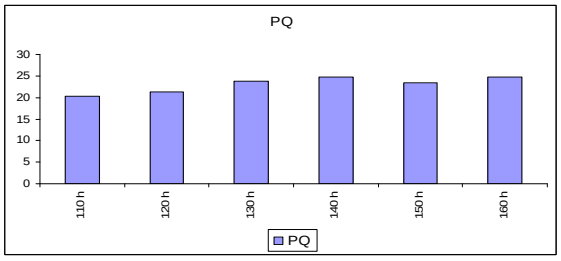

Fig 4: Particle Quantifier (PQ) analysis results during different running hours

Table 4 showed that the value of PQ after 160 running hours was between the average of PQ plus two times of standard deviation of data those gotten during different running hours. Also the results of this table showed that the variation percent of PQ after 160 running hours was not more than 50 percent.

Table 4: Value, warning zone and variation percent of PQ of oil in 160 running hours

Wear index	Value (ppm)	Average + 1* standard deviation (ppm)	Average + 2* standard deviation (ppm)	Variation percent
PQ	24.75	24.88	26.75	7.5

3.5. Viscosity

In this study the viscosity has been investigated by ASTM D-445 standard. The viscosity of industrial oils, by contrast, is mostly measured at 40ºC. The viscosity can be decreased by adding more fluid oil, or as a result of high water content, of by shearing of the VI-improver. The viscosity can be in creased by adding a more viscous oil, and by oil oxidation (e.g. as a result of overheating). The viscosity characteristics of oils at 40 ºC have been shown in table 6. The viscosity of industrial oils, by contrast, is mostly measured at 40ºC. Results of oils analysis for viscosity don't give us a reliable consequence. Figure 5 shows the amount of viscosity in the investigation between 110 and 160 running hours.

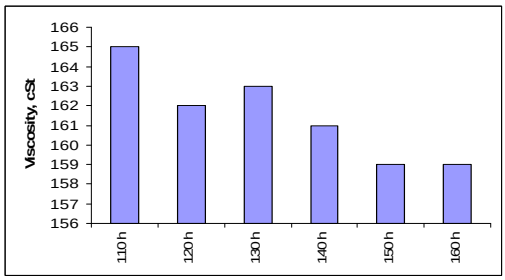

Fig 5: Viscosity analysis results during different running hours

3.6. Total Base Number (TBN)

In this study TBN has been investigated by ASTM D-2896 standard. Engines operating on heavier residual fuels are exposed to a more corrosive regime, as fuel sulphur levels are typically 2 to 4%. Here the TBN levels are typically between 20 and 40 dependent on fuel sulphur level. Maintaining a correct alkaline reserve is critical in preventing unnecessary corrosion of the upper piston, piston rings and top end bearing. Additionally, low TBN is indicative of reduced oil detergency (Ahmadi et al., 2009b).

The oil is continuously exposed to acidic combustion products and these must be neutralized before they could corrode engine parts (Ahmadi et al., 2009).

Too low a TBN volume can be due to: heavy oxidation of the oil, when the oil has been in service for too long, the oil level was insufficient, or due to a defective cooling system, producing overheating; use of a fuel containing a high sulphur content; use of an inappropriate lubricant; or contamination of the oil by fuel or water. The lowest recommended TBN for oil according to our fuel in Iran is 6. Figure 6 has shown the TBN change during the running hours of samples. Results showed that there wasn't significant difference between samples. The TBN level of each sample had more than recommended level of TBN. Figure 6 and table 5 shows the variation percent of viscosity and TBN at 40 ºC. According to the TBN analysis, the oil had an acceptable function until 150 running hours.

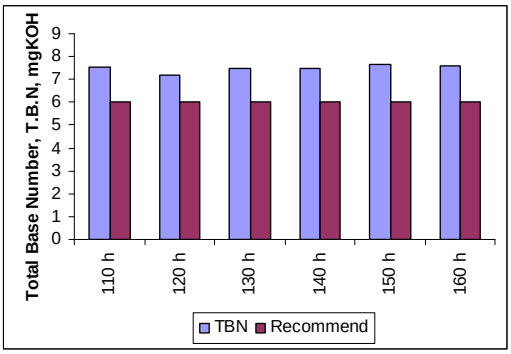

Fig 6: TBN changes during the different running hours

Table 5: Value, average minus standard deviation, average minus two times of standard deviation and variation percent of physical & chemical indices of oil in 150 and 160 running hours

Physical & chemical indices	Value (ppm)	Average - 1* standard deviation (ppm)	Average - 2* standard deviation (ppm)	Variation percent
VIS40	159	159.15	156.81	1.5
TBN	7.57	7.33	7.17	1.1

4. Conclusions

The objective of this research is to choose and investigate the best oil replacement time by oil condition monitoring for crane Liebherr model D9408 diesel engine. This is achieved by investigating different oil sample analyses of crane Liebherr model D9408 diesel engine. Right now, the oil of the diesel engine is replacing every 125 hours. According to the majority indices results of the oil analysis, But not for all of them, they had an acceptable function after 160 running hours. The variation percent of plumb in wear debris analysis was above 50 percent. Additive depletion results showed that the oil had an acceptable function after 150 running hours, and absolute variation percent of each additive material after 160 running hours were not more 50 percent. Pollutant materials results of this table showed that the variation percent of each pollutant material after 160 running hours was not more than 50 percent. Also the Particle Quantifier (PQ) results showed that the variation percent of PQ after 160 running hours was not more than 50 percent. Results of oils analysis for viscosity didn't give us a reliable consequence. According to the Total Base Number (TBN) analysis, the oil had an acceptable function until 150 running hours. Overall the best time for replacing the oil for this engine has been calculated as 150 running hours.

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20/10/2009

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Cold Laser as a Complementary Drug in the Treatment of Osteoarthritis

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Abstract: Osteoarthritis is a common cartilage condition and a major cause of pain and disability in older adults. Osteoarthritis most often occurs at the ends of the fingers, thumbs, neck, lower back, knees, and hips. Osteoarthritis hurts people in more than their joints: their finances and lifestyles also are affected**.** Magnetic susceptibility, dielectric relaxation in the frequency range 100 KHz up to 10 MHz of Hb molecule of osteoarthritic patients receiving anti inflammatory drugs were compared to those received drugs and subjected to soft laser emitted from He-Ne laser with two IR diodes. In addition SOD and whole blood ATP concentration enzyme were measured. The dielectric results indicated that the molecular shape tends to deviate from the non spherical form in patients treated with non steroidal anti inflammatory drugs, to spherical one in those receiving soft laser as an additive drug. Low power laser has significant ability to decrease the pain and suffering of arthritis as well as reducing the disease symptoms. Side effects of medications were reduced in patients received cold laser as a complementary drug. [Journal of American Science 2010;6(5):142-152]. (ISSN: 1545-1003).

Keywords: Osteoarthritis, dielectric properties, soft laser, hemoglobin, magnetic susceptibility.

Introduction

Osteoarthritis (OA, also known as degenerative arthritis, degenerative joint disease), is a group of diseases and mechanical abnormalities results from many metabolic, genetic, chemical, and mechanical factors (Valdes and Spector, 2008), involving degradation of joints (Brandt et al., 2008), including articular cartilage and the subchondral bone next to it. Clinical manifestations of OA may include joint pain, tenderness, stiffness, creaking, locking of joints, and sometimes local inflammation, OA is the most common form of arthritis (Brandt et al., 2009) and a major cause of impaired mobility and disability for the ageing populations (Kokebie and Block 2008 ; Lozada 2009). The greater the degree of inflammation, the greater the complaint of stiffness (Lin EHB et al, 2003). There are a number of drugs under development for symptomatic and disease modification, and several studies are also evaluating alternative therapies. There are several drugs on the market whose clinical effectiveness and long-term safety still need to be determined. This assessment is especially important since OA requires long-term disease management and the disease primarily affects people over the age of 60 who are most prone to drug toxicity, and for whom the potential for drug interactions are high. Information on the impact of the disease to society and the cost of disease management (including pharmacological and non-pharmacological treatments) needs to be re-evaluated. (Bradley et al., 1991; Stamm et al., 2002; Watkins et al., 2006 ; Towheed et al., 2006; Zhang et al., 2007)

At present, there is no cure for OA. The management of OA is broadly divided into non-pharmacological, pharmacological, and surgical treatments. Surgical management is generally reserved for failed medical management where functional disability affects a patient's quality of life. Pharmacological management includes control of pain and improvement in function and quality of life while limiting drug toxicity. Experts in this field suggest that appropriate therapy for OA combines one or more pharmacological agents with exercise, weight loss and physical therapy (i.e. non-pharmacological therapy) (Song et al., 2006).

The therapeutic aim is to treat pain, maintain motor strength, joint range of movement, mobility and therefore function (Dieppe PA, 2004; Dominick et al., 2004; Foster NE, et al., 2007; Reichenbach S., et al., 2007). Strategies include a pharma-cological and non-pharmacological approach. Surgical intervention requires strict indications (Rozendaal RM, et al., 2008 ; Sawitzke AD, et al., 2008 ; Zhang W, et al., 2008).While medicines, injections, and surgery all have their place and are valuable, there is still a need for

potentially useful adjunctive modalities that might speed up recovery and reduce pain faster (Messier SP, et al., 2000; Goldkind L, and Simon LS, 2006).

Lasers for treating osteoarthritis are approved by the FDA (Kipshidze N, et al., 2000), and there are a number of surveys that point to the laser's effectiveness. Low – energy lasers are now widely used to treat a variety of musculoskeletal conditions. Although controlled evaluation of these treatment is limited, quite enthusiastic claims are made for pain, arthritic and wound healing applications .Since these devices are safe, easy to use, and relatively inexpensive.(Ozdemir F., et al., 2001; Stergioulas, A., 2004; Ozcelik O., et al., 2008; and Kuhn A, et al., 2009)

Low level therapeutic laser, better known as phototherapy, is a relatively new form of treatment. Its premise is that certain wavelengths of light have effects on living tissue. This effect is termed "photobiomodulation." (Markovic AB, et al., 2006; Huang, Y.Y et al., 2009). Certain wavelengths of light at certain intensities (or irradiance to use the technically correct term) delivered by laser, LED or another monochromatic source may affect tissue regeneration, inflammation, or pain. (Gupta AK, et al., 1998; Schubert, V, 2001; and Bjordal JM, et al., 2006).

Laser treatment, unlike traditional surgical methods, is non-invasive and leaves no scarring. Laser treatments are often preferred over medicinal treatment, as lasers do not have the side effects pain pills often do. (Karu, T, 1999 ; Minatel DG, et al., 2009; Lapchak PA, et al., 2010). Laser therapy was introduced as a non-invasive treatment option for osteoarthritis .The effect produced by laser therapy is not thermal (heat) instead, it has to do with photochemical reactions in cells (Albert, Y. and Banerjee 1975; Karu T, 1988; Lohr NL, et al., 2009).

Many of the pharmacological agents used in the treatment of osteo arthritis can interfere with the various aspects of the inflammatory response. However, deform abilities is still occurred with low rate to some extent. Searching for new trends of treatments to achieve the therapeutic aim of the maximum benefit with minimal toxicity was the aim of the present study.

Material and methods

This study was conducted on 60 osteoarthritic patients, with age range from 47 to 76 years old. They were classified into two subgroups according to the type of treatment received. G_1 (25 patients, 15 females and 10 males) treated with non steroid anti inflammatory drugs. G_2 (35 patients, 20 females, 15 males) treated with non steroid anti inflammatory drugs and subjected to soft laser produced from mid laser infra red medical instrument. Patients received laser sessions along four weeks every other day. We irradiated the trigger points, access points to the joint, and striated muscles adjacent to relevant nerve roots. Patients were subjected to soft laser and detailed clinical examination in the Air Force Hospital. Blood samples were collected from the patients once after the last session of laser irradiation.

All patients were subjected to detailed clinical history, past history and laying stress on compliant of patients, onset and course of diseases, the pattern of joint involvement and extra articular affection. Pregnant women and patients suffering from other inflammatory diseases were excluded. A pulsed diode laser, He-Ne mid laser with IR manufactured by space laser SRI was used. Turin with continuous emission visible light 632.8 nm wavelength (Out put power 5 mw, output divergence after lens 60 mRad), in coaxial associated with 2 infra red diodes of wave length 904 nm, each with the following specification:

1-Infra- red laser emitters:

Peak output power = 5×10 w. Average output power = $5 \times (0.3 + 5)$ mw. Pulse width 180 nsec. Pulse frequency min. 200 Hz - Max. 4000 Hz. Output beam divergence 70 m Rad.

Number of diodes = 5

2- I.R. Handles:

Peak power = 10 W. Average output power = 3 mW (min.). Pulse duration 180 nsec. Pulse frequency 4000 Hz. Output beam divergence 70 mRad.

Magnetic susceptibility of hemo-globin is measured using the well known Alert method (Takizawa and Horie, 1986). The force excreted on the sample tube in the magnetic field is determined with a commercial semimicro-balance. This rests upon a mechanical stage, adjustable in two directions, which permits adjustment of the sample between the shoes. Thermal disturbances of the magnetic field (3 to 10 KG) are provided by a water-cooled Wiess magnet. The air gap is 3.8 cm. Fluctuations in the laboratory supply direct current voltage frequently call for a special supply for the magnet. The alternating current supply is fed via a magnetic stabilizer (constant voltage transformer) to an adjustable transformer and rectifier in a two-way rectifier stage. The magnet current can be controlled by

the regulating transformer. The components of the magnetometer are:

1- AC source .
2- Thermocouple
3- Variable transformer with control
4-Two-way rectifier
5- Magnet
6- Semi-micro balance
7- Power supply for the heating jacket
8- Magnetic constant voltage supply.

Calculation:

Volume magnetic susceptibility is given from :

$$K_S = \frac{\delta S}{S}\frac{W}{\delta W}\frac{\rho}{d}K_w + [1 - \frac{\delta S}{S}\frac{W}{\delta W}\frac{\rho}{d}K_a) \ldots (1)$$

Where

K_S : volume magnetic susceptibility

δS : weight change of the sample in and outside the field

δW : same parameter of the water

S : weight of sample

W : weight of water

ρ : density of sample

d : density of water

K_W is the volume magnetic susceptibility of water at the temperature of the measurements.

$K_W = -0.72145 \times 10\text{-}6 - 0.000108 (t\text{-}20) \times 10^{-6}$ (2)

K_a is the volume magnetic susceptibility of air which is 0.029×10^{-6}

Molar magnetic susceptibility is given from:

Molar Mag. Sus. = $K_S \times MW$ …….(3)

Determination of hemoglobin oscillator strength is done by using a spectrophotometer UV-Visible 240 Shimadzu (200-700 nm) at room temperature. The extinction coefficient at the wavelength ranged from 310 to 700 nm are taken and then oscillator strength per molar heme was calculated on the following equations:

$$f = \frac{2.303MC}{Na\,e^2} N_o \left(\frac{3}{N_o^2 + 2}\right)^2 \int \varepsilon \partial \upsilon \quad .(4)$$

$$= 1.44 \times 10^{-19} \times 0.841 \int \varepsilon \partial \upsilon \ldots . \quad (5)$$

$$= 1.44 \times 10^{-19} \times 0.841 \times 3 \times 10^{10} \times \ln \lambda \times \varepsilon . \quad (6)$$

Super oxide dismutase (SOD) and Adenosine triphosphate (ATP) enzymes activities were measured spectroph-otometrically. The reagents used were RANSOD by RANDOK kits (Randox .Crumlin.UK). The instrument used was a double beam spectrometer (UV Model 2410, shimadzu, Japan).

Dielectric Measurements:

The dielectric dispersion for 5% aqueous solution of Hb was measured at 25°C in frequency range 0.1 and 10 MHz for SLE patients compared to normal control using a Loss Factor meter type 1033, RFT, Funkwerk Erfurt. Gerny. The Hb samples were measured by the cell type pw 9510/60, manufactured by Philips. The sample cell has two squared platinum black electrodes each having an area of 0.8x0.8 cm^2 with an intermediate distance of 1 cm. The cell with the sample is kept at 25^0C ± 0.1 in a temperature controlled incubator Kotterman type 2771 Germany. The value of ε' (Relative permittivity of the sample in the cell) was calculated at each frequency from the constant K (the cell constant that depend on the cell dimensions) and C_o (The residual capacitance) and the measured values of C, also the loss tangent (tan $\boldsymbol{\delta}$) was obtained from the measured values of the resistance R and C in farad as:

$$\tan\delta = \frac{1}{2\pi f RC} = \frac{\varepsilon''}{\varepsilon'}$$ the dielectric loss ε" was calculated from the relation $\varepsilon'' = \varepsilon' \tan\delta$, the conductivity (σ) was then calculated from the relation

$$\sigma = 2\pi f \varepsilon'' \varepsilon_o = C/K$$

For spherical macromolecules the dielectric relaxation time depends on the viscosity of the liquid η and its absolute temperature T. Viscosity measurements of each Hb solution was carried out with an ostwald viscometer at concentration of 5% and 25 °C bi-distilled water was used first at fixed volume to pass through certain height of the Ostwald's capillary, then the efflux of water t_2 is determined three times and an and an average value was taken also the averaged efflux times t_1 for both G_1&G_2were determined, then the viscosity coefficient η_1 of each sample was calculated as

$$\frac{\eta_1}{\eta_2} = \frac{(f_1 t_1)}{(f_2 t_2)}$$

Where η_2 is the viscosity coefficient of water, f_1 and f_2 are the densities of water and solute molecules respectively.

Data were analyzed statistically by using the common T-test.

Results

Table (1) : Oscillator strength, molar magnetic susceptibility of osteoarthritic patients received anti inflammatory drugs G_1 as compared to those received soft laser G_2

	G_1 (n = 25)	G_2 (n= 35)
Oscillator strength	3.368 ± 0.65 ***	2.985 ± 0.48
Molar magnetic susceptibility	- 0.7321 ± 0.12 x 10^{-6}	- 0.8962 ± 0.14 x 10^{-6}***

*** $P < 0.001$

Table 1 illustrates a significant decrease in the oscillator strength per molar heme concomitant with Molar magnetic susceptibility of Hb of G_2 as compared to G_1.

Table (2) : Serum ATP concentration and SOD activity of osteoarthritic patients received anti inflammatory drugs G_1 as compared to those received soft laser G_2

	G_1 (n = 25)	G_2 (n= 35)
ATP conc. mg/100 ml	20.173 ± 0.58	21.285 ± 0.46 ***
SOD conc. mg/ml	83.23 ± 0.61	81.83 ± 4.67 ***

* $P < 0.01$ *** $P < 0.001$

Whole body ATP concentration revealed a significant increase in patients treated with anti-inflammatory drugs and irradiated with soft laser G_2 as compared to those received anti inflammatory drugs G_1. Highly significant decrease in the concentration of Super oxide dismutase activity was observed in G_1 as compared to G_2.

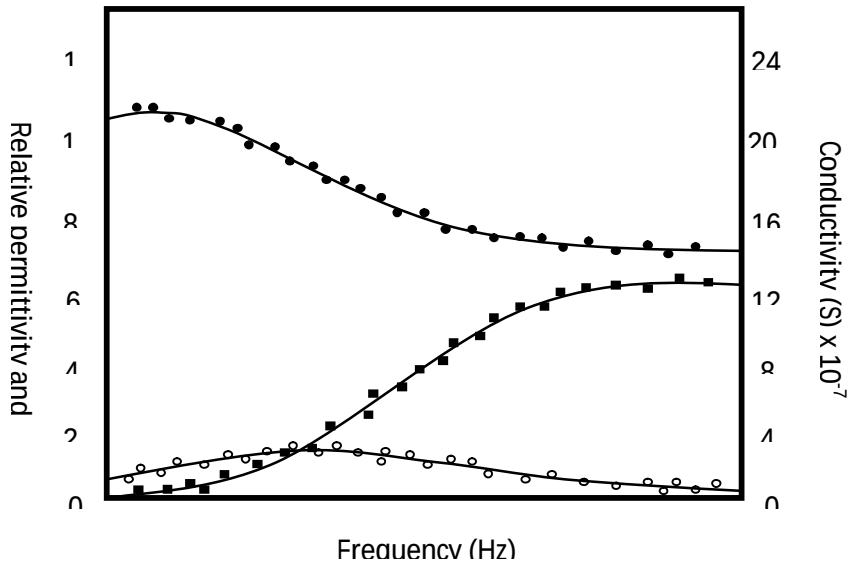

Fig. (1) Variation of relative permittivity (ε'), (°); dielectric loss (ε''), (°); conductivity (S), (') with frequency for 5 % aqueous G1 Hb solution at 25 °C.

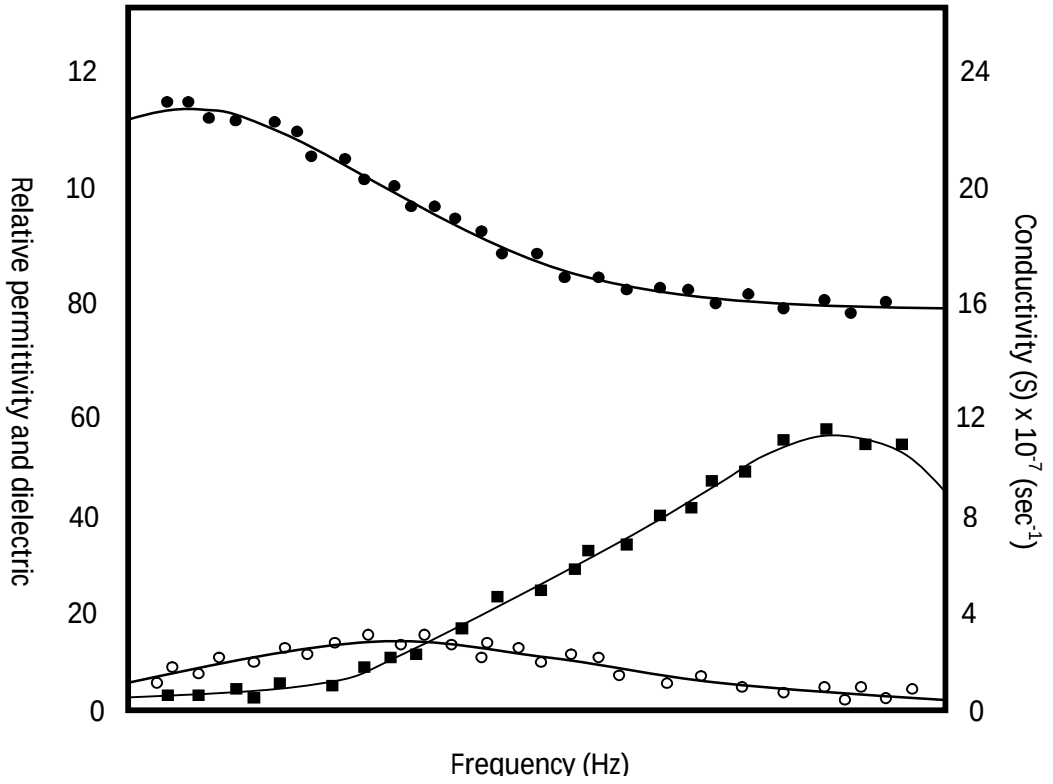

Fig. (2) Variation of relative permittivity (ε'), (°); dielectric loss (ε''), (°); conductivity (S), (˙) with frequency for 5 % aqueous G2 Hb solution at 25 °C.

Figure 1, 2 illustrate The results of the relative permittivity ε', the dielectric loss ε'' and conductivity S were measured in the frequency range 0.1 to 10 MHz for (G_1 and G_2) respectively .These figures indicate that Hb has a critical frequency f_c ranging from0.5 to 0.6 MHz at 25°C & 5% aqueous solution of hemoglobin

Table (3): Values of the static ε_s and infinite ε_∞ , dielectric constant, dielectric increment per gm per 1000 ml, cole-cole parameter α , relaxation time τ_β in *M* sec., viscosity coefficient η in poise and molecular radius in nm

	ε_∞^*	ε_s^*	Δ_β	α^*	τ_β^*	H	r
G_1	74.26±0.71	103.37±0.67	$0.874\pm3\times10^{-3}$	$0.1148\pm3.6\times10^{-4}$	$0.496\pm2.7\times10^{-3}$	$0.0152\pm3\times10^{-5}$	$3.706\pm4.3\times10^{-3}$
G_2	77.16±0.52	101.31±0.62	$0.63\pm2\times10^{-3}$	$0.0121\pm4\times10^{-4}$	$0.308\pm2.8\times10^{-4}$	$0.0224\pm2.4\times10^{-4}$	3.341±0.0015

* Fitted parameters from the computer program

Table 3 illustrates the values of the static ε_s and infinite ε_∞, dielectric constant, dielectric increment per gm per 1000 ml, cole-cole, the relaxation time ζ_β, viscosity coefficient η in poise and molecular radius in nm for G_1&G_2. The molecular radius of Hb (r) was calculated from the data of relaxation time ζ_β through $\tau_\beta = \frac{4\pi r^3 \eta}{k\tau}$.

The results indicated that the radius of Hb molecule decreased as well as the relaxation time in G_2 compared to G_1. The dielectric increment (Δ_β) per gm per liter was calculated from $\tau_\beta = \frac{\varepsilon_s - \varepsilon_\infty}{C}$ where C is the concentration of Hb solution in gm/liter. The results of the dielectric increment indicated a higher value in G_1 compared to G_2. Figures 3, 4 show cole- cole plot (ε" vs ε') for (G_1, G_2) respectively. From these figures, the values of the cole-cole parameter (α) for all samples are deduced and given in table 3, these results revealed that there is a wide distribution of the relaxation times of Hb molecules of G_1 compared to G_2. The curve fitting analysis has shown that, the cole –cole model gave a better fit for the dielectric data.

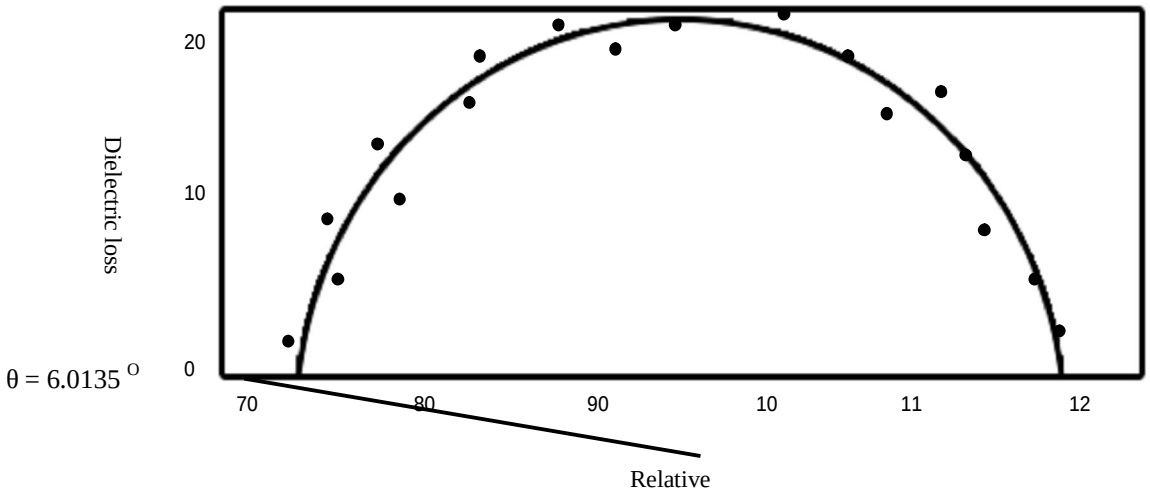

Fig. (3) : Cole-Cole plot of patients treated with non steroid anti-inflammatory drugs

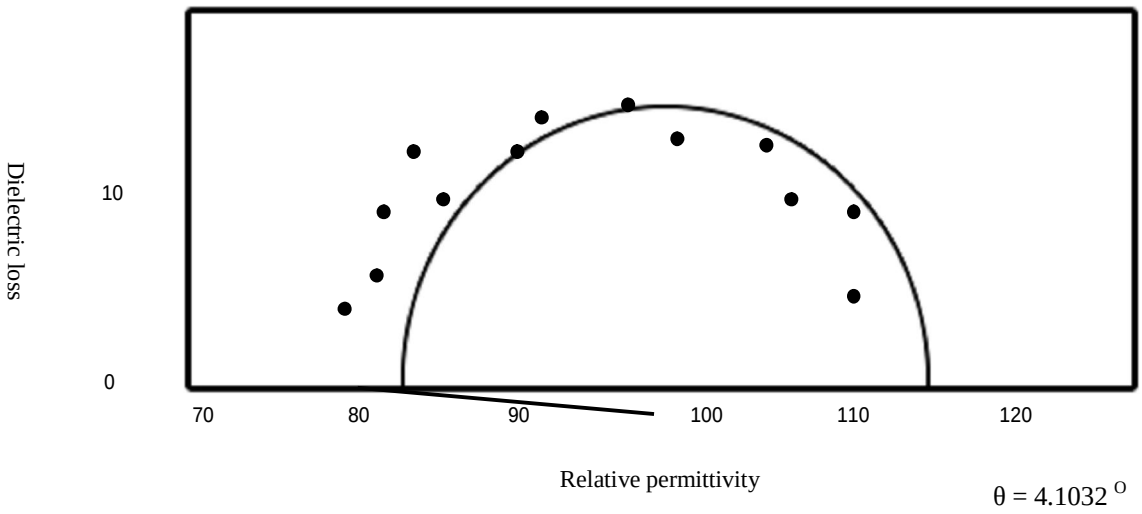

Fig. (4) : Cole-Cole plot patients treated with ant-inflammatory drug and subjected to soft laser

Discussion

The present study represents a preferable improvement due to the line of interaction treatment by using common anti inflammatory drugs coupled with cold laser in the treatment of osteoarthritis disease.

Oscillator strength is a characteristic constant that reflects the total light absorption of an electronic transition. It provides quantitative information on the electronic states of the heme-prosthetic group. Significant decrease in oscillator strength of hemoglobin of osteoarthritic patients subjected to soft laser (G_2) as compared to those treated with anti inflammatory drugs (G_1) confirm the stabilization of hemoglobin as a folding process (Freedman DE, et al., 2010).

Since the ferric atom in any form of chemical binding has an odd number of electrons. Thus, the increment of magnetic susceptibility of hemoglobin of osteoarthritic patients G_1 compared to those receiving soft laser G_2 seems to be related to a degree of oxidation instead of oxygenation (Regan E, et al., 2005).

SOD in general is known to be produced within the RBC by the spontaneous oxidation of oxy hemoglobin of isolated Hb chains, and in particular would appear to be an indicator of disease response (Regan EA, et al., 2008**).**

The inhibitory effect of low power laser on the emergence of chemotactic factors, which appeared as decrement SOD concentration in the RBC of G_2 compared to G_1. Another possibility is that a low power laser may interfere with the effect of chemical mediators or super oxide induced by inflammation causing re absorption of exudates and facilitating the elimination of algogenic substances (Cho HJ, et al., 2004; Benedicenti S, et al., 2008; Hegedus B, et al., 2009)

An abnormal release of activated species of oxygen in RBC, is believed to be responsible for extensive cellular damage such as the Hb precipitation as Hinz bodies and peroxidase of erythrocyte membrane. Damage by oxygen free radicals within the RBC is prevented by curpo -zinc. SOD, this enzyme catalyzes the dismutation of O_2^* by forming O_2 and H_2O_2 which is subsequently catabolized by catalase and / or glutathione peroxidase (Gen Dent, 2008; Yamaura M, et al., 2009; Rubio CR, et al., 2009).

The photochemical and / or photo physical process of low power laser irradiation on cells, appeared from the increase of ATP in blood of patients exposed to soft laser (G_2) compared to (G_1) (Blanco FJ, et al., 2004; Kassák P, et al., 2006 ; Toncheva A, et al., 2009).

Light is absorbed by enzymes in the mitochondria, which activates the respiratory chain by accelerating the electron transfer in the redox pairs in some sections of the respiratory chain, which promotes proton influx through ATP synthetase. This may result in enhanced ATP synthesis. These changes then initiate a cascade of molecular events leading to a final cellular response**,** suggested that irradiation with a different wave length (specially 904 nm) could initiate the same final cellular response at a different point in the sequence of molecular events. It is believed that the small changes in concentration of adenine nucleotides (ATP, ADP and AMP) induce considerable changes in cellular metabolism since the nucleotides act as allosteric effectors (activators and inhibitors) of the several key enzymes of energy metabolism (Kujawa J, et al., 2004 ; Kocer I, et al., 2007).

Dielectric relaxation technique, gives more useful informations about some biophysical properties of the molecule such as the relaxation time ζ_β, the shape of the molecule and the viscosity coefficient η. The variation of the conductivity S as a function of frequency can be considered as another viewpoint for treating the dispersion data in the β region. It was shown that at high frequency end, the conductivity curve is still elevated for G_2 while flattening appeared in patients treated with anti-inflammatory drugs(G_1).

It is clear from the dielectric relaxation data in table 3, that both the relaxation time & radius of the Hb molecules increased for G_1 as compared to G_2. The shift towards lower or higher frequencies f_c, as indicated from the β dispersion in fig (1-4) is attributed to changes in molecular radius. Since smaller molecules have shorter relaxation times and hence larger critical frequencies (Debye, P., 1929 ; K.S Cole, and R.H Cole. 1941; Srivastova, A, et al., 1997; Samiha T. Bishay. 2000 ; De Morais NC, et al., 2009).

There is a marked increase in the dielectric increment for G_1 as compared to G_2, it may be presumed that the activity of the disease may result in the variation of the dielectric increment.

Theoretical treatment of the dielectric relaxation data to calculate the Cole –Cole parameter α illustrates another form of the conformational changes in the hemoglobin, the values of α show a very wide distribution of relaxation time. Cole-Cole plot (ε" vs ε') is nearly semi circle. The shape of Hb molecule tend to shift from the non spherical form to spherical one (Fig; 3,4) i.e. a decrement in the unfolding process in G_2 as compared to G_1. The change in the tertiary structure of Hb molecule results in a change in its molecular shape from non spherical form (G_1) to nearly spherical form in G_2 with different values of the parameter α.

These conformational changes may be attributed to direct effect of laser on Hb molecules, or to an indirect effect with many enzymes systems related to Hb functions in erythrocyte. There is a hypothesis that cell components may be re-oriented by the linear polarization of laser and as a result its metabolic may becomes activated (Obay BD, et al., 2008; Karu, TI, 2008; Chow, RT et al., 2009).

The indirect effect of soft laser, on Hb conformation may be through interaction with these erythrocyte enzymes. Reaction rate of H_2O_2 decomposition by catalase increased may therefore reduce the extent of the side reactions that are destructive to the protein moiety, preserving the stable (native) tertiary structure of hemoglobin molecule. The magnitude of this effect therefore enhanced with the laser irradiation. (Lubart R, et al., 2006; Tumilty, et al., 2009).

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Association of serum Leptin and Adiponectin with Atherosclerosis in obese and non-obese Type 2 Diabetes Mellitus patients

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Abstract: Obesity is a major risk factor for insulin resistance, type 2 diabetes, heart disease, and many other chronic diseases The current study was designed to investigate the endogenous mechanism by which obesity may increase the risk of CVD by examining whether serum adiponectin, Leptin or insulin mediate the association of obesity and type2 diabetes and cardiovascular risk factors in Egyptian adult patients. **Patients and Methods:** This study included 82 subjects, 30 patients suffering from type 2 diabetes and 52 patients suffering from type 2 diabetes together with coronary artery disease (CAD) together with another group having CAD without diabetes. They were classified according to their body mass index (BMI) into obese and non-obese groups, also 25 healthy volunteers were considered as controls. All patients were subjected to anthropometric assessment and laboratory determination of serum Adiponectin, Leptin, insulin and glucose. Insulin resistance was established by homeostasis model assessment (HOMA-IR) Differences in clinical or laboratory parameters among groups were compared by using one-way ANOVA test. **Results** revealed highly significant decrease in Adiponectin levels and highly significant increase in serum Leptin in non obese groups (G1 (T2D), G2 (CAD) and G3 (T2D+ CAD) as compared to controls. However, there were no statistical variations between non obese groups when compared to each others. HOMA-IR showed highly significant increase in non obese groups as compared to both controls and each other. Also, the results showed high significant decrease in Adiponectin and highly significant increase in Leptin in obese groups (G4 (T2D), G5 (CAD) and G6 (T2D+CAD) when compared to controls. However, there were no statistical variations between obese groups when compared to each others as regard Adiponectin, while Leptin showed statistical increase between (G4) and (G5) groups when compared to each others, HOMA-IR showed highly significant increase in the two obese groups only (G4 and G6) as compared to controls, while there was no significant variation in (G5) when compared to controls. Moreover, there was a significant increase in all obese groups when compared to each other. Also, there was significant correlation between serum Adiponectin and Leptin in obese DM patients. **Conclusion:** The coexistence of correlation between serum leptin and Adiponectin levels in addition to increase of serum leptin and decrease serum Adiponectin levels in obese DM patients in the current study; support the hypothesis of their susceptibility to atherosclerosis. [Journal of American Science 2010;6(5):153-164]. (ISSN: 1545-1003).

Keywords: Type2 Diabetes, Cardiovascular disease, Adiponectin, Leptin, HOMA-IR

1. Introduction

Obesity is a major risk factor for insulin resistance (IR), type 2 diabetes, heart disease and many other chronic diseases .These associations are influenced by adipose tissue (AT) distribution (Marcus et al., 1999). IR is characteristically more severe in T2DM than in similarly obese non-diabetics, but whether this difference is related to differences in body composition is unclear (Azuma et al., 2007; Marinou et al., 2009).

Type 2 diabetes mellitus (T2D) is considered one of the major metabolic diseases of 21st century (Kowalska,2007, Thévenod 2008). The excessive intake of food, sedentary life style and lack of physical activity are responsible for the growing epidemic of obesity, together with the increasing rate of T2D in many parts of the world (Zimmet, et al., 2001) The main burden of T2D is connected with development of vascular complications, which are a consequence of accelerated atherogenesis (Cooper et al., 2001; Salas-Salvadó et al., 2007).

Atherosclerotic cardiovascular complications are the major causes of morbidity and mortality in type 2 diabetic patients. Cardiovascular disease is the main cause of premature mortality and two to six time's greater morbidity of T2D patients than of non-diabetic people. There are several mechanisms which could play a role in the pathogenesis of vascular complications and most of them are triggered by hyperglycemia and hyperglycemia-induced oxidative stress (Qasim et al., 2008).

However, during the past few years a lot of attention has been paid to the potential role of adipose

tissue in the development of vascular complications of diabetes. As obesity is considered to be a major risk factor for atherosclerosis, understanding of the underlying mechanisms leading to obesity and linking obesity with atherogenesis is necessary, for the development of therapeutic strategies against atherosclerosis. The pathophysiology of CVD linked to obesity is an area of intensive research (Marinou et al., 2009). The relation between obesity and CVD is indeed complicated. Some investigators suggest that the connection is indirect and dependent on the increased prevalence of diabetes, hypertension and dyslipidemia, whereas others have demonstrated an independent association between obesity (and especially abdominal obesity) and CVD risk (Frayn 2005). The relationship between obesity and CVD appears to develop at a relatively young age. Obesity in young men, aged 15 to 34 years, is associated with accelerated coronary atherosclerosis (McGill et al. 2002).

Excessive adiposity is the most important risk factor in the development of insulin resistance and type 2 diabetes mellitus (Bloomgarden.,2002). The pathophysiology linking obesity to type 2 diabetes is not completely understood, but adipokines are thought to be involved (Jazet et al., 2003). Adipose tissue produces several proteins (adipocytokines) such as leptin, adiponectin, resistin, tumor-necrosis factor-a, and interleukin (IL)-6, that modulate insulin sensitivity and appear to play an important role in the pathogenesis of insulin resistance, diabetes, dyslipidemia, inflammation, and atherosclerosis (Trujillo & Scherer , 2005, Arita et al., 1999 Kershaw and Flier 2004; Arner ,2005; Fantuzzi .,2005; Hotamisligil and Spiegelman 1994; Hotamisligil et al., 1993, Hotamisligil et al.,1994). However, the mechanisms by which fat tissue induces insulin resistance and the role of adipocytokines in the pathogenesis of type 2 diabetes mellitus have not been well established.

Adipokines are fat-secreted biomolecules with diverse signaling effects that modulate insulin resistance, hepatic lipoprotein production, and vascular inflammation . Two in particular, Adiponectin and leptin, are almost exclusively fat derived and have antithetic actions in insulin resistance and in vascular signaling. Because of these properties, Adiponectin and leptin have been proposed as biomarkers of adipose function that may add value in predicting cardiovascular disease (CVD) risk and provide targets for therapeutic interventions (Qasim et al., 2008).

Levels of Adiponectin, an insulin-sensitizing hormone with anti-inflammatory properties, are reduced in obesity, type 2 diabetes, and coronary artery disease (CAD) compared with controls (Behre., 2007) Indeed, several (Schulze et al., 2005, Pischon et al., 2004]) but not all (Lawlor et al., 2005, Lindsay et al., 2005) epidemiologic studies suggest that reduced plasma Adiponectin levels are independent predictors of

Leptin, on the other hand, is a pleiotropic adipokine that modulates innate immune functions and vascular signaling in addition to its central role in regulation of appetite and energy expenditure (Hamann & Matthae, 1996) In contrast to adiponectin, leptin levels directly correlate with insulin resistance, obesity (Chu et al.,2000) and several CVD risk factors (Haynes., 2005) Leptin levels have been associated with CVD beyond body mass index (BMI) in some studies (Reilly et al.,2004) Several large population based studies have suggested strong positive associations between leptin and insulin resistance and its components including hyperlipidemia and hypertension, independent of body mass index (Koerner et al.,2005).

The current study was designed to investigate the endogenous mechanism by which obesity may increase the risk of CVD by examining whether fasting Adiponectin ,leptin or insulin mediate the association of obesity and type2 diabetes, and thrombogenic cardiovascular risk factors in a national population-based cohort of Egyptian adults. A second purpose was to determine whether Adiponectin, leptin and insulin are associated with these CVD risk factors, independent of obesity and type2 diabetes.

Patients and Methods

Patients

This study included 81 subjects, 29 patients suffering from type 2 diabetes selected from National Institute of Diabetes and Endocrinology, 52 patients suffering from type 2 diabetes together with coronary artery disease (CAD) and another group with CAD without diabetes were selected from the National Institute of Heart Disease, they were classified according to their body mass index (BMI) into obese and non-obese groups, obesity was defined according to the WHO criterion (BMI>30kg/m2), also 25 healthy volunteers were considered as controls.

Type 2 diabetes mellitus was diagnosed based on the criteria of World Health Organization criteria (Alberti and Zimmet, 1998). The duration of diabetes was 9.1+ 0.7 years (mean+ SD). No subject had clinical or laboratory signs of acute infection and none had a history or presence of clinically evident cardiovascular disease. They do not receive insulin therapy.

The CAD inclusion criteria were: angiographic evidence with >50% occlusion of ≥1 major coronary arteries, old myocardial infarction, or angina pectoris, but any possible non-Atherogenesis occlusions such as osteal stenosis and spasm were excluded. Electrocardiogram (ECG)) abnormality was

defined when the interpretation was of a trial fibrillation, a major ST-T segment change, left ventricular hypertrophy or a ventricular conduction defect.

All subjects provided informed consent and the study protocol was approved by the Ethics Committee of the Institutional Review Board of National Institute of Diabetes & Endocrinology and National Heart Institute.

All patients were classified according to their body mass index (BMI) into obese and non-obese groups, obesity was defined according to the WHO criterion (BMI>30kg/m2).

- G1: included 10 non-obese suffering from type II diabetes mellitus patients without history of cardiovascular complications (T2DM)
- G2: included 19 non-obese patients suffer from Coronary Artery Disease without suffering from type II diabetes mellitus (CAD)
- G3: included 10 non-obese patients suffer from T2DM+CAD
- G4 included 19 obese suffering from type II diabetes mellitus patients without history of cardiovascular complications
- G5: included 10 non-obese patients suffer from CAD without suffering from type II diabetes mellitus
- G6: included 13 obese patients suffer from T2DM+CAD

Beside reference group included (25) healthy as controls. Criteria for normal controls included (1) absence of history of CHD. (2) Absence of hypertension, diabetes mellitus, or impaired renal function, and (3) Normal ECG and chest x-ray.

Anthropometric assessments

Body mass index:

Body mass index calculated as the body weight divided by the square of the height (kg/m2) was used as a marker of obesity. Weight and height were measured on the third or fourth day after admission while the subjects were fasting and wearing only their undergarments. Patients were designated as obese where BMI exceeded 30 kg/m2 and were considered non-obese where BMI was below 30 kg/m2.

Blood samples and Laboratory assessments:

Venous blood samples were obtained from all the patients on admission to the hospital. Venous blood specimens were collected without EDTA-treated and plain tubes after a 12-h fast. The tubes were immediately placed on ice until they arrived at the laboratory room (within 1–3h) and were stored at −70 °C until analysis. The CVs of the biomarker measurements were all <6%.

For glucose tests, blood was collected in fluorinated tubes and plasma was immediately separated and kept refrigerated at 4° C for up to 48 hours. Plasma glucose was determined by the glucose-oxidase method.

Assessment of insulin resistance

Fasting plasma glucose (FPG) was measured using an enzymatic colorimetric method with glucose oxidase. Insulin was measured by micro particle enzyme assay (Abbott, Chicago, IL). .Homeostasis model assessment (HOMA) or (HOMAIR) was used to detect the degree of insulin resistance (Matthews et al., 1985). In each subject, the degree of insulin resistance can be assessed from the fasting glucose and insulin concentrations by the formula: resistance (HOMA-IR.)

HOMA IR (%) = fasting blood glucose (mg/dL)/18 × fasting insulin (μU/mL)/22.5.

Measurement of serum Adiponectin and Leptin

Serum samples for measurement of Adiponectin and leptin were stored at - 80°C until subsequent analysis. The quantitative determination of human Leptin was conducted in serum by solid phase ELISA techniques, using commercially available kit, purchased from R&D Systems Inc., (Minneapolis, USA). Adiponectin was determined by ELISA (Human Adiponectin ELISA Kit, Linco Research, Inc., St. Charles, MO; intra-assay coefficient of variation, 1.0–7.4%; intra-assay coefficient of variation, 2.4–8.4%; sensitivity, 0.5 ng/ml).

Statistical analyses

Statistical analyses were conducted using SPSS for Windows, Version 11.0 (SPSS, Chicago, IL). Data are expressed as means ± standard deviations

Significant differences between groups were evaluated using ANOVA with post hoc testing. Possible associations between variables were tested using Pearson's correlation .Differences were considered statistically significant at $p < 0.05$.

Results

Table (1a &b) and figure (1&2) summarizes data and Multiple Comparisons (Post Hoc Tests) among the different studied non-obese groups.

-Serum Adiponectin:

In non obese groups

The results revealed that the mean values ± S.E.M of Adiponectin in controls (C) and non obese patients groups T2D (G1), CAD (G2),and T2D+CAD (G3)] were 15.43± 0.57, 10.86 ±0.45, 11.29±0.4,and 11.158±0.68 ng/ml respectively with ranges of 10.8-

20.6, 8.14-12.7, 7.86-14.62,and 7.7-14.38 ng/ml (Table 1). The result showed that the total serum Adiponectin levels were lower than the upper limit in 10 cases (100%), 19 cases (100%) and 10 cases (100%).in group (G1, (G2) and (G3) respectively. The statistical analysis revealed highly significant decrease in non obese groups (G1, G2 and G3) as compared to controls (P<0.001).However, there was no statistical variation between non Obese groups when compared to each others (Table 2a).

In obese groups

The results revealed that the mean values ± S.E.M of Adiponectin in controls (C), obese patient groups [T2D (G4), CAD (G5), and T2D+CAD (G6)] were15.43± 0.57, 9.99 ±0.71, 11.69±0.67,and 9.9±0.4. ng/ml with ranges of 10.8-20.6, 5.18-15.91 , 8.3-14.4, and 7.9-12 ng/ml respectively (Table 1a)

The result showed that the total serum Adiponectin levels were lower than the upper limit in 19 cases (100%) 10 cases (100%) and13 cases (100%) in group G4, G5 and G6. The statistical analysis revealed high significant decrease in obese groups (G4, G5, and G6) when compared to controls (P<0.001).However, there were no statistical variations between obese groups when compared to each others.

-Serum Leptin:

In non Obese

The results revealed that the mean values ± S.E.M of Leptin in controls (C),T2D (G1), CAD (G2),and T2D+CAD (G3) were 3.87± 0.19, 5.66±.0.29, 5.92±.0.45 ,and 6.645±0.57 ng/ml with ranges of 2.17-5.6, 4.1-6.9, 3.4-9.88,and 4.18-9.22 ng/ml. respectively (Table 1a)

The result showed that serum Leptin levels were higher than the upper limit in 5 cases (50%), 10 cases (53%) and 6 cases (32%) in group G1, G2 and G3 respectively.

The statistical analysis revealed highly significant increase in non Obese groups (G1, G2, and G3) as compared to controls (P<0.001).However, there was no statistical variation between obese and non obese.

In Obese

The results revealed that the mean values ± S.E.M of Leptin in controls (C), obeseT2D (G4), obese CAD (G5), and Obese T2D+CAD (G6) were, 3.87±0.19, 5.05±.0.41, 6.56±0.99 and 7.8±0.40 ng/ml respectively with ranges of 2.17-5.6, 2.43-9, 2.36-11.84, and 5.2-10.04 ng/ml (Table 1a). Serum Leptin levels were higher than the upper limit in 11 cases (85%) in group (G4), 5 cases (50%) in group (G5), 7 cases (36%) in group (G6). The statistical analysis revealed high significant increase in obese groups (G4, G5, and G6) as compared to controls (P<0.001).Moreover, there was statistical increase between (G4) and (G5) groups when compared to each others, but there was no significant variation between (G5) and (G6) groups when compared to each others (P>0.05) (Tablevariation between non Obese groups when compared to each others (Table 2a).

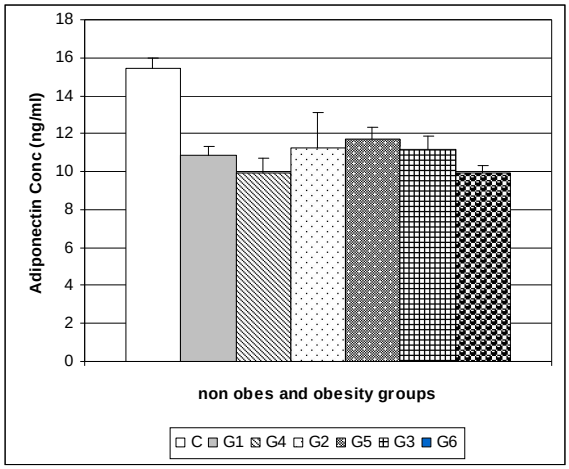


Fig. 1. Adiponectin Conc. (ng/ml) in Different Studied Groups (non obese and obese groups).
C: Controls; G1: T2DM (non obese); G4: T2DM (obese); G2: CAD (non obese); G5: CAD (obese); G3: T2DM+CAD (non obese); G6: T2DM+CAD (obese)

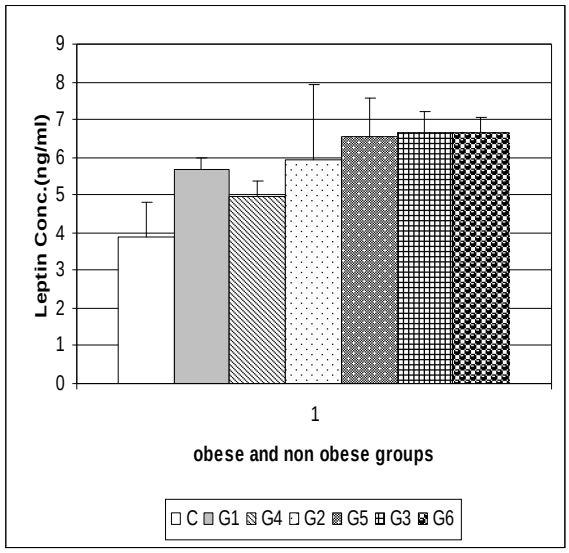


Fig. 2. Leptin Conc. (ng/ml) in Different Studied Groups (non obese and obese groups).
C: Controls; G1: T2DM (non obese); G4: T2DM (obese); G2: CAD (non obese); G5: CAD (obese); G3: T2DM+CAD (non obese); G6: T2DM+CAD (obese)

Table (1a). Collective Data for Serum Adipokines (Adiponectin, Leptin) in non Obese, obese and Healthy Control Groups

Parameters / Groups		**Controls (C)**	**T2DM**		**CAD**		**T2DM +CAD**	
			Non-obese (G1)	Obese (G4)	Non-obese (G2)	Obese (G5)	Non-obese (G3	Obese (G6)
BMI	N	25	10	19	19	10	10	13
	Range	21.70- 25.10	20.00 -29.50	5.18-15.91	22.00-30.00	31.00-36.00	25.00-30.00	31.51-44.00
	Mean	23.0720	26.0500	9.9579	26.3842	32.6000	27.9330	36.0438
	S.D.	1.04184	2.96695	3.25528	2.13027	1.71270	1.87806	3.88264
	S.E.	.20837	.93823	.74681	.48872	.54160	.59389	1.07685
Adiponectin	N	25	10	19	19	10	10	13
	Range	10.80-20.60	8.14-12.71	2.43-9.00	7.86- 14.62	8.30-14.40	7.68-14.38	7.90-12.00
	Mean	15.4300	10.8560	4.9358	11.2858	11.6900	11.1579	9.9231
	S.D.	2.86669	1.41221	1.81995	1.82027	2.11474	2.14920	1.35040
	S.E.	.57334	.44658	.41752	.41760	.66874	.67964	.37453
Leptin	N	25	10	19	19	10	10	13
	Range	2.17-5.60	4.10-6.93	2.43-9.00	3.40-9.88	2.36-11.84	4.18-9.22	5.20-10.04-
	Mean	3.8696	5.6901	4.9358	5.9247	6.5610	6.6450	7.8477
	S.D.	.95263	.92908	1.81995	1.97883	3.13274	1.79425	1.52043
	S.E.	.19053	.29380	.41752	.45397	.99066	.56739	.42169

Table (1b). Collective Data for Serum Glucose, Insulin and HOMA-IR in non Obese, obese and Healthy Control Groups

Parameters / Groups		Controls (C)	T2DM		CAD		T2DM +CAD	
			Non-obese (G1)	Obese (G4)	Non-obese (G2)	Obese (G5)	Non-obese (G1)	Obese (G4)
Glucose	N	25	10	19	19	10	10	13
	Range	75.00-105.00	123.00-311.00	110.00-357.00	82.00-127.00	75.00-114.78	123.00-228.00	141.25-265.00
	Mean	86.2000	198.6000	230.3684	99.6316	93.1780	169.5000	184.8715
	S.D.	9.21954	51.84421	67.55344	12.20751	12.49912	33.67904	40.68162
	S.E.	1.84391	16.39458	15.49782	2.80060	3.95257	10.65025	11.28305
Insulin	N	25	10	19	19	10	10	13
	Range	3.60-8.90	6.11-19.21	2.70- 10.90	1.09-17.66	4.30-55.31	5.24-17.72	6.42-27.21
	Mean	5.4904	10.1360	5.9842	7.8221	11.6450	11.3830	16.5623
	S.D.	1.48685	4.22863	2.35118	4.81128	15.47841	3.72399	5.92084
	S.E.	.29737	1.33721	.53940	1.10378	4.89470	1.17763	1.64214
HOMA-IR	N	25	10	19	19	10	10	13
	Range	.70-2.20	2.80-9.60	2.70-10.90	.30-4.10	.80-11.50	2.30	4.00
	Mean	1.1840	4.8500	5.9842	1.8632	2.5900	4.7800	7.4923
	S.D.	.41501	2.18187	2.35118	1.12852	3.19668	1.94867	3.43765
	S.E.	.08300	.68997	.53940	.25890	1.01088	.61623	.95343

Table 2 (a): Multiple Comparisons (Post Hoc Tests) Between Age, BMI, Adiponectin and Leptin in non obese Patients and healthy control Groups

Dependent Variable	(I) DIAGNOSIS	(J) DIAGNOSIS	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
BMI	C	G1	**-2.9780***	.71436	.000	-4.4069	-1.5491
		G2	**-3.3122***	.58108	.000	-4.4745	-2.1499
		G3	**-4.8610***	.71436	.000	-6.2899	-3.4321
	G1	C	**2.9780***	.71436	.000	1.5491	4.4069
		G2	-.3342	.74589	.656	-1.8262	1.1578
		G3	**-1.8830***	.85382	.031	-3.5909	-.1751
	G2	C	**3.3122***	.58108	.000	2.1499	4.4745
		G1	.3342	.74589	.656	-1.1578	1.8262
		G3	**-1.5488***	.74589	.042	-3.0408	-.0568
	G3	C	**4.8610***	.71436	.000	3.4321	6.2899

		G1	**1.8830***	.85382	**.031**	.1751	3.5909
		G2	**1.5488***	.74589	**.042**	.0568	3.0408
ADIPONEC	C	G3	**4.2721***	.92511	**.000**	2.4051	6.1391
		G1	**4.5740***	.92511	**.000**	2.7070	6.4410
		G2	**4.1442***	.69890	**.000**	2.7462	5.5422
	G3	C	**-4.2721***	.92511	**.000**	-6.1391	-2.4051
		G1	.3019	1.10572	.786	-1.9295	2.5333
		G2	-.1279	.89714	.887	-1.9224	1.6667
	G1	C	**-4.5740***	.92511	**.000**	-6.4410	-2.7070
		G2	-.4298	.89714	.634	-2.2243	1.3648
		G3	-.3019	1.10572	.786	-2.5333	1.9295
	G2	C	**2.0551***	.44628	**.000**	1.1624	2.9478
		G2	.2346	.57286	.684	-.9113	1.3805
		G3	-.7203	.57286	.214	-1.8662	.4256
LEPTIN	C	G3	**-2.7754***	.44167	**.000**	-3.6667	-1.8841
		G2	**-2.0551***	.44628	**.000**	-2.9478	-1.1624
		G1	**-1.8205**	.44167	**.000**	-2.7118	-.9292
	G3	C1	**2.7754***	.44167	**.000**	1.8841	3.6667
		G1	.9549	.52790	.078	-.1104	2.0202
		G2	.7203	.57286	.214	-.4256	1.8662
	G1	C	**1.8205***	.44167	**.000**	.9292	2.7118
		G3	-.9549	.52790	.078	-2.0202	.1104
		G3	-.2346	.57286	.684	-1.3805	.9113
	G2	C	**2.0551***	.44628	**.000**	1.1624	2.9478
		G1	.2346	.57286	.684	-.9113	1.3805
		G3	-.7203	.57286	.214	-1.8662	.4256

Based on observed means.

***The mean difference is significant at the .05 level.

N.B.: C=controls G1= T2DM

G2 = CAD G3= CAD+T2DM

Table 2 (b): Multiple Comparisons (Post Hoc Tests) Between Glucose, Insulin and HOMA.IR in non obese Patients and healthy control Groups

Dependent Variable	(I) DIAGNOSIS	(J) DIAGNOSI	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
GLUCOSE	C	G1	**-112.400***	9.55422	**.000**	-131.5113	-93.2887
		G2	-13.4316	7.77160	.089	-28.9771	2.1139
		G3	**-83.3000***	9.55422	**.000**	-102.4113	-64.1887
	G1	C	**112.4000***	9.55422	**.000**	93.2887	131.5113
		G2	**98.9684***	9.97593	**.000**	79.0136	118.9232
		G3	**29.1000***	11.41947	**.013**	6.2576	51.9424
	G2	C	13.4316	7.77160	.089	-2.1139	28.9771
		G1	**-98.9684***	9.97593	**.000**	-118.9232	-79.0136
		G3	**-69.8684***	9.97593	**.000**	-89.8232	-49.9136
	G3	C	**83.3000***	9.55422	**.000**	64.1887	102.4113
		G1	**-29.1000***	11.41947	**.013**	-51.9424	-6.2576
		G2	**69.8684***	9.97593	**.000**	49.9136	89.8232
INSLUIN	C	G1	**-4.6456***	1.32769	**.001**	-7.3014	-1.9898
		G2	**-2.3317***	1.07997	**.035**	-4.4920	-.1714
		G3	**-5.8926***	1.32769	**.000**	-8.5484	-3.2368
	G1	C	**4.6456***	1.32769	**.001**	1.9898	7.3014
		G2	2.3139	1.38630	.100	-.4591	5.0869
		G3	-1.2470	1.58690	.435	-4.4213	1.9273
	G2	C	**2.3317***	1.07997	**.035**	.1714	4.4920
		G1	-2.3139	1.38630	.100	-5.0869	.4591
		G3	**-3.5609***	1.38630	**.013**	-6.3339	-.7879
	G3	C	**5.8926***	1.32769	**.000**	3.2368	8.5484
		G1	1.2470	1.58690	.435	-1.9273	4.4213
		G2	**3.5609***	1.38630	**.013**	.7879	6.3339
HOMA	C	G3	**-3.5960**	.52011	**.000**	-4.6456	-2.5464
		G1	**-3.6660**	.52011	**.000**	-4.7156	-2.6164
		G2	-.6792	.40085	.095	-1.4810	.1227
	G3	C	**3.5960**	.52011	**.000**	2.5464	4.6456
		G1	-.0700	.62165	.911	-1.3245	1.1845
		G2	**2.9168***	.51455	**.000**	1.8876	3.9461

G1	C	**3.6660**	.52011	**.000**	2.6164	4.7156
	G2	**2.9868***	.51455	**.000**	1.9576	4.0161
	G3	.0700	.62165	.911	-1.1845	1.3245
G2	C	.6792	.40085	.095	-.1227	1.4810
	G1	**-2.9868***	.51455	**.000**	-4.0161	-1.9576
	G3	**-2.9168***	.51455	**.000**	-3.9461	-1.8876

Based on observed means.

***The mean difference is significant at the .05 level.

N.B.: C=controls G1= T2DM

G2 = CAD G3= CAD+T2DM

Table 3 (a). Univariate analysis of correlation between Adipokines (Adiponectin & Leptin) in T2DM groups and other variables (all results are two-tailed)

Variable	***Adiponectin***				***Leptin***				***HOMA-IR***			
	Non obese		**obese**		**Non obese**		**obese**		**Non obese**		**obese**	
	R	***P* value**	**R**	***P* value**	**R**	***P* value**	**R**	***P* value**	**R**	***P* value**	**R**	***P* value**
BM	-.491	.149	.373	.116	.470	.171	.269	.265	**.698***	**.025**	.427	.069
Adiponectin	1	.	1	.	.228	.526	**.523***	**.021**	-.472	.168	.109	.658
Leptin	.228	.526	**.523***	**.021**	1	.	1	.	-.087	.812	.431	.066
Glucose	-.062	.866	-.004	.988	-.185	.608	.142	.561	.424	.222	.412	.080
Insulin	-.349	.323	.102	.676	.081	.823	.218	.370	**.786****	**.007**	**.594****	.007
HOMA-IR	-.472	.168	.109	.658	-.087	812	.431	.066	1	.	1	.

Table 3 (b). Univariate analysis of correlation between Adipokines (Adiponectin & Leptin) in CAD groups and other variables (all results are two-tailed)

Variable	***Adiponectin***				***Leptin***				***HOMA-IR***			
	Non obese		**obese**		**Non obese**		**obese**		**Non obese**		**obese**	
	R	***P* value**	**R**	***P* value**	**R**	***P* value**	**R**	***P* value**	**R**	***P* value**	**R**	***P* value**
BM	-.328	.170	.014	.969	.038	.876	-.121	.738	.215	.377	-.200	.580
Adiponectin	1	.	1	.	.443	.058	.378	.282	.224	.356	.539	.108
Leptin	.443	.058	.378	.282	1	.	1	.	.418	.075	.246	.493
Glucose	-.250	.303			**-.604****	**.006**	-.206	.569	-.326	.173	.780	-.102
Insulin	.247	.308	.514	.128	**.499***	**.030**	.272	.447	**.985****	**.000**	.996	.000
HOMA-IR	.224	.356	.539	.108	.418	. .075	.246	.493	1	.	1	.

Table 3 (C). Univariate analysis of correlation between Adipokines (Adiponectin & Leptin) in T2DM+CAD groups and other variables (all results are two-tailed)

Variable	***Adiponectin***				***Leptin***				***HOMA-IR***			
	Non obese		**obese**		**Non obese**		**obese**		**Non obese**		**obese**	
	R	***P* value**	**R**	***P* value**	**R**	***P* value**	**R**	***P* value**	**R**	***P* value**	**R**	***P* value**
BM	.533	.113	-.094	.759	-.222	.538	.113	.713	**-.716***	**.020**	-.202	.508
Adiponectin	1	.	1	.	.376	.285	**.660***	**.020**	-.288	.420	-.202	.509
Leptin	.376	.285	**.660***	**.020**	1	.	1	3	.517	.126	-.046	.882
Glucose	.165	.649	.253	.404	.565	.089	.273	.367	.524	.120	.367	.218
Insulin	-.340	.336	.265	.509	.382	.276	-.197	.520	**0.902****	**.000**	**0.868***	**000**
HOMA-IR	-.288	.420	-.202	.509	.517	.126	-.046	.882	1	.	1	.

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

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

HOMA-IR Ratio:

In non obese

The results revealed that the mean values ± S.E.M of HOMA-IR Ratio in controls (C),T2D (G1), CAD (G2),and T2D+CAD (G3) were1.8±0.082, 4.85±0.69, 1.86±0.26,and 4.78±0.62 with ranges of, 0.7-2.2, 6.1-19.21, 0.3-4.1,and 2.3-8.2 respectively (Table 1b). The result showed that the HOMA-IR Ratio was higher than the upper limit in 10 cases (100%) ,6 cases (32%) and 6 cases (60%) in group G1,G2and G3 respectively. The statistical analysis revealed highly significant increase in non obese groups (G1,G2and G3) as compared to both controls and each other (P<0.001).(Table 2b).

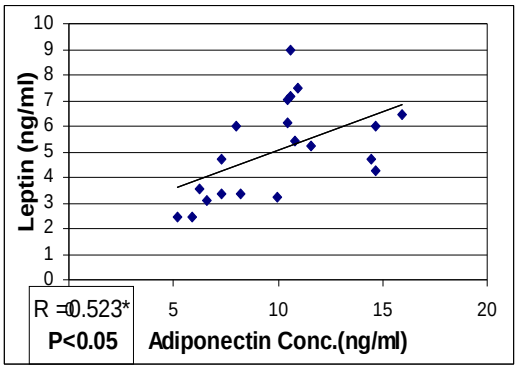
In Obese

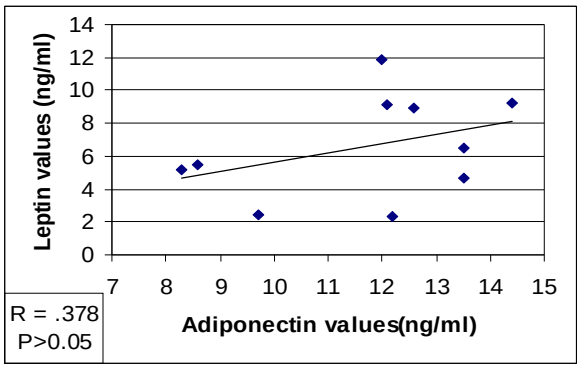
The results revealed that the mean values ± S.E.M of HOMA-IR Ratio in controls (C),T2D (G4), CAD (G5),and T2D+CAD (G6) were1.18±0.082, 5.96±0.51, 2.59±1.01,and 7.5±1.0 respectively with ranges of, 0.7-2.2 , 2.7-10.9,0.8-11.5,and 4-16.5. The result showed that the HOMA-IR Ratio was higher than the upper limit in 19 cases (100%) in group (G4),2 cases (20%) in group (G5),and 13 cases (100%) in group (G6).

The statistical analysis revealed highly significant increase in the two obese groups only (G4, and G6) as compared to controls (P<0.001). While there was no significant variation in (G5) when compared to (C) (P>0.05).Moreover, there was a significant increase in all obese groups when compared to each others (P<0.05). The current study showed also a significant correlation between serum adiponectin and leptin in obese DM patients (Figure2).

Relationship between adipocytokines and other parameters

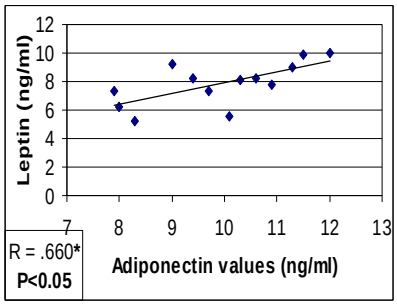
Data on the relationship between Adiponectin and the other variables in non obese and obese patient groups are shown in Tables (2a, b, c). Serum Adiponectin levels were poor associated with Leptin CAD (obese) {r=0.378, P=0.282) (Table (3a, C) & Fig 3) Whereas Adiponectin were associated with Leptin in both of obese T2DM without and CAD (r=0.0.523; P=0.0021; r=0.660; P=0.020) respectively (Table 3b & Fig 3). Leptin Levels were good associated with Insulin in CAD (non obese) (r=0.499, P<0.03).





(G4)T2DM (Obese) **(G5) CAD (obese)**

Fig 3a. Correlation between Adiponectin and Leptin in obese T2DM and CAD Groups.



(G6) T2DM+CAD (obese)

Fig 3b. Correlation between Adiponectin and Leptin in obese T2DM+ CAD Group. Correlation is significant at the 0.05 level.

Discussion

The current study demonstrated that serum leptin concentration was significantly higher in obese DM + CAD patients than obese patients with CAD alone. These findings suggested that the hyperinsulinaemia that accompanies obesity and diabetes in our DM+CAD patients probably results in increased ob gene expression and higher plasma leptin concentration This agreed with previous studies that showed that leptin is part of the adipoinsular axis, as insulin stimulates leptin production in adipocytes and leptin interacts with the pancreatic B-cell through various effects on insulin secretion (Bullo et al. 2002) It has been shown in animal studies , insulin increases the expression of the ob gene and results in increased leptin concentration, while relative or absolute deficiency of insulin results in reduced ob gene expression(Friedman and Halaas 1998)

The increased leptin levels in obese DM+CAD, obese DM, and obese CAD patients in current study might represent an integrated marker of adiposity, insulin resistance, and vascular dysfunction in Egyptian patients This integrity agrees with previous studies by (Wallace et al. 2001) who postulated that hyperleptinimeia has been recognized as a mediator between obesity and cardiovascular disease.The effect of obesity and high leptin levels on vascular system likely due to several various factors such as increasd sympathetic activity,enhanced platelet aggregation, increased oxidative stress, and cardiac hypertrophy(Konstantinides et al. 2001; Bodary et al. 2002; Paolisso et al. 1999) Besides, increased adipose tissue in obesity requires an increased vascular bed to maintain its baseline circulation (Bouloumie et al. 1998; Sierra-Honigmann et al. 1998) Thus this adaptation may, conversely, promote arteriosclerosis over long periods of time.

Higher leptin concentrations were shown to be associated with an impaired arterial distensibility(Singhal et al. 2002), and patients with a restenosis after coronary stenting had higher leptin levels than those without a restenosis(Piatti et al. 2003) Furthermore, the direct influence of leptin on the vascular biology is supported by the *ob/ob* mice, which lacks leptin and consequently becomes hyperphagia and obesity but is nevertheless resistant to atherosclerosis(Schafer et al. 2004) However,the administration of leptin removes this protective effect against atherosclerosis.The atherosclerosis risk in heterozygote and the control mouse,which suggest a dose-response relation between the leptin levels and the atherosclerotic process(Nishina et al. 1994)

Our findings also showed increased levels of insulin in studied obese patients.The increase in leptin levels in these groups did not show significant correlation with insulin resistance This agreed with evidence that plasma leptin may vary much more as a function of the circulating insulin concentrations than of the degree of the insulin resistance itself (Mohamed-Ali et al. 1997; Lonnqvist et al. 1995) The current study also showed a significant decrease in serum adiponectin levels in obese and non obese CAD, and DM+CAD patients compared to controls, consistent with our findings, previous studies that showed a decreased levels of adiponectin in a variety of insulin-resistant states, including obesity , diabetes, and cardiovascular diseases(Hotta et al. 2000; Weyer et al. 2001; Kumada et al. 2003; Trujillo and Scherer 2005) .

There were some possibilities explained the decrease in serum levels of adiponectin in different studied obese and non obese groups. One of them is elevated insulin levels in diabetic subjects may have been responsible for the decreased adiponectin concentrations as insulin regulates the secretion of various proteins from adipose tissue(Yu et al. 2002; Mohlig et al. 2002). There is evidence that insulin may have direct effect on adiponectin gene expression and adiponectin concentrations in vitro (Fasshauer et al. 2002; Halleux et al. 2001; Motoshima et al. 2002). Another possibility was that accumulation of adiponectin in atherosclerotic vascular walls may accelerate its half-life in plasma, resulting in the reduction of the plasma concentration of adiponectin in subjects with CAD (Hotta et al. 2000) . The current study showed significant correlation between serum adiponectin and leptin in obese DM patients, similar results were obtained by Satoh et al. 2004; Kotani et al. 2005).

Conclusion

The coexistence of a correlation between serum leptin and Adiponectin levels in addition to increase of serum leptin and decrease serum Adiponectin levels in obese DM patients in the current study, support that hypothesis of their susceptibility to atherosclerosis. Although the small sample size does

not enable us to make a definitive conclusion, our study revealed that there were significant differences in plasma levels of Adiponectin between CAD patients with and without DM. DM has an additional effect on CAD, that of decreasing plasma Adiponectin levels. We speculate that people who have very low plasma Adiponectin levels may be at increased risk of developing both CAD and DM.

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Effects of L-carnitine on growth performance of Nile tilapia (*Oreochromis niloticus)* fingerlings fed basal diet or diets containing decreasing protein levels

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Abstract: The effects of L-carnitine on growth rate, feed utilization efficiency and protein sparing of Nile tilapia (*Oreochromis niloticus* L.) fingerlings were investigated in two consecutive experiments. In experiment 1, triplicate groups of 10 fingerlings (4.16 ±0.07) each were stocked in 85 L glass aquaria, filled with 70 L dechlorinated tap water. Five levels of L-carnitine (0, 75, 150, 300, 450 mg/kg) were separately added to the basal diet (30% crude protein and 18.74 Mj GE/kg). The fish were fed the diets, at a daily rate of 5% BW, twice a day for 70 days. The results revealed that fish growth rates, feed utilization and whole body protein and lipid levels were increased with increasing L-carnitine levels. In experiment 2, Nile tilapia fingerlings (4.3±0.1 g) were fed diets containing decreasing levels of protein (30, 25, and 20%) and supplemented with 450 mg L-carnitine/kg diet, for 84 days. Fish performance was not significantly affected with decreasing dietary protein levels up to 20%. These results suggest that dietary inclusion of L-carnitine in Nile tilapia diets may significantly reduce dietary protein requirements and may facilitate the use of fatty acids for obtaining energy and consequently, can spare dietary protein for somatic growth. [Journal of American Science 2010;6(5):165-172]. (ISSN: 1545-1003).

Keywords: L-carnitine, Nile tilapia, performance, protein sparing.

1. Introduction

Tilapia culture has been growing at an outstanding rate during the past two decades. As a result, the production of farmed tilapia has witness a 6-fold increase during the past 15 years, jumping from 383,654 mt in 1990 to 2,348,656 mt in 2006 (FAO, 2008). In addition, there has been a gradual shift in tilapia culture from traditional semi-intensive to more intensive farming systems. This has created an increasing demand for artificial feed.

The replacement of fishmeal with other plant- or animal-based protein sources in tilapia feeds is well documented (El-Sayed, 1990; 1992; 1998; 1999; Ebrahim and Abou-Seif, 2008). However, most of these protein sources contain different antinutrients and are deficient in certain essential amino acids, and may therefore lead to retarded performance (Francis *et al*., 2001). Supplementing these sources with certain compounds may improve their nutritive values for fish and accordingly reduce the feed costs.

L-carnitine (l-h-hydroxy-g-N,N,N-trimethylaminobutyric acid) is one of those compounds that may play a significant role in fish nutrition. It is a lysine derivative, hygroscopic, water soluble organic compound (Harpaz, 2005). It is also non essential since can be synthesized from lysine and methionine in animal and human liver, brain and kidney (Emaus and Bieber, 1983). L-carnitine acts as a cofactor for the transport and oxidation of long chain fatty acids by the mitochondria by facilitating the use of fatty acids for obtaining energy, and thus sparing dietary protein for anabolic processes (Bilinski and Jonas, 1970; Emaus and Bieber, 1983).

The use of L-carnitine as a growth promoter in fish is controversial. Many studies revealed significant effects of dietary L-carnitine on the performance of European sea bass (Santulli and D'Amelio, 1986); African catfish (Torreele *et al*., 1993); red sea bream (Chatzifotis *et al*., 1995); Indiana major carp rohu (Keshavanath and Renuka, 1998); hybrid striped bass (Twibell and Brown, 2000); Beluge sturgeon

(Mohsni *et al.*, 2008); Mossambique tilapia (Jayaprakas *et al*. 1996) and Hybrid tilapia *Oreochromis niloticus* × *O. aureus* (Becker *et al*., 1999) and Nile tilapia (Yang *et al*, 2009)..

In contrast, no effects of L-carnitine supplementation was reported in European sea bass (Dias *et al*., 2001), channel catfish (Burtle and Liu, 1994), Atlantic salmon (Ji *et al*., 1996), Rainbow trout (Rodehutscord, 1995; Chatzifotis *et al*. 1997) , Hybrid striped bass (Gaylord and Gatlin, 2000 a,b), African catfish (Ozorio *et al*. 2001 a,b) and Hybrid tilapia *O. niloticus* × *O. aureus* (Schlechtriem *et al*., 2004).

It appears from this discrepancy that the response of fish to supplemental L-carnitine is species specific. This response may also be affected by other dietary components being, carnitine levels, culture conditions and water quality. Therefore, more work is urgently needed to verify the effects of L-carnitine supplementation on fish performance and health.

The present study was carried out, in two consecutive experiments. The first experiment aimed at investigating the effects of L-carnitine supplementation on the performance and body composition of Nile tilapia (*Oreochromis niloticus)* fingerlings fed basal diets. The second experiment investigated the effect of L-carnitine supplementation on protein sparing effect in Nile tilapia fingerlings fed decreasing dietary protein levels.

2. Material and Methods

Fish and culture facilities

The present study was carried out at fish research unit, Department of Animal Production, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt. Mono sex Nile tilapia *Oreochromis niloticus* fingerlings used in the present study were obtained from a private hatchery at Kafr El-Sheikh Governorate. The fish were adapted to the lab conditions for 7 days, during which they were fed the test diets assigned to each treatment. At the ends of the conditioning period, the fish were netted, reweighed collectively, and the average initial weight was recorded. Culture aquaria were continuously provided with aeration through an air compressor. Tanks were cleaned every morning, before the first feeding, and the faeces were removed then, water was replaced by 10% of new fresh, dechlorinated water. Natural photoperiod was adopted throughout the study. Water quality parameters, including temperature (T), pH, dissolved oxygen (DO), total ammonia (NH_3), and nitrites (NO_2-N) were monitored weekly. The average values of these parameters throughout the study were; T 27± 0.5° C, pH 7.03± 0.38, DO 6.8± 0.63 mg^{-1}, NH_3 0.4± 0.1 mg^{-1} and NO_2 0.2±0.02 mg^{-1} .

Experimental Design

Experiment 1

The first experiment was designed to study the effects of supplemental L-carnitine on growth performance, feed conversion efficiency and body composition of Nile tilapia (*Oreochromis niloticus)* fingerlings fed basal diet contain 30% crude protein and 18.74 MJ GE/kg. Five levels of L-carnitine (0, 75, 150, 300, 450 mg/kg) were separately added to the basal diet to formulate 5 experimental diets (Table 1). Proximate analysis of the basal diet was performed according to AOAC (1998). Fish with an initial average weight of 4.16 ± 0.07 g were stocked in triplicates into 85 L glass aquaria, filled with 70 L dechlorinated tap water, at a density of 10 fish/aquarium. The fish were fed the diets, at a daily rate of 5% BW, twice a day for 70 days. The fish were weighed collectively at 15-day intervals, their average weights recorded and daily feeds were rations readjusted accordingly. At the ends of the experiment, fish were netted, weighed individually and the average final weight of each replicate was recorded.

Experiment 2

In experiment 2, triplicate groups of Nile tilapia fingerlings (4.3±0.1 g) were fed diets containing decreasing levels of protein (30, 25, and 20%) and supplemented with 450 mg L-carnitine/kg diet, for 84 days (Table 2). Lysine and methionine were added to the diets to adjust the amino acids required by Nile tilapia according to NRC (1993) proximate analysis of the experimental diets was done according to AOAC (1998). The fish were exposed to the same culture conditions and feeding regime adopted in experiment 1.

Growth rates and feed efficiency

Growth rates and feed conversion efficiency were calculated as follows:

Average daily gain (ADG) = Weight gain (g)/time of experiment (days),

Specific growth rate (% SGR) = 100 (ln average final weight–ln average initial weight)/time (days),

Feed conversion ratio (FCR) = g dry feed given/g wet weight gain.

Protein efficiency ratio (PER) = g wet weight gain/g protein fed.

Net protein utilization (NPU) = 100×(final body protein –initial body protein)/protein fed.

Body composition

Initial body composition of fish was analyzed from samples of 50 fish which were frozen (at -20ºC) prior to the study. At the end of the study, the fish in each aquarium were netted, their total weight (g) were recorded and frozen for final chemical analysis. Chemical analyses of fish and test diets were performed according to standard AOAC (1998) methods.

Statistical analysis

Simple linear and non-linear regressions were performed to correlate the obtained results. A one-way analysis of variance (ANOVA) was conducted to test the effect of dietary treatments on the performance of Nile tilapia fingerlings, using the computer program SPSS (SPSS Version11.0.0, 2003). Least significant difference was used to compare between means at P=0.05, as described by (Gill, 1981).

3. Results

Experiment 1

Fish performance in Experiment 1 was significantly affected by dietary treatments (P<0.05). Growth rates were significantly increased (P<0.05) with increasing L-carnitine (Table 3). Feed conversion ratio (FCR), protein efficiency ratio (PER) and net protein utilization (NPU) were also improved with increasing L-carnitine levels in the diets (P<0.05). However, fish fed diets containing 300 and 450 mg/kg L-carnitine showed similar gain and SGR (P>0.05).

Body composition of Nile tilapia fed the experimental diets in Experiment 1 was significantly affected by L-carnitine supplementation (Table 4). Body moisture and ash contents were negatively correlated with L-carnitine, while crude protein and lipid contents significantly increased (P<0.05) with increasing L-carnitine in the diets.

Experiment 2

Fish performance in Experiment 2 was significantly affected by decreasing dietary protein levels and supplementation of L-carnitine (Table 5). Growth rates of fish fed diets containing 20-30% CP were not significantly different (P>0.05). Similarly, FCR was not significantly affected by dietary treatments (P>0.05). In contrast, PER and NPU were improved (P<0.05) with decreasing dietary protein levels.

Body composition was also significantly affected by decreasing dietary protein levels and supplementation of L-carnitine (Table 6). Body water and ash contents slightly increased with decreasing dietary protein levels (P>0.05), while body protein and lipids content were negatively correlated (P>0.05) with decreasing dietary protein level.

4. Discussions

It has been reported that carnitine is closely associated with lipid metabolism, through promotion of fatty acid oxidation. Therefore, it is expected that carnitine administration in fish diets may improve energy utilization and spare protein for somatic growth. Several studies have investigated this assumption in recent years. However, controversial results have been reported, depending on fish species and size, carnitine levels, dietary composition and culture conditions.

In the present study, we investigated the response of Nile tilapia fingerlings to diets supplemented with L-carnitine levels. The results clearly revealed that fish growth rates, feed utilization and whole body protein and lipid content were significantly increased with increasing dietary L-carnitine levels.

Similarly, studies on other tilapias including Mozambique tilapia (*Oreochromis mossambicus*) (Jayaprakas *et al*., 1996), hybrid tilapia *Oreochromis niloticus* × *O. aureus* (Becker *et al* ., 1999) and Nile tilapia (Abou-Seif, 2006) revealed that dietary L-carnitine supplementation resulted in improved growth rates and feed efficiency. Similar results have also been reported with several other species reared on diets supplemented with L-carnitine, including European sea bass (Santulli and D'Amelio, 1986), African catfish (*Clarias gariepinus*) (Torreele *et al* ., 1993; Ozorio *et al*., 2001b), red sea bream (Chatzifotis, *et al* ., 1995), Indian major carp rohu (Keshavanath and Renuka, 1998); hybrid striped bass (Twibell and Brown, 2000).

However, the scale of improvement in growth rates in the present study (18.2, 29.3, 45.5 and 54.5% at 75, 150, 300 and 450 mg carnitine kg^{-1}, Respectively) was much higher than those previously reported on *O. mossambicus* (16%) (Jayaprakas *et al*., 1996) and *O. niloticus* × *O. aureus* (18.5%) (Becker *et al*., 1999).

The significant differences of FCR in experiment 1, may suggest that the improved growth was related to feed consumption and better fed utilization efficiency. Therefore, the improved performance of Nile tilapia in experiment 1 with L-carnitine supplementation is presumably due to the enhancement, caused by carnitine, of energy

utilization from fatty acid oxidation (through β-oxidation) (Torreele *et al*., 1993; Chatzifotis *et al.*, 1995). It has also been reported that L-carnitine facilitates the removal of short chain organic acids from the mitochondria, leading to freeing coenzyme A to participate in β-oxidation and tricarboxylic acid cycle pathways (Harpaz, 2005).

In contrast, no effects of L-carnitine supplementation was reported in European sea bass (Dias *et al*., 2001), Channel catfish (Burtle and Liu, 1994), Atlantic salmon (Ji *et al* ., 1996), Rainbow trout (Rodehutscord 1995; Chatzifotis *et al* ., 1997), hybrid striped bass (Gaylord and Gatlin 2000 a,b), African catfish (Ozorio *et al* ., 2001 a,b) and hybrid tilapia *O. niloticus* × *O. aureus* (Schlechtriem *et al* . 2004). The discrepancies in these results may have been due to the differences in culture conditions, diets composition and fish species and sizes.

Dietary protein requirement of fish is affected by dietary energy. In other words, fish growth is sustained from the energy supplied from dietary protein or energy sources. Therefore, the relationship between dietary protein and energy in fish feeds should be considered if cost-effective and environmentally friendly diets are formulated. Many studies have indicated that at inadequate energy level, dietary protein may be used as an energy source, whereas at high protein level, a proportion of this protein will be deaminated and the carbon skeleton used as an energy source (Garling and Wilson, 1976). At adequate energy level, dietary protein can be spared for anabolic functions (Garling and Wilson, 1976; El-Sayed, 1987; El-Sayed and Kawanna, 2008). Thus, the design of practical fish diets is a compromise between a protein level that will permit good growth with little conversion to energy, and an energy level concomitant with a high rate of protein synthesis, without excessive deposition of carcass lipid.

In experiment 2 of the present study, the inclusion of L-carnitine in the diets has significantly enhanced the growth, PER and NPU of Nile tilapia despite the reduction of dietary protein from 30% to 20%. Once again, these results suggest that carnitine enhanced energy utilization through promotion of fatty acid oxidation and accordingly, sparing dietary protein for somatic growth. This may explain the increase of PER and NPU at lower dietary protein levels. The results of experiment 2 are in agreement with the result of Ozorio *et al.* (2001b), who found that dietary carnitine supplementation in African catfish diets improved growth rates and FCR when protein energy (PE) – to – non protein energy (NPE) was low (i.e., when dietary protein was in shortage), leading to increasing body protein : fat ratio. Those authors suggested that low dietary PE:NPE may lead to higher enzymatic activity and elevated availability of free carnitine in fish tissues, which, in turn, leads to improved utilization of dietary lipid for energy fuel and spare protein for growth.

5. Conclusion

The present study indicated that a significant improvement in fish performance was observed when the diets were supplemented with 450 mg L-carnitine/kg. The study revealed also that the inclusion of L-carnitine in tilapia diets may significantly reduce dietary protein requirements. Moreover, the results suggest that dietary inclusion of L-carnitine in Nile tilapia diets may facilitate the use of fatty acids for energy and consequently, spare dietary protein for somatic growth.

Table 1. Formulation and proximate composition (%) of the basal diet used in Experiment 1.

Ingredients	%
Fish Meal (72%)	24.00
Soybean meal (44%)	21.00
Yellow corn	51.00
Corn oil	2.50
Vit & Min mix[1]	1.50
Total	100
Proximate composition	
Crude protein	29.62
EE	7.90
Ash	6.92
CF	5.11
NFE[2]	50.45
GE[3]	18.74

[1]Contains per kg: vitamin A, 4.8 m. I.U; vit D3 , 0.8 m.I.U; vit E, 4.0 g; vit. K, 0.8 g; vit B1, 0.49, vit. B2, 1.6 g; vit. B6, 0.6 g; vit. B12, 4 mg; Pantothenic acid, 4 g; Nicotinc acid, 8 g; Folic acid, 400 mg; Biotin, 20 mg; Choline chloride, 200 mg; Copper, 4.0 g ; Iodine, 0.4g ; Iron, 12 mg ; Manganese, 22 g; Zinc, 22 g and Selenium 0.04 g.

[2]Nitrogen free extract, determined by difference.

[3]Gross energy (MJ/kg), calculated based on 0.17, 0.237, 0.398 MJ/g for carbohydrate, protein and lipid, respectively (Jobling, 1983).

Table 2. Formulation and proximate composition (%) of the diets used in Experiment 2.

	Diets		
Ingredients	1	2	3
Fish meal (72%)	8	8	8
Soybean meal (44%)	46	36	22
Yellow corn	39.14	49.34	56.34
Corn oil	2	2	3
Vit & Min Mix[1]	2	2	2
Wheat bran	0.5	0.5	7
L-Lysine HCL	1.86	1.60	1.26
DL-Methionine	0.50	0.46	0.40
Total	100	100	100
Proximate composition			
CP	29.54	24.62	20.48
EE	11.42	11.52	11.36
CF	4.20	3.70	10.30
Ash	5.59	5.43	5.52
NFE[1]	49.25	54.73	52.34
GE (MJ/kg)[1]	19.92	19.72	18.28
P/E ratio[2]	14.82	12.48	11.20
PE/NPE[3]	0.54	0.47	0.39

[1]Gross energy (MJ/kg), calculated based on 0.17, 0.237, 0.398 MJ/g for carbohydrate, protein and lipid, respectively. [2]Protein-to-energy ratio (g protein/MJ). [3]Protein energy/non protein energy

Table 3. Performance (mean ± SE) of Nile tilapia fingerlings fed different levels of L–carnitine(Experiment 1).

Carnitine (mg/kg)	IW	FW	SGR	% gain	FCR	PER	NPU
0	4.16 ^{a} ±0.01	14.08 ^{c} ±0.55	1.74 ^{d} ±0.05	239 ^{d} ±13	2.66 ^{e} ±0.04	1.27 ^{e} ±0.02	19.82 ^{e} ±0.51
75	4.23 ^{a} ±0.02	16.64 ^{b} ±0.36	1.96 ^{c} ±0.03	293 ^{c} ±8	2.41 ^{d} ±0.02	1.40 ^{d} ±0.01	22.58 ^{d} ±0.44
150	4.09 ^{a} ±0.02	18.20 ^{b} ±0.23	2.13 ^{b} ±0.02	345 ^{b} ±6	2.22 ^{c} ±0.02	1.52 ^{c} ±0.01	24.53 ^{c} ±0.61
300	4.19 ^{a} ±0.01	20.48 ^{a} ±0.99	2.27 ^{ab} ±0.08	389 ^{ab} ±25	2.01 ^{b} ±0.01	1.68 ^{b} ±0.01	28.05 ^{b} ±0.32
450	4.11 ^{a} ±0.02	21.75 ^{a} ±0.47	2.38 ^{a} ±0.03	429 ^{a} ±12	1.82 ^{a} ±0.06	1.86 ^{a} ±0.07	31.38 ^{a} ±0.92

Values in the same column with different superscripts are significantly different ($p< 0.05$).

Table 4. Effect of L-carnitine Levels on body composition (% dry weight) of Nile tilapia fingerlings (Experiment 1).

Carnitine (mg/kg)	Moisture	Protein	Lipid	Ash
0	72.55 ^{a} ±0.30	53.79 ^{d} ±0.19	18.92 ^{d} ±0.20	27.07 ^{a} ±0.12
75	72.02 ^{a} ±0.18	54.53 ^{cd} ±0.29	20.66 ^{c} ±0.31	24.59 ^{b} ±0.47
150	72.24 ^{a} ±0.36	55.58 ^{bc} ±0.33	22.93 ^{b} ±0.33	21.16 ^{c} ±0.42
300	71.62 ^{a} ±0.28	56.04 ^{b} ±0.38	23.04 ^{b} ±0.17	20.43 ^{c} ±0.34
450	71.78 ^{a} ±0.30	57.14 ^{a} ±0.46	24.45 ^{a} ±0.30	18.00 ^{d} ±0.80
Initial	74.89 ±0.38	51.21 ±1.11	24.98 ±0.34	23.50 ±0.62

Values in the same column with different superscripts are significantly different ($p< 0.05$).

Table 5. The Effect of L-carnitine supplementation (450 mg/kg) on growth rates and feed utilization efficiency of Nile tilapia fingerlings fed decreasing dietary protein levels (Experiment 2).

Item	Dietary protein level (%)		
	30	25	20
IW	4.29 ±0.02	4.30 ±0.01	4.29 ±0.01
FW	50.00 [a] ±0.40	49.54 [a] ±1.03	48.69 [a] ±0.70
SGR	2.92 [a] ±0.14	2.91 [a] ±0.19	2.89 [a] ±0.21
ADG	0.54 [a] ±0.06	0.54 [a] ±0.05	0.53 [a] ±0.01
FCR	1.99 [a] ±0.13	1.96 [a] ±0.11	1.95 [a] ±0.13
PER	1.70 [c] ±0.17	2.07 [b] ±0.19	2.34 [a] ±0.23
NPU	28.87 [c] ±1.57	33.52 [b] ±1.19	36.86 [a] ±0.41

Values in the same column with different superscripts are significantly different ($p < 0.05$).

Table 6. The Effect of L-carnitine supplementation (450 mg/kg) on body composition (% dry weight) of Nile tilapia fingerlings fed decreasing dietary protein levels in (Experiment 2.)

Dietary protein (%)	Moisture	Protein	Lipid	Ash
30	74.61 [a] ±0.24	65.03 [a] ±0.27	22.86 [a] ±0.34	10.91 [a] ±0.32
25	75.42 [a] ±0.14	64.24 [a] ±0.25	22.75 [a] ±0.42	11.49 [a] ±0.17
20	75.92 [a] ±0.23	63.94 [a] ±0.14	22.16 [a] ±0.19	12.55 [a] ±0.15
Initial	78.18 ±0.89	58.42 ±0.58	21.90 ±0.18	17.26 ±0.43

Values in the same column with different superscripts are significantly different ($p < 0.05$).

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ESTIMATION OF TOXIC METALS IN CANNED MILK PRODUCTS FROM UNLAQUERED TIN PLATE CANS.

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Abstract: Branded canned milk (B1, B2, B3 and B4) were selected in triplicate, using market basket approach. The samples were pre-treated and analysed for heavy metals. Their physicochemical variables were estimated. The metal concentration (in μgg^{-1},using AAS)of some toxic metals compared to those of uncanned dairy products include: 0.02±0.008 (006±0.003); 1.61±0.21 (0.01±0.01); 1.47±0.73 (0.01±0.01); 1.64±0.66 (0.05±0.03) and 1.75±0.29(1.54±1.2) for Cd, Co, Cr, Ni, and Pb found in canned and (uncanned) milk products respectively. Further analysis revealed that Nickel contents in milk is less, compared to those of canned fish products. Unlike Cd contents, Cr and Pb concentration were above the threshold limit values (TLV) of $2.0\mu gg^{-1}$. . [Journal of American Science 2010;6(5):173-178]. (ISSN: 1545-1003).

Key words: Toxic metals, canned milk, Corrosion, Health

1. Introduction.

Research achievements that contribute to prevention of disease are not as dramatic as the discovery or implementation of cures for existent disease but are certainly no less important. Research regarding the health effects of toxic metals may make a small contribution that helps millions of people, but is probably unknown to them. While these epi-beneficial effects may not be apparent to the individuals who are spared the problems of toxicity, the implementation of measures to reduce exposure to these toxins are clearly visible when examined on a population basis (Robert,1996). It is necessary to keep food preserved for a considerable period such that when required, it will be in a state, fit for consumption. That is, the food is kept free of mould and bacteria growth. Food canning implies the storing of food in airtight containers. The food is preheated to bring about the destruction of organisms (Kenneth and Helen; 1947). The lethal effect of heat on micro organism has been used for food preservation long before the microbial causes of food spoilage was discovered. Pasteur showed that the spoilage of milk could be prevented by holding the milk for a few minutes at a temperature between 50-70^0C (Ngoddy and Ihekomonye, 1992). Canning of food product is the sealing of the food products after heat treatment (Itodo *et al.,* 2009).

Tin plate is light gauge, steel sheet or strip coated on both sides with pure tin. (Steve *et al.,* 2003). They are important containers for packaging of food, including milk and for heat

processing. The properties of open tin plates which make it an ideal canning material are; Strength and rigidity, Ability to be worked at high speed for the construction of can bodies and ends, formation of a double side seams, good corrosion resistance under normal storage condition, attractive appearance, ability to withstand high pressure and high processing temperature and ease of shaping and decorations. Tin plate cans usually consist of central core of base steel covered on each surface by a layer of oxide and finally a thin film of oil. Lacquered tin plate (tin plate with synthetic substance that form a hard protective coating) has another layer, usually on one side (Ngoddy and Ihekomonye, 1992).When the Tin plate cans are not lacquered, alloying metals which make up the electrolytic composition of the cans may easily leach out of corroded cans.

Mechanism of Metal Toxicity: Metallic toxicant in canned milk, through exposure or ingestion may find their way into the body, at such, may act through one or more of these possible mechanisms

(a) **Inhibition of Enzymatic Activities**: This is so because some metals such as Pb, Hg and Cd have affinity for sulphur and therefore attack sulphur bonds in enzyme, thus immobilizing them. Other site of attack include the free amino ($-NH_2$) and carboxyl (-COOH) groups in protein (Ademoroti, 1996 ; Alka, 2000).

(b) **Attacks on Cell Membrane and Receptor**: The heavy metals bind to cell membrane and receptor, thereby altering their structures. This affect transport and other inter or intra cellular processes in the body. Cd inhibits oxidative phosphorylation in the body (Alka, 2000).

(c) **Interference with Metabolic Cations**: Heavy metals interfere with the metabolism

of essential cations such as absorption, transportation, decomposition and storage. Cd follows the pathway of Zn and Cu metabolisms. Pb replaces Ca in bones (Michael and Peter, 1990). This is the most crucial aspect of metals that leads to manifestation of toxic effect.

(d) **Action on the Artery**: Heavy metals can increase the acidity of the blood. The body draws Ca from the bones to help restore blood pH. Further toxic metals set up conditions that lead to inflammation in arteries and tissues, causing more Ca to be drawn to the area as a buffer. The Ca, coats the inflamed area in the blood vessel but creating another by the hardening of the artery walls and its progressive blockage of the arteries. This leads to osteoporosis (Michael and Peter, 2003)

Heavy metals in canned food had its source traced to the untreated water, chemical residue in processed foods, leachates, bio – accumulation in aquatic animals and industrial emission into food before packaging or canning (Goyer, 1991).

pH and Leachates Concentration: Most proteins exhibit maximum stability in cans but not at the pH in which they naturally occur. Most protein are denatured at pH < 3 or > 10. At this stage, the leachate is either enriched with heavy metals within the food material or cans depending on the pH of the medium. Direct uptake of heavy metal by the food material before canning, as a result of processing or as a result of storage and distribution. In other words, leachate enrichment with heavy metals can be from the food or from the containers all depending on pH and other denaturing agents.

Chemistry Involved In Leaching of Metals from Cans: The chemistry involved in heavy metal delamination into canned food involves the following step: Detinning/corrosion, rusting of cans and leaching of the toxic metal from the corroded cans. Detinning is the removal of the tin coating. It occurs at pH > 2, rendering the inner container unprotected. This process is due to the action of oxygen

$1/_2O_{2(g)} + H_2O_{(g)} + 2e^- \longrightarrow 2OH^-$

$NO^-_{3(aq)} \longrightarrow NO_{2(g)} + O_{2(g)}$

$Sn \longrightarrow Sn^{2+} + 2e$

$Sn^{2+} + 2OH^- \longrightarrow Sn(OH)_2$

The $Sn(OH)_2$ has low solubility and can cause gastro-intestinal disorders,

Rusting of the tinplate cans begins during and after the detinning following the reaction below:

$Fe_{(s)} \longrightarrow Fe^{2+}_{(aq)} + 2e^-$ (oxidation)

$2H^+ + 2e^- \longrightarrow H_2$ (reduction)

$Fe + 2H^+ \longrightarrow Fe^{2+} + H_2$

$Fe^{2+} + 2H_20 \longrightarrow Fe(OH)_2 + 2H^+$

$Fe_{(s)} + 2H^+ \longrightarrow Fe^{2+} + H_2O \xrightarrow{[O]} Fe(OH)_2$

$CO_2 + H_2O \longrightarrow H_2CO_3$

$Fe(OH)_2 \xrightarrow{\text{(corrosion)}} Fe_2O_3 .xH_20$

Brown hydrated iron (iii) oxide.

The above reactions render the can plate, porous thereby effecting the ease of leaching of alloying metals (Charles, 1999; Ngoddy and Ihekomonye, 1992; Steve and Wallace, 2003). This present work was embarked upon to estimate the heavy metal contents of various canned milk from village markets in Sokoto state, Nigeria, for the purpose of providing data for future compilation of Nigerian food consumption table, display of health implications for the populace and for use by the Nigerian Standard Organizations.

2. Materials and Methods

Triplicates of four brands of canned milk stored in Unlacquered cans were randomly bought from village markets in Sokoto state, Nigeria. Two analytical techniques were employed viz: the flame photometer, used for preliminary analysis followed by the Atomic Absorption spectrophotometer.

Sample Preparation for Protein Determination (Kjeldahl Method): 2.00g each of the blended material was placed into a Kjeldahl flask and 20cm^3 of water was added. The flask was shaken for two minutes and was allowed to stand. One tablet of mercury catalyst or Kjeldahl digestion tablet was added followed by 30cm^3 of concentrated H_2SO_4. The contents in the digestion tubes were heated continuously at 100^{0}C in the digestion block. When water was removed and frothing ceases, the heating was continued for two hours for complete digestion indicated by the disappearance of original canned food to give a clear solution. This is followed by cooling of the samples and dilution to 50cm^3 with water (AOAC, 1990).

(b) Procedure for Protein Analysis

20 cm^3 of the digest was pipetted into another microKjeldarhl digestion and distillation apparatus. 25 cm^3 of boric acid indicator was added in the

conical flask placed under the condenser of the apparatus. 20 cm^3 of 10M NaOH was added to the content of the flask by opening the cock through the distillation flask. The heat supplied was regulated to avoid sucking back. The condenser was kept cooled by passing cold water. When all the available distillate has been collected in 25 cm^3. of boric acid, the distillation was stopped and set for further analysis. The nitrogen in the distillate was determined by titrating with either 0.01M or 0.1M HCl. The colour change at end point was from green to blue (Ceirwn, 1995).

Physicochemical Variables

(a) Determination of Moisture Content

5g each of the blended food sample was weighed in a previously weighed porcelain dish. The samples was dried in the Galenkamp side two oven operated at 105^0C, for three hours, cooled in a dessicator containing silica gel overnight before re-weighing (AOAC, 1990)

(b) Determination of Ash Contents

The dried sample was used for ash determination. The sample in the porcelain dish was fed into the furnace at a temperature of 700^OC and was allowed to normalize. Ashing was done for four hours after which the sample was removed and placed in a dessicator containing silica gel. Alternatively, the fresh sample is left overnight in the furnace and later weighed. The percentage ash was calculated. Wet digestion, including the Nitric Acid – Hydrogen Peroxide (HNO_3/H_2O_2) and Nitric Acid – Sulfuric Acid (HNO_3-H_2SO_4) methods (earlier described by AOAC, 1990; Itodo *et al*., 2009 and Ademoroti, 1996) was adopted to leach the metals into the analytical solutions.5 cm^3 of water was added to the digest and was allowed to cool, followed by filtration using the Whatman filter paper No.42. The solution was neutralised with conc. NH_4OH, transferred into a 25ml volumetric flask and diluted to the mark (Ademoroti 1996).The Unicam 969 AAS was set up according to manufacturer's instruction with the wavelength corresponding to that of the element under investigation. The spectrometer was set to zero absorbance using the blank solution. The absorbance of each sample was read with an automatic calculation of the average (μgg^{-1}) .The machine (Windaus L.F. 2400 photometer) was also set up according to manufacturer's instruction with the wavelength corresponding to that of the element under analysis. The photometer was set to zero using the 0 mg /cm^3 solution. The absorbance of each sample in the sample cell was measured in duplicate.

3. Results and discussion.

The sampling data (Table1) and results of the physicochemical variables (Table 2) were presented below. The entire brands are legislatively accepted into markets, thus, tagged with the NAFDAC numbers. In their Market Basket Surveys ,Nigerian Food and Drug Administration and Control(NAFDAC), indicate federal law which provides a Medicaid benefit package for the populace through the Early and Periodic awareness campaign, Screening, Diagnosis and Treatment for toxic metal poisonings, including chelation therapy, vitamin and mineral supplementation, medical care, environmental investigations, education services, and nutritional developmental assessments.

Table 1: Sampling and Nutritional Data of Various Canned Food as at November, 2005.

Canned milk products	Net wt.	Man. Date	Exp. date	Shelf life (months)	Food duration in cans	Ingredients
B1	170g	Oct, 2004	Oct 2005	12	1 month	Milk fat, cow milk, Veg. oil, Vit A, D3 and E and 28 other vitamins
B2	170g	Oct. 2004	Oct. 2005	12	1 month	Skimmed cow milk, veg. oil, vit. A,D3 and E
B3	170g	Aug. 2003	Aug. 2005	24	15 months	Full cream cow milk, H_20, Vit A and D3
B4	170g	Oct. 2003	Oct, 2005	24	13 months	Cow milk, Vit D2 and stabilizer

Two important parameters that enrich leachates metal concentration were measured (Table 2). Mean value of 16.69±1.25% and 69.50±1.91% were reported for the protein and moisture contents respectively. Most
proteins exhibit maximum stability in cans but not at the pH in which they naturally occur. protein are denatured at pH < 3 or > 10. At this stage, the leachates are either enriched with heavy metals within the food material or cans depending on the pH of the medium. Many protein foods precipitate at their isoelectric point. (Ngoddy and Ihekomonye, 1992; Cesar *et al.,* 2001; Danute,2001).

Table 2: pH, Conductivity, Protein, Moisture, Ash and Organic Matter Content of Canned Milk Products

	Metal Concentration (μgg^{-1}),using (i)AAS								(ii) Photometric analysis.							
Canned Milk	Cr (i)	Pb (i)	Cd (i)	Fe (i)	Ni (i)	Co (i)	Zn (i)	Mg (i)	Cr ii)	Pb (ii)	Cd (ii)	Fe (ii)	Ni (ii)	Cu (ii)	Al (ii)	Mn (ii)
B1	1.68	0.01	0.01	0.95	2.03	2.00	1.80	12.53	0.43	4.38	0.78	4.25	3.38	1.66	3.38	4.50
B2	0.45	0.02	0.02	1.58	1.53	1.60	1.63	19.78	0.63	4.50	0.55	3.88	3.38	1.38	3.38	4.38
B3	2.20	0.02	0.02	0.80	0.75	1.60	1.28	16.30	0.48	.13	0.71	4.38	3.30	2.04	3.30	4.25
B4	1.48	0.03	0.03	1.53	2.23	1.53	2.30	13.90	0.53	5.75	0.78	5.23	3.25	1.38	3.25	4.00
Mean	1.45	1.12	0.02	1.22	1.64	1.68	1.75	15.63	0.53	4.69	0.71	4.44	3.33	1.62	0.22	4.26
±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
S.D	0.73	0.48	0.008	0.40	0.66	0.21	0.29	3.18	0.07	0.72	0.11	0.57	0.06	0.31	0.14	0.07

Table 3 presents mean value of triplicate analysis for the level of heavy metals in four brands of canned milk products. It is evidence that the levels of toxic metals falls within ranges for those reported in literature. The values of heavy metals measured in this study were compared with those in literature. The results for micronutrients like Cu, Fe, Mn and Zn indicate higher concentration for Fe and Zn than those of Cu and Al. This is in agreement with result obtained by Cesar (Cesar *et al.*, 2001).

The mean range for values of Cd contents ($0.02 \pm 0.008 \mu gg^{-1}$ using AAS and 0.71 ± 0.11 μgg^{-1} using photometer) is low compared to that of any other metals analysed. Cadmium concentration, according to Kent ,(2003), is generally low in canned food The high Cd concentration in milk and fish product could be traced to contaminated water waste into river with subsequent flow through the food chain (Abdulrahman and Itodo, 2006).

Table 3: Toxic metal concentration of canned milk in Unlacquared tin plate cans, using (i) AAS and (ii) photometric analysis.

CANNED MILK PRODUCTS	**PHYSICOCHEMICAL VARIABLES**					
	pH	Conductivity (µs/Cm)	Protein content (%)	Moisture content (%)	Ash Content (%)	Organic solid (%)
B1	6.05	1.00	15.69	70.00	2.00	28.00
B2	6.16	1.00	17.29	72.00	2.00	26.00
B3	5.77	1.00	15.63	68.00	2.00	30.00
B4	5.52	1.00	18.18	68.00	2.00	30.00
Mean	5.87	1.00	16.69	69.50	2.00	28.50
±	±	±	±	±	±	±
S.D	0.29	0.00	1.25	1.91	0.00	1.91

The lead (Pb) content level in canned food depends on the method used to seal the cans. Contrary to this is the use of welded or lacquered cans with low lead content (Danute, 2000). Mean lead value (1.12 ± 0.48 μgg^{-1}) was obtained. A blood Pb level greater than $1.0 \mu g/cm^3$ is dangerous to health (Adekunle, 2003).Value from the photometric analysis of Pb in milk ($1.45 \pm 0.73 \mu gg^{-1}$). This value is above recommended dose ($0.10 \mu gg^{-1}$) by the existing legislation (Cesar *et al,* 2001).Food is the central source of Cu as an essential element available to man (WHO, 1996). From this study, the mean level of Cu is 1.62 ± 0.31 μgg^{-1}.

Comparative study

Two sets of comparative studies were carried out to justify the accumulation of toxic metals due to canning processes. Seven different metals estimated in canned milk (Table 4) presented values that are higher than those of their corresponding uncanned Diary products in the mean ratio of 1:3.3, 1:17, 1:14.7, 1:5, 1:32.8, 1:10 and 1:1.1 for Cd, Co, Cr, Ni, Pb, and Zn respectively. It thus follows that Nickel, a toxic metal in canned milk presented values that is 33 times higher than Ni in uncanned Diary products. It also implies that comparing Ni in canned milk to those of other canned food is critical for study.

Nickel (Ni) in canned milk and other canned products.

Nickel is trace in the environment but in higher concentration in a number of mineral ores like nickel sulphide, oxides and silicates (ATSDR, 1993). They are also found in hydrothermal veins-channels and surface deposit formed by erosion and weathering of rocks, volcanic eruption, meteorites concentrate. Trace amount of Ni are found in household products from faucet to shampoo.

Most form of Ni do not pose any threat to human health, however, large doses of it such as accidental ingestion, have been recognized with

effects as stomach ache, heart failure, lung tumors, cancer, allergic skin reactions and dermatitis(ATSDR, 1993). Other research have shown that workers who inhaled Ni dust in metal processing and refining industries and workers who inhaled Ni-containing fumes from welding stainless steel can impose more serious health threat (ATSDR, 1993).

Pulmonary absorption is the major route of Ni exposure. It may be absorbed as the soluble nickel ion (Ni^{2+}) while soluble Ni compounds may be phagocytized by macrophages (ATSDR, 1988). The kidney and lung are the primary sites of its accumulation. Other organs such as the kidney, liver, heart and testis also accumulate the metal but to a lesser extent (Coogan *et al.*, 1993).

Figure 1, presents the concentration of Ni (in µg/g) for canned milk and various canned foods and drinks using AAS and photometric method. The chat below clearly indicates that the two analytical techniques (Viz AAS and photometric analysis) may not be used interchangeably for analysis of meat and fish products. The AAS values for sardine (5.11± 1.68 µg/g), canned geisha (5.70±0.95) and corned beef (3.63±1.68 µg/g) are respectively and to a greater extend lower compared to the use of photometer which gave their corresponding values as 3.09± 1.40µg/g, 0.79± 0.29 µg/g and 1.00± 0.14µg/g respectively. The metabolism of Ni is viewed in light of it's binding to form ligand and it's transport through the body (Coogan *et al.*, 1993).Although data are not available to possibly identity which Ni compound are responsible for inducing carcinogenic response, nickel oxides and soluble Ni are carcinogenic (Coogan *et al.*, 1989). This is as a result of either genetic change such as mutation of the DNA sequence and the epigenetic changes that affect gene expression without altering DNA sequence (ATSDR, 1993). Summarily, Ni contents in canned milk are higher than those of canned alcoholic, fruit and carbonated beverages but lower, compared to those of semi solid foods, including canned Geisha, tomatoes, sardine, baked beans and canned vegetable salad (Figure 1).

CONCLUSION AND RECOMMENDATION

This present analysis showed that alloying metals contents, their eruption, leaching and movement into food is critical for estimating the level of metals in semi- liquid food drink. Low pH and high conductivity resulting from increased CO_2 contents, low oxygen accompanied by alkalinity or high oxygen content in acidic medium, high temperature during processing and storage e.t.c are combined factor affecting the attenuation of heavy metals. A comparative study also revealed that the level of metals in canned milk exceed, to a greater extent, the corresponding uncanned products. The low electrical conductivity values reported in this work shows its insignificance in the metal transport .In view of this deductions, it may be necessary for milk manufactures to avoid the use of low pH or acidic water avoid excessive heating, stored and transported within favorable temperature range, reduced shelf life to avoid oxygen intake by rusted cans, use of internally lacquered cans for packaging ,use of materials made up of glass, paper and polymers, else, powdered or uncanned dairy products may be preferred.

Table 4: Comparing some Heavy Metal Concentration for (a) Canned and (b) Uncanned milk products Marketed in Nigeria.

samples	Toxic metals concentrations(µgg^{-1})						
	Cd	Co	Cr	Cu	Ni	Pb	Zn
(a)	0.02±0.008	1.61±0.21	1.47±0.73	1.62±0.31	1.64±0.66	1.12±0.48	1.75±0.29
(b)	0.006±0.003	0.09±0.05	0.01±0.01	0.33 ±0.31	0.05±0.03	0.11±0.08	1.54±1.2

Sources/Key: (a)-current work,(b)-uncanned Diary products by Onianwa *et al.*, 1999.

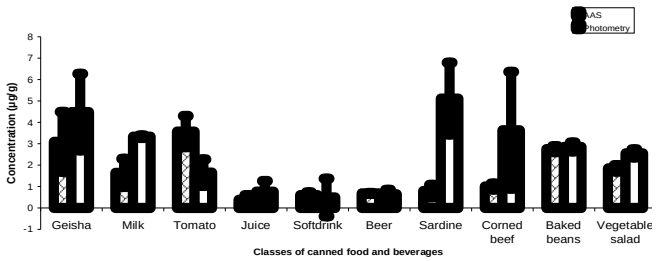

Fig. 1: Comparison of nickel in canned milk and other canned foods and beverages.

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Green Tea Extract Ameliorate Liver Toxicity and Immune System Dysfunction Induced by Cyproterone Acetate in Female Rats

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Abstract: Green tea, consumed worldwide since ancient times, is considered beneficial to human health. The present study aimed to evaluate the effect of green tea extract (GTE) on liver damage and immune system function in female rats treated with cyproterone acetate (CPA). Forty healthy female adult albino rats were randomly assigned to four groups. Group (1) was fed on a standard diet as a control. Group (2) was fed on a standard diet and received an intraperitoneally injection of 25mg/Kg/day. Group (3) was fed on a standard diet supplemented with 1 g GTE% and received a daily injection. Group (4) was fed on the supplemented diet for 7 days prior to receiving the daily injection. The experimental duration lasted for 3 weeks initiated from the first injection. The results showed CPA alone led to diminish liver function, hepatic antioxidant enzyme activities and elevated hepatic oxidative stress and serum IgG and IgM levels comparing with the control group of rats. However, the ingestion of GTE either along with or prior to the CPA treatment could significantly improve the function of liver, hepatic oxidative stress and hepatic antioxidant status and elevate the IgG and IgM levels. These data suggested that, GTE possesses a protective effect on the liver against the induced CPA toxicity by increasing auto immunity and countering the hepatic oxidative stress. [Journal of American Science 2010;6(5):179-185]. (ISSN: 1545-1003).

Key words: Cyproterone acetate, green tea extract, liver toxicity, oxidative stress, immunity

1. Introduction

Cyproterone acetate (CPA) is a derivative of 17α-hydroxyprogesterone (Pregnanes) (Fig.1). In addition to the 6, 7 double bond, the 1,2α- methyl group is present.

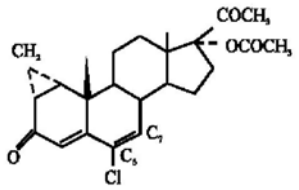

Figure (1): Cyproterone acetate

CPA is a potent steroidal antiandrogen with progestational activity. It is used alone or in combination with ethinylestradiol or estradiol valerate in the treatment of woman suffering from disorders associated with androgenization, e.g., acne or hisuitism. CPA competes with dihydrotestosterone for the ondrogen receptor and inhibits translocation of the hormone receptor complex into the cell nucleus (Siddique and Afzal, 2008). The bioavailability is nearly 100%. CPA has no binding affinity to sex hormone binding globulin and corticosteroid binding globulin in the serum but 93% of compound is bound to serum albumin. It is stored in fat tissue and excreted slowly. The important metabolic steps are hydroxylation reaction and de-acetylation. The metabolite 15β hydroxycyproterone acetate shows only 10% of the progestogenic potency of cyproterone itself. The bio-activation of the CPA involves the reduction of the keto group at carbon-3, which is followed by sulfonation of the hydroxy steroid. The resulting sulfoconjugate is supposed to be very unstable and can decompose to a reactive DNA binding carbonium ion (Schindler et al., 2003).

The International Agency on Cancer (IARC), mainly on the basis of epidemiological studies classifies steroidal estrogens and estrogen progestin combinations among agents carcinogenic to human (Group 1), progestins as possibly carcinogenic (Group 2) and androgenic anabolic steroids as probably carcinogenic (Group 2A). Carcinogenicity to human of sex steroids has been evaluated, and is reported that high dose of estrogen-progestin combinations can cause liver cancer of human (Siddique et al., 2008). In a very recent "Multi Center Study" on oral contraceptives and liver cancer the "Project Team" came to the conclusion that oral contraceptives may enhance the risk of liver carcinomas. CPA is not only a tumor promoting

agent but also a genotoxic chemical (Joosten et al., 2004).

CPA is a tumor initiating agent in the liver of female rats (Deml et al., 1993). It induced micronucleus in rat liver cells, chromosomal aberrations in V79 cells, and human peripheral blood lymphocytes, and also sister chromatoid exchanges in human peripheral blood lymphocyte in vitro (Siddique and Afzal, 2005a). The genotoxic effects of synthetic progestins can be reduced by the use of antioxidants (Ahmad et al., 2002), natural plants products (Siddique and Afzal, 2005b) and herbal health products (Romero-Jimenez et al., 2005).

Tea is one of the most popular beverages consumed worldwide. Tea, from the plant *Camellia sinensis*, is consumed in different parts of the world as green, black, or oolong tea. Green tea (GT) is favored in Japan and China, and initial research on the benefits of GT was carried out in these countries because of local customs (Crespy and Williamson, 2004).

Tea contains many compounds, especially polyphenols, a heterogeneous group of chemicals characterized by hydroxylated aromatic rings. Polyphenols contained in teas are classified as catechins, and are collectively reffered to as GT catechins. GT contains six primary catechins compounds: catechin, gallaocatechin, epicatechin, epigallocatechin, epicatechin gallate and epigallocatechin gallate. These constituents posses potent antioxidant action, although to vary degree and are considered as potent scavengers of reactive oxygen species (ROS) such as superoxide, hydrogen peroxide, hydroxyl radicals and nitric oxide produced by various chemicals (Khan and Kour, 2007).

GT catechins and their derivatives are shown to contribute beneficial health effects ascribed to tea by their antioxidant, antimutagenic and anticarcinogenic properties. GT consumption has been linked to lowering of various forms of cancer. GT constituents also have been shown to have cardioprotective, neuroprotective, antidibetic, and antimicrobial properties. In addition, GT has been found to be useful in the treatment of arthritis, high cholesterol levels, infection, and impaired immune function. GT consumption also has resulted in improved kidney function in animal models of renal failure (Yokozawa et al., 2005).

The present study is an effort to investigate the role of green tea extract (GTE) in overcoming the hepatotoxic effects of CPA administration of adult female albino rats.

2. Material and Methods

Materials:

- CPA was purchased from Schering S.A., France as compressed tablets each containing 50 mg CPA.
- GTE was obtained from El Obour Pharma (Reg. No. 2958/2002) as tablets, each containing 1000 mg extract.

Animals:

Forty adult female albino rats "Sprague-Dawley strain" weighing 100-126 g were obtained from Research of Bilharizia Institute. Academic of Scientific Research and Technology. Cairo, Egypt. The animals were kept individually in wire cages in an environmentally controlled room (temperature 20±2^{o}C, 12 h dark/ 12 h light cycles), with free access to water and diets.

Experimental protocol:

All animals were allowed to acclimatize for 7 days prior to initiation of the experiment. The rats were divided into four groups with the same average body weight. The rats of group (1) (control group) were fed on a standard diet which is prepared from fine ingredients according to AIN (1993). The rats of groups (2) and (3) (CPA group) and (CPA+GTE group), respectively were injected intraperitoneally 25 mg/Kg/day (Siddique et al., 2006) from the beginning of the experimental duration. Rats of CPA group were fed on a standard diet, whereas those of the CPA+GTE group were fed on a standard diet supplemented with 1 g GTE/100 g diet (Sai et al., 1998). Rats of group (4) (GTE+CPA) were fed on the GTE supplemented standard diet for 7 days prior to receiving an intraperitoneally injection of CPA.

At the end of the experimental duration (3 weeks; initiated from the first injection), animals were scarified after overnight fasting. Blood samples were collected from the hepatic portal veins for serum separation. Liver was removed, rinsed in saline and weighed. All samples were stored at -80^{o}C until analysis be done.

Biochemical measurements:

Serum was analyzed for the following biochemical parameters: alanine transaminase (ALT), asparate transaminase (AST), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) by using Kits (BioMerieux). Both of IgG and IgM were determined by using radial immunodiffusion plates specific for rats (The binding site Ltd., Birmingham, UK), which contained anti-serum specific to the antigen. The recommended amount of serum was put into the wells of plates and incubated for 72-96 h at room temperature. The diameter of the precipitation

ring was then measured and the concentrations of Igs were determined by using standard calibration curve.

Accurately weighed pieces of liver tissue were treated differently for the separation and estimation of the liver parameters. A portion of liver tissue was per fused with a phosphate buffered saline solution, pH 7.4 containing 0.16 mg/ml heparin to remove any red blood cells and clots. Then tissues were homogenized in 5-10 ml cold 50 mμ potassium phosphate buffer, pH 7.4. Centrifuge at 4000 rpm for 15 min at 4°C, then malondialdehyde (MDA) value was determined in the supernatant colorimetrically by using Kits. A second portion of liver was homogenized in 3% sulfosalicylic acid (5% homogenate), centrifuged at 1000 rpm at 4°C for 20 min and the resultant supernatant was used for the assay of glutathione (GSH). The third portion of liver was homogenized in 5% metaphosphoric acid, centrifuged at 3000 rpm at 4°C for 15 min and the resulted supernatant was used for the estimation of LDH activity. The fourth portion of liver was homogenized in cold 50 mM potassium phosphate buffer pH 7.4 containing 1mM EDTA for catalase activity determination. The homogenate is centrifuged at 4000 rpm for 15 min at 4°C. The supernatant was collected for the assay. The fifth portion of liver was homogenized in Tris-sucrose buffer pH 7.4 (10% homogenate), and centrifuged at 105000 rpm at 4°C for 15 min. Then the supernatant was used for the determination of gamma glutamyl transferase (GGT) activity. Protein concentration of the above supernatants was estimated.

Statistical analysis:

The data were subjected to statistical analysis using one way classification (F-test) and least significant difference (LSD).

3. Results

Table (1): Effect of GTE administration on liver weight and relative weight of adult female albino rats treated with CPA injection.

Groups / Parameters	Control group	CPA group	CPA+ GTE group	GTE+ CPA group
Relative liver weight (g%)	3.94 ± 0.313	4.86 ± 0.405	4.49 ± 0.234	4.44± 1.41
Liver weight(g)	6.27 ± 0.498	7.58 ± 0.398	7.43 ± 0.62	6.76± 1.07

Data are expressed as means ± S.D.; n= 10.

It is clearly observed from table (1) that, both the absolute and relative liver weights of the female rats treated with CPA were increased significantly compared with the other experimental groups.

Table (2): Effect of GTE administration on liver function parameters (U/L) adult female albino rats treated with CPA injection.

Groups / Parameters	Control group	CPA group	CPA+ GTE group	GTE+ CPA group
Serum ALT	42.33 ± 4.4	148.34 ± 2.97	63.83 ± 3.71	81.04 ± 3.31
Serum AST	260.70 ± 4.66	386.06 ± 3.59	341.85 ± 4.75	312.2 ± 2.64
Serum ALP	2432.49 ± 4.4	4203.53 ± 6.2	3939.89 ± 3.0	3962.5 ± 3.8
Serum LDH	3834.06 ± 4.5	8702.95 ± 5.7	6324.58 ± 4.2	6520.6 ± 3.1

Data are expressed as means ± S.D.; n= 10

All the estimated liver function parameters; serum ALT, AST, ALP, and LDH activities; illustrated in table (2) shows a highly significant difference ($P< 0.01$) in these parameters in adult female rats treated with CPA, whereas these increments were monitored by the administration of GTE.

Table (3): Effect of GTE administration on hepatic MDA, GSH, LDH, and GGT of adult female albino rats treated with CPA injection.

Groups / Parameters	Control group	CPA group	CPA+ GTE group	GTE+ CPA group
Hepatic MDA (nmol/g)	5.87± 0.039	9.67± 0.046	5.24± 0.042	5.74± 0.047
Hepatic GSH (mg/g)	25.36± 1.78	34.93± 2.09	31.26± 1.98	27.31± 1.85
Hepatic Catalase (U/g)	8.89± 0.026	7.60± 0.024	8.58± 0.037	8.81± 0.031
Hepatic LDH (μmoles/mg Protein/min	1.34± 0.35	1.32± 0.123	2.18± 0.193	2.26± 0.148
Hepatic GGT (U/mg protein)	2.24± 0.197	5.35± 0.147	2.64± 0.10	2.85± 0.193

Data are expressed as means ± S.D.; n= 10

Table (3) shows increased activities of hepatic MDA and GGT of female rats injected by CPA compared with both the control and treated groups with GTE. Whereas, there was a significant decrease in hepatic GSH catalase and LDH activities of the CPA group comparing with the other groups.

Table (4): Effect of GTE administration on serum IgG and IgM values of adult female albino rats treated with CPA injection.

Groups / Parameters	Control group	CPA group	CPA+ GTE group	GTE+ CPA group
Serum IgG (ng/dL)	652.16 ± 4.84	842.90 ±4.73	874.19 ± 2.74	863.89 ±3.53
Serum IgM (ng/dL)	167.20 ± 4.24	262.89 ±3.94	255.05 ± 3.70	264.80 ± 3.82

Data are expressed as means ± S.D.; n= 10.

Table (4) shows that, there were a significantly elevation in both serum IgG and IgM values in all the experimental groups treated with CPA and administered with GTE either before or along with the treatment comparing with the control group values.

4. Discussions

Hepatocellular carcinoma is considered the main cause of cancer death all over the world. ALT, AST, ALP, LDH, liver weight and relative liver weight are reliable references, widely used in animal study, to indicate poor hepatic function; hepatic damage and malignancy (Cantoni et al., 2003). In the present study, all the previously mentioned parameters were increased significantly in the CPA administered rats, which are an indication of liver cell proliferation. These increases in serum activities of ALT, AST and ALP of female rats injured by CPA were consistent with the results of Ali (2008). Hence, CPA is considered a tumor initiating agent in the liver of female rats by Deml et al. (1993). This was elucidated later that, the bio-activation of the CPA involves the reduction of the keto group at carbon-3, which is followed by sulfonation of the hydroxyl steroid. The resulting sulfoconjugate is supposed to be very unstable and can decompose to a reactive DNA binding carbonium ion (Wolff et al., 2001). GTE at 1g% either before or along with CPA administration gave a high hepatoprotective effect by suppress the increment of serum ALT, AST, ALP and LDH activities, liver weight and relative liver weight values. The observed decrease in these parameters showed that, GTE, to some extent, had liver injury-preventative effects and preserved the structural integrity of the liver from the toxic effect. The hepatoprotective effect of GT polyphenols was confirmed against microcystin-LR (Xu et al., 2007) and chlorpyriphos in rats (Khan and Kour, 2007) as a feature of lowering the increased activities of ALT, AST and ALP induced by the different treatments.

Results of the present study revealed a significant increase in liver MDA level in CPA-received rats. This result could be attributed to excessive generation of free radicals during the metabolism of CPA. The possible mechanism and cause of the genotoxicity of CPA has been studied by using one of the genotoxic doses (i.e., 30 µM of CPA) with different doses of superoxide dismutase (SOD) and catalase (10 and 20 µg/ml). SODs are family of metal enzymes that covert O^{-}_{2} to H_2O_2 according to the reaction of (Figure 2).Since the treatment with SOD increases the chromosomal aberrations and sister chromatoid exchange frequency, so that there is a possibility somehow CPA is generating oxygen species. Further the treatment with catalase separately and in combination of SOD results in the significant decrease in chromosomal aberrations and sister chromatoid exchange, approving the production of H_2O_2. Because catalase catalyses the decomposition of H_2O_2 to water and oxygen according to the reaction of (Figure 2).In the light of above results, a suggestion can be made for the possible mechanism of generating ROS by CPA (Siddique and Afzal 2008).

Figure (2) shows the structure of CPA with (a) a1,2α-methylene group, (b) a keto group at carbon-3, (c) two double bonds, C_4=C_5 and C_6=C_7, and (d) C_1 at carbon-6, promote the tendency toward free radical formation. XOOH can also give rise to $XOO^{\cdot}$ (alkoxyl) and $^{\cdot}OOH$ (peroxyl) radicals. The presence of $XOO^{\cdot}$ appears to be a very remote possibility in view of the highly polar nature of the living system because for that the X has to be essentially an alkyl group (Siddique and Afzal, 2005a).

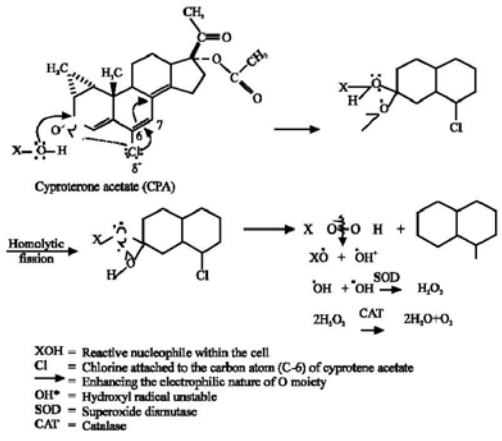

Figure (2): Possible mechanism of generating free radicals by CPA

The present study revealed significant increase in hepatic GGT and GSH activities accompanied with a significant decrease in hepatic catalase and LDH activities in the CPA administered

female rats. These results are in harmony with those of Ahmad et al. (2002). The ubiquity of elevated GGT level in many rodent and human hepatic and extra hepatic carcinomas have led to the hypothesis that GGT provides a growth advantage to focal cells during carcinogenesis. The advantage may be due to the role of GGT in hydrolysis of GSH to gamma-glutamyl moiety and cysteinylglycine in GSH and GSH conjugate catabolism. This transport in GSH constituents, leading to increase in cellular GSH, which is required for proliferation and resistance to chemotherapy (Csanaky and Gregus, 2005).

The decrease in the hepatic catalase activity in the injured rat group may be explained according to its function as a free radical scavenger enzyme, which suppress the formation of the ROS and/or oppose their action. The by-products of oxygen metabolism initiate different sub cellular outcomes. The superoxide radical has been shown to directly inhibit the activity of catalase. Likewise, singlet oxygen and peroxyl radicals have been shown to inhibit catalase activity (Escobar et al., 1996). These observations manifest and explain the significant inhibition of catalase activity in the CPA administered group of rats.

The result also revealed a significant decrease in hepatic LDH activity in the CPA injured female rats. This may be elucidated according to Sauer and Dauchy (1985) who indicated that tumors in vivo have a large capacity for its production.

Most beneficial health effects ascribed to GT are considered to be mediated by potential antioxidant properties of its constituents that scavenge free radicals and reduce oxidative damage. Several lines of evidence suggest that prooxidant and antioxidant actions of plant polyphenols may be important mechanisms for their anticancer properties. In this study, GTE consumption to female rats either along with or before the treatment with CPA doses exert a noticeable significant decrease in hepatic MDA level, GSH and GGT activities with a significant increase in hepatic catalase and LDH activities. The decrease in hepatic MDA level in the GTE consumed rats is consistent with that of Yokozawa et al. (2002). Also, the increase in hepatic catalase and GSH activities according to the consumption of GTE is consistent with that of Khan and Kour (2007) and Xu et al. (2007). However there is a disagreement of the result of hepatic LDH activity with that of Hasegawa et al. (1995) who found that hepatic LDH activity of rats administered GTE 2% wt/v and injured with 2-nitropropane had a diminished activity.

It has been reported that GT exert its biological effects on the basis of the redox state of a particular cell/tissue and according to the level of GT polyphenols accumulated in the tissues. It is hypothesized that GTP help in the protection against ROS damage by contributing, along with antioxidant vitamins (i.e., vitamins C and E) and enzymes (i.e., SOD and catalase), to the total antioxidant defense system (Crespy and William, 2004). GTP, water-soluble antioxidants, have been demonstrated to inhibit iron-induced oxidation of synaptosomes by scavenging hydroxyl radicals generated in lecithin/lipoxidase system. On the other hand, GTP can penetrate the lipid bilayer, decreasing free radicals concentration or influencing antioxidant capability in biomembranes. On the other hand, they could reduce the mobility of free radicals into the lipid bilayers as well. Moreover, GTP can interact with phospholipid head groups, particularly with those containing hydroxyl groups, so they could decrease the fluidity in the polar surface of phospholipid bilayer. In addition, GTP can prevent the loss of the lipophilic antioxidant α-tocopherol, by repairing tocopheryl radicals, and protection of the hydrophilic antioxidant ascorbate (Skrzydlewska et al. 2002). So that, the results of this study made a speculation that GTE administration can modulate the susceptibility to lipid peroxidation coupled with up-regulation of the antioxidant status and block tumor development in liver of female rats treated with CPA doses.

In this study, there is a markedly increase in the serum IgG and IgM of female rats either administered with CPA alone or treated with GTE (either with or before the CPA doses). This elevation in CPA administered group of rats may be due to the increasing of hepatic GSH and GGT activities comparing with control rats. This elevation in activities may activate T-lymphocytes in white blood cells, leading to increase immune parameters. The steroid hormones affect upon cytokine production which is mediated by the nuclear factor-KB (NF-KB). This is an inducible transcription factor that positively regulates the expression of pro-immune and pro-inflammatory genes. It has been shown that the steroid/receptor complex can physically interact with NF-KB and inhibits its transactivation activity (Mc Kay and Cidlowski, 1999). Via this mechanism esterogens, progesterone and testosterone can inhibit pro-inflammatory cytokine expression in immune cells expressing the respective receptor. The mechanism by which steroid binding with membrane receptors affect immune cell function remains obscure. A proposed explanation by their lipophilic nature, sex steroid can integrate into the membrane and alter membrane properties, such as fluidity and thereby changing the function of the immune cells (Lamche et al. 1990). Flavonoids exert a prime function in the most important weapon in the body

defense (i.e., immune system). They stimulated both of the immune branches; the humoral and the cellular. Flavonoids stimulate the production of antibodies in a yet poorly known fashion. However, it is likely that they do so by altering cytokine production, since this is assisted by protein P-kinase cascades, which are known to be under the influence of flavonoids. Besides, flavonoids prevent the synthesis of PGs that suppress the T-cells (Havsteen, 2002). Ingestion of GT improved the depressed immune functions either humoral or cell immunity in mice treated with diethylnitrosamine to basal levels. Moreover, the transplantation of Lewis lung carcinoma cells into mice decreased the CD^+ positive to lymphocytes and CD_4^+: CD_8^+ ratio. So, it could be concluded that GT ingestion improved immune functions and inhibited tumor growth (Zhu et al. 1999).

Conclusion

In conclusion, GTE could potentially attenuate the hepatic injury induced by CPA treatment as evidenced by: (I) Restoration of almost all enzymatic liver function tests. (II) Normalization of the oxidative stress biomarker, MDA. (III) Balancing of the oxidant/antioxidant status of the liver cells. (IV) Improving of the immune system function. Thus daily moderate levels of GT consumption can inhibit the activation of some types of environmentally encountered injures.

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Screening of some Biopesticides for the control of *Callosobruchus chinesis* in Stored Black Beans (*Vigna mungo*) in Imo State.

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Abstract: The influence of some oil extracts comprising Cashew nut oil (CNO), Coconut oil (CONO), Udara nut oil (UDNO),and Neem leaf oil in controlling stored black beans weevil (*Callosobruchus chinesis*) was investigated. The experiment was laid down in the laboratory using Completely Randomized Design (CRD). The results showed that the number of eggs and exit holes of C.chinesis were not significant at 5 % probability level before treatment with the extracts. Then after two months in storage the black beans were treated with the extracts and there was significant reduction of rate of oviposition and number of exit holes. The plots treated with coconut oil extract proved more effective than other oils and was therefore recommended for use by farmers for black beans storage under our agro-ecological zone. [Journal of American Science 2010; 6(5):186-188]. (ISSN: 1545-1003).

Keywords: Sceening, biopesticides, black beans, *callosobruchus chinesis*, extracts

1,Introduction

Black beans (*Vigna mungo*) belong to the family Leguminoseae. It probably originated from *Vigna soblobatus* which occur wild in India. It has been introduced to other areas in the tropics mainly by Indian immigrants (Taylor, 1971). It is utilized for human consumption and forms about 50 to 70 % of livestock feed (Adesuyi, 1978). It could be consumed as fresh or dried pod, boiled or roasted pods. In south eastern Nigeria where the beans are cultivated and consumed extensively insect pests have been the major constraints to the production and storage of the beans. The major post harvest losses and quality deterioration caused by storage pests on stored products are major problems facing agriculture in Nigeria (Adedire and Ajayi, 1996). In some countries the damage on stored products may be exceeded by rodents (Gwinner *et. al.*, 1990). *Callosobruchus chinensis* (L.) is an important pest of black beans in stores and seems to attack other stored pulses such as cowpea, groundnut, chickpea bambara groundnut, jack bean, and Pigeonpea (Adedire and Ajayi,1996)

The use of toxic metabolites such as gammalin, actellic dusts, Pilf palf, etc have been employed by farmers in Southeastern Nigeria for protecting black beans against damage by *C.chinensis* Though these pesticides have positive effect on the pests, they have continued to remain hazardous to man and his environment . In South eastern,Nigeria there are many cases of deaths resulting from consumption of black beans stored with pilf palf and actellic dusts. As a result of the interest of general public for consumption of organic food , the interest of researchers have been directed to finding alternative pesticide that should be environmentally friendly and which will not possess dangers to man.
Many botanicals have been shown to have great potentials as alternatives to the synthetic insecticides. For instance, Osisiogu and Agbakwuru, (1978), reported that the essential oil of *Denneltia tripetala* was effective in the control of cowpea bruchid *Callosobruchus maculatus* (F.), cowpea weevil *C.chinesis* (L.), and maize weevil *Sitophilus zeamais.* The heavier spray oil fractions are more effective at killing insects than the lighter oils (Luik,et. al.,2000).Therefore the main purpose of this research is to isolate an effective biopesticide for the control of weevil of black beans in storage.

2. Materials and Methods.

The research on storage of black beans was conducted at the Department of Crop Science and Technology, Postgraduate Teaching and research Laboratory, Federal University of Technology, Owerri, starting from May, 2007 to December, 2007.
The materials used were black beans bought from Ogbete market in Enugu state, cashew seeds, coconut, Udara seed and neem leaves.

Methods of preparing the Extracts.

The neem leaves were collected from the neem plant (*Azadirachta indica* (A.juss). They were air dried under shade for 3-4 days and crushed into a powder using a mortar and pestle. 1 kg of the powder was soaked in petroleum spirit and oil extracted with

a soxhlet extractor after heating at a temperature of 40 to 60^{o}c and kept for sometime to allow the spirit to evaporate. Coconut (Cocos *nucifera* (L.), cashew seeds (*Anacardium occidentalis*) and udara seeds (*Chrysophyllum albidum*) were cracked to expose the kernel and nuts and were later dried in the sun for three days and oil extracted .

The experiment will be laid down in the laboratory using Complete Randomized Design (CRD) with four replications. There were five treatments comprising cashew nut oil, coconut kernel oil, Udara oil, neem leaf oil, and control. These five treatments were replicated four times completely at random using plastic container with a cover to give a total of 20 treatment combinations. Five (5) ml of each of the oils were admixed with 1kg of black beans. Data was first collected on number of eggs present on the seeds before and after treatment and secondly on number of exit holes before and after treatment. Observations on or before treatment were made weekly at eight weeks intervals. 100 seeds were selected at random and examined with hand lens for presence of eggs and exit holes.

Analysis of data

Separation of means for statistical significance was by the use of Least Significance Difference as outlined by Obi, (1986).

3. Results

Table **1** and **2** show that there were no significant difference with respect to the number of eggs laid by the *C.chinesis* and their exit holes before extracts were applied. Tables **3** and **4** show there were significant difference at 5 % probability level after the application of oil extracts. On the average the coconut oil extract reduced the level of oviposition and exit holes by C.chinesis when compared with other plant oils.

4. Discussions

The non-significant effect observed with respect the no of eggs laid and their holes before the botanical extracts were applied is to be expected as the pests were moving and feeding freely without restriction. The efficacy of coconut oil in reducing the level of egg production and exit holes by C.chinesis better than other oils tested agreed with the findings of Alkolifi, (1989), who reported that coconut oil when applied to rice protected it against lesser grain borer (*Rhizopertha dominica*).

The coconut oil probably possessed strong ovicidal properties which may have depleted the oxygen level available to the eggs in the grains thus preventing the development of the eggs and causing their mortality.

Summary and Recommendations:

The oil extract from coconut kernel could be of great advantage in preventing damage caused by *C.chinesis* to stored black beans. However, further research need to carried out to assess the toxicological profile, ovicidal properties and appropriate rate of application.

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Neurobehavioural, neurochemical and neuromorphological effects of cadmium in male rats

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Abstract: AS Cadmium is a widespread toxic environmental and industrial pollutant. The present study was carried out to investigate the possible effect of cadmium chloride ($CdCl_2$) on memory, exploratory motor activity (EMA)and motor balance in male rats. Forty five male Wistar rats weighing (100-120 gm) were administered $CdCl_2$ in drinking water at one of three concentrations; 0 ,5 and 50 mg/ L dissolved in water for a period of 60 days. Memory retention was evaluated through open-field habituation test (non associative learning), classic maze test (associative learning) as well as working spatial memory in a Y-maze.Moreover, exploratory motor activity and motor coordination were evaluated. Brain tissue specimens, representing all treatment groups, were taken for histopathological and biochemical examination. The average body weight significantly lower in group of rats exposed to high $CdCl_2$ doses. Open field revealed marked impairment in habituation with noticed influence on both anxiety and fear in rats exposed to high $CdCl_2$ .Moreover, learning and memory assessed during classic maze test and Y-maze test showed reduced memory retention in cadmium exposed animals as compared to control group .In novelty acquisition test ,a reduced exploratory motor activity in rats exposed to high $CdCl_2$ was noticed .Additionally ,complex motor behaviour (motor coordination)was significantly impaired due to cadmium intoxication. Furthermore, ,histopathological and biochemical evaluation revealed distinct neurodegenerative changes of nerve cells especially in hippocampus , inhibition of cholinesterase activity ,as well as decrease in the antioxidant enzymes activity (GST and SOD). Overall, these results suggest that intoxication with cadmium chloride has potentially deleterious effects on brain as reflected in impairment learning and memory. Also exploratory motor activity and motor coordination were reduced. [Journal of American Science 2010; 6(5):189-202-]. (ISSN: 1545-1003).

Keywords: Cadmium intoxication; learning and memory; motor activity; hippocampus, AChE; SOD;GST;Rats.

1. Introduction

Humans and animals interact with their environments on a daily basis and as a consequence are exposed to a broad spectrum of synthesized chemicals present in the food they eat, the air they breathe and the water they drink (Wade et al., 2002). It is a widespread toxic environmental and industrial pollutant. Cadmium has been released into the environment through human activities and is routinely found as a contaminant in tissues collected from the human population throughout the world (Newsome et al., 1995).

Cadmium is unique among the other metals because of its toxicity at a very low dosage and long biologic half life (30 years in human) and its low rate of excretion from the body (Jones and Cherian, 1990). It is listed by the U.S. Environmental Protection Agency as one of 126 priority pollutants.

Acute-Cd exposure results in pulmonary edema and respiratory tract irritation, whereas chronic exposure to Cd often leads to renal dysfunction, anemia, osteoporosis, and bone fractures (Friberg et al.;1986 , Goering,et al.;1995), Cd is carcinogenic for a number of tissues (Waalkes;2000) and is classified by IARC(1993) as a human carcinogen.

In laboratory animals, acute Cd poisoning produces primarily hepatic and testicular injury, whereas chronic exposure results in renal damage, anemia, and immuno- and osteotoxicity (Goering, et al.; 1995, Klaassen, et al.; 1999). Cadmium can enter into the brain parenchyma and neurons (Nishimura et al., 2006) causing neurological alterations in humans (Rose et al., 1992) and animal models (Lukawski et al., 2005) leading to lower attention, olfactory dysfunction and memory deficits. Additionally , there are studies showing the neurotoxicity of cadmium on cell culture models like neurons and glial cells (Im et al.,2006; Lo'pez et al.,2006; Nishimura et al., 2006). In contrast, there are few studies discussing the effect of cadmium on learning and memory in rats.

Regarding the locomotor activity and motor balance,decrease in distance traveled, stereotypic time and movements, ambulatory time and vertical

movements were observed in Cd-exposed rats (Ali et al .,1990).

A variety of neurobehavioural and biochemical effects are produced on the nervous system of rodents given repeated doses of cadmium (Murphy, 1997).It has been suggested that the mechanism of Cd toxicity involves production of reactive oxygen species and free radicals (Manca, et al.; 1994, Stohs et al.; 2001). In animals, the various toxic effects induced by cadmium may be due to increased lipid peroxidation (Manca et al., 1991; Calderoni et al., 2005). The increase in lipid peroxidation may be attributed to alterations in the antioxidant defense system (Ognjanovic et al., 1995). This defense system includes the enzymes glutathione peroxidase, thioredoxin reductase as well as the reduced glutathione (GSH), which normally protect the biological system against free radical toxicity (Sarkar et al., 1998; El-Sharaky et al., 2007). Neurotoxicity was still not regarded to certain specific reason, as Cd exhibits several effects on neural level concerning with neurochemical mediators like catecholamines, serotonin (Antonio and Leret 2003) and cholinergic transmission (De Castro *et al*., 1996). Assembly of cell membrane proteins and phospholipids may also be affected under Cadmium toxicity (Gerak-Kramberger and Sabolic 2001).

To our knowledge no literatures are available to address the effect of cadmium , on learning and memory in rats .Furthermore , measurements of both associative and non associative learning abilities as well as spatial working memory in rats are not well implemented .

So, the objective of the current study, was to evaluate the effects of Cadmium chloride solution (5 or 50mg) intake on two memory tasks in adult male rats as measured by open –field habituation (non associative learning) and classic maze (associative learning). Also, spatial working memory performance was measured in Y- maze. As the hippocampus and cholinergic system are greatly involved in the process of learning and memory, histopathological and biochemical examination were also carried out in order to detect neurodegenerative changes in brain. Additionally, exploratory motor activity (EMA) and motor coordination were evaluated as a result of neurodegenerative deficits.

2. Materials and Methods

2.1.: Animals:-

Forty five Wistar male albino rats weighing about 100-120 gm were used in this study. Animals were raised in the Animals House Unit in Faculty of Veterinary Medicine, Cairo University. They were maintained in plastic cages with stainless steel wire lids; (bedded with wood shavings); on a standard laboratory feed diet. Animals were housed at constant room temperature (20-22 °C) ,60% humidity and light cycle of 12h. /day.

2.2.: Administration of Cadmium

The animals (45 male rats) were divided randomly into three groups of 15 animals each. The first group served as the control and the animals were allowed ad libitum normal tap water during the experiment without any added cadmium. The other two groups of rats (experimental), were allowed ad-libitum tap water containing 5mg cadmium chloride/L dissolved in water (low dose) and 50 mg cadmium chloride / L dissolved in water (high dose), respectively (Waalkes et al., 1999).All animals were exposed to ad-libitum supply of low doses and high doses $CdCl_2$ for 60 days in drinking water till completing all assessments of learning and memory behaviour test.

2.3. Open-field test:

Habituation , a form of non associative learning, was measured in the open –field test (Kelly, 1993; Mello e' Souza et al; 2000 and Lea et al; 2008). The open field used was a square arena (90 cm x 90 cm x 25 cm), built from wood. The wood of the apparatus is covered with plastic laminate (Formica), which prevents absorption of fluids (urine of rats). The floor was divided by black lines into 36 small squares (15x15 cm) .The rats were gently placed in the corner of the arena and left to explore for 3 minutes. Crossings of the black lines and rearings performed were counted for three consecutive days. Also,number of fecal pellets in the arena were recorded. The open field was cleaned with 10 % alcohol and water solution prior to behavioral testing to remove residues left by previously tested rats. The decrease in the number of crossings and rearings was taken as a measure of the retention of habituation (Lea et al; 2008).

2.4. Maze learning test (classic maze):

Associative learning was assessed using classic maze test. The base measure of the maze was 100x60 cm and the walls were 25 cm high. The entire maze was made of wood with a glass cover to prevent escape of animals and allow observation. Testing was carried out between 09:00 and 15:00, where all groups were randomly allowed for testing at the same day. Male rats were deprived of feed for a 23 hours period before start of testing. Rats were given their daily amount of food as a reward at the end of the maze. Animals were given one trial per day for five consecutive days. Time elapsed to locate the feed at the end and numbers of entries of blind alleys were recorded according to Staddon (1983) .

2.5. Spatial Y-maze memory:

Spontaneous alternation in a single session was assessed in a Y –maze, which is used as a measure of

short- term memory performance (Maurice et al; 1994 and Roghani et al; 2006).

Each arm of Y –maze was 40 cm long, 30 cm high and 15 cm wide (Roghani et al; 2006) and converged in an equilateral triangular central area with 15 cm at its longest axis. Rat was placed at the end of one arm and allowed to move freely through the maze for eight minutes. The sequence of each arm entry recorded manually.

Measure of spatial memory, was defined as the entry into all three arms on consecutive choices in overlapping triplet sets. The percent of spontaneous alternation beaviour was calculated as the ratio of actual to possible alternation.

2.6. Novelty exploration test:

To investigate exploratory motor activity (EMA) in rats in the three studied groups, a "mini-holeboard" was designed that could be inserted into the base of a wooden box, with a floor (40 x 40 cm) and walls 50 cm. The mini-holeboard consisted of a dark platform (40 x 40 cm) which contained a hole (diameter 5.5, depth 5 cm) in each quadrant. A small object, which differed from in scent and texture, was placed in each hole (stimulus- rich). Exploratory behaviour of rats including numbers of rears and head-dips (to examine the interior of ,or the objects within the four holeboard holes)were counted during the 15-min. exposure period of the rats to the holeboard (Vaughan and Braunewell, 1999).

2.7. Psychomotor testing (Motor complex behaviour):

Animals of the three groups were examined with two different motor tests (rod walking and plank walking).

Rod walking: The ability of rats to balance on a stationary, horizontal rod, measures psychomotor coordination. Male rats were placed in the center of a rod (100 cm long , 26 mm in diameter , positioned 23cm above the table surface), parallel to it , and their latency to fall off the rod onto a cushion below was recorded (max . score = 60 s) .

Plank walk test : Balance and coordination were measured by exposing the rats to one trial on each of two horizontal planks (wide= 25 mm and narrow = 13 mm) , each 100 cm long , placed 34 cm above the table top .Distance traveled (in cm) and number of turns on the planks were recorded and averaged for each trial (Barbara et al , 1998).

2.8. Body weight and brain weight:

All male rats per group were weighed at the onset of treatment and weekly throughout the study. At the end of the study, five rats from each group were sacrificed by decapitation; brain of each animal was removed, cleaned and weighed.

2.9. Biochemical examination*:*

At the end of experiment five rats from each group were sacrificed by decapitation. Brain of each animal was taken on ice cold. Then homogenized in phosphate buffer with PH 8 (W/V), centrifuged at 1500 rpm for 10 min. The supernatant fluid froze at -20°C until assayed for further analysis.

- Acetylcholine esterase (AChE) activity was determined using acetylcholine iodide as a substrate according to the method of Elman *et al*., (1961).

- Estimation of lipid peroxidation. Enzymatic activity for oxidative stress were estimated including Glutathione-s-transferase (GST) and superoxide dismutase (SOD) according to methods of Habig *et al*., (1974) and Giannopolitis & Ries (1977), respectively.

- Total protein (TP) was determined by lowery's method (Lowry *et al*., 1951).

2.10. Histopathological examination:

Tissue specimens from brain of all experimental rats were collected at the end of the study and fixed in neutral buffered formalin, processed by conventional method, embedded in paraffin, sectioned at 4-5 um and stained by Haematoxylin and Eosin (Bancroft et al., 1996).

2.11. Animal Care

All animals received humane care as well as the approved ethical rules .Animal care was in compliance with applicable guidelines from Cairo University policy on Animal Care and Use.

2.12. Statistical analysis*:*

Statistical analyses were performed by using SPSS statistical software package. Data are presented as means with their standard error. Normality and homogeneity of the data were confirmed before ANOVA, differences among the experimental groups were assessed by one-way ANOVA followed by Duncan's test (SPSS,2006).

3. Results

3.1.Open field test :

In the open- field habituation (Table 1.), a significant effect of Cd regarding the number of crossed squares, number of rearings and number of faecal pellets was observed .

These parameters exhibited significant differences between high dose group (50 mg Cd) and control one. Where the group of rats treated with high dose of Cd showed a significant increase in the locomotor behaviour in the field (crossing of squares & rearings) ($p< 0.01$), the mean values were 57.90±8.26 and 19.61±3.13 when compared to the control group (26.70±2.22 and 7.02±0.82). So, over the three test sessions , impairment in habituation was markedly

seen in high $CdCl_2$ group compared to other treatments . Also, there was a significant increase in motor activity in the field ($p<0.05$) in male rats treated with low doses of cadmium .

Concerning the number of faecal pellets in the field (vegetative behaviour), there was significant differences between the high dose group and the control one, as the mean values were (5.03±0.26 and 2.22±0.31) respectively. This indicates that, with habituation impairment, fear and excitation increased in the group treated with high dose of cadmium, as rats defecated more frequently.

3.2. Maze learning Test : (classic maze)

Learning and memory assessed over five days of maze test ,showed that group of animals exposed to high concentrations of $CdCl_2$ took longer time to locate feed (Table .1) (1.68 ± 0.44 minutes , $p <0.05$) , with higher frequency for entering blind alleys (3.53±0.42).

These results demonstrating poor memory retention relative to cadmium intoxication. Regarding mean values of time elapsed and number of errors of low dose group, showed a non significant differences (1.34± 0, 29 minutes and 3.31±0.58) .

3.3. Spatial Y-maze memory:

In Table (1), the mean percent of spontaneous alternation behavior for high dose Cd, low dose Cd and control group were (36.99±3.45, 39.21±4.25and 60.70±3.36 respectively). There were significant differences in working spatial memory observed among the examined groups ($P< 0.01$ and $P< 0.05$ respectively). Furthermore, there were significant difference $(p< 0.01)$ in the mean of total number of times the animals entered arms (19.80±1.82, 19.09±1.73 and 12.13±0.63) for high dose, low dose and control group respectively.

3.4. Novelty exploration test:

A significant result in exploratory activities was found between treatments during novelty exposure. Number of both rearing and head dipping were significantly lower in high treated group (Table.1) ($p<0.05$) in comparison with the low dose Cd group and control group. Thus, a less degree of exploration was noticeably showed in rats with high doses of Cadmium intoxication in the novel environment.

3.5. Performance on psychomotor testing:

Complex motor behaviour (motor coordination), as measured by rod walk and plank walk, declined significantly in rats exposed to high concentration of Cd .(Table ,2) .

3.6. Biochemical examination :

There was a significant decrease ($p < 0.05$)in acetylcholinesterase activity in the brain of Cd treated groups (Table 3). Concerning brain oxidative state, significant decline was noted markedly in SOD , while no changes were recorded in GST level in brain of Cd intoxicated groups. .Also, there was a significant reduction ($p<0.05$) in the brain total protein of $CdCl_2$ treated groups (Table 3).

3.7. Histopathological examination:

The brain of rats treated with low dose and high dose of cadmium were macroscopically slightly congested. Microscopically, brain sections of rats treated with low dose of cadmium revealed neuronal degeneration, pyknosis of neurons (Fig.1) and neuronphagia of pyknotic neurons (Fig.2). Moreover, brain of rats treated with high dose of cadmium showed congestion of blood vessels , necrosis of neurons (Fig 3) , neuronphagia , focal gliosis (Fig .4) as well as hemorrhage in Virchow space (Fig.5) and necrosis of Purkinje cells of the cerebellum (Fig.6) .In hippocampus the pyramidal cells appeared atrophied and necrosed (Fig .7) . Meanwhile, brain of control, untreated rats, showed no histopathological changes (Fig.8).

3.8. Body weight and Brain weight:

Body weight was significantly ($p < 0.05$) low in groups of animals exposed to high concentrations of $CdCl_2$, compared to those exposed to low doses of $CdCl_2$ and in control group (Table.3).

Lower brain weight was significantly ($p<0.05$) seen in rats exposed to high and low concentration of $CdCl_2$ (1.82±0.13 gm and 1.82±0.23 gm) compared to rats in control group (2.38±0.63 gm) (Table 3) .

Table 1. Effect of exposure to different doses of $CdCl_2$ on measurements of Open-field test, Classic maze test, Y-maze and Novelty acquisition tests.

Group	Control	Low dose	High dose
Parameter			
Open –field test			
• No. of squares	26.70±2.22	36.16±1.59 [b]	57.90±8.26 [a]
• No. of rearing	7.02±0.82	9.78±1.68 [b]	19.61±3.13 [a]
• No. of pellets*	2.22±0.31	2.90±0.21	5.03±0.26 [b]
Maze test			
• time elapsed (min)	1.21±0.27	1.34 ±0.29	1.68 ±0.44 [b]
• No. of errors	3.11 ± 0.64	3.31 ± 0.58	3.53 ± 0.42
Spatial Y-maze			
• No. of arms	12.13±0.63	19.09±1.73 [a]	19.80±1.82 [a]
• % of s.alternations	60.70±3.36	39.21±4.25 [a]	36.99±3.45 [a]
Novelty exploratory test			
• No. of rearing	34.32±2.11	32.72± 2.33	27.70±2.22 [b]
• No. of head dips	18.91±1.22	18.14± 1.18	16.36±1.18 [b]

Figures in the same row with different letters are statistically significantly different (compared with the control group). [a] ($P<0.01$) and [b] ($P<0.05$), * :Fecal pellets.

Table 2. Effect of exposure to different doses of $CdCl_2$ on complex motor behavior (motor coordination).

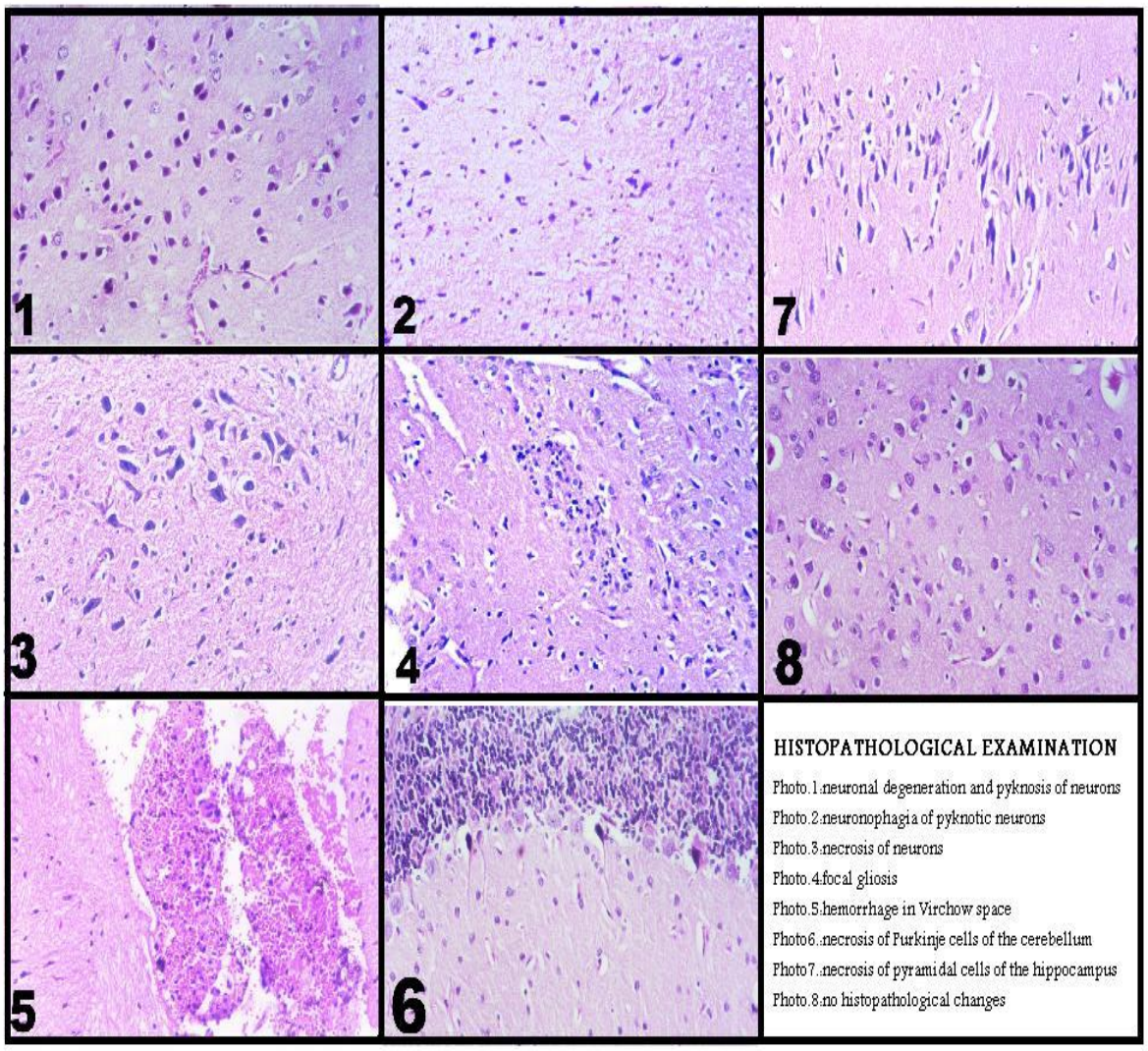
Group / Parameter	Control	Low dose	High dose
Rod walking (Latency to fall,sec)	26.58±3.77	25.61±4.55	20.15±4.58 [b]
Plank walk			
• Plank 1.3 mm			
1- No. of turns	3.50±0.27	2. 40 ±0.18 [b]	2.38 ±0.44 [b]
2- Dist.trv (cm)*	133.20±8.50	134.50±9.40	70.8±4.33 [a]
• Plank 2.5 mm			
1- No. of turns	2.90± 0.12	2.70±0.11	2.78±0.32
2- Dist.trv (cm)*	164.30± 4.22	112.14±5.44 [b]	134±6.22 [b]

Figures in the same row with different letters are statistically significantly different (compared with the control group). [a] ($P<0.01$) and [b] ($P<0.05$). * Dist.trv (cm):Distance traveled on the plank

Table 3. Brain, body weight and biochemical examination (acetylcholine esterase, Lipid peroxidation and total protein) of brain in rats exposed to $Cdcl_2$ through drinking water.

Group	Control	Low dose	High dose
Parameter			
Biochemical examination			
• Acetylcholine esterase (nmol/mg protein/min)	0.802±0.08	0.672±0.05 [b]	0.504±0.02 [b]
• lipid peroxidation			
- GST (unit/mg protein/min)	0.372±0.02	0.359±0.03	0.337±0.1
- SOD(unit/mg protein)	0.071±0.008	0.058±0.001 [b]	0.044±0.003 [a]
• Total protein (mg/gm brain tissue)	62.49±1.71	44.59±2.40 [b]	39.16±0.54 [b]
Brain weight(gm)	2.38±0.63	1.82±0.23 [b]	1.82±0.13 [b]
Body weight (gm)	260±6.27	238 ±4.29 [b]	226 ±4.44 [b]

SOD (superoxide dismutase enzyme) and GST (glutathione- s – transferase) were selected for measuring lipid peroxidation level in the brain (endogenous antioxidant defense). Figures in the same row with different letters are statistically significantly different (compared with the control group). [a] ($P<0.01$) and [b] ($P<0.05$).



Figures

(1): Microphotograph of brain of rat treated with low dose of cadmium showing neuronal degeneration and pyknosis of neurons (H & E stain X 200).

(2): Microphotograph of brain of rat treated with low dose of cadmium showing neuronophagia of pyknotic neurons (& E stain X 200).

(3): Microphotograph of brain of rat treated with high dose of cadmium showing necrosis of neurons (H & E stain X 200).
(4): Microphotograph of brain of rat treated with high dose of cadmium showing focal gliosis (H & E stain X 200).
(5): Microphotograph of brain of rat treated with high dose of cadmium showing hemorrhage in Virchow space (H & E stain X 200).
(6): Microphotograph of brain of rat treated with high dose of cadmium showing necrosis of Purkinje cells of the cerebellum (H & E stain X 200).
(7): Microphotograph of brain of rat treated with high dose of cadmium showing atrophy and necrosis of pyramidal cells of the hippocampus (H & E stain X 200).
(8): Microphotograph of brain of control, untreated rat showing no histopathological changes (H & E stain X 200).

4. Discussions

The main findings of the present study are learning impairment in the open – field habituation, maze learning test and spatial Y- maze memory which induced by higher cadmium chloride intake in male rats.

The results are in agreement with animal data that showed memory impairment in cadmium intoxication (Lehotzky et al; 1990). In the present study , the open field test provides simultaneous measures of both habituation and anxiety .Long term habituation to a novel environment is one of the most elementary forms of non – associative learning . In this study, where reduction in spatial exploration during test session was taken as an index for memory habituation (Montag-Sallaz et al .,1999). An impairment in the open field habituation was noticed in $CdCl_2$ treated groups. Moreover, animals treated with high cadmium were more fearful and highly anxious. Supportive evidence derived from increasing number of fecal boil. The latter was considered the most credible criteria for judging anxious animals.

In addition, associative learning in classic maze, declared that, rats with high doses of cadmium, demonstrated higher latency with increased numbers of errors in the maze reflecting a poorer memory retention relative to other treatments.

In Y- maze test, the treated groups of rats showed significant decrease in alternation behaviour scores in comparison with the control group and there was a significant difference in total number of times the animals entered the arms. Where groups of animals exposed to high concentrations of cadmium, showed higher frequency for entering arms. A proof that there was impairment in working spatial memory. These results confirmed that cadmium intoxication impair learning and memory. In a cadmium toxicity study for Baranski et al , (1983) , a decreased acquisition of avoidance behaviour and alterations in behaviour in open field in adult rats was noticed .

The neurotransmitters in the central nervous system have important roles in normal functioning and behaviour of the adult individual. They interact with each other in complex networks in the process of learning and memory, in which acetylcholine is proposed to have a central role (Decker and McGaugh ;1991). Acetylcholinestrase (AChE) is an enzyme that responsible for hydrolyzing and so deactivating acetylcholine in the body. It is a good indicator of sublethal toxicity by heavy metals (Forget *et al.*, 1999). Brain contains 2 forms of AChE, membrane bound forms constitute 90% of the enzyme and soluble form represents the rest 10% (Atack *et al*., 1986 and Mortensen *et al*., 1998). Level of the soluble form considered a simple and sufficient indicator of relative change of AChE in the brain (Muller *et al*., 1985 and Zakut *et al*., 1985) which measures the turnover of ACh activity (Sastry *et al*., 1983). Alterations in this enzyme level are indicative to impairment of cholinergic function (Slechta and Pokora, 1995). Results in this study revealed significant inhibitory effect on AChE activity in brain tissue which is in accordance with previous investigations of Gupta *et al*., (1993) as well as Antonio *et al*., (2003). Additionally, Murphy (1997) reported that exposure to cadmium generally impairs enzymes involved in the synthesis of neurotransmitters. Our results confirm the presence of an association between the cholinergic innervations and memory. Similar data reported by Flicker et al . (1983), where impairment of learning was evidenced by decreased cholinergic activity in brain .

Oxidative stress caused by different metals may damage certain tissues and liberate various transaminases into the plasma (Jackim et al ., 1970).Cadmium posses the ability to affect the activation of various signaling pathways and produce reactive radicals, which lead to oxidative stress state, resulting in DNA damage and lipid & protein oxidation (Ognjanovic et al ; 2008 ,Valko et al,2005). Also, Cadmium may be associated with the production of reactive oxygen species (ROS) (Szuster- Ciesielska et al, 2000; Liu et al 2002) .As lipid peroxidation was involved in the memory impairment , SOD and GST were selected for measuring lipid peroxidation level in the brain (endogenous antioxidant defense). In the present study, significant decrease in SOD enzymatic activities in brain tissues of rats administered $CdCl_2$ (high dose and low doses), which evidenced oxidative damage of brain tissues . The oxidative damage mechanism caused by Cd intoxication might be related to it's displacement to iron ((Fe^{+2}) and copper (Cu^{+2}) from cytoplasmic and cell membrane proteins with consequent elevation in their ions inside the cell leading to free radical generation . These like hydroxyl radicals , superoxide anions , nitric oxide and H 2O 2 (Koizumi et al .,1996, Casalino et al.; 1997, Ognjanovic et al .,1995 and Waisberg et al ., 2003). Those deplete the endogenous antioxidant defense (GST ,SOD,GSH , Peroxidase and Catalase) resulted in increased lipid peroxidation and DNA damage (Ognjanovic et al.,2003). Therefore, a significant oxidative stress caused by cadmium intoxication ,may be related to impaired learning ability .

Since. De novo protein synthesis and neurotransmitter system are critical event in memory formation (Davis and Squire ,1984; Milner et al; 1998; schafe et al ;1999; Wang et al ;2008), total protein content (TP)of brain tissues were measured in the three treated groups . Results revealed a significant decrease in total protein level in both low and high

doses of Cd. treated groups. Similar finding was recorded for rat's liver and kidney tissues in the study of Jadhav *et al*., (2007). This reduction in TP might be regarded to decreased protein synthesis due to hepatic dysfunction under heavy metal exposure (Ayensu and Tchounwou 2006, Goswami *et al*., 2005 and Mousa 2004). Also chronic renal diseases associated with heavy metal toxicity resulted in excessive loss of protein (Barbier *et al*., 2005, Madden and Fowler 2000). Moreover Cd binds to sulfhydryl group (SH) of many enzymes and inhibit the protein synthesis resulted in inhibiting of many enzymatic activities (Shaikh *et al*., 1999 and Waisberg *et al*., 2003).

The hippocampus and the cerebral cortex are the key structures of memory formation (Shirai and Suzuki; 2004), because the hippocampus is especially indispensable in the integration of spatial information. Since cadmium is classified as neurotoxic substance, our histopathological examination of the brain confirmed that hippocampus is the most affected region due to cadmium intoxication, as well as significant reduction in wet brain weight. Results showed congestion of blood vessels , neuronal degeneration , necrosis of neurons and neuronphagia (Fig.1,2,3) ,focal gliosis (Fig,4) as well as hemorrhage in Virchow space (Fig, 5) and necrosis of Purkinje cells of cerebellum (Fig,6).Moreover , the pyramidal cells appeared atrophied and necrosed (Fig,7). Jadhav et al (2007) , observed dose-dependent vascular , degenerative and necrotic changes in the brain of male rats exposed via drinking water to a mixture of metals (arsenic , cadmium ,lead, mercury ,chromium ,manganese , iron and nickel).The impairments of behaviours in relation to learning and memory may be due to the disturbance of the hippocampal circuit and its vast connections through cortical and subcortical pathways (Skutella and Nitsch,2001 **).** Also Deacon et al (2002) ,. has accounted that hippocampal lesions in general produce impairment in spatial memory.

Holland et al ;(1999) recorded that hippocampal lesions in general produce changes in rat's activity levels. In novelty acquisition during exploration, our results revealed a significant reduction in exploratory motor activity (EMA)in high $CdCl_2$ treated animals .This can be interpreted on the basis of increase emotionality in high concentration animals . Moreover, the animals in the novel environment were highly anxious and fearful .In Cadmium toxicity for Nation et al. (1990), a decreased movement and increased rest time was noticed. Also, Hans, (2006) observed skeletal deformations and flaccidity of muscles produced by cadmium in rats. The Agency for Toxic Substances and Disease Registry (2008) reported that acute oral exposure of cadmium in rats and mice resulted in weakness and muscle atrophy . This could be attributed to the symptoms of fatigue and disturbance of sensory motor function in individuals exposed to cadmium (Murphy, 1997). Desi et al. (1999) related the decrease of exploratory activity and a significantly lower exploration frequency of the open field centre in rats, to cadmium, which affects the bioelectrical and higher order functions of the nervous system .

In the present study, complex motor behaviour (motor balance)as measured by rod-walking and plank walking were significantly impaired in rats exposed to high concentration of Cd . Since these behavioural tests require the execution of complex coordinated movements, balance and strength, so this impairment may be attributed to the effect of cadmium on sensory motor capability. Supportive results derived from Viaene et al., (2000), who recorded that, workers suffered from peripheral neuropathy and complains about equilibrium in chronic occupational exposure to cadmium. Also, Ali et al. (1990) observed significant decrease in distance traveled, stereotypic time and movements, ambulatory time and vertical movements in Cd-exposed rats. Intermediate – duration oral exposure to cadmium caused weakness and muscle atrophy and significant decrease in motor activity .In addition , Murphy (1997) reported that individuals exposed to cadmium, showed increased symptoms as fatigue and disturbance of sensory and motor function. Since, Cadmium (Cd) is a neurotoxic metal, which induces oxidative stress and membrane disturbances in nerve system. . Claudia and Maria,(2005) confirmed that, Cadmium chloride increases oxidative stress in the skeletal muscle cell line c2 c12 and production of reactive oxygen species (ROS) in tissues and inhibits the activity of some enzymes of the antioxidative defense system (Sikic et al, 1997).

In the final, lower body weight was observed in our study in rats exposed to high daily doses of cadmium. Similar results derived from other studies with Cd treated animals (Smith et al .1985, and Gupta et al, 1993).

In conclusion, where, developing brain is greatly targeted to damage by toxic agents. Along with evidence derived from our study where exposure to cadmium constitutes a great threat being associated with neural injurious effects. Hence, concern should be directed to limit the inadvertent incorporation of cadmium in human – consumed products.

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Antioxidative properties of flavonoids from *Cheilanthes anceps* Swartz.

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Abstract: Antioxidative guided chromatographic fractionation of BuOH fraction from aqueous-ethanolic extract of fern fronds of *Cheilanthus anceps* gave flavonol glycosides, Quercetin-3-0-δ–L-rhamnopyranosyl(1→2)-β-D-glucopyranoside-7-O-β-D-glucopyranoside, Kaempferol-3-O- δ-L-rhamnopyranosyl (1→2)-β -D-glucopyranoside-7 O-β -D-glucopyranoside, Quercetin-3-O-β -D-glucosyl (1→2)-β -D-galactoside-7-O-β -D-glucoside, Quercetin-3-methyl ether-5-O-glucoside, Quercetin-3-O-glucoside and Kaempferol-3-O-glucoside. Of these flavonol-glycosides, the glycosides of Quercetin showed prominent antioxidative activity compared to Kaempferol glycosides. [Journal of American Science 2010; 6(5):203-207]. (ISSN: 1545-1003).

Keywords: *Cheilanthes anceps*, Flavonol glycosides, Antioxidative activity.

1. Introduction

Cheilanthes Swartz, a genus of fern's family psinopteridaceae and a group of leptosporangiate ferns of flora of filicinae, comprises 180 species with cosmopolitan in distribution. Fifteen species of *Cheilanthes* have been reported from sub-tropical and temperate Kumaun Himalayas (Pandey et al., 2002). Members of genus *Cheilanthes*, commonly known as silva back ferns, have widely been used as traditional medicines by the local inhabitants of Kumaun Himalayas. *Cheilanthes anceps* Blanford, a common fern constituent of sun facing hills and pine forest areas of Kumaun hills, nearly an altitude ranging from 1000 to 2000m, is characterized morphologically to bear 20-30 cm long and 10-12 cm broad lanceolate fronds. Various species of *Cheilanthes* have been recognized as a traditional medicine and extracts derived from them have been screened for various biological activities (Chopra et al., 1958; Banerjee and Sen, 1980; Lal et al., 1994). Flavonoids, a highly diversified group of natural products and a dietary health promoting substances, have widely been reported from Cheilanthoideae of filicinae group of ferns (Wollenweber et al., 1997). *Cheilanthes* have been characterized for the presence of methoxylated flavones, flavonols and glycosides of Kaempferol and Quercetin (Salatino and Prado, 1998).

Cheilanthes anceps, a fern native to low reaches of Central Himalaya and a constituent of Pine forests of the region, has been used as traditional medicines by the tribal folk of Kumaun Himalaya, and has widely been recommended as medicines to cure cough, asthma, tuberculosis and joint pain. Literature survey revealed that the fern is still awaited for chemical investigation. Present chemical investigation revealed the presence of mono and 3, 7-di-O-glycosides of Kaempferol and Quercetin from antioxidative guided chromatographic fractionation of aqueous-ethanolic extracts of *Cheilanthes anceps.*

2. Materials and Methods

The fresh fronds of *Cheilanthes anceps* were collected from Kumaun hills, altitude ranging from 2500m to 3,000m. The species was identified and deposited (Herbarium specimen No.18) in the Department of Chemistry, Kumaun University campus, Almora (UK).

Extraction and Isolation of flavonoidal compounds

About 2.5 kg of air dried and powered fronds of *Cheilanthes anceps* was extracted sequentially with 80% and 50% EtOH by cold percolation methods. Two extracts were combined and concentrated under reduced pressure at 60ºC in Rota evaporator. The residue was partitioned between dichloromethane and H_2O (1:1). After separating lower dichloromethane layer, the upper H_2O layer was further partitioned with BuOH. The BuOH fraction was concentrated under reduced pressure and residue was adsorbed on cellulose (Merck grade) CC and eluted initially with H_2O then increasing polarity with AcOH. On eluting column with 20% aq. AcOH three dark purple fluorescent bands observed with UV light (360 nm) and each was eluted and collected separately. 2DPC examination of the eluents of faster moving, middle and slower moving bands, using BAW (n-BuOH-AcOH-H20, 4:1:5, V/V, upper layer) and 30% HOAc as a developing solvent system, afforded three, five and two flavonoidal positive

constituents respectively. Each fraction was resolved into pure components by RPPC using BAW (n-BuOH-AcOH-H2O, 4:1:5, V/V, upper layer) as a developing solvent. The pure compounds obtained were finally passed over sephadex LH-20 using MeOH: H_2O (1:1) for final purification.

3. Results and Discussion

Three flavonol glycosides 1, 2 and 3 were isolated from FRAC –1, an eluent derived from faster moving dark purple UV fluorescent band. Compound **4**, a dull blue fluorescent, was isolated from the eluents of middle band. Compounds **5** and **6** were isolated from the eluent of slower moving band (FRAC-III). Compounds **2** and **3** were identified as Quercetin-3, 7-di O-glycosides and compound **1** was identified as a Kaempferol-3, 7-di O-glycoside on the basis of chromatographic behavior and UV spectral methods (Mabry et al., 1970; Markham et al., 1975). Complete acid hydrolysis of compounds **1**, **2** and **3** gave two aglycones which exhibited a dull yellow colour under UV light indicating a release of sugar moiety from the 3-position. H_2O_2 oxidation of these compounds gave two monoglycoside, which exhibited bright yellow fluorescent colour with and without the presence of NH_3 vapours indicated the Flavonol-3, 7-dioglycoside nature (Willians and Grayer, 2004).

Compound 1, showed a $[M + H]^+$ peak m/2 779 $[M + Na]^+$ in FABMS, 757 $[M + H]^+$, and other prominent ions at 611 $[(M + H)-146]^+$, 449 $[(M + H)-308]^+$ and 287 $[(M + H)-470]^+$, supported the 3,7-dioglycoside of Kaempferol and successive elimination of rhamnose and two hexoses respectively. Further complete acid hydrolysis of the compound afforded Kaempferol (CoPC), Glucose (CoPC) and Rhamnose (CoPC). H_2O_2 oxidation of the compound gave Kaempferol-7-O-β-D-glucoside and a disaccharide sugar on PC at Rf 14 BAW solvent. Complete acid hydrolysis of the compound **1** gave Kaempferol (CoPC), Glucose and Rhamnose (CoPC). Compound **1** appeared as a purple fluorescent spot on a paper chromatogram under UV light and changed to yellow – green with ammonia, indicating the presence of free 5- and 4`-hydroxyl groups. When a cellulose TLC plate was sprayed with Naturstoffreagenz (A) reagent, the spot turned yellow indicating a 4`-hydroxyl but no ortho-dihydroxy group in the B-ring. Compound **1** exhibited UV maxima in methanol at 265 (band II) and 354 (band I) and shifts obtained with diagnostic reagents : NaOMe, 245, 270, 350 sh, 390 (it) ; $AlCl_3$, 275, 302, 356, 400 ; $AlCl_3$ + HCl : 275, 300, 355, 400 ; NaOAc : 267, 360, 405 sh ; and NaOAc + H_3BO_3: 266, 320 sh, 353 indicated the presence of free hydroxyls at C-5 and C-4`.

^{1}HNMR of the compound (DMSO-d_6, 400MHz) gave two meta coupled doublets at δ 6.40 and δ 6.77, each with J=20 Hz, indicated the presence of H-6 and H-8 of 7-O-substituted flavone moiety. Two symmetrical ortho coupled doublets each with J=8.5 Hz at 6.84 and 8.02 represent p-di substituted β-ring for H-3`, 5` and H-2`, 6`. Three anomeric protons signals observed at δ 5.62 (d, J=7.5 Hz), δ 5.06 (1 H,d,J=7.5 Hz) and a broad singlet at 5.03 (1H, brS) assignable to the glucose moieties at C-3, C-7 and terminal rhamnose unit respectively. An overlapped multiples for remaining protons of three sugar units observed between 3, 15 – 4.0. A downfield broad singlet observed at 12.50 represents 5-OH (chelated). A high field doublet at 0.80 (3H, d, J=6.5 Hz) for rhamnose-CH_3 group (**Table 1**).

Table 1: ^{1}HNMR of Compound 1

Shift (δ)	Multiplicity, J=Hz	Identification
6.40	1H, d, 2.0	H-6
6.77	1H, d, 2.0	H-8
6.84	2H, d, 8.5	H-3` and 5`
8.02	2H, d, 8.5	H-2` and H-6`
5.62	1H, d, 7.5	glucose anomirz (C-3)
5.06	1H, d, 7.5	glucose anomeric at C-7
5.03	1H, br.S	rhamnosyl terminal sugar
3.15-4.0	m	remaing protons of rhamnose + two glucose moieties
0.80	3H, d, J=6.5	-CH3 rhamnose
12.50	5 (br)	5 –OH

The recognizable down-field shift (Δδ=0.4 ppm) which was detected on comparing the chemical shifts of the anomeric glucoside proton signals with those of Kaempferol-3-glucoside proved that the terminal α-rhamnosyl moiety is attached to C-2 of the primary β-glucoside moiety (Altona and Haasnoot, 1980). Thus, the compound **1** was identified as Kaempferol-3-O-β-(rhamnosyl 1→2) glucoside-7-O-β-D-glucoside.

Compound 2, Chromatographic isolation of the compound gave yellow amorphous powder, which exhibited a molecular ion in FABS, m/e at773 $[M + H]^+$ consistent with a molecular formula $C_{33}H_{40}O_{21}$. The signals found at m/e 627 $[(M + H) – 146]^+$, 465 $[(M + H) – 308]^+$ and 303 $[(M + H) – 470]^+$ indicated successive elimination of rhamnose and two hexoses respectively. These finding supported a

3, 7-glycosylated flavonol structure. Chromatographic behavior and UV spectral data of the compound in MeOH at (λmax, nm) 256 (band I) and shifts obtained with diagnostic reagents, NaOMe, 269, 400; $AlCl_3$, 275, 330sh, 433; $AlCl_3$ + HCl, 269, 300sh, 360sh, 400, NaOAc, 262, 408 and NaOAc + H_3BO_3, 260, 378, suggested a flavonol triglycoside with a free hydroxyls at C-5, C-3` and C-4` [7-8]. ^{1}HNMR of the compound (in DMSO-d_6, 400MHz) gave five aromatic signals at (δ) 6.40 (1H, d, J=2Hz), 6.75 (1H, d, J=2Hz), 6.80 (1H, d, J=7.5Hz), 7.51 (1H,d, J=2.0 Hz) and 7.75 (1H, dd, J=2.0 and 7.5 Hz) for H-6, H-8, H-5`, H-2` and H-6` respectively. Three anomeric proton signals appeared at 5.62 (1H, J=7.5 Hz), 5.06 (1H, d, J=7.5) and 5.03 (1H, S) identified for C-3 (glucose), C-7 (glucosyl) and a terminal rhamnosyl respectively. Complete acid hydrolysis of the compound gave Quercetin (CoPC), Glucose (CoPC) and Rhamnose (CoPC). The recognizable downfield shift (Δδ=0.3 ppm) which was detected on comparing the chemical shifts of the anomeric glucoside proton that the terminal α-rhamnosyl moiety is attached to C-2 of the inner β-D-glucoside moiety [10]. The ^{1}HNMR of compound **2** in sugar region was found similar to the compound **1**. Thus, the compound **2** was identified as Quercetin 3-O-β (rhamnosyl 1→2 glucoside) 7-O-β-D-glucoside.

Compound 3, Chromatographically isolated grey amorphous powder gave a molecular ion in FAB-MS, m/e at 811 $(M + Na)^+$ and other prominent

ions, 465 $[M + H - 324)^+$ and m/e 303 $[M + H - 486]^+$, indicated successive elimination one and two hexose moiety respectively. These findings supported a 3,7di-glycosylated flavonol structure. Chromatographic behaviour and UV spectral data of the compound which were found similar to compound **2** suggested a flavonol triglycoside with a free hydroxyls at C-5, C-3` and C-4` [7-8]. Complete acid hydrolysis of compound gave quercetin, glucose and galactose. ^{1}HNMR of compound (DMSO-d6, 400 MHz) gave five aromatic protons signals at (δ) 640 (1H, d, J=2.0 Hz), 6.74 (1H, d, J=2.0 Hz), 6.85 (1H, d, J=8.5 Hz), 7.56 (1H, d, J=2.0 Hz) and 7.65 (1H, dd, J=8.5 and 2.0 Hz) assignable to H-6, H-8, H-5`, H-2` and H-6` respectively. Three aliphatic anomeric proton signals appeared at 4.57 (1H, d, J=7.5 Hz), 5.07 (1H, d, J=8.0 Hz) and 5.64 (1H, d, J=7.5 Hz) representing for terminal glucose, C-7- glucose and C-3 primary galactose respectively. The recognizable down field shift of (Δδ=1.17) which was detected on comparing the chemical shifts of the anomeric galactoside proton signals in the spectrum of 3 with those of Quercetin-3-galactoside proved that the terminal glucosyl moiety is attached to C-3 of the inner β-galactoside moiety. Consequently 3 was identified as Quercetin-3-O-β-glucosyl (1-2) glactoside-7-O-β-D-gluoside.

Compound 4, FABMS of the compound gave a molecular ion m/e at 477 $[M - H]^-$ calculated for $C_{22}H_{22}O_{12}$. It appeared as a dull blue florescent spot on paper chromatogram under UV light and changed to yellow-green with ammonia, indicating the presence of free 5- and 4`-hydroxyl groups. When a cellulose TLC plate was sprayed with Naturstoffreagenz (NA), the spot turned orange

indicating the presence of ortho-dihydroxyl group in the B-ring. ^{1}HNMR of the compound (in DMSO-d6, 400 MHz) gave two meta coupled doublets at 6.45 (1H, d, J=2.0 Hz) assignable to H-6 and H-8 of A-ring. An ABX system for three B-ring protons observed at 6.89 (1H, d, J=8.5 Hz), 7.43 (1H, dd, J=2.0 and 8.5 Hz) and 7.53 (1H, d, J=2.0 Hz) identified for H-5`, H-6` and H-2` respectively. The dull blue florescence of compound on PC under UV light and down field shift of C-6 and C-8 protons of A-ring, indicating either algence of 5-OH group or 5-OH group is substituted. Further, two prominent ions in MS, m/e at 461 (M-CH_3-H) and 301 (M-176-H) showing the attachment of sugar moiety at C-5 position of flavones. ^{1}HNMR spectra (**Table. 2**) indicated the attachment of glucose unit at C-5 position (Mabry et al., 1970).

Table 2: ^{1}HNMR (DMSO-d_6) 400 MHz of COMPOUND 4

Shift (δ)	Multiplicity, J=Hz	Identification of protons
6.45	1H, d, 2.0	H-6
6.75	1H, d, 2.0	H-8
6.89	1H, d, 8.5	H-5
7.43	1H, dd, 8.5, 2.0	H-6
7.53	1H, d, 2.0	H-8
4.76	1H, d, 7.5	H-1``
3.2-4	6H, m	glucosyl
3.90	3H (s)	protons –OCH_3

In aliphatic region of ^{1}HNMR spectra 4, gave a doublet at δ 4.76 (1H, J=7.5 Hz) indicated β-configuration in pyranose form of glucose (Altona and Haasnoot, 1980). A three proton sharp singlet observed at 3.90 for OCH_3 group attached at C-3 position. Except anomeric proton, remaining five protons of glucose appeared as a multiplet between

3.2 to 4.0. Complete acid hydrolysis of compound 4 gave a dark purple florescent aglycone on PC and a dull brown spot of sugar at Rf 23 (BAW) after spraying with benzidine reagent. The sugar component was identified as glucose by comparing with its standard on PC. The structure of aglycone was identified as follows:

The aglycone, representing structure 4(a) was isolated from acid hydrolysed mixture of 4 by RPPC using 30% HOAc as a developing solvent. It crystallized as deep yellow fine needles, mp, 282. The MS of 4(a) exhibited a molecular ion at m/e 316 $[M]^+$ (100%), 315 (70%), 301 $[M –CH3)^+$, 287 $(M – HCO)^+$, 153 $(A + H)^+$ (23%), 144 (10%), 137 $[B2]^+$ (21%) and 121 [B1 – COMe] (10%). Cellulose TLC of aglycone when sprayed with methanolic solution of Naturstoffreagenz reagent, the dark purple fluorescence of compound turned to orange, indicating the presence of ortho-dihydroxyl compound in the B-ring. ^{1}HNMR of 4(a) gave two meta coupled doublets at 6.24 (1H, J=2.0 Hz) and 6.48 (1H, J=2.0) assignable to H-6 and H-8 of A-ring. A three protons ABX system was observed at 7.00 (1H, d, J=8.5), 7.59 (1H, dd, 2.0 and 8.5 Hz) and 7.24 (1H, d, J=2.0 Hz) representing H-5`, H-6` and H-2` respectively. A three proton singlet observed at δ 3.90 attached at C-3 position. A high field singlet observed at 12.80 indicated presence of chelated 5 – OH in the A-ring. The compound 4(a) was hydrolyzed with H1 in presence of base, form a dull yellow fluorescent compound on PC under UV light (CoPC). It was identified as Quercetin. Thus, 4(a) was identified as Quercetin-3-OCH_3. On the basis of UV, ^{1}HNMR, MS and hydrolytic methods, the compound 4 was identified as Quercetin-3-OCH_3-5-O-β-D-glucoside.

Compund 5, FABMS (NBA) of compound gave a molecular ion at m/e 447 $[M – H]^-$ and a prominent ion observed at 285 $[M – 162 – H)^-$ showing loss of glucose moiety from aglycone, kaempferol. ^{1}HNMR (DMSO –d6, 400 MHz) of 5 gave four doubles at 6.20 (1H, d, J=2.0 Hz), 6.42 (1H, d, J=2.0 Hz), 6.86 (2H, d, J=8.5 Hz) and 8.03 (2H, d, J=8.5 Hz) assignable to H-6, H-8, H-3`,5` and H-2`, 6` respectively of flavonoid nucleus. A doublet observed at 5.20 (J=7.5 Hz) represent anomeric proton of glucose and β-configuration of pyranose form of sugar (Altona and Haasnoot, 1980). Complete acid hydrolysis of compound with 2NHCl gave glucose (CoPC) and kaempferol (CoPC). On the basis of above spectral evidences the 5 was identified as Kaempferol-3-O-β-D-glucoside.

Compound 6, FABMS (NBA) of 6 gave a molecular ion m/e, 463 $[M – H]^-$, and other prominent ion at 301 $[M – 162 – H]^-$ showing loss of glucose moiety from aglycone, quercetin. It has also been supported by the acid hydrolysis of compound with 2NHCl as it produced quercetin (CoPC) and glucose (CoPC). ^{1}HNMR of the compound in aglycone region gave two meta coupled doublets each with J=2.0 Hz at 6.20 and 6.40 for H-6 and H-8 of phloroglucosiol type A-ring and ABX system for three protons at 6.86 (1H, d, J=8.5 Hz), 7.58 (1H, dd, 2.0 and 8.5 Hz) and 8.01 (1H, d, J=2.0 Hz) assignable to H-2`, H-6` and H-2` respectively of B-ring. The ^{1}HNMR of compound in sugar region was found similar to the compound **5**. The compound **6** was identified as Quercetin-3-O-β-D-glucoside.

Antioxidative activity of flavonoidal compounds isolated from BuOH fraction

Six flavonol glycosides 1, 2, 3, 4, 5 and 6 were isolated from FRAC-1, FRAC-2 and FRAC-3 derived from 20% HOAc cellulose CC fractionation of BuOH fraction of aqueous-ethanolic extract of *Cheilanthus anceps*. Methanolic solution of each isolate was examined for antioxidative activity by the standard method of thin layer autobiography using SiO_2 as an adsorbant and methanolic solution of DPPH (0.02%, 2,2 diphenyl-1 (2,4,6-trinitrohydrazide) as a spraying reagent (Cuendet et al., 2000; 2001; Chacha et al., 2005). Thin layer autobiography of compound **1** to **6** revealed that the compounds **2**, **3**, **4** and **6** gave active spots as they produce yellow spots against purple background.

Finally the antioxidative activity of compounds **2**, **3**, **4** and **6** confirmed by UV-VIS spectrophotometer. 30µl methanolic solution of each isolate and 200µl of MeOH were added to 50µl of a 0.02% MeOH solution of DPPH. Absorbance at 517 nm was determined after 30mins. and the percentage of activity was calculated. The decreasing sequence of activity among the active compounds was found in the order 6>3>2>4. The compounds 1 and 5, the glycosides of Kaempferol, did not show any activity while the glycoside derivatives of Quercetin were found to be active. Among the active glycosides of Quercetin, the compound 6, a Quercetin-3-O-β-D-glucoside was identified as a prominent antioxidative compound. It has further been established that Quercetin-3-O-monoglycosides are comparatively more active than Qurecetin-3-O-oligosaccharides and Quercetin-3,7-dioglycosids. Quercetin, a non glycosidic flavonol derivative and a widely distributed naturally occurring pigment of various food and fodder plants, has a more pronounced antioxidative activity compared to its glycosides (Yamashita and Kawanishi, 2000; Rietjens et al., 2005; Cantero et al., 2006). It was concluded that

Quercetin attached with more sugar moieties is less antioxidative.

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Effect of Treatment with Antifibrotic Drugs in Combination with PZQ in Immunized *Schistosoma mansoni* Infected Murine Model

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Abstract : The main problem in schistosomal hepatic morbidity is fibrosis and extensive scarring induced by living eggs. In this study, we tried to study the effect of treatment using antihelminthic drug (PZQ) and/or antifibrotic drugs (PTX and silymarin) in combination with immunization. The parasitological parameters, the dynamics of serum-specific immunoglobulins and splenic cytokines associated with changes in granuloma diameter were assessed. Naïve mice were immunized intravenously with 10 ug of SEA in three doses at 2 days intervals 6 weeks before infection. Animals were infected by tail immersion with 100 cercariae and divided into several groups. Three groups were treated with PZQ, PTX or silymarin administered alone. Another two groups were treated with PZQ combined with PTX or silymarin. All treated animals and respective controls were sacrificed 12 weeks post infection. Immunization did not affect worm reduction , but slight decrease in granuloma diameter, increase in immunoglobulins and cytokines was observed . Reduction in worm burden was associated with reduction in ova count and changes in oogram pattern which were mainly due to PZQ treatment. Increasing reduction in granuloma diameter, elevation of immunogloulins and cytokines levels were observed in the groups treated with PZQ alone or cmbined with PTX or silymarin. In conclusion, in this study, treatment with PZQ complemented with immunization resulted in significant reduction of parasitological parameters and rise of specific Igs. Addition of antifibrotic drugs PTX or silymarin to PZQ, potentiated an antipathology effect which minimized and ameliorated liver fibrosis by inhibition of HSC activation and accentuation of the effect of suppressor Treg cells. [Journal of American Science 2010; 6(5):208-216]. (ISSN: 1545-1003).

Key word: *Schistosoma mansoni*, Praziquantel, Pentoxifyllin, silymarin.

1- Introduction

Schistosomal pathology is a direct consequence of the immunological response to ovideposition in host tissue especially liver. Liver injury is typically associated with infiltration of inflammatory cells leading to fibrosis (Friedman, 2003).

Liver fibrosis results from chronic damage of the liver and activation of hepatic stellate cells (HSC) which leads to excess production of extracellular matrix (ECM) components (Friedman and Arthur, 2002 ; Friedman, 2003 ; Bartley et al., 2006). Various investigators have focused on the protective immunization against schistoromiasis using several soluble egg antigen (SEA) fractions which were identified and tested in experimental models with the induction of variable levels of protection against infection (Tendler et al., 1996). Immunization of mice stimulates specific immunity which causes reduction in worm burden, intestinal egg load and liver pathology (Romeih et al., 2008 ; Garcia et al., 2008). Until recently, non of immunizing fractions was able to induce more than 67% protection, but the existence of at least partially protective immunity would make a logical complement to drug therapy (Bergquist et al., 2008 ; Maher et al., 2003 , Zovain et al., 2001). Praziquental (PZQ) is the drug of choice for all species of *Schistosoma* as an effective antischistosomal drug (Utzinger and Keiser, 2004). Although treatment with this drug is effective, but frequent schistosome reinfection occurs after treatment due to relative resistance to schistosomicide drugs (Silva et al., 2003). At the same time, it is stated that it is preferable to develop combination of PZQ and anti-fibrotic drugs in the treatment of murine schistosomiasis which could minimize liver fibrosis simultaneously with worm elimination (Mahmoud et al., 2002 ; Doenhoff et al., 2002). Pentoxyfilline (PTX) has been identified as an antifibrotic drug which can interfere on a large spectrum of cytokines with proinflammatory action and causes inhibition of ECM synthesis (Bienvenu et al., 1995 ; Reis et al., 2001). Antioxidants such as silymarin have received attention as potential antifibrotics which inhibit HSC activation and protect hepatocytes from undergoing apoptosis (Leiber et al.,

2003). In this work, parasitological parameters and the dynamics of serum-specific immnoglobulins and splenic cytokines associated with changes in hepatic pathogenesis and granuloma diameter, were assessed in an attempt to study the effect of treatment with PZQ alone and in combination with PTX or silymarin in immunized infected mice model.

2- Material and Methods:

Animals: C57 BL/6 mice (6-8 weeks old), (18-20g) were bred and maintained at Schistosome Biology Supply Center (SBSC) of Theodor Bilharz Research Institute (TBRI) and kept under standard housing conditions. The animal experiments have been carried out according to the internationally valid guidelines in an institution responsible for animal ethics (TBRI) (Nessim et al., 2000).

Preparation of *S. mansoni* soluble egg antigen (SEA):

SEA was prepared (Boros and Warren, 1970, Carter and Colley, 1978) and purified from host antigen by affinity chromatography using cyanogen bromide activated sepharose-4B beads (Nordon & Strand, 1984). SEA was sterilized by filtration and protein content was estimated using Bio-Rad kit (Bradford, 1976).

Drug and doses:

a) Praziquental (PZQ) (Distocide ®, Epico Pharma Cairo, Egypt) was orally administered 7 wks p.i. at a dose of 500 mg/kg body weight for 2 consecutive days. It was freshly prepared before use as a 2% suspension in Cremophor-El (Sigma chemicals Co. St. Louis, Missouri).

b) Pentoxifylline (PTX) (Trental ®, Aventis Pharma, Cairo, Egypt), was orally administered 4 wks post infection (PI) 5 days/wk at dose of 400 mg/Kg body weight. The treatment was continued until the date of sacrifice.

c) Silymarin: (SEDICO Pharmasetuical-co) was orally administered starting from the day of infection at dose of 140 mg / kg three times / week until the day of sacrifice.

Experimental design: 140 mice were immunized with SEA (10 ug X 3). Six weeks later, they were infected by tail immersion with 100 cercariae of an Egyptian strain of *S. mansoni* supplied from SBSP, TBRI and were divided into 6 groups. Three groups were treated with PZQ, PTX or silymarin as described before. Another two groups were treated with PZQ combined with PTX or Silymarin. The sixth group- immunized, infected untreated mice were used as immunized infected control. Infected not immunized, untreated animals were used as infected control. Clean uninfected, untreated animals were used as normal control. All animals were sacrificed 12 weeks post infection.

Parasitological Parameters:

1- Worm burden: Infected animals were perfused to recover hepatic and portomesenteric worms for subsequent counting (Duvall and DeWitt, 1967).

2- Tissue egg load: The number of eggs per gram tissue (liver and intestine) was studied according to the procedure described by Cheever (1968).

3- Oogram pattern: The percentages of immature, mature and dead eggs in the small intestines were computed from a total of 100 eggs per intestinal segment and classified according to categories previously defined by Pellegrino et al. (1962).

Immunological Study:-

Determination of anti-SEA immunoglobulin subclasses IgG1, IgG2 and IgG4 were measured using indirect ELISA, based on the method of Engvall and Perlman (1971). ELISA microtiter plates were coated with 100 ul / well of 30 ug/ml of SEA. Sera were diluted 1:20 and anti-mouse IgG subclasses (Binding site, Birmingham, UK) were used at a dilution of 1:500. Absorbance at 492 nm was measured.

Cytokine assay: Serum IFN-γ, IL-4 and IL-10 levels were measured by a sandwich ELISA technique. Briefly, plates were coated with capture antibodies and 100 ul of serum samples or recombinant cytokines were added. Following addition of the biotinylated detection antibody and streptavidin-alkaline phosphatase conjugate, the reaction was developed with paranitrophenyl phosphate (Sigma) and absorbance was measured at 405 nm.

Granuloma measurement: Hepatic granuloma diameter was measured according to Von Lichtenberg (1962). The percent reduction in granuloma diameter relative to infected control was calculated as follows:

% reduction of granuloma diameter = mean diameter of controls - mean diameter of test groups / mean diameter of control group x 100.

Statistical analysis: The data were presented as mean ± standard error of the mean (X ± SE). The means of the different groups were compared globally using the analysis of variance ANOVA. Data were considered significant if p values were less than 0.05.

3- Results:

Parasitological parameters:

The total number of worms and the percent reduction of worm burden showed no significant difference between infected control and the immunized infected control. On the other hand, the groups treated with PZQ alone or combined with PTX or silymarin showed highly significant decrease ($P<0.001$)

compared to immunized infected control. It showed no significant or slight decrease (p<0.05) in groups treated with PTX or silymarin respectively compared to immunized infected control. The mean ova count in intestine and liver showed significant reduction (P<0.01) in immunized infected control compared to infected control, while all treated groups showed highly significant reduction (P<0.001) compared to immunized infected control (Table (1)). As regards oogram pattern, there was no significant change between the infected control and immunized infected control. On the other hand, highly significant decrease was shown only in the groups treated with PZQ alone or combined with PTX or silymarine (P< 0.001) compared to immunized infected control (Table (2).

Granuloma diameter:

Granuloma diameter showed slight decrease in immunized infected control compared to infected control (P<0.05), while in all treated groups, it showed highly significant decrease (P<0.001) except the group treated with PZQ alone which showed no significant change compared to immunized infected control (Table (3)).

Immunological Parameters:

Serum-specific immunoglobulin isotypes:

In infected control group, there was no significant change in IgG isotypes compared to normal control. However in immunized infected control there is significant increase in IgG1 (P< 0.01) and IgG4 (P<0.05) compared to the infected control. The level of IgG1 showed no significant change in the treated groups except in the groups treated with PTX combined with PZQ or silymarin alone which showed slight decrease (P<0.05) compared to immunized infected control. On the other hand , there was highly significant increase in IgG2 level in all treated groups (P<0.001), while the increase in IgG4 level was shown only in the groups treated with PZQ alone or combined with PTX or silymarin (P<0.05, P<0.05 and P<0.01 respectively) compared to immunized infected control (Table (4).

Serum cytokines level:

The profile of Th-1 related cytokine IFN-γ showed significant increase in infected control (P < 0.001) compared to normal control. On the other hand it showed slightly significant decrease in immunized infected control compared to infected control (P<0.05). In treated groups, the groups treated with PZQ alone or combined with PTX or silymarin showed significant increase (P < 0.05) compared to immunized infected control. On the other hand, significant decrease in IFN-γ level was observed in groups treated with silymarin or PTX alone (P<0.01 – P< 0.001 respectively) compared to immunized infected control. The Th-2-related cytokines IL-4 showed highly significant increase in the infected control compared to normal control (P< 0.001). At the same time, it showed significant decrease in the immunized infected control (P<0.01) compared to infected control. Also, it showed no significant change in all treated groups except groups treated with PTX or PZQ alone which showed slight decrease (P<0.05) compared to immunized infected control. On the other hand, the Treg-related cytokine IL-10 level showed significant highly increase in infected control (P<0.001) compared to normal control and slight increase in immunized infected control (P<0.05) compared to infected control. In the treated groups, it showed slightly significant increase in the groups treated with PZQ, silymarin or PTX alone (P<0.05) compared to immunized infected control (Table 5).

Table 1: Worm burden and tissue load in mice immunized with SEA (10 µg X3) 6 wks before infection and treated with different types of drugs then sacrificed 12 wks post infection.

Animal group	Mean no. of worms ± SEM	% reduction	Mean no. of ova count ± SEM / g tissue			
			Intestine	% reduction	Liver	% reduction
Infected control	31.6 ± 0.33		17234 ± 1291		3011 ± 374	
Immunized infected control	29.5 ± 0.17	6.65%	7450 ± 114 **	56.8%	1420 ± 210 **	53%
Treated groups						
PTX	27.1 ± 0.29	14.2%	#4158 ± 234	75.9%	##988 ± 277	67.3%
PTX + PZQ	###1.3 ± 0.30	95.9%	###612 ± 133	96.4%	###158 ± 33	94.8%
Sily	# 21.6 ± 0.21	31.6%	##3889 ± 303	77.4%	###812 ± 90	73.1%
Sily + PZQ	###1.1 ± 0.21	96.5%	###500 ± 125	97.1%	###99 ± 11	97.1%
PZQ	###1.2 ± 0.3	96.2%	###638 ± 131	96.3%	###112 ± 10	96.3%

 # # # P<0.001, # # *** P < 0.001 , ** P < 0.01, * P <0.05 relative to infected control.
P<0.01, # P<0.05 compared to immunized infected control.

Table 2: Oogram pattern in mice immunized with SEA (10 µg x3). 6 wks before infection and treated with different types of drugs then sacrificed 12 wks post infection.

Animal Group	Oogram pattern (% ova)		
	Immature	Mature	Dead
Infected control	67.2 ± 5	33.1 ± 2.6	1.7 ± 0.3
Immunized infected control	62.3 ± 2.4	31.8 ± 3.5	21.6 ± 0.7*
Treated groups			
PTX	# 47.9 ± 3.9	29.9 ± 3.1	22.2 ±1.1
PTX + PZQ	### 4.9 ± 5.4	###3.3 ± 1.4	###91.8 ± 7.4
Sily	## 22.1 ± 3.8	40.8 ± 3	37.1 ± 2
Sily + PZQ	### 9.5 ± 1.2	###8.8 ± 1.1	## 81.7 ± 4.1
PZQ	### 2.0 ± 0.3	###1.7 ± 0.2	## 96.3 ±4.9

***** P < 0.001 , ** P < 0.01, * P <0.05 relative to infected control.**
 # # # P<0.001, # # P<0.01, # P<0.05 compared to immunized infected control.

Table 3: Hepatic granuloma diameter and % reduction in mice immunized with SEA (10 µg x3). 6 wks before infection and treated with different types of drugs then sacrificed 12 wks post infection

Animal Group	Hepatic granuloma diameter(a) Mean µm ± SEM	% Reduction (b)
Infected control	299.8 ± 12.5	
Immunized infected control	254 ± 14.3*	15.3%
Treated groups		
PTX	## 139.9 ± 20.1	53.3%
PTX + PZQ	### 103.4 ± 17.5	65.4%
Sily	## 164.4 ± 13.2	45.2%
Sily + PZQ	### 128.6 ± 20.7	57.1%
PZQ	225.1 ± 19.3	24.9%

(a) The mean granuloma diameter per group was calculated from the mean values (10-15 granulomas per mouse).
(b) Percentage reduction was calculated relative to infected control.
***** P < 0.001 , ** P < 0.01, * P <0.05 relative to infected control.**
 # # # P<0.001, # # P<0.01, # P<0.05 compared to immunized infected control.

Table 4: Serum anti-SEA IgG subclasses levels in mice infected with SEA (10 µg x3). 6 wks before infection and treated with different types of drugs then sacrificed 12 wks post infection

Animal Group	X` O.D ± SEM Ig G1	X` O.D ± SEM Ig G2	X` O.D ± SEM Ig G4
Normal control	0.202 ± 0.178	0.294 ± 0.21	0.321 ± 0.02
Infected control	0.462 ± 0.103	0.606 ± 0.207	0.99 ± 0.126
Immunized infected control	0.954 ± 0.34 **	0.521 ± 0.383	1.12 ± 0.135*
Treated groups			
PTX	0.815 ± 0.251	###0.939 ± 0.178	1.08 ± 0.204
PTX + PZQ	# 0.783 ± 0.187	### 0.998 ±0.231	# 1.58 ± 0.179
Sily	# 0.791 ± 0.245	###1.049 ± 0.159	1.21 ± 0.321
Sily + PZQ	0.833 ± 0.213	###1.180 ± 0.256	## 1.69 ± 0.155
PZQ	0.802 ± 0.421	###0.897 ± 0.534	# 1.43 ± 0.254

***** P < 0.001 , ** P < 0.01, * P <0.05 relative to infected control.**
 # # # P<0.001, # # P<0.01, # P<0.05 compared to immunized infected control.

Table 5: Serum cytokine level in mice immunized with SEA (10 µg x3) 6 wks before infection and treated with different types of drugs the sacrificed 12 wks post infection

Animal Group	IFN – γ Pg/ml ± SEM	IL – 4 Pg/ml ± SEM	IL – 10 Pg/ml ± SEM
Normal control	235 ± 26.5	15.6 ± 0.57	90 ± 14.5
Infected control	611 ± 34 ♣	69.6 ± 12 ♣	510 ± 29.1 ♣
Immunized infected control	420 ± 22.4 *	35.4 ± 9.77 **	625 ± 16.5*
Treated groups			
PTX	###140 ± 51	# 28.8 ± 5.1	#733 ± 21.9
PTX + PZQ	# 587 ± 94	40.2 ± 11.7	655 ± 40
Sily	## 221 ± 59	# 25.5 ± 14.4	# 719 ± 46.5
Sily + PZQ	# 613 ± 72.2	45.1 ± 14.2	685 ± 50.3
PZQ	# 633 ± 53.4	39.8 ± 13.3	# 723 ± 45.5

♣ P<0.001 relative to normal control
***** P < 0.001 , ** P < 0.01, * P <0.05 relative to infected control.**
 # # # P<0.001, # # P<0.01, # P<0.05 compared to immunized infected control.

4- Discussion:

Schistosomal infection is cryptic as it frequently goes undetected and developed significant pathology before chemotherapy administration (Wilson et al., 2006). The combination of protection using SEA fractions and treatment was recommended in several studies as it provided many complementary goals, a reduction of egg- induced pathology, minimal parenchymal changes and the eradication of worms. Therefore, the assessment of the effect of treatment of immunized infected mice is important by studying several criteria related to the parasitic intensity, stages and distribution through the tissues of the host for the evaluation of the magnitude of infection and efficacy of the treatment (Abdel-Ghaffar et al., 2005).

The present study revealed that the immunization schedule used did not cause any significant change in worm burden but slightl significant reduction in tissue egg load which agreed with Botros et al. (1996). The treatment of PZQ alone or combined with PTX or silymarin in immunized infected animals caused almost similar high percentage of eradication of worms and tissue egg load which also agree with the work of Suleiman et al. (2004). The death of the worms due to the treatment with antischistosomal drugs was attributed to metabolic disorders, mechanical destruction and muscular contraction of the treated worms (Doenhoff et al., 2002). At the same time, percent reduction in the egg count in both immunized infected and treated groups was found to be higher in the intestinal tissue than in hepatic tissue. This variation was attributed to excretion of some ova from the intestine prior to digestion and to hepatic shift of worms after treatment (Abdel-Ghaffar and Qurtam, 2001, Abdel –Ghaffar, 2004). On the other hand, the treatment with PZQ alone or combined with PTX or silymarin caused decrease in immature egg stages and number of mature eggs with high increase in number of dead eggs which agree with the findings of Botros et al.(1996). The parasitological improvement is due to antiparasitic drug (PZQ) which causes direct or indirect toxic effect in combination with the effect of immunization with SEA which lead to reduction in tissue egg load. This may be attributed to marked decrease in the worm number or fecundity due to hindering the process of oviposition (Guirguis, 2003).

The manifestations of schistosomiasis are mainly attributed to granulomatous inflammation around parasite eggs (Abath et al., 2006). The formation of granulomas depends predominantly on CD_4^+ T cell specific for egg antigen and represents a delayed – type hypersensitivity (Stadecker, et al., 2004 ; Pearce, 2005; Garcia et al., 2008). At the same time, hepatic stellate cells (HSCs) comprise 10-15% of all hepatic cells and they are recruited to areas of hepatic injury and become activated (Cassiman et al., 2002). They adopted a myofibroblast–like phenotype, secreting extracellular matrix components (Iredale, 2003; Mann et al., 2009).

In this work, although all treated groups revealed significant diminution of granuloma diameter, but the groups treated with PTX or silymarin alone or combined to PZQ revealed more diminution in granuloma diameter. This is because PTX reduces transdifferentiation of HSC to myofibroblasts and inhibits HSC proliferation that

leads to inhibition of extracellular matrix synthesis, beside the effect of PZQ which reduces CD4 T cells and increase CD8 cells (Raetsch et al., 2002; El-Ahwany et al., 2006; El-Lakkany and Nosseir, 2007). At the same time, the groups treated with silymarin or PTX alone revealed lower pattern than the other treated groups and this many be due to the effect of previous immunization of the infected animals before treatment. This effect is considered complementary to the immunization effect which modulate the immune response by limiting the immunopathological reactions against schistosome eggs trapped in the liver. In this study, immunization before infection increased the levels of production of IgG1 and IgG4 . All treated groups had increased levels of IgG2 , but slight increase in the level of IgG4 was observed in the groups treated with PZQ alone or combined with PTX or silymarin. This increase in the production of immunoglobulins have an important role in the improvement of the pathology and the reduction in the ova count and worm burden (Soren et al., 2009; El-Ahwany et al., 2006).

Cytokines which act on lymphocytes are of special interest because of their role in regulating cells of the immune response (Kim et al., 1997). During schistosomal infection, both Th1 and Th2 responses directed against egg antigen and produce IFN- γ , IL-4, IL-5 and IL-13 (Hoffman et al., 2002; Sadler et al.,2003; Stadecker et al., 2004). In this study, the diminished production of Th1-cytokine IFN-γ and Th2-cytokine IL-4 in the immunized group may be implicated in the down modulation of the granulomatous response due to immunization (Chensue et al., 1992). Groups treated with PTX or silymarin alone showed significant decrease in IFN- γ and IL-4 . On the other hand, groups treated with PZQ alone or combined with PTX or silymarin showed increase in Treg cell cytokine IL-10. Recent studies suggest that Treg cells play a pivotal role in suppressing Th1 cell development as well as limiting the magnitude of Th2 response directed against egg antigen by a process dependent upon IL-10 (Hori et al., 2003; Wynn, 2004; Stadecker et al., 2004). The increasing level of IL-10 is probably implicated in the down regulation of granuloma formation as it reduces the intrahepatic inflammatory response and hence it has an antifibrotic effect (Nelson et al., 2003; Thompson et al., 1998). These results indicate the importance of the effect of PTX as it has a potent antifibrogenic role. Also using silymarin as an antioxidant drug can inhibit HSC activation and slow down the progression of liver fibrosis (Afdhal and Nunes, 2004; Jhy-wen et al., 2009). Recent studies recommended using PTX in early stage of infection and in a long-term treatment. Also, it can be used as an adjuvant therapeutic tool when combined with PZQ in treatment of schistosomiasis (El-lakkany and Nosseir, 2007). In conclusion, in this study, treatment with PZQ complemented with immunization resulted in significant reduction of parasitological parameters and rise of specific Igs. Addition of antifibrotic drugs PTX or silymarin to PZQ, potentiated an antipathology effect which minimized and ameliorated liver fibrosis by inhibition of HSC activation and accentuation of the effect of suppressor Treg cells.

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3/25/2010

FMSIND: A Framework of Multi-Agent Systems Interaction during Natural Disaster

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Abstract: Multi-agent systems have a potential to collaborate with each other using their language but the challenge is to make them work intelligently during the situation of catastrophic disaster. In such situations, it is extremely viable to diagnose and dispose resources like ambulances, volunteers, etc. timely, in order to help out people and reduce casualties. We studied the existing frameworks and methodologies in this area but none of them satisfy the requirements on the whole. If one lacks the coordination between agents then other has deficiency of decision support system. This was a motivation for us to propose a framework that covers all aspects of the problem. In this paper, we propose an algorithm to find out the plans of other collaborative agents for coordination and a complete architecture of the framework. The decision support system has been incorporated in the framework for taking optimized decisions. We take a scenario as a case study to verify and validate the proposed framework. . We also show the implementation of interaction among the agents. [Journal of American Science 2010; 6(5):217-224]. (ISSN: 1545-1003).

Keywords: Agents; multi-agent systems, JADE; decision support system

1. Introduction

The gigantic earthquake of Pakistan (Admin, 2006) disturbed the country not only economically but also with the loss of precious lives, urged us to contribute our skills in order to mitigate the consequences. We could reduce casualties by having the correct information in-time or in advance.

Multi-agent systems (MAS) are useful from the real world applications to the graphical applications e.g., computer games. They are also used for coordinated defense systems. MAS can be very useful in the scenario of natural disaster where multiple physical or logical entities need to coordinate in very critical situation. MAS make the agents to work collaboratively and intelligently, which is the key requirement for the selected application (Sadik et al., 2006).

Traditional rescue teams and disaster management departments take decision, for rescuing people and handling casualties, according to the situations but they are either far-away to sense the situation properly or the geographic factors failed their decision ability. The consequences of this lead them with higher number of casualties and infrastructure loss. By using the MAS systems and the other accessories like sensor system, decision support system, neural system, etc., we can have an infrastructure upfront that have better coordination among the agents. The coordination part can be done using the existing frame-works of agent communication e.g., Java Agent DEvelopment Framework (JADE) (Caire, 2007).

MAS, the Decision Support System (DSS), repositories, and the interaction between MAS are the desired components of Disaster Management System. Scalable Agent Grooming Environment (SAGE) (Sadik et al., 2006) (Farooq et al., 2005) offers scalability and fault tolerance but it lacks DSS and the collaboration between agents. The EQ-Rescue (Fiedrich, 2006) does not have DSS. The Earthquake Management System and Knowledge Oriented Sensors Web (Sia et al., 2008) do not have the collaboration between agents. None of the system is providing the complete solution upfront. We integrate all necessary components in our solution.

In Section 2, we analyze existing systems/frameworks. We present materials and methods for the proposed Framework of Multi-agent Systems Interaction during Natural Disaster (FMSIND) in Section 3. In Section 4, results and discussions are presented. Finally in Section 5, we conclude our work and present the future work.

2. Related Work

In this section, we give a brief review of previously proposed prominent frameworks:

2.1 Scalable Fault Tolerant Agent Grooming Environment (SAGE)

SAGE is a fault tolerant, Scalable and decentralized environment (Sadik et al., 2006) (Farooq et al., 2005). It consists of following components.

Agent Management System (AMS) is the core component of SAGE and has the supervisor level control over the platform and its components. It can either request agents to perform some function or forcefully deploy them on any task. AMS also performs management functions i.e., create, suspend, kill, and resume along with control of various agents' platform parameters.

Directory Facilitator (DF) provides the services of yellow pages. Agents can register their services to DF. They can query DF as well to find what services are provided by the other agents. Agents can also de-register or update their registration contents.

Visual Management Agent (VMA) provides the services of visualization of the platform. It provides the Graphical User Interface (GUI) for administration and monitoring services. Apart from GUI, it provides tools as well that can be used to interact with other components like AMS, DF and to test application agents administratively.

Message Transport Service (MTS) is responsible for sending and receiving messages between system and application agents. The agents involved can belong to single local platform or multiple platforms. There are two modes of communication in SAGE: Inter Platform and Intra Platform.

Agent Communication Language (ACL) provides the message format and the description of the agent intention. It is responsible for the creation of messages that are understandable by all of the components involved in MAS.

SAGE gives the advantage of decentralization and distribution with message priority queue to achieve fault tolerance and scalability. However, in SAGE, agents lack coordination which results in the non -cooperative system that cannot work in timely manner.

2.2 Earth Quake Rescue

Earth Quake Rescue (EQ-Rescue) is a distributed simulation system that models the initial response activities after earthquake disaster (Fiedrich, 2006). It consists of following three models:

Disaster World Model is used for the representation and simulation of disaster area. The simulators in the model have complete knowledge of the actual situation. The information includes the real damage of buildings, infrastructure, roads, the number of injured and trapped persons and the buildings that are on initial stages of catching fire. Therefore, the information world resulting from these simulators is called as complete information world.

Resource Model - Resources in the field have limited access to the information world provided by the Disaster World Model. Each resource has specified radiant in which it can sense the disaster world for pre-defined resolution. The assignment of task to the resources can only be devised by emergency operation center members or agents. The further processing then takes place on the devised assignment by the resource simulator itself.

Emergency Operation Center (EOC) agents or members do not have the complete information about the disaster world. Rather they have access to the Incomplete Information World (IIW) that populates the information from the pre-disaster database. This is the decision making component of EQ-Rescue.

The present version of EQ-Rescue does not include an agent-interface to the decision makers / EOC agents. The communication mechanism between the EOC agents and the working agents is lacking.

2.3 Knowledge Oriented Sensor Web

Knowledge oriented sensor web is also an approach in this stream where agents work intelligently not only to sense the situation but also to take action (Sia et al., 2008). They deal with the updated information as well as the decision making part as per their sense. They use multi-agent system in two parts: Sensing and Response. They link these parts through knowledge-base and the decision support system. The core part of the knowledge oriented sensor web is the interaction between sensor agent (SA) and disaster response agent (DRA). The interaction between these agents depends upon specific environment and is carried out through communication protocol.

But, if the interaction is through web, the communication channel such as gateway is required. The architecture uses rule-based expert system, MAS and Sensor Web; however, it does not describe about the gateway for communication between different components of the system.

2.4 Earthquake Management System

In Earthquake Management System (EMS), policy based migration of mobile agents is

used for movement of agents in disaster management system (Sadik et al., 2006). Policies consist of rule-based statements which are the set of pre- post conditions and action statements. These operations have the mobility and task execution strategy. The operations can be picked from the set of operations list, for instance, relocating the agent. The user of the mobile agent picks a goal to perform. The goal first needs to satisfy all condition operations associated with the pre-conditions. Then, control will be handed over to Do-Action part. That will have the mobility strategy and the execution methods. After that post-conditions must need to be satisfied before completion.

The architecture deals only with the mobility and interaction of agents. The other necessary parts like decision support system are lacking in the system. The agents in this architecture lack coordination and collaboration.

2.5 The ALADDIN Project

Autonomous Learning Agents for Decentralized Data and Information Networks (ALADDIN) is a multi-disciplinary project that deals with the dynamic nature of uncertain distributed and decentralized Intelligent Agent Systems (Padhy et al., 2006). The project deals with the communication and interaction between the multiple agents to achieve the individual and collective goal. It concerns with three main tasks:

- How the interaction between agents can be structured?
- How the class of methods and agents can be used to coordinate for solving the problem during the operations?
- How interactions between these agents can be modeled and simulated?

To achieve the above tasks the project exhibits the following methods:

- Auction Method. Resource allocation in MAS is also an extensive research area especially if we come in the domain of natural disasters. Multiple approaches have been used to fulfill this task like the Bidding Strategy, in which there would be an auction over the resource allocation. The project uses neural networks to optimize the resource allocation and to train the system (Enrico et al., 2008)
- Coalition Detection Method. Coordination and interaction between MAS is done through the agent communication languages but in case of multiple MAS, the coordination would be a bigger and complex problem because every MAS would have its own protocol to communicate. Less number of resources and their deployment according to the situation is an enigma. Anytime Coalition Formation is an algorithm which forms the team of resource agents and assigns them weight-age and assists the system to pick the suitable coalition (Rahwan et al., 2007).

3. Materials and Methods

3.1 Framework of Multi-agent System Interaction during Natural Disaster (FMSIND)

The key components of our framework are the following (see Figure 1).

- Sensor System senses the situation (e.g., seismic reading of earthquake or the smoke sensors etc.) and if it is crossing the threshold then it intimates to the Sensor Agents. The sensor devices are used for this purpose e.g., for earthquake detection; the Seismic Reading device can be used, and for fire detection; the usual Fire Alarms can be used.
- Sensor Agents sense the situation on-site. These agents have link with sensor devices that give them activation signals, if threshold limit crosses. The sensor device is responsible for activation of sensor agent. Once the sensor reading crosses the threshold, at the same time, it produces the alarm along with the signals to the sensor system.

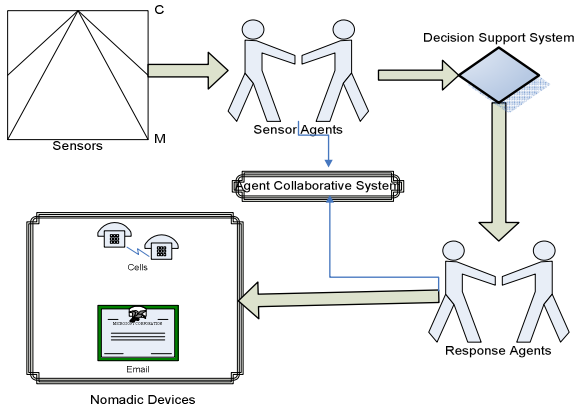

Figure-1. Architecture of FMSIND

- Interactive MAS consists of agent communication framework like Java Agent Development Framework (JADE)
- Decision support system includes learning agents, neural networks, and the components of data warehousing to find the pattern.
- Response agents are activated as per instruction of the decision support system.

Nomadic Devices like personal digital assistants, email systems, mobile phones are used for intimations and warnings for taking actions.

Our main focus in this framework is on the interaction between the agents at disaster site and resource site (like hospitals, fire-brigade offices, rescue services, etc.). The messages between the agents are sent using agent communication language (ACL) (Fornara and Colombetti, 2002). Research in the project of ALADDIN has developed such an algorithm, and it offers several orders-of-magnitude improvement on current state of the art (Rahwan et al., 2007). We use this algorithm in the coordination part of on-site and resource-agents.

3.2 FMSIND Architecture: A Detailed View

This section gives a detailed view of the architecture of FMSIND. The on-site part and the remote-site part are included in the Figure 2. Following are the key components of this architecture:

Sensor Agent (SA) has the responsibility of activating the suspended agents as well as getting information from the sensor system about the loss, and providing this information to the database and decision support system. It also has the responsibility to get data from the decision support system about the on-site required resources.

Supervisor agent has responsibility to get the required resources data from the sensor agent and assign tasks to the on-site coordinating agents. For example, if there is a need of 5 ambulances, the supervisor agent passes the information that the site requires 5 ambulances and assigns duty to the on-site agent to coordinate with the remote-site agents to get this done.

On-site agents get the resources required on on-site from supervisor agents and check their availability by interacting with the disaster database management system. Then, they provide this data to the decision support system to get the plan. They also provide the information about the resources required on on-site to the remote-site agents.

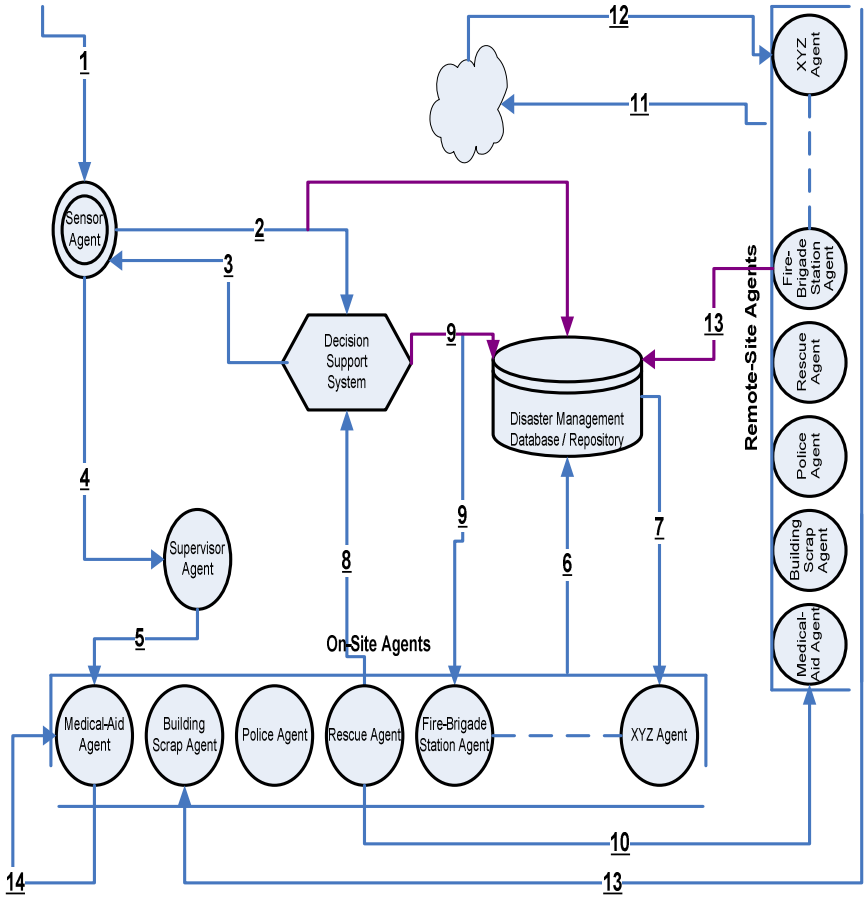

Figure-2. Detailed architecture of FMSIND

Remote-site agents send the required resource information to the resource handlers as per the addresses provided by the on-site agents. The resource handlers send the reply to the remote-site agents. The remote-site agents then compile the information and send back to the on-site agents.

Decision Support System (DSS) has the responsibility to decide about the number of resources required by the site as per the information provided by the sensor agent. We use the decision support system for devising a plan to assign resources to the on-site agents.

Disaster Management Database System / Repository (DB) has the data about disasters, allocated and deployed resources. It also has the data about the resources availability and their addresses. The resource coordinators have rights to update this information in the database.

3.3 Step wise Description of FMSIND

1. Sensor input data: Number of casualties, injuries, building damage area, fire losses, damage of infra-structure, etc.
2. SA sends data to DSS and DB
3. DSS sends number of resources required e.g. ambulances, doctors, beds, volunteers, fire-brigades, policemen etc. to SA
4. SA sends requirement of resources data to supervisor agent.
5. Supervisor agent devises the duties to the relevant on-site agent.
6. The on-site agents check the availability of resources and their addresses
7. DB sends the required data with resources' addresses and their status.
8. The on-site agents send data to DSS to get the resource allocation plan.
9. Resources with allocation plan are sent to the on-site agents and saved in the DB as well
10. A request is made by the on-site agents to the relevant remote-site agents to perform a task.
11. The remote-site agents send a message to the resources' coordinators (RC) on their preferred information media e.g., email, short messages on cell phones etc. to get the required resources.
12. RC allocates the required resources and informs the remote-site agents.
13. The remote sire agents give information about the allocated resources to the on-site agents. For example, how many ambulances and doctors have been sent to the on-site? The remote-site agents also update DB with the allocated resources to the on-site agents.
14. A loop from step no. 6 to onward until the fulfillment of requirements.

3.4 A Detailed Functional Description of the On-Site and Remote-Site Agents of the FMSIND

On-Site Part

The on-site part of our FMSIND comprises of multiple agents, the decision support system and the disaster management repository system. The agents include the sensor, supervisor and categorically named coordinating agents. System starts when sensors cross some threshold and activate / awake the sleeping / suspended sensor agent that has responsibility to signal the agents of the whole system to activate them. Sensor agents get information from the sensor system about the casualties, damage, loss of infrastructure, etc. It compiles the information and sends to the database and to the decision support system as well. The decision support system provides the information about the resource requirement according to the raw-information provided by the sensor agent. The data is then sent to the sensor agent.

The sensor agent provides the resource requirement data to the supervisor agent. The supervisor agent has the responsibility to devise the duties to the relevant category on-site agent. The on-site agents then communicate with the database that has information about the resources availability and their addresses.

This information is sent to the agent back as the results of query and the agent passes the requirement of resources and the available resources information to the decision support system. The decision support system then takes decision how these resources should be allocated and sends the plan to the agent as well as to the database to keep it up-to-date. The on-site agent then coordinates with the remote agent to implement the provided plan.

Remote-Site Part

Agents of this site become operational when any call is sent to these agents by the on-site coordinating agents. The on-site agents advise different duties to these agents according to their operation. For example, on-site rescue agent will send the information to the concerning remote rescue agents to send the number of rescuers on the disaster place.

The remote-site agent has responsibility to directly communicate with the resources' coordinators (RC). They send information to the RC that this number of resources required at the required place. The information is sent to the RC

on their preferred information media e.g., the hospital coordinator choose the media Short Message Service (SMS) to coordinate with the agents. The remote-site agent waits for the response from the coordinator. They send intimation again if they get no response after certain time.

After receiving response from RC the remote-site agents update the database with the information that how many resources are available and how many has started work on the devised task. The remote-site agents also update the on-site agents about the information which becomes the cause of starting the operation of on-site agents.

4. Results and Discussions

4.1 Agents Coordination

The coordination part is very important in our architecture of FMSIND. The agents plan to perform a task. This is their default behavior. While working with MAS, they need to collaborate with one another in order to achieve the broad goal of the system. There are two things that come across while going deep into interaction/collaboration:

1. Planning comprises of how to perform a task locally. Each agent by default does planning whenever it needs to perform a task. In MAS they need to know the plans of others in order to perform the tasks in synchronous way.

2. Scheduling is the actual strategy of performing a task with synchronicity. The scheduling is done after devising a plan. The agents need to do the planning on two levels:

a. Local – they need to form their own plan to perform the task

b. Global – they need to know the plans of other collaborative agents for coordination

For the global scheduling and planning we present an algorithm in Figure 3.

4.2 Validation

As a case study we are taking the earthquake of 2005 which came in northern areas of Pakistan and its magnitude was recorded as high as 7.6 (Admin, 2006). The northern areas have a lot of mountains, hills, glaciers, etc. which made the help difficult to reach there. The outside help takes significant delays because of the geographical condition and the hazards on the way to these areas. The rate of casualties increased enormously because of this problem. We place our system and see how we can reduce the casualties and other hazards. Sensor agent, supervisor agent and all other on-site agents such as medical-aid agent, building scrap agent, police agent, rescue agent and fire brigade station agent are installed on the on-site i.e. Muzaffarabad, Azad Kashmir to perform relative duties. Relative remote agents are installed in Islamabad and Rawalpindi.

Suppose the earthquake has occurred. The seismic (Carrington et al., 2008) instrument has touched the threshold value. Sensor agent receives data from the sensor devices, installed on the on-site, and provides it to the decision support system. DSS calculates that the on-site requires 2 fire brigade vehicles. Now the supervisor agent assigns this duty to the fire brigade station agent of the on-site. The on-site fire brigade station agent sends this requirement to corresponding remote fire brigade station agent (as shown in Figure 4). The remote fire brigade station agent sends an email or makes a phone call for a concerned person about this emergency. The concerned person sends the required fire brigade vehicles to the on-site and inform to the remote fire brigade agent as well. The remote fire brigade station agent then informs the concerned agent of the on-site. During this process, database is updated accordingly. This process is automatic, very fast that and the on-site is provided with the required resources immediately and number of causalities and other losses of property is minimized and saved at an enormously rate.

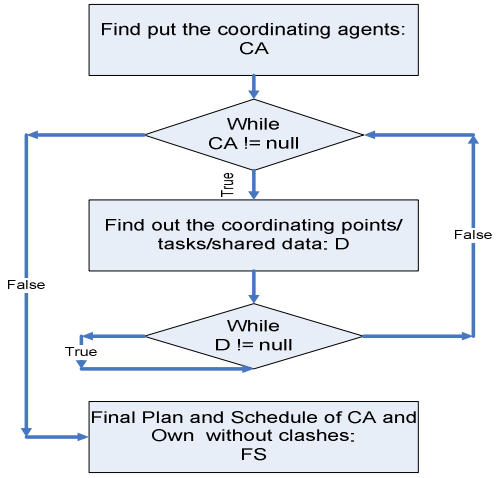

Figure-3. FMSIND Interaction Algorithm Flow Chart

5. Conclusion and Future Work

We integrate all necessary components of a disaster management system into the agent based architecture. The architecture has complete information about what should be included in a disaster management system. We implemented our system's agent interaction part using JADE. The solution is compatible with any system as it is based on Java.

The framework needs to be checked with more real world case studies to test the potential problems of communications and coordination among the agents.

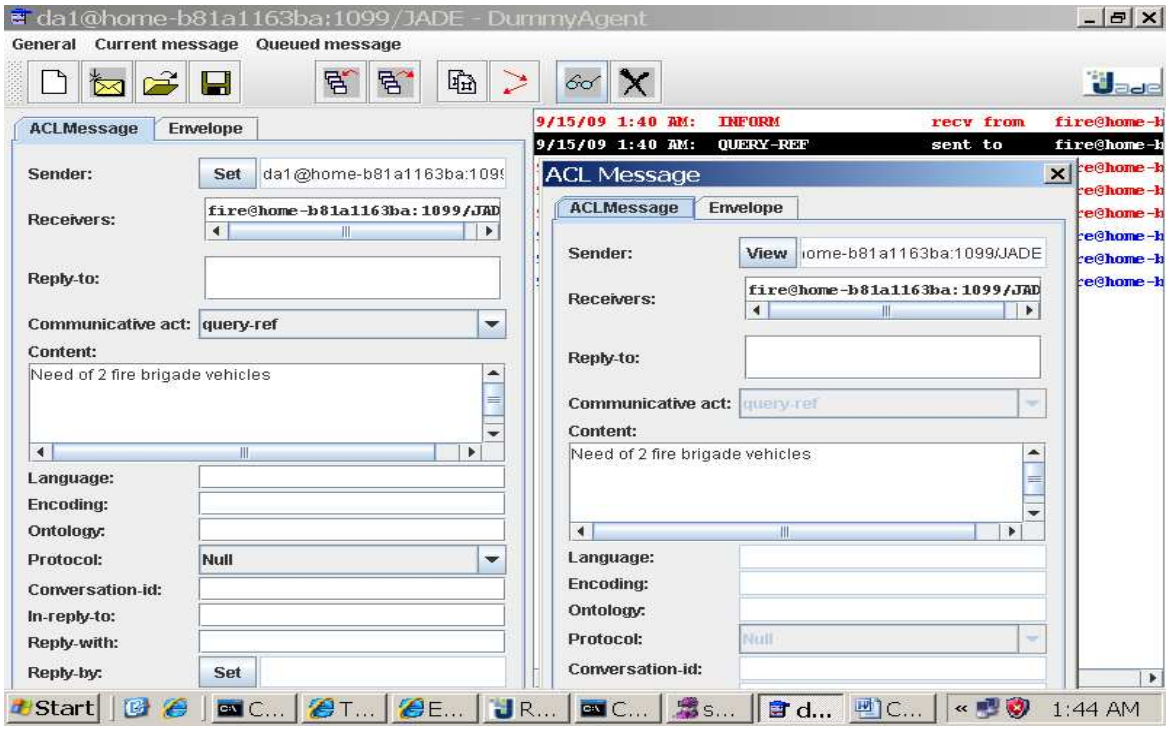

Figure-4. Interaction between on-site and remote site fire brigade agents

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3/26/2010

Postpartum Performance Of Buffaloes Treated With Gnrh To Overcome The Impact Of Placenta Retention

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Abstract: This study aimed to investigate impacts of GnRh treatment on post-partum productive and reproductive performance of buffaloes subjected to placenta retention. A number of 30 female buffaloes were used in the study among them 20 buffaloes were detected with retained placenta (RP), while 10 buffaloes were normally calved (NRP). Buffaloes with RP were divided into two groups (10 buffaloes each) where group (RPT) were injected with 10 ml GnRH at the 7th day postpartum and group (RPC) served as control group. Blood samples were collected twice weekly from each buffalo cow during late pregnancy and postpartum period for determination of progesterone (P4), estradiol 17β (EST) as well as some blood metabolites. Placental tissue samples were taken from four animals with normal and retained placenta for histological examination. Postpartum loss in live body weight was greater (P <0.01) in NRP buffaloes than animals with RP. Differences between groups in calf birth weight (CBW) were insignificant while differences between newborn males and females were highly significant (P < 0.01). Volume of fetal fluids was greater in NRP group comparing with the other groups (P < 0.01) whereas no significant differences were detected in weight of fetal membranes between groups. Time elapsed for placenta expulsion in was 4.23, 17.26 and 18.7 hr. in NRP, RPT and RPC groups, respectively. Sex of newly born calf had only a significant effect (P < 0.01) on CBW and CBW/DAM. The normal group of buffaloes (NRP) achieved the least (P < 0.01) calving interval (CI) and days open (DO) as compared with buffalo groups with RP. However, GnRH treatment had significantly (P < 0.05) reduced CI and DO for group RPT than that for group RPC by 10.41% and 28.33%, respectively. No. of services per conception declined in response to GnRH treatment (2.6) when compared with RPC group (3.5). Differences between the studied groups in milk traits (total milk yield, days in milk and daily milk yield) were highly significant (P < 0.01) not only in the current milking season but also in the previous and next milking season. Buffaloes treated with GnRH (RPT group) achieved greater milk productivity (13.27%) than RPC group. Post partum concentrations of P4 were significantly (P <0.05) greater in NRP animals than that in buffaloes with RP throughout the experimental months. GnRH treatment increased significantly (P < 0.05) postpartum EST concentrations during 5th to 8th week as compared with non-treated animals. Concentrations of all studied metabolic parameters were relatively lees in RP groups than that in non retained group (NRP). GnRH treatment had relatively ameliorated the metabolic function in treated buffaloes via increasing concentrations of blood total protein, glucose, creatine, creatinine, clacium and inorganic phosphorus. The histological sections revealed dismaturation of the RP denoted by limited number of trophblastic giant cells, decomposition and fragmentation of the placental tissue and chorionic villi concomitant with hyperplasia in the chorionic epithelial cell of the villi. [Journal of American Science 2010; 6(5):225-233]. (ISSN: 1545-1003).

Keywords: Buffaloes , retained placenta, GnRH, productive and reproductive traits.

1. Introduction

Buffaloes represent the main dairy animals raised in small or medium size holdings and play an important role in the animal agriculture eco-system in Egypt. However, buffaloes are characterized by low reproductive efficiency as they achieve longer calving intervals (El-Rigalaty, 1995). This factor compromises a major impediment for buffalo productive performance of milk and meat. Placenta retention is one of the reproductive disorders affecting profitability of buffalo production since it delays uterine involution, predisposes females to ovarian cystic degeneration and reduces fertility. El-Malky (2007) demonstrated that retained placenta (RP) was observed in 4.6% cases of buffaloes over three years of study. The higher levels of progesterone (P4) concomitant with a significant lower level of oestradiol (EST17β) at delivery leads to incidence of RP (Hashem and Amer, 2008). Several investigators pointed out some factors that predispose for RP such as pre partum metabolic disturbances (Michal *et al*. 2006) season of the year (El-Malky (2007), dystocia (Thompson *et al.,* 1983), animal age (Erb and Martin, 1980) and hormonal levels (Bous *et al.,* 1984). The retained placenta (RP) frequently results in a secondary bacterial infection and subsequently depresses fertility (Hashem and Amer, 2008), prolonged calving interval, loss of milk production (Rajala and Grohn, 1998) and higher costs of veterinary treatment.

Several attempts have been made to adopt exogenous treatment with gonadotrophic releasing hormone (GnRH) for inducing ovulation and

resumption of ovarian activity in animals-suffering from reproductive disorders (Aboul-Ela *et al*. 1985 and Barkawi and Aboul-Ela 1987). The present study aimed to investigate impacts of GnRh treatment on post-partum productive and reproductive performance of RP buffaloes. In addition, the study aimed to compare levels of some hormones and blood constituents of buffaloes during pre-partum and post-partum periods to elucidate possible association between those parameters and incidence of RP as well as to examine physiological and histological changes in intact and retained placenta taken from buffaloes.

2. Material and Methods

The experimental procedures:

This work was conducted at the Research Station of Mehallet Mousa belonging to the Animal Production Research Institute. A number of 30 female buffaloes were chosen to carry out the experimental work, among them 20 buffaloes were detected with retained placenta (RP), while 10 buffaloes were normally calved (Group NRP). As a general rule in the study, placenta was considered retained if it remained for a postpartum period longer than 12 hours (Laven and Peters, 1996). Buffaloes with RP were divided into two groups (10 buffaloes each) where group (RPT) were injected with 10 ml GnRH (Receptal™) at the 7th day postpartum and group (RPC) served as control group. During the experimental period, close observation was undertaken at the late pregnancy (one month before calving) and continued three months after delivery. Birth weight of the offspring (CBW) was recorded and the time elapsed for placenta expulsion was determined for each dam. Also, dam weights before and after parturition were recorded. Weight of completely intact (PTW) or damaged placenta from buffaloes with RP was also recorded. As regular farming system, the experimental buffaloes were included in mating groups one month after parturition, where pregnancy diagnosis through rectal palpation was adopted subsequently after two months postpartum.

Management and feeding:

Buffaloes at late pregnancy period were kept in their shed until time of delivery then they were transferred to the maternity unit. After delivery the newly born calves were kept for one week with its dam for colostrums feeding. The dam was then transferred to the milking unit. The experimental buffalo cows were housed in open sheds and subjected to regular managerial practices of the breeding stock.

Animals were fed according to their live weight and milk production, feed allowances were offered for animals as recommended by APRI (1997). The animals were fed on concentrate feed mixture (CFM) twice daily along with wheat or rice straw and clover hay, when available. Buffalo cows were hand milked twice daily and milk yield was recorded at each milking. Water was freely available in water troughs except at the milking time.

Blood sampling:

Blood samples were collected twice weekly via the jugular vein from each buffalo cow during late pregnancy and postpartum period. Blood plasma was separated after centrifugation at 3000 r.p.m. for 15 minutes, and then stored at -20 C^{o} until analysis for the different blood parameters. Using ready made kits, plasma was used for determination of total protein (TPR), albumin (ALB), Glucose (GLU), blood urea nitrogen (BUN), creatine (CRT), creatinine (CRTN), aminotransferases (AST and ALT), total cholesterol (TC), calcium (Ca) and inorganic phosphorus (PHOS). Direct radioimmunoassay technique was performed for determination of progesterone (P4) and estradiol (EST17β) in representative plasma samples. Kits of ‘‘Diagnostic Products Corporation, (DPC) Los Angles, USA’’ with ready antibody coated tubes were used according to the procedure outlined by the manufacturer.

Measurement of reproductive parameters:

One month after parturition, all animals were observed twice daily for heat detection at 8.00 a.m. and 3.00 p.m. by using a fertile bull. Mating procedure was conducted naturally after 12 hours of heat detection. The number of days open (DO) and number of services per conception (NSPC) were determined for each dam.

Histological examination:

Placental tissue samples were taken from four animals with normal and retained placenta for histological examination. Tissue samples were dehydrated by ascending graded series of ethyl alcohol 70, 80, 90, 96 and 100 %. Toluene was used as a clearing agent for 8-10 hours. The samples were impregnated in two successive baths of melted paraffin wax, then embedded in melted wax blocks. Finally the paraffin blocks were cut into thin section (5-7 micron) by rotary microtome. From each sample, 20 sections at least had been mounted and stained by haematoxylin and eusin. The slides were examined by means of light microscope

Statistical analysis:

Statistical analysis was carried out using the General Linear Model Program (GLM) of SAS (2000). Differences were subjected to Duncan’s Multiple Range Test (1955). Data concerning productive traits of different groups of buffaloes were analyzed using the model: $Y_{ijk} = \mu + T_I + Sj + e_{ijk}$

Where:
$\mathbf{Y_{ijk}}$ is the observation taken on the recorded animal ij,
μ is the overall mean,
$\mathbf{T_I}$ is the effect of group
Sj is the effect of born calf sex
$\mathbf{e_{ijk}}$ is the random error.
Data concerning blood constituents of the experimental buffaloes were analyzed using the following model:

$$Y_{ijk} = \mu + M_i + T_j + MT_{ij} + e_{ijk}$$

Where:
$\mathbf{Y_{ij}}$ is the observation taken on the experimental animal **ij**,
μ is the overall mean,
$\mathbf{M_i}$ is the effect of month of sampling,
$\mathbf{T_j}$ is the effect of group,
$\mathbf{MT_{ij}}$ is the interaction between month of sampling and group effect
$\mathbf{e_{ijk}}$ is the random error.

3. Results and Discussion

Post-partum traits of the experimental groups:

As shown in table (1), postpartum loss in LBW had the same trend of LBW being significantly ($P < 0.01$) greater in NRP buffaloes than animals with RP. Percentages of calving loss in LBW of buffaloes in proportion to the pre-partum weight were 10.30%, 11.22% and 11.16 % for NRP, RPT and RBC groups, respectively. Awara (2006) working on buffaloes found that loss in weight was 9.30 to 10.41% depending on pre-partum feed supplementation. Differences between groups in calf birth weight were insignificant while differences between newborn males and females were highly significant ($P < 0.01$). However, relative weights of calves to their dams (CBW/DAM) were significantly higher ($P <0.05$) in RP groups comparing with NRP group probably due to decline in LBW of RP group. This finding was in contrary to that obtained by Bhalaru *et al.* (1983) who indicated that percentages of RP decreased significantly, when (CBW/DAM) increased. Karen (1996) found that differences in CBW from cows with RP were insignificant. Joosten *et al.* (1988) observed that higher incidence of RP was associated with greater CBW. Bhalaru *et al.* (1985) found that dams LBW at calving and CBW were significantly affected the time elapsed until expulsion of placenta among 234 normal parturitions of buffaloes. Volume of fetal fluids reached its maximum value in NRP group comparing with the other groups ($P <0.01$) whereas no significant differences were detected in weight of fetal membranes between groups (Table 1). Time elapsed for placenta expulsion in NRP was 4.23 hr. while it was 17.26 and 18.7 hr. in RPT and RBC groups, respectively. Greater values of placental tissue weight (PTW), being 4.7 - 5.5 kg and foetal fluids (FF) 16.9- 17.4 L in NRP buffaloes were recorded by Awara (2006). Janakiraman (1981) reported that the time spent for normal placenta expulsion varied from 4.08 to 5.38 h and PTW from 3.01 to 3.40 kg denoting that the risk of RP was increased as PTW increased and that of FF volume was decreased. It seems that FF facilitates the fast expulsion of the placenta; meanwhile the detachment of placental membranes seems to be delayed with the excess in PTW. Sex of newly born calf had only a significant effect ($P < 0.01$) on CBW and CBW/DAM whereas, a significant effect ($P < 0.05$) was noticed on the dam's loss in weight and CBW due to interaction of groups and calf sex. The NRP buffaloes that calved males recorded greater ($P < 0.05$) CBW and PTW in comparison with their group mates that calved females. Meanwhile, differences in CBW/DAM were significantly ($P < 0.01$) affected by sex of the born calf or incidence of RP. El-Malky (2007) observed a significant effect of the calf sex on calf LBW in RP or NRP buffaloes.

Histological features of non retained and retained placentome :

As shown in figure (1), histological examination of the NRP revealed presence of intact basal decidua and chorionic epithelium with normal trophoblastic giant cell. The normal allantochrion was bearing simple epithelium on the side facing the allantoic cavity and the mesenchymal layer was containing blood vessels. Histological examination of RP showed decomposition and fragmentation of the placental tissue and chorionic villi. Such alteration was concomitant with hyperplasia in the chorionic epithelial cell of the villi. The number of trophblastic giant cells in RP was limited denoting placental dysmaturity while, focal areas of trophoblastic degeneration and desquamation was remarkable. The section also revealed necrosis of the lining chorionic epithelium with inflammatory cell aggregation. Hemorrhages and congestion of most blood vessels was noticeable accompanied with necrosis and calcification of the lining endothelial capillaries. Hence, thickening in the wall of blood vessels with edema in the connective tissue layer was expected. In agreement, Al-Sadi et al. (1994) observed presence of compacted degenerating deciduas, extensive necrosis and numerous clumps of bacterial colonies in placentomes of cows with RP including vascular changes (edema, thrombosis and vasculitis). The author noticed also presence of numerous binucleate cells, infiltration of polymorphonuclear cells in the connective tissue of the villi.

Table (1): Pre- and post-partum weights of buffalo dams, calf birth weight, placental tissue weight, weight of fetal fluids and time of placenta expulsion in the different experimental groups.

	Experimental groups						Level of Significance		
	NRP		RPT		RPC				
Sex of newborn calf	♂	♀	♂	♀	♂	♀	Group	Sex	Group* Sex
No. of animals	3	7	7	3	6	4			
Dam body weight (kg):									
pre-partum	607.8	572.2	490.6	494.3	482.2	511.3	**	NS	NS
post-partum	544.4	513.6	433.7	443.3	425.9	451.4	**	NS	NS
Loss in weight (kg)	63.40 a	58.60 b	56.86	51.00	56.25	59.88	**	NS	*
CBW (kg)	41.80 a	36.80 b	41.00 a	36.33 b	40.17	39.75	NS	**	*
CBW/Dam	7.68	7.22	9.51 a	8.16 b	9.45	8.83	**	**	NS
Fetal fluids (L)	16.90	17.92	12.33	10.90	12.17	11.25	**	NS	NS
Placenta tissue (kg)	4.70 a	3.88 b	3.36	3.37	4.38	3.92	**	NS	NS
Expulsion time (hr)	4.21	4.24	17.22	17.28	18.64	18.71	**	NS	NS

Reproductive performance of treated groups:

The normal group of buffaloes (NRP) achieved the least ($P < 0.01$) calving interval (CI) and days open (DO) as compared with buffalo groups with RP (Table 2). However, GnRH treatment had significantly ($P < 0.05$) reduced CI and DO for RPT buffalo group than that for group RPC by 10.41% and 28.33%, respectively. Despite, number of services/ conception (NSPC) declined in response to GnRH treatment (2.6) when compared with RPC buffaloes (3.5), the hormonal treatment failed to attain 1.3 services/ conception that recorded by the NRP group. The shorter interval from calving to 1st detected ovulation and date of conception recorded by NRP group may explain the negative impact of RP on post-partum reproductive performance. Incidence of RP causes a deleterious effect on fertility and milk productivity of cows (Jainudeen and Hafez, 1992). Swiefy (2003) found that Friesian cows with RP under Egyptian conditions had significantly ($P <0.05$) longer intervals of postpartum uterine involution, first ovulation, first estrus and days open. There are an earlier evidence that postpartum GnRH treatment shortened the interval from ovulation to peak P4 (Aboul-Ela *et al*. 1985 and Barkawi and Aboul-Ela 1987) and reduced significantly the intervals from parturition to 1st ovlulation and 1st detected oestrus (Aboul-Ela *et al*. 1985). Our finding agree with Hashem and Amer (2008) who found that GnRH treatments reduced significantly ($P <0.05$) the interval from calving to 1st insemination and NSPC, followed by a significantly ($P <0.05$) higher conception rate. However, Risco *et al* (1994) found that cows affected with RP and treated with GnRH (100 µg at d 12 postpartum) did not improve the reproductive performance.

Milk productivity of treated groups:

As shown in table (3), differences between the studied groups in milk traits (total milk yield, days in milk and daily milk yield) were highly significant ($P < 0.01$) not only in the current milking season but also in the previous and next milking season. Estimates of milking traits were greater in NRP group than the other groups with RP at the current season indicting severe negative effect of placenta retention on milk productivity of buffaloes. The decline in total milk yield of current season compared with that of the preceding season was 7.84%, 40.02% and 48.60% in the studied groups NRP, RPT and RPC, respectively. Meanwhile, the milking traits were positively maintained in the next season for buffaloes with RP. Moreover, it was noticed that buffaloes treated with GnRH (RPT group) achieved greater milk productivity (13.27%) than RPC group. These results are in agreement with those of (Paisley *et al.*,1986 and Rajala and Grohn, 1998) who reported that RP resulted in a significantly negative effect on milk yield of dairy cows for several weeks after calving. El-Malky (2007) noticed that buffaloes group with RP reduced its milk yield by 15.79% than the preceding season while NRP group increased milk productivity by 6.99% over that of the preceding season.

Table (2): Estimates of buffalo reproductive parameters in the different experimental groups.

	Treatments				
	NRP	RPT	RPC	± SE	Sign
No. of animals	10	10	10		
Calving interval (days)	411.9 c	489.9 b	541.0 a	10.96	HS
Days open	101.9 c	179.9 b	231.0 a	10.96	HS
No. services/ conception	1.3 c	2.6 b	3.5 a	0.38	HS

Interval from calving to:					
1st detected ovulation	49.9 b	85.1 a	93.6 a	6.97	HS
Conception	70.10 c	90.10 b	107.10 a	5.82	HS

Table (3): Estimates of milk productivity in the different experimental groups of buffaloes.

	Season *	Treatments			
		NRP	RPT	RPC	Significance
No. of animals		10	10	10	
Total milk yield	P	2538.6 a ± 129.97	1762.2 b ± 141.66	1815.2 b ± 114.69	HS
	C	2339.6 a ± 97.45	1056.9 b ± 106.21	933.1 b ± 85.99	HS
	N	2668.8 a ± 2668.7	1281.5 c ± 73.55	1371.3 b ± 59.55	HS
Days in milk	P	236.9 ± 9.59	227.9 ±10.46	219.9 ± 8.47	NS
	C	209.1 a ±8.47	178.7 b ± 9.23	153.2 b ±7.47	HS
	N	232.3 a ±6.72	179.2 b ± 7.33	209.1 a ±5.93	HS
Daily milk Yield	P	10.7 a ±0.49	7.8 b ±0.53	8.2 b ±0.44	HS
	C	11.2 a ±0.43	5.9 b ±0.46	6.1 b ±0.37	HS
	N	11.5 a ±0.32	7.3 b ±0.35	6.6 c ±0.28	HS

P= Previous milking season, C= Current milking season, N= Next milking season.
Means bearing different superscripts in the same raw are significantly (P <0.05) different.

Changes in levels of progesterone and estradiol hormones:

Differences between treatments in progesterone (P4) concentration were insignificant while differences between experimental months were highly significant (P<0.01) taking into account a significant interaction (P <0.01) between treatments and month of sampling. Averages of P4 concentrations were 1.97, 1.95 and 1.84 ng/ml in plasma of NRP, RPT and RPC groups, respectively. Pre-partum concentrations of P4 were almost similar between groups (3.06- 3.62 ng/ml) then it reached the minimum level at the 1st month after parturition (1.11 ng/ml) followed by a relative increase in the 2nd month (1.65 ng/ml) and a relative decrease in the 3rd month (1.46 ng/ml) for all groups. As shown in Figure (2), P4 concentration was significantly (P <0.05) greater in NRP animals than that in buffaloes with RP throughout the experimental months particularly beyond the 5th week postpartum. The diminished levels of P_4 before parturition in NRP animals was stated by several authors (El-Malky, 2007 and Amjad Ali *et al.,* (2009). It was evidenced that P_4 level was significantly higher in cows suffering from RP compared to NRP cows (Sabry *et al.* 1997 and Harendra *et al.,* 2001).

Differences in concentrations of E2 due to treatments or month of sampling were highly significant (P <0.01) with noticed interaction (P <0.01) between those effects. Averages of E2 concentrations were 60.46, 55.4 and 52.29 pg/ml in plasma of NRP, RPT and RPC groups, respectively. Pre-partum concentration of EST were almost similar among groups (103.9-109.0 pg/ml). Concentration of E2 minimized in the 1st month after parturition (22.47 pg/ml) followed by a gradual increase up to the 3rd month (52.72 pg/ml). In addition to the elevated level of E2 in NRP group over that of group RPC, the results indicated a positive effect of GnRH treatment by increasing E2 concentration significantly (P < 0.05) in the 2nd month (5th to 8th week) after parturition when compared with that of non-treated animals (Figure 1). El-Malky (2007) found that prepartum concentration of E2 was relatively higher in NRP buffaloes than that of RP group. In contrary, Harendra *et al.* (2001) did not observe difference in E2 level between RP and NRP animals. The sharp increase of E2 and decrease of P_4 just before parturition in NRP cows was reported by Gordon (1996). Such finding was evidenced also by Hashem and Amer (2008) working on cattle and Amjad Ali *et al.* (2009) working on buffaloes. Normal calving requires softening and dilation of the cervix,

particularly during LP due to the influence of relaxin and estrogen when P_4 dominance decline and uterine prostaglandin production is increasing (Taverne, 1992). On the other hand, normal expulsion of fetal membranes requires a rise in E2 before calving accompanied by a gradual and sustained fall in P_4 (El-Wardani *et al.*, 1998).

The ratio of E2/ P_4 was similar among treated groups of buffaloes. However, the respective ratio had greater (P <0.01) estimate during prepartum period in comparison with postpartum period. On the other side, that ratio was greater at the 3rd month after delivery than that at the 1st and 2nd month postpartum.

Changes in blood constituents of treated groups:

Generally, concentrations of blood proteins (TPR), glucose (GLU), blood urea nitrogen (BUN), creatine (CRT), alanine transferase (ALT), total cholesterol (TC), calcium (CA) and inorganic phosphorus (PHOS) increased significantly (P < 0.01) with advancement of month of sampling (Table 4). On the other hand, TPR, CRT, amino transferases, TC and PHOS showed higher concentrations in pre-partum month than that in postpartum months. In accordance, Some studies attributed the differences between pre-partum and post-partum periods in level of blood TPR and GLU to milk protein synthesis (Rakesh Kumar *et al.*, 2001) or increased proteins break down required for gluconeogensis (Abdul Gani *et al.*, 2003). The differences in TPR due to treatment or month of sampling and their interaction were highly significant (P <0.01). It was observed that concentrations of all studied metabolic parameters were relatively less in RP groups than that in non retained group (NRP). Malnutrition may have a significant role influencing pre-parturient hormonal balance particularly those hormones mediated in energy metabolism leading to placenta retention. In agreement with this finding, Sabry *et al.*, (1997) observed that TPR and GLU in NRP cows were greater than that in cows with RP while,. Choudhury *et al.*, (1993) did not detect appreciable change in plasma TPR of cattle or buffaloes due to RP. In contrary, Deyab (2000) working on Friesian cows noticed an increase of TPR and GLU in animals with RP. Harendra *et al.*, (2001) did not found a significant change in serum glucose in NR or RP cows. Hashem and Amer (2008) noticed that concentration of liver enzymes was significantly (P <0.05) higher, while serum GLU and TC levels were significantly lower in cows with RP compared with the NRP group.

The hypocalcaemia detected in RP buffaloes could be considered as another indicator of metabolic disorder due to malnutrition. These findings were in accordance with results reported earlier by Sabry *et al.* (1997), Deyab (2000) and Patel *et al.* (2003). However, Harendra *et al.* (2001) did not observe any significant change in concentration of CA and PHOS in blood serum of RP compared to NR cows. Hashem and Amer (2008) concluded that low serum electrolytes levels and hormonal imbalance might predispose to RP in dairy cows while, Rakesh Kumar *et al.* (2001) attributed the low CA level during late pregnancy to its diversion toward fetal skeletal formation throughout the gestation period. El-Malky (2007) noticed 26.14% and 16.94% increase in CA and PHOS content in blood of NRP than RP groups, respectively. In the current work, GnRH treatment had relatively ameliorated the metabolic function in treated buffaloes via increasing concentrations of TPR, GLU, CRT, CRTN, ALT, CA and PHOS. Hashem and Amer (2008) observed that RP cows treated with GnRH showed a higher (P<0.05) levels of GLU and total lipids, with lower liver enzyme concentrations than controls adding that protein profile returned close to control level in the treated groups.

Table (4): Least square means of concentrations of blood metabolites in the experimental buffaloes.

Blood parameter	Unit	Experimental groups			
		NRP	RPT	RPC	± SE
Total protein (TPR)	g/dl	8.48 [a]	7.35 [b]	6.94 [c]	0.04
Albumin (ALB)	g/dl	3.82 [a]	3.31 [b]	3.31 [b]	0.03
Globulin (GLOB)	g/dl	4.66 [a]	4.04 [b]	3.64 [c]	0.03
Glucose (GLU)	mg/dl	58.80 [a]	51.18 [b]	49.07 [c]	0.30
Blood urea nitrogen (BUN)	mg/dl	42.82 [a]	39.84 [b]	39.44 [b]	0.14
Creatine (CRT)	mg/dl	50.37 [a]	48.02 [a]	43.20 [b]	1.50
Creatinine (CRTN)	mg/dl	2.82 [a]	2.71 [b]	2.54 [c]	0.02
AST	(U/L)	50.53 [a]	48.17 [b]	48.04 [b]	0.20
ALT	(U/L)	21.29 [a]	21.24 [a]	20.20 [b]	0.14
Total cholesterol (TC)	mg/dl	88.53 [a]	84.32 [b]	85.67 [b]	0.52
Calcium (CA)	mg/dl	9.57 [a]	8.65 [b]	8.22 [c]	0.05
Inorganic phosphorus (PHOS)	mg/dl	5.39 [a]	5.18 [b]	4.45 [c]	0.03

Means bearing different superscripts in the same raw are significantly (P <0.05) different.

Conclusion

The current study indicated different negative impacts due to incidence of RP in buffaloes. Among those impacts, the prolonged DO and CI in addition to reduced animal milk productivity. The higher P4 with lower E2 concentrations during the week before parturition seems to be an important crucial factor predisposing to RP in cattle or buffaloes. The decreased level of E2 may be indicated as a factor enhancing RP (El-Nemer *et al.*, 2000). Prepartum hypoglycemia may act as a predictive indicator of RP risk (Markiewicz *et al*., 2001).

The current study revealed that GnRH treatment had significantly ($P < 0.05$) reduced CI and DO for group RPT than that for group RPC by 10.41% and 28.33%, respectively. Also, NSPC declined in response to GnRH treatment (2.6) when compared with RPC group (3.5). Improvement of reproductive efficiency in GnRH treated buffaloes may result from normalization of P4 level (Foote and Riek, 1999), the treatment may be helpful in hastening ovulation and establishment of a *corpus luteum.* In addition, buffaloes with RP that treated with GnRH (RPT group) achieved greater milk productivity (13.27%) than RPC group. Such response may be referring to amelioration of the metabolic activities in treated buffaloes. The role of GnRH to overcome impacts of placenta retention as well as to sustain fertility of the affected animal may be a dose dependant. Further studies are needed to focus on appropriate doses of gonadotrpins to re-maintain the indigenous hormonal balance.

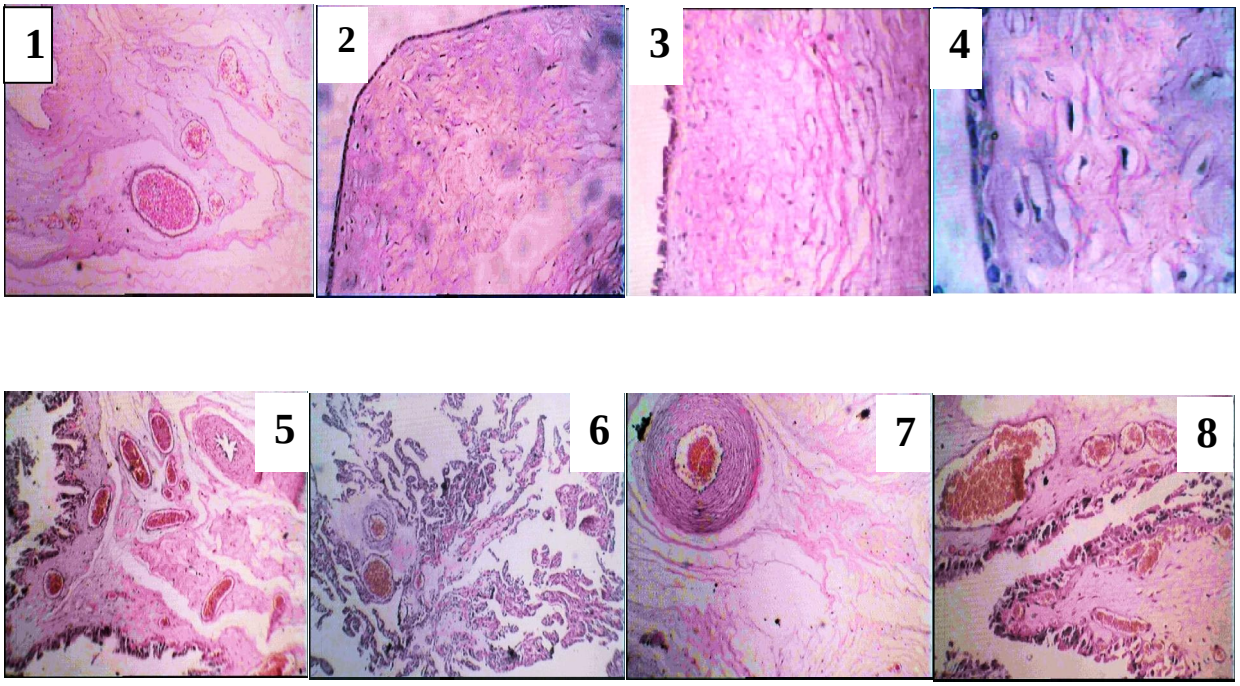

Fig. (1): Sections (H&E staining) from non retained placenta (1,2,3 and 4) of buffaloes, revealing normal allantochorion which bears simple epithelium on the side facing the allantoic cavity (1). The mesenchymal layer contains normal blood vessels (X50). The chorionic epithelium (2) contained numerous mono nucleated cells(X100) and regular arrangement of trophoblast (3)(X250). The basal decidua is intact (4) with presence of normal trophoblastic giant cell(X400). Sections through the retained placenta (5, 6, 7 and 8) showed hyperplasia in the chorionic epithelial cell of the villi (5) with congestion of most of chorionic blood vessels (X100). Decomposition and fragmentation of the placental tissue (6) and chorionic villi is abundant (X100), accompanied with thickening in the wall of blood vessels (7) with edema in the connective tissue layer (X 250). Focal areas of trophoblastic degeneration and desquamation (8) with inflammatory cell aggregation, hemorrhages and congestion of most blood vessels (X 250).

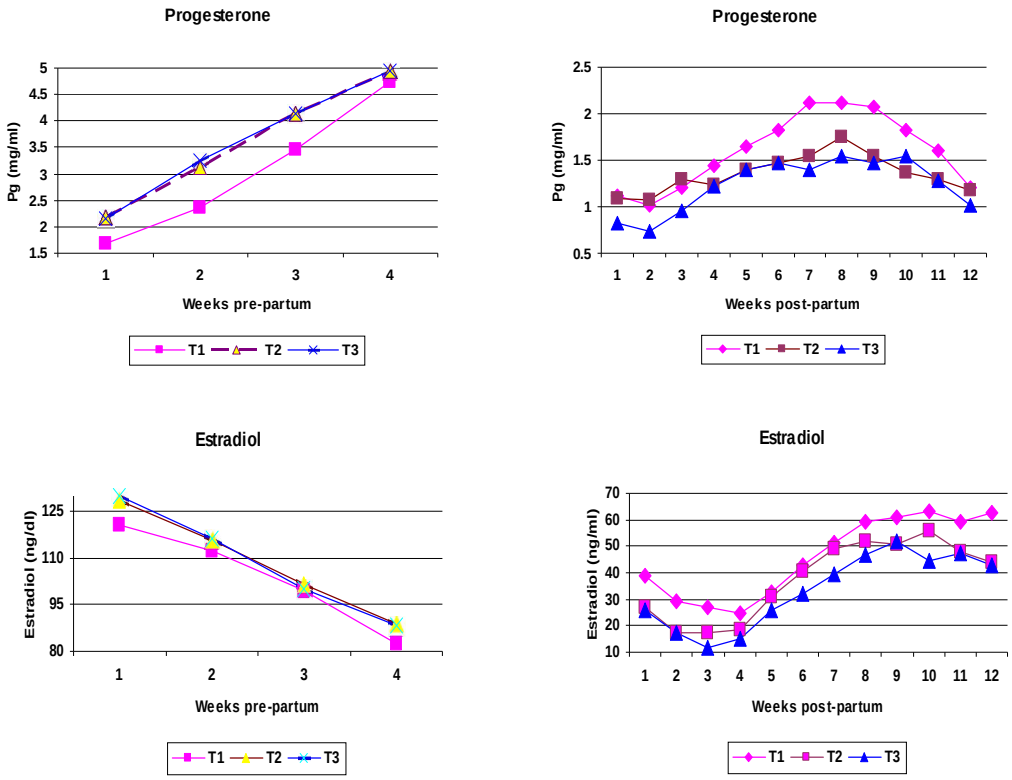

Fig. (2): Pre partum and postpartum weekly levels of progesterone and estradiol in blood of buffaloes with retained placenta (RPC), with retained placenta and treated with GnRH (RPT) and without retained placenta (NRP).

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Physico-Chemical Characteristics, Microbial Assessment And Antibiotic Susceptibility Of Pathogenic Bacteria Of Ismailia Canal Water, River Nile, Egypt

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Abstract: Thirty two water samples were collected during four successive seasons during the period between August, & April 2006 along the area extending from El-Mazallat square to Mostorud area (River Nile, Egypt). These samples represented the effect of the factories effluent discharge onto the canal on water quality. Physical characteristics (air and water temperature, transparency and electrical conductivity) and chemical characteristics (pH, DO, BOD, COD, CO_3^-, HCO_3^-, SO_4^{--}, NO_2^-, NO_3^-, NH_3 and PO_4^{3-}) were measured to identify the Ismailia Canal water quality. These measurements showed slight variations during different seasons at different stations. Additionally, the bacteriological analysis for water samples was done and included the total viable bacterial counts at 22 and 37 ^{0}C and the bacterial indicators of faecal pollution (*total coliformas, faecal coliforms* and *faecal streptococci*). The pathogenic bacteria were identified as *E.coli, Salmonella, Choleraesuis, Streptococcus faecium and Pseuedomones aeruginosa.* Antibiotics susceptibility testing was selected, the families Beta-lactams (amipicillin & cefeprime), Aminology cosides (gentamycin & Kanamycin), Macrolides (erythromycin, spiramycin, tylosin and spectinomycin), Tetracyclines (oxytetracycline base, doxycycline HCl and chlorotetracycline HCl) and Amino acids (neomycin & streptomycin). Pathogenic bacterial isolates revealed resistance against most applied antibiotics pathogenic bacterial isolates were also subjected to fifteen herbal extracts. The test herbal extract extended an antimicrobial activity, *P. aeruginosa* was sensitive to coriander and *E. coli* was sensitive to Cinnamon. [Journal of American Science 2010; 6(5):234-250]. (ISSN: 1545-1003).

Key words: Physico-chemical characteristics, microbial diversity, antibiotic susceptibility, Ismailia Canal, Egypt

1. Introduction

Ismailia Canal is one of the most important irrigation and water supplies in Egypt. It was constructed in 1862 and transports fresh water from River Nile at north of Cairo at El-Mazalet region to Ismailia, Port Said and Suez governorates. The original canal dimensions were average 2.1 and 18 m of depth and width respectively. It extends for about 128 km and has more than one regulator bridges constructed along the total length. It is worthy to note that some factories constructed at this area discharge their wastes into the canal and cause dramatic changes in water quality (Abdo, 1998).

Water quality now is the concern of all countries in the world. The decision of WHO's 29th session (May, 1976) emphasized that the water delivered to the consumer should meet the high requirements of modern hygiene and should at least be free from pathogenic organisms and toxic substance (Voznaya, 1983 and Abdo, 2005).

Aquatic ecosystems are much more complex and integrated to be simply regulated by a single nutrient. Metabolism, growth, productivity and behavior are certainly regulated by many organic compounds in addition to traditional macro factors controls e.g. nutrients (Wetz *et al.*, 2000).

In fact, attention has been paid for monitoring and assessing the microbiological quality of water resources all over the world (Lindskog and Lindskog, 1988; Fernandez-Alvars *et al.*, 1991; Khalafalla *et al.*, 1993). The industrial wastewater represents the main source of water pollution in different parts of the world, e.g. Egypt (Abu-Shady *et al.*, 1996; Sabae *et al.*,2006; Sabae *et al.*, 2008); Poland (Niewolak, 2000); Nigeria (Ekhaise and Anyasi, 2005; Akaninwor *et al.*, 2007) and Brazil (Gunkel *et al.*, 2007).

A powerful monitoring program is needed to provide reliable information about the current water quality. Therefore, the present study was conducted to assess the physico-chemical, the microbial water quality of Ismailia canal and to estimate the antibiotic sensitivity against the isolated pathogenic bacteria. Screening for its sensitivity against some herbal extracts was also done.

2. Material and Methods

Sampling and analysis:-

Water samples were collected seasonally from Ismailia Canal during the period between August 2005 and April 2006. Four stations were selected to represent the different effluents discharged into the canal. Eight water samples were collected during each season (one sample before and the other after each station to know the effluents effect on the canal) water quality. The sampling stations in the map of the Ismailia Canal, Fig. (1).

The water samples were collected from the subsurface layer of the midstream of the canal (at depth 30 cm) by polyvinyl Van Dorn plastic bottles. The water samples were kept in well stopper polyethylene plastic bottles transferred immediately in an ice-box to be analyzed.

Physical and chemical analyses of water samples were measured according to (APHA, 1998). Air and water temperatures, Electrical conductivity as well as pH were measured using Hydro-Lab., Model "Multi 340 II SET". Water transparency was measured using Secchi-Disc diameter 25 cm in the field. CO_3^{--} and HCO_3^- were measured by titrimetric method against 0.025 N H_2SO_4 and using phenol phethaline and methyl orange as indicators. SO_4^{--} was determined by turbidimeteric method. The dissolved oxygen (DO) was performed by azide modification, COD by potassium permanganate oxidation and BOD by the 5-day incubation method. The concentrations of NO_2^-, NO_3^-, NH_3 and PO_4^{3-} were determined by colorimetric techniques with formation of reddish purple azo-dye, Cu and hydrazine sulphates reduction, nesslerization and stannous chloride reduction methods.

All absorbance measurements were determined using spectrophotometer Model LKB Biochem Ultraspec II.

Bacterial Examinations:

For the bacteriological assessment, the water sample were collected in sterile glass bottles and delivered to the laboratory in an ice- box to be analysed within 6 hours.

The bacteriological analysis were carried out to determine the following parameters:

- Heterophic bacteria developing at 22 ˚C and parastic bacteria at 37 ˚C on plate count agar medium using spread plate method (APHA, 1998).
- Feacal indicators; total coliform (TC), faecal coliform (FC) and feacal streptococci (FS) were determined by the most probable number (MPN) method (APHA, 1998) MacConky broth medium was used to estimate MPN of TC and FC at 37 ˚C and 44.5 ˚C for 48 and 24 hrs., respectively. On the other hand, azid dextrose broth medium was used to determine FS at 37 ˚C for 48 hrs.

Isolation and identification of pathogenic bacteria:

Pathogenic bacteria that were isolated from selective media, plate count agar, MacConky broth, EMB and L-aspargine media were used for isolation and purification of the tested bacteria. Bacterial identification was done according to the guidelines of Bergey,s Manual of Determinative Bacteriology (Holt et al., 1994) with the following tests, gram stain, motility, indole reaction, methyl red test, citrate utilization, blood haemolysis, urea and arginine hydrolysis, production of fluorescent pigment, catalase test, oxides test and utilization of certain carbon sources.

Antibiotic assay:

All experiments were conducted using the original nutrient agar stock culture slant avoid to the spontaneous loss of antibiotic resistance sometime associated with frequent sub culturing. The bacterial isolates were transferred to 4-5 ml of Müller Hinton broth. The culture was incubated at 35 ˚C for 2-6 hrs to develop a turbidity of approximately 1-2 x 10^8 cfu/ml. Müller Hinton agar plates were inoculated with the organism grown in the broth by streaking with sterile swap. After streaking, the plates were left for 15 minutes to allow the complete adsorption for the inoculun before applying the antibiotic discs. Then, the discs were applied and the plates were incubated for 16 – 18 hrs. After incubation, inhabitation zone diameters were measured and compared to the table of inhibition zones to be reported as resistant, intermediate or susceptible (Barry, 1991).

Different antibiotic families were applied against all bacterial isolates. The antibiotic families were Beta-lactams (ampicillin & cefepime), Aminoglycosides (gentamycin & kanamycin), Macrolides (erythromycin, spiramycin, tylosin and spectinomycin), Tetracyclines (oxytetracyline base, doxycycline HCl and chlortetracycline HCl), and Amino acids (neomycin & streptomycin). Also, some antibiotic mixtures are also applied against all isolates these mixed antibiotics are; Ticarcillin/Clavulanic acid, Pipracillin/Tazobactam, Ampicillin/ Sulbactam and Ceftazidime/Clavulanic acid.

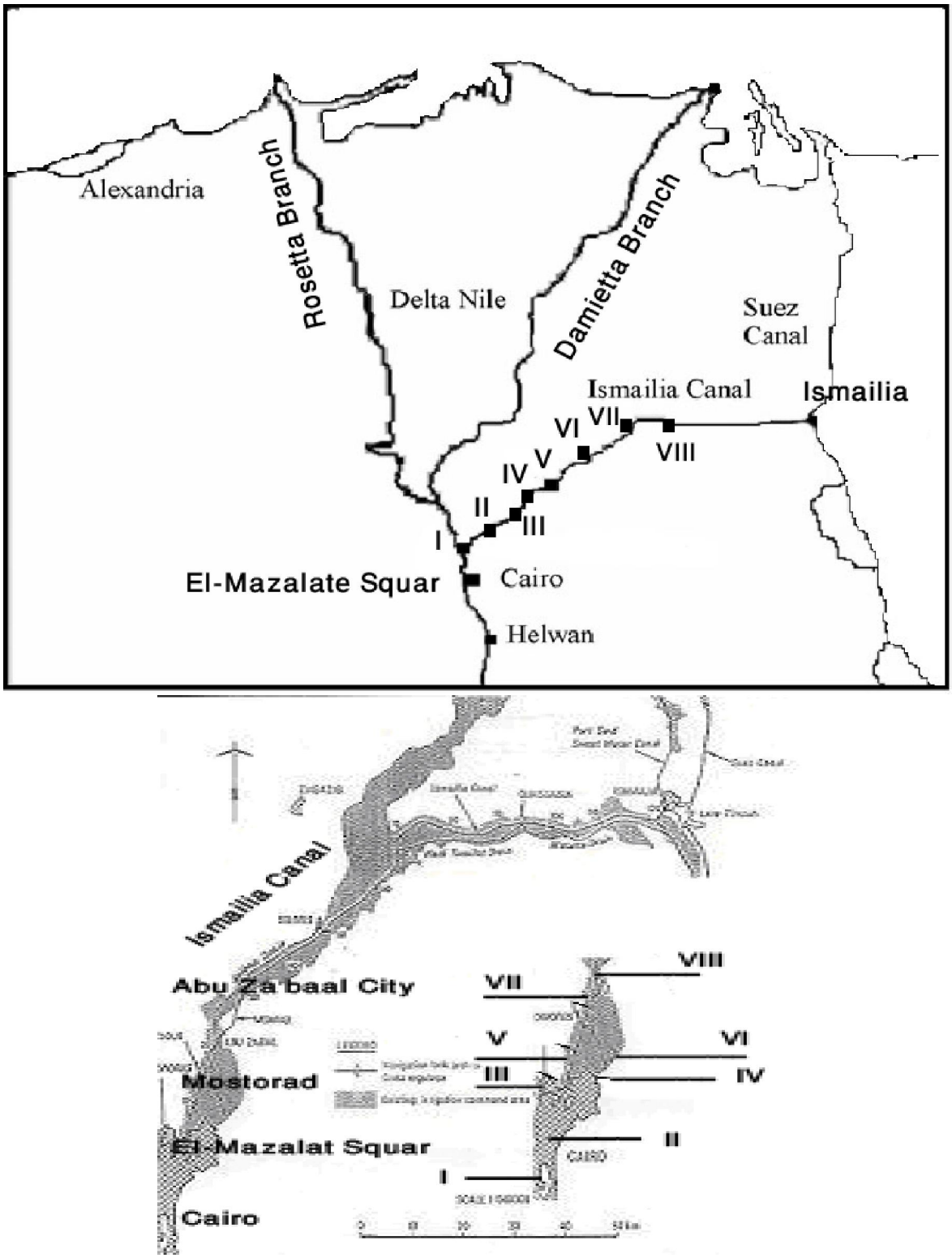

Fig. (1): The sampling locations of Ismailia Canal.

Herbal study:

Plant extracts from some herbs were tested against the most resistant isolates for the detection of antibacterial activity of these extracts. The plants used are; Dill (*Anethum graveolens*), Parsley (*Petroselinum crispum*), Peppermint (*Menthax piperta*), Cinnamon (*Cinnamomum verum*), Anise (*Pimpinella anisum*), Hibiscus, ginger, Absinth , Coriandrum, Black Pepper, Chamomile, Nigella, Licorice, Sage, Rosemary. The extraction method was carried out by grinding the plant well using electrical blender, add 20 ml pure ethyl alcohol on 5 gm of the plant (except for; Sage, Chamomile, Rosemary add 50 ml of ethyl alcohol), soak for 2 hours with shaking and then filter the mixture by filter paper Watman No.1. and exposed for saturation with the extracts and let them to dry.

Three similar colonies were transferred to 4 - 5 ml of Müeller Hinton broth. The culture was incubated at 35 ^{0}C for 2 - 6 hours to develop a turbidity of approximately 1 to 2 x 10^8 MPN/ml. Müeller Hinton agar plates were incubated with the organism grown in the broth by streaking with sterile swab, and the plates were left for 15 minutes to allow the complete absorption for the incubation before applying the plant extracts discs. The plates were incubated for 16 - 18 hours. Clear zone around the disc indicates that the plant has antibacterial activity, otherwise it has no effect.

Statistical analysis:-

Correlation coefficient "r" between physico-chemical, bacteriological as well as between investigated bacteria and hydrological parameters was calculated for testing the relationships between variables using Microsoft Excel (2003).

3. Results

Variations of physico-chemical characteristics of Ismailia Canal water during summer, autumn, winter and spring 2005 were shown in Tables (1 – 4).

Air and water temperatures were found to be 30.5 - 33.9 , 26.5 - 35.4 , 19 - 23 , 19 – 27, 18 - 19.5 , 17.5 – 29, 23.8 - 30 and 23.8 – 32.4 ^{0}C during summer, autumn, spring and winter seasons respectively.

Water transparency ranged between 65 - 130, 50 - 125, 70 - 140 and 60 - 100 cm during summer, autumn, winter and spring seasons respectively. The highest value reached to 140 cm at station II during winter while the lowest value was 50 cm at station VIII during autumn.

Seasonal variations in EC values fluctuated between 264 - 412, 311 - 464, 284 - 407 and 291 - 510 µmohs/cm during summer, autumn, winter and spring seasons respectively. The highest value was recorded at station VIII in autumn, where the lowest value at station II in summer.

Seasonal variations in DO concentrations fluctuated between 9.6 -12.6, 9.2 - 13.6, 8.4 - 12.8 and 9.2 - 11.8 mg/l during summer, autumn, winter and spring seasons respectively. The highest value (13.6 mg/l) was observed at station VII and the lowest value (9.2 mg/l) at stations II and VIII.

Seasonal variations in BOD concentrations were found to be 0.8 – 6.0, 1.0 – 4.4, 1.2 - 5.2 and 1.0 – 3.9 mg/l during summer, autumn, winter and spring seasons respectively. The minimum value was 0.8 and the maximum was 6.0 mg/l at stations II and VI respectively during summer.

Seasonal variations in COD concentrations fluctuated between 4.2 - 27.4, 6.7 - 33.5, 8.4 - 35.6 and 6.6 - 34.9 mg/l during summer, autumn, winter and spring seasons respectively. The maximum value was 35.6 mg/l at station VIII and the minimum was 4.2 mg/l at station II.

Seasonal variations in pH values were fluctuated between 7.7 - 8.17, 7.84 - 8.16, 7.81- 8.12 and 7.8 - 8.15 during summer, autumn, winter and spring seasons respectively. The highest and lowest values were recorded in summer at station V and station II respectively.

The carbonate concentrations were not detected during summer and spring seasons. The ranges of CO_3^{--} during autumn and winter were found to be 3.8 -15.0 and 1 - 4 mg/l respectively. The maximum and minimum values 15 & 1 mg/l were recorded at stations VIII and III respectively.

The seasonal variations in HCO_3^{--} concentrations fluctuated between 116 - 251, 219 - 272, 186 - 208 and 211 - 245 mg/l during summer, autumn, winter and spring seasons respectively. The maximum and minimum values 272 and 166 mg/l at stations VI and VII during autumn and summer seasons respectively.

The seasonal variations in sulphate concentrations were found to be in the ranges of 21.8 - 36.7, 36.6 - 47.7, 31.3 - 42.1 and 21.6 - 43.0 mg/l during summer, autumn, winter and spring respectively. The lowest value reached was 21.6 mg/l at station IV in spring and the highest (47.7 mg/l) was recorded at station VIII during autumn.

Seasonal variations in nitrite concentrations fluctuated in the ranges of 5.1 - 14.38, 4.36 - 11.77, 6.30 - 12.14 and 2.36 - 11.24 µg/l during summer, autumn, winter and spring seasons respectively. The minimal value was recorded in spring at station II and was 2.36 µg/l, while the highest value was 14.38 µg/l at station V during summer.

Seasonal variations in nitrate concentrations were found to be 13.02 - 27.83, 18.02 - 29.0, 14.14 - 19.15 and 12.28 – 19.65 µg/l during summer,

autumn, winter and spring seasons respectively. The minimal value (13.02 µg/l) was found at station II and the maximal value (27.83 µg/l) at station VII during summer.

Seasonal variations in ammonia concentrations of the area under investigation fluctuated between 1.25 - 2.24, 0.67 - 1.64, 0.93 - 1.21 and 1.11 – 2.00 mg/l during summer, autumn, winter and spring seasons respectively. The highest concentration value was (2.24 mg/l) and the lowest was (0.67 mg/l) and were recorded at stations VI, VII during summer and autumn seasons respectively.

The seasonal variations in orthophosphate concentrations were found to be 18.4 - 57.2, 49.0-131.0, 62.3 - 128.8 and 30.0 - 100.0 µg/l during summer, autumn, winter and spring respectively. The minimal value (18.4 µg/l) was recorded in summer at station VI, and the maximal value (131.0 µg/l) at station VIII during autumn.

Bacterial investigations:

Fig. (2) showed that, the bacterial counts of the area under investigation at 22 ^{0}C ranged between 2.9 x10^6 - 78.2 x10^6 cfu/ml, 111.3 x 10^6 cfu/ml - 360 x 10^6 cfu/ml and 41.0 x 10^6 cfu/ml - 481.8 x 10^6 cfu/ml during summer, autumn, winter and spring respectively. The minimum count was 2.9 x10^6 cfu/ml at station II in summer, and the maximum count was 481.8 x10^6 cfu/ml in spring at station IV. Also, the bacterial counts at 37 ^{0}C ranged from 10 x10^6/ml to 75 x10^6 cfu/ml, 15.6 x10^6 cfu/ml - 280 x10^6 cfu/ml, 90 x10^6 cfu/ml - 620 x10^6 cfu/ml and between 24.9 x10^6 cfu/ml - 413 x10^6 cfu/ml during summer, autumn, winter and spring seasons respectively. The results also showed that the lowest count was recorded in summer at station II and was 10.0 x10^6 cfu/ml, while the highest count was 413.0 x10^6 cfu/ml in spring shown in Fig.(3).

Count of total coliforms by most probable number are illustrate in Fig. (4). Total coliforms densities varied between 7 – 110000, 93l – 110000, 4 - 110000 and 460 - 4300 MPN/100ml during summer, autumn, winter and spring seasons, respectively. The lowest count (4 MPN/100 ml) was recorded at station II in winter, and the highest count 11x10^4 MPN/100 ml at station VIII.

Seasonal variations in faecal coliforms of the area under investigation illustrated in Fig. (5). The ranges fluctuated between 3 - 46 x10^3, 0.0 - 3.9 x10^3, 0.0 - 7.5 x10^3 and 120 - 4.3 x10^3 MPN/100 ml during summer, autumn, winter and spring respectively. The minimum counts were recorded during autumn and winter, and the maximum counts during summer.

The most probable numbers of fecal streptococci are represented in Fig. (6). The ranges were found to be 0.0 - 460, 11 - 460, 3 - 210 and 7 - 120 MPN/100 ml during summer, autumn, winter and spring seasons respectively.

The counts of *P. aeruginosa* were represented in Fig. (7). The counts of *P aeruginosa* ranged between 0.0 - 93, 7 - 120, 0.0 - 53 and 6 - 93 MPN/100 ml during summer, autumn, winter and spring seasons respectively.

The results of salmonella–shigella bacteria are illustrated in Fig. (8). The S.S. bacterial counts ranged between 0.0 - 24, 0.0 - 8, 0.0 - 40 and 0.0 - 19 MPN/ml during summer, autumn, winter and spring seasons respectively.

Identification of most resistant isolates:

The most resistant isolates from the above antibiotic screening were characterized using Bergy's Manual. The results of characterization and identification declared that the isolated indicators of pollution are E. *coli, P. aeruginosa, S. faecium and S. choleraesuis* and the applied tests are represented in Tables (5, 6, 7 & 8).

Herbal extracts:

Table (9), represents, the applied herbal extracts against the identified pathogenic isolates. *P. aeruginosa* was sensitive to Coriandrum, Black Pepper, Chamomile, Nigella, Ginger, Sage and Rosemary. *E. coli* isolate was sensitive only against Cinnamon and Licorice extracts. Also, *S. faecium* isolate revealed sensitivity against Sage and Rosemary extracts. *Salmonella choleraesius* isolate was sensitive against Ginger and Licorice extracts. On the other hand dill, Absinth, Peppermint, Anise, Parsley and hibiscus extracts didn't affect any of the subjected isolates.

4. Discussion

The quality of drinking water has been decreased during this century due to discharge of wastewater into water resources as well as due to environmental pollutants. The major global health problems, cross adaptation of microbial population to structurally related chemicals, may play an important role in the practical development and application of bioremediation techniques (Liu and Jones, 1995).

The decrease or increase in water temperature of the canal depends mainly on the climatic conditions, sampling times, the number of sunshine hours and also affected by specific characteristics of water environment such as turbidity, wind force, plant cover and humidity (Mahmoud, 2002 and Tayel, 2002). Air and water temperatures showed positive correlation (r = 0.73) during different seasons, indicating the importance of the heat budget water canal. Also, there is a positive correlation between water temperature and total coliform, faecal coliform and *P. aeruginosa* (r = 0.44, 0.47 and 0.44 respectively). This indicats the

strong effect of water temperature on bacterial growth. These results are concordant with that reported by Sabae *et al.,* (2006). The relative increase in water temperature at station VIII during all seasons especially summer is due to the thermal pollution discharged to the canal from the petroleum companies located on the canal bank.

Water turbidity is caused by suspended matter such as clay, silt, finely divided organic and inorganic matters, soluble colored organic compounds, planktons and water microscopic organism. The clarity of a natural body of water is a major determinant of the condition and productivity of the sustain (APHA, 1998). The turbidity degree of stream water is an approximate measure of the intensity of the pollution (Siliem, 1995). The transparency lower values of recorded during spring may be due to the flourishing of phyto-zooplankton during this season, while the high values during winter may be related to the decrease in water level during drought period (Abdo, 1998). Lower transparency values during different seasons at station VIII were mainly attributed to the effluents discharged from petroleum companies into the canal water at this area.

EC measure of the ability of aqueous solution to carry electric current. Solutions of most inorganic compounds and more abundant ions have higher conductivity (APHA, 1995). The increase in EC values at station VIII reflects the strong effect of petroleum companies pipelines effluent discharge at this area. EC were positively correlated with different studied anions e.g. SO_4^{2-}, CO_3^{2-} and HCO_3^- (r = 0.44, 0.56 and 0.42 respectively). As well as with TVC at 37 ^{0}C, TC, *P. aeruginosa* (r = 0.45, 0.56 and 0.43 respectively). Our result is in accordance with that reported by Sabae *et al.,* (2006).

Hydrogen ion concentration (pH) is the master that controls all aquatic chemical and biological processes. Changes in pH values beyond the optimum range may affect microbial physiology (Hassanin, 2006). Also, the pH of natural waters affects biological and chemical reactions, control the solubility of metal ions, and affect natural aquatic life. The desirable pH for fresh - water aquatic life is in the range of 6.5 - 9.0, and 6.5 - 8.5 for aquatic life (Chin, 2000). As regard the pH values of Ismailia Canal water. (Tables 1 – 4) our results revealed that, the canal water were on the alkaline side during the investigation period (pH > 7). The pH values slightly fluctuated at most stations during different seasons. However, the seasonal variations in pH was mainly affected by temperature, carbonate and bicarbonate system, rather than the photo synthetic activity of the primary procedures (Ezz El-Din, 1990 and Abdo, 2005).

Dissolved oxygen is a very important factor to the aquatic organisms, because it affects their biological processes, respiration of animal and oxidation of the organic matter in water and sediments. In this latter process, complex organic substances are converted to simple dissolved inorganic salts which could be utilized by the micro - and macrophyte (Okbah and Tayel, 1999). Our results showed that, the Ismailia Canal water was oxygenated during all seasons, Tables (1 – 4). The highest values were recorded in both winter due to the decrease in water temperature, and spring, corresponding to the flourishing of phytoplankton (Anon, 2007). Generally, the DO at most stations of canal water was within normal guideline values cited by USEPA, (1999) for the protection of aquatic life [for warm water biota: early life stage = 6 mg/l, other life stages = 5.5 mg/l. For cold water biota: early life stages = 9.5 mg/l, other life stages = 6.5 mg/l].

The minimum value of BOD was at station II and this may be due to the treated water discharged in the canal in this area which decreases the bacterial load in water in this area. On the other hand, the maximal BOD concentrations frequently recorded at stations VI & VIII could be related to the high bacterial load in the water at VI opposite to the discharge of the water treatment of Al-Amyeria water purification plant. Also at station VIII could be due to the high organic matter discharged into the canal from petroleum companies and also due to the relative high temperatures which enhance the enumeration of bacteria. The higher regional average was in autumn and winter 3.3 mg/l and may be due to the increase in the dissolved oxygen content as a result of decreasing in water temperature, and this result is agreed with the results of Sabae *et al.,* (2006) on River Nile water.

The maximum values of COD were recorded during winter (Table 3) and was mainly related to drought period effect during this season (Abdo, 1998). Our maximum values 35.6 and 21.3 mg/l at stations VIII and VI respectively may be attributed to the effluents discharge of petroleum pipelines and Al-Amyeria water purification plant at these areas. These results are in accordance with those obtained by El-Haddad, (2005) on the same area. Correlation coefficient "r" between COD and TVC at 37 ^{0}C, TC, FC, FS and *P. aeruginosa* were (0.46, 0.57, 0.47, 0.49 and 0.57 respectively), these correlations revealed the important role played by organic matter in control of bacteriological parameters.

The absence carbonate during hot seasons, (Tables 1, 4) may be due to the flourishing of phytoplankton during these seasons (Abdo, 1998). On the other hand, the detection of CO_3^{2-} during cold

seasons, Tables (2,3) could be attributed to the decaying and decomposition of phytoplankton during drought period and liberation of CO_2 (El-Haddad, 2005).

The increase in bicarbonate concentrations during hot seasons, (Tables 1, 4) may be attributed to the fact that the increase in temperature accelerates the organic matter accessible to bacterial decomposition, where the HCO_3^- is the final product of this decomposition (Abdo, 2002). CO_3^{2-} and HCO_3^- were dependent on each other with an (r:0.7). Also, showed positive correlation with FS and *P. aeruginosa* (r = 0.61 and 0.50, respectively). This revealed the important role of CO_3^{2-} and HCO_3^- played in bacteriological assessment.

The relative increased in sulphate concentrations at all water stations during winter (Table 3) may be due to death and decomposition of aquatic microorganisms then oxidation of liberated sulphur into sulphate in presence of high dissolved oxygen concentration during drought period in this season. This results are coincident with those reported by Abdo, (1998) and El-Hadad, (2006) on the same area. The positive correlation "r" between bacteriological parameters e.g. TVC at 37 ^{0}C, TC, FS and *P. aeruginosa* with SO_4^{2-} (r = 0.43, 0.48, 0.47 and 0.41, respectively) showed the important role of sulphate concentration played in bacterial count in the area under investigation.

The results of NO_2^- concentrations (Tables 1 – 4) showed that NO_2^-decreased during hot seasons (summer – spring) 7.76 and 7.95 µg/l and increased during cold seasons (winter – autumn) 9.20 and 10.26 µg/l respectively. The increase in NO_2^- during cold seasons might be attributed to low consumption by phytoplankton as well as the oxidation of ammonia by nitrifying bacteria and biological nitrification (Rabeh, 2001). The relative increase in NO_2^- concentration at stations VI & VII during different seasons is not only related to the effluents discharge of Amyeria water purification plant and petroleum companies but also due to decomposition of organic matter present in waste water where nitrosomonas bacteria oxidize ammonia into nitrite (Mason, 1991).

NO_3^- concentrations were increased at all stations during autumn season and the regional value reached was 22.84 µg/l. This is mainly attributed to the leaching water effect during this season. Also, the high values of NO_3^- at stations VII & VIII are mainly attributed to the effluents discharge of petroleum companies at this area. These results are in accordance with that obtained by Abdo, (1998) and Sabae *et al.*, (2006) on the same area. The correlation was slightly positive between total coliform (TC), faecal coliform (FC) and *P. aeruginosa* with NO_3^- (r = 0.57, 0.50 and 0.57, respectively).

The average values of ammonia concentrations, (Tables 1 – 4) revealed that the minimal value in winter was 1.03 mg/l and the highest value was 1.94 mg/l in summer. This is due to the fact that the high temperature accelerates the reduction rate of nitrate into ammonia. The regional average showed that the lowest average value was 1.17 mg/l at station II and the highest was 1.66 mg/l at station VIII. This is due to the accumulations of organic matter in the sediment at the discharge of petroleum companies where the transformation process takes place in sediment causing an increase in ammonia (Kepinska & Wypch, 1990, Bolalak & Frankowaski, 2003 and Engy, 2005). The toxicity of ammonia is pH dependent, so the average values of pH in present study ranged from 7.93 to 8.05 and ammonia concentrations from 1.025 to 1.94 mg/l. Our results were found to be in agreement with normal limits guidelines (1.27 – 3.88 mg/l) at pH (8.0 – 8.1) cited by USEPA, (1999).

The cycling of phosphorus within lakes and rivers is a dynamic and complex process, involving adsorption and precipitation reactions, interchange with sediments and uptake by aquatic biota. PO_4^{3-} represents the major content of dissolved phosphorus in aquatic environment and the other inorganic phosphorus are not well soluble and their solubility is pH dependent (Broberg and Persson, 1988). The increase in PO_4^{3-} concentrations at most stations during cold seasons (Tables 2, 3) can be related to the complete mixing of the water column and more phosphorus release from the bottom sediment to water in presence of high dissolved oxygen as reported by Abdo, (2005). On the other hand, the decrease in PO_4^{3-} concentration during hot seasons, (Tables 1, 4) is probably due to the distinct drop in phytoplankton biomass, on which nutrient regeneration process depends (Lehman, 1980). The maximum values of PO_4^{3-} were recorded at station VIII e.g. 100, 30.6, 131 and 103.2 µg/l and could be attributed to the effluents discharged from petroleum companies at this area. Also, at station IV PO_4^{3-} concentrations were 86.6, 57.2, 104 and 128.8 µg/l and is mainly related to the domestic wastes discharged from the police company into Ismailia Canal water at this area. The significant positive correlation between bacteriological parameters e.g. TVC at 22 ^{0}C and 37 ^{0}C and PO_4^{3-} (r = 0.45 and 0.57, respectively) shows a good evidence to the strong relationship between PO_4^{3-} concentration and total bacterial count at the points discharge e.g. IV and VII.

The total bacterial counts showed minimal count of 35.8 x10^6 MPN/ml in summer and this is due

to flood period which dilutes the organic matter used as food for the bacteria, while maximal mean count was 251.7 $x10^6$ MPN/ml in spring. The regional averages ranged from 42.2 x 10^6 MPN/ml at station II to 229.8 $x10^6$ MPN/ml at station IV, this may be explained by the effect of domestic waste discharged from water station treatment. Our results agree with the results of Sabae and Rabeh (2006).

The highest values of total coliform bacteria were recorded in summer with seasonal mean value 16139 MPN/100 ml. In contrast with summer, spring showed the lower mean value of total coliforms 1529 MPN/100 ml, this might be attributed to the high temperature and the effluents discharge into the canal, this agrees with the results of Sabae and Rabeh (2006).

With respect to faecal coliform bacteria and faecal streptococci, station II recorded the minimal regional average value 61 MPN/100 ml, while station VIII recorded the maximal regional average value 15425 MPN/100 ml. The data showed that there is gradual increase in the bacterial indicators from up-to-down stream, which might be attributed to the domestic and agriculture effluents discharge into the canal, this agrees with the results of Sabae and Rabeh (2006). Also, the pathogenic bacteria (*P. aeruginosa* and *Salmonella- shigella*) increased from up-to-down stream, this increase might be due to the effect of the sewage, agricultural and industrial effluents discharged into the canal.

A lot of antibiotics of different families and combinations were used to carry out the assay. The inhibitory actions of the used antibiotics varied from one organism of the isolated genera to the other according to the mechanism of action of the antibiotic and the susceptibility of the isolated organism towards the antimicrobial agents.

Susceptibility of pathogenic bacteria to antibiotics is an important problem because of the diversity of resistant mutant among bacterial pathogens and is due to high rates of the prescriptions of antibiotics. The increased prescriptions of ampicillin has led to lowering its effect on pathogenic bacteria (Nascimento *et al.*, 2003 and Orrett, 2004).

Results of antibiotic combinations against isolates of *P. aeruginosa* are as follows; Ticarcillin/Clavulanic acid (75/10 µg/ml) has an effect on all isolates. Pipracillin/Tazobactam (100/10 µg/ml) has an effect on all isolates. Ceftazidime/Clavulanic acid (30/10 µg/ml) affected 16 isolates and ampicillin/Sulbactam (10/10 µg/ml) has an effect on 15 isolates, and our results are in line with the results of Hassanin, (2006).

Isolates of FS bacteria gave different responses against antibiotic mixes and our results showed that all FS bacterial isolates were sensitive to Ticarcillin/clavulanic acid and Pipracillin/Tazobactam at concentrations of (75/10 µg/ml) and (100/10 µg/ml) respectively. While 9 isolates were sensitive to Ceftazidime/Clavulanic acid (30/10 µg/ml), only one FS isolate was sensitive to Ampicillin/Sulbactam (10/10 µg/ml).

Isolates of FC bacteria gave different responses against antibiotic mixes and the results showed that all FC bacterial isolates were sensitive to Ticarcillin/clavulanic acid, Pipracillin/Tazobactam and Ceftazidine/Clavulanic acid at concentrations of (75/10 µg/ml), (100/10 µg/ml) and (30/10 µg/ml) respectively. However seven FC bacterial isolates were sensitive to Ampicillin/Sulbactam (10/10 µg/ml).

All pathogenic bacterial isolates which revealed resistance against most applied antibiotics were subjected to fifteen herbal extracts. The investigation was ended to the following result: *P. aeruginosa* was sensitive to Coriander and this is in line with the results of Ayfer and Ozlem, (2003), Chamomile, Nigella, Ginger, Sage, Rosemary and this also agrees with Tamara *et al.*, (2006), and Black Pepper and this agrees with the results of Mazia and Perween, (2006). *E. coli* isolate was sensitive only to Cinnamon and Licorice extracts this agrees with the results of Sema *et al.*, (2007), and Suree and Pana, (2005). While *S. faecium* isolate revealed sensitive to Sage and Rosemary extracts. *Salmonella choleraesius* isolate was sensitive to Ginger and Licorice extracts and this disagrees with the results of Suree and Pana, (2005) and Onyeagba *et al.*, (2004).

Conclusion

- The main pollution sources of Ismailia Canal were due to the domestic and effluents of police camp and petroleum companies. Therefore, the wastewater effluents should be treated before its drainage in the canal.
- Because of resistance of bacterial isolates against most applied antibiotics, the use of traditional antibiotics should be decreased.
- The expansion of using some herbal extracts is a recommended substitution to the traditional antibiotics.

Table (1): Variations of physico-chemical characteristics of Ismailia Canal water during summer 2005

Parameters \ Stations	I	II	III	IV	V	VI	VII	VIII	Regional Average
Air Temp. (^{0}C)	33.9	33.9	30.8	30.5	30.5	34.2	34.0	32.5	32.53
Water Temp.(^{0}C)	26.5	26.3	27.1	27.8	28.8	28.7	27.3	35.4	28.36
Trans. (cm)	120	130	90	85	95	75	85	65	93
EC (µmohs/cm)	324	264	317	295	337	320	367	412	329.5
pH	7.98	7.70	7.98	7.94	7.95	7.99	7.99	7.96	7.93
DO (mg/l)	10.2	10.2	9.6	10.4	11.0	11.4	12.6	10.2	10.70
BOD (mg/l)	1.3	0.8	2.6	3.2	2.8	6.0	4.7	4.4	3.22
COD (mg/l)	11.1	4.2	8.8	17.8	14.7	19.9	15.5	27.4	14.92
CO_3^{--} (mg/l)	ND	ND	ND	ND	ND	ND	ND	ND	ND
HCO_3^- (mg/l)	190	199	207	195	200	211	166	253	202
SO_4^{2-} (mg/l)	32.7	21.8	29.1	34.6	31.1	36.7	30	33.1	31.13
NO_2^- (µg/l)	7.19	5.10	8.06	6.54	14.38	9.15	4.8	7	7.76
NO_3^- (µg/l)	15.40	13.02	16.03	19.56	21.20	18.42	27.83	23.12	19.31
NH_3 (mg/l)	1.93	1.25	1.99	2.20	2.16	2.24	1.81	1.95	1.94
PO_4^{3-} (µg/l)	26.6	28.6	35.8	57.2	23.5	18.4	24.5	60.6	30.650

ND: Not Detected

Table (2): Variations of physico-chemical characteristics of Ismailia Canal water during autumn 2005

Parameters \ Stations	I	II	III	IV	V	VI	VII	VIII	Regional Average
Air Temp. (^{0}C)	21.0	21.8	20.5	19.0	21.0	20.5	21.6	23.0	21.05
Water Temp.(^{0}C)	19.0	19.5	18.5	18.8	18.0	17.9	17.6	27.0	19.53
Trans. (cm)	110	125	90	80	90	70	95	50	88.75
EC (µmohs/cm)	356	311	326	318	339	341	398	464	356.62
pH	8.15	7.92	8.10	8.16	7.94	8.15	8.00	7.84	8.03
DO (mg/l)	10.0	9.2	11.2	10.4	11.2	11.9	13.6	10.8	11.03
BOD (mg/l)	3.2	1.0	3.6	3.6	3.0	3.9	3.6	4.40	3.28
COD (mg/l)	10.8	6.7	11.0	17.6	16.1	21.3	18.1	32.5	16.88
CO_3^{--} (mg/l)	11.5	3.8	11.0	13.0	11.8	12.4	11.6	15.1	10.01
HCO_3^- (mg/l)	219	271	255	250	241	272	261	233	190.6
SO_4^{2-} (mg/l)	37.0	39.9	37.0	36.6	40.1	45.2	41.3	47.7	31.1
NO_2^- (µg/l)	9.80	4.36	11.65	10.24	11.11	11.37	11.80	11.77	10.26
NO_3^- (µg/l)	21.10	18.02	18.65	16.60	20.0	28.42	27.80	23.12	22.84
NH_3 (mg/l)	1.18	0.91	1.00	1.28	0.83	0.80	0.67	1.64	1.042
PO_4^{3-} (µg/l)	49	87	75	104	80	95	101	131	90.25

Table (3): Variations of physico-chemical characteristics of Ismailia Canal water during winter 2005

Parameters \ Stations	I	II	III	IV	V	VI	VII	VIII	Regional Average
Air Temp. (^{0}C)	18.0	18.3	19.2	19.5	19.0	18.6	18.3	19.0	18.73
Water Temp.(^{0}C)	17.0	17.2	16.5	17.0	17.5	18.5	18.5	29.0	18.9
Trans. (cm)	120	140	100	110	100	70	85	70	99.27
EC (µmohs/cm)	310	284	319	300	327	313	337	407	324.6
pH	8.12	8.06	8.08	8.00	8.07	8.10	7.98	7.81	8.03
DO (mg/l)	12.40	12.8	11.6	12.4	10.5	10.0	11.2	8.4	11.16
BOD (mg/l)	2.0	1.20	4.0	5.20	3.3	3.6	2.90	3.8	3.25
COD (mg/l)	18.9	8.40	9.8	17.3	12.7	20.4	20.5	35.6	18.00
CO_3^{--} (mg/l)	3.1	1.1	1.0	3.3	2.8	3.9	2.4	4.0	2.7
HCO_3^- (mg/l)	200	186	194	202	212	184	200	208	198.25
SO_4^{2-} (mg/l)	37.4	40.9	34.5	41.5	31.3	42.1	34.7	40.2	37.87
NO_2^- (µg/l)	9.10	6.30	7.40	10.5	9.32	10.0	9.53	12.14	9.30
NO_3^- (µg/l)	18.10	16.53	18.10	19.15	14.14	15.22	18.10	17.40	17.10
NH_3 (mg/l)	0.93	1.21	0.91	1.05	1.14	0.94	0.97	1.05	1.025
PO_4^{3-} (µg/l)	79.70	62.3	115.5	128.8	71.5	83.8	76.6	103.2	90.17

Table (4): Variations of physico-chemical characteristics of Ismailia Canal water during spring 2005

Parameters \ Stations	I	II	III	IV	V	VI	VII	VIII	Regional Average
Air Temp. (^{0}C)	23.8	27.0	30.0	30.0	24.0	25.0	27.0	26.0	26.6
Water Temp.(^{0}C)	23.9	23.8	23.40	24.0	24.0	25.0	24.6	32.4	25.13
Trans. (cm)	90.0	100	85	90	85	60	80	70	82.5
EC (µmohs/cm)	332	291	315	304	344	321	366	510	347.87
pH	8.11	8.06	8.10	8.10	8.15	8.00	8.10	7.80	8.05
DO (mg/l)	10.5	10.7	11.3	11.5	10.9	11.0	11.8	9.20	10.86
BOD (mg/l)	2.5	1.0	3.2	3.40	2.40	3.5	2.10	3.9	2.70
COD (mg/l)	13.4	6.6	8.7	11.5	10.2	18.3	16.70	34.9	15.0
CO_3^{--} (mg/l)	ND	ND	ND	ND	ND	ND	ND	ND	ND
HCO_3^- (mg/l)	225	220	238	242	229	231	245	211	230.75
SO_4^{2-} (mg/l)	34.3	24.9	21.6	27.20	29.6	38.30	30.6	43.0	31.20
NO_2^- (µg/l)	8.26	2.36	7.66	6.89	8.56	9.48	9.21	11.24	7.95
NO_3^- (µg/l)	16.25	15.36	19.45	12.28	18.96	19.41	20.60	19.65	17.75
NH_3 (mg/l)	1.11	1.32	1.26	1.63	1.69	1.79	1.81	2.00	1.58
PO_4^{3-} (µg/l)	55.2	30.0	60.0	86.6	71.6	80.9	61.6	100.0	67.41

ND: Not Detected

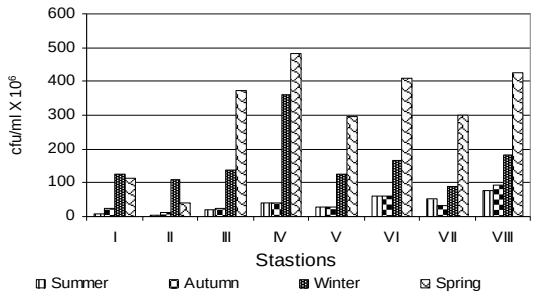

Fig. (2): Seasonal variations of bacterial count x10^6 cfu/ml in water of the area under investigation of Ismailia Canal at 22 ^{0}C.

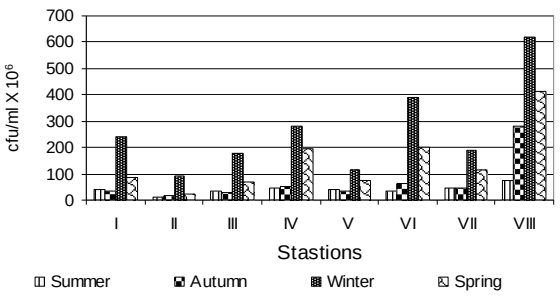

Fig. (3): Seasonal variations of bacterial count x10^6 cfu/ml in water of the area under investigation of Ismailia Canal at 37 ^{0}C.

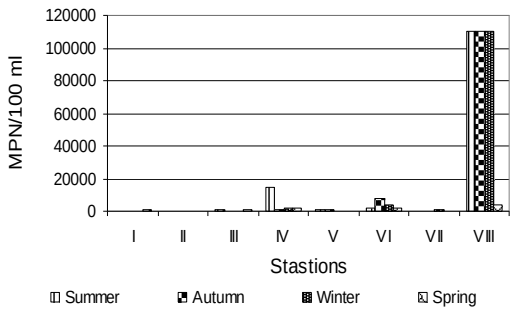

Fig. (4): Seasonal variations of total coliform bacteria MPN/100 ml in water of the area under investigation of Ismailia Canal.

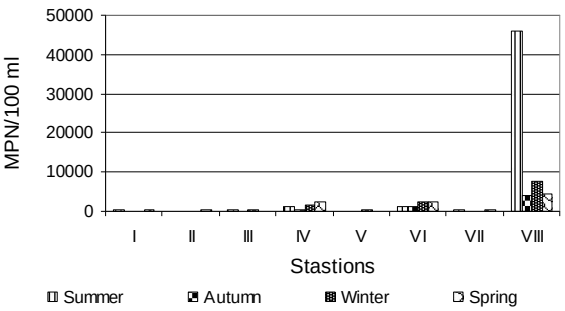

Fig. (5): Seasonal variations of fecal coliform bacteria MPN / 100 ml in water of the area under investigation of Ismailia Canal.

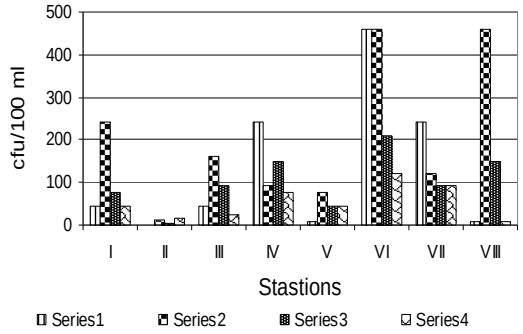

Fig. (6): Seasonal variations of fecal streptococci bacteria MPN / 100 ml in water of the area under investigation of Ismailia Canal.

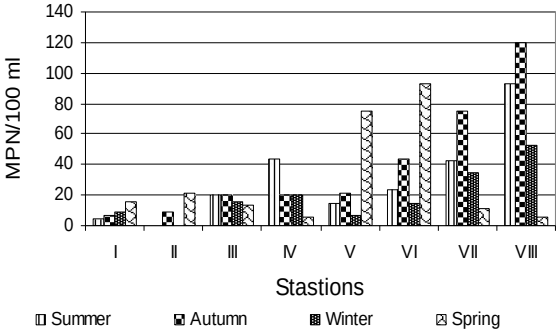

Fig. (7): Seasonal variations of *P. aeruginosa* bacteria MPN / 100 ml in water of the area under investigation of Ismailia Canal.

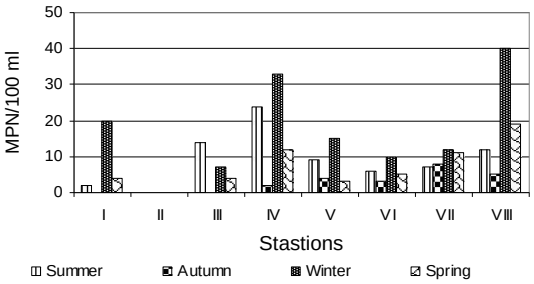

Fig. (8): Seasonal variations of *S. shigella* bacteria MPN/100 in water of the area under investigation of Ismailia Canal.

Table (5): Characteristic properties of isolate No. 56. (*E. coli*).

Characteristics	Results
Morphological characteristics	
Gram stain	-ve
Cell shape	Rods
Motility	+ve
Biochemical characteristics	
Catalase test	+ve
KOH reaction	+ve
Fermentation of	
* D-Manitol	+ve (acid & gas)
* D-Glucose	+ve (acid & gas)
* Lactose	+ve (acid & gas)
Oxidase test	-ve
Indole test	+ve
Methyl red test	+ve
Citrate utilization	-ve
Gelatin hydrolysis	-ve

Table (6): Characteristic properties of isolate No. 18 *(P. aeruginosa).*

Characteristics	Results
Morphological characteristics	
Gram stain	-ve
Cell shape	Rods
Motility	+ve
Biochemical characteristics	
Catalase test	+ve
KOH reaction	+ve
Fermentation of Lactose	-ve
H_2S production	+ve
Oxidase test	+ve
Production of diffusible pigment	+ve
Hydrolysis of Arginine	+ve
Growth on :	
* D-Glucose	-ve
* D-Manitol	-ve
Growth at :	
* 4 ^{0}C	-ve
* 41 ^{0}C	+ve

Table (7): Characteristic properties of isolate No. 43. *(Streptococcus faecium).*

Characteristics	Results
Morphological characteristics	
Gram stain	+ve
Cell shape	coccus
Endospore	-ve
Motility	-ve
Biochemical characteristics	
Catalase test	-ve
KOH reaction	-ve
Citrate utilization	-ve
Ammonia utilization	-ve
Growth in 6.5 % NaCl	-ve
Growth in Sodium azide 0.02%	+ve
Growth on :	
* Sucrose	-ve
* Lactose	+ve

Table (8): Characteristic properties of isolate No. 3. *(Salmonella choleraesuis).*

Characteristics	Results
Morphological characteristics	
Gram stain	-ve
Cell shape	Rods
Motility	+ve
Biochemical characteristics	
H_2S production	**+ve**
KOH reaction	**+ve**
Citrate utilization	**-ve**
Arginine hydrolysis	**+ve**
Growth in Sodium azide 0.02%	**+ve**
Growth on :	
* D-Manitol	**-ve**
* Maltose	**+ve**
* D-xylose	**+ve**

Table (9): Antibacterial activity of plant extracts against the identified isolates

S.	Plant	*E. coli*	*P. aeruginosa*	*S. faecium*	*S. choleraesius*
1	*Coriandrum sativum* (Coriander)	- Ve	+Ve	- Ve	- Ve
2	*P. aromatic* (Black Pepper)	- Ve	+Ve	- Ve	- Ve
3	*Anethum foeniculum* (Dill)	- Ve	- Ve	- Ve	- Ve
4	*Absinithium vulgare* (Absinth)	- Ve	- Ve	- Ve	- Ve
5	*Chamomilla officinalis* (Chamomile)	- Ve	+Ve	- Ve	- Ve
6	*M. piperita* (Peppermint)	- Ve	- Ve	- Ve	- Ve
7	*Grostemma githago* (Nigella)	- Ve	+Ve	- Ve	- Ve
8	*Amomum zingiber* (Ginger)	- Ve	+Ve	- Ve	+Ve
9	*Laurus cinamonum* (Cinnamon)	+Ve	- Ve	- Ve	- Ve
10	*Anisum vulgare* (Anise)	- Ve	- Ve	- Ve	- Ve
11	*Liquiritia offecinalis* (Licorice)	+Ve	- Ve	- Ve	+Ve
12	*Salvia triloba* (Sage)	- Ve	+Ve	+Ve	- Ve
13	*Rosmarinus offecinalis* (Rosemary)	- Ve	+Ve	+Ve	- Ve
14	*C. petroselinum* (Parsley)	- Ve	- Ve	- Ve	- Ve
15	*H . abelmoschus* (Hibiscus)	- Ve	- Ve	- Ve	- Ve

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New Synthesis of Furochromenyl Imidazo [2a-1b] Thiazole Derivatives, Studies on Their Antitumor Activities.

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Abstract 4, 9-Dimethoxy-5-oxo-5H-furo [3, 2-g] benzopyran-6-carboxaldehyde 1 was condensed with 2-thiox-4-imidazolinone 2 to form 3. Treatment of 3 with α-chloroacetyl chloride gave 4. Cyclization of 4 with acetic anhydride took place by heating to give 5. Condensation of 5 with aromatic aldehydes gave the arylidene derivatives 6a-c. Coupling of 5 with diazonium salts gave azo derivatives 7a-c. The work was further extended to investigate the behavior of 3 with 1, 2-dichloroethane to give (4Z)-2-(2-chloroethylthio)-4-((4, 9-dimethoxy-5-oxo-5H-furo [3, 2-g] chromen-6-yl) methylene)-1H-imidazol-5(4H)-one 8. Then 8 was cyclized with acetic anhydride to give (6Z)-2, 3-dihydro-6-[(4, 9-dimethoxy-5-oxo-5H-furo-[3, 2-g] chromen-6-yl) methylene] imidazo [2, 1-b] thiazol-5-(6H)-one 9. [Journal of American Science 2010; 6(5):251-256]. (ISSN: 1545-1003).

Key words: Furochromon; arylidene derivatives; azo; antitumor activity

1. Introduction

2-Thioxo-4-imidazolinone derivatives posses mathematical correlation of plasma levels of anticonvulsant drugs in epileptic patients which introduced in 1978 by Abarbanel [(1)] and use of it as antiasthmatic drugs [(2)]. On the other hand, furochromen and flavones were known to possess coronary dilator activities [(3)]. Some derivatives of furochromen composite for treating chronic skin or eye diseases which used in ophthalmic drugs and in dermatological diseases[(4)] Recently some furochromone derivatives showed potent antispasmodic , and antitumor activities [(5-10)].

A compound having both imidazolinone and furochromone moieties could expect to posses marked biological activities. This prompted us to design and synthesis new furochromen imidazo [2, 1-b]thiazole derivatives to study their antitumor activities.

2. Material and Methods

1-Chemistry

Experimental

All melting points were uncorrected. IR spectra recorded on a Pye Unicam SP- 1100 spectrophotometer using KBr discs. The ^{1}HNMR spectra were recorded on a Varian EM-390-90 MHz spectrometer using DMSO-d_6 as a solvent and TMS as an internal standard. Chemical shifts expressed as δ ppm units. The micro analytical Centre at Cairo University performed the microanalysis. The antitumor activity of the newly compounds were tested at Cancer Biology Department, National Cancer Institute Cairo, Egypt.

General procedure for preparation of 4 and 8

A solution of *(4Z)*-2-mercapto-4-[(4,9-dimethoxy-5-oxo-*5H*-furo-[3,2-*g*]chromen-6-yl)methylene]-1-*H*-imidazol-5*(4H)* one (3) (0.01 mole) in a mixture of 2% potassium hydroxide (56 ml) and absolute ethanol(40 ml) was added α-chloroacetyl chloride (0.01 mole) The reaction mixture was reflux on a steam bath for 3hrs then left to cool at room temperature. It acidified with dilute hydrochloric acid. The solid obtain was filtered off and crystallized from ethanol as yellow crystals of 4.

S-(4Z)-4, 5-dihydro-4-[(4, 9-dimethoxy-5-oxo-5H-fuoro [3, 2-g] chromen-6-yl) methylen]-5-oxo-1H-imidazol-2-yl-2-chloroethanethioate (4)

4 :m.p 261^{0}C yields 75%.

Analysis : $C_{19}H_{13}ClN_2O_7S$

Calculated :C, 50.84; H, 2.92; N, 6.24; S, 7. 14; Cl, 7.90

Found : C, 50.61; H, 2.73; N, 6.11; S, 7. 31; Cl, 8.01.

IR (Cm^{-1}): 3380 (NH); 1720 (ring C=O); 1690 (γ-pyrone C=O); 1640 (C=N).

MS : m,z 448,450

^{1}H NMR (DMSO-d_6) (δ ppm): 3..8, 3.7 (2s, 6H, 2OCH_3); 4.5 (s, 2H, CH_2); 6.3-6.8 (2s, 2H, 2CH=C); 8.0, 7.2 (2d, 2H, H-2, H-3 furan); 9.2 (s, 1H, NH exchangeable with D_2O).

(4Z)-2-(2-Chloroethylthio)-4-[(4, 9-dimethoxy-5-oxo-5H-furo [3, 2-g]chromen-6-yl) methylen]-1H-imidazol-5(4H)-one (8) crystallized from ethanol as a yellowish green *of 8.*

8 : m.p. 243^{0}C yield 65%.

Analysis : $C_{19}H_{15}ClN_2O_6S$

Calculate: C, 52.46; H, 4.39; N, 6.44; S, 7, 38; Cl, 8.16

Found : C, 52.23; H, 4, 12; N, 6.23; S, 7, 50; Cl, 8.45

IR (Cm^{-1}): 3440 (NH); 1740 (ring C=O); 1665 (γ-pyrone C=O); 1640 (C=N); 1243 (C-S).

MS : m/z 434, 436
^{1}H NMR (DMSO-d_6) (δ ppm) : 3.8, 3.9 (2s, 6H, 2OCH_3); 3.3 (t, CH_2-S); 4.1 (t, CH_2-Cl);
6.2, 7.1 (2s, 2H, 2CH=C); 8.1, 7.2 (2d, 2H, H-2, H-3 furan); 8.7 (s, 1H, NH exchangeable with D_2O).

General procedure for preparation of 5 and 9

A suspension of each of 4, 8 (0.01mole) in acetic anhydride (30 ml) was refluxed for 4hrs. The reaction mixture allowed cooling, and then the mixture poured into cold water. The product obtained was filtered off and crystallized from ethanol as yellowish green, dark brown crystals for 5 and 9 respectively.

(6Z)-6-[(4,9-dimethoxy-5-oxo-*5H*-furo[3,2-*g*]chromen-6-yl)methylen]imidazo [2,1-b]thiazol-2,5(*3H*,*6H*)-dione (5) crystallized from ethanol as a yellowish green of 5.

5 : m.p. 272^{o}C yield 70%.
Analysis : $C_{19}H_{12}N_2O_7S$
Calculate : C, 55.34; H, 2.93; N, 6.79; S, 7.78
Found : C, 55.60; H, 2.72; N, 6.54; S, 8.03
IR (Cm^{-1}): 1720-1710(two ring C=O); 1680 (γ -pyrone C=O); 1640 (C=N).
MS : m/z 434, 436
^{1}HNMR (DMSO-d_6)
(δppm):3.7,3.6(2s,6H,2OCH_3);4.1(s,2H,CH_2);6.4,6.7(2s,2H,2CH=C);7.1,7.9 (2d,2H,H-2,H-3furan)

(6Z)-2, 3-dihydro-6-([(4, 9-dimethoxy-5-oxo-*5H*-furo [3, 2-*g*]chromen-6-yl) methylen] imidazo[2,1-b]thiazol-5(*6H)*-one (9) crystallized from ethanol as dark brown of 9.

9 : m.p. 255^{o}C yield 75%.
Analysis : $C_{19}H_{14}N_2O_6S$
Calculated : C, 62.86; H, 3.07; N, 6.11; S, 7.00
Found : C, 63.01; H, 2.93; N, 5.89; S, 7.40
IR (Cm^{-1}) : 1736 (ring C=O); 1655 (γ -pyronC=O); 1648(C=N); 1253(C-S
MS : m/z 398
^{1}HNMR (DMSO-d_6)
(δppm):3.6,3.9(2s,6H,2OCH_3);3.2,3.7(2t,2CH_2);7.O,6.3 (2s, 2H, 2CH=C);8.1,7.2 (2d,2H,H-2,H-3 furan).

Reaction of (5) with aromatic aldehydes

A mixture of 5 (0.005 mole) fused sodium acetate (2.5g) and a slight excess of aromatic aldehyde (0.005 mole) benzaldehyde, chlorobenzaldehyde, bromobenzaldehyde in (25ml) glacial acetic acid were refluxed for 2 hrs. The reaction mixture poured over cooled water, and then separated solid filtered off washed with water and crystallized from acetic acid to give greenish yellow, olive green and dark brown for 6a-c respectively.

(3E,6Z)-3-benzylidene-6-[(4,9-dimethoxy-5-oxo-*5H*-furo[3,2-*g*]chromen-6-yl)methylen] imidazo[2,1-b]thiazol-2,5(*3H*,*6H*)-dione (6a) crystallized from acetic acid as greenish yellow of 6a.

6a : m.p. 281^{o}C yield 65%.
Analysis : $C_{26}H_{16}N_2O_7S$
Calculated : C, 62.34; H, 3.19; N, 5.59; S, 6.39
Found : C, 62.50; H, 3.12; N, 5.82; S, 6.25
IR (Cm^{-1}) : 1715, 1700 (two ring C=O); 1680 (γ -pyron C=O); 1635(C=N)
MS : m/z 500
^{1}HNMR(DMSO-d_6)(δppm):
3.8,3.6(2s,6H,2OCH_3);6.3,6.5,6.8(3s,3H,3CH=C);7.1,7.8(2d,2H,H- 2,H-3furan);7.3-7.6 (m,5H,aromatic protons).

(3E,6Z)-3-(7-chlorohepta-2,4,6-triynylidene)-6-[(4,9-dimethoxy-5-oxo-*5H*-furo [3,2-*g*]chromen-6-yl)methylen]imidazo[2,1-b]thiazol 2,5(*3H*,*6H*)-dione (6b) crystallized from acetic acid as olive green of 6b.

6b : m.p. 259^{o}C yield 70 %.
Analysis : $C_{26}H_{15}ClN_2O_7S$
Calculated :C, 58.38; H, 2.83; N, 5.24; S, 5.99; Cl, 6.63
Found : C, 58.21; H, 3.10; N, 5.41; S, 6.23; Cl, 6.42
IR (Cm^{-1}) : 1720, 1710 (two ring C=O); 1690 (γ -pyrone C=O); 1640 (C=N).
MS : m/z 534,536

(3E,6Z)-3-(7-bromohepta-2,4,6 triynylidene)-6-[(4,9-dimethoxy-5-oxo-*5H*-furo [3,2-*g*]chromen-6-yl)methylen]imidazo[2,1-b]thiazol 2,5(*3H*,*6H*)-dione (6c) crystallized from acetic acid as dark brown of 6c.

6c : m.p. 247^{o}C yield 75%
Analysis : $C_{26}H_{15}BrN_2O_7S$
Calculated :C, 53.90; H, 2.61; N, 4.84; S, 5.53; Br, 13.79
Found : C, 54.17; H, 2.86; N, 5.11; S, 5.81; Br, 13.53
IR (Cm^{-1}) : 1715, 1705 (two ring C=O); 1685 (γ -pyrone C=O); 1635(C=N).
MS : m/z 578,580

Reaction of 5 with diazotised aromatic amines:-

In a very cold condition, a solution of (0.01 mole) of the appropriate diazotised aromatic amines (prepared from the equivalent amounts of the amine, HCl and $NaNO_2$) was gradually added to a cold solution of 5 (0.01 mole) in aqueous sodium hydroxide solution (2%, 20 ml) in about 15 min. The reaction mixture kept in the ice-chest for 2hrs with constant stirring; the solid product collected by filtration, washed with water then crystallized from appropriate solvent to give reddish brown, dark brown and brown crystals of compounds 7a-c respectively.

(6Z)-3-(2-phenyldiazenyl)-6-[(4,9-dimethoxy-5-oxo-*5H*-furo[3,2-*g*]chromen-6-yl)methylen] imidazo[2,1-b]thiazol-2,5(*3H,6H*)-dione (7a) crystallized from acetone as reddish brown of 7a.

7a : m.p. 278°C yield 70%
Analysis : $C_{25}H_{16}N_4O_7S$
Calculated :C, 58.13; H, 3.12; N, 10.84; S, 6.21
Found : C, 57.91; H, 3.32; N, 11.00; S, 6.13.
IR (Cm^{-1}):3370 (NH); 1720,1700 (two rings C=O);1680 (γ - pyroneC=O);1635(C=N)
MS : m/z 516
^{1}HNMR(DMSO-d_6)(δppm) : 3.7, 3.6(2s,6H,2OCH_3 ; 6.4, 6.6(2s,2H,2CH=C);7.2, 7.7(2d,2H,H-2,H-3 furan); 7.3-7.6 (m,5H,aromatic protons and 12.4(s,1H,NH).

(6Z)-3-(2-(6-chlorohexa-1,3,5-triynyl)diazenyl)-6-[(4,9-dimethoxy-5-oxo-*5H*-furo[3,2-*g*]chromen-6-yl)methylen]imidazo[2,1-b]thiazol-2,5(*3H,6H*)-dione (7b)crystallized from chloroform as dark brown of 7b.

7b : m.p. 263°C yield 70%
Analysis: $C_{25}H_{15}ClN_4O_7S$
Calculated: C, 54.49; H, 2.72; Cl, 6.44; N, 10.17; S, 5.82.
Found : C, 54.27; H, 2.95; Cl, 6.2;.N, 10.34; S, 6.11.
IR (Cm^{-1}):3390 (NH); 1725,1710 (two rings C=O);1690 (γ pyroneC=O);(C=N)
MS : m/z 551,553

(6Z)-3-(2-(6-bromohexa-1,3,5-triynyl)diazenyl)-6-[(4,9-dimethoxy-5-oxo-*5H*- furo[3,2-*g*]chromen-6-yl)methylen]imidazo[2,1-b]thiazol-2,5(*3H,6H*)-dione (7c) crystallized from chloroform as brown of 7c.

7c : m.p. 262°C yield 75%
Analysis : $C_{25}H_{15}BrN_4O_7S$
Calculated: C, 50.41; H,2.54; Br,13.42; N,9.41; S,5.93
Found : C, 50.67; H,2.76; Br,13.16; N,9.59; S,5.63.
IR (Cm^{-1}) :3380 (NH); 1716, 1700 (two ring C=O); 1685 (γ -pyrone C=O); 1635 (C=N).
MS : m/z 595,593

2-Antitumor

Different concentration of the tested compounds between 1-10 μg/ml were added to the cell monolayer using SRB ASSAY (Sulfrohodamine B stain), and compared with the standard drug Doxorubicin DXR[19] using the method of Skehan et al[20].

The antitumor activity of the new formed compounds were tested at Cancer Biological Department, National Cancer Institute, Cairo, Egypt .

Results and Discussion

1-Chemistry

4,9-Dimethoxy-5-oxo-5H-furo[3,2-g]benzopyran-6-carboxaldehyde (1) [11] condensed with 2-thio-4-imidazolinone (2) [12] to give (4Z)-2-mercapto-4-[(4,9-dimethoxy-5-oxo-5-H-furo[3,2-g]chromen-6-yl)methylene]-1-H-imidazol-5-(4H)-one (3). The reaction product (3) was formed via the condensation of the formyl group of (1) with active methylene group of (2).

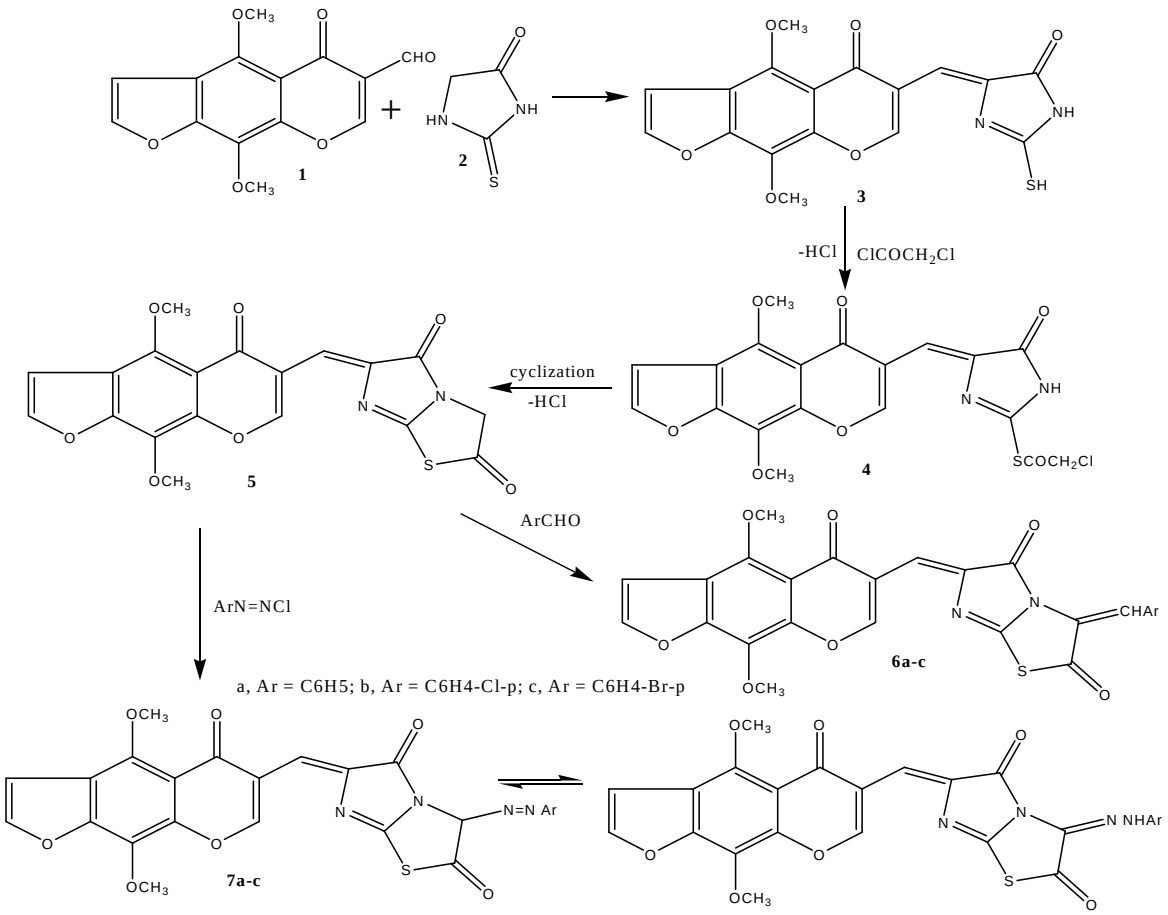
Treatment of (3) with α-chloroacetyl chloride gave S-(4Z)-4, 5-dihydro-4-[(4, 9-dimethoxy-5-oxo-5H-furo [3, 2-g] chromen-6-yl) methylene]-1H-imidazol-2-yl-2-chloro-ethanethioate (4). Compound (4) confirmed by elemental analysis and spectral data.

When compound (4) was heated with acetic anhydride, cyclization took place and (6Z)-6-[(4,9-dimethoxy-5-oxo-5H-furo[3,2-g]chromen-6-yl)methylene]imidazo[2,1-b]thiazole-2,5(3H,6H)-dione (5) was obtained via loss of HCl, the IR and ^{1}H NMR spectrum of (5) was characterized by the absence of NH proton. The cyclic structure proposed for compound (5) was the favor one.

Moreover, compound (5) having an active methylene group adjacent to carbonyl group was condensed with aromatic aldehydes (benzaldehyde chlorobenzaldehyde, bromobenzabenzaldehyde in glacial acetic acid in presence of fused sodium acetate at 140°C to give (3E,6Z)-3-benzylidene)-6-[(4,9-dimethoxy-5-oxo-5H-furo[3,2-g]chromen-6-yl)methylene]imidazo[2,1-b]thiazole-2,5-(3H,6H)-dione derivatives (6a-c). The arylidene derivatives (6a-c) showed the correct analytical values, their UV absorption spectra were studied (6a) absorbed at 358 nm (ε= 1553). This absorption is compatible with the benzene ring conjugated with C=C bond which in turn is conjugated with carbonyl group[13] Exhibiting another property of active methylene groups. Compound (5) reacted in sodium hydroxide solution with aromatic diazonium compounds to give (3E,6Z)-3-(aryl-hydrazone)-6-[(4,9-dimethoxy-5-oxo-5H-furo[3,2-g]chromen-6-yl)methylene]imidazo[2,1-b]thia-zole-2,5(3H,6H)-dione derivatives (7a-c). However, it is generally assumed that hydrazon is the stable form, whenever, coupling occurs at a methylene carbon atom (Scheme 1).

Wlley and Jarboe[14] and Tanner[15] have presented the IR absorption data which corroborated the above view, and in addition the presence of maximum band at 410 nm in the UV spectrum of (7a) which proved that (7a) exists in the hydrazone form rather than the azo form [16,17] (Scheme 1).

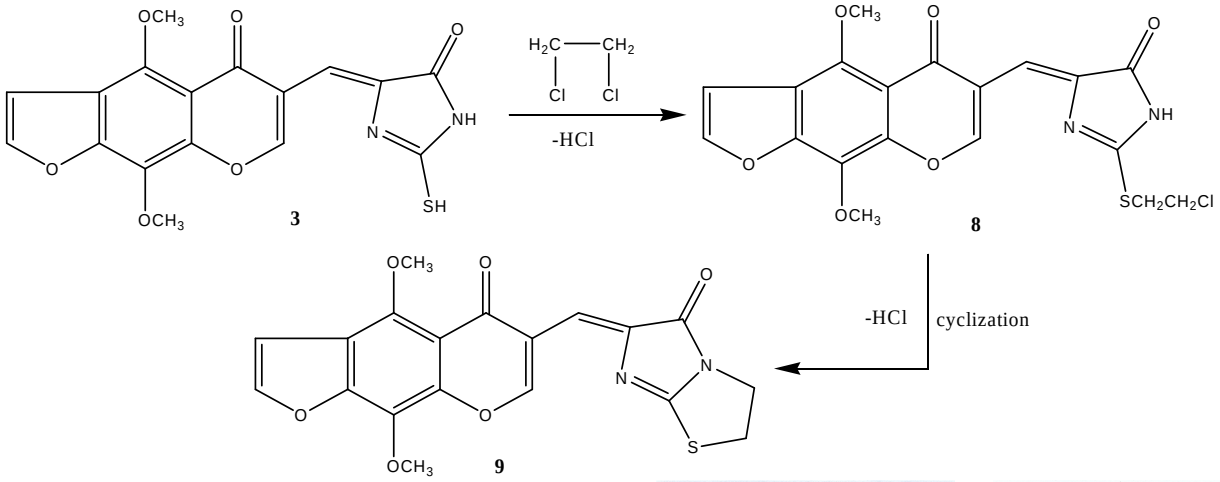
The work was further extended to investigate the behavior of 3 with 1,2-dichloroethane to give (4Z)-2-(2-chloroethylthio)-4-[(4,9-dimethoxy-5-oxo-5H-furo[3,2-g]chromen-6-yl)methylene]-1H-imidazol-5(4H)-one(8) which upon cyclization with acetic anhydride gave (6Z)-2,3-dihydro-6-[(4,9-dimethoxy-5-oxo-5H-furo[3,2-g]chromen-6-yl)methylene]imidazo[2,1-b]thiazol-5(6H)-one(9) by elemination of HCl (Scheme 2). The structure assigned for the cyclized product based on the correct analytical and spectral data. The ^{1}HNMR spectrum of the above compound revealed the absence of the NH group.



Azo-form Hydrazo-form

a,Ar=C_6H_5; b,Ar=C_6H_4-Cl-$_p$; c,Ar=C_6H_4-Br-$_p$

Scheme-1-



Scheme 2

Antitumor activity
The cytotoxic activity:

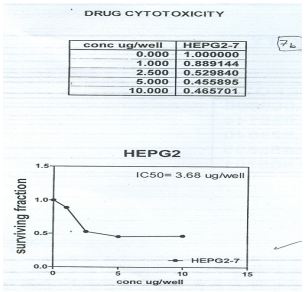
All the newly synthesized compounds were tested for their cytotoxic activity using tumor cell Lines [18] ,[HEPG2 (Human Liver Carcinoma Cell Line) and MCF7 (Human Breast Carcinoma Cell Line)].

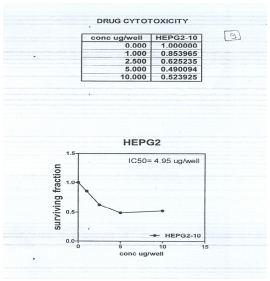
2-Antitumor

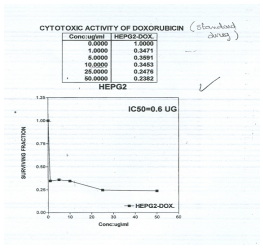
The cytotoxic activity of the tested compounds on HEPG2 and MCF7 were expressed as IC50, table (I), where IC50 (UM) is the dose of compound which reduces survival to 50%. The relation between the surviving fraction and drug concentration plotted to get the survival curve of the tumor cell line. The tested compound showed this activity only at the specified concentration and this cell lines.c.f.Table (I)

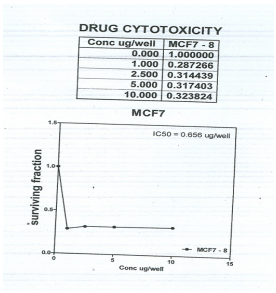
Table (I):

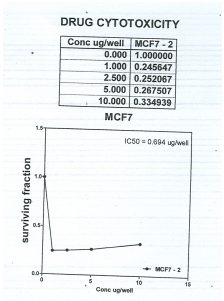
Compound No.	Cell lines	
	HEPG2 IC50	MCF7 IC50
1	-ve	0.769
2	-ve	0.694
3	-ve	0.731
4	-ve	0.769
5	-ve	0.694
6a	-ve	0.656
6b	-ve	0.806
7b	3.68	0.769
8	-ve	0.769
9	4.95	0.731

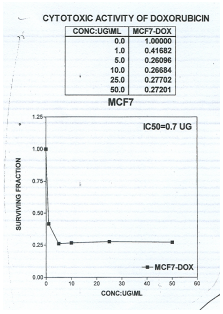












The standard curves for the most active compounds and the standard drugs Doxorubicin (DXR) are given below.

Conclusion:

All the tested compounds showed remarkable antitumor activity against human MCF7 cell line. Compound 6b was the most potent one comparing with the standard drug DXR.

The following compounds 6_a<2, 5<3, 9<1,4,7_b,8 showed varying activity in an increasing order.

When those compounds tested against human HEPG2 cell line, compounds 7_b, 9 showed moderate activity. Compounds 1, 2, 3, 4, 5, $6_{a,b}$, 8 has no activity, comparing with the standard drug DXR.

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3/5/2010

The effect of *Botrytis Cinerea* and *Rhizopus Stolonifer* on pre-harvest energy losses of strawberry production in Iran

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Abstract: It is well accepted that agricultural production must be increased considerably in the foreseeable future to meet the food and feed demands of a rising human population and increasing livestock production. Crop protection plays a key role in safeguarding crop productivity against competition from weeds, animal pests, pathogens and viruses. The aim of this study was to evaluate the amount of energy losses caused by pre-harvest strawberry losses in the Kurdistan province of Iran. These losses were caused by *Botrytis cinerea* (Gray Mold) and *Rhizopus stolonifer*. The average pre-harvest losses of strawberry production were found to be 6% in this study, thus the average losses were found to be about 544.3 kg ha^{-1}. The total energy losses of strawberry production in the study area are estimated to be 2.585 TJ. This amount of losses is equal to 422.5 BOE (Barrel of Oil Equivalent), also the total pre-harvest strawberry losses are equal to 1,673,412.3 $. Tools and techniques are needed to assist in developing strategies that can lead to higher food production, prevent crop production losses, and ensure minimal greenhouse gas emissions while maintaining soil fertility. [Journal of American Science 2010;6(5):257-260]. (ISSN: 1545-1003).

Key words: *Botrytis*; energy losses; Kurdistan; Iran; *Rhizopus*; strawberry

1. Introduction

The increasing world population has led to increased demand for food and reduced per capita availability of arable land and irrigation water. Compounding this problem is the fact that most farmers in the developing world own only small plots of land that have the potential to feed one family and generate income. Low soil fertility and crop losses from pests and droughts have reduced harvests to below subsistence levels (Vasil, 1998; Conway and Toenniessen, 2003). Small-scale farmers in developing countries are faced with many problems and constraints. Pre- and postharvest crop losses due to insects, diseases, weeds, and droughts result in low and fluctuating yields, as well as risks and fluctuations in incomes and food availability (Tonukari, and Omotor, 2010).

Significant to the goal of increasing productivity per unit area is the need to reduce crop losses occurring at different stages of production. Crop losses occur during the pre-harvest period mainly due to either the wrong application of cultivation techniques or natural factors such as frost, flood, plant diseases and pests. These are potential losses and generally are reported as low productivity (Tatlidil et al., 2005).

Assessments of crop losses despite actual crop protection strategies are required to demonstrate where action is needed and for decision making (Smith et al., 1984).

Most often, losses due to pests are the major limiting factor for sustaining the increase in crop productivity and production. On average, the avoidable crop losses caused by pests, such as insects, diseases, weeds and others in India, have been estimated to range from 10% to 30% of the total production. To keep pace with the demand for food commodities, adoption of appropriate strategies that include effective, economical, safe and environmentally sound plant protection technology in sustainable agriculture is a critical requirement (Chandurkar, 2001).

In the United States, the pre-harvest crop losses to pests including arthropods, weeds, diseases, and nematodes, were estimated to be about 37 % of the maximum potential yield (Pimentel et al. 1993).

When soil conditions are altered so that the overall soil community that buffers the ecosystem is influenced negatively, soilborne pests and pathogens proliferate and cause tremendous yield losses. To ensure long term sustainable, effective land use management is essential (Abawi and Widmer, 2000).

Strawberry is an important small fruit, grown throughout the world. It is deep red in color with unique shape and flavor. The major strawberry

producing countries of the world are USA, Spain, Japan, Poland, Korea and Russian Federation. The estimated production of strawberries in the world during 2007 was 5822 thousand tons (Sharma et al., 2009).

Losses can be categorized on the basis of cause into three classes: Mechanical damage, physiological damage (storage disorders), and biological damage (insect and pathogen diseases) (Ferguson et al., 1999). Biological damage is the most important portion of pre-harvest losses of strawberry production in Iran.

The most common decay of strawberry is Botrytis rot, also called Gray Mold, caused by *Botrytis cinerea* (Ceponis et al., 1987). The disease can begin pre-harvest, remaining as latent infections, or begin postharvest. This fungus continues to grow at 0 °C (32 °F). However, growth is slow at this temperature. Rhizopus rot caused by *Rhizopus stolonifer* is another important disease of strawberry. This fungus cannot grow at temperatures below 5 °C (41 °F) (Sommer et al., 1973). Depending on the cultivar, untreated strawberries can quickly become infected, damaging both yields and fruit quality. Of all the diseases, perhaps the most important is Botrytis fruit rot (Botrytis cinerea), causing pre-harvest losses of up to 15% of the fruit on susceptible cultivars (Legard and Chandler, 1998, 2000; Legard et al., 2000).

Crop diseases caused by fungi, bacteria, viruses, and plant parasitic nematodes inflict a significant amount of losses on crops. For instance, according to the field study by Holeta Agricultural Research Station (1986), losses on field crops ranged between 32-52%. Similarly the average loss on industrial crops ranged between 22 and 44%, and on horticultural crops ranged between 35 and 62% (Amera and Abate, 2008).

Life is a continuous process of energy conversion and transformation. The accomplishment of civilization has largely been accomplished due to the increasing efficient and extensive harnessing of various forms of energy to extend human capabilities and ingenuity. Energy is thus one of the indispensable factors for continuous development and economic growth (Mohamed et al., 2006).

In developing countries like Iran, agricultural growth is essential for fostering the economic development and meeting the ever-higher demands of the growing population. Energy in agriculture is important in terms of crop production and agro processing for value adding (Karimi et al. 2008).

Energy use in agriculture has been developed in response to increasing populations, limited supply of arable land and desire for an increasing standard of living. In all societies, these factors have encouraged an increase in energy inputs to maximize yields, minimize labor-intensive practices or both (Esengun et al. 2007).

The aim of this paper was to estimate the amount of energy losses caused by pre-harvest strawberry losses in the Kurdistan province of Iran. These losses were caused by *Botrytis cinerea* (Gray Mold) and *Rhizopus stolonifer*.

2. Materials and methods

In this study, the data were collected from 110 farmers in 13 villages growing strawberry in Kurdistan province, Iran by using a face-to-face questionnaire in August-September 2009. The province is located in the west of Iran, within 34° 44´–36° 30´ north latitude and 45° 31´–48° 16´ east longitude. The total area of the Kurdistan province is 2,820,300 ha. The average rainfall of the province is 450 millimeters (Salami et al., 2009).

The total land area cultivated for strawberry crop was 3800 ha in Iran and this amount was 2500 ha in Kurdistan province in 2007. In this year, the total production of strawberry was 38500 tones, while this amount was 30951 tones in Kurdistan province, thus about 80% of total strawberry production in Iran was obtained from Kurdistan province (FAO, 2007; Ministry of Jihad-e-Agriculture of Iran, 2007).

The amounts of strawberry losses were calculated per hectare and then, these data were multiplied with the coefficient of energy equivalent. Energy equivalent of strawberry is equal to 1.9 MJ kg^{-1} (Singh and Mittal, 1992).

3. Results and discussion

The average annual yield of strawberry farms was 9071.6 kg ha^{-1} in the study area. The average pre-harvest losses of strawberry production were found to be 6% in this study, thus the average losses is about 544.3 kg ha^{-1}. The total land area cultivated for strawberry crop in Kurdistan province is 2500 ha, so the total pre-harvest strawberry losses in Kurdistan province are evaluated as 1360.75 ton. As the energy equivalent of strawberry is equal to 1.9 MJ kg^{-1}, thus the total energy losses of strawberry production in Kurdistan province are estimated to be 2.585 TJ. This amount of losses is equal to 422.5 BOE (Barrel of Oil Equivalent). Also the total pre-harvest strawberry losses are equal to 1,673,412.3 $.

Oerke and Dehne mentioned the estimates of the worldwide loss potential of fungal and bacterial pathogens in wheat, rice, maize, barley, potatoes, soybeans, sugar beet, and cotton totaled 16%, 16%, 11%, 15%, 22%, 11%, 14%, and 9%, respectively (Oerke and Dehne, 2004).

Van Leeuwen et al. evaluated the pre- and post-harvest yield losses caused by *M. fructigena* in two susceptible apple cultivars. They notified that the final pre-harvest yield losses in both apple cultivars ranged from 2.7% to 4.4% over both years. In conclusion, yield loss caused by *M. fructigena* did not exceed 5% in the pre-harvest stage (Van Leeuwen et al., 2000).

Total crop loss to tomato farmers in the Ayaş district represented 25.92% of production; whereas in the Nallihan district it represented 27.51%.The breakdown of this total crop loss for Ayaş involved 14.78% loss during the seedling period, 5.99% during the field production period, and 5.15% during the harvest period. The breakdown for Nallihan was 12.76% for the seedling period, 4.92% for the field production period, and 9.83% for the harvest period, with the harvest period breakdown being 4.44% represented by fruit cracking, 2.9% by fruit rotting, and 2.49% by sun scalding (Tatlidil et al., 2005).

This study evaluated the amount of energy losses caused by pre-harvest strawberry losses. It seems that the amount of post-harvest strawberry losses is much higher than pre-harvest losses, thus another study is needed to determine the energy losses caused by post-harvest strawberry losses.

4. Conclusions

The average pre-harvest losses of strawberry production were found to be 6% in this study. As the average annual yield of strawberry farms was 9071.6 kg ha^{-1}, thus the average losses are about 544.3 kg ha^{-1}. As the total land area cultivated for strawberry crop is 2500 ha in the study area, so the total pre-harvest strawberry losses are evaluated as 1360.75 ton, thus the total energy losses of strawberry production in Kurdistan province are estimated to be 2.585 TJ. This amount of losses is equal to 422.5 BOE (Barrel of Oil Equivalent). Also the total pre-harvest strawberry losses are equal to 1,673,412.3 $.

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3/27/2010

Acid Washing Of "Zeolite A": Performance Assessment And Optimization

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Abstract Zeolite A has been developed, characterized and tested for the removal of chromium (III) from solutions. To facilitate the industrial manufacture of zeolite A, numerous experimental trials and testing procedures have been undertaken to develop prediction methodology for the synthesis of zeolite A with specified characteristics. However, conditioning of the prepared zeolite to the required pH and purity necessitated extensive washing cycles and time. In this paper an approach has been developed, through modeling and optimization techniques, to predict the range of operating parameters governing the washing of zeolite A using oxalic acid and elucidate the mechanism governing the acid washing process in a batch stirred tank reactor. Several washing parameters have been addressed comprising acid gram equivalent, liquid to solid ratios, temperature and stirring speed. It is thus possible through this prediction methodology to define the conditions required for minimum washing costs and high chromium (III) uptake. Determination of the effectiveness factor indicates that the chemical reaction controls the washing rate. The results of this paper indicate that the amount of water required for washing decreased from 310 m^3 to 20 m^3 per ton zeolite A using oxalic acid, consequently the washing cost decreased by about 18%. The results of optimization indicate that the acid washed zeolite A was able to adsorb 179 mg Cr^{3+}/g as compared to 184 mg Cr^{3+}/g for the conventionally washed zeolite A. [Journal of American Science 2010; 6(5):261-271]. (ISSN: 1545-1003).

Key words: zeolite A, washing, prediction, optimization, chromium

1. Introduction

Zeolite A has been in environmental practice for decades as an effective tool for the removal of heavy metals. [1-5] With the rapid increase in demand, synthetic routes to zeolites synthesis have been widely explored [6-13] with the aim of setting cost effective approach for the preparation of tailored zeolite [14-19]. According to the conventional manufacturing processes, zeolite A is synthesized in an aqueous environment in presence of NaOH. For the structure of zeolite A to be stable and to be handled safely in several applications such as detergents and adsorbents for heavy metals, etc…, the excess alkalinity should be minimized or eliminated. [20-22]

According to the reported experience, this operation is normally performed by washing with demineralized water in a filter press or centrifuge until almost complete removal of NaOH.[23-25] There are many disadvantages of this procedure, for example, the excessive amount of wash water used and the intensive energy requirements in the filtration and/ or centrifugation of the wash water which increase the cost of this separation process. Further, it is difficult to reach a pH value < 9.5 even by using excessive amounts of washing water. The recovery or treatment of large amounts of washing water represents another disadvantage. Moreover, after drying, aggregate powders having low apparent density, which are difficult to be handled, are obtained [20].

To improve these short comings several approaches have been reported. Prepared zeolite was separated from the mother liquor, without or by partial washing followed by neutralization of the residual NaOH in the cake by a suitable acid. This solution presents practical difficulties due to the extreme instability of the zeolite in a strong acid environment, with possibilities of dealumination and structure degradation. [20,22]

Acid conditioning has been attempted by different workers. N.G. Vasilev, et.al, used HCl acid treatment of zeolites NaX, NaY, mordenite Na(NaM), and natural aluminosilicate clinoptilolite (NaK). The results of this research confirm that during zeolite treatment by hydrochloric acid there is destruction of the aluminum-oxygen tetrahedra to yield aluminum ions and to form silanol groups. [22]

Sulphuric acid washing has been described in a Japanese patent[26]. The inventors concluded that this process requests substantially critical working conditions. Neutralization has been performed using a suitable fluidizer and sulphuric acid at a concentration greater than 70%. This process did not

give rise to significant alterations in the stability of the neutralized zeolite A.
Moreover, the use of tridecyl benzene sulphonic acid has proved to be very expensive, due to the very large amount of the acid requested for the neutralization step.[27]
In this paper a modified cost effective acid washing method has been developed for neutralization of zeolite A with significant reduction of wash water and complexation and precipitation removal mechanisms of chromium associated with highly alkaline zeolite A. [28]
Oxalic acid has been chosen being an organic weak acid to avoid the strong acidity and severity of acid treatment on zeolite structure. The formed soluble sodium oxalate salt is easily separated with the wash solution. In addition, sodium oxalate is considered as a good dispersant for silicates in solution which aids strongly in the homogeneity and the efficiency of acid washing at all zeolite washed sites. This avoids localization and accumulation of highly acidic sites which may cause structure damage.[20,22
This paper also addresses the development of an approach for the prediction of optimum zeolite A washing conditions required to fulfill high chromium uptake and minimum washing cost based on a model developed from the experimental data followed by optimization. Physical interpretation of the washing data is introduced through diffusion with chemical reaction models and calculation of Thiele modulus.[29,30]

2. Material and Methods

2.1 Materials

Synthesis materials comprise aluminum hydroxide (Panareac Quimicasa), sodium meta silicate (Arabic Laboratory Equipment Co. GPR), sodium hydroxide (Modern Lab) and oxalic acid dihydrate (POch S.A). Zeolite A has been synthesized following the predicted synthesis conditions and the starting molar ratios as described in previous work. [31] Briefly, the synthesis gel has been prepared by addition of the silicate solution to the aluminate solution at 65^{o}C, and mixing for 1 h at 500 rpm with seeding by a previously prepared zeolite of about 7.5 wt%, followed by crystallization for 2 h at 95^{o}C and 250 rpm.

2.2 Washing

Even though solids' washing was achieved by traditional cake washing and displacement techniques, this work introduces a new technique for washing, which is applied in a batch stirred tank reactor. A certain weight of zeolite cake has been placed in oxalic acid solution of varying composition which is expressed as acid gram equivalent AGE (0.8-1.2). One AGE was determined by using 4.6 ml of 1N oxalic acid dihydrate per gram of zeolite A to reach pH 8.5. The liquid to solid ratio (water ratio WR) was varied in the range (20-120). WR is defined as the water volume (ml) used to wash one gram zeolite A. The mixture was mixed using a mechanical stirrer (VELP Scientifica DLS) with stirring speed varying from 50 to 500 rpm and at temperature ranging of (22 – 60) ^{o}C. After the specified washing time (2 h), the solution has been separated by a centrifuge (Flyng Pharma Apparate TDL-5A). The samples were then dried and sieved.

2.3 Chromium adsorption

The adsorbent (0.1 g), with particle size <75 microns, was left in contact with 100 ml of the chromium sulphate solutions of 1000 ppm with an initial pH value of about 3. The experiments were carried out at room temperature for one hour under constant shaking. The filtered solution was then analyzed to determine the final chromium concentration using an atomic absorption flame spectrometer (GBC Avanta). The adsorption capacity is expressed as the amount of ions adsorbed per unit mass of adsorbent

2.4 Characterization

The chemical formula, phases formed, have been characterized by X-ray diffraction analysis (XRD) using a computer controlled X-ray diffractometer (made by Diano Corporation, USA) of a measuring range (2θ) from -20º to +150º. Target X-ray tube operated at 45 kV and 6 mA. The prepared samples have been also analyzed by X-ray fluorescence analysis (XRF) using AXIOS, WD-XRF Sequential Spectrophotometer (Panalytical, 2005) for determination of the Si/Al molar ratio. The FTIR spectra of washed zeolite samples was recorded using FTIR spectrophotometer (FT/IR-6100 type A) between 400-1600 cm^{-1}). Zeolite surface morphology has been determined using Scanning Electron Microscope (SEM) images Model JEOL: JXA-840A Electron Probe Micro-analyzer coupled with Energy Dispersive Analysis by X-ray (EDEX). All samples were gold coated prior to measurement. The particle size distribution was determined with LASER scattering particle size distribution analyzer (Horiba LA 950). Zeta potential has been used to characterize the zeolite used at various pH conditions. It was measured using a laser zetameter, Malvern instruments, (Zeta sizer 2000).

2.5 Theoretical analysis

2.5.1 Theoretical models & Optimization

The experimental results have been theoretically analyzed assuming diffusion with chemical reaction mechanism. It is assumed that the occurring reaction was a first order neutralization reaction between the sodium hydroxide which has been entrapped and the added oxalic acid. Calculation of the reaction rate constant and diffusion coefficient were necessary to estimate the value of Thiele modulus and effectiveness factor [29]. The rate equation for a first order reaction could be written as follows:

$$r = k[C - Ce] \quad (1)$$

Where r is expressed as (mol/sec. g zeolite), k represents the first order reaction rate constant (cm3/sec. g zeolite), C and Ce represent the hydrogen ion concentration at different times and at equilibrium respectively (mol/cm^3). The reaction rate constant was determined by parameter estimation using the first order reaction rate equation by firstly performing numerical integration of the rate equation for an initial assumption of the rate constant using the method of Runga-Kutta-Fehlberg [32]. The square of the difference between the calculated and the actual acid concentrations in the range of variation of time is then used as the objective function to calculate an improved value of the rate constant using Marquardt algorithm [33]. This procedure is iteratively repeated using tailored software until the value of the rate constant that provides acceptable error between the calculated and measured acid concentration is obtained. The diffusion coefficient, De (m^2/sec), can be estimated by the Wilke–Chang equation [34,35]

$$De=7.4E\text{-}15\frac{T(\xi M)\text{^}0.5}{\eta(\upsilon*10\text{^}6)\text{^}0.6} \quad (2)$$

Where T is the washing temperature (K), ζ represents the association factor of solvent (water), M represents the solvent's molecular weight, υ represents the solute (sodium hydroxide) molar volume (m^3/mol) and η represent the solvent dynamic viscosity (Pa sec). Thiele modulus was then calculated using the following equation:

$$\Phi = \frac{rs}{3}\sqrt{\frac{k\rho}{De}} \quad (3)$$

Where Φ is the dimensionless Thiele type modulus for spherical pellet, rs represents zeolite pellet diameter (m), ρ represents the zeolite density (g/cm^3) and De is the diffusion coefficient of sodium hydroxide from the zeolite pores out to the washing solution (m^2/sec). The effectiveness factor ή was then determined according to Smith. [29]

2.5.2 Empirical models formulation

Empirical models were formulated representing the effect of the different washing parameters on the final washing pH and on the chromium uptake. The experimental results have been correlated by applying relevant analysis methods, such as multiple non linear regression software and curve fitting to develop empirical models governing the washing of zeolite A. Typical software used for the purpose include Labfit (V.7.2.37) and Microsoft Excel.

2.5.3 Optimization

BOX Complex Routine [36] has been used to predict and define the optimum washing conditions required to fulfill minimum washing cost and high chromium uptake. This has been achieved through formulation of a cost objective function constrained with the formulated empirical equations relating the final washing pH and the chromium uploading to the washing conditions.

3. Results and Discussion

3.1 Characterization

3.1.1 XRD

Figure 1 represents a typical XRD chart for 3 of the prepared zeolite samples and acid washed at different conditions and a sample washed with water till pH 10.4. The XRD chart for the acid washed samples is typical of that of zeolite prepared via the prediction route and washed with distilled water till pH 10.4. The charts show sharp peaks of high intensity indicating highly crystalline and pure zeolite A with the following composition and characteristics: Product name: Na_2O. Al_2O_3. $2SiO_2$. $4.5(H_2O)$—Sodium aluminum silicate hydrate (Zeolite LTA), molecular weight: 2191.06System: cubic with unit cell parameter a=24.61.[37,38]

This indicates that the acid washed zeolite samples retains the intact zeolite A structure and the identified chemical structure as that of the samples washed with water until pH 10.4

3.1.2 XRF

XRF was also used to emphasize the performed zeolite structure. It resulted in Si/Al ratio of almost 1 for all the acid washed and the conventionally water washed samples, which agrees with the XRD identified Si/Al molar ratio.

3.1.3. Inrfa red spectrometry

Figure 2 displays the FTIR spectra of the washed zeolite samples between (400-1300 cm^{-1}) [6,37]. The IR spectra shows the structure sensitive bands, as follows: asymmetric stretch 995 cm^{-1}, symmetric stretch 600 cm^{-1}, double rings D4R 550 cm^{-1} which is characteristic of zeolite A [28,37] and T-O bend 464

cm^{-1} . The presented bands are typical for all washed zeolite A samples, which indicate that the zeolite structure remains intact even after acid washing.

3.1.4 SEM

The synthesized zeolite samples have been characterized by (SEM) as shown in figure 3 as typical for the washed samples at 1.2 AGE and 500 rpm (a, b, c) and (d) for water washed zeolite sample at room temperature. The SEM figures show that the highly crystalline small zeolite agglomerates formed have the size from a minimum of 0.2 to a maximum of 2 microns as compared to literature [4, 23]. This indicates that the acid washed zeolite samples retain the same surface morphology. It is clear that sample (1) has a wide particle size distribution, while the other samples have a narrow distribution.

3.1.5 Laser particle size analysis

The following table represents the particle size distribution for all the zeolite washed samples. It is clear that sample (1) has a wide particle size distribution. Also, sample 2 has narrower particle size distribution as indicated in the SEM.

Table (1) Laser particle size analysis of the washed zeolite samples

Sample	Mode size (µm)	Size range (µm)
1	10.1	0.9-101.5
2	4.5	1.2-13.2
3	10.1	1-29.1
4	5.5	1-20

3.1.6 Zeta potential

The zeta potential for the zeolite sample washed at 60°C, 80 WR, 500 rpm and 1.2 AGE, and the zeolite sample conventionally washed is manifested in figure (4). The point of zero charge (PZC) is almost the same (at pH 5.4) for both zeolite samples.

3.2 Effect of washing parameters on pH

3.2.1 Time dependence of solution pH at different acid concentrations

Time dependence of solution pH at different acid concentrations along the 2 hours washing period is represented in figure 5 as typical for all the other parameters.

A sharp fall in pH is observed after the first (5-10) minutes. A typical trend was also observed in the time dependence of solution pH relations at different water ratios, temperature and rpm. indicating rather rapid neutralization of entrained alkalinity with oxalic acid. It is assumed that the accessible alkali under the prevailing conditions has almost been entirely neutralized since minimal pH change has been observed after 24 h as shown in figure 6.

3.2.2 Effect of water ratio

Effect of water ratio (WR) on the final solution pH after 2 and 24 h is shown in figure 7. Inspection of the data shown in figure 7 reveals the almost insignificant change of the final pH for water ratio more than 20. These findings would help reducing the water ratio used to minimum.

3.2.3 Effect of temperature

Effect of temperature on the final solution pH after 2 and 24 h is shown in figure 8. Increasing the temperature of the acid washing solution reveals 2 distinct zones. The first is related to sharp linear decrease of pH up to 30°C. The second zone (segment) manifests moderate linear decrease of pH up to 60°C, the immediate implication of these results is that most of the unreacted alkali is neutralized in the moderate temperature zone (20-30°C). While the remainder is neutralized between 30-60°C. Thus, in industrial practices using washing solution of temperature 30°C is suffice to bring the final washing pH (after 2 h) from 8.5 to about 7.6. These findings also enable thorough monitoring of the final zeolite pH through simple, but, cost effective tools such as acid concentration, water ratio and temperature.

3.2.4 Effect of stirring speed

Effect of stirring rpm on the final solution pH after 2 and 24 h is shown in figure 9.The final pH is almost constant with the variation in the rpm after 2 and 24 h, which confirms as will be shown later, that the neutralization reaction is not diffusion controlled.

3.3 Performance analysis

Acid washed zeolite samples have been tested for the chromium removal. Figures 10(a-d) show the results of the adsorption tests for zeolites subjected to acid wash under the specified conditions.

Figure 10a manifests a rapid fall of chromium loading from 0.8 to 0.9 AGE followed by almost restoration of a high loading capacity in the range of 0.9 to 1.2 AGE The first segment reflects the effect of pH on the surface of zeolite which decreases the extent of complexation mechanism. Further decrease of pH leads to probable improvement of access to zeolite pores and cavities with subsequent improvement of loading capacity. The value of 1.2 AGE was been chosen to be the appropriate acid gram equivalent one.

Figure 10b manifests an increase of chromium loading from 165 mg/g at 20 WR to 183 mg/g at 60 WR. The chromium loading then decreases to 181

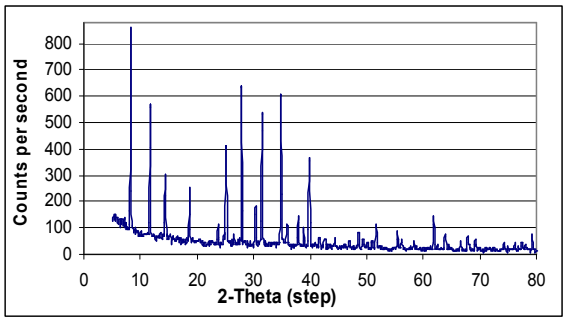


Fig. 1 XRD representing four zeolite samples: the acid washed zeolite samples sample 1 (20 WR, 22°C),sample 2 (80 WR, 60°C), sample 3 (80 WR, 22°C) at 1.2 AGE and 500rpm, sample 4 (water washed)

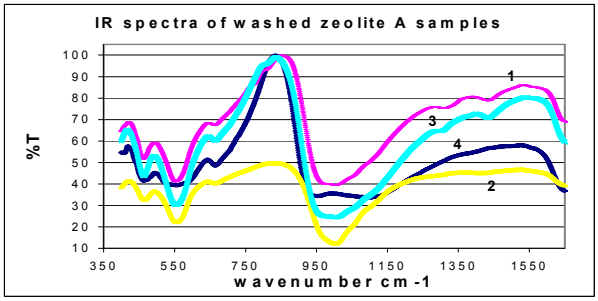
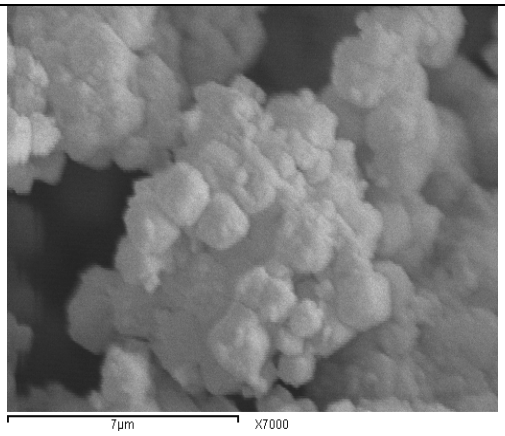
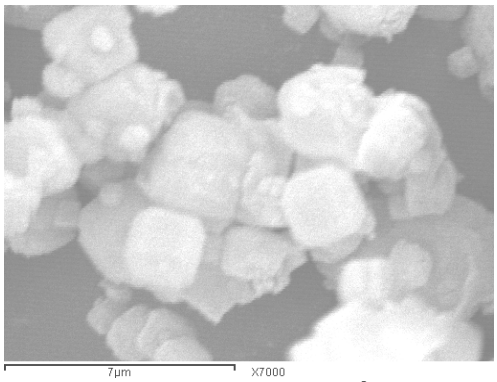


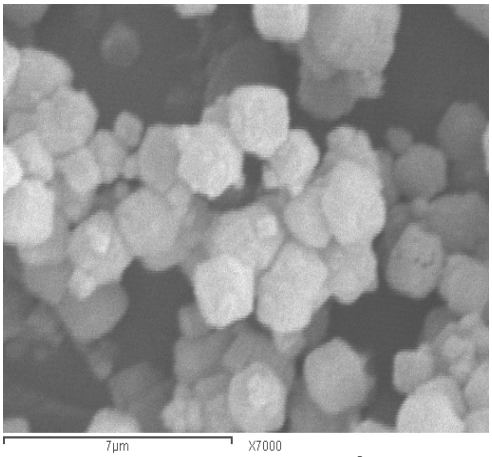
Fig. 2 IR spectra of washed zeolite samples: acid washed (1-3) and water washed (4)



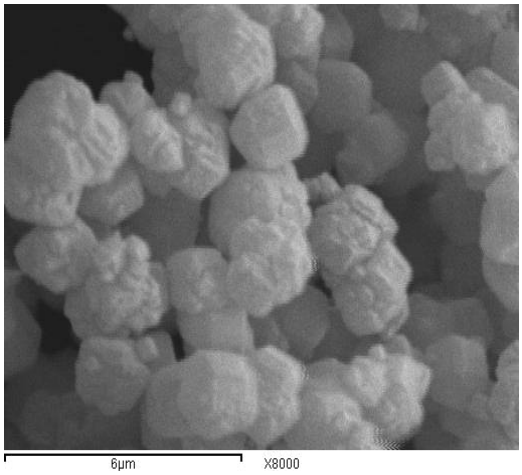
a) sample 1 (20 WR, 22°C)



b) sample 2 (80 WR, 60°C)



c) sample 3 (80 WR, 22°C)



d) sample 4 (not acid washed)

Fig. 3 SEM of the acid washed zeolite samples (a),(b),(c), and water washed sample (d)

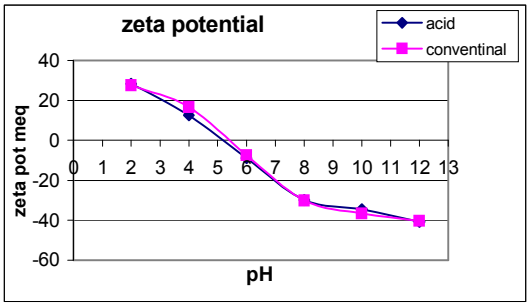


Fig4: Zeta potential measurement of zeolite washed samples

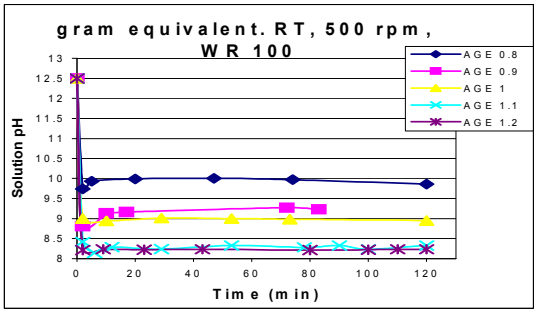


Fig.5: Time dependence of solution pH at different acid gram equivalents at room temperature, 500 rpm and 100 WR

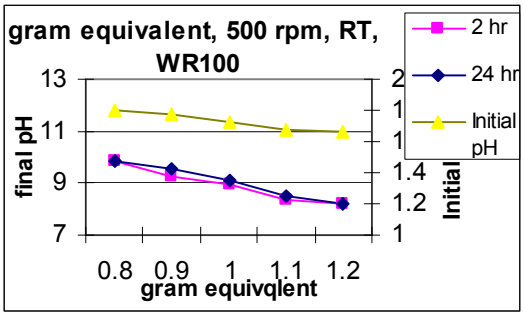


Fig.6: Effect of acid gram equivalent on the final washing pH after 2, 24 hr

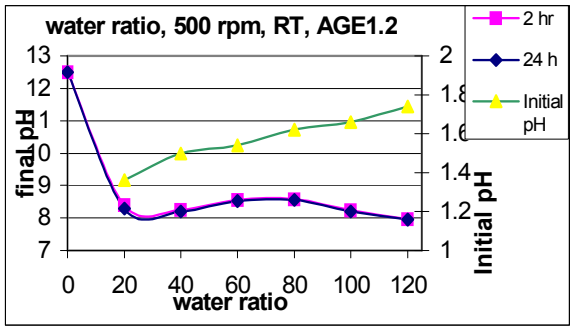


Fig.7: Effect water ratio on the final washing pH after 2, 24 hr

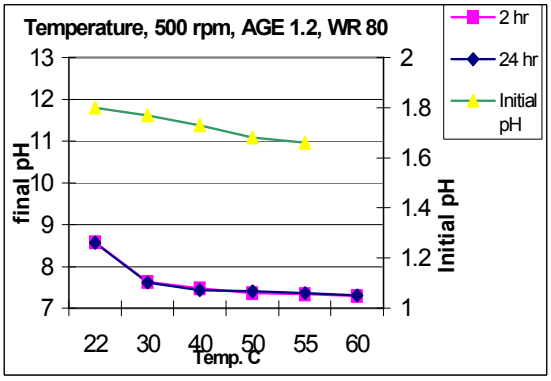


Fig.8: Effect temperature on the final washing pH after 2, 24 hr.

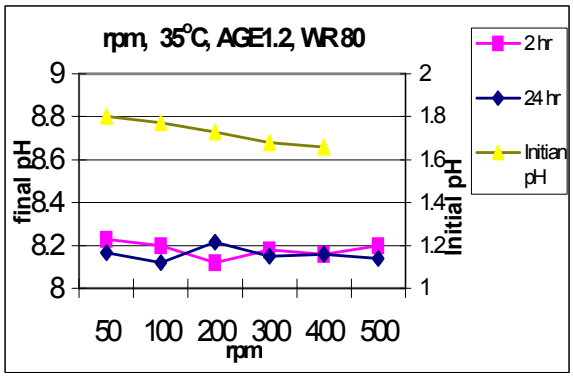


Fig.9: Effect stirring speed on the final washing pH after 2, 24 hr.

mg/g at 80 WR. The value of 80 WR has been chosen to be the most appropriate water ratio. The impact of water ratio is related to simultaneous pH changes and dilution effect. The minor inaccuracies associated with the sensitivity of measurements should be ruled out taking into consideration the narrow range of chromium loading variation (183-170 mg/g).

Figure 10c manifests the decrease of chromium loading from 25°C to 50°C by 7% then it remains almost constant till 60°C. This may be compared with the final pH curve, since the final pH shows moderate to minor decrease above 30°C. Temperature accelerates the alkali neutralization rate which increases the acid washing efficiency. The 7% decrease in the value of chromium loading may be considered minor indicating the limited effect of temperature on the washing process. The value of 35°C has been chosen to be the most appropriate washing temperature.

Table (2) Thiele modulus & effectiveness factor values

Parameter		Value
I. Input		
T	Temperature (K)	298
M	Water molecular weight (g/mol)	18
ζ	Water association factor	2.6
υ	Solute molar volume (m^3/mol)	20 E-06
rs	Zeolite pellet diameter (m)	2 E-06
ρ	Zeolite hydrated density (g/cm^3)	2
II. Calculated		
k	Reaction rate constant (cm^3/sec. g zeolite)	5.12 E-04
De	Diffusion coefficient (m^2/sec)	2.5 E-09
Φ	Thiele modulus	4.3 E-04
ή	Effectiveness factor	1

Figure 10d manifests the sharp increase of chromium loading from 166 mg/g at 50 rpm to 179 mg/g at 200 rpm followed by sharp decrease in the chromium loading for higher rpm. This may be attributed to increasing the ion mobility and homogeneity in mixed solutions up to 200. By increasing the rpm higher than 200 rpm, adsorption and desorption take place simultaneously reducing the over all chromium uptake. The corresponding final washing pH remains almost constant indicating the negligible effect of stirring on the final washing pH.

Figure 11 represents the over all effect of the final pH along the washing study on the chromium loading washing pH. The highest reported value of chromium loading was recorded at final pH value of 9.88. The effect of pH on the Cr(III) adsorption is quite complicated as mentioned by [39]. It was mentioned that the presence of chromium hydroxide complex is low up to pH 6 then it increases and then decreases again by increasing the pH more than 12. In this study it is worth mentioning that the starting pH of adsorption bulk solution was 3 and it ended at about 4.5-5, which eliminated the complexation precipitation removal mechanism. On the other hand, the actual pH inside the zeolite pores was that of the final washing pH resulting in different forms of chromium complexes other than in bulk solution and enhancing the complexation precipitation removal mechanism inside the pores. The water washed zeolite (sample 4) recorded 184 mg Cr/g zeolite adsorption capacity. It was found that the complexation mechanism has decreased by 14% for some of the acid washed samples.

3.4 Diffusion with chemical reaction

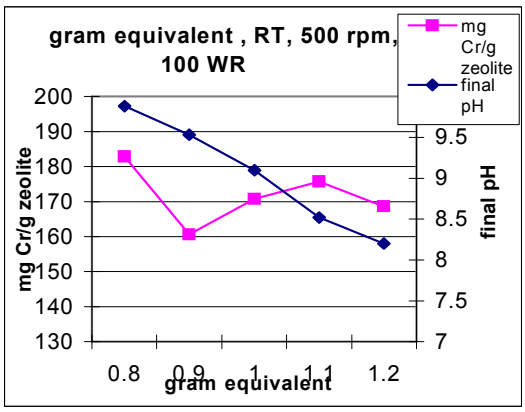
The diffusion with chemical reaction concept was applied to a chosen set of washing conditions as an example for the process. The operating conditions are 1.2 AGE, WR 80, 500 rpm stirring speed and 25°C temperature. The hydrogen ion concentration has been varied from 0.95E-13 to 2.7E-12 mol/cm^3 starting from 1 min washing time up to 34 min.. The parameters in the previous equations (1-3) were calculated considering a spherical zeolite pellet. The results are shown in the table (2).

The results manifest that the effectiveness factor is considered 1, since Thiele modulus value is less than 1 [29]. This indicates that the rate for the whole zeolite pellet is the same as the rate if all of the surface were available to reactant; i.e., the rate at the center is the same as the rate at the outer surface, consequently the entire surface is considered fully effective and the intraparticle diffusion resistance is minimum. Thus the intraparticle mass transport has no effect and the chemical reaction controls the washing rate. This indicates that the effect of the mixing speed on the washing process is limited for values above 200 rpm. This result was emphasized experimentally as demonstrated in figure (10d).

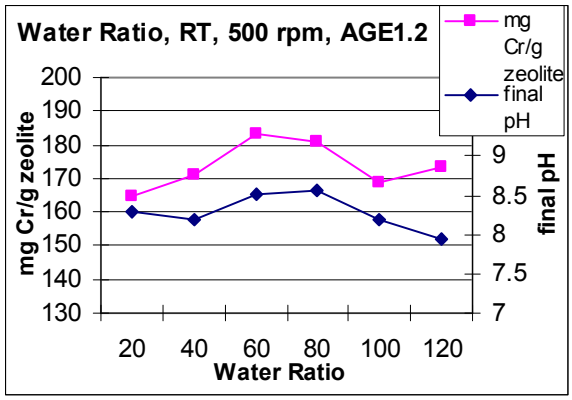
3.5 Empirical Modeling and optimization

Data analysis has resulted into the formulation of the following empirical equations governing the relationship between the final washing pH, chromium loading and the washing operating conditions and the with R^2 ranging from 0.9 to 1,

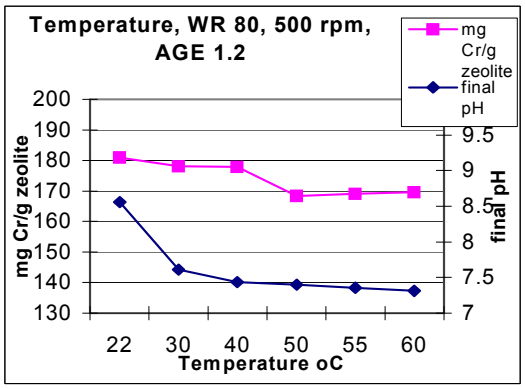
$$pH_F = (1.8637 - 0.9643\ AGE - 0.0626\ WR - 0.25\ T - 0.0649\ S)^{0.5} \quad (4)$$



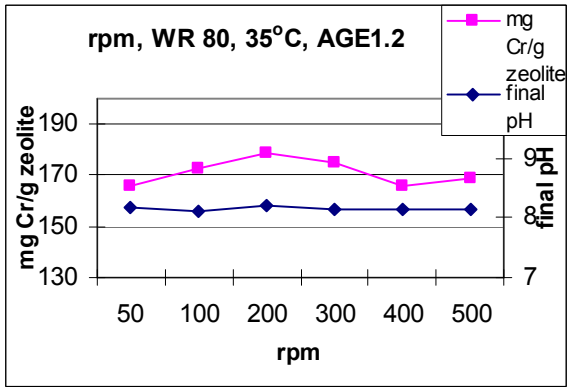
a)



b)



c)



d)

Fig.10: Chromium adsorption on acid washed zeolites: a) effect of acid gram equivalent, b) effect of water ratio, c) temperature dependence and d) influence of rpm

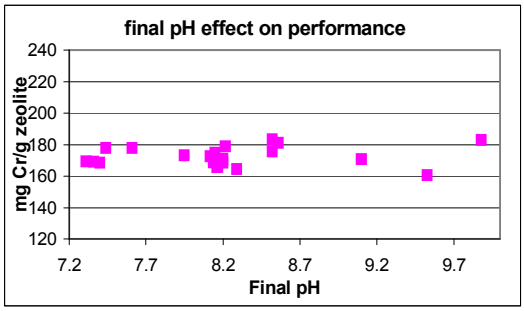


Fig 11: Effect of the final washing pH on the chromium adsorption capacity along the entire washing region

$$L = 30.18\ pH_F^5 - 1283.47\ pH_F^4 + 21771.52\ pH_F^3 - 184166.19\ pH_F^2 + 776905.33\ pH_F - 1307394.33 \quad (5)$$

BOX COMPLEX routine has been adopted to find the values of the washing conditions that would minimize the washing process costs while maintaining high zeolite chromium loading. This has been achieved through formulation of a cost objective function, equation (6), constrained by equation (4) starting with the pH_F value corresponding to maximum loading calculated from equation (5). Consequently, equations (4) have been used to calculate other parameters using the defined optimum for the independent variables. The objective function is expressed based on the oxalic acid price of 0.2 USD/kg, water price of 0.2 USD/m^3, and 0.08 USD/kWh:

$$C = 0.059\ AGE + 2E\text{-}04\ WR + (4.3E\text{-}04\ AGE + 9E\text{-}05\ WR)\ (T\text{-}22) \quad (6)$$

Results are presented in table (3)

Table (3) Predicted Washing Variables

PARAMETER		RANGE OF VALIDITY	PREDICTED VALUE
pH_F	Final washing pH	(7.31-9.88)	9.87
AGE	Oxalic acid gram equivalent/ g zeolite	(0.8-1.2)	0.8
WR	Water ratio ml/ g zeolite	(20-120)	20
T	Washing temperature (^oC)	(22-60)	22
S	Stirring rpm	(50-500)	50
L	Chromium loading mg Cr^{3+}/g zeolite	(160.6-183)	178.9
C	Washing cost USD/ kg zeolite		0.051
CT	Washing cost USD/ ton zeolite		51

Table (3) indicates that the oxalic acid demand is about 200 kg/ ton zeolite and the wash water demand was 20 m^3/ ton zeolite. The results show that 51 USD are needed for acid washing of one ton zeolite to a final pH of 9.88. On the other hand 62 USD, represent the price of water only (310 m^3/ ton zeolite), for washing of one ton zeolite with water to a pH of 10.4. The net savings are considered to be about 11 USD per ton of zeolite, in addition to other technical benefits as discussed in the previous sections.

4. Conclusions

Zeolite A washed by oxalic acid has been characterized by XRD, XRF, FTIR, SEM, laser particle size analysis and zeta potential measurements. Further, the adsorption capacity of chromium has been assessed The results indicate that the proposed acid washing scheme retained the intact zeolite structure and surface morphology and also high chromium loading as compared to zeolite washed with distilled water. The complexation effect decreased by 14%. The intraparticle mass transport has no effect and the chemical reaction controls the washing rate. This indicates that the effect of the mixing speed on the washing process could be considered negligible above 200 rpm. Based on the adopted optimization procedure, the amount of water required for washing has been decreased from about 310 m^3/ ton zeolite to reach pH value of 10.4 to about 20 m^3/ ton zeolite with the proposed acid washing scheme to reach pH value range of 9.87. This results in the decrease of the cost & energy requirements of washing process. Consequently the net savings in washing cost has also decreased by about 18% as compared to the conventional zeolite washing conditions. In addition, the formulated rational scheme has proved to be a powerful tool for the prediction of optimum zeolite A washing conditions to fulfil minimum washing cost and high chromium loading.

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Effect of Spearmint Essential Oil on Chemical Composition and Sensory Properties of White Cheese

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Abstract: Spearmint (*Mentha spicata*) the most common herb in the Mediterranean region, widely used as a source of essential oil for flavoring, spearmint essential oil contains about 24 constituents representing 98.45 % of the total essential oil; the main compounds are carvone (68.58%) and limonene (16.42 %). Two lipid model systems (DPPH scavenging activity and β-carotene bleaching test) were used to determine the antioxidant activity of spearmint essential oil. White cheeses with different concentrations of spearmint essential oil (0.5 to 2.5 ml/kg retentate) were prepared and stored at (7 ^{0}C ± 2) for 5 weeks. The chemical composition and ripening index of spearmint white cheese were determined. Obtained results showed that lower concentrations of spearmint essential oil increased titratable acidity values significantly, while ripening index was increased significantly by increasing the concentration of essential oil. Prolonging the cold storage period for five weeks increased these values significantly. Panel study showed that lower concentration of essential oil got the highest total acceptability scores. [Journal of American Science 2010; 6(5):272-279]. (ISSN: 1545-1003).

Key words: white cheese, spearmint, essential oil, sensory evaluation, antioxidant activity, ripening index

1. Introduction

Spearmint is an herbal plant that is found abundantly in the Mediterranean region. Its byproduct, spearmint oil, provides plenty of health benefits. Obtained from the spearmint leaves, this oil is well known for its medicinal properties. This minty oil is used to treat a variety of ailments. Spearmint oil is loaded with nutrients such as vitamin A and vitamin C, and has a sweet taste. The characteristic feature of this oil is its refreshing fragrance that provides therapeutic benefits when inhaled. There are numerous health benefits of spearmint essential oil such as it can reduce fever, provide relief from depression and asthma (El-Moghazy, 2008).

Recently, there is a growing interest in substances exhibiting antimicrobial and antioxidant properties that are supplied to human and animal organisms as food components or as specific pharmaceutics. It has been well-known that essential oils have antimicrobial and antioxidant effects (Özer *et al.,* 2007). In addition to, common need is availability of natural extracts with a pleasant taste or smell combined with a preservative action, aimed to avoid lipid deterioration, oxidation and spoilage by microorganisms (Sacchetti *et al.,* 2005). The most common herb in the Mediterranean region is spearmint (*Mentha spicata*), widely used as a source of essential oils for flavoring and recently has been used as a valuable source of the potent antioxidant for the nutraceuticals and cosmetic industries (Wang and Weller, 2006). Proteolysis and lipolysis are the main biochemical reaction in the development of flavour in cheese during the ripening. Exogeneous and endogeneous enzymes of microflora contribute to cheese proteolysis and lipolysis during processing and ripening (Cinbas and Kilic, 2006). Lipolysis is an important biochemical event occurring during cheese ripening and varied from cheese type to another and Free Fatty Acids (FFA) are important precursors of catabolic reactions, while produce compounds that are volatile and contribute to flavor (McSweeney and Sousa, 2000). Spearmint essential oil was analyzed previously by the authors, results showed that it contained about 24 constituents were identified representing 98.45 % of the total essential oil; the main compounds, which identified by GC-MS spectrometer, were carvone (68.58%) and limonene (16.42 %) (Foda *et al.,* 2009 b).

The objectives of the present study were: evaluation of spearmint essential oil, study its effect on the chemical composition and consumers acceptability when applied to white cheese.

2. Material and Methods

Dried spearmint (*Mintha spicata* L.) was obtained from Medicinal and Aromatic Plant Research Dept., Agriculture Research Center, Giza, Egypt. Buffalo's milk retentate was purchased from Dairy Industry Unit, Animal Production Research Institute, Ministry of Agriculture, Cairo, Egypt. The milk retentate

contained 64.0 % moisture content; 15.5 % fat; 10.5% total protein and 0.09% titrable acidity. Microbial rennet (*Mucor mehiei)* was obtained from Novo, Denmark.

2. 1 Extraction of the essential oil

Essential oil was extracted according to Tepe *et al.*, (2005), by submitting dried spearmint to water distillation for 3 hrs using a Clevengar-type apparatus. The obtained essential oil was dried over anhydrous sodium sulphate and after filtration stored at 4^{o}C for further analyzed. The yield of the essential oil was determined.

2.2 Evaluation of antioxidant activity:

Free radical scavenging activity was measured by 2,2-diphenyl-1 picryl-hydrazil (DPPH) according to the method which described by Tepe *et al.,* (2005). Essential oil concentration providing 50% inhibition (IC_{50}) was calculated using the graph by plotting inhibition percentage against extract concentration. Total phenolic compounds were determined with Folin-Cioalteu reagent according to Slinkard and Singleton (1977), the results were expressed as milligrams of gallic acid equivalent/g of dry extract. β–Carotene bleaching test was used according to Miller *et al.,* (1993), with modifications of Wanasundara *et al.,* (1994). Synthetic antioxidant reagent butylated hydroxy anisole (BHA) was used as a positive control. All determination was carried out in triplicate.

2.3 Cheese making:

White cheese was prepared according to Foda *et al.,* (2008). Milk retentate was divided into 7 portions; each portion was salted to a concentration of 3 %, well mixed and pasteurized at 73 °C for 15 sec. First portion was served as control and for the other six portions, different concentrations of spearmint essential oil (0.5, 0.75, 1.0, 1.5, 2.0, and 2.5 ml / kg retentate} were added at 40°C to prepare cheese treatments A, B, C, D, E and F respectively. Curds were hold at the same temperature after adding the rennet, cheeses samples were taken fresh and every week during stored under refrigerator temperature (5°C±1) for 5 weeks. Three replicates were prepared for each cheese to determine their chemical composition and organoleptic properties.

2.4 Chemical analysis

Cheese samples were analyzed for moisture and fat contents and titrable acidity according to Ling, (1963). Total nitrogen (TN %) was determined using Kjeldahl method according to AOAC, (2000). Protein content was obtained by multiplying the percentage of total nitrogen by 6.38. Water-soluble nitrogen (WSN) was extracted, Trichloroacetic acid soluble nitrogen (TCA-SN) and phosphotungstic acid soluble nitrogen (PTA-SN) were determined according to the method which described by Coskun and Tuncturk (2002). Ripening index (%) of white cheese was calculated according to the equation:

$$\text{Ripening index (\%)} = \frac{\text{WSN}}{\text{TN}} \times 100$$

Total volatile free fatty acids (TVFFA) were determined according to Kosikowski, (1978).

2.5 Sensory analysis:

Fifteen panelists (7 male and 8 female, aged between 25 and 45 years) who have experience with white cheese and regularly used its descriptive vocabulary, were participated. They scored the cheeses for appearance & color (20 points), body & texture (20 points), odor ((20 points), taste (20 points), and overall acceptability (20 points). Panel members were also instructed to report any defects or unpleasant flavor. Water and no salted crackers were provided to clean their palates between tasting.

2.6 Statistical analysis

Statistical analysis of experimental data was performed by analysis of variance (ANOVA) producers using SAS PROC GLM / STAT (1998). Differences among means were identified using Duncan multiple range test.

3. Results and Discussion

Yield of spearmint essential oil using Hydrodistillation was 2.0 (%). Edris *et al*., (2003) mentioned that yield of spearmint essential oil was varied from 1.28 – 3.9 % depending on cultivation locations.

3.1 Antioxidant properties

DPPH scavenging activity (%) of different concentrations of spearmint essential oil is shown in Table (1). It could be noticed that spearmint radical scavenging was increased significantly (P> 0.05) with increasing the essential oil concentrations from 5 to 25 µl/ml and maximum inhibition reached 71.07 %. The concentration of spearmint essential oil which provide 50% inhibition of DPPH (IC_{50}) was 10 780 µg/ml, which indicated that spearmint essential oil has very low inhibition effect compared with the synthetic antioxidant reagent (BHA). Also, total phenolic content in spearmint essential oil was very low (1.04 ± 0.02) mg (gallic acid/ g) dry extract.

Antioxidant activity (%) of spearmint essential oil was determined at intervals of incubation time by β-carotene test compared with BHA is shown in Table (2). It can be seen that spearmint essential oil did not exhibit any antioxidant activity at the concentrations of 50 or 100 µg/ml during the experimental period (100

min), only the concentration of 200 µg/ml showed 1.04 % inhibition of the linoleic acid oxidation. This study showed that spearmint essential oil had poor free radical scavenging compared with the synthetic antioxidant BHA, while previous antioxidant activity results showed that spearmint water and ethanolic extracts ranged from 59 – 62 % and 70 – 37 % respectively with 200 µg/ml during the same incubation period 20 - 100 min. (Foda *et al.,* 2009a). Ollanketo *et al.,* (2002) reported that the antioxidative performance of plant extracts depends not only on the extraction method, but also on the quality of the original plant, its geographic origin, the harvesting date, its storage and processing prior to extraction.

3.2 Effect of spearmint essential oil on chemical composition of white cheese

The changes of chemical composition of control and white cheese with different concentrations of spearmint essential oil (0.5, 0.75, 1.0, 1.5, 2.0, and 2.5 ml / kg retentate) which ordered as treatment A, B, C, D, E, and D respectively, are shown in Table (3). It could be noticed that although there is revealed variations in moisture content in white cheese made by essential oil compare to control samples, increasing the essential oil concentrations did not affect the moisture content significantly. While, significant ($P<0.05$) differences were appeared after two weeks of cold storage compared with fresh cheese. Increasing the concentrations of essential oil increased the protein content of white cheese significantly ($P<0.05$). Also, protein content increased significantly after 4 weeks of cold storage.

It could be noticed that essential oil has no significant effect on cheese fat content, while prolonging the cold storage affected this content significantly. Spearmint essential oil had no significant effect on the moisture of white cheese; this contradicts earlier findings by Ayar, (2002) while the results of fat and protein content were matched by his finding. Juven *et al.,* (1994) and Ultee *et al.,* (1998) reported that protein content in the food may also have an influencing factor in the effectiveness of the essential oils, bovine serum albumin neutralized the antimicrobial action of thymol (a major component of oil of thyme), and form a complex between phenolic components and proteins or other components of cell envelope especially the cell membrane which is widely regarded as one of the primary target sites for plant essential oil. Titratable acidity values (TA) in Table (3) shows that lower concentration of essential oil (0.5, 0.75 or 1.0 ml) caused significant increasing ($P<0.05$) in acidity values compare with control cheese. While higher oil concentrations had no effect on titratable acidity values (TA) this could be due to its antimicrobial activity (Foda *et al.,* 2009b). Similar results were observed in Feta cheese by Vafopoulou, (1989) and Ayar (2002) reported that pH and acidity values varied due to moisture and microbial activity of cheese samples. Cold storage increased the titratable acidity values significantly ($P<0.05$).

3.3 Effect of spearmint essential oil on biochemical characters of white cheese

Water Soluble Nitrogen (WSN) fraction is commonly used in cheese as an index of ripening (Lopez-Fandino *et al.,* 1994), it contains small & medium sized peptides and free amino acids separated from proteins and large peptides mainly as a result of the activity of chymosin and a lesser extent plasmin (McSweeney and Fox, 1997). Figure 1(a-c) shows the effect of different concentrations of spearmint essential oil on the ripening index of white cheese. It could be noticed that the WSN/TN ratio was increased by increasing the concentrations of spearmint essential oil. Higher concentrations (≤1.0 ml) increased this ratio significantly, while lower concentration 0.5 ml caused significant reduction ($P<0.05$) compared with control sample (Table-4). Prolonging the cold storage more than two weeks increased the WSN/TN ratio significantly ($P<0.05$). Also, increasing the essential oil concentrations increased the trichloroacitic acid soluble nitrogen (TCA-SN**),** and higher concentration (≤1.5 ml) caused significant increase compared with control cheese. TCA-SN values increased significantly ($P< 0.05$) only after 4 weeks of cold storage.

Fig. (1-c) shows effect of essential oil on the change of phosphotungestic acid soluble fraction (PTA-SN) in fresh cheese and during the cold storage period for 5 weeks. It could be noticed that lower concentration of essential oil (≥ 1.0 ml) caused significant decrease in PTA-SN, while higher concentration (≤1.5 ml) increased these values significantly ($P< 0.05$) compared with control sample (Table- 4). Yvon *et al.,* (1989) reported that trichloroacitic acid soluble nitrogen (TCA-SN**)** expresses small molecules of peptides (lower than 20 amino acid residue) and free amino acid its levels regarded to ripening depth index. Also, the phosphotungestic acid soluble fraction (PTA-SN) contains tri-, di,- peptides and free amino acids are soluble state (Fialaire and Postaire, 1994). The changes of total volatile free fatty acids (TVFFA) in control and spearmint cheeses are shown in Fig.2. Adding essential oil with any concentrations affected the TVFFA values significantly ($P<0.05$) compared with control sample (Table-4). Moreover, increasing essential oil concentration, more than 1.0 m/kg retentate, increased these values significantly. Data indicated also, that prolonging the cold storage for 5 weeks increased (TVFFA) values significantly compared with fresh cheese. These results in agreement with those obtained by Abdel-Salam *et al.,* (1993) who reported that most

of the changes in (TVFFA) occur during the first 15-30 days of storage which coincide with maximum bacterial growth and high concentration of total volatile free fatty acids in cheese would contribute significantly to the total acidity.

3.4 Sensory analysis:

Effect of different concentrations of spearmint essential oil on the organoleptic properties of white cheese during cold storage for 5 weeks is shown in Table (5). Increasing spearmint essential oil from 0.5 to 2.5 ml/ kg retentate did not affect the appearance of white cheese during cold storage, while after 4 weeks body & texture scores decreased significantly ($P<0.05$). Cheese odor, taste and overall acceptability scores decreased significantly ($P<0.05$) by higher concentration of essential oil ($\leq$ 1.5 ml), while prolonging the cold storage had no significant effect. Similar results were obtained by Ayar, (2002) and Hussein (2004).

Table (1): DPPH scavenging activity (%) by different concentrations of spearmint essential oil

Spearmint essential oil (µl/ml)	DPPH scavenging activity (%)
5	32.5 ± 1.9 [d]
10	46.4 ± 0.29 [c]
15	53.4 ± 0.4 [c]
20	59.6 ± 0.87 [b]
25	71.07 ± 2.9 [a]
*IC_{50}	10 780 ± 95
BHA	5 ± 0.47
**Total phenolic	1.04 ± 0.02

Means ± Stander Deviation
Means with the same letter are not significantly different ($P<0.05$)
*IC_{50} values of DPPH assay as µg/ml; ** Given as mg gallic acid/g dry extract

Table (2): Antioxidant activity (%) of different concentration of essential oil by β-carotene test compared with BHA

Time (minutes)	Inhibition of linoleic acid oxidation (%)					
	BHA (µg/ml)			Essential oil (µg/ml)		
	50	100	200	50	100	200
20	90.39	93.73	97.22	0.0	0.0	0.0
40	92.19	95.02	97.25	0.0	0.0	0.0
60	92.19	95.03	97.37	0.0	0.0	0.0
80	91.85	94.58	96.70	0.0	0.0	0.0
100	92.47	94.39	96.89	0.0	0.0	1.04 ± 0.02

Table (3): Effect of spearmint essential oil on the gross chemical composition of white cheese during cold storage period.

Cheese samples*	Moisture (%)	Fat (%)	Protein (%)	Acidity
Control	68.22 ± 0.59^{BC}	15.22±0.27^{A}	10.52±0.14^{BC}	0.22±0.10^{B}
A	67.68 ±0.26^{E}	15.22 ±0.36^{A}	10.98 ±0.04^{AB}	0.26 ±0.14^{A}
B	67.16 ±1.01^{DE}	15.18 ±0.32^{A}	11.03 ±0.16^{AB}	0.28 ±0.15^{A}
C	68.35 ± 0.16^{BC}	15.15 ±0.40^{A}	11.13 ±0.16^{A}	0.28 ±0.15^{A}
D	68.60 ±0.93^{A-C}	15.18 ±0.30^{A}	10.46 ±0.34^{D}	0.21 ±0.10^{B}
E	68.11 ±0.47^{CD}	15.17 ±0.28^{A}	10.50 ±0.15^{D}	0.22 ±0.11^{B}
F	68.40 ±0.53^{BC}	15.12 ±0.29^{A}	10.57 ±0.19^{DC}	0.20 ±0.11^{B}
Storage period (weeks)**				
Fresh	68.86 ±0.58^{A}	14.75 ±0.58^{E}	10.67 ±0.28^{B}	0.12 ±0.04^{F}
1	68.91 ±0.71^{A}	15.07 ±0.71^{D}	10.61 ±0.26^{B}	0.14 ±0.03^{E}
2	68.63 ±0.82^{A}	15.15 ±0.82^{C}	10.58 ±0.28^{B}	0.19 ±0.04^{D}
3	67.94 ±0.66^{B}	15.22 ±0.66^{C}	10.61 ±0.31^{B}	0.26 ±0.06^{C}
4	67.94 ±0.53^{B}	15.35 ±0.53^{B}	10.72 ±0.33^{B}	0.31 ±0.07^{B}
5	67.62 ±0.63^{B}	15.63 ±0.63^{A}	11.03 ±0.27^{A}	0.38 ±0.06^{A}

A= 0.5 ml essential oil, B= 0. 75 ml, C= 1.0 ml, D = 1.5 ml, E = 2.0 ml, and F = 2.5 ml. Means ± Standard Division, different letters are significantly different ($P< 0.05$). *Each value represents 18 values, **Each value represents 30 values.

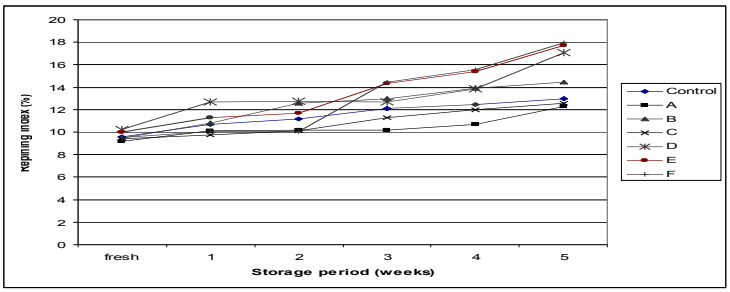
Table (4): The changes of biochemical characteristics of control and whit cheese with spearmint essential oil during cold storage

Cheese samples	WSN/TN%	TCA-TN%	PT-TN%	TVFFA
Treatment *				
Control	11.51±1.26^{C}	3.44 ± 0.25^{B}	2.35±0.17^{B}	2.41±0.40^{D}
A	9.19±0.06^{D}	3.91± 0.39^{AB}	1.97±0.09^{C}	2.26±0.19^{E}
B	12.36±1.90^{B}	3.40 ± 0.30 ^{C}	1.89±0.08^{C}	2.18±0.24^{E}
C	10.86±1.29^{C}	3.35 ± 0.05 ^{C}	1.79±0.03^{C}	2.24±0.74^{E}
D	13.72±1.72^{A}	3.70 ± 0.20 ^{B}	3.12±0.30^{A}	2.88±0.47^{B}
E	13.57±2.7^{A}	3.87 ± 0.59^{AB}	3.20±0.46^{A}	2.57±0.26^{C}
F	12.94±3.54^{AB}	4.08 ± 0.14^{A}	3.17±0.41^{A}	2.58±0.32^{C}
Storage period (weeks)**				
Fresh	10.53±1.34^{D}	3.56±0.46 ^{C}	2.44±0.50^{D}	2.12±0.48^{F}
1	10.88±1.00^{D}	3.63±0.37^{BC}	2.48±0.46^{CD}	2.21±0.46^{E}
2	11.15±1.14^{D}	3.62±0.30^{BC}	2.62±0.55^{B-D}	2.37±0.43^{D}
3	12.83±1.66^{C}	3.65±0.38^{BC}	2.77±0.68^{A-C}	2.60±0.41^{C}
4	13.59±1.94^{B}	3.84±0.43^{AB}	2.88±0.78^{AB}	2.81±0.43^{B}
5	15.25±2.88^{A}	3.96±0.41 ^{A}	3.02±0.89 ^{A}	3.05±0.40^{A}

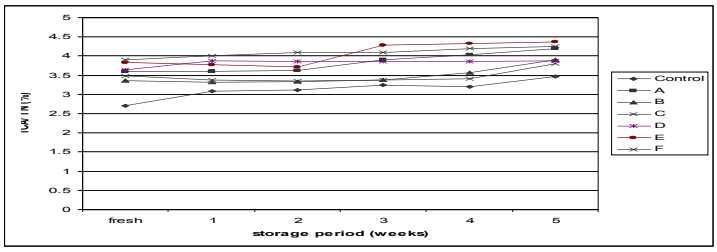
Table (5): Effect of spearmint essential oil on some organoleptic properties of white cheese during cold storage

Cheese samples	Organoleptic properties of white cheese				
	Appearance & color (20)	Body &Texture (20)	Odor (20)	Taste (20)	Overall acceptability (20)
Treatment *					
Control	19.06±0.76^{A}	18.61±0.35^{A}	18.15±1.34^{AB}	17.72±0.99^{AB}	18.59±1.23^{A}
A	18.89±0.62^{A}	18.65±0.53^{A}	18.54±0.68^{A}	18.72±0.86^{A}	18.72±0.76^{A}
B	19.00±0.39^{A}	18.78±0.21^{A}	18.46±0.31^{A}	18.15±0.38^{AB}	18.59±0.41^{A}
C	19.09±0.21^{A}	18.46±0.51^{A}	17.85±0.76^{AB}	17.52 ±1.25^{B}	18.00±0.96^{A}
D	19.07±0.67^{A}	18.30±0.45^{A}	16.20±0.76^{C}	14.02 ±1.25^{D}	14.37±1.04^{C}
E	19.00±0.80^{A}	18.20±0.52^{A}	15. 30±0.93^{C}	12.72 ±1.00^{E}	12.81±1.39^{D}
F	19.07±0.68^{A}	18.37±0.52^{A}	14.28±0.86^{D}	11.85 ±1.27^{E}	11.67±0.94^{E}
Storage period (weeks)**					
Fresh	17.50±3.65^{A}	18.31±0.79^{A}	17.07±1.49^{AB}	16.08±2.72^{A}	16.04±2.90^{A}
1	17.41±3.36^{A}	18.24±0.73^{A}	16.84±1.75^{A-C}	16.27±2.22^{A}	16.19±2.89^{A}
2	17.24±3.49^{A}	18.12±0.64^{A}	16.81±1.72^{A-C}	16.06±2.43^{A}	15.93±2.64^{AB}
3	17.58±3.06^{A}	18.20±1.04^{A}	17.48±1.38^{A}	16.29±1.81^{A}	16.43±1.95^{A}
4	17.32±1.70^{A}	17.74±0.76^{AB}	16.14±1.76^{C}	15.40±2.68^{AB}	15.69±2.26^{AB}
5	17.50±1.85^{A}	17.43±1.00^{B}	16.58±1.62^{BC}	15.02±3.16^{B}	15.14±3.05^{B}

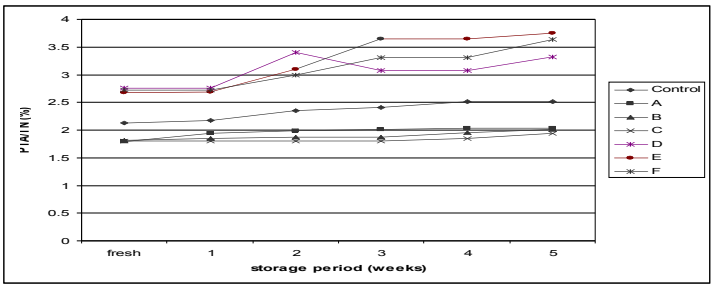
A= 0.5 ml essential oil, B= 0. 75 ml, C= 1.0 ml, D = 1.5 ml, E = 2.0 ml, and F = 2.5 ml.
TVFFA = (Total Volatile Free Fatty Acids) 0.1N of Na OH /10g cheese. Means ± Standard Division, different letters are significantly different ($P< 0.05$). *Each value represents 18 values, **Each value represents 30 values.
A= 0.5 ml essential oil, B= 0. 75 ml, C= 1.0 ml, D = 1.5 ml, E = 2.0 ml, and F = 2.5 ml. Means ± Standard Division Different letters are significantly different ($p< 0.05$). *Each value represents 18 values, ** Each value represents 30 values.



(a) Water-soluble nitrogen (WSN/TN)



(b) Trichloroacetic acid-soluble (TCA-SN)



(c) Phosphotungstic acid-soluble nitrogen (PTA-SN)

Fig. 1: Effect of spearmint essential oil on biochemical characters of white cheese during cold storage 5 weeks. A = 0.5 ml essential oil, B= 0.75 ml, C= 1.0 ml, D= 1.5 ml, E= 2.0 ml, F= 2.5 ml.

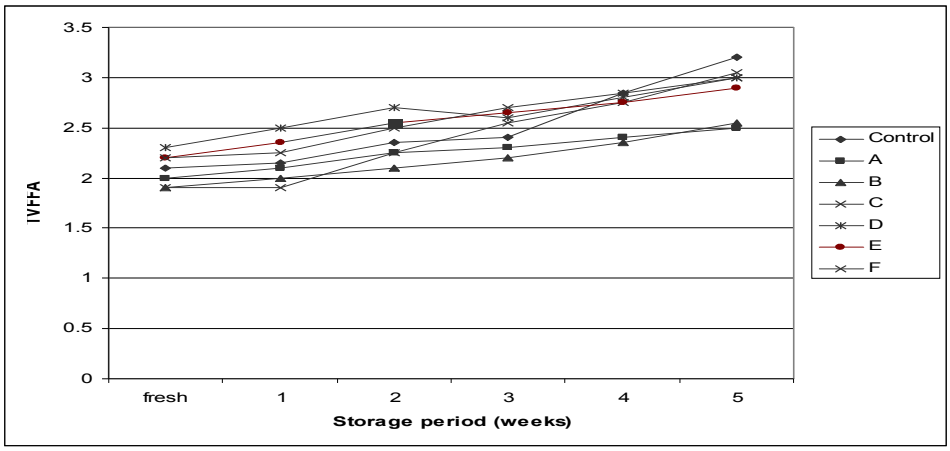


Fig. (2): Effect of spearmint essential oil on Total Volatile Free Fatty Acids (TVFFA) of white cheese during cold storage 5 weeks. A = 0.5 ml essential oil, B= 0.75 ml, C= 1.0 ml, D= 1.5 ml, E= 2.0 ml, F= 2.5 ml.

4 Conclusions

Lower concentration of spearmint essential oil would be recommended for white cheese because the use of spearmint essential oil at high concentration, required to be effective in cheese quality, could raise concerns regarding changes in the organoleptic properties. A number of options can be considered to overcome this problem, such as to view the essential oil not only as a preservative but also as a flavour component. Alternatively, it could be incorporated into products which already have a strong flavour, or to use the most active components instead of the whole oil. This would hopefully reduce the changes in the organoleptic properties, whilst retaining antimicrobial activity.

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Alginate/ Polyvinyl Alcohol - Kaolin Composite for Removal of Methylene Blue from Aqueous Solution in a Batch Stirred Tank Reactor

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Abstract: The investigation of possible use of Alginate/ polyvinyl alcohol -kaolin composite instead of free kaolin in the removal of methylene blue from aqueous solutions was studied. Various experiments have been carried out using batch adsorption technique to study the effects of the process variables, which include contact time, beads diameter, beads swelling, organic-kaolin composite dosage, initial dye concentration, pH, agitation speed and solution temperature on the adsorption process. In the batch kinetic study of methylene blue, the order of the reaction, the half-life and the rate constant were determined. Numerical correlations using regression analysis for maximum percentage removal of dye with operating condition of the process were presented. The result showed that the adsorption attained to equilibrium in 360 min and the kinetics followed first order in nature. [Journal of American Science 2010; 6(5):280-292]. (ISSN: 1545-1003).

Keywords: Adsorption, Cationic dye, Isotherms, Kaolin composite, binding polymers.

1. Introduction

The effluents from textile, leather, food processing, dyeing, cosmetics, paper, and dye manufacturing industries are important sources of dye pollution [1]. Many dyes and their break down products may be toxic for living organisms [2]. Therefore, removal of dyes is important aspect of wastewater treatment before discharge. It is difficult to remove the dyes from the effluent, because dyes are not easily degradable and are generally not removed from wastewater by conventional wastewater systems [3]. Adsorption of dye at the solid/liquid interface has been extensively studied the last years. Activated carbon has been successfully used in removing colored organic species with being the most widely used adsorbent due to its high capacity for the adsorption of organic materials [4–6]. However, due to its high cost and the difficulty of regeneration, a search for cheap, effective adsorbents such as bentonite clay derivatives is needed.

Clay has received particular attention as an economical adsorbent for removing heavy metals from wastewater due to its abundance and easy availability but it is still difficult to be separated from the wastewater [7]. In order to overcome the handling problem, organic binding polymers for the granulation of kaolin have a lot of advantages such as simplicity of preparation procedure and excellent physicochemical properties [8]. Recently, Alginate, agar and carrageenan, are known as the major binding biopolymers. However, the mechanical strength of agar is rather weak, also carrageenan, has the economical disadvantage of a high removal cost for carrageenan causing the gel to become weak. Consequently, it may be impractical to apply these polymeric materials to wastewater treatment as immobilization carriers. Many studies have been carried out about the application of alginic acid to the aqueous phase separation of heavy metals, and the possibility of alginic acid for the adsorbent material has been suggested [9,10]. In such applications, alginic acid is not rigid enough to be used in a down flow packed-bed column operation and presents an unacceptable pressure drop [11]. The use of polyvinyl alcohol (PVA) as an immobilization carrier was initiated about 10 years ago [12]. PVA is a raw material of vinylon and can be produced industrially rather cheaply. PVA also offers various advantages over the conventional immobilization methods, such as low cost, high durability and chemical stability. Immobilization using PVA can be used only PVA crosslinked with boric acid [13]. But the formed beads had a strong tendency to agglomerate into a mass of polymer which was very difficult to break up. This

agglomeration problem appears to be due to the relatively slow crosslinking of the PVA by boric acid. Droplets of PVA, which have not been sufficiently crosslinked, tend to agglomerate. This problem persisted even with vigorous stirring of the boric acid solution to keep the beads suspended [14]. So, many attempts to form spherical beads, which are the preferred shape for application, from PVA took place using alginate to prevent agglomeration. Mixed solution of PVA and sodium alginate were used by few researchers. Remmers and Vorlop [15] reported that the fracture strength for alginate beads is smaller than PVA–alginate beads, in addition the PVA–alginic beads were also stable under strong acidic (below pH 1.0) and high temperature (above 170°C) conditions. On the other hand Dave and Madamwar [16] mentioned that the beads produced from mixed polymers (alginate & PVA) exhibited rubber like elastic properties, PVA contributed strength and durability to the beads, whereas calcium alginate improved the surface properties, reducing the tendency to agglomerate.

The present work deals with the preparation of organic- kaolin composite from kaolin and polymer mixture of alginate & PVA. It was also focusing on the factors that might affect the removal of cationic dye (methylene blue) using batch stirred reactor. From the present work the order of the reaction, the half-life and the rate constant of the reaction were determined. Finally, Langmuir & Freundlich isotherms were performed for interpretation of results.

2. Material and Methods

2.1. Materials

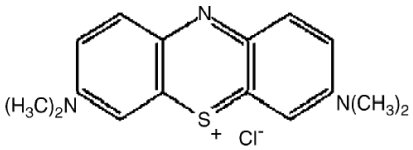
Kaolin,hydrated aluminum silicate, (SIGMA-Aldrich, Germany).
Poly vinyl alcohol (PVA) (MERCK, Germany), Alginic Acid sodium salt (SIGMA, USA.), Methylene Blue (NICE CHEMICALS Pvt. Ltd., COCHIN).

2.2. Preparation of basic dye solution

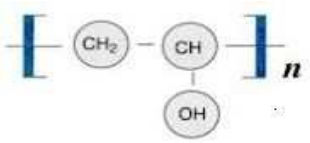
Methylene Blue, $C_{16}H_{18}N_3SCl.3H_2O$, is a cationic dye. The structure of this dye is shown in Fig.1.a. The stock dye solution was prepared by dissolving 1g of methylene blue in 1000 ml distilled water. The samples were filtered and the dye concentration in the supernatant solution was estimated by measuring absorbance using UV-Vis double Beam spectrophotometer (LABOMED. Inc) using 1 cm light-path cell at λ max of 665 nm.the residual dye concentration was also determined using the same technique.

2.3. Preparation of composite Solution

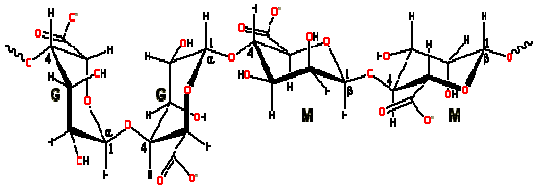
4% PVA solution was prepared by dissolving 20g of PVA (its molecular weight 72000) that has alcoholic structure as indicated in Fig.1.b. in demineralized water by heating the solution around 60ºC with continuous stirring to dissolve PVA. 1% alginate solution that has linear unbranched polymers containing β-(1⟶4)-linked D-mannuronic acid (M) and α-(1⟶4)-linked L-guluronic acid (G) as indicated in Fig.1.C. was prepared by dissolving alginic acid sodium salt (medium viscosity ~3500cps) in water with gentle heating and stirring.



a. Structure of Methlyene Blue



b. PVA structure



c. Sodium alginate structure

Fig.1. a. Structure of Methlyene Blue, b. PVA structure and c. Sodium alginate structure

2.4. Preparation and characterization of Organic-Kaolin Composite

Kaolin (9g) was mixed thoroughly with an aqueous solution of 5% PVA containing 0.5% sodium alginate using the homogenizer for 5min at 14000rpm. This mixture was pumped by a peristaltic pump at 10 ml/min and then dropped into a gently stirred 6% boric acid solution containing 3% $CaCl_2$ to form spherical beads. In order to complete gelation inside beads, these beads were stirred gently in the boric acid-$CaCl_2$ solution for 24h. The beads were then removed and washed with distilled water. Finally the washed beads were dried at 40ºC for 24 h. The dried composite was used for detailed studies. The prepared composite was characterized using FTIR, SEM and TGA techniques and also tested for swelling and turbidity.

2.5. Setup

The apparatus used in this study consists of a cylindrical Pyrex jacked reactor, with outer diameter 12.3 cm, wall thickness 1.6cm and 18.2 cm in height. The mixer consists of a stainless steel shaft fitted with four blade propellers, with a diameter 5cm. The stirrer is coated with epoxy resin and is driven by a 35 watt motor which is fixed firmly against a steel frame to prevent vibrations. The rotation speed is controlled automatically by LED display. The jacked reactor connected with water bath model-ultra term (J.P.SELECTA Co., Spain) to control the solution temperature.

2.6. Batch adsorption experiments

2.6.1. Effect of binding polymers

A sample of polymer mixture (alginate+ PVA) 3.2g compared with 4.8g sample of kaolin and 8g sample organic-kaolin composite (each 8g sample of composite contains 3.2g polymer mixture and 4.8g kaolin) with 2mm dry beads diameter was added to each 800ml of methylene blue aqueous solution having an initial concentration of 20 mg/L for investigation of the effect of binding polymer for a constant stirring speed, 500 rpm, initial pH= 9. The experiments were carried out at 22ºC in the jacketed reactor. Samples at different time intervals were taken from the reactor for dye analysis using UV-Vis double Beam spectrophotometer.

2.6.2. Effect of contact time

A dry sample of organic-kaolin composite (8 g) with 2mm beads diameter was added to each 800 ml of methylene blue aqueous solution having an initial concentration of 20 mg/L with a constant stirring speed, 500 rpm, initial pH= 9 and the experiments were carried out at 22ºC in the jacketed reactor. Samples at different time intervals were taken from the reactor for dye analysis using UV-Vis double Beam spectrophotometer.

2.6.3. Effect of beads Diameter

A dry sample of organic-kaolin composite (8 g) with different diameters (0.5, 1&2mm) was added to each 800 ml of methylene blue having an initial concentration of 20 mg/L at constant stirring speed, 500 rpm, initial pH= 9 and the experiments were carried out at 22ºC in the jacketed reactor. Samples at different time intervals were taken from the reactor for dye analysis using UV-Vis double Beam spectrophotometer.

2.6.4. Effect of beads swelling

A sample of either dry and swollen organic-kaolin composite (8 g) with 2mm dry beads diameter was added to each 800 ml of methylene blue aqueous solution having an initial concentration of 20 mg/L for a constant stirring speed, 500 rpm, initial pH= 9 and the experiments were carried out at 22ºC in the jacketed reactor. Samples at different time intervals were taken from the reactor for dye analysis using UV-Vis double Beam spectrophotometer.

2.6.5. Effect of organic-kaolin composite dosage

Effect of organic-kaolin composite at various doses, which are 2, 4, 8, and 10g on percentage dye removal was studied. In the experiments, a 2mm dry beads diameter of composite was added to each 800 ml of methylene blue aqueous solution having an initial concentration 20 mg/L for a constant sorption time, 360 min at constant stirring speed, 500 rpm, initial pH= 9 and the experiments were carried out at 22ºC in jacketed reactor.

Effect of initial dye concentration

A sample of 2mm dry organic-kaolin composite (8 g) was added to 800mL of methylene blue solution. The initial concentrations of dye solution tested were 10, 20, 125,225, and 500 mg/L and the experiments were carried out for a constant sorption time, 360 min at constant stirring speed, 500 rpm, initial pH= 9 and the experiments were carried out at 22ºC in jacketed reactor.

2.6.7. Effect of pH

Effect of initial dye pH was investigated at various pH, which are 2, 7, 9, and 12. In the experiments, 8 g of 2mm dry composite was added to each 800 ml of methylene blue aqueous solution having an initial concentration 20 mg/L for a constant sorption time, 360 min and the experiments were carried out at constant stirring speed, 500 rpm, and the experiments were carried out at 22ºC in jacketed reactor.

2.6.8. Effect of agitation speed

A sample of 2mm dry organic-kaolin composite (8 g) was added to 800 ml of methylene blue aqueous solution having an initial concentration of 20 mg/L for investigation of the effect of mixing rate at various stirring rate (0,100, 250, 500,750 and 1000 rpm). The experiments were carried out for a constant sorption time, 360 min at constant stirring speed, 500 rpm, initial pH= 9 and the experiments were carried out at 22ºC in jacketed reactor.

2.6.9. Effect of solution temperature

A sample of 2mm dry organic-kaolin composite (8 g) was added to each 800 ml of methylene blue aqueous solution having an initial concentration 20 mg/L. The experiments were carried out at a constant temperature stirred jacketed reactor which controlled the temperature to 22, 35, 50, and 65 ºC within ±1. The experiments were carried out for a constant sorption time, 360 min at constant stirring speed, 500 rpm, and initial pH= 9.

3. Results and discussion

3.1. Characterization of adsorbing material.

3.1.1. Fourier Transform Infrared Spectroscopy FTIR

The FTIR spectrum of Kaolin, Polymer mixture and also the prepared polymer-kaolin composite were measured using the disc technique with KBr as a matrix. And the FTIR analysis was performed using Fourier Transform Infrared Spectrophotometer FTIR-8400 Shimadzu- Japan. Fig.2. shows the pattern of Kaolin in which a broad band at 3600 cm^{-1} was which contain ~ 14% water. Also there are two bands at 1022 cm^{-1} and 465 cm^{-1} could be attributed to the stretching and bending vibrations of the SiO_2 in the structure of Kaolin. Polymer mixture pattern shows significant broad band corresponding to OH group at 3200-2500 cm^{-1} corresponding to both alcoholic and acidic groups. Also there is a band at 2900 cm^{-1} could be attributed to CH stretching for aliphatic chain. At 1720 cm^{-1} there is a band corresponding to C=O of the carboxylic group. There is also band at 1120 cm^{-1} corresponding to C-C bond. Figure (2) also depicts the pattern of the prepared polymer- kaolin composite in which a broad band at 3400 cm^{-1} appeared and it could be assigned to the interstitial water in the Kaolin structure and also the OH group of the used polymers. Also a band at 1620 cm^{-1} was appeared and it could be attributed to the carboxylic group of Alginate [17]. the C-H stretching in PVA shows an absorption band at 1440 cm^{-1} [18] and the C-C stretching in PVA appeared at 1118 cm^{-1} [19].

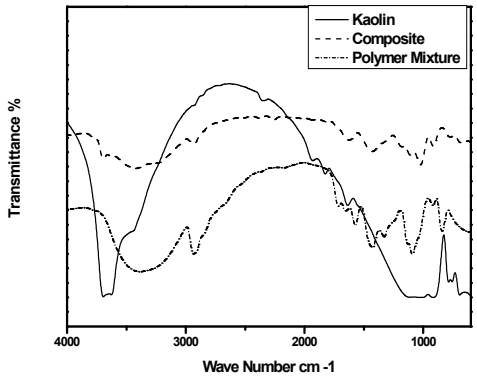


Fig.2. FTIR spectrum of (a) Kaolin and (b) Organic- Kaolin Composite

3.1.2. Thermogravemetric analysis TGA

The thermal stability of the polymer- kaolin composite was evaluated by Thermo Gravimetric Analysis TGA using Thermo Gravimetric Analyzer Shimadzu TGA-50 Japan. Fig. (3-a) shows the TGA of kaolin, the main step began at 427 ^oC is attributed to the loss of interstitial water in kaolin structure and the weight loss is 11.363% in accordance with the known water ratio in kaolin. Fig. (3-b) shows the TGA pattern of the prepared composite which contained three main steps. The first step began at 41 and ended at 335 ^oC which was explained by the removal of external water molecules together with degradation of alginate chain

and it is interpreted with second degradation temperature due to elimination of side-groups of PVA. [20].

The third step is obviously due to the complete loss of the organic components and it started at 432 ^{o}C and ended at 712 ^{o}C [21].

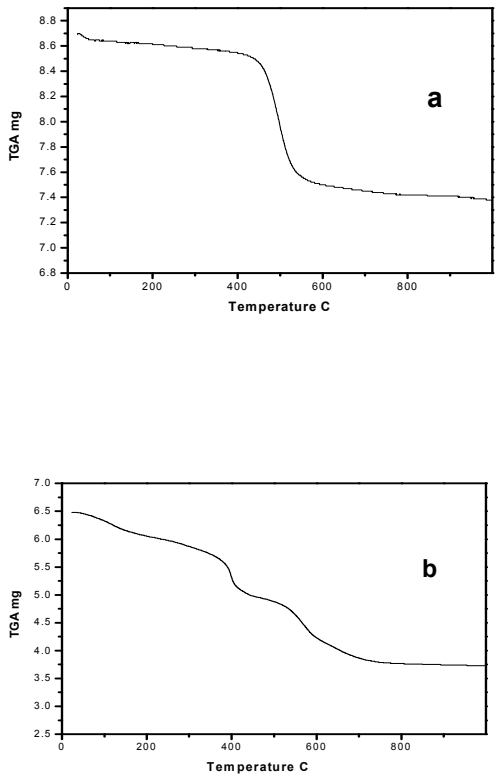


Fig.3. TGA analysis of (a) Kaolin and (b) Organic-Kaolin Composite

3.1.3. Scanning Electron Microscope SEM

The morphology of the prepared composite was investigated using Jeol JSM-6360 LA analytical Scanning Electron Microscope SEM. The samples were stocked over a holder and sprayed with gold. The sample was scanned to identify the structure and estimate the diameter. It was shown in Fig. 4. that the composite prepared from kaolin and PVA& alginate mixture has almost uniform porosity with pore diameter range 36-75 µm. The diameter of the composite beads ranged from 1.9- 2.4 mm.

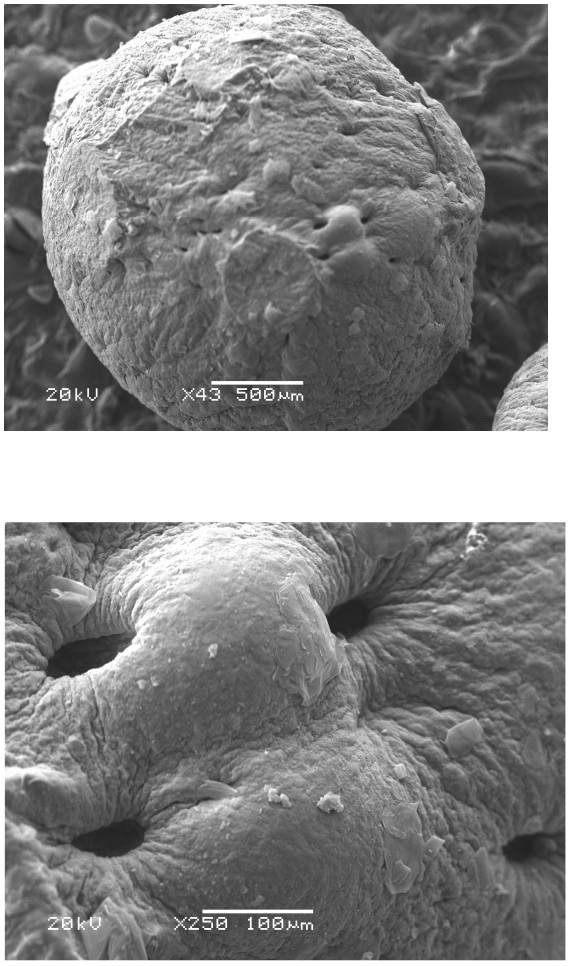


Fig.4. SEM of Organic – Kaolin composite

3.1.4. Turbidity measurements as a factor in mechanical strength

The beads produced from kaolin with mixed polymer (PVA & alginate) exhibited rubber like elastic properties, PVA contributed strength and durability to the beads whereas alginate improved the surface properties, reduced the tendency to agglomerate [16]. The mechanical properties of the composite were studied to determine its availability for the column operations. From water turbidity measurements after stirring for different time intervals, it is obvious that the beads of the prepared composite have excellent mechanical strength under stirring for 72 hours at 1000 rpm.

3.1.5. Swelling measurements

The swelling behavior of the beads was studied in order to test its suitability for column operation. It is concluded that the composite beads swelling in water increase with time till reached equilibrium after 5 hours. So the produced polymer-kaolin composite suitable to be used in column operation after being swollen in water for 5 hours to avoid column clogging during the treatment process.

3.2. Adsorption experiments

The % removal of blue dye was calculated according to the following equation:
The removal percentage (% removal) =
((Co –C)/Co) * 100) (1)
Where C_O and C (both in mg/L), are the initial concentration and the concentration at any time respectively.
% removal is defined as the ratio of difference in dye concentration before and after adsorption (Co –C) to the initial dye concentration in the aqueous solution.
The removal capacity (q)
q (mg/g)= (C_O-C) * (V/M) (2)
Where V is the solution volume (L),
M is the adsorbent amount (g).

3.2.1. Effect of binding polymers

It was observed from Fig .5. that the alginate & PVA matrix has a small dye uptake may be due to their hydroxide groups that are suitable for cationic dyes removal. On the other hand, the presence of the polymer matrix enhance the percentage dye removal than the free kaolin due to the presence of hydroxide groups associated with the polymer matrix as indicated in Fig.1. The percentage removal increased from 70.3% to 92.7% after 360 min, upon using composite beads instead of free kaolin. However the adsorption rate decreased through the first adsorption hour may be due to the diffusion limitation of dyes through the composite beads to reach to the active adsorption sites (kaolin).

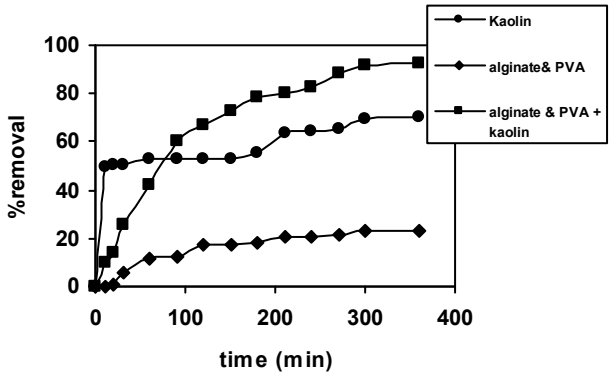


Fig.5. Effect of binding polymer on the percentage dye removal

3.2.2. Effect of contact time

The effect of contact time on the % removal of dye adsorbed was investigated as shown in Fig. 6. The % removal of Methylene Blue by organic-kaolin composite was found to increase, reach a maximum value with increase in contact time. In some cases it almost become constant with increase in contact time, after 360 min. based on these results, 360 min was taken as the equilibrium time in adsorption experiments. The removal of methylene blue from aqueous solutions by adsorption on organic-kaolin composite increases with time, till equilibrium is attained. Similar results have been reported in literature for removal of dyes [2, 22].

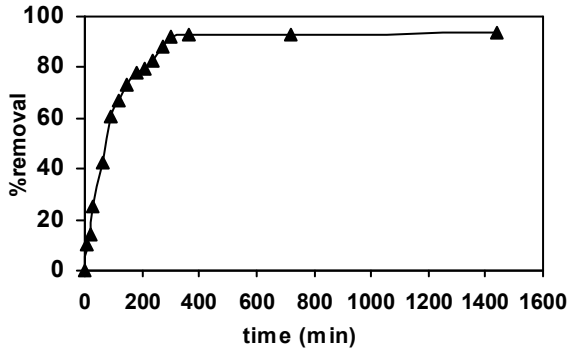


Fig.6. Effect of contact time on the percentage dye removal

In batch type adsorption process monolayer of adsorbate is generally formed on the surface of adsorbent [23] and removal rate of adsorbate species from aqueous solution is controlled especially by the transport rate of the adsorbate species form the outer sites to interior sites of the adsorbent particulars [2].

In a kinetic study of methylene blue adsorption on organic-kaolin composite was investigated**,** Fig.7. shows a plot of (ln C_o / C) against time (t) was applied up to 360 min. The data gave straight lines, which indicated that the reaction was classified as a first order [24]. In a first order reaction the rate is directly proportional to the concentration of the reacting substance. According to the first order equation (3) the slope of the straight line is = K, where K is the rate constant (min^{-1}) that equal $7.9x10^{-3}$ min^{-1} .

$$\ln C_O/C = K^* t \qquad (3)$$

The above equation is the integrated form of the equation:

$$-V_S\,(dc/dt) = KC \qquad (4)$$

The half time,$t_{0.5}$, for first order reaction is calculated according to the following equation [5]:

$$t_{0.5} = 0.693/K \qquad (5)$$

Where, $t_{0.5}$ (min) is the time for the removal of half the amount of dye that equal 87.7min.

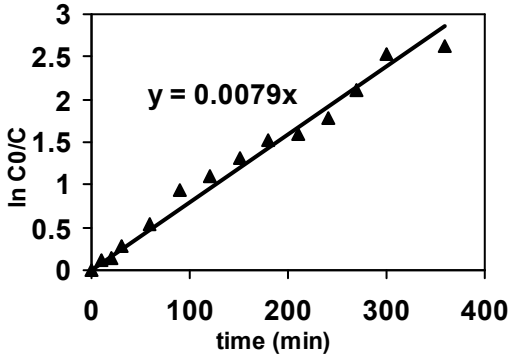


Fig.7. Plot of $\ln C_0/C$ Vs. time for methylene blue removal

3.2.3. Effect of beads Diameter

There is no significant increase in the percentage dye removal as the beads diameter increased from 0.5 mm to 2mm (Fig.8.)

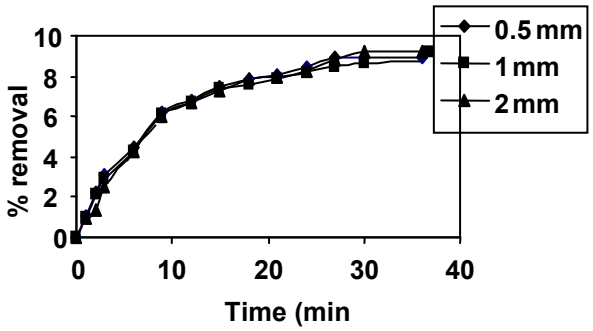


Fig.8. Effect of beads diameter on the percentage dye removal

3.2.4. Effect of beads swelling

In spite of the composite beads have swelling characteristics there is no variation on both the rate and the percentage dye removal for the swollen beads than the dry ones. Which is may be due to the effect of the other different operating variables in the batch process that eliminate the swelling effect.

3.2.5. Effect of organic-kaolin composite dosage

Fig.9. illustrates the effect organic-kaolin composite dosage that is added to methylene blue solution. It is observed that the removal of dye increases as the dosage of composite increases. For instance, the removal of dye increases from 41.8 to 95.5% on increasing the mass of composite from 2 to 10 g after 360 minutes. Increasing the adsorbent dosage at constant methylene blue concentration provided more available adsorption sites for dye and thus increased the extent of dye removal [25-26]. However the ratio of dye adsorbed to composite (mg/g) decreased with the increasing composite dosage from 3.37 to 1.53 mg/g. The relationship between % removal and the adsorbent dosage can be modeled using the following equation:

Max.% removal =

$$-1.2333\,(\text{composite dosage})^2 + 21.05\,(\text{composite dosage}) + 6.6333 \qquad (6)$$

$R^2 = 0.984$

Where: $2 \le composite dosage \le 10$

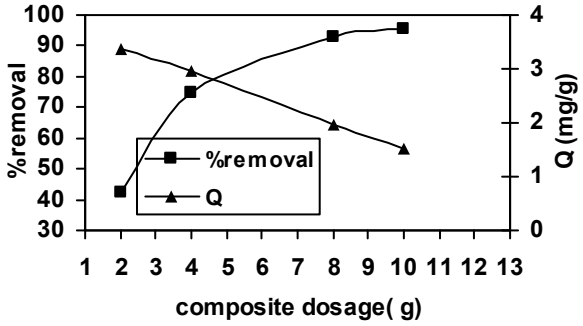


Fig.9. Effect of composite dosage on the percentage dye removal and removal capacity

3.2.6. Effect of initial dye concentration

It is observed from Fig. 10 that the % removal is inversely proportional to the initial dye solution concentration. The removal of dye decreased from 100 to 61.6% on increasing the initial dye concentration from 10 to 500 mg/L after 360 minutes.

This may be attributed to the increase of dye molecules adsorption onto the external surface of the organic-kaolin composite, increases significantly the local dye concentration, giving rise to the formation of aggregates of the dye on the composite particles. However the increase at initial dye concentration leads to an increase in the adsorption capacity. As the initial dye concentration increases from 10 to 500 mg/L, the adsorption capacity of dye onto composite changes from 1 to 30.8 mg/g. This indicates that the initial dye concentration plays an important role in the adsorption capacity of dye.

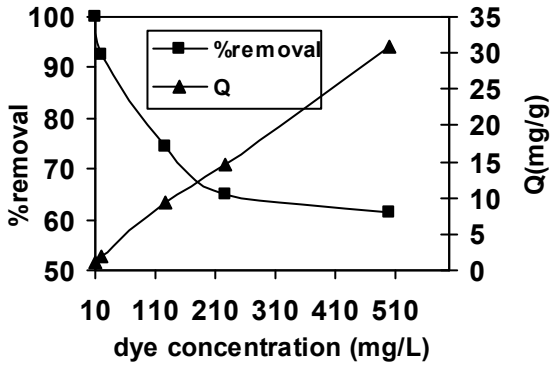


Fig.10. Effect of initial dye concentration on the percentage dye removal and removal capacity

The relationship between removal and initial dye concentration can be modeled using the following equation:

Max.% removal =

$$0.003*(conc.)^2 - 0.2288*(conc.) + 99.53 \quad (7)$$

$R^2 = 0.9856$

Where $0 \le conc. \le 500$

Two important physiochemical aspects for the evaluation of the adsorption process as a unit operation are the equilibrium of the adsorption and the kinetics. Equilibrium studies give the capacity of the adsorbent [27]. The equilibrium relationships between adsorbent and adsorbate are described by adsorption isotherms, usually the ratio between the quantity adsorbed and that remaining in solution at a fixed temperature at equilibrium. There are two types of adsorption isotherms: Langmuir adsorption isotherms and Freundlich adsorption isotherms.

Analysis of equilibrium data is important for developing an equation that can be used for design purposes. Classical adsorption models, such as Langmuir and Freunlich models have been extensively used to describe the equilibrium established between the adsorbed dye on the prepared samples and the dye remaining in solution.

The Langmuir adsorption isotherm is often used for adsorption of a solute from a liquid solution. The Langmuir adsorption isotherm is perhaps the best known of all isotherms describing adsorption and is often expressed as:

$1/qe = 1/b + 1/abC_e$ (8)

Where q_e is the mass of dye adsorbed per gram adsorbent at equilibrium, C_e is the equilibrium dye solution concentration, mg of dye/L, a is Langmuir

constant, l/mg of dye, b is the monolayer coverage, mg of dye/g of adsorbent.
A plot of ($1/q_e$) versus ($1/C_e$) should indicate a straight line of slope (1/ab) and an intercept of (1/b).
The linear Langmuir equation for Methylene Blue on organic kaolin composite was determined to be:

$$1 / q_e = 0.7066/ C_e + 0.0577 \quad (9)$$

The correlation coefficients R^2 value (0.9904) suggests that the Langmuir isotherm provides a good model of the sorption system. a, and b are equal to 0.0817 liter/mg of Methylene Blue and 17.33 mg of dye/g of adsorbent respectively.
The essential features of the Langmuir isotherm can be expressed in terms of a dimensionless constant separation factor or equilibrium parameter (R_L), which is defined by the following relationship:

$$R_L = 1/ (1+a C_o) \quad (10)$$

Calculating the dimensionless equilibrium parameter (R_L= 0.058) for Methylene Blue shows favorable adsorption since it is less than 1 and greater than zero [27]. Accordingly it can be concluded that the adsorption of Methylene Blue on organic kaolin composite obeys Langmuir isotherm. A similar result was reported for the adsorption of MB on hydrolyzed Oak sawdust [28].
The Freundlich isotherm is the earliest known relationship describing the adsorption equation and is often expressed as:

$$\log q_e = \log K_f + 1/n \log C_e \quad (11)$$

Where (K_f) and (n) are constants incorporating all factors affecting the adsorption process such as adsorption capacity and intensity of adsorption .A plot of ($\log q_e$) versus ($\log C_e$) gives a straight line of slope (1/n) and an intercept of ($\log K_f$) .
The linear Freundlich equation for methylene blue on organic kaolin composite was determined to be:

$$\log q_e = 0.1614 + 0.5533 \log C_e \quad (12)$$

The correlation coefficients coefficient R^2 value (0.9916) suggests that the Freundlich isotherm provides a good model of the sorption system. The related constants were K_f and n are equal to 1.45 and 1.81 respectively, also n is greater than 1 which indicates good adsorption of methylene blue on organic kaolin composite.

3.2.7. *Effect of pH*

The effect of pH of the dye solution on the amount of dye adsorbed was studied by varying the initial pH under constant process parameters as shown in Fig. 11. The percentage removal of methylene blue solutions after an adsorption period of 360 min was increased from 76 to 93.4% between pH values 2 to 12. The removal capacity show the same behavior, where it increased from 1.52 to 1.87 mg/g. The lower adsorption of methylene blue at acidic pH, it could be due to the presence of excess H^+ ions that competed with the dye cation for adsorption sites. As the pH of the system increased (pH > 8), the number of positively charged available sites decreased while the number of the negatively charged sites increased. The negatively charged sites favored the adsorption of dye cation due to electrostatic attraction. The final pH of the solution was found to decrease only slightly (by 0.5–0.7 pH units) after adsorption of methylene blue (in cationic form) with the release of H^+ ions from the active site of the adsorbent surface. The results were in agreement with other literature reports [2, 29-30].
The relationship between % removal and pH can be modeled using the following equation:

$$\text{Max.\% Removal} = 1.8358*(pH) + 73.781 \quad (13)$$

$$R^2 = 0.9186$$

Where: $2 \le pH \le 12$

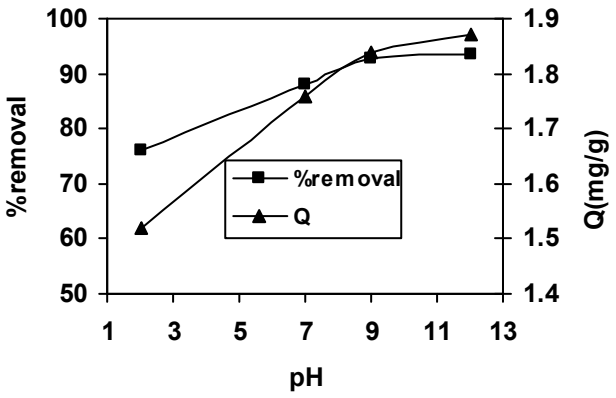


Fig.11. Effect of pH on the percentage dye removal and removal capacity

3.2.8. Effect of Agitation speed

Agitation is an important parameter in sorption phenomena, influencing the distribution of the solute in the bulk solution and the formation of the external boundary film. The effect of stirring speed (in rpm) on the %removal of the original dye concentration was investigated. Fig. 12. illustrates that the % removal seemed to be affected by the agitation speed for values between 0 and 100 rpm, thus confirming that the influence of external diffusion on the sorption kinetic control plays a significant role. In contrast, the small effect of agitation in the range of

100–750 rpm. It is clear that while increasing mixing rate from 750 to 1000 rpm, % removal decreased from 93.3 to nearly 91.1 %. The decrease may be attributed to an increase desorption tendency of dye molecules and/or having similar speed of organic-kaolin composite particles and adsorbate ions (i.e. the formation of a more stable film around the organic-kaolin composite particles). Thus, it can be conducted that the rising of mixing speed to 750 rpm may cause deformation of the stable film and so disappearance of film diffusion control resulted from the organic-kaolin composite particles and adsorbate ions that move at the same speed [31]. The results were in agreement with Batzias F.A., and D.K. Sidiras [32].

The relationship between % removal and rpm can be modeled using the following equation:

Max.%removal=

$$-1E-05*(rpm)^2+0.0111*(rpm)+89.905 \quad (14)$$

$$R^2=0.871$$

Where: $100 \le rpm \le 1000$

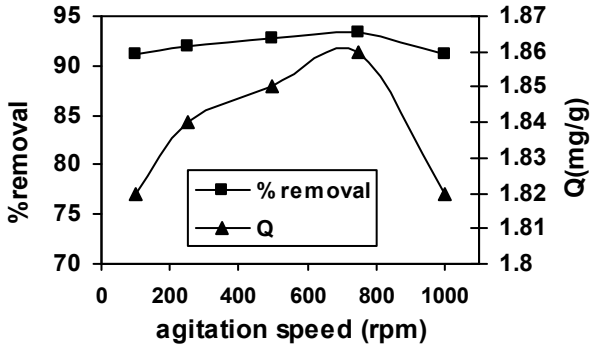


Fig.12. Effect of agitation speed on the percentage dye removal and removal capacity

3.2.9. Effect of Temperature

Temperature has important effects on the adsorption process. As the temperature increased, the rate of diffusion of adsorbate molecules across the external boundary layer and internal pores of the adsorbent particle increased [33]. Changing the temperature will change the equilibrium capacity of the adsorbent for particular adsorbate [33-34].

Fig. 13. depicts effects of different temperatures for methylene blue adsorption on organic-kaolin composite. The% removal by adsorption on organic-kaolin composite increases from 92.7 to 99.2 % by increasing temperature of the solution from 22 to 65 ºC, indicating that the process to be endothermic.

The relationship between % removal and temperature can be modeled using the following equation

Max.% removal =

$$-0.0036*(temp)^2+0.4613*(temp)+84443 \quad (15)$$

$$R^2=0.9889$$

Where: $22 \le Temp. \le 65$

Finally the overall model that can be related to the operating condition such as agitation speed, initial dye concentration, temperature, adsorbent dose and pH of dye solution can be represented by the following equation using regression analysis:

Max. % removal =

0.176*rpm+0.0752*conc+0.2681*temp+2.855*pH+7.523*amount of absorbent (16)

R^2 =0.99

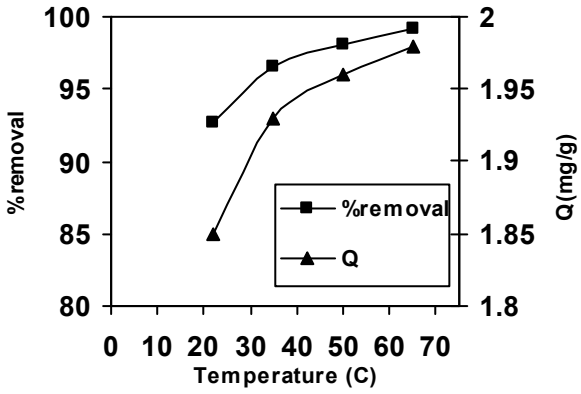


Fig.13. Effect of solution temperature on the percentage dye removal and removal capacity

4. Conclusions

With the presented results and discussions it is clear that Alginate/ polyvinyl alcohol -kaolin composite, can be successfully used as adsorbent for removal of the cationic dye, methylene blue from its aqueous solutions in a stirred tank reactor. Batch studies indicate that Alginate/ polyvinyl alcohol -kaolin composite can adsorb almost 61.6 to 100 % of the methylene blue from its aqueous solutions in the concentration range 500 to 10 mg/L, at 22°C. As the initial ion concentration increased the amount of Methylene Blue adsorbed per gram composite increased. The maximum adsorbed amount of methylene blue was 30.8mg/g organic composite. However, no significant effect was noticed on the percentage dye removal with the variations of both beads diameter and their swelling. Also from this study it was concluded that, the percentage removal of methylene blue increased with increased in the amount of composite, contact time, agitation speed up to 750rpm, initial dye pH, and temperature, which means that an endothermic process tookplace. Adsorption process was mainly chemisorptions, followed first order kinetics, and the half time was 87.7 min. The kinetic studies indicated that equilibrium of methylene blue adsorption on the composite was reached in 360 minute. Isothermal data of methylene blue sorption on Alginate/ polyvinyl alcohol -kaolin composite can be obeyed both Freundlich and Langmuir isotherms.
Numerical correlations that can be used to predict the maximum percentage removal of dye by knowing the operating conditions were developed.

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3/2/2010

Assessment Of Cell Kinetics In The Tissues Of Brownbanded Bamboosharks (*Chiloscyllium Punctatum*) By Using Bromodeoxyuridine (Brdu) And Anti-Brdu Monoclonal Antibody

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Abstract: The 5-bromo-2'-deoxyuridine (BrdU) labeling method has been used to assess the quantity of proliferative potential in organs and tissues in various mammals. For application of this method in fish, it was necessary to determine conditions that optimize the detection of the BrdU epitope. In the present investigation, we investigated the localization of proliferative cells as well as various conditions for detection of S-phase cells in the tissues of adult brownbanded bamboosharks by means of the BrdU immunohistochemical method. Our results demonstrated that BrdU-positive cells were satisfactorily demonstrated in the tissues of brownbanded bamboosharks treated with BrdU at a dose of 6 mg/kg or higher. However, there was no difference in BrdU reactivity between routes of administration, including intravenous, subcutaneous and intraperitoneal injections. BrdU-incorporated cells were detected both in formalin-fixed and 70% ethanol-fixed tissues with enzymatic treatment and acid hydrolysis in the shark tissues, while formalin-and ethanol-fixed brownbanded bambooshark tissues that did not undergo the enzymatic procedure showed no BrdU reactive cells. Importantly, samples were quickly fixed in heated formalin solution and treated with 5N HCL and 0.01% Nagarase at 37 C for 30 seconds to one minute. In conclusion, the BrdU labeling method was useful in a cell kinetic study detecting S-shaped cells in sharks, as in other mammals. [Journal of American Science 2010;6(5):293-299]. (ISSN: 1545-1003).

Keywords: BrdU, IHC, Labeling method, Brownbanded bambooshark (*Chiloscyllium punctatum*)

1. Introduction

Evaluation of cell proliferation in animal tissues is essential for various biomedical studies, among them embryology, histology and oncology. In cell kinetic studies,5-bromodeoxyuridine(BrdU)immunohistochemistry (IHC) has been applied as a standard method, as well as proliferating cell nuclear antigen (PCNA) or Ki-67 IHC.

BrdU is an analog of thymidine which is incorporated into replicating nuclear DNA. It can be detected immunocytochemically by a specific anti BrdU monoclonal antibody (Gratzner 1982). The BrdU IHC method has been used to detect DNA-synthesizing cells, instead of 3H-thymidine autoradiography, in cell cultures and in whole mount preparations (Hamada 1985 and Plickert & Kroiher 1985). This method was applied to teleost fish as an excellent proliferating marker in vivo. Given such factors, BrdU has been manifested for studying the developmental biology of various organs and tissues. It has been detected in the retinas of zebra fish (Hitchcock and Raymond 2004), in the testes of mosquito fish (Koya and Iwase 2004), in the skin of channel catfish (Zhao et al. 2008) and in the gills of killifish (Pierre et al. 2006). Although several lines of evidence have been reported in teleosts, to our knowledge there have been almost no data available in application to shark tissues. Basic techniques for BrdU IHC in sharks, including fixation methods and appropriate pretreatment for antigen retrieval, have not been validated.

The purpose of this study was to establish the optimal BrdU labeling method, including effective administration doses and tissue fixation, in sharks.

2.Materials and Methods

Animals

Six adult brownbanded bamboosharks (*Chiloscyllium punctatum*) were used in this study (Table 1). Two sharks (Nos. 1 and 3) were females, while the others (Nos. 2, 4, 5 and 6) were males. All were sexually mature. The average total body length and body weight of the sharks were 103 cm (range 97-110 cm) and 4.3 kg (range 3.6-5.6 kg), respectively. The sharks were housed in an open circular tank at the Okinawa Churaumi Aquarium (Motobu Town, Okinawa Prefecture, Japan) and fed filleted fish (*Scomber australasicus, Scomber japonicas, Spratelloides gracilis* and others) and loliginids

(*Loligo sp.*) twice a week. Seawater was circulated every two and a half hours and the water temperature was adjusted based on the ambient sea temperature (20-30 C). The sharks exhibited no abnormal features at the time of examination.

BrdU solution dispensation and administration

Five-bromo-2'-deoxy-uridine (BrdU) was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). An amount of 1,000 mg was dissolved in 2 ml of dimethyl sulfoxide (Nakalai Tesque, Kyoto, Japan), and 8 ml of physiological saline solution was added. The resulting solution was put in hot water to dissolve completely. The solution was dispensed at the time of use.

Three of the sharks (Nos. 1-3) were given 6-100 mg/kg of BrdU via a vessel at the axilla of the first dorsal fin under manual restraint, without anesthesia. The other three sharks (Nos. 4-6) were administered 100-200 mg/kg of BrdU subcutaneously or intravenously under general anesthesia. For the anesthesia, we administered 2.0 mg/kg of propofol (Rapinovet®; Schering-Plough K.K., Osaka, Japan) according to Miller et al. (2005) and 5 μg/kg fentanyl (DAIICHI SANKYO PROPHARMA CO., Tokyo, Japan) intravenously. After faint respiration (weak opening and closing of the gill slits) was confirmed, the sharks were taken out of the water and a small incision was made in the skin of the abdomen to insert a needle. To administrate the BrdU, we used a sterilized 50 cm^3 syringe (TERUMO Co. Tokyo, Japan) with a 20-gauge indwelling needle (TERUMO Co. Tokyo, Japan). After injection, the incision was stitched with needle-tipped suture thread (Johnson & Johnson Inc., USA), and the sharks were placed back in the water. Three sharks (Nos. 1-3) were humanely euthanized with an excessive dose of propofol (25-72 mg/kg), while the other three sharks (Nos. 4-6) were euthanized by immersion in an excessive dose of phenoxine (over 2,000 ppm) (2-phenoxyethanol; KANTO CHEMICAL CO., Tokyo, Japan) 2-3 hours after the administration of the BrdU.

Optimization of fixation

To evaluate the sample conditions, including fixation methods, for BrdU-immunoreactivity, the spiral intestine, testis and epigonal organ of each shark were collected, sliced at a thickness of 5 mm, and fixed in 10% neutral buffered formalin at room temperature (RT) for 24 hours, 1 and 2 weeks, or fixed in 70% ethanol at RT for 24 hours. The samples were also immersed in 10% neutral buffered formalin, heated without boiling for 15-30 minutes for rapid fixing, and left overnight.

BrdU immunohistochemistry

The fixed samples were embedded in paraffin wax, and serial sections (4 μm) were cut and stained with hematoxylin and eosin.

Immunostaining of BrdU-incorporated cells was performed as described previously by Moran et al. (1985) and Yanai et al. (1996)**,** and the pretreatment was selected to decide the optimal method in sharks. For BrdU-immunostaining, serial sections were cut and mounted on glass slides coated with 3-aminopropyltriethoxysilane (Sigma, USA) and dried overnight at 50 C. After dewaxing and rehydrating, the serial sections were hydrolysed with HCl (2N or 5N) at RT for 30 minutes to denature the DNA, and neutralized in Palitisch's boric acid-NaCl-borate buffer (pH 7.6) at 4 C for 5 minutes, repeated 3 times. Some of the sections were then subjected to protease digestion using 0.01% or 0.05% protease (Nagarase, Sigma-Aldrich Co.; 37 C; 30 seconds, 1, 3 and 5 minutes) in PBS, 0.04% Pepsin (Wako Pure Chemical Industries, Osaka, Japan) in 0.01N HCl or 0.05% Trypsin (Life Technologies Inc., MD, USA) in Tris solution, while the other sections were not subjected to this treatment. All sections were inactivated with endogenous peroxidase by 0.3% hydrogen peroxide in methanol at RT and subjected to blocking of nonspecific antibody binding (Protein Block, Serum-Free; Dako Cytomation, CA, USA). The primary anti-BrdU monoclonal antibody (Bu20a, Dako Cytomation, CA, USA) was diluted by 1:200 with PBS and the sections were mounted and incubated overnight at 4 C. After being rinsed three times with PBS for a total of 1 hour, the sections were then incubated with biotinylated anti-mouse IgG goat polyclonal antibody (EnVision+® System Labelled Polymer-HRP Anti-Mouse, Dako Cytomation, CA, USA) for 30 minutes at RT. Following this, they were rinsed three times with PBS for a total of 30 minutes. Images of the labeled cells were created using a diaminobenzidine commercial kit (Liquid DAB+ Substrate Chromogen System; Dako Cytomation, CA, USA). Finally, the sections were counterstained using Mayer's hematoxylin.

Assessment of immunolabeling

To compare intensities in BrdU-positive reaction in the nuclei under various conditions, the degrees of reactions were classified into four grades (++, intense; +, moderate; +/-, slight; -, negative). Sstaining of nuclei counterstain was evaluated on the basis of three grades (+, well; +/-, minimum; -, no), which reflected any damage to the tissue caused by the treatment procedures. The samples from shark No. 3 (minimum BrdU dose; of 6 mg/kg) which had been fixed in 10% neutral buffered formalin for 24 hours and those from shark No. 6 (maximum BrdU dose of 200 mg/kg) which had been heated in formalin

solution were used to evaluate the effect of pretreatment for BrdU-immunoreactivities and nuclear stainability. 5N HCl hydrolysis with or without 0.05% Nagarase treatment for 1 minute was performed to evaluate the effect of the dose, the administration routes of the BrdU, and the fixation method for BrdU-immunoreactivities.

3. Results

Optimal pretreatment condition

The cell nuclei incorporating BrdU were easily recognized in the tissue sections by immunohistochemisity for BrdU, and showed upin a mixture of punctuated and diffuse patterns. Tables 2 and 3 show the effects of pretreatment for BrdU-immunoreactivity and on the stainability of nuclear reactivity. Hydrolyse with 5N HCl and protease treated with 0.01% Nagarase proved to produce the best results among all treatment conditions. However, it was found that the longer the protease treatment time, the poorer the nuclear stain ability. The combination of hydrolyse with 2N HCl and protease treatment with 0.05% Nagarase showed good reactivity in almost all samples, particularly in Shark No. 6, while longer-time protease treatment resulted in poor nuclear stain ability. The samples treated with 5N HCl and 0.05% Nagarase exhibited a strong positive reaction in shark No. 6, but pretreatment was so strong that the tissues and nuclei were damaged and denatured. When protease treatment was excluded and only hydrolyse with HCl was used, the positive reaction was weak (Fig. 1). Protease digestion at 37 C for 30 seconds to 1 minute produced the best reactivity condition. A longer digestive treatment resulted in severe damage to the samples; for example, nuclear counterstaining intensity was lower, and there was denaturing of tissues.

Optimal fixing conditions and the effects of doses and routes on BrdU-immunoreactivities

Details of the various doses and administration routes of BrdU, along with the fixing conditions, are shown in Table 4. Frequent BrdU incorporating nuclei could be detected in samples at doses of more than 6 mg/kg of BrdU in all routes when fixed for no less than 24 hours and treated with protease. However, the immunoreactivities became weaker as the fixation time became longer (Fig. **3**). Both the formalin fixation and ethanol fixation were useful for BrdU immunohistochemistry when treated with protease.

4. Discussion

BrdU is a halogenated nucleotide analogue of thymidine that is incorporated into DNA during the S-phase of the cell cycle (Gratzner 1982). Pulse-labeling of DNA with BrdU and subsequent immunohistochemical (IHC) detection of labeled nuclei is increasingly being used to study the rates of cell proliferation in normal and malignant cells in vivo and in vitro (Sapino et al.1990 and Plickert & Kroiher 1985). Although the BrdU method is similar in specificity and sensitivity to the autoradiographic detection of [^{3}H]-thymidine incorporated into DNA, the BrdU method has the advantage of speed, and analysis is more convenient. Additionally, it obviates the need to use radioisotopes (Thornton et al. 1988 and Sapino et al. 1990). BrdU has been used in conventional laboratories for cell kinetics. The ratio of BrdU-incorporated nuclei to the total number of cell nuclei is defined as the labeling index (Nagashima et al. 1985 and Yanai et al. 1996) in human and cattle tissues (Schulz et al. 2005 and Brodeur et al. 2003) and in fish tissues.

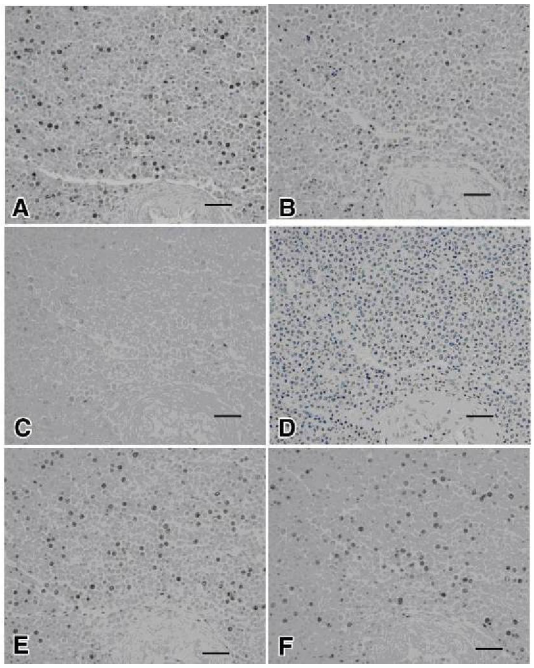

Figure 1. BrdU-immunoreactivities in the epigonal organ of shark C (BrdU 6 mg/kg)fixed with 10%neutral buffer formalin. A: Combination of hydrolyses with 5N HCL and protease treatment with 0.01%nagarase for 30 sec. B:2N HCL and 0.05%nagarase for 30 sec. Intensity of BrdU-reactivities became week.C:5NHCL and 0.05% nagarase for 30 sec.The staining intensity with hematoxylin counterstained also decreasd.D:2N HCL and0.01%nagarase for 30 sec.It could'nt observe the BrdU-positive cells.E:5NHCL and0.01%nagarase treatment for 3 min. F:5NHCL and 0.01%nagarase for 5 min. Long period of protease treatment resulted in decrease of nucleic stainability .Bar=30um.

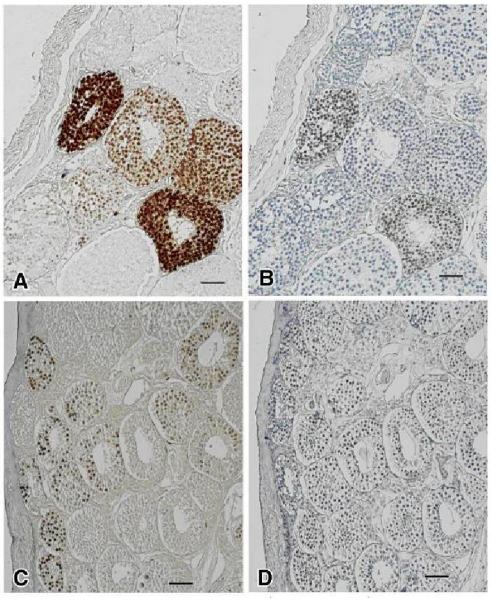

Figure 2. BrdU-possitive cells in the testis of shark D (BrdU 200 mg/kg s.c). A: Sample was fixed in 10%neutral buffer formalin, and performed in combination of hydrolyse with 5N HCL and protease treatment with 0.01% nagarase. B:Fixed in10%formalin and no protease treatement.C:Fixed in 70%ethanol, 5NHCL and 0.01% nagarase treatment were done. D: Fixed in 70% ethanol, without protease treatment. The stainabilities of BrdU were markedly decrease. Bar=30um.

In the present study, BrdU-positive cells were satisfactorily demonstrated in the tissues of brownbanded bamboosharks treated with BrdU at doses of 6 mg/kg or higher. In laboratory animals such as mice and rats, optimal doses for BrdU immunohistochemistry were reported to be 10 mg/kg （Sapino et al, 1990）and 100 mg/kg （Nagasawa 1983）respectively. Yanai *et al* (1996) reported that administration of BrdU at doses of 2 mg/kg produced sufficient reactivity in cattle. However, Alfei et al (1993 and 1994) and Elger et al (2003) applied BrdU to fish tissues at doses of 100 mg/kg for common carp (*Cyprinus carpio*) and 150 mg/kg for skate (*Leucoraja erinacea*). BrdU application is thought to be a compound with a lower level of genetic damage. In one study, human patients with brain tumors were administered BrdU at doses of 500-1,000 mg/day for 4-6 weeks without any serious side effects (Hoshino 1991). The challenge now is to decrease the doses administered in order to reduce costs, as well as to avoid gene damage.

The present investigation revealed that there were no differences in BrdU reactivity among various routes of administration, including intravenous, subcutaneous and intraperitoneal injections. With all three routes, it was possible to examine cell kinetics using BrdU immunohistochemistry in shark tissues.Other BrdU administration routes have also been reported with respect to intramuscular application (Schulz et al. 2005) and oral application (Jecker et al. 1997).

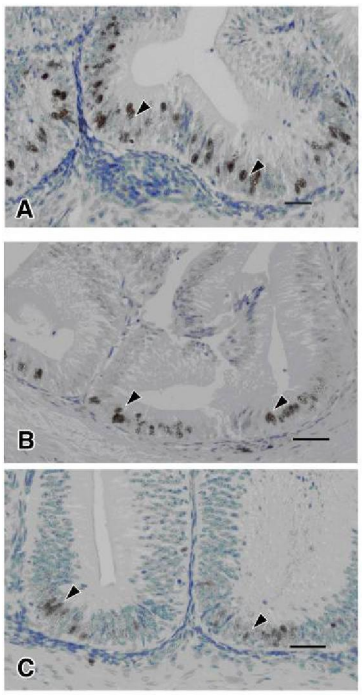

Figure 3 .BrdU-positive cells in the spiral intestine of shark A(BrdU 100 mg / kg i.v.)Samples were fixed with 10% neutral buffer formalin, treated with 5N HCL and 0.01% Nagarase. A : Fixed for 24 h. Fixed for 1 week. C: Fixed for 2 weeks. Nuclear positive reactions were observed in epithelial cells in crypts(arrow heads).Bar =30um.

Many factors have been reported to influence the immunoreactivity of the BrdU epitope; prominent among these is the method of tissue fixation (Mitchell et al. 1985; Schutte et al. 1987).

Our findings revealed that BrdU-incorporated cells were detected both in formalin-fixed and 70% ethanol-fixed tissues with enzymatic treatment and acid hydrolysis in the shark tissues. However cattle, dog and cat tissues fixed in 70% ethanol followed by hydrolysis alone showed sufficient reactivity (Yanai et al. 1996 and Ishikawa et al. 2005). Some reports recommended the addition of acetic acid to the alcohol to decrease shrinkage artifact, soften the tissue, and aid in the preservation of nucleoproteins (Carson 1990). In another effort to achieve a balance between the advantages and limitations of alcohol fixation, the use

of mixtures of methanol, acetic acid, and chloroform (methacarn) has been proposed as producing both excellent preservation and increased immunoreactivity (Mitchell et al. 1985).

The present study revealed that ethanol or formalin-fixed brownbanded bambooshark tissues that did not undergo the enzymatic procedure did not exhibit BrdU reactive cells. Consequently, the optimal condition for superior BrdU reactivity as well as improved histological preservation was obtained when the samples were quickly fixed in heated formalin solution and treated with 5N HCl and 0.01% Nagarase at 37 C for 30 seconds to 1 minute. Taken together, the samples fixed with formalin for a longer period of time resulted in less active BrdU immunoreactivities, and a longer digestion time resulted in severe histological damage.

Our investigation revealed that the most important factor in improved BrdU immunohistochemistry is thought to be quick and sufficient fixation. As an alternative to enzymatic digestion, heat-induced epitope retrieval (HIER) is useful in recovering immunoreactivity of BrdU epitopes in formalin-fixed tissue. This concurs with the findings of Shi et al. (1991) and Lan et al. (1995). The required duration of enzyme digestion for unmasking varies according to the length of fixation, but this approach can be accompanied by an increase in nonspecific staining, resulting in false-positive staining (Bak and Panos 1997).

Finally, the present study revealed that the BrdU labeling method may be useful in cell kinetic studies aimed at detecting S-phase cells in sharks, as in other animals and in humans.

Table 1: Sharks, doses of bromodeoxyuridine and routes of administration.

Shark No.	Sex*	Sexual Maturity	Total length(cm)	Body weight(Kg)	Dose (mg / Kg)	Route**
1	F	mature	97	4.1	100	i.v.
2	M	mature	100	4.0	25	i.v.
3	F	mature	110	5.6	6	i.v.
4	M	mature	104	3.9	200	s.c
5	M	mature	107	4.9	100	i.p
6	M	mature	100	3.6	200	i.p .

*M, male; F, female

** i.v., intravenous; s.c., subcutaneous; i.p., intraperitoneal.

Table 2 : The effects of pretreatment on BrdU-immunoreactivities

Protease	Protease Treatment Time	Shark No.3*		Shark No. 6 **	
		5N HCl	2N HCl	5N HCl	2N HCl
0.05%	30 sec	+	+	++	++
Nagarase	1min.	+/-	+	++	++
	3min.	N.T.#	+	++	++
	5min.	N.T.	+	N.D# #	++
0.01%	30 sec	++	-	++	+
Nagarase	1min.	++	-	++	+
	3min.	++	+	++	+
	5min.	++	+	++	+
0.05% Trypsin	1min.	N.T.	N.T.	++	+
0.04% Pepsin	1min.	N.T.	N.T.	+/-	+/-
No treatment	-	+/-	N.T.	+/-	+/-

++, intense; +, moderate; +/-, slight; - ,negative

N.T.#; Not tested.

N.D# #;Not detected because of heavy tissue denaturation by treatment procedure.

Shark No.3*Minimum dose of BrdU(6mg/Kg i.v.).

Shark No. 6 **; maximum dose of BrdU(200mg/Kg i.p.).

Table 3 : The effects of pretreatment on nuclear stainability.

Protease	Protease Treatment Time	Shark No.3*		Shark No. 6 **	
		5N HCl	**2N** HCl	**5N** HCl	**2N** HCl
0.05% Nagarase	30 sec	-	+	+/-	+
	1min.	-	+/-	-	+
	3min.	N.T.#	+/-	-	+
	5min.	N.T.	+/-	-	+
0.01% Nagarase	30 sec	+	+	+	+
	1min.	-	+	+	+
	3min.	-	+	+	+
	5min.	-	+	+	+
0.05% Trypsin	1min.	N.T.	N.T.	-	+
0.04% Pepsin	1min.	N.T.	N.T.	+	+
No treatment	-	+	N.T	+	+

+; good stained, +/-; poorly stained, -; not stained.
N.T.#; not tested.

Table 4: The effects of Dose, Route and Fixation on BrdU-immunoreactivities.

Shark No.	Dose (mg/Kg)	Route	Fixation*	10% neutral buffered formalin		70% ethanol	
				Protease**	No protease	Protease**	No protease
1	100	i.v.	24hr	++	-		
			1w	+	N.T.		
			2w	+/-	N.T		
2	25	i.v.	24hr	++	N.T.		
			1w	++	N.T.		
			2w	+	N.T.		
3	6	i.v.	24hr	++	+/-		
			1w	+	N.T.		
			2w	+/-	N.T.		
4	200	s.c.	heat***	++	+/-	++	+/-
5	100	i.p.	heat	+	N.T.	++	N.T.
6	200	i.p.	heat	++	+/-	++	+/-

++, intense; +, moderate; +/-, slight; -,negative
*; Fixation time or method.
** ; 0.05% Nagarase treatment for 30 sec.
***; The samples heated in formalin for 15-30 min. and fixed overnight.

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