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CONTENTS

Effect of Exposure to Mercury on Health in Tropical *Macrobrachium Rosenbergii*

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Abstract: The effects of Hg on mortality, resistance and bioconcentration in the tropical giant freshwater *Macrobrachium rosenbergii* were studied. Mortalities of prawns exposed to mercury doses below 100 µg L^{-1} were significantly lower than those exposed to higher doses. After 96 hours prawns exposed to >400 µg $L^{-1}$of mercury had a greater reduction in total haemocyte count and phagocytic activity than those exposed to lower concentrations. Bioconcentration of mercury (Hg) in the gills, hepatopancreas and muscles was variable. Mercury accumulated in gills and hepatopancreas but Hg accumulation in the muscles only increased marginally. *Macrobrachium rosenbergii* manifested histopathological alterations in gills, hepatopancreas and muscles when exposed to different concentrations of mercury.

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Keywords: *Macrobrachium rosenbergii*, mercury, total haemocyte count, phagocytic activity

1. Introduction:

The contamination of fresh water with a wide range of pollutants has become a matter of concern over the last few decades (Voegborlo *et al.*, 1999; Dirilgen, 2001; Vutukuru, 2005). The natural aquatic systems may extensively be contaminated with heavy metals released from domestic, industrial and other man-made activities (Conacher, *et al.*, 1993; Velez and Montoro, 1998). Heavy metal contaminations may have devastating effects on the ecological balance of the recipient environment and a diversity of aquatic organisms (Ashraj, 2005; Vosyliene and Jankaite, 2006; Farombi, et al., 2007).

The toxic effects of heavy metals have been reviewed, including bioaccumulations (Adami *et al.*, 2002; Waqar, 2006). Heavy metals are surrounded with great care and special importance due to their highly toxic effects on fish as they affect survivability, growth and reproduction. The immune system in all living creatures and the immune response come about as protective mechanism to protect the fish from attack by various microorganisms and parasites (Vorkamp et al., 2004; Andreji et al., 2005). Suppression of immune system and immune response may result from action of several pollutants including heavy metals which provide opportunities for the entering of many pathogens.

Mercury (Hg) is one of the most toxic heavy metals in our environment including the lithosphere, hydrosphere, atmosphere and biosphere (Barbosa *et al*., 2001). So, Hg was the most toxic of all metals in *Penaeus monodon*, followed by Cu, Cd and Zn and that Cd toxicity was the most rapid (Chen, 1979).

In freshwater prawn, *Macrobrachium malcolmsonii*, both Hg and Cu had inhibitory effects on the functions of the hemocytes but, the difference between the two metals is the time and concentration at which the effects become apparent (Alcivar-Warren *et al.,* 2006). In decapod crustaceans, 3 types of circulating hemocytes are recognized: hyaline, semi-granular and large granular cells (Tsing *et al*., 1989). They are involved in cellular immune responses that include phagocytosis and constitute the primary method of eliminating microorganisms or foreign particles (Bayne, 1990). In addition to phagocytosis, hemocytes are involved in the process of coagulation and production of melanin via the prophenoloxidase system (Johansson & Söderhäll 1989, Söderhäll *et al*., 1996). Enzymes for the prophenoloxidase system are present in the granular hemocytes and released as proenzymes upon stimulation by microbial cell components such as 1, 3-glucan or lipopolysaccharide from fungal cell walls, and activated by a serine protease (Söderhäll 1983, Smith *et al.*, 1984, Söderhäll *et al*., 1996). Several physico-chemical parameters and environmental contaminants have adverse effect on the immune response of crustaceans (Le Moullac & Haffner, 2000). Environmental toxicants have been reported to cause a reduction in hemocyte count in the common shrimp *Crangon crangon* (Smith & Johnston 1992).Moreover , Heavy metals like mercury and cadmium are known to be accumulated

in marine organisms, and cause rapid genetic changes (Nimmo *et al.,* 1978, Nevo *et al*., 1986).

Histopathological examination has been increasingly recognized as a valuable tool for field assessment of the impact of environmental pollutants on fish (Heath, 1995; Teh et al., 1997). Specific lesions occurring in organs of fish exposed to toxic substances under laboratory conditions help to identify biomarkers of exposure. Many authors have studied the histopathological effects of mercury on fish exposed to water-borne inorganic mercury (inorganic Hg) on liver, kidneys, gills, olfactory epithelium, and spleen (Oliveira-Ribeiro et al., 2002; Samson and Shenker, 2000).

Knowledge of the toxicity of mercury will be helpful to water quality management in fish farms so; this study evaluates the impact of the short-term mercury exposure on survival, resistance, tissue bioconcentration and histopathological alterations in gills, hepatopancreas and muscles in tropical freshwater prawn (*Macrobrachium rosenbergii*).

2. Materials and Methods

Experimental design

Experiments were carried out in Department of Veterinary Hygiene and Management, Faculty of Veterinary Medicine, Cairo University

Fresh water was adjusted with the desired parameters as follow: temperature of 20-28 °C, pH 7-7.8, dissolved oxygen 5-8 mgL^{-1}, salinity 2 ppt, hardness 100-150 ppm Ca(CO)3, total ammonia less than10 ppm, nitrate 20 ppm and nitrite1 ppm) according to, New (1995).

A stock mercury solution was prepared as follow: 135.3 mg Hg Cl_2 salt dissolved in a solution composed of 700 mL water plus 1.5 mL concentrated nitric acid and then diluted up to 1000 mL with water (1.00 mL = 100 µg Hg). Seven different concentrations of Hg were then prepared from the stock solutions (10, 50, 100, 200, 300, 400, 500 μgL^{-1}).

Macrobrachium rosenbergii were obtained from commercial farms in Alexandria and Al-Kalubia, Egypt, and acclimated in the laboratory for two days before the experiment was done.

The toxicity tests were conducted according to the standard procedures of FAO (1985). Seven concentrations of Hg (10, 50, 100, 200, 300, 400 and 500 μgL^{-1}) and a negative control were set up. Ten shrimps each of 13.2 to 16.5 g body weight with an average of 14.85±0.15 g were transferred from the holding tanks into the control and experimental tanks. Three trials were carried out for each concentration. The aquaria were aerated continuously, while the test solution in each tank was changed with the appropriate fresh solution every 24 hrs to maintain the definite concentration of Hg for 96 hrs. Observations for mortality were made twice (10.0 am and 6.0 pm) daily.

Analysis

The 96 hrs LC_{50} values were calculated using probit analysis according to Finney (1971).

Cell counts

Hemolymph (100 µl) was sampled individually at the beginning of each test and at 96 h post exposure to Hg. It was withdrawn from the ventral sinus of each prawn into a 1 mL sterile syringe containing 0.9 mL anticoagulant solution (trisodium citrate 0.114 M, sodium chloride 0.1 M, pH 7.45, osmolality 490 mOsm kg^{-1}). A drop of the anticoagulant-hemolymph mixture was placed on a hemocytometer to measure total hemocyte count (THC) using an inverted-phase contrast microscope.

Culture of *Lactococcus garvieae*

The bacterial strain *L. garvieae* isolated from diseased *Macrobrachium rosenbergii* after artificial infection was used in this study. The bacterium was cultured on tryptic soya agar (TSA) for 24 h at 28 °C before being transferred to 10 mL of tryptic soya broth (TSB) for 24 h at 28 °C as a stock culture. The stock cultures were centrifuged at 7155 x g for 15 min at l4 °C and then the supernatant fluid removed and the sediment resuspended in a saline solution (0.85 NaCL) and adjusted at 10^{10} cfu mL^{-1} as stock bacterial suspensions for testing.

Phagocytic activity of *M. rosenbergii* to *L. garvieae*

After 96 hrs of Hg exposure in each treatment, prawns were injected in the cephalothorax with 20 µl of the bacteria suspension (10^{10} cfu mL^{-1} in 0.85% NaCI) resulting in 2 x 10^8 cfu prawn l^{-1}. After injection, the prawns were held in their respective solutions for 3 hours. Hemolymph (200 µl) was collected from the ventral sinus and mixed with 200 µl of sterile anticoagulant containing sodium citrate (0.8 g), EDTA (0.34 g), Tween 80 (10 µl) and distilled water (100 mL with pH of 7.45).

Phagocytic activity was measured using the method described by Weeks-Perkins et al., (1995) where 200 µl of diluted hemolymph sample was mixed with 0.2 mL of 0.1% paraformaldehyde for 30 min at 4 °C to fix the hemocytes. They were then centrifuged at 800x g at 4 °C, washed and resuspended in 0.4 ml of sterile phosphate buffer

solution. The suspension (50 µl) was spread onto a slide glass and air-dried and stained with Diff-Quick stain according to Skipper R and DeStephano (1989). 200 hemocytes were counted using light microscope and the phagocytic rate was estimated as follows: PR=[(phagocytic hemocytes) /(total hemocytes)] x 100.

Preparation and analysis of tissue samples

Were carried out in two procedures. Procedure A: Each sample was represented by 0.5 gram of tissues dissected from the gills, hepatopancreas, and muscles, then placed in a clean screw-capped tube and digested according to the method described by Finerty *et al.,* (1990).

Procedure B: The measurement of the mercury concentration in examined tissue samples was carried out at minimal temperature for all samples where 0.5 gram macerated tissues was digested according to the technique described by Diaz *et al.,* (1995). 5 ml stannous chloride solution were added to the obtained solutions to reduce mercury to elemental form and then analyzed by using Atomic Absorption Spectrophotometer equipped with mercury hydride system "MHS" "Cold Vapour Technique".

Histopathological examination

Tissue specimens from gills, hepatopancreas and muscles of experimental *M rosenbergii* were taken and fixed in 15 % buffered neutral formalin. Tissues were processed to obtain five micron thick paraffin sections then stained with hematoxylin and eosin, (H&E) according to the methods described by Bancroft *et al.,* (1996) and examined under light microscope.

Bioconcentration factor (**BCF**) is the concentration of a particular chemical in a biological tissue per concentration of that chemical in water surrounding that tissue. That is, a dimensionless number representing how much of a chemical is in a tissue relative to how much of that chemical exists in the environment (Chiou, 2002).

$$BCF = \frac{Concentration_{Organism}}{Concentration_{Environment}}$$

Tissues with BCF greater than 1,000 are considered high, and less than 250 low, with those between classified as moderate.

Statistical analysis

Data were analyzed using Analysis of Variance (ANOVA) and means were separated by the Duncan post-hoc test at a probability level of < 0.05 (SAS, 2000).

3. Results

Mortality

Tropical *Macrobrachium rosenbergii* exposed to Hg had significantly lower THC and Phagocytic activity than the control ones. After 96 hours, mean (±SD) mortality of prawns in control tanks (0 Hg) was 4±2.20 % and significantly lowers *(P < 0.05)* than that of prawns in all other treatments as shown in Table 1 and Figure 1-a. 96 h(s) post-exposure, mortality rates of prawns exposed to 10-50 μgL^{-1} concentrations of mercury were significantly lower *(P < 0.05)* than those exposed to higher concentrations (100 μgL^{-1} or greater), but were not significantly different from each other *(P < 0.05)*. In Table 1 and Figure 1-a,mortality rates of prawns exposed to 100, 200, 300, 400 and 500 μgL^{-1} of mercury were significantly higher *(P < 0.05)*, with means of (±SD) 23 ± 0.70%, 40 ± 0.70%, 43 ±0.70%, 60 ± 0.20% and 70 ± 0.21%, respectively .

Resistance

Table 1 and Figure1-b,show the significant reduction (P < 0.05), in THC and Phagocytic activity for prawns exposed to 100, 200, 300, 400 and 500 μgL^{-1} of mercury with means of (±SD) 170±19 , 70±7.00 ; 160±28 , 62±7.00; 145±21, 50±2.70; 138±19, 40±0.70 and 132±16 , 0 respectively.

Bioconcentration and residues of mercury (Hg) in different tissues of tropical *M. rosenbergii*

Table 2 and Figure 2, show the residues of mercury (Hg) in gills, hepatopancreas and muscle tissues of tropical *M. rosenbergii* which were higher in the hepatopancreas> gills > muscles. The BCF for 96 h(s)-exposure were less than 250 in hepatopancreas, gills and muscles respectively.

Hepatopancreas:

The rate of accumulation of mercury was maximum in hepatopancreas of exposed prawns and no detectable amount of mercury was observed in the hepatopancreas of control prawns as well as in exposed prawns to very low concentration of mercury (10-50 μgL^{-1}). The rate of accumulation increased along with the increasing of mercury concentration reaching 11.12± 0.032 at 400 μgL^{-1}.

Gills:

As with the case of hepatopancreas, mercury could not be traced in the gills of the control prawn as well as in exposed prawns to very low concentration 10 μgL^{-1}, even though the quantity of accumulated mercury was relatively less in the case of gills (9.14± 0.042 at 400 μgL^{-1}) when

compared to hepatopancreas, the pattern of accumulation showed a more or less continuous increasing trend.

Muscles:

The rate of accumulation of mercury in muscle increased along with exposure to increased mercury concentration. The mean quantity of mercury residue at 400 $\mu gL^{-1}$was 1.025± 0.002. The rate of accumulation was less as compared with other tissues.

The LC50 of Hg in tropical *M. rosenbergii*

The 96-hour LC_{50} values of mercury in tropical *M. rosenbergii* were calculated using probit analysis, to be 430 $\mu g\ L^{-1}$ (Fig.3).

Histopathological alterations in different tissues of tropical *M. rosenbergii*

Results of the present study revealed that, tropical M. rosenbergii manifested histopathological changes in gills, hepatopancreas and muscles. Exposure to concentration 0.4 mg L^{-1} for 96 hours resulted in profound structural changes as shown in Figures 4, 5 and 6.

Gills showed mild congestion, swelling and edema at low doses of Hg exposure. Severe edema, hyperplasia, at highest doses of intoxication was observed. Moreover, accumulation of hemocytes in the hemocoelic space; swelling of the lamellae; abnormal gill tips; and hyperplastic, necrotic, and clavate–globate lamellae in the gills (Fig.4).

Hepatopancreas showed hemocytic infiltration in the interstitial sinuses, an increased number of hemocytes, thickening and ruptures of the basal laminae, and necrosis of the tubules were observed in the hepatopancreas (Fig.5 a&b).

Muscular tissues showed several histopathological alterations. The pathological findings included degeneration in muscles with infiltration and aggregations of hemocytes between them and focal areas of necrosis. Also, atrophy of muscle bundles, edema, hyaline degeneration and splitting of muscle fibers were seen (Fig.6 a&b).

Table 1: Effect of mercury 96 hrs-exposure on mortality, total hemocyte count (THC) and phagocytosis in tropical freshwater prawns, *Macrobrachium rosenbergii.*

Hg[1] Conc.	Mortality %	Immune response	
		T H C[2]	Phagocytic%
0	4±2.20	186±60	91±7.50
0.01	14±1.70	185±36	90±.9.78
0.05	14±1.60	179±22*	88±6.00
0.10	23 ± 0.70*	170±19*	70±7.00*
0.2	40 ± 0.70*	160±28 *	62±7.00*
0.3	43 ±0.70*	145±21*	50±2.70*
0.4	60 ± 0.20*	138±19*	40±0.70*
0.5	70 ± 0.21*	132±16 *	0

[1]: Hg^{2+} mg L^{-1} , [2]: x $10^5 mL^{-1}$, *Significant ($P < 0.05$). Values are means± SD (n = 4 prawns in each case).

Table 2. Bioaccumulation of mercury in tissues of tropical freshwater prawns, *Macrobrachium rosenbergii,* exposed to mercury for 96 hours.

Bioaccumulation in tissues[2]			
Conc. of Hg[1]	Gills	Hepatopancreas	Muscles
0	0	0	0
0.01	0.005 ±0.00	0.002±0.006	0
0.05	0.01 ±0.009	0.025±0.008	0.001±0.0001
0.10	2.15 ±0.018	5.03±0.012	0.42±0.013
0.2	5.066 ±0.021	7.14±0.098	0.52±0.005
0.3	7.28± 0.032	10.06±0.011	0.83±0.31
0.4	9.14± 0.042	11.12± 0.032	1.025± 0.002
0.5	9.00 ±0.011	11.12 ±0.022	1.015±0.021

[1]: Hg^{2+} mg L^{-1} , [2] :Hg^{2+} $\mu g\ gm^{-1}$ = mg kg^{-1} = ppm. Values are means± SD.

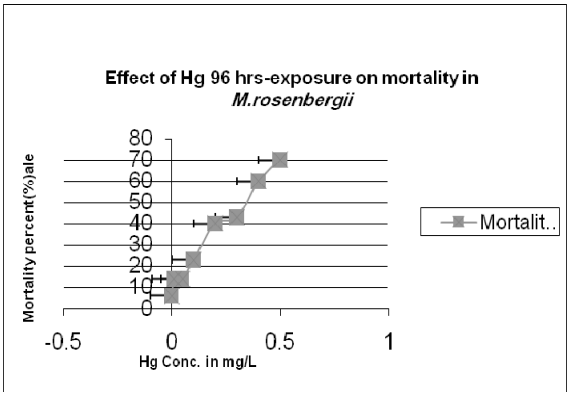

Fig.1-a: mortality rates of prawns exposed to 100, 200, 300, 400 and 500 µg L^{-1} of mercury were significantly higher ($P < 0.05$), with means of (±SD) 23 ± 0.70%, 40 ± 0.70%, 43 ±0.70%, 60 ± 0.20% and 70 ± 0.21%, respectively.

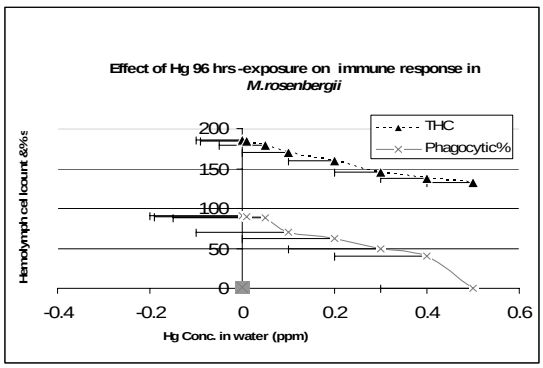

Fig.1-b :the significant reduction ($P < 0.05$), in THC and Phagocytic activity for prawns exposed to 100, 200, 300, 400 and 500 µg L^{-1} of mercury with means of (±SD) 170±19, 70±7.00 ; 160±28 , 62±7.00; 145±21, 50±2.70; 138±19, 40±0.70 and 132±16 , 0 respectively.

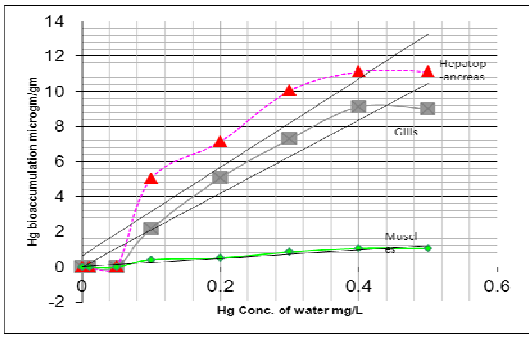

Fig.2: The residues of mercury (Hg) in gills, hepatopancreas and muscle tissues of tropical *M. rosenbergii* which were higher in the hepatopancreas> gills > muscles. The BCF for 96 h(s)-exposure were less than 250 in hepatopancreas, gills and muscles respectively.

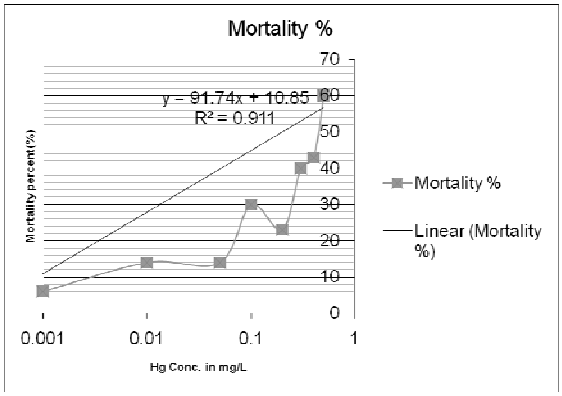

Fig.3: The 96-hour LC_{50} values of mercury in tropical *M. rosenbergii* were calculated using probit analysis, to be 430 µg L^{-1}.

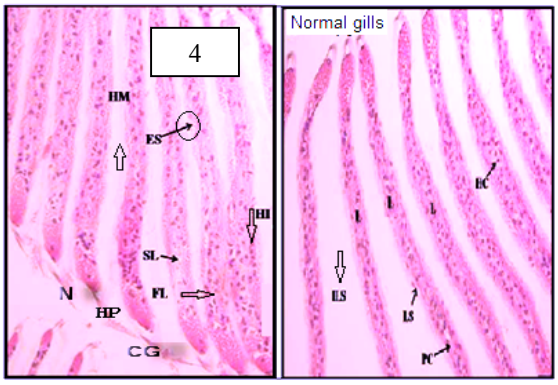

Fig.4 Cross-sections of gill lamellae of M. rosenbergii after 96 h of exposure to a control solution and Hg . Control prawn showing normal lamellae (L) with uniform interlamellar spaces (ILS), the lamellar sinus (LS), and pillar cell (PC) and hemocyte (HC) in the lamella. Mercury exposed prawn showing hemocytic infiltration (HI), swollen (SL) and fused (FL) lamellae, enlargement of the lamellar sinuses (ES) and hyper-mucus (HM) in the interlamellar spaces, necrosis (N) and hyperplasia (HP) tip of lamellae. H&E stain, (x200).

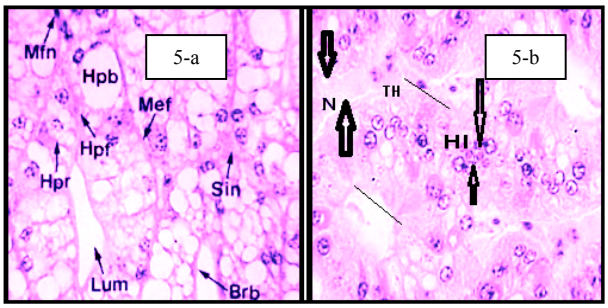

Fig .5-a: Cross sections of hepatopancreatic tubules: normal lumens (Lum), tubule tissues (Hpf: F-cell; Hpb: B-cell; Hpr: R-cell; Mfn: myoepithelial cell nuclei; Mef: myoepithelial layer; Brb: microvillus brush borders) and hemal sinuses (Sin) between tubules. Fig.5-b: Cross sections of hepatopancreatic tubules: showed hemocytic infiltration (HI) in the interstitial sinuses, an increased number of hemocytes, thickening (TH) and ruptures of the basal laminae, and necrosis (N) of the tubules (arrowheads). H&E stain, (x200).

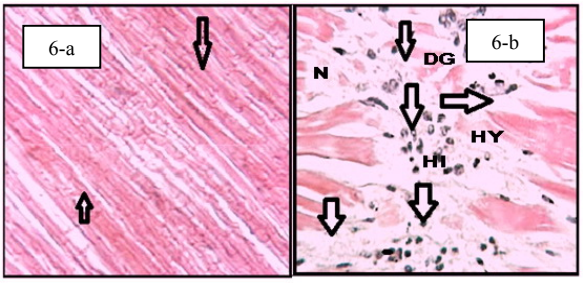

Fig.6-a: Longitudinal sections of muscle tissue Healthy prawn tissue showing normal muscle fibres. Fig.6-b: Cross section showing degeneration (DG), focal areas of necrotic musculature (N) infiltrated by hemocytes (HI) (arrowheads). Also, atrophy of muscle bundles, edema, hyaline degeneration (HY) and splitting of muscle fibers were seen. H&E stain, (x200).

4. Discussion:

After 96 h(s) post-exposure, the survival of tropical *M. rosenbergii* exposed to 10-50 μgL^{-1} concentrations of mercury were significantly greater *(P < 0.05)* than those exposed to higher concentrations (100 μgL^{-1} or greater).

Cheng (1979) tested Hg, Cu, Cd and Zn in *Penaeus monodon* and found that, Hg was the most toxic of all metals, followed by Cu, Cd and Zn. In our study in which prawns exposed to 100, 200, 300, 400 and 500 μgL^{-1} of mercury concentrations had significantly greater reduction in THC and phagocytic activity than prawns exposed to lower concentrations (10 – 50 μgL^{-1}), *(P* < 0.05). Our findings may confirm the results reported by Cheng (1979).

Several scientists have investigated the effects of environmental contaminants on crustacean defense mechanisms. Carolina (2009) studied the effect of Mn on the immune system of marine invertebrates and found that Mn severely suppresses the number of circulating hemocytes in *Nephrops norvegicus* by inducing apoptosis. However, Mn increased the number of circulating hemocytes in *Asterias rubens* and at the same time affected their ability to phagocyte. Circulating hemocytes can be affected by extrinsic factors in several species of decapods crustaceans (Truscott & White 1990, Le Moullac et al. 1998, Le Moullac & Haffner 2000, Cheng & Chen2001).

In freshwater prawn, *Macrobrachium malcolmsonii* , Alcivar-Warren, (2006) reported that both Hg and Cu had inhibitory effects on the functions of the hemocytes, the difference between the two metals being the time and the concentration at which the effects become apparent and suppression of total counts of hemocytes (hemopoeisis) appears to involve metal transport (96 h LC50 for Hg = 0.145 mgL^{-1} when 0.024 mgL^{-1} Hg (1/6th of LC50) was used, the total hemocyte count, percentile phagocitosis and superoxide anion production was significantly lower than the controls.

The immune system in all living creatures and the immune response come about as protective mechanism to react and protect the fish from attack by various microorganisms and parasites (Vorkamp et al., 2004; Andreji et al., 2005). Suppression of immune system and immune response may results from action of several pollutants including heavy metals which provide opportunities for entering of many pathogens.

In the present study the highest bioaccumulation of mercury was observed in the organs mainly implicated in metal intoxication (hepatopancreas). Mercury (Hg) in tissues was high in the hepatopancreas> gills > muscles.

Tropical M. rosenbergii manifested histopathological changes in gills, hepatopancreas and muscles. Exposure to concentration 400 μgL^{-1} for 96 hours resulted in profound structural changes as shown in Figures 4, 5 and 6.

Victor et al., (1990) studied the effect of HgCl2 on Macrobrachium idae exposed to 1×10^{-3} mg/L of HgCl2 and found that there were Hyperplastic gill lamellae engorged with hemocytes as a specific toxic reaction in these prawns, then hemocytes were released into the interlamellar

spaces through necrotic regions and blanketed the entire gill lamellae.Also, Piyan et al (1985) revealed that stage 1 larvae had the lowest threshold lethal concentration (TLC) of mercury, 0.041 ppm Hg, while the post-larvae had a TLC of 0.325 ppm Hg.

Similar results were observed by Mela et al. (2007) and Frías-Espericueta et al., (2008) who studied the effect of three concentrations of Cu (3.512, 1.756 and 0.877 mg L^{-1}) on juvenile Litopenaeus vannamei and found that there were severe time- and dose-dependent structural damages, such as necrosis, loss of regular structure and infiltration of hemocytes in the gill tissues, as well as atrophy, necrosis and irregular tubular structure in the hepatopancreas, similar to that reported by Li et al., (2009).

The higher Hg concentration in the hepatopancreas suggested that this organ plays a role in metal storage and/ or in detoxification process by a metal binding component (White and Rainbow 1986). In crustacean, the hepatopancreas is the primary organ responsible of absorption and storage of ingested materials (Vogt et al., 1989; Johnston et al., 1998). Also, this organ is involved in the synthesis of digestive enzymes and the detoxification of xenobiotics (Barker and Gibson, 1979; Icely and Nott, 1992) were apparent as the hepatospleen consider the organ of detoxification, excretion and binding proteins such as metallothionein (MTs). The metal-binding proteins that present in the nuclei of hepatocytes suggested an increase in cell damage (De Smet and Blust, 2001).

Liver of fish is sensitive to environmental contaminants because many contaminants tend to accumulate in the liver and exposing it to a much higher levels than in the environment, or in other organs (Heath 1995). Pandey et al., (1994) described the alterations in liver and intestine of Liza parsia exposed to Hg Cl_2 (0.2 mg Hg L^{-1}) for 15 days. Similarly, Oliveira Ribeiro et al. (2002) reported serious injuries in gills and olfactory epithelium of Salvelinus alpinus exposed to 0.15 mg Hg L^{-1}.

M. rosenbergii are able to tolerate low levels of mercury pollution but, high levels lead to cellular injury and tissue damage in hepatopancreas. Hg^{2+} disrupted the histostructures of the hepatopancreas, causing decreases in activities of pepsin, tryptase, amylase, and cellulose, which are synthesized in the hepatopancreas, and worst survival rate of the crabs in 0.30 mgL^{-1} (Zhao et al., 2010).

Mean mercury accumulation in muscles of M. rosenbergii at 400 µg L^{-1} was 1.025 µg $g^{-1}$and the maximum permissible limits recommended by WHO, (1984) is 1µg g^{-1}. The recorded results of mercury concentrations in muscles of M. rosenbrgii were higher than the permissible limits intended by Spain: Boletin Official del Estado (1991), Schumacher and Domingo (1996) in Spain [l µg g^{-1}], FAO/WHO (1992) [0.5 µg g^{-1}] and Quality Control (E.O.S.Q.C) (1993) [0.1 µg g^{-1}].

Frías-Espericueta et al. (2009) found that the mean contents of Cd, Cu, Pb and Zn of the white shrimp (Litopenaeus vannamei) were lower in the muscle than in the corresponding hepatopancreas samples, which is in agreement with most literature on the metal contents in the tissues of different aquatic organisms because the hepatopancreas is the main organ for metal accumulation (Roesijadi and Robinson 1994, Yang et al. 2007).

5. Conclusion:

This study reveals an important precaution for prawn cultivation. Knowledge of the toxicity of mercury will be helpful to water quality management in fish farms with reference to prawn culture since it affected the immune response and cause a reduction in hemocyte count in Macrobrachium rosenbergii. Mercury (Hg) caused a decrease in hemocyte-related functions of tropical freshwater shrimp, including hemocyte count and percentile phagocitosis. Caution should be exercised against water source contamination and exposure to industrial pollution. For this reason, the assessment of risk and the safe levels of toxic substances added to any natural environment through human or natural sources, should not neglect the effects on biological systems caused by exposure to minute amounts of toxicants.

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6. References:

1. Adami G. M., Barbieri P., Fabiani M., Piselli S., Predonzani, S., Reisenhofer E., (2002). Levels of cadmium and zinc in hepatopancreas of reared *Mytilus galloprovincialis* from the Gulf of Trieste (Italy). Chemosphere, 48 (7), 671 – 677.
2. Alcivar-Warren A., Primavera J. H., Leobert D. de la Pena L D., Pettit P. , Xu Z.(2006). Heavy Metals, Pcbs And Pahs In *Penaeus Monodon* Shrimp From The Philippines: Indicators Of Environmental Contaminants Exposure

3. Andreji J., Stranai I., Massanyi P., Valent M., (2005). Concentration of selected metals in muscle of various fish species. J Environ Sci Health. 40A:899–912.
4. Ashraj, W., (2005). Accumulation of heavy metals in kidney and heart tissues of *Epinephelus microdon* fish from the Arabian Gulf. Environ. Monit. Assess., 101 (1-3), 311-316.
5. Bancroft J.D, Stevens A., Turner D.R. .(1996). Theory and Practice of Histological Techniques. 4th Ed. New York, Churchill, Livingstone.
6. Barbosa A.C., Jardim W., Dorea J.G., Fosberg B., Souza J., (2001). Hair mercury speciation as a function of gender, age and body mass index in inhabitants of the Negro River basin, Amazon, Brazil. Arch Environ Contam Toxicol 2001; 40: 439–44.
7. Bayne, C. J., (1990). Phagocytosis and non-self recognition in invertebrates. Phagocytosis appears to be an ancient line of defense. Bioscience 40:723–731
8. Boletín Oficial del Estado (1991). Además, las distintas editoriales jurídicas (el BOE, Cívitas, Aranzadi o Tecnos, por ejemplo) suelen hacer ediciones reducidas de las principales leyes agrupadas por bloques temáticos, ediciones que resultan muy asequibles y son de fácil manejo.
9. Chen, C.A., (1979). Preliminary report on the Gonadal Development and Induced Breeding of *Penaeus monodon Fabricius*. M.S. Thesis. Institute of Oceanography, National University Taipei, Taiwan
10. Chiou, C.T.,(2002). Bioconcentration of Organic Contaminants, *in* Partition and Adsorption of Organic Contaminants in Environmental Systems: Hoboken, NJ, John Wiley & Sons, Inc., p. 257.
11. Conacher, H. B., Page, B. D., Ryan, J. J., (1993). Industrial chemical contamination of foods [Review]. Food Addit. Contam., 10 (1), 129-143.
12. DeSmet H,, Blust R., (2001). Stress responses and changes in protein metabolism in carp (*Cyprinus carpio*) during cadmium exposure. Ecotoxicology and Environmental Safety 48: 255-62.
13. Diaz, V.R., (1995). Preliminary results of acute toxicity tests for mercury and cadmium on Milkfish (*Chanos chanos Forsskal*) juveniles. In: Watson D, Ong KS, Vigers G. (eds). ASEAN Criteria and Monitoring: Advances in Marine Environmental Management and Human Health Protection. Proceedings of the ASEAN-Canada Midterm Technical Review Conference on Marine Science (24-28 October 1994), Singapore. EVS Environment Consultants, Vancouver, and National Science and Technology Board, Singapore.
14. Dirilgen, N., (2001). Accumulation of heavy metals in freshwater organisms: Assessment of toxic interactions. Turk. J. Chem., 25 (3), 173-179.
15. Carolina O., (2009). Immunotoxicology in marine invertebrates: effects of Mn on the immune responses. MD thesis, School of Life Sciences, Heriot-Waff University, Edinburgh, UK.
16. Cheng W,, Chen J .C., (2001). Effect of intrinsic and extrinsic factors on the haemocyte profile of the prawn, *Macrobrachium rosenbergii*. Fish Shell fish Immunol 11:53–63.
17. Cheng, H.C., (1979). Acute toxicity of heavy metals to some marine prawns. China Fish. Mon. 316:3-10. In Chinese with English abstract.
18. Egyptian organization for standardization and quality control (E.O.S.q.c.) (1993): Maximum residue limits for heavy metals in food. Ministry of Industry.No.2360/ 1993. pp.5, Cairo, Egypt.
19. FAO, (1985). Manual of methods in aquatic environment research. Part 4. Bases for selecting biological tests to evaluate marine pollution: FAO fish tech paper 164.
20. FAO/WHO,(1992). Joint Expert Committee on food Additives, WHO Technical ReportseriesNo.505(1972);No.555(1974c);No.683(1982);No751(1987)andNo.776(1989)Evaluation of certain food additives and contaminants, Geneva.
21. Farombi, E. O., Adelowo, O. A., Ajimoko. Y. R., (2007). Biomarkers of oxidative stress and heavy metal levels as indicators of environmental pollution in African Cat fish (*Clarias gariepinus*) from Nigeria ogun river. Int. J. Environ. Res. Public Health., 4 (2), 158-165.
22. Frías-Espericueta, M.G., J.I. Osuna-López, D. Voltolina, G. López-López, G. Izaguirre-Fierro & M.D. Muy-Rangel. (2008). The metal content of bivalve molluscs of a coastal lagoon of NW Mexico. Bull. Environ. Contam. Toxicol., 80: 90-92.
23. Frías-Espericueta, M.G., J.I. Osuna-López, D. Voltolina, M.A. Beltrán-Velarde, G. Izaguirre-Fierro, G. López-López, M.D. Muy-Rangel & W. Rubio-Carrasco. (2009). The content of Cd, Cu, Pb and Zn of the white shrimp *Litopenaeus vannamei* of six coastal lagoons of Sinaloa, NW Mexico. Rev. Biol.Mar. Oceanogr., 44: 197-201.
24. Finerty M.W., Madden J.D., Feagly S.E., Grodner R.M., (1990). Effect of environs and seasonality on metal residues in tissues of wild and pond raised Cray fish in Southern Louisiana. Archives of Environmental Contamination and Toxicology 19: 49-55.

25. Finney D.J., (1971). Probit Analysis. Cambridge University Press 1971.
26. Gibson R. , Barker P. (1979).The decapod heptopancreas. Oceanoger. Mar. Biol. Ann. Rev. 17, 285-346.
27. Heath A.C., (1995). Water pollution and fish physiology. 2nd edn., Lewis Publishers, Boca Raton. pp. 125-140.
28. Icely JD, Nott JA (1992). Digestion and absorption: digestive system and associated organs. In: Microscopic anatomy of invertebrates: Decapod, Crustacea. Vol. 10. F.W. Harrison and A.G. Humes, Eds. Wily-Liss Inc., N.Y., pp 147-201.
29. Johansson M.W, Söderhäll K., (1989). Cellular immunity in crustaceans and the proPO system. Parasitol. Today. 5:171–176.
30. Johnston, J. A., Ward, C. L., Kopito, R. R., (1998). Aggresomes: A cellular response to misfolded proteins. The Journal of Cell Biology, 143, 1883–1898.
31. Le Moullac G., Haffner P., (2000). Environmental factors affecting immune response in Crustacea. Aquaculture 191: 121–131
32. Le Moullac G., Soyez C., Saulnier D., Ansquer D., Avarre J.C., Levy P., (1998). Effect of hypoxia stress on the immune response and the resistance to vibriosis of the shrimp *Penaeus stylirostris*. Fish Shell fish Immunol 8:621–629
33. Li , X., Cai, W., An, J., Kim, S., Nah, J., Yang, D., Piner, R., Velamakanni A., Jung I., Tutuc, E., Sanjay, K., Banerjee, K., Colombo, L and Ruoff, R. S. (2009). Large-Area Synthesis of High-Quality and Uniform Graphene Films on Copper Foils. Science, 324(5932): 1312-1314.
34. Mela M.R., Ventura .F, Carvalho D.F, Pelletier C.E, Ribeiro C.A., (2007). Effects of dietary methyl mercury on liver and kidney histology in the neotropical fish *Hoplias malabaricus*. Ecotoxicology and Environmental Safety 68: 426 - 35.
35. Nevo E., Noy R., Lavie B., Beiles A., Muchtar S., (1986). Genetic diversity and resistance to marine pollution. Biological Journal of the Linnean Society 29: 139-144.
36. New, M.B., (1995). Status of freshwater prawn farming: a review. Aquatic Research 26: l-54.
37. Nimmo D.R., Rigby R.A., Bahner L.H., Sheppard J.M., (1978). The acute and chronic effects of cadmium on the estuarine mysid, *Mysidopsis bahia*. Bulletin of environmental Contamination and Toxicology 19: 80–85.
38. Oliveira-Ribeiro .CA., Belger L., Pelletiter E., Rouleau C., (2002). Histopathological evidence of inorganic mercury and methyl mercury toxicity in the arctic charr (*Salvelinus alpinus*). Environmental Research 90: 217-225.
39. Pandey A,K,, Mohamed M.P., George K.C., (1994). Histopathological alterations in liver and intestine of *Liza parsia* (Hamilton-Buchanan) in response to mercury toxicity. Journal of Advanced Zoology 15: 18-24.
40. Piyan B. T., Law A. T. , Cheah S. H.,(1985). Toxic levels of mercury for sequential larval stages of *Macrobrachium rosenbergii* (de Man). Aquaculture Volume 46, Issue 4, 15 . 353-359
44. Roesijadi G., Robinson W.E., (1994). Metal regulation in aquatic animals: mechanisms of uptake, accumulation, and release. In: Malins DC, Ostrander GK (eds). Aquatic toxicology: molecular, biochemical and cellular perspectives; pp. 387-420. Lewis Publishers, Boca Raton.
45. SAS Institute, (2000). SAS User's Guide: statistics, SAS Institute, Cary, NC.
46. Schuhmacher M., Domingo J.L., (1996). Concentrations of selected elements in oysters (*Crassostrea angulata*) from the Spanish Coast. Bulletin of Environmental Contamination and Toxicology, 56, 106–113.
47. Skipper R, , DeStephano D,, (1989). Diff-Quik stain set. J.Histotechnol., 12 (4), 303.
48. Smith V.J., Johnston P.A., (1992). Differential haemotoxic effect of PCB congeners in the common shrimp, *Crangon crangon*. Comp. Biochem. Physiol. C 101: 641–649.
49. Smith V.J., Söderhäll K., Hamilton M., (1984). 1, 3-glucan induced cellular defense reaction in the shore crab, *Carcinus maenas*. Comp Biochem Physiol. A. 77: 636–639.
50. Söderhäll K., (1983). 1, 3-glucan enhancement of protease activity in crayfish hemocyte lysate. Comp Biochem Physiol B 74:221–224
51. Söderhäll K., Cerenius L., Johansson M.W., (1996). The prophenoloxidase activating system in invertebrates. In: Söderhäll K, Iwanaga SGR, Vasta GR (eds) New directions in invertebrate immunology. SOS Publications. Fair Haven NJ, p 229–253
52. Truscott R., White K.N., (1990). the influence of metal and temperature stress on the immune system of crabs. Funct Ecol4:455–461
53. Tsing A., Arcier J.M., Brèhèlin M., (1989). Haemocytes of penaeids and palaemonid shrimps: morphology, cytochemistry and hemograms. J Invertebr Pathol 53:64–77
54. Velez D., Montoro R., (1998). Arsenic speciation in manufactured seafood products: a review. J. food. Protect, 61 (9), 1240-1245.
55. Victor B., Narayanan M., Nelson D. Jones., (1990). Gill pathology and hemocyte response in

mercury exposed *Macrobrachium idae* (Heller) Journal of Environmental Biology 11(1), 61-5
56. Voegborlo R. B., Methnani A. M. E., Abedin M. Z., (1999). Mercury, cadmium and lead content of canned Tuna fish. Food Chem., 67 (4), 341 – 345.
57. Vogt G., (1994). Life cycle and functional cytology of the hepatopancreatic cells of *Astacus astacus*. Zoomorphology 114, 83-101.
58. Vorkamp K., Christensen J.H, Riget F., (2004). Polybrominated diphenyl ethers and organochlorine compounds in biota from the marine environment of East Greenland. Sci Total Environ. 331:143–55.
59. Vosyliene M. Z., Jankaite A., (2006). Effect of heavy metal model mixture on rainbow trout biological parameters. Ekologija., 4, 12-17.
60. Vutukuru S. S., (2005). Acute effects of Hexavalent chromium on survival, oxygen consumption, hematological parameters and some biochemical profiles of the Indian Major carp, *Labeo rohita*. Int. J. Environ. Res. Public Health., 2 (3), 456- 462.
61. Waqar A., (2006). Levels of selected heavy metals in Tuna fish. Arab. J. Sci. Eng., 31 (1A), 89–92.
62. Weeks-Perkins, B.A., Chansue, N., Wong-Verelle, D. (1995). Assay of immune function in shrimp phagocytes: techniques used as indicators or pesticide exposure. In: Stolen, J.S., Fletcher, T.C., Smith, S.A., Zelikoff, J.T., Kaattari, S.L., Anderson, R.S., So¨derha¨ll, K., Weeks-Perkins, B.A. (Eds.), Techniques in Fish Immunology-4. SOS Publications, Fair Haven, USA, pp. 223–231.
63. White S.L., Rainbow P.S., (1986). Accumulation of cadmium by *Palaemon elegans* (Crustacea: Decapoda). Marine Ecology Progress Series 32: 17-25.
64. World Health Organisation (WHO), (1984). Guidelines for drinking water quality. WHO, Geneva, No.111.
65. Yang Z.B., Zhao Y.L., Li N., Yang J., (2007). Effect of waterborne copper on the microstructure of gill and hepatopancreas in *Eriocheir sinensis* and its induction of metallothionein synthesis. Archives of Environmental Contamination and Toxicology 52: 222-228.
66. Zhao Y., Wang X., Qin Y. , Zheng B., (2010). Mercury (Hg^{2+}) effect on enzyme activities and hepatopancreas histostructures of juvenile Chinese mitten crab *Eriocheir sinensis* . Chinese Journal of Oceanology and Limnology Volume 28, (3): 427-434.

Evaluation of the sustainability of Different Desensitizing Agents after in-Office Bleaching

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Abstract: Objective: The purpose of present study is to compare the effect of different desensitizing agents in the management of dentinal hypersensitivity after the application of in-office bleaching. Methods: This study was conducted on 27 patients complaining of tooth hypersensitivity and seeking for their teeth whitening. Gluma, Seal & Protect and Fluoride varnish desensitizing agents were used in this study then in-office bleaching was applied. The Verbal Rating Scale (VRS) was used to record scores before and after desensitization, and then was used again to record scores before and after in-office bleaching. The data compiled was statistically analyzed. Results: A remarkable reduction in dentinal hypersensitivity to both air blast and cold water stimuli was noted at the end of the application of the three desensitizing agents. However, the differences in effectiveness of the desensitizing effect after the application of the bleaching material were recorded. Conclusion: Within the limits of this study, it could be implied that for relieving hypersensitivity, all three desensitizing agents were almost equally effective, but it was concluded that the sustainability of the desensitizing effect was detected with the resin-based desensitizing agents rather than the Fluoride-based desensitizing agents.
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1. Introduction:

Dentinal hypersensitivity (DH) is characterized by short sharp pain arising from exposed dentine in response to stimuli typically thermal, evaporative, tactile, osmotic or chemical and which cannot be ascribed to any other form of dental defect or pathology [(1)].

The difficulty found in treating DH is expressed by the enormous number of techniques and therapeutic alternatives to relieve it. Several methods and materials, such as varnishes, liners, restorative materials, dentinal adhesives, dentifrices and mouthwashes are used to reduce dental sensitivity [(2).] During the past several years, patients have become increasingly interested in the esthetic benefits available from dental treatment. In Periodontics, esthetic outcomes can be enhanced with crown lengthening, edentulous ridge augmentation or root coverage by means of a variety of surgical techniques [(3,4).] Restorative procedures that modify the shape, position or shade of teeth are used widely to accomplish esthetic goals [(5).]. Non-restorative procedures such as enamel microabrasion [(6)] and tooth bleaching [(7)] are popular alternatives to restorative treatment when the goal is to achieve a lighter shade of enamel. Internal bleaching of endodontically treated teeth is done to reverse the darkening that frequently occurs in conjunction with pulpal necrosis. Bleaching of vital teeth has been performed in the dental office from many years [(8,9,10,).]

A variety of products have been reported to successfully reduce dentinal hyper-sensitivity. These products generally occlude and seal the dentinal tubules. Resin-based materials have been reported to successfully reduce dentinal hypersensitivity [(11,12).] Thus, the purpose of this study was to investigate the clinical efficacy of some desensitizing agents to sustain its desensitizing effect after in-office bleaching.

2. Methodology:

This study was conducted on 27 patients specially complaining from teeth hypersensitivity and seeking for teeth whitening. Signed informed consent were obtained from all patients who participated in this study. The selected examined teeth were incisor, canine and premolar for every patient. Each tooth was isolated by cotton rolls and operator's fingers and subjected to air blast and cold water tests as follows:
Air blast test: The nozzle tip of an air syringe was kept about 1- 2 cm away from the isolated tooth and then a blast of air was directed on the tooth for one second. Cold water test: A disposable syringe was filled with ice-cold water and the water was applied on the suspected isolated tooth surface drop by drop. Therefore, VRS (Verbal Rating Scale) was used to record scores:-

0 – Was recorded for those patients with No discomfort
1 – Was recorded for patients complaining from Mild discomfort

2 – Was recorded for patients complaining from Moderate discomfort
3 – Was recorded for patients complaining from severe pain only during application of stimulus
4 – Was recorded for patients complaining from severe pain persisting after removal of stimulus

Only patients with teeth recorded a discomfort score of two or more were included in this study. (VRS) Records were recorded and tabulated. Then the teeth were cleaned, dried, and isolated with cotton rolls. Then the subjects included in this study were grouped into three groups of nine patients each according to the desensitizing agent received as follows;

Group I: A few drops of Gluma Desensitizer (Heraeus Kulzer, Armonk, NY, USA) (Dentsply, 5% Glutaraldytes and 35% hyroxyethyl methacrylate (HEMA) were applied with a cotton pellet using a gentle but firm rubbing motion. After 30 seconds, the area was dried thoroughly until the fluid disappeared and the surface was not shinny. Group II: A few drops of Seal & Protect (Di-and Trimethacrylate resins, PENTA, Silica, Triclosan, Cetylamine hydrofluoride and acetone) were applied to the dentin surface with an applicator tip. The surface was left undisturbed for 20 seconds and the excess solvent removed by gently airing for a few seconds and cured using Bluelex-LED (BlueLex, LD-105, San-Chong city, Taiwan) with constant mode of full intensity 800 mW/cm2 for 10 seconds. With a cotton pellet, the oxygen-inhibited layer was removed and the excess checked with a periodontal probe. Group III: Fluoride varnish (Fluoride Varnish, Dentsply Professional, York, Pa.) was applied for 1 minute. Excess gel was removed with a cotton pellet and the patients were advised not to drink or eat for the next hour after the application of the product in each group.

After the application of the three desensitizing agents, again the sensitivity test was done based on the (VRS) as previously mentioned to record the amount of reduction in the dentinal hypersensitivity.

In-office bleaching was applied for the patients of the three groups after application of the three desensitizing agents using the White smile Power bleaching system kit with 38% hydrogen peroxide. Bleaching procedures have been followed according to the manufacturer`s instructions and teeth subjected to the desensitizing agents were exposed also to the bleaching material.

White smile after bleaching Mousse containing potassium nitrate, fluoride and Xylitol was applied for all teeth exposed to bleaching procedures.

Once again, the sensitivity assessment test using the (VRS) was done after bleaching for the same specific examined teeth which were treated using the three different desensitizing agents. Hypersensitivity (VRS) scores before and after bleaching were recorded and statistically analysed using Chi square-test between groups. Statistical analysis was performed using Graphpad Prism-4 statistics software for Windows. P values ≤ 0.05 are considered to be statistically significant in all tests.

3. Results

The criteria for evaluation of the degree of sensitivity were based on verbal rating scale. Results revealed that the dentinal hypersensitivity reduced significantly ($P<0.05$) after the application of the three different desensitizing agents as revealed by Chi square test (Chi value = 14.88, $p<0.05$). Yet, group of teeth treated with Fluoride varnish recorded the least reduction of the hypersensitivity in comparison with the two other desensitizing agents (Gluma and Seal & Protect) (Table 1 and Figure 1).

Table (1): Verbal rate scores of dentinal hypersensitivity before and after application of three different desensitizing agents

Group / Score	Group 1 (Gluma)		Group 2 (Seal &protect)		Group 3 (Fluoride varnish)	
	Before	After	Before	After	Before	After
Score0	0%	50%	0%	66.67%	0%	5.56%
Score1	0%	50%	0%	33.33%	0%	94.44%
Score2	38.89%	0%	11.11%	0%	38.89%	0%
Score3	38.89%	0%	50%	0%	44.44%	0%
Score4	22.22%	0%	38.89%	0%	16.67%	0%
Chi square test	Chi value	36	Chi value	36	Chi value	36
	p value	<0.0001*	p value	<0.0001*	p value	<0.0001*

ns; non-significant ($p>0.05$) *; significant ($p<0.05$)

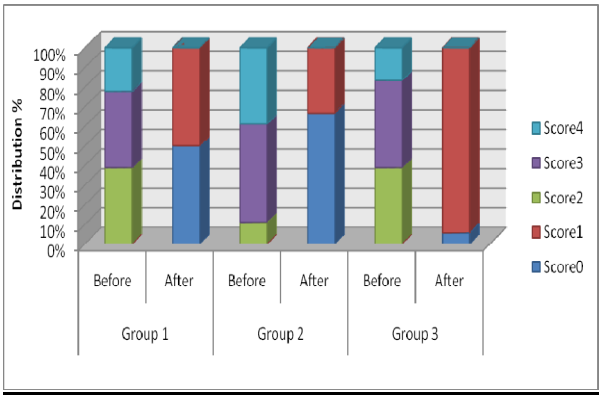

Figure (1) A stacked column chart of verbal rate scores before and after application of the three desensitizing agents

On the other hand, after application of the three desensitizing agents, it was found that the difference in dentinal hypersensitivity between teeth received Gluma and Seal & Protect before and after in-office bleaching was statistically non-significant as revealed by Chi square test ($p>0.05$). While, for those teeth received the Fluoride varnish, a statistical significant difference was obtained before and after in-office bleaching (increased of the dentinal hypersensitivity) as revealed by Chi square test ($p< 0.05$) (Table 2 and Figure 2,3,4)

Table (2): Verbal rate scores of dentinal hypersensitivity after the application of the desensitizing agents before and after in-office bleaching

Group / Score	Group 1	In-office Bleaching	Group 2	In-office Bleaching	Group 3	In-office Bleaching
Score0	50%	50%	66.67%	66.67%	5.56%	0%
Score1	50%	50%	33.33%	33.33%	94.44%	11.11%
Score2	0%	0%	0%	0%	0%	61.11%
Score3	0%	0%	0%	0%	0%	27.78%
Score4	0%	0%	0%	0%	0%	0%
Chi square test	Chi value	0.00	Chi value	0.00	Chi value	28.8
	p value	1ns	p value	1ns	p value	<0.0001*

ns; non-significant ($p>0.05$) *; significant ($p<0.05$)

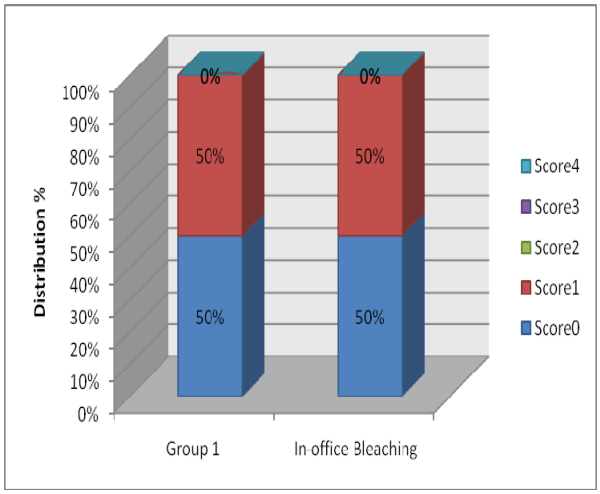

Figure (2) A stacked column chart of verbal rate scores after application of Gluma desensitizing agent before (Group 1) and after in-office bleaching

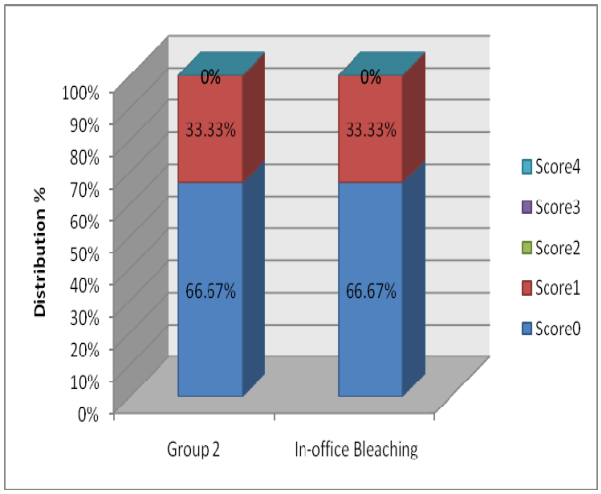

Figure (3) A stacked column chart of verbal rate scores after application of Seal & Protect desensitizing agent before (Group2) and after in-office bleaching

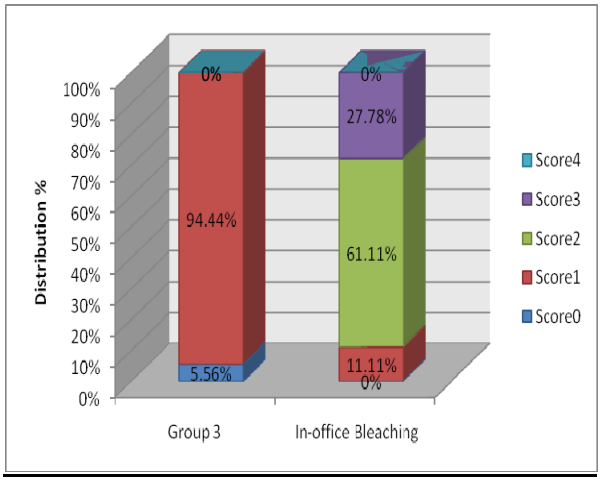

Figure (4) A stacked column chart of verbal rate scores after application of Fluoride varnish desensitizing agent before (Group3) and after in-office bleaching

4. Discussion:

Cervical dentin hypersensitivity is a significant clinical problem in dentistry because it affects a large percentage of the population due to erosion, abfraction, abrasion, ext,. Also, as life expectancy increases and patients retain their natural teeth longer because of more effective treatments for caries and periodontal disease, the risk of developing cervical dentin hypersensitivity increases as a result of physiological gingival recession and exposure of cervical dentin.

The new delivery system for the desensitizing agents proved to be effective and convenient for a single-patient application, with no drawbacks regarding handling and/or ease of application. In addition to their desensitizing effect, topical fluoride varnishes was found to play an important role for prevention of caries [13,14]. In this study, the results revealed that Gluma and the Seal & Protect showed superior results over the Fluoride varnish which showed less stability as a desensitizing agent after the application of the in-office bleaching agent.

The Gluma Desensitizer product contains 5% glutaraldehyde and 35% hydroxyethyl methacrylate (HEMA). The hypothesis for the immediate occlusion of the dentin tubules is an effect of glutaraldehyde on the proteins of the dentinal fluid. It was proposed that amino group-containing substances in dentin react with glutaraldehyde and start the formation of a HEMA polymer. It is conceivable that the ε-amino groups in these amino acids of a collagen molecule react with glutaraldehyde-derived aldehyde, forming cross-links where the two groups of aldehydes present in glutaraldehyde interlock themselves with the amino groups of dentin collagen, leading to a fixing of proteins, forming a protein precipitate resulting in partial or total occlusion ([15, 16]. The results of Gluma Desensitizer presented in this study are in agreement with the literature.[17,18,19,20]

While, the desensitizing agent Seal & Protect showed similar results to those shown by Gluma. The agent Seal & Protect is derived from the adhesive system Prime & Bond NT that has an anti-microbial characteristic, resulting from the incorporation of triclosan, and acid monomers, which are self-conditioning[21,22].

It was revealed from the results of this study that the in-office bleaching using the white smile bleaching system had no effect on the desensitizing effect of the Gluma and the seal & Protect desensitizing agents. And these findings were in accordance with the literature as it was concluded that the percentages of 38% hydrogen peroxide when applied according to the manufacturer instructions do not lead to increase in microleakage. Klukowsha [23] and White [24] also found that bleaching agents based of hydrogen, carbamide peroxide, and perborate did not cause an increase in microleakage at the interface adhesive.

But, on the other hand, the reduced effect of desensitization of Fluoride varnish after the application of in-office bleaching could be attributed to the effect of the high concentration of the hydrogen peroxide used in such type of in-office bleaching systems that might lead to the dissolution of the Fluoride varnish. However, the usage of fluoride varnish as a desensitizing agent could compromise the bleaching efficiency applied on the hypersensitive teeth.

5. Conclusion:

It is recommended to use resin-based desensitizing agents for treatment of the dentinal hypersensitivity when teeth are indicated for in-office bleaching to guarantee the sustainability of the desensitizing effect.

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6. References:

1. Dowell P, Addy M (1983) Dentine hypersensitivity – a review. Aetiology, symptoms and theories of pain production. J Clin Periodontol 10, 341-350.

2. Kazemi RB, , Sen BH, Spångberg LSW (1999) Permeability changes of dentine treated with titanium tetrafluoride. J Dent 27, 531-538.
3. Miller PD Jr. Periodontal plastic surgery. Curr Opin Periodontol. 1993:136–43.
4. Jorgensen MG, Nowzari H. Aesthetic crown lengthening. Periodontol 2000 2001;27:45–58.
5. Bello A, Jarvis RH. A review of esthetic alternatives for the restoration of anterior teeth. J Prosthet Dent 1997;78:437–40.
6. Weinstein AR. Esthetic applications of restorative materials and techniques in the anterior dentition. Dent Clin North Am 1993;37:391–409.
7. Croll TP. Enamel microabrasion: observations after 10 years. JADA 1997;128:45S–50S.
8. 8-Christensen GJ. Bleaching teeth: practitioner trends. JADA 1997;128:16S–18S.
9. Ames JW. Removing stains from mottled enamel. JADA 1937;24:1674–7.
10. Cohen S, Parkins FM. Bleaching tetracycline-stained vital teeth. Oral Surg Oral Med Oral Pathol 1970;29:465–71.
11. Duran I, Sengun A. The long-term effectiveness of five current desensitizing products on cervical dentine sensitivity. J Oral Rehabil. 2004;31(4): 351-356.
12. Kakaboura A, Rahiotis C, Thomaidis S, Doukoudakis S. Clinical effectiveness of two agents on the treatment of tooth cervical hypersensitivity. Am J Dent. 2005;18(4):291-295.
13. Attin T, Grieme R, Paque F, Hannig C, Buchalla W, Attin R. Enamel fluoride uptake of a novel water-based fluoride varnish. Arch Oral Biol 2005;50:317–22.
14. Strohmenger L, Brambilla E. The use of fluoride varnishes in the prevention of dental caries: a short review. Oral Dis 2001;7(2):71–80.
15. Dijkman GEHM, Jonebloed WL, de Vries J, Ogaard B, Arends J. Closing of dentinal tubules by Glutaraldeyde treatment, a scanning electron microscopy study. Scand Dent Res. 1994;102(3):144-50.
16. Schüpbach P, Lutz F, Finger WJ. Closing of dentinal tubules by Gluma Desensitizer. Eur J Oral Sci. 1997;105(5):414-21
17. Kakaboura A, Rahiotis C, Thomaidis S, Doukoudakis S. Clinical effectiveness of two agents on the treatment of tooth cervical hypersensitivity. Am J Dent. 2005;18(4):291-5.
18. Olusile AO, Bamise CT, Oginni AO, Dosumu OO. Short-term clinical evaluation of four desensitizing agents. J Contemp Dent Pract. 2008;9(1):22-9.
19. Camps J, Pizzanti S, Dejou J, Franquin JC. Effects of desensitizing agents on human dentin permeability. Am J Dent. 1998;1(6):286-90.
20. Davidson DF, Suzuki M. The Gluma bonding System: a clinical evaluation of its various components for the treatment of hypersensitive root dentin. J Can Dent Assoc. 1997;63(1):38-41.
21. Pamir T, Dalgar H, Onal B. Clinical evaluation of three desensitizing agents in relieving dentin hypersensitivity. Oper Dent. 2007;32(6):544-8.
22. Azzopardi A, Bartlett DW, Watson TF, Sherriff M. The meas-urement and prevention of erosion and abrasion. J Dent. 2001;29(6):395-400.
23. Klukowska MA, White DJ, Gibb RD, Garcia-Godoy F, Garcia-Godoy C, Duschner H. The effects of high concentration tooth whitening bleaches on microleakage of Class V composite restorations. J Clin Dent 2008;19:14-7.
24. White DJ, Duschner H, Pioch T. Effect of bleaching treatments on microleakage of class I restorations. J Clin Dent. 2008;19:33-6.

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Antioxidant Activity of Leek towards Free Radical Resulting from Consumption of Carbonated Meat in Rats

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Abstract: Forty -two albino male rats were randomly classified into six groups (7 rats each). The first group kept as control negative fed basal diet only. The other five groups fed on basal diet with 10% extreme grilled meat and classified into non treated group and treated groups with water leek extract, methanol leek extract, 2.5 % leek powder and 5% leek powder. Consumption of carbonyl meat only in non treated group decreased final weight, weight gain, food intake ,feed efficiency ratio(FER) , hemoglobin , packed cell volume ,blood glutathione peroxidase (GPX) , blood super oxide dismutase (SOD) , liver super oxide dismutase (SOD), liver glutathione peroxidase (GPX) and liver glutathione transferase (GST) but significant increase in AST enzyme, creatinine ,urea nitrogen blood free radical and serum lipid peroxide (LPX) and liver malondialdehyde (MDA) compared to normal control group. The treated groups with water ,methanol extracts, 2.5 % and 5% leak showed that the values of final weight, weight gain, food intake ,FER, hemoglobin, serum alanine amino transferase (ALT) enzyme, creatinine, urea nitrogen , blood GPX , blood SOD and blood GST around the values of normal control group. The treated groups with water ,methanol extracts, 2.5 % and 5% leak showed significant decrease in packed cell volume , liver SOD and GPX but significant increase in AST, blood free radical , LPX ,liver MDA compared to normal control group. The values of final weight, weight gain, food intake , FER hemoglobin , packed cell volume ,blood GPX , blood SOD, liver SOD, liver GPX and liver GST were increased but the values of AST enzyme, creatinine ,urea nitrogen, blood free radical, serum lipid peroxide and liver MDA were decreased in all treated groups compared to non treated group.

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Key wards: leek, free radical, antioxidant, carbonated meat& rats

1. Introduction:

The extreme grilled meat is usually consumed as (Cabab). Such food contains nitrated polycyclic aromatic hydrocarbons free radicals which have very high risk for body health (Solyakov and Skog 2002). Free radicals have unpaired electrons and so they try to steal them from other molecules in a process called oxidation. These oxidation damages the cell membranes, genetic material in cells (DNA), fatty acids and other body structures (Abd El-Ghany et al ., 2007). The antioxidative effects of natural phenolic compounds in pure forms or in their extracts from different plant sources such as vegetables, fruits and medicinal plants were studied in vitro using different model systems of oxidation. Antioxidants may be of great value in preventing the onset and/or the propagation of oxidative diseases (Pietta et al., 1998, and Garth and Rita 2006).

Fruits and vegetables contain different phytochemicals with biological activity that can be of valuable therapeutic index. Phytochemicals have an excellent antioxidant activity, including the ability to neutralise potentially harmful free radicals that helps prevent a number of chronic diseases (Franca and Harri 2001 and Liu 2003).

The allium group is one of the world's most widely cultivated vegetable groups, with their culinary and medicinal uses. Equally varied are their health benefits, for they contain a range of phytochemicals with an array of biological effects. Evidence shows they play an important role in protecting against major lifestyle chronic diseases as well as health problems associated with ageing. Their antimicrobial activity, long recognized in folk remedies, has also now been scientifically validated. The Allium genus includes approximately 500 species, the most widely used of which are onions, garlic, and leeks (Malairajan et al., 2006).

Leeks (Allium porrum or A. ampeloprasum var. porrum), sometimes called "the gourmet's onion" have flat leaves instead of tubular and relatively little bulb development and is native to Western Asia and the Mediterranean countries. The thick leaf bases and slightly developed bulb look like a giant green onion, and are eaten as a cooked vegetable. Leeks contain saponins and the major flavonoid in leeks is kaempferol, with only a small amount of quercetin, carotenoids and chlorophyll mainly in the green tops (Onyeagba et al., 2004 and Garth and Rita 2006).

The aim of this study was to investigate the antioxidant effect of leeks either powder or extract on free radical producing agents

2. Materials and Methods

A – Materials:

Carbonated meat and leek:

Meat was extremely grilled in hot oven until be carbonated then crushed and mixed in basal diet in 10 % all over the period of experiment. Leek was collected from the local market. Part of leek leaves was dried at 60°C and then crushed to powder. The leek powder added in 2.5 % and 5% to diet. The other part was used for preparation of water and methanol extract.

Basal diet:

The experimental diet composed of casein (200g/kg), corn starch (497g/kg), sucrose (100g/kg) , cellulose (30 g/kg) ,corn oil (50g/kg), mineral mixture (100g/kg) , vitamin mixture (20g/kg) and DL-methionine (3g/kg).The standard diet was performed according to NRC (1995).

Experimental animals:

42 adult male of white albino rats (Sprague dawley strain) weighing 115 ±5g were provided from of National Research Center, Cairo, Egypt.

Biochemical kits:

BioMeriuex Kits were purchased from Alkan Co. for Chemicals and Biodignostics, Dokki, Egypt.

B- Methods

1-Preparation of water and methanol leek extracts

270 g of fresh leek were peeled and then was chopped with both 300 mL distilled water and ethanol by using a blender for 1 min at average speed. The mixture were macerated during 24h at the + 4ºC. After that, resulting extracts were filtered using a 0.45 µm pore size cellulose acetate membrane filter. The extracts were used directly (Irkin and Korukluoglu 2007).

2-Experimental design:

Rats were housed in wire cages under the normal laboratory conditions and fed on basal diet for a week as adaptation period. Food and water were provided ad-libitum. The rats were randomly classified into six groups (7 rats each). The first group kept as normal control fed basal diet only. The other five groups fed on basal diet with 10% of extreme grilled meat and classified into

1-Non treated group.

2- Treated group with water leek extract.

3- Treated group with methanol leek extract.

4- Treated group with 2.5 % leek powder.

5- Treated group with 5% leek powder.

The experiment continued for eight weeks. The food intake was calculated daily and the body weight gain was recorded weekly. Feed efficiency ratio (FER) was determined by Chapman et al., (1950) as following: FER = weight gain (g)/ feed intake (g).

3-Collection of blood and liver samples:

At the end of experiment, rats were anesthetized, blood sample were collected from hepatic portal vein in clean centrifuge tubes. The liver of sacrificing rats was removed by careful dissection, blotted frees of adhering blood, washed with cold saline solution, and dried between two filter papers. Livers perfuse with 50 to 100 of ice cold 0.9%NaCL solution for some analyses.

4- Blood analysis:

Part of blood was heparinized for estimation of hemoglobin and packed cell volume (Drabkin, 1949 and Mc Inory, 1954) .The rest of blood was left to coagulate then centrifuged at 3000 rpm for 15 minutes to obtain serum. Serum biochemical analyses were estimated colorometriclly . Serum alanine and aspertate aminotransferase (ALT, AST) enzymes activity, creatinine and urea nitrogen were estimated colorometriclly according to Reitman and Frankel (1957), Husdan and Rapoport (1968) and Fawcett and Scott (1960), respectively. Blood glutathione peroxidase (GPX), superoxide dismutase (SOD) and also serum lipid peroxide (LPX) were estimated by BioMeriuex Kits according to Beuther et al., (1987), Beuchamp and Fridovich, (1971), and Botsoglou et al., (1994), respectively. Blood free radical was estimated according to Borg, (1976) by an Electron Spin Resonance spectroscopy National Research Center.

5- Liver analysis:

Liver superoxide dismutase (SOD), glutathione peroxidase (GPX) , glutathione S-transferase (GST) and malondialdehyde (MDA) were estimated according to Beuchamp and Fridovich,(1971) , Weiss et al., (1980) , Habig et al.,(1974) and Uchiyama and Mihara (1978), respectively.

8-Statisticl analysis:

Collected data were presented as mean ±SD and statistically analyzed using one way analysis of variance (ANOVA).Student "t" test was used for significance according to Artimage and Berry (1987).

3. Results and Disscusion

Consumption of carbonyl meat only in non treated group decreased final weight, weight gain, food intake and FER at $p<0.05$, 0.01 &0.001 compared to normal control group. The treated groups with water, methanol extracts, 2.5 % and 5% leak showed the values of final weight, weight gain, food intake and FER around the values of normal control group. The values of final weight, weight gain, food intake and FER of all treated groups were increased compared to non treated group as shown in table (1).

Common cooking heat processing of protein-rich foods such as boiling, frying, flame-grilling, induce the formation of potent mutagenic and carcinogenic hydrocarbons HCAs .The grilled meat contain heterocyclic amines as phenylimidazo [4,5] pyridine which as potent mutagens (Solyakov and Skog 2002). Vegetables are an important source of mineral and phenolics which play an important role in nutritive value. Allium vegetables and related organosulfur compounds inhibit of mutagenesis, modulation of enzyme activities, inhibit of DNA adduct formation, scavenge of free-radical, and effect on cell proliferation and tumor growth (Garth and Rita 2006).Leeks are a good source of dietary fiber, folic acid, calcium, potassium, and vitamin C. Leeks are easy to digest and have laxative, antiseptic, diuretic, and anti-arthritic properties. Leeks support healthy digestion by promoting the growth of usefull bacteria in the gut due to contain prebiotics carbohydrates that serve as fuel for good bacteria in the digestive tract. These probiotic bacteria fortify the immune systems and keep digestive processes running smoothly. Leeks fiber energizes the human body to perform many types of biological functions like digestion and metabolism (Dorant et al., 1996 and Riley et al., 2001).

Consumption of carbonyl meat only in non treated group decreased hemoglobin and packed cell volume at $p<0.01$ compared to normal control group. The treated groups with water ,methanol extracts ,2.5 % and 5% leak showed non significant decrease in hemoglobin at $p>0.05$ but significant decrease in packed cell volume at $p<0.05$ compared to normal control group. The treated groups with water, methanol extracts, 2.5 % and 5% leak showed increase in hemoglobin and packed cell volume compared to non treated group as shown in table (2).

Leeks are a good source of allyl sulfides and also rich in the flavonoid especially kaempferol. Leeks contain excellent amounts of vitamin C, as well as folate, and some useful amounts of B vitamins, vitamin E, copper, potassium and iron. These vitamins and minerals work together to help stabilize blood. Calcium in leeks is also used for the proper clotting of blood in the human body. Allium species also have immune enhancing actions that include promotion of lymphocyte synthesis, cytokine release, phagocytosis and natural killer-cell activity (Merchant, 2003 and Fortin, 2004)

Consumption of carbonyl meat only in non treated group increased serum aspartate amino transferase (AST) enzyme, creatinine and urea at $p<0.01$&0.001 compared to normal control group. The treated groups with water ,methanol extracts ,2.5 % and 5% leak showed non significant increase in serum alanine amino transferase (ALT) enzyme ,creatinine and urea nitrogen at $p>0.05$ but significant increase in AST at $p<0.05$ compared to normal control group. The treated groups with water,methanol extracts ,2.5 % and 5% leak showed decrease in AST enzyme, creatinine and urea nitrogen compared to non treated group as shown in table (3).

These results were in agreement with the fact that ALT was widely distributed in cells throughout the body and found predominantly in the cytoplasm of hepatic parenchymal cells. ALT is widely considered to be specifically for the liver increase in blood and indicative for liver damage as hepatitis, cirrhosis or hepatic tumors. If cells were damage, ALT will excreted into the blood (Thoma, 2000).Creatinine was a waste product in the blood created by the normal breakdown of muscle during activity. Healthy kidneys take creatinine out of the blood and put it in the urine to leave the body. Creatinine builds up in the blood in kidney disease. The significant increase in creatinine and urea in the present study may be attributed to renal damage due to administration of carbonated meat. Phenylimidazo [4,5] pyridine (PhIP) was naturally formed in meats during the cooking process at least in part due to heat dependent condensation of creatinine and phenylalonine which are the two natural components of muscle meats (Sinha et al., 1995). Dietary onions partailly reversed the abnormalities in blood urea and creatinine in streptozotocin induced diabetes mellitus rats. The significant decrease in creatinine and uric acid in the present study may be due to the higher antioxidants activities of leek. The non-nutrient constituents influence biotransformation enzymes involved in activation and detoxification of xenobiotic compounds (Block 1999 and Bordia, et al., 2002).

Consumption of carbonyl meat only in non treated group decreased blood glutathione peroxidase (GPX) and superoxide dismutase (SOD) at $p<0.01$ but increased blood free radical and serum lipid peroxide (LPX) at $p< 0.001$ compared to normal control group. The treated groups with water ,methanol extracts ,2.5 % and 5% leak showed non

significant decrease in blood glutathione peroxidase and super oxide dismutase at p>0.05 but significant increase in blood free radical and serum lipid peroxide at p< 0.01 compared to normal control group.

The treated groups with water, methanol extracts, 2.5 % and 5% leak showed increase in blood glutathione peroxidase (GPX) and super oxide dismutase but decrease in blood free radical and serum lipid peroxide compared to non treated group as shown in table (4).

Free radicals are highly reactive compounds that are created in the body during normal metabolic functions or introduced from the environment. Free radicals are inherently unstable since they contain "extra" energy. To reduce their energy load, free radicals react with certain chemicals in the body and in the process interfere with the cells ability to function normally. Free radicals are believed to play a vital role in more than sixty different health conditions, including the aging process, cancer, and atherosclerosis. Fatty acyl side chains peroxidation in biological membranes by reactive oxygen species and transition metal ions in a free radical chain reaction can be deleterious for membrane permeability as it can produce toxic compounds, such as malonaldehyde and acetaldehyde, which in turn produce abnormal adducts with biological substances, including DNA and RNA (Wei and Lee 2002).

Most living organisms possess enzymatic and nonenzymatic defence systems against excess production of reactive oxygen species. However, different external factors such as smoke, diet, alcohol and some drugs and aging could decrease the capability of such protective systems resulting in disturbances of the redox equilibrium that is established in healthy conditions. Superoxide dismutase (SOD) is, an enzyme, found in the cytosol and mitochondria. SOD is responsible for decreasing superoxide levels. Glutathione peroxidase occurs in the mitochondria and cytosol and reduces organic hydroperoxides and hydrogen peroxide in reaction that involves glutathione (Kanno et al., 2004).Reduced glutathione (GSH) is not only a cofactor for GST but also serves as a reductant for glutathione peroxidase (GPX), an enzyme involved in natural protection by free radicals, in addition to superoxide dismutase and catalase. Reducing exposure to free radicals and increasing intake of antioxidants nutrients has the potential to reduce the risk of free radical-related health problems. Phytochemicals are molecules of plant origin (such as carotenoids, flavenoids, phytosterols, chlorophylls, terpenoids, indoles and allylic compounds) consumed in the diet that are not true vitamins but can be very potent antioxidant nutrients (Ames et al., 2002 and Garth and Rita 2006).

Consumption of carbonyl meat only in non treated group decreased liver superoxide dismutase (SOD), glutathione peroxidase (GPX) and glutathione transferase (GST) at p<0.001 but showed significant increase in liver malondialdehyde (MDA) at p<0.001 compared to normal control group. The treated groups with water ,methanol extracts ,2.5 % and 5% leak showed significant decrease in liver superoxide dismutase (SOD) and glutathione peroxidase (GPX) at p<0.05 but significant increase in liver malondialdehyde (MDA) at p< 0.05 and non significant decrease in glutathione transferase (GST) compared to normal control group. The treated groups with water ,methanol extracts ,2.5 % and 5% leak showed increase in liver superoxide dismutase (SOD), glutathione peroxidase (GPX) and glutathione transferase (GST) but decrease in liver malondialdehyde (MDA) compared to non treated group as shown in table (5).

Organosulfur compounds enhance glutathione-S-transferase enzyme system, which are biochemical pathways involved in the liver's detoxification of carcinogenic substances. *Allium* vegetables and related organosulfur compounds inhibit mutagenesis modulate of enzyme activities, inhibit of DNA adduct formation, scavenge free-radical, and effect on cell proliferation and tumor growth (Takahashi et al., 1992 and Franca and Harri 2001). The antioxidant properties of *Allium* vegetables result from the contributions of various sulfur components. Allyl derivatives of leek oils stimulate the activity of GPX and inhibited the decreased ratio of reduced to oxidized glutathione produced by 12-*O*-tetradecanoylphorbol-13- acetate in epidermal cells. Diallyldisulfide increase GPX activity in animal tissues with increased the activity of glutathione reductase, and superoxide dismutase (Jin and Baillie 1997 and Riley et al., 2001).

It is recommended to consume leek as a good source of antioxidant especially with grilled meat for scavenger of free radicals.

Table (1): Mean values ± SD of body weight gain, food intake and feed efficiency ratio (FER) of the experimental rat groups.

Groups / Variables	Normal control	Non treated	Treated groups			
			Leek water extract	Leek methanolic extract	Leek powder (2.5%)	Leek powder (5%)
Initial weight	117.33 ± 3.71[a]	118.55 ± 3.60[a]	115.99 ± 4.25[a]	119.41 ± 4.14[a]	118.75 ± 3.18[a]	117.39 ± 3.61[a]
Final Weight (g)	201.41 ± 8.61[a]	160.71 ± 7.11[b***]	200.41 ± 9.21[a]	202.35 ± 13.78[a]	199.91 ± 9.30[a]	198.89 ± 10.22[a]
Weight Gain (g)	84.08 ± 6.11[a]	42.16 ± 4.36[b***]	84.42 ± 5.61[a]	82.94 ± 6.14[a]	81.16 ± 6.18[a]	81.50 ± 5.19[a]
Food Intake (g/d))	16.38 ± 1.45[a]	14.33 ± 1.39[b*]	16.24 ± 1.25[a]	16.57 ± 1.14[a]	16.01 ± 1.31[a]	16.11 ± 1.29[a]
FER	0.085 ± 0.001[a]	0.049 ± 0.001[b**]	0.086 ± 0.003[a]	0.083 ± 0.004[a]	0.084 ± 0.002[a]	0.084 ± 0.001[a]

Significant with control (-ve) group * P<0.05 ** P<0.01 *** P<0.001
Mean values in each raw having different superscript (a, b, c) denote significant difference.

Table (2): Mean values ± SD of blood hemoglobin (HB) and packed cell volume (PCV) of the experimental ratgroups.

Groups / Variables	Normal control	Non treated	Treated groups			
			Leek water extract	Leek methanol extract	Leek powder (2.5%)	Leek powder (5%)
HB (gm/dl)	**13.01 ± 2.11[a]**	**8.11 ± 1.21[b**]**	**11.34 ± 1.51[a]**	**11.81 ± 1.17[a]**	**10.91 ± 1.98[a]**	**11.45 ± 2.20[a]**
PCV %	**39.40 ± 6.17[a]**	**25.98 ± 5.11[c**]**	**34.61 ± 7.01[b*]**	**33.81 ± 6.14[b*]**	**32.11 ± 6.38[b*]**	**35. 19 ± 5.18[b*]**

Significant with control (-ve) group * P<0.05 ** P<0.01 *** P<0.001
Mean values in each raw having different superscript (a, b, c) denote significant difference.

Table (3): Mean values ± SD of serum amino transferase enzymes (ALT & AST), creatinine and urea nitrogen of the experimental rat groups.

Groups / Variables	Normal control	Non treated	Treated groups			
			Leek water extract	Leek methanol extract	Leek powder (2.5%)	Leek Powder (5%)
ALT (µ /ml)	18.53 ± 5.81[a]	23.79 ± 5.45[a]	22.14 ± 4.33[a]	21.16 ± 3.22[a]	22.81 ± 3.69[a]	21.91 ± 4.01[a]
AST (µ /ml)	38.41 ± 6.13[c]	51.31 ± 6.22[a***]	45.32 ± 4.15[b*]	44.14 ± 5.22[b*]	48.32 ± 5.38[b*]	46.34 ± 4.87[b*]
Creatinine (mg/dl)	0.88 ± 0.02[b]	1.21 ± 0.33[a**]	0.98 ± 0.12[b]	0.89 ± 0.11[b]	0.97 ± 0.03[b]	0.87 ± 0.04[b]
Urea nitrogen (mg/dl)	35.41 ± 3.24[b]	55.32 ± 6.71[a**]	39.14 ± 4.12[b*]	40.21 ± 4.91[b]	38.33 ± 3.77[b]	41.32 ± 4.81[b]

Significant with control (-ve) group * P<0.05 ** P<0.01 *** P<0.001
Mean values in each raw having different superscript (a, b, c) denote significant difference.

Table (4): Mean values ± SD of blood glutathione peroxidase (GPX), superoxid dismutase (SOD) enzymes and free radical and serum lipid peroxide (LPX) of the experimental rat groups.

Groups / Variables	Normal control	Non treated	Treated groups			
			Leek water extract	Leek methanol extract	Leek powder (2.5%)	Leek powder (5%)
GPX (µ /ml)	7.13 ± 1.10[a]	3.31 ± 0.44 [b**]	6.66 ± 1.14 [a]	6.17 ± 1.13 [a]	5.49 ± 1.12 [a]	5.79 ± 1.01 [a]
SOD (µ /l)	20.55 ± 2.34 [a]	10.88 ± 1.67 [b**]	18.49 ± 1.73[a]	17.99 ± 2.21 [a]	18.41 ± 2.34 [a]	18.91± 1.98 [a]
free radical	20266.45± 3230.57 [c]	73464.67 ± 4532041 [a***]	41320.87 ± 3357.22 [b**]	39421.77 ± 3567.22 [b**]	45425.11 ± 4034.67 [b**]	43334.35± 2947.41 [b**]
LPX (mg/dl)	1.30 ± 0.22[c]	5.45 ± 1.71 [a***]	2.99 ± 0.55[b**]	1.89 ± 0.93 [b*]	2.61 ± 0.65 [b**]	2.85 ± 0.67 [b**]

Significant with control (-ve) group * P<0.05 ** P<0.01 *** P<0.001
Mean values in each raw having different superscript (a, b, c) denote significant difference.

Table (5): Mean values ± SD of liver superoxid dismutase (SOD), glutathione peroxidase (GPX), glutathione transferase (GST) and malondialdehyde (MDA) of the experimental rat groups.

Groups / Variables	Normal control	Non treated	Treated groups			
			Leek water extract	Leek methanol extract	Leek powder (2.5%)	Leek Powder (5%)
SOD (µ /mg)	115.71 ± 10.25[a]	45.11 ± 5.14 [b***]	85.17 ± 7.34 [c*]	89.36 ± 6.99 [c*]	86.33 ± 8.23 [c*]	88.34 ± 8.13 [c*]
GPX (µ /mg)	110.33 ± 11.21 [a]	52.18 ± 6.13 [b***]	87.75 ± 7.81[c*]	90.12 ± 10.10 [c*]	88.66 ± 9.54 [c*]	86.32 ± 8.22 [c*]
GST (µ /mg)	1.22 ± 0.13 [a]	0.44 ± 0.02 [b***]	1.12 ± 0.06 [a]	o.99 ± o.13 [a]	1.01 ± 0.33 [a]	1.21 ± 0.52 [a]
MDA (nmol/g)	13.14 ± 2.21[c]	31.21 ± 3.71 [a***]	19.81 ± 2.14[b*]	18.31 ± 1.41 [b*]	17.99 ± 1.49 [b*]	19.33 ± 2.01 [b*]

Significant with control (-ve) group * P<0.05 ** P<0.01 *** P<0.001
Mean values in each raw having different superscript (a, b, c) denote significant difference.

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6. References:

1. Abd El-Ghany, M. A., Farouk, M. El Tellawy., Mohamed, A. H., Hatem, M. F And Hanaa, F. El-Meheiry. (2007): Effect Of Some Vegetables Intake On The Precarcinogenic Risk Resulting From Extreme Grilled Meat On Experimental Rats. 2 TH Specific Education Scientific Conference, Mansoura Univ.11-12 April: 959-982.
2. Ames, B.N., Shigenaga , M.K. And Hagen, T.M. (2002): Oxidants, Antioxidants, And The Degenerative Diseases Of Aging. Proc Natl Acad Sci, 90:7915-22.
3. Artimage, G.Y And Berry, W.G. (1987): Statistical Methods 7th Ed. Ames, Iowa Stata University Press, 39-63.
4. Beuchamp, C. And Fridovich, J. (1971): Superoxide Dismutase. Improved Assay An Assay Applicable To Acryloamide Gels. Anal Biochem. 44: 276-287.
5. Beuther, E., Duron, O. And Kelly, B.M. (1987): Improved Method For The Determination Of Total Blood Glutathione. J.Lab.Clin. 43 (5)365-371.
6. Block, E. (1999): The Organosulfur Chemical Of The Genus Allium-Implications For The Organic Chemistry Of Sulfur. Angew. Chem.Int.Ed. Engl.31:1145-1178.

7. Bordia, A., Bansal, H. C., Arora, S. K. And Singh, S. V. (2002): Diuretic Effect And Mechanism Of Action Of Onions. J. Ethnopharmacol.; 79:353–357.
8. Borg, D.C. (1976): Application Of Electron Spinreasonance In Biology. WA., Ed. Free Radicals In Biology, Vol .1:69. New York : Academic Press.In Pryor .
9. Botsoglou, N.A., Fletouris, D.J., Papageorgiou, G.E., Vassilopoulos,V.N., Mantis, A.J. And Trakatellis, A.G., (1994): Rapid, Sensitive, And Specific Thiobarbituric Acid Method For Measuring Lipid Peroxidation In Animal Tissue, Food And Feedstuff Samples. J. Agric. Food Chem. 42, 1931–1937.
10. Chapman, D.G., Gastilla R. And Campbell, T.A. (1950): Evaluation Of Protein In Food. I. A. Method For The Determination Of Protein Efficiency Ratio. Can. J. Biochem. Physio. I (37) 679-686.
11. Drabkin, D.L. (1949): The Standardization Of Hemoglobin Measurement. Am.J. Med. Sc., 217-710.
12. Dorant , E., Van Den, .P.A .And Goldbohm, R.A. (1996): A Prospective Cohort Study On The Relationship Between Onion And Leek Consumption, Garlic Supplement Use And The Risk Of Colorectal Carcinoma In The Netherlands. Carcinogenesis Mar;17(3):477-84.
13. Fawcett, J.K. And Scott, J.E. (1960): A Rapid And Precise Method For The Determination Of Blood Urea .J .Clin .Pathol. 13,156-159.
14. Fortin, F. (2004): Protection Against Co-Carcinogenesis By Antioxidants. Editorial Director. The Visual Foods Encyclopedia. Macmillan, New York. 22:116.
15. Franca, B. And Harri, V. (2001): Allium Vegetables And Organosulfur Compounds: Do They Help Preventcancer? Environmental Health Perspectives. 109 (9):893.
16. Garth, L. N. And Rita, E. (2006): Lipid Replacement And Antioxidant Nutritional Therapy For Restoring Mitochondrial Function And Reducing Fatigue In Chronic Fatigue Syndrome And Other Fatiguing Illnesses. Journal Of Chronic Fatigue Syndrome; 13(1): 57-68.
17. Habig, W.H., Pabst, M.J. And Takob, W.B. (1974): Glutathione S-Transferase. The First Enzymatic Step In Meracapturic Acid Formation. J.Biol. Chem .249(22):7130-7139.
18. Husdan, H And Rapoport, A. (1968): Estimation Of Creatinine By Jaffe Reaction. Clin.Chem, 138,149-150.
19. Irkin, R. And Korukluoglu, M. (2007): Control Of Aspergillus Niger With Garlic, Onion And Leek Extracts. African Journal Of Biotechnology Vol. 6 (4), 384-387.
20. Jin, L. And Baillie, T.A. (1997): Metabolism Of The Chemoprotective Agent Diallyl Sulfide To Glutathione Conjugates In Rats. Chem Res Toxicol 10:318–327.
21. Kanno, T., Sato, E.E., Muranaka ,S., Fujita, H., Fujiwara, T., Utsumi, T., Inoue, M. And Utsumi, K.(2004): Oxidative Stress Underlies The Mechanism For Ca(2+)-Induced Permeability Transition Of Mitochondria. Free Radical Res; 38(1):27-35.
22. Liu, R.H. (2003): Health Benefits Of Fruit And Vegetables Are From Additive And Synergistic Combinations Of Phytochemicals. Am. J. Clin. Nutr., 78: 517 -520 .
23. Malairajan, P., Geetha, G., Narasimhan, S. And Jessi, K. (2006): Analgesic Activity Of Some Indian Medicinal Plants. J. Ethnopharmacol., 19: 425-428.
24. Mc Inory, R.A. (1954): Amicro Hematocrit For Determining The Packed Cell And Hemoglobin Concentration On Capillary Blood. J. Clin Path., 7; 32.
25. Merchant, R.E. (2003): Dietary Chlorella Pyrenoidosa For Patients With Malignant Glioma: Effects On Immunocompetence, Quality Of Life, And Survival. Phytother. Res. 2003; 4(6):220-31.
26. NRC (1995): National Research Council: Nutrient Requirements Of Laboratory Animals, Fourth Revised Edition, PP.29-30 National Academy Press. Washington, DC.
27. Onyeagba, R.A., Ugbogu, O.C., Okeke, C.U And Iroakasi, O. (2004): Studies On The Antimicrobial Effects Of Garlic (Allium Sativum Linn), Ginger (Zingiber Officinale Roscoe) And Lime (Citrus Aurantifolia Linn). Afr. J.Biotechnol. 3:552-554.
28. Pietta P, Simonetti P And Mauri P (1998): Antioxidant Activity Of Selected Medicinal Plants. J Agric Food Chem 46, 4487 - 4490.
29. Reitman, S. And Frankel, S. (1957): Enzymatic Determination Of Liver Function. Am. J .Clin. Path., 28:56-63.
30. Riley, D.M., Bianchini, F. And Vainio, H.(2001): Allium Vegetables And Organosulfur Compounds: Do They Help Prevent Cancer. Environ Health Perspect. Sep; 109(9):893-902.
31. Sinha, R ., Rothman; N., Brown, E.D., Salmon CP., Knize MC., Swanson ,C.A., Rossi, S.C. , Mark, S.D., Levander OA. And Felten JS. (1995): High Concentrations Of The Carcinogen 2 – Amino -1- Methyl -6- Phenylimidazo [4-5-B] Pyridine (Phtp Occurin Chicken But Are Dependent On The Cooking Method. Cancer Res., 55, 4516-4519.

32. Solyakov, A. And Skog, K. (2002): Screening For Heterocyclic Amines In Chicken Cooked In Various Ways Food And Chemical Toxicology. 40, 1205-1211.
33. Takahashi, S., Hakoi, K., Yada, H., Hirose, M., Ito, N. And Fukushima, S. (1992): Enhancing Effects Of Diallyl Sulfide On Hepatocarcinogenesis And Inhibitory Actions Of The Related Diallyl Disulfide On Colon And Renal Carcinogenesis In Rats. Carcinogenesis 13:1513–1518.
34. Thoma, L. (2000): Alanin-Aminotransferase (ALT), Aspartate Aminotransferase (AST) In: Labor And Diagnose. The Books Verlagsgesell Schaft. Mbh, Pp. 56-67.
35. Uchiyama, M And Mihara, M. (1978): Determination Of Malondialdhyde Precursor In Tissues By Thiobarbituric Acid Test. Anal. Biochem .,86 (1),271-278.
36. Wei, Y.H And Lee, H.C. (2002): Oxidative Stress, Mitochondrial DNA Mutation And Impairment Of Antioxidant Enzymes In Aging. Exp Biol Med; 227:671-682.
37. Weiss, C., Marker, H.S. And Lehrer, G.M. (1980): Sensitive Fluorometric Assays For Glutathione Peroxidase And Reductase. Anal Biochem 106: 512–516.

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Effect of Different Media and Growth Regulators on the *in vitro* Shoot Proliferation of Aspen, Hybrid Aspen and White Poplar Male Tree and Molecular Analysis of Variants in Micropropagated Plants

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Abstract: Among other *in vitro* factors like temperature and light, concentration of plant growth regulators and medium constituents are two of the most important aspects of successful micropropagation. With the aim of optimization of *in vitro* multiplication of *Populus alba* L., *Populus tremula* L. and *Populus tremula* L. *x Populus. tremuloides* Michx , the effect of MS and WPM media with various concentrations of BAP and 2iP was studied. The following multiplication parameters were monitored: number of shoots regenerated/explant, explant height, and explants weight were determined. MS medium proved to be the most effective one, resulting in better and morphologically superior microshoots as compared to WPM medium in the case of *Populus alba*. However in *Populus tremula* and *Populus tremula x Populus Tremuloides* the highest number of shoots was found when grown on WPM medium. In all three poplar lines, the highest shoot multiplication was obtained on MS and WPM media supplemented with BAP at (0.1 and 0.2 mgl^{-1}). Very poor multiplication was achieved on media with 2iP. Shoot tips were isolated and induced to root on MS medium supplemented with IAA, IBA and/or NAA (0.0, 0.1, 0.2 and 0.4 mg l^{-1}). About 90% of the rooted plantlets tested have successfully established in soil. *In vitro* derived plants were genetically analyzed using RAPD fingerprints. RAPD analysis confirmed that all the *in vitro* derived plant which tested were genetically identical to their donor plants, suggesting the absence of detectable genetic variation in the regenerated plants.

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1. Introduction:

Forest trees are of great environmental and economic importance and also display remarkable developmental traits (Groover *et al*., 2004). Fast-growing poplar trees are widely used in a variety of climate zones for applications to stabilize soils, and to decrease windblown dust and the vertical migration of pollutants (Schnoor, 2000) *Populus* species are economically and ecologically important because of their suitable wood properties (i.e for paper making and timber), fast growth rate and high biodiversity of organisms dependent on living or decaying trees of *Populus* (Ranua, 1996, 2001; Rautio *et at*., 2001; Karl**,** 1988). *Populus* has been the second most used tree genus in biotechnology studies in general (after *Pinus*) and the most used in genetic modification worldwide (Marchadier and Sigaud, 2005). Aspen and white poplar belong to the genus ***Populus*,** section *Populus*, family Salicaceae. They are dioecious, with male and female flowers (in catkins) occurring on separate trees.

Hybrid aspen grow even faster than European aspen and can reach a height of 20 meters in just 25 years (Hynynen and Karlsson, 2002). White poplar (*Populus alba* L.) have been introduced and widely used in commercial scale in a number of countries from Europe, North Africa, Near and Middle East during the second half of the twentieth century (Confalonieri *et al*., 2000). *Populus alba* is a species important for its resistance to disease, dryness, and sea breezes, and it has been widely used as a designated source of hybrid poplars (Chiba, 1971). Male clones in some *Populus* spp. tended to have longer internodes, higher plant dry weight and heavier wood as compared to the female clones (Khosla and Deol**,** 1984).

Poplar trees propagated through sucker shoots that arise from horizontal roots, and their rapid growth (Bradshaw *et al*., 2000). Aspen (*Populus tremula* and *P. tremuloides*) are, however, difficult to root from woody cuttings (Ahuja, 1983). Therefore, an efficient *in vitro* propagation system for aspen is highly needed. Recently, many techniques have been developed to detect and identify genetic variations of vegetatively propagated plants (Ruibal-Mendieta and Lints, 1998). Molecular techniques such as RAPD, for instance, are a quick and reliable method that could significantly detect small genetic changes in plants (Williams *et al*., 1990). RAPD analysis using PCR in association with short primers of arbitrary sequence has been demonstrated to be sensitive in detecting variation among individuals (Rani *et al*.

1995, Soniya *et al*. 2001). According to results of Kiss *et al*., (2001), Liu and Furnier (1993) and Lu *et al*., (2006) RAPDs are powerful for fingerprinting individuals in *Populus*. The main aim of the present study was to find an efficient and simple method of *in vitro* clonal propagation using shoot tip explant for producing large numbers of male (aspen, hybrid aspen and white poplar) trees plants for forest plantations and for further phytoremidiation studies and detect if it was somaclonal variations in *in vitro* derived plants with the aim to monitor the uniformity of plants multiplied *in vitro*.

2. Material and Methods

2.1 Plant material

The plant material used in my experiments was kindly provided by the Institute for Forest Genetics, Grosshansdorf, Germany. It had already been introduced into the culture so that in this paper I only conducted the procedures of shoot multiplication, rooting of shoots and their *ex vitro* acclimatization.

The following clones were used: W52 (*Populus tremula* L.), (*Populus alba* L.) and the hybrid aspen clone T89 (*Populus. tremula* L. x *Populus tremuloides* Michx.).

2.2 Culture medium

Shoot proliferation medium: Shoot tips and stem cuttings were used as explants for shoot multiplication. The explants were cut into small pieces (about 10-15 mm long). Then explants were inoculated aseptically on MS (Murashige and Skoog, 1962) and WPM woody plant media (Lloyd and McCown 1980). Media supplemented with either 6-Benzyladenopurine (BAP) or 2-isopentenyladenine (2iP) varying concentrations (0.0, 0.1, 0.2 and 0.4 mgl^{-1}) were prepared for shoot proliferation. The MS and WPM media were supplemented with 2% (w/v) sucrose and solidified with 0.6% agar. The pH of the medium was adjusted to 5.8 before autoclaving at 121°C and 1.2–1.3 kg/cm^2 pressure for 20 min. 400 ml culture vessels (containing 60 ml of medium) were used to maintain shoot cultures. Every jar contained two explants. The cultures were incubated under growth room conditions (22 ± 2 °C, 16 h photoperiod and light intensity of 4000 lux provided by cool white fluorescent lamps (Phillips TLM 40W/33RS). After 4 weeks of plant culture the data were recorded on number of shoots regenerated/explants, explants height and explants weight. Each treatment had ten replicates.

2.3 Rooting and transplantation

For root formation, shoots developed on MS and WPM multiplication media with different levels of BAP or 2iP were transferred and cultured in 400 ml jars containing 60 ml full strength MS basic medium supplemented with 2% (w/v) sucrose, 6 g/L agar with different concentrations of 3-indolebutyric acid (IBA), indole acetic acid (IAA) or naphthalene acetic acid (NAA) at (0.0, 0.1, 0.2 and 0.4 mgl^{-1}). Every jar was inoculated with four shoots (about 10-15 mm long). The shoots were maintained for 4 weeks under the same culture conditions as for development of shoots. After this time, the number of root, root length, plant weight and plant height were recorded. Each experiment was repeated three times and ten replications per treatment were taken into account. Rooted plantlets were taken out from the culture flasks and carefully washed with tap water to remove agar and they were transplanted to pots filled with sterilized mixture of compost and sand (1:1) and grown for 4 weeks in greenhouse conditions to determine the percentage of plants that survived. The potted plants were irrigated with distillation water twice a day for 4 weeks. Established plantlets were then transferred in 30 cm pots.

2.4 Genomic DNA extraction

DNA was extracted from fresh leaves of five *in vitro* derived plants of the three populus species as well as control plants using a standard CTAB extraction procedure (Wolff *et al.* 1994, modified after Saghai-Maroof *et al.* 1984). Cleaning with ammonium acetate was necessary. Samples were diluted with half the volume of 7.5 M, cold ammonium acetate, cooled in a fridge for 15 min, followed by spinning for 15 min at 5000 rpm. The supernatant was taken and two volumes of cold 96% ethanol gently mixed and left for 30 min in a freezer. After spinning for 15 min, the precipitate was taken, and 500 µl of cold 70 % ethanol was added for washing. The supernatant was removed and the precipitate left to air-dry at room temperature for 10-20 min, and then dissolved in a suitable volume of TE buffer. DNA concentration was determend by NanoDrop 3300 (Thermo Scientific)

2.5 Random Amplified polymorphic DNA (RAPD)

RAPD analysis was performed in 25 µl volume reactions according to Wolff and Peters Van Rijn (1993). A reaction mixture (17.5 ng genomic DNA, 12.5 REDTaq ReadyMix (Sigma) [20 mM Tris-HCl, pH 8.3, 100 mM KCl, 3mM $MgCl_2$, 0.002% gelatin, 0.4mM mix dNTP (dATP, dCTP, dGTP, dTTP) and 0.06 unit/ µl Taq DNA polymerase] and 0.4 p mole was prepared for each primer sufficient for all samples plus one negative control to which water was added instead of DNA.

All reagents were centrifuged and kept on ice during the preparation of the master mix. Amplifications were carried out in a Mastercycler gradient programmed according to Wolff (1996) [the initial denaturation for 3 min at 94 °C was followed by 45 cycles of denaturation (30 sec. at 94°C), annealing (45 sec. at 36 °C), extension (1.5 min at 72°C)]. PCR products were analyzed by gel electrophoresis on 1.4% agarose gel prepared in 0.5 X TBE buffers, DNA ladder (Fermentas) was used as a standard with molecular sizes of 1000, 900, 800, 700, 600, 500, 400, 300, 200 and 100 bp. The gel was stained with ethidium bromide for 20 min and examined using UV cabinet unit and photographed with a Polaroid camera connected to a computer system with analytical software (GelDocu Advanced version).

Ten different oligonucleotide random primers were used for RAPD analysis (A1) 5'AGACGTCCAC3', (A2) 5ACGCGCATGT3', (A3)5'AATGGCGCAG3', (A4) 5'GAATCGGCCA3', (A5) 5'GGGAGACATC3'), (A6) 5'GGAAGTCGCC3' – (A7) 5'ACGCGCATGT3' (A8) 5'GGTCGGAGAA3' – (A9) 5'CCTACGTCAG3' and (A10) 5' CTGACCAGCC3' were used for RAPD.

2.6. Statistical analysis

Experiments were set up in completely randomized design. Data were statistically analyzed using ANOVA\MANOVA of Statistica 6 software (Statsoft, 2001), the significance of differences among means was carried out using the Least Significant Test (L.S.D) at $p = 0.05$.

3. Results and Discussion

3.1 Shoot multiplication

From our previous work, we found that shoot multiplication from shoot tips yielded much higher number of shoots than from stem cuttings. Shoot tips proved an effective explant for micropropagation, and provided more than one explant. Therefore, we used shoot tips to find optimal culture conditions for proliferation. The growth rate of the plant material of three poplars under *in vitro* conditions was proportionate to the multiplication rate in both types of multiplication mediums.

Among two cytokinins tested, BAP proved to be more effective than 2iP for initiating shoots per explant (Table 1). From this experiment it was evident that best result obtained from WPM medium amended with BAP (0.1 and 0.2 mgl^{-1}) which produced 51.3 and 28.3 shoots/explant in the case of *Populus tremula* and *Populus tremula x Populus tremuloides*), respectively. In *Populus alba* BAP at 0.2 mgl^{-1} in MS medium seemed to be the best formula, since it facilitated a high rate of proliferation (14 shoots/explant) and shoot development without altering their elongation. Besides, no callus formation occurred at all BAP concentrations. Further increase in the concentrations of BAP had no effects on the number of multiple shoots of *Populus alba* and *Populus tremula* grown on MS or WPM (Table1). In contrast, with *Populus tremula x Populus tremuloides*, the elevated level of BA had stimulating effects on the total number of regenerated plantlets which grown on MS. Micropropagation is, thus, similar to the traditional method of vegetative propagation using cuttings has the distinct advantage of producing greater number of identical plants in a much shorter time (Barakat, 2008). The positive effect of BAP on the capacity to induce plant regeneration in *Populus alba* has been reported previously Pintaric' (2008) reported a range from 5.36 and 5.86 shoots per explant after 28–35 days from culture on MS and WPM media, respectively.

When the present results on this species were compared with the previous studies it was seen that each species needs appropriate culture medium, with appropriate concentration of growth regulators. *In vitro* shoot multiplication had been reported in *Populus spp* by using stem cutting as explant (Špela *et al*; 2009, Rahman and Rajora, 2001, Sung *et a*l., 1991).

3.2 Root formation

Data presented in Table (2) and Fig. (1D and E) show the effect of IAA, IBA and /or NAA on the *in vitro* rooting of proliferated shoots of the *Populus alba*, *Populus tremula* and *Populus tremula x Populus. tremuloides*. Root formation was 100% in response to the application of IAA, IBA and /or NAA to the culture media. The MS basic medium without IAA, IBA and /or NAA also revealed root formation. No callus formation was observed in the cultures. The MS medium with IBA (0.4 mgl^{-1}) provides the highest number of roots per explants (9.5 roots/explants in *Populus alba*)**,** Table (2). However, the highest number of roots per plant (7.0 and 6.4) were recorded in IAA (0.2 $mgl^{-1)}$ and NAA (0.1 mgl^{-1}) in the case of *Populus tremula* and *Populus tremula x Populus. tremuloides*, respectively**.** Further increase in the concentrations of IAA, IBA and /or NAA had no effects on the number of roots in the case of *Populus tremula.* The maximum root growth was recorded on MS medium with 0.1 mgl^{-1} IBA, 0.4 mgl^{-1} IAA and 0.1 mgl^{-1}NAA in the case of *Populus alba, Populus tremula* and *Populus tremula x Populus. tremuloides*, respectively. Although excessive auxin is commonly characterized by callus formation, no callus formation was detected in rooting stages of *Populus alba* and *Populus tremula.* The absence of callus at shoot base is an important observation

because it can be excluded that auxin treatments were supplied in improper high supplements (Nerman *et al*., 2009).

Table (1): The effect of medium type and cytokinin concentrations on shoot induction, elongation and plant fresh weight of *Populus alba, Populus tremula* and *Populus tremulax Populus tremuloides*

			P. alba			*P. tremula*			*P. tremula x P. tremuloides*		
Medium	Growth regulators mgl^{-1}		No of shoots/ explant	Length of the longest shoot (cm)	explant fresh weight (g)	No of shoots/ explant	Length of the longest shoot (cm)	explant fresh weight (g)	No of shoots/ explant	Length of the longest shoot (cm)	explant fresh weight (g)
	BA	2iP									
	0.0	0.0	1.5d	5.5b	0.39bcd	2.3d	8.7abc	1.5cd	5.0f	8.7ab	1.3ab
	0.1	0.0	7.75bc	3.25cd	0.30cd	16.8cd	7.6bcd	1.4cd	8.2ef	6.5bcd	1.2bc
MS	0.2	0.0	14.0a	4.67bc	0.51bcd	19.8c	11.1d	2.9ab	14.8cd	6.7bc	1.7a
	0.4	0.0	12.0ab	5.0bc	0.79bcd	15.7cd	6.2bcd	1.4cd	24.8ab	4.3def	0.84b
	0.0	0.1	1.0d	5.0bc	0.63bcd	4.7cd	4.7de	0.4d	5.7f	9.1a	0.93bcd
	0.0	0.2	1.0d	5.0bc	2.28a	8.0cd	8.2bc	2.0bc	5.4f	6.7ab	0.68cd
	0.0	0.4	1.0d	3.4cd	0.3cd	6.3cd	6.3bcd	1.0cd	2.7f	6.3bcde	0.7bcd
	0.0	0.0	1.0d	7.67a	1.21b	1.0d	11.3a	1.5cd	6.2f	5.2cde	0.6d
	0.1	0.0	11.25a	4.75bc	1.26b	51.3a	6.9bcd	1.7cd	16.4cd	3.6ef	0.8bcd
WPM			b								
	0.2	0.0	4.75cd	4.25bc	0.19d	21.3bc	6.3cde	1.6cd	28.3a	3.7ef	1.4ab
	0.4	0.0	8.25bc	2.38d	0.48bcd	33.5b	4.5e	1.1cd	19.3bc	2.8f	0.9bcd
	0.0	0.1	1.0d	5.33b	0.20cd	7.3cd	8.9abc	1.7bcd	12.3de	8.5cde	1.2b
	0.0	0.2	4.25cd	4.25bc	0.97bc	5.8cd	8.8abc	1.9bc	7.3ef	7.7ab	1.1bc
	0.0	0.4	1.0d	4.33bc	0.49bcd	4.0d	10.8a	3.1a	6.5f	4.9cde	0.9bcd

Means followed by the same letter within a column are not significantly different at 0.05 level of probability according to L.S.D. test

Table (2): Effect of different concentrations of IAA, IBA and NAA on number of roots , root length, Plant height and Fresh weight of *Populus alba, Populus tremula* and *Populus tremula x Populus tremuloides*

			P. alba				*P. tremula*				*P. tremula x P. tremuloides*			
Growth regulators mgl^{-1}			Root number	Length of the Longest root (cm)	Plant height (cm)	Plant Fresh weight (g)	Root number	Length of the Longest root (cm))	Plant height (cm)	Plant Fresh weight (g))	Root number	Length of the Longest root (cm)	Plant height (cm)	Plant Fresh weight (g)
IAA	IBA	NAA												
0.0	0.0	0.0	2.6b	2.0c	4.5de	0.4b	2.2b	5.2a	7.5bc	1.5ab	0.7d	0.8bc	7.3ab	0.7bcd
0.1	0.0	0.0	3.25b	6.0ab	7.0bcd	0.5b	4.7ab	3.7a	7.9bc	0.6c	1.0d	0.4c	6.7ab	0.5cd
0.2	0.0	0.0	3.33b	6.0ab	5.5de	0.8ab	7.0a	5.6a	8.8ab	1.2b	2.0cd	0.7bc	5.0d	0.54bcd
0.4	0.0	0.0	5.0ab	8.25a	8.8abc	1.04ab	6.5a	5.3a	10.3a	1.5ab	0.2d	0.6c	6.4abcd	1.6a
0.0	0.1	0.0	6.0ab	2.0c	11.0a	1.5ab	3.3b	3.9a	9.0ab	1.3b	1.6cd	0.7c	7.1ab	0.82b
0.0	0.2	0.0	6.2ab	2.8bc	9.4ab	1.7a	3.5b	3.7a	7.1bc	1.9a	2.9c	1.1bc	6.4abcd	0.8bc
0.0	0.4	0.0	9.5a	2.5bc	5.5cde	1.7ab	3.3b	3.4a	7.3bc	1.0bc	4.4b	2.4a	6.5abc	0.45d
0.0	0.0	0.1	4.5ab	0.5c	3.7e	0.9ab	3.1b	3.2a	6.8c	1.2bc	6.4a	2.9a	5.5cd	0.7bcd
0.0	0.0	0.2	4.0b	2.0bc	5.0de	0.26b	3.4b	3.2a	10.2a	1.2bc	0.7d	0.3c	7.8a	0.45cd
0.0	0.0	0.4	3.30b	4.3bc	7.3bcd	1.0ab	2.8b	2.9a	6.5c	0.9bc	1.0cd	1.9ab	5.8bcd	0.6bcd

Means followed by the same letter within a column are not significantly different at 0.05 level of probability according to L.S.D. test

3.3 Acclimatization

The rooted plantlets were transferred to a mixture of Sand: Peat-moss (1:1, v/v) in the portrays for further development and hardening (Fig.1F). Humidity was maintained by covering the trays with rigid plastic cover and frequently spraying of water. Similar process of maintaining humidity was practiced for hardening (Jasrai *et al*., 1999; Rolf and Ricardo 1995). Plants transferred to the field after 4 weeks showed good growth. Almost 90% of the

regenerated plants of the three species survived and showed a vigorous growth.

3.4 RAPD analysis

Maintaining genetic stability in regenerated plants is essential for species conservation (Quiala *et al.* 2009). In order to confirm whether somaclonal variation was detectable in the regenerated plants. . RAPD was employed to analyze the genetic fidelity of five plants from each species randomly selected from *in vitro* derived plants as well as control donor plants. Two primers (A6 and A7) of the ten primers tested gave bands in RAPD analysis. Fig 2 shows the *in vitro* derived plants shared the same banding patterns as those of the donor plants, implying that they were possibly genetically identical to each other. Generally, it is important to make sure that the regenerants were genetically true-to-type of their donor plants with respect to genetic fidelity. In order to know if there is any aberration in the regenerated plants, the RAPD marker system was employed for this purpose. This system has been revealed to be a potential marker for distinguishing genetic variation (Piccioni *et a*l; 1997; Raimondi *et al*; 2001). These results confirm that all of the regenerants showed genetic stability in our regeneration system. Therefore, we can conclude that direct regeneration from shoot tip explants did not induce any somaclonal variation that has been depicted in other explants-mediated culture (Cassells and Curry, 2001; S˘us˘ek *et al*; 2002).

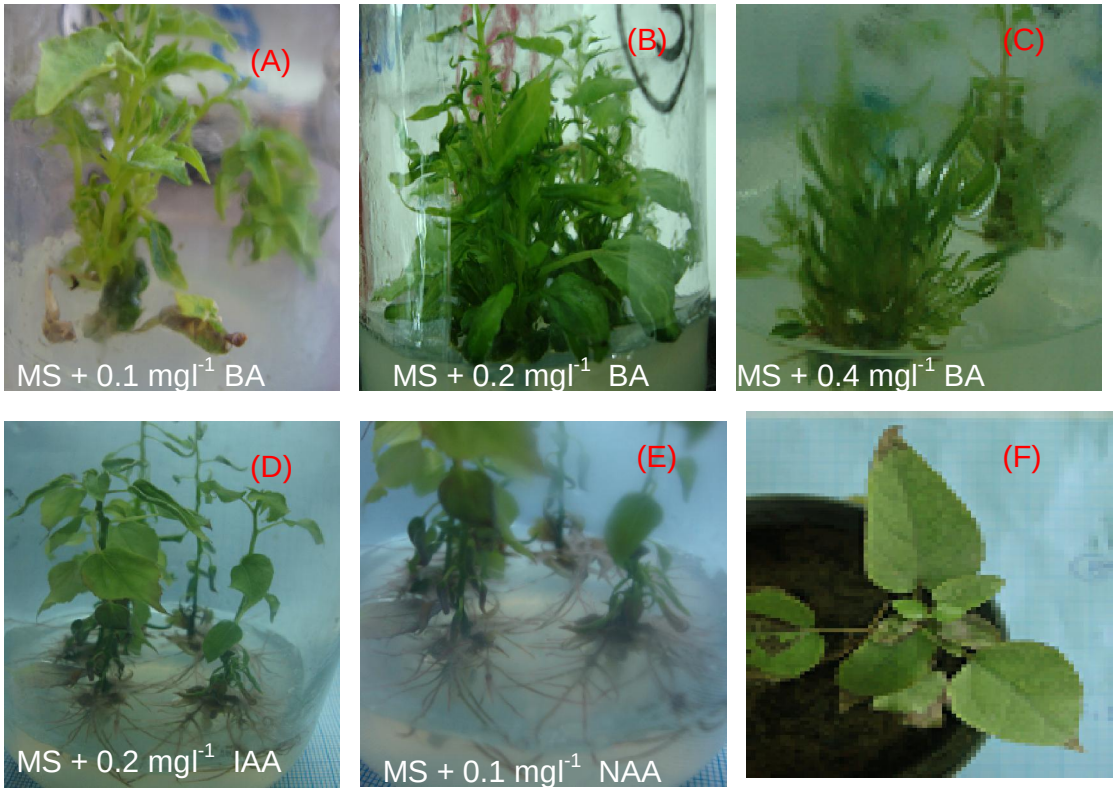

Figure (1): Modified micropropagation method of *Populus spp*., (A, B and C) Shoot regeneration of *Populus alba*, *Populus tremula* and *Populus tremula x Populus. Tremuloides* respectively. (D and E) Root formation of *Populus tremula* and *Populus tremula x Populus. Tremuloides* respectively, (F) transplantation of regenerated plantlets of *Populus tremula x Populus. Tremuloides* in plastic pots after eight weeks.

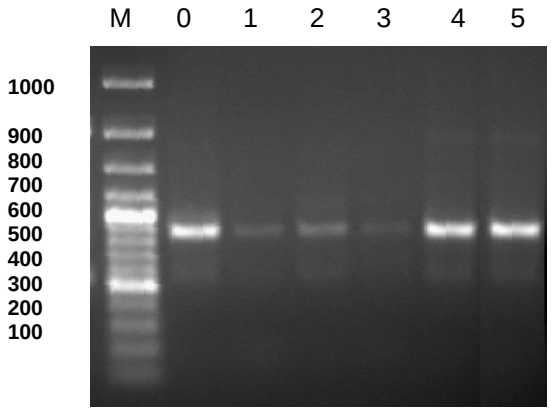

Fig. (2): RAPD pattern of aspen with primer A6 (1, 2, 3, 4 and 5) refer to sample plants, (0) is the control plant of aspen and (M) refers to the DNA marker

4. Conclusions

Plant regeneration through *in vitro* shoot tip is a rapid and simple method for clonal and mass propagation of male aspen, hybrid aspen and white poplar tree. Using this technique, regenerated plants were obtained within 4 weeks at an average of 51.3 and 28.3 shoots/explant in the case of *Populus tremula* and *Populus tremula x Populus tremuloides*), and (14.0 shoots/explant) in the case of *Populus alba.* In the current work I used two different media to induce shoots from poplar shoot tips explants. These media, with various combinations of cytokinin and auxin, triggered direct shoot organogenesis, for forest plantations and for further phytoremidiation studies. Maintaining genetic stability in regenerated plants is essential for conservation of endangered species.

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5. References:

1. Ahuja, M.R. (1983): Somatic cell differentiation and rapid clonal propagation of aspen. Silvae Genet. 32:131-135.
2. Bradshaw, H.D., Jr., R. Ceulemans, J. Davis, and R. Stettler. 2000. Emerging model systems in plant biology: poplar (*Populus*) as a model forest tree. Journal of Plant Growth Regulators 19:306-313.
3. Cassells, A.C. and Curry, R.F. (2001): Oxidative stress and physiological, epigenetic and genetic variability in plant tissue culture: implications for micropropagators and genetic engineers. Plant Cell, Tissue and Organ Culture. 64, 145–157.
4. Chiba, S. (1971): In trans new forestry. 45, Chikyu press, Tokyo. Confalonieri, M., Belenghi, B., Balesirazzi, A., Negri, S., Facciotto, G., Schenone, G. and Delledonne, M. 2000. Transformation of elite white poplar (*P. alba* L.) cv. "Villafranca" and evaluation of herbicide resistance, Plant Cell Reports, 19: 978-982.
5. Groover A., Joseph R. F., Gayle D., Caiping M., Robert M., Steven S. and Richard M. (2004) Gene and Enhancer Trap Tagging of Vascular-Expressed Genes in Poplar. Trees Plant Physiol 134: 1742-1751
6. Hynynen J. and Karlsson K. (2002): Intensive management of hybrid aspen in Finland. In Hynynen J. and Sanaslahti A. (eds.). Management and utilization of broad leaved tree species in Nordic and Baltic countries-Birch, aspen and alder. Finnish Forest Research Institute. Research Papers 847:99-100.
7. Jasrai, Y.T., Kannan, V.R., Remakanthan, A. and George, M. M. (1999): *Ex vitro* survival of *in vitro* derived banana plants without greenhouse facilities. Plant Tissue Culture. 9: 127-132.
8. Karl, W. (1988): Aspen: quickly becoming today's preferred wood species for pulp. Pulp and Pap. J. 41(5): 119-123.
9. Khosla, P. K. and Deol, G. S. 1984. Growth response of Male and Female clones of *Populus Ciliata* "Wall." Ex royle to nitrogen fertilizer .Proc. Indian main. Sci. Acad. B50 (6). 603-606.
10. Kiss, J., Kondrák, M. , Törjék , O. , Kiss, E. , Gyulai ,G. , Mázik-Tökei , K. and Heszky , L.E. (2001): Morphological and RAPD analysis of poplar trees of anther culture origin Euphytica ,8, (2), 213-221.
11. Lioyd, G. and McCown, B. 1980. Commercially-feasible micropropagation of mountain laurel, *Kalmia latifolia*, by use of

shoot-tip culture. Inter Plant Prop. Soc. Proc. 30: 421–427 (Publ. 1981).
12. Liu, Z. and Furnier, G. R. (1993): Comparison of allozyme, RFLP, and RAPD markers for revealing genetic variation within and between trembling aspen and bigtooth aspen. TAG Theoretical and Applied Genetics 87, (1-2), 97-105.
13. Lu Z., Wang Y., Peng Y., Korpelainen H., Li C. (2006):. Genetic diversity of populus cathayana Rehd populations in southwestern china revealed by ISSR markers. *Plant Science*, 170 (2), 407-412.
14. Macleod, M. (1987): Aspen provides a new challenge. Pulp Pap. J. 5/6: 38-39.
15. Marchadier, H. and P. Sigaud, (2005): Poplars in biotechnology research. Unasylva, N 221, vol. 56, 2. In: http://www.fao.org/docrep/008/a0026e/a0026e00.htm.
16. Murashige, T. and Skoog, F. (1962): A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 13, 473–497.
17. Nerman, M., Souad E. and Hussein T. (2009)*: In vitro* mass propagation of the endangered Sainai Hawthorn *Crataecus* Sinanica boiss. International Journal of Academic Research 1 (1): 24-29.
18. Piccioni, E; Barcaccia, G; Falcinelli, M. and Standardi, A. (1997): Estimating alfalfa somaclonal variation in axillary branching propagation and indirect somatic embryogenesis by RAPD fingerprinting. International Journal of *Plant* Sciences. 158, 556–562.
19. Pintaric, B. (2008): Microprpagation of white poplar (Populus alba L.). umarski list br. 7–8, CXXXII, 343-354.
20. Rahman, M. and Rajora, O. (2001): Microsatellite DNA somaclonal variation in micropropagated trembling aspen (Populus tremuloides). Plant Cell Report, 20 (6) 531-536.
21. Raimondi, J.P; Masuelli, R.W; Camadro, E. L. (2001): Assessment of somaclonal variation in asparagus by RAPD fingerprinting and cytogenetic analyses. Scientia horticulturae. 90, 19–29.
22. Ranua, J. (2001): Industrial use of aspen fibers. In Pulkkinen, P., Tigerstedt, P. M. A. and Viirros, R. (eds.) Aspen in paper making. University of Helsinki, Department of Applied Biology Publication. 5:1-4.
23. Ranua, J. (1996): Miksi haapa kiinnosta metsäteollisuutta? (Why is the Finnish forest industry interested in Aspen?). Sorbifolia 4: 178-180.
24. Rani V, Parida A, Raina SN (1995): Random amplified polymorphic DNA (RAPD) markers for genetic analysis in micropropagated plants of *Populus deltoids* Marsh Plant Cell Report 14, 459-462.
25. Rautio, M., Kangas, T., Auterinen, T., Alen, R. and Pulkkinen, P. (2001): Wood quality components of hybrid aspen for paper making .In Pulkkinen, P., Tigerstedt, P. M. A. and Viirros, R. (eds.) Aspen in paper making University of Helsinki, Department of Applied Biology Publication. 5: 19-26.
26. Rolf, D. I. and Ricardo, T. F. (1995): Micropropagation of *Alpinia purpurata* from inflorescence buds. Plant Cell Tissue and Organ Culture 40: 183-185.
27. Ruibal-Mendieta, N.L. and Lints, F.A. (1998): Novel and transgenic food crops: Overview of scientific versus public perception. Transgenic Research. 7(5) 379-386.
28. Saghi-Maroof, M. A., Soliman, K. M., Jorgensen, R. A. and ALLARD. R. W. (1984) Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proc. Natl. Acad. Sci. 81, 8014-8018.
29. Schnoor, J.L. (2000): Phytostabilisation of metals using hybrid poplar trees.In: Raskin,I., Ensley, B.D. Eds.), phytoremediation of Toxic Metals: Using plants to clean up the environment. John Wiley and Sons.Inc.,NY.
30. Soniya, E.V; Banerjee N.S. and Das, M.R. 2001. Genetic analysis of somaclonal variation among callus-derived plants of tomato. Current Science. 80,1213-1215.
31. Špela, P., Karin, G., Nada, G. and Marjana, R. (2009): *I n vitro* propagation of European aspen (*Populus tremula* L.) from axillary buds via organogenesis. Scientia Horticulturae, 121 (1)109-112.
32. Statsoft, Inc. (2001): STATISTICA fur Windows (software-system fur Datenanalyse) Version 6. http:\www.statisoft.com.
33. S˘us˘ek, A; Javornik, B. and Bohanec, B. 2002. Factors affecting direct organogenesis from flower explants of *Allium giganteum* Plant Cell, Tissue and Organ Culture. 68, 27–33.

34. Sung, H. S., Young W. C. and Richard B. H. (1991): Cold storage of in vitro cultures of hybrid poplar shoots (Populus alba L. x P. grandidentata Michx.). Plant Cell, Tissue and Organ Culture 27(2) 161-168.
35. Williams, J.G.K; Kublik, A.R; Livak, K.J; Rafalski, J.A. and Tingey, S.V. (1990): DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research. 18, 6531-6535.
36. Wolff, K 1996. RAPD analysis of sporting and chimerism in Chrysanthermum. Euphytica, 89, 159-164.
37. Wolff, K; Peters-Van Rijn, J. and Hofstra, H. (1994): RFLP analysis in chrysanthemum I. Probe and primer development. Theoretical and Applied Genetics. 88,472-478.

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Amelioration the Toxic Effects of Cadmium-Exposure in Nile Tilapia (*Oreochromis Niloticus*) by using *Lemna gibba L*

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Abstract: The effect of cadmium (Cd) toxicity and its impact on histopathology, haematological, and biochemical changes in Nile tilapia (*Oreochromius niloticus*) were studied. Fish (35-45 grams) were exposed to Cd that resulted in significant reduction ($p< 0.05$) of the erythrocyte count (RBCs), haemoglobin content (Hb) and haematocrit value (Hct). Significant increases in plasma aspartate aminotranseferase (AST) and alanine aminotransferase (ALT) were observed in fish exposed to Cd. The obtained results indicate that *Lemna gibba L* weed and their extract are effective in removing Cd from water and reducing Cd bioaccumulation in fish. The addition of *Lemna gibba L*-extract reduced significantly ($p<0.05$) the Cd level in water and the metal uptake as compared to fish exposed to Cd alone. The Cd concentration in water was 9.620±0.198 mg L^{-1} and it decreased significantly ($p< 0.05$). The Cd accumulation in liver and muscles of fish exposed to Cd alone was higher than that of *Lemna gibba L*-extract treatment. The addition of *Lemna gibba L*-extract improves the haematological parameters (RBCs, Hb and Hct) and ameliorates the toxic effect of Cd which indicating the capability of *Lemna gibba L*-extract to chelate Cd from the media. Subsequently, the Cd toxicity was reduced. This study indicates that Cd poisoning cause structural damage in the fish organs. It is also demonstrated that *Lemna gibba L*-extract, weed or the weed plus the extract provided protection against the degenerative action of Cd and increased the chance of tissue regeneration.
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Keywords: Cadmium**;** histopathology*; Lemna gibba L***;** amelioration; *Oreochromis niloticus*

1. Introduction:

Cadmium (Cd) is a well known heavy metal toxicant with a specific gravity 8.65 times greater than water (Lide, 1992). Cadmium is an extremely toxic metal. It is widely used in mining, metallurgical operations, electroplating industries manufacturing vinyl plastics which used in metallic and plastic pipes. Effluents from such activities are sources of cadmium into aquatic environments. Most aquatic organisms have the capability of concentrating metals by feeding and metabolic processes, which can lead to accumulation of high concentrations of metals in their tissues. Metals interact with legends in proteins particularly, enzymes and may inhibit their biochemical and physiological activities (Passow *et al*., 1961).

The levels of contamination by cadmium in fish are of considerable interest because fish consumption is an important source of intake cadmium for the general population. Most of the cadmium content in fish or other seafood is highly absorbable in humans; the efficiency of gastrointestinal absorption of cadmium has been reported to be approximately 3–8% of the ingested load. Cadmium is particularly accumulated in kidney and to lower extent in muscles (ATSDR, 2003).

Bio-enhancement of Cd transfer along a food chain was studied by Seebaugh & Wallace (2005) and fish are reported to be used as biological indicators to assess water pollution (Rashed, 2001). In aquatic systems, as fish occupy the upper trophic level, there are greater chances of transferring Cd to higher organisms particularly to human. The European Commission (2001) established the maximum levels permitted of cadmium in seafood as follows 0.05 mg/kg in fish, 0.5 mg/kg in crustaceans (except crab), 1.0 mg/kg in molluscs and crab. Moreover, the Joint FAO/ World Health Organization have recommended the provisional tolerable weekly intake (PTWI) as 0.007 mg/kg for cadmium (0.420 mg/g/week for a 60-kg person) (FAO/WHO, 2005).

Metal bioaccumulation can occur via complexation, coordination, chelating, ion exchange and other processes of greater or lesser specificity. It

is a strictly aggressive process in which metal ions are sequestered by metal binding site in the interior of the cell. The removal of toxic elements from contaminated water has potential advantages over the conventional treatment process. The reduction of toxic elements like cadmium in aquatic environments is needed by any acceptable method. The most widely used technique for the removal of toxic elements involves the process of neutralization and metal hydroxide precipitation (Hiemesh & Mahadevaswamy, 1994).

The use of aquatic plants in water ecosystems and terrestrial plants in hydroponic systems has high potential to clean up the metal contaminated water through phytoextraction and phytostabilization. Phytostabilization utilizes the plant production of compounds, which immobilize contaminants at the entrance of roots. An example of this method is where root exudates cause the precipitation of metals and reducing their bioavailability. Phytodegradation (also known as phytotransformation) is the enzyme-catalysed metabolism of contaminants, typically organics, within plant tissues. The enzymes are usually dehalogenases, oxygenases and reductases (Black, 1995).

Biosorption potential of *Prosopis juliflora* seed powder (PJSP) for Pb (II) from aqueous solution has been investigated by Jayaram & Prasad (2009) where they found that the maximum Pb (II) adsorbed was found to be 40.322 mg/g and the adsorption process was spontaneous and exothermic in nature. Removal of certain heavy metals from waste water by *Lemna gibba L*. has been reported by Kwan & Smith (1991); Buckley (1994); Miranda & IIangovan (1996) and Wafaa (2007).

In the present study, short and long-term bioassays were designed to evaluate the influence of *Lemna gibba L*- plant and/or its extract on the reduction of cadmium in water as well as to investigate the amelioration effect of *Lemna gibba L* on some blood parameters, enzymes and histopathological alterations induced by Cd exposure on Nile tilapia (*Oreochromis niloticus*).

2. Materials and Methods:

Fish culture management

Healthy *Oreochromis niloticus* of 35-45 grams were collected during the late August and early September, 2010 from the ponds of the Central Laboratory for Aquaculture Research at Abbassa, Abo-Hammad, and Sharkia, Egypt (belonging to a single population) .They were collected locally and confined to large plastic aquaria bearing tap water for up to 7 days in the laboratory for acclimation.

Cadmium chloride

Technical grade cadmium chloride (99% purity) was obtained from El-Nasr Chemical Company (Cairo, Egypt) and prepared in aquatic solution to provide the required concentrations of cadmium. Control test without cadmium was performed.

Determination of Lc_{50}

Acute Toxicity Assays

Laboratory bioassays were conducted to determine the 24 hrs, 48 hrs, 72 hrs and 96 hrs LC50 values for tilapia exposed to $CdCl_2$. The experimental design and calculations for the acute toxicity were based on well-known procedures given by Finney (1978) and Sparks (2000). The tests were carried out in 50 Litres rectangular fibreglass aquaria filled with well-aerated tape water (pH 6.5–7.0). Dissolved oxygen in each tank was maintained at close to saturation by aeration. The temperature in each aquarium was maintained at 25.5-27°C using submerged heaters. The photoperiod was 12 hrs light length/day. The fish were visibly free of any deformities, lesions, or disease and were acclimated in tap water for 1 week prior to the experiment. The nominal concentrations of $CdCl_2$ tested were 0 (control), 1, 2, 5, 10, 20, 40, and 80 mg/ L (Chung, 1983). Gross fish mortality of each concentration was recorded every 1 h for the first 12 hrs and every 2 hrs thereafter for 96 hrs while the dead fish were removed every 3–8 hrs.

Tilapias were not fed throughout the test. The control and each test concentration were tested in duplicate.

LC_{50} value of cadmium chloride for *T. nilotica* was determined by the simple graphic method, Probit graphic method and the un-weighted regression method (Finney, 1971).

The tested weed

The duckweed species used was *Lemna gibba L* which was taken from Ganabiet-Tersa drain, Giza, Egypt. The duckweed was acclimatized to the laboratory conditions for one week before starting the experiments.

Plant extracts

Dried plant materials were extracted twice with 50% and 100% methanol as well as 50% and 100% acetone in v/v proportions (200 ml/5g plant) for 2 hrs with constant stirring. The collected filtered extracts were dried in a rotary evaporator (Büchi: Rotavapor-R114 and water bath B-481) at 40^{o}C under reduced pressure (Ghobrial *et al*., 2009).

Cadmium reduction

Tilapias were distributed randomly in 120 Litres rectangular fibreglass aquaria filled with well-aerated tape water (pH 6.5–7.0) at a rate of 15 fish / aquarium. Dissolved oxygen in each tank was maintained at close to saturation by aeration. The temperature in each aquarium was maintained at 27±1°C by means of thermostats. The photoperiod was 12 hrs light-length/days. These aquaria were divided into five groups with three replicates each per group. The first group was free from Cd and *Lemna gibba L* and maintained as a control. The second groups were exposed to 10 mg of $CdCl_2$ only (Equivalent to 1/4 96 h LC_{50}). The third, fourth and fifth group were exposed to 10 mg $CdCl_2$ L^{-1} and 0.1, 1 and 0.1 plus 1 g L^{-1} extract, plant and extract plus plant of *Lemna gibba L*, respectively (Table I).

Fish were fed frequently on a diet containing 30% crude protein at a rate of 3% of live body weight twice daily for 7 and 25 days. Siphoning three quarters aquariums was done every day for waste removal and replacing it by an equal volume of water containing the same concentration of Cd and *Lemna gibba L*. Dead fish were removed and recorded daily.

Hematological, enzymatic and histopathological investigations

After 7 and 25 days of the experiment, blood samples were taken from three Fish from each aquarium. Fish were not fed for 24 hrs before sampling and were anaesthetized with buffered MS222 (50 mg L^{-1}) and blood samples were taken from the caudal vein of an anaesthetized fish by sterile syringe containing EDTA solution as anticoagulant. These blood samples were used for determining erythrocyte count (Dacie & Lewis, 1984) and hemoglobin content (Van Kampen & Zijlstra, 1961). Haematocrit value (Hct) was calculated according to the formulae mentioned by Britton (1963).

Plasma was obtained by centrifugation of blood at 3000 rpm for 15 min and non haemolysed plasma was stored in a deep freezer for further biochemical analyses. After decapitation of fish, samples of gills, liver, spleen, kidney, stomach, intestine and brain were taken and frozen for histopathological investigations. Plasma activities of aspartate amninotransferase (AST) and alanine aminotransferase (ALT) were determined calorimetrically according to Reitman & Frankel (1957)

Cd residue

Cadmium residues were measured in water, liver and muscles according to method of Eaton & Stinson (1983). The water samples were preserved by the addition of one mL of concentrated nitric acid per litre until the time of analysis. The water samples were filtered through 0.45µl membrane filter. The required volume (100 mL) of the filtrate was collected to measure cadmium levels in water samples by using Air/Acetylene Flame Atomic Absorption Spectrophotometer (UNICAM 696 AA Spectrometer).

The analysis of tissue sample was represented by one gram of tissues dissected from the liver and muscles, then placed in a clean screw-capped tube and digested according to the method described by Finerty *et al*. (1990). The obtained solutions were then analyzed by using Air/ Acetylene Flame Atomic Absorption Spectrophotometer (UNICAM 696 AA Spectrometer).

Statistical analysis

The obtained data were subjected to analysis of variance according to Snedecor & Cochran (1982). Differences between means were done at the 5% probability level, using Duncan's new multiple range test (Duncan, 1955).

3. Results:

The LC_{50} values from the three methods (the simple graphic method, Probit graphic method and the un-weighted regression method) are 40.47 mg/L, 40.99 mg/L and 40.13 mg/L ,respectively. Hence the calculated average LC_{50} is 40.533 mg/L. The equation for the dose mortality regression line was found to be $Y = 2.65X+3.368$.

Hematological parameters

Table II shows that the RBCs , HB and HCT were reduced in fish exposed to Cd at both periods and they were lower than that of the control ($p<0.05$). The RBCs count also decreased significantly in fish exposed to Cd at 7 and 25 days. On the other hand, these parameters were return to the normal values and increased significantly in fish exposed to Cd with *Lemna gibba L*- weed and/or its extract for 7 and 25 days. Blood parameters were improved in fish exposed to Cd with different levels of *Lemna gibba L*.

Biochemical parameters

Table III showed that AST activity increased significantly in plasma of fish exposed to Cd alone. The addition of *Lemna gibba L*-extract decreased significantly the AST activity to be lower than that of Cd alone ($p<0.05$). The AST activity in fish exposed to Cd with *Lemna gibba L* plus their extract became nearly similar to that of control at 7 days and 25 days. The plasma ALT activity increased significantly in fish exposed to Cd alone at 7 and 25 days. The

addition of *Lemna gibba L* enhanced ALT activity to be nearly as in the control especially the groups exposed to Cd with *Lemna gibba L*- weed plus their extract at both periods.

Histopathological alterations

The histopathology of different Tilapia tissues revealed that there are several histopathological changes in different Tilapia organs as shown in our figures.

Gills (Figure 1(A)) showed necrosis and atrophy of the gill lamellae. Severe edema, hyperplasia, fusion and focal desquamation of the epithelial lining of the secondary lamellae also were detected. The gill arch showed numerous mononuclear leucocytic infiltration, edema, congestion and the apex of gill filaments showed congestion, hyper activation of the mucous and chloride cells.

Liver of tilapia treated with cadmium showed degeneration of the hepatocytes with nuclear pyknosis in the majority of the cells as well as the accumulation of the metal binding proteins in their nuclei. Intravascular hemolysis is seen in blood vessels and sinusoids with necrosed hepatocytes (Figures 1 (B, C, D, E)).

Spleen The microscopic examination of Tilapia's spleen revealed lymphoid depletion (LD) associated with congestion and necrosis of splenic sinusoids and activation of Melanomacrophage center (MMC). Large sub-capsular areas of necrosis were also observed (Figure 1(F), 2(A)). In the control fish, liver contains few melano macrophage centers (MMC) and free macrophages.

Stomach The sub-mucosal tissues were fully vacuolated with degeneration of the serosal layer, degeneration of columnar epithelium, goblet cell and basement membrane as well as the secretory cells were damaged and fully distorted (Figure 2(B)).

Intestine Intestine of tilapia treated with cadmium showing necrosed mucosa, sub-mucosal hemorrhage, muscle fibers were loosely arranged with the degeneration of sub-mucosal tissue and each villus facing the lumen showed cell degeneration and the cells did not show distinct nuclei and cytoplasmic boundaries. There was a distortion of basement membrane of the villi and blood vessel, and lymphocytes were fully distorted and there was a degeneration of columnar epithelium of the intestine (Figure 2 (C, D, E, F)).

Muscular tissues degeneration in muscle bundles with aggregations of inflammatory cells (leucocytic infiltration) between them with focal areas of necrosis (Figure 2 (F)). Atrophy and edema of muscle bundles as well as splitting of muscle fibers and hyalinized muscles tissue were seen (Figure 3 (A, B)).

Brain edema was the main characteristic histopathological change in the brain tissues. The lesion also characterized by vacuolation of brain tissue in addition to encephalitis that characterized microscopically by congestion of cerebral blood capillaries associated with neuronal degeneration, severe necrosis and demylination of brain tissue with extravasations of free RBCs as well as aggregation of EGCs in brain tissue (Figure 3 (C, D, E, F)).

Kidney showed hydropic swelling of the renal tubules, sometimes with pyknotic nuclei and many necrotic areas as well as swollen proximal epithelial cells with necrotic nuclei (Figure 4 (A)).

Abnormal blood cells Tilapia treated with cadmium showing abnormal blood cells (Aminocytosis) (Figure 4 (B, C)). Gills of tilapia treated with cadmium showing atrophy and necrosis of gill lamellae (arrows) (H & E X 400).

Cd Bioaccumulation

The highest bioaccumulation of cadmium was observed in the organs mainly implicated in metal intoxication and so it was higher in the liver followed by muscles.

Addition of *Lemna gibba L*-extract to the Cd polluted media reduced significantly ($p<0.05$) the Cd level in aquarium's water as compared to that of Cd alone, Table I. The Cd concentration in water exposed Cd alone was 9.620±0.198 mg L^{-1} and declined significantly ($p<0.05$) to 7.8 ±0.276, 4.58 ±0.208 and 0.89 ±0.105 mg L^{-1} with 0.1, 1 and 0.1 plus 1 g L^{-1} extract, weed and extract plus weed of *Lemna gibba L*, respectively. The highest amount of Cd residue was found in the liver after 7 days of exposure. Table I also, showed that the uptake of Cd in the liver of fish exposed to Cd alone was 2.350±0.18 and 5.88±0.540 mg g^{-1} dry weight for 7 and 25 days, respectively. It declined significantly to 1.392±0.095, 4.500±0.320 and 0.990±0.015, 3.792 ±0.320 and 0.510 ±0.021, 1.850 ±0.150 mg g^{-1} dry weight in fish group exposed to Cd with 0.1, 1 and 0.1 plus 1 g L^{-1} extract, weed and extract plus weed of *Lemna gibba L* at 7 and 25 days, respectively. Similar trends were observed in fish muscles.

4. Discussion:

The present study reveals that the fish exposed to Cd alone showed significant reduction in RBCs, Hb and HCT than those exposed to Cd with different level of *Lemna gibba L*-extract and plant. The reduction of these parameters in *O niloticus* at sub-lethal levels of cadmium might be due to the destruction of mature RBCs and the inhibition of erythrocyte production due to reduction of

Table I: Changes in cadmium residue in water (mg Cd L^{-1}), liver and muscles (mg Cd g^{-1} dry weigh) of Nile tilapia *(O. niloticus*) exposed to Cd with and without *Lemna gibba L* plant

Group	Water	Liver		Muscles	
Days		**7**	**25**	**7**	**25**
Control (metal free water)	0.032±0.004[a]	0.038±0.003[a]	0.040±0.006[a]	0.021±0.004[a]	0.018±0.001[a]
Cadmium alone (10mg) L^{-1}	9.620±0.198[b]	2.350±0.18[b]	5.88±0.540[b]	0.485±0.11[b]	1.097±0.058[b]
Cadmium (10mg)+0.1g ext. L^{-1}	7.8 ±0.276[b]	1.392±0.095[b]	4.500±0.320[b]	0.343±0.086[a]	0.868±0.088[b]
Cadmium (10mg)+1g P L^{-1}	4.58 ±0.208[c]	0.990±0.015[c]	3.792 ±0.320[c]	0.380±0.098[b]	0.383±0.077[b]
Cadmium (10mg)+ 0.1g ext. L^{-1}+1g P L^{-1}	0.89 ±0.105[d]	0.510 ±0.021[d]	1.850 ±0.150[d]	0.216±0.086[a]	0.217±0.076[a]

The same letter in the same column is not significantly different at P<0.05.
The first group was free from Cd and *Lemna gibba L* and maintained as a control.
The second groups were exposed to 10 mg of $CdCl_2$ only (Equivalent to 1/4 96 h LC_{50}).
The third was exposed to 10 mg Cd L^{-1} and 0.1 extract.
The fourth group was exposed to 10 mg Cd L^{-1} and 1 g L^{-1} *Lemna gibba L* plant.
The fifth group was exposed to 10 mg Cd L^{-1}+ 0.1g extract L^{1} +1 g L^{-1} *Lemna gibba L* plant.

Table II: Changes in erythrocyte (count $x10^6/mm^3$), hemoglobin content (g 100 mL^{-1}) and haematocrit value (%) in the blood of Nile tilapia (*O. niloticus*) exposed to Cd with and without *Lemna gibba L* plant.

Group	Erythrocyte count (RBCs)		Hemoglobin(HB)		Haematocrit value (Hct)	
	7	**25**	**7**	**25**	**7**	**25**
Control (metal free water)	1.560 ±0.040[a]	1.622±0.080[a]	5.590±0.354[a]	6.422±0.454 [a]	14.900±0.334[a]	16.332±0.454[a]
Cadmium alone (10mg) L^{-1}	1.190±0.070[b]	1.160±0.050[b]	4.320±0.354[b]	4.212±0.345[b]	13.220 ±1.454[b]	13.026±0.354[b]
Cadmium (10mg) +0.1g ext. L^{-1}	1.482±0.080[a]	1.370±0.060[c]	4.440±0.534[b]	5.340±0.452 [c]	14.110 ±0.432[a]	15.065±0.765[c]
Cadmium (10mg) +1g P L^{-1}	1.56 ±0.080[a]	1.677±0.070[a]	5.270±0.454[a]	6.112±0.457[a]	14.320±0.359[a]	15.688±0.655[c]
Cadmium (10mg)+ 0.1g ext. L^{-1} + 1g P L^{-1}	1.678±0.070[a]	1.625±0.050[a]	5.476±0.65[a]	6.450±0.554[a]	14.800±0.365[a]	16.112±0.732[a]

The same letter in the same column is not significantly different at P<0.05.
The first group was free from Cd and *Lemna gibba L* and maintained as a control.
The second groups were exposed to 10 mg of $CdCl_2$ only (Equivalent to 1/4 96 h LC_{50}).
The third was exposed to 10 mg Cd L^{-1} and 0.1 extract.
The fourth group was exposed to 10 mg Cd L^{-1} and 1 g L^{-1} *Lemna gibba L* plant.
The fifth group was exposed to 10 mg Cd L^{-1}+ 0.1g extract L^{1} +1 g L^{-1} *Lemna gibba L* plant.

Table III: Changes in aspartate aminotransferase activity (AST) and alanine aminotransferase (ALT) activity (IU L^{-1}) in plasma of Nile tilapia (*O. niloticus*) exposed to Cd with and without *Lemna gibba L* plant.

Group	AST		ALT	
Days	**7**	**25**	**7**	**25**
Control (metal free water)	50.390±1.62[a]	88.690±2.343[a]	30.966±2.532[a]	41.65±2.688[a]
Cadmium alone (10mg) L^{-1}	96.480±1.822[b]	117.770±2.564[c]	50.966±2.854[b]	73.80±2.966[b]
Cadmium (10mg)+ 0.1g ext. L^{-1}	95.690±1.666[b]	107.870±2.644[b]	48.966±2.674[b]	71.68±2.845[b]
Cadmium (10mg)+ 1g P L^{-1}	71.793±1.688[c]	95.790±2.865[a]	35.966±2.727[a]	52.67±2.754[c]
Cadmium (10mg)+ 0.1g ext. L^{-1} + 1g P L^{-1}	53.570±1.995[a]	90.780±2.986[a]	34.966±2.568[a]	42.77±2.876[a]

The same letter in the same column is not significantly different at P<0.05.
The first group was free from Cd and *Lemna gibba L* and maintained as a control.
The second groups were exposed to 10 mg of $CdCl_2$ only (Equivalent to 1/4 96 h LC_{50}).
The third was exposed to 10 mg Cd L^{-1} and 0.1 extract.
The fourth group was exposed to 10 mg Cd L^{-1} and 1 g L^{-1} *Lemna gibba L* plant.
The fifth group was exposed to 10 mg Cd L^{-1}+ 0.1g extract L^{1} +1 g L^{-1} *Lemna gibba L* plant.

Figure 1

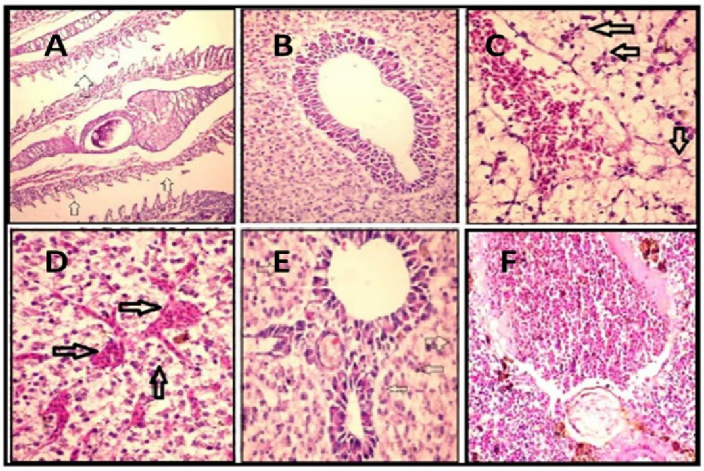

Figure 1.

A. Gills of tilapia treated with cadmium showing atrophy and necrosis of gill lamellae (arrows) (H & E X 400).
B. Liver of tilapia treated with cadmium and Plant-ext showing apparently healthy liver tissue (H & E X 200).
C. Liver of tilapia treated with cadmium showing congestion (C) with severely degenerated and necrosed hepatocytes (arrows) (H & E X 200).
D. Liver of tilapia treated with cadmium showing congested sinusoids with necrosed hepatocytes (arrows) (H & E X 200).
E. Liver of tilapia treated with cadmium and Plant-ext showing normal portal tract with slightly degenerated hepatocytes (arrows) (H & E X 400).
F. Spleen of tilapia treated with cadmium and Plant-ext showing apparently normal spleen (H & E X 200).

Figure 2

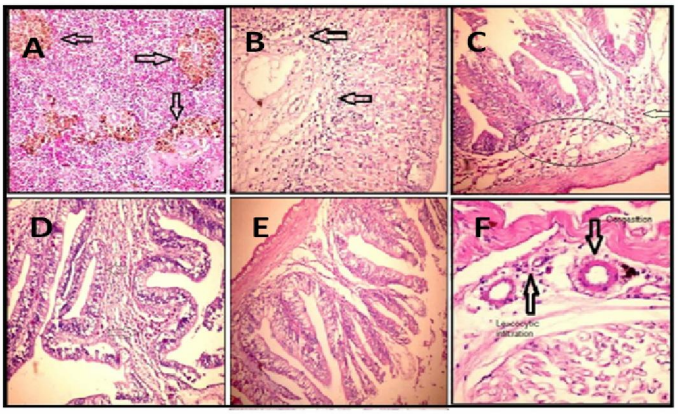

Figure 2.

A. Spleen of tilapia treated with cadmium showing hyperplasia in the melanomacrophage cells (arrows) (H & E X 200).
B. Stomach of tilapia treated with cadmium showing leucocytic infiltration in the sub mucosa (arrow) together with congestion (C) (H & E X 400).
C. Intestine of tilapia treated with cadmium showing necrosed mucosa (n), sub mucosal haemorrhage (arrow) (H & E X 200).
D. Intestine of tilapia treated with cadmium showing higher power of the previous lesion (H & E X 400).
E. Intestine of tilapia treated with cadmium and Plant-ext showing apparently intestinal tissue (H & E X 200).
F. Muscles of tilapia treated with showing congestion (C) and leucocytic infiltration (arrow) (H & E X 200).

Figure 3

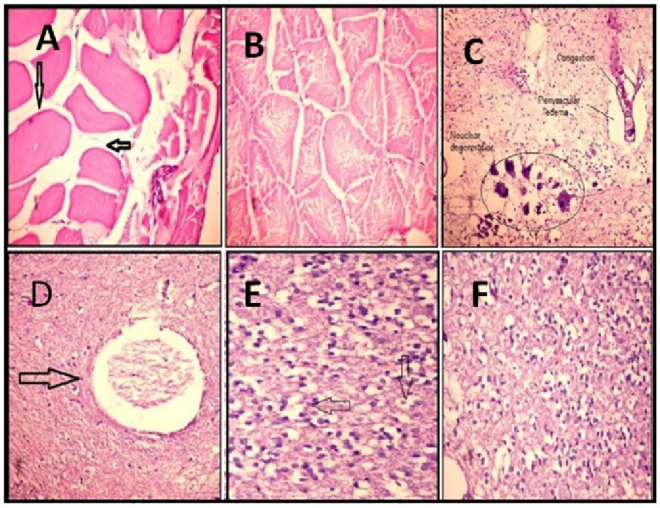

Figure 3.
A. Muscles of tilapia treated with cadmium showing hyalinised muscles tissue (arrow) (H & E X 200).
B. Muscles of tilapia treated with cadmium and Plant-ext showing apparently healthy muscular tissue (H & E X 200).
C. Brain of tilapia treated with cadmium showing congestion (C), perivascular edema (e) and neural degeneration (n) (arrow) (H & E X 200).
D.Brain of tilapia treated with cadmium showing osteomalacia (arrow) (H & E X 200).
E. Brain of tilapia treated with cadmium showing intracellular brain edema (arrow) (H & E X 200).
F. Brain of tilapia treated with cadmium and Plant-ext showing apparently healthy tissue (H & E X 200).

Figure 4

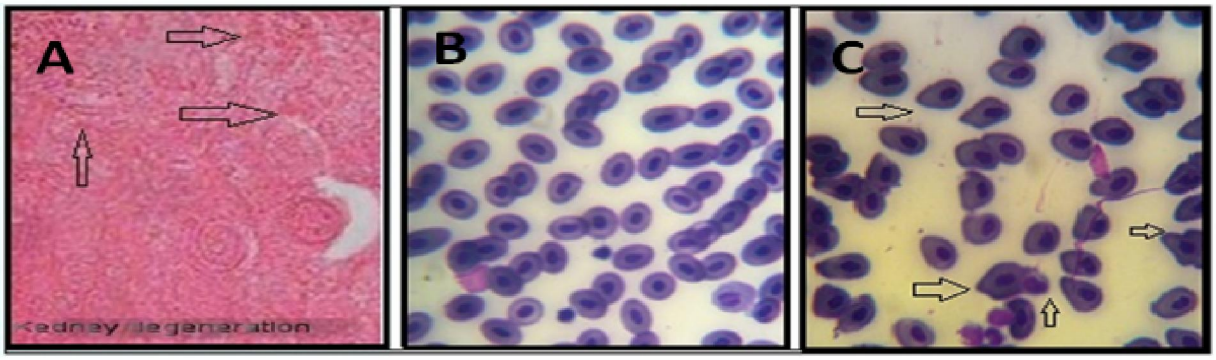

Figure 4.
A. Kidneys showed hydropic swelling of tubules with pyknotic nuclei and many necrotic areas as well as swollen proximal epithelial cells with necrotic nuclei.
B. Tilapia treated with cadmium and Plant-ext showing apparently healthy blood cells (X 1000).Tilapia treated with cadmium showing abnormal blood cells (Amioscytosis) (X 1000).

haemsynthesis that affected by pollutants (Wintrobe, 1978). Also, the decrease in RBCs count may be attributed to haematopathology that results in sever anemia in most vertebrates including fish species exposed to different environmental pollutants (Khangarot & Tripathi, 1991). The decrease in the RBCs may be attributed to reduction of growth and other food utilization parameters which results in

sever anemia (James and Sampath, 1999). Also Gill & Epple (1993) found a significant reduction in the RBCs, Hb and HCT in American eel *(Anguilla rostrata*) after exposure to 150 ug Cd L^{-1}. Karuppasamy *et al*. (2005) found a significant decrease in total erythrocyte count, haemoglobin content, haematocrit value and mean corpuscular haemoglobin concentration in air breathing fish, *Channa punctatus* after exposure to sub-lethal dose of Cd (29 mg Cd L^{-1}).

The decrease in RBCs, Hb and HCT values may be due to the exaggerated disturbances that occurred in both metabolic and hemopoietic activities of fish exposed to sub-lethal concentration of pollutants (Moussa, 1999).

The activity of AST and ALT enzymes in blood may also be used as a stress indicator. The significant changes in the activities of these enzymes in blood plasma indicate tissue impairment caused by stress (James *et al*., 1991; Svoboda, 2001). In the present study, there were significant changes in AST and ALT activities in plasma of fish exposed to cadmium compared to the control group. The increase in concentration of AST and ALT in blood plasma indicates impairment of parenchymatous organs mainly liver. In addition, the increase of plasma AST and ALT may be attributed to the hepatocellular damage or cellular degradation in liver, spleen or muscles (Yamawaki *et al*., 1986). These results are in agreement with those reported by Shalaby (1997) who found that sub-lethal concentration of Cd caused significant increases in AST and ALT of Common Carp after 7 and 25 days.

Histopathological biomarkers have been largely used in fish to identify and evaluate the toxic effects of pollutants exposure (Rabitto *et al*., 2005; Oliveira Ribeiro *et al*., 2006). The presence of necrosis is in fact one of the most visible damages in tissues affected by a pollutant (Rabitto *et al*., 2005). According to Manahan (1991) the occurrence of necrosis is also a consequence of enzymatic inhibition, damages in the cellular membrane integrity, and disturbances in the synthesis of proteins and carbohydrate metabolism.

Mallat (1985) reported that the pathological changes of fish gills are induced by elevated heavy metals and low oxygen content in water. The hyperplasia induced by any pollutant may be due to the simple response to cellular necrosis as previously mentioned by Marie *et al*. (1998). Moreover, Shaker *et al*. (2000) reported that the epithelial hyperplasia is known as a protective and defense mechanism of fish gills. In addition to, the congestion in branchial blood vessels may be due to the counter irritation and stimulation of vasodilators (Marie *et al*., 1998).

Structural damage due to Cd toxicity in fish gills has been reported by Voyer (1975); Karlson-Norrgren *et al*. (1985) and Kothari & Saxena (1988). Narayan & Singh (1991) observed extensive degeneration of cytoplasm with pyknosis of nuclei and loss of glycogen in liver tissue of *Heteropneustes fossils* while subjecting them to acute thiodan toxicity. Recent studies have demonstrated the links between exposure to pollutants and the development of hepatic lesions. Stentiford *et al*. (2003) and Stehr *et al*. (2004) observed that toxicopathic liver lesions in fish species are effective biological markers of chemical exposures.

Melano macrophages are pigmented cells that can appear isolated or arranged in clusters forming the MMCs in the spleen. These cells are positive indicator for the presence of neutral carbohydrates (P.A.S. stain) and melanin (Masson–Fontana stain) (Rabitto *et al*., 2005).

The pathological findings in the intestine included atrophy in the muscularis, degenerative and necrotic changes in the intestinal mucosa and sub-mucosa with necrotized cells aggregated in the intestinal lumen, edema and atrophy in the submucosa. These findings are similar in *Chana punctatus* exposed to Cadmium (Stromberg *et al*., 1983) and lead (Sastri & Gupta, 1978).

The present results indicate that *Lemna gibba L* weed and extract are effective in removing Cd from water and reducing Cd bioaccumulation inTilapia fish. The addition of *Lemna gibba L*-extract reduced significantly ($p<0.05$) the Cd level in water and the metal uptake as compared to fish exposed to Cd alone. The Cd concentration in water was 9.620 ± 0.198 mg L^{-1} and it decreased significantly ($p< 0.05$). The Cd accumulation in liver and muscles of fish exposed to Cd alone was higher than that of *Lemna gibba L*-extract treatment group. These results suggest that *Lemna gibba L weed and/or* extract could chelate Cd ions producing a stable complex, thus reducing the chance for metal uptake by tissues. These results are in agreement with Santschi (1988) who reported that any agent that can remove Cd from water helps to reduce the bioaccumulation of this metal in fish.

The addition of *Lemna gibba L*-extract improves the haematological parameters (RBCs, Hb and Hct) and ameliorates the toxic effect of Cd in Tilapia fish which indicating the capability of *Lemna gibba L*-extract to chelate Cd from the media. Subsequently, the Cd toxicity was reduced. These results are in agreement with those recorded by Jayaram & Prasad (2009) who observed the biosorption potential of *Prosopis juliflora* seed powder (PJSP) for Pb (II) from aqueous solution at pH 6.0. Findings in fish also indicated that

degenerative changes were less when *Lemna gibba L*-extract or the weed and their extract were added to the rearing water.

The present study shows that addition of *Lemna gibba L*- weed and/or its extract to Cd contaminated media reduced significantly the Cd level in the water and helped to eliminate metal from the fish body and in turn improved the biochemical parameters as compared to fish exposed to Cd alone.

Finally, we could conclude that Cd poisoning cause structural damage in Tilapia organs. It is also demonstrated that *Lemna gibba L*-extract, weed or the weed plus the extract provided protection against the degenerative action of Cd and increased the chance of tissue regeneration.

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5. References:

1. ATSDR. (2003). Toxicological Profile for Selenium. Atlanta, GA:Agency for Toxic Substances and Disease Registry.
2. Black, H. (1995). Absorbing possibilities: phytoremediation. *Environmental health perspectives* 103, 1106–1108.
3. Britton, C. L. (1963). Disorders of the Blood. 9th ed. Achur-Chill. Ltd., London.
4. Buckley, J.A. (1994). The bioavailability of copper in wastewater to *Lemna minor* with biological and electrochemical measures of complexation. *Water research* 28, 2457-2467.
5. Chung, K.S. (1983). Lethal Effects of cadmium in Tropical Fish. *Bulletin of the Japanese Society of Fisheries Oceanography* 49, 1565-68.
6. Dacie, J.V. & Lewis, S.M. (1984). Practical Hematology. 6th edition. Churchill
7. Livingstone. Edinburgh. London. Melbourne and New York. 22 pp.
8. Duncan, D.B. (1955). Multiple ranges and multiple F test. *Biometrics.* 11, 1-42.
9. Eaton, D.L. & Stinson, M.D., (1983). Concentration of lead, cadmium, mercury and copper in the cray fish (*Pacifasticus leniusculus*) obtained from a lake receiving urban runoff. *Archive of Environmental Contamination and Toxicolology* 12, 693- 700.
10. European Commission. (2001). Commission Regulation (EC) No, 466/2001 of 8 March 2001, setting maximum levels for certain contaminants in foodstuffs.
11. FAO / WHO (Food and Agriculture Organization/ World Health Organization). 2005. Summary and conclusions of the sixty-four meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). <ftp://ftp.fao.org/es/esn/jecfa/jecfa64_summary.pdf>.
12. Finerty, M.W. Madden, J.D., Feagly, S.E. & Grodner, R.M. (1990). Effect of environs and seasonality on metal residues in tissues of wild and pond-raised Crayfish in southern Louisiana. *Archive of Environmental Contamination and Toxicolology* 19, 49-55.
13. Finney, D.J. (1971). Probit Analysis. Cambridge University Press.
14. Finney, D.J. (1978). Statistical method in biological assay. London. Griffin.
15. Ghobrial, I., Roccaro, A., Chauhan, D., Aderson, K. & Palladino, M. (2009). Methods of using [3.2.0] heterocyclic compounds and analogs thereof in treating waldenstrom's macroglobulinemia. Nereus Pharmaceuticals, INC. San Diego, CA, US.
16. Gill, T.S. & Epple, A. (1993). Stress-related changes in the hematological profile of
17. the American eel (*Anguilla rostrata*). *Ecotoxicology and Environmental Safety* 25, 227-35.
18. Hiemesh, S. & Mahadevaswamy, M., (1994). Sorption potential of biosorban: For the removal of copper. *Indian Journal of Environmental Health* 36, 165- 169.
19. James, P., Thorpe, I.J., Kokkinos, N., Morkot, R. & Frankish, J. (1991). Centuries of Darkness: context, methodology and implications. *Cambridge Journal Archive* 1, 228-35.
20. James, R. & Sampath, K. (1999). Effect of the ion- exchanging agent, Zeolite, on reduction of cadmium toxicity: an experimental study on growth and elemental uptake in *Heteropneustes fossilis* (Bloch). *Journal of* Aquaculture *in the Tropics* 14, 65- 74.
21. Jayaram, K. & Prasad, M.N.V. (2009). Removal of Pb(II) from aqueous solution by seed powder of *Prosopis juliflora* DC. *Journal of Hazardous Materials* 169 (1-3), 991-7.
22. Karlson-Norrgren, L., Runn, P., Haux, C. & Forlin, L. (1985). Cadmium-induced changes in gill morphology of zebra fish *Brachydanio rerio* (Hamilton-Buchaman) and rainbow trout *Salmogiardneri, Richardson. Journal of Fish Bioliology* 27, 81.
23. Karuppasamy, R., Subathra, S. & Puvaneswari, S. (2005). Haematological responses to exposure to

sublethal concentration of cadmium in air breathing fish, *Channa punctatus* (Bloch). *Journal of Environmental Biology* 26, 123-8.
24. Khangarot, B.S. & Tripathi, D.M. (1991). Changes in humoral and cell mediated immune responses and in skin and respiratory surfaces of catfish, *Saccobranchus fossilis* following copper exposure.*Ecotoxicology and Environmental Safety* 22, 291.
25. Kothari, S. & Saxena, G. (1988). Histological and histochemical studies in the gills of *Puntius sophore* (Ham.) exposed to cadmium chloride. *Journal of Hydrobiologica* 27, 81
26. Kwan, K.H.M. and Smith, S., (1991). Some aspects of the kinetics of cadmium and thallium uptake by fronds of *Lemna minor L*. New Phytol. 117, 91-102.
27. Lide, D. (1992). CRC Handbook of Chemistry and Physics, 73rd Edition 1992. Boca Raton, FL: CRC Press.
28. Mallat, J. (1985). Fish gill structural changes induced by toxicants and other irritants: a statistical review. *Canadian Journal of Fisheries and Aquatic Science* 42, 630-648.
29. Manahan, S. E. (1991). Water Pollution Environment Chemistry, first ed. Lewis Publishers, London
30. Marie, M.A.S., Haggag, A.M. & EI-Badawy, A.A. (1998). Physiological and biochemical responses of the common carp; *Cyprinus carpio* to an organophosphorus insecticide Profenofos. *Egyptian Journal of Zoology* 13, 279-302.
31. Mary, G.G., Omar, S. & Tarek, A. (2007). Potential for finding new bioactive agents from selected aquatic plant extracts against enteric bacteria. *Egyptian Journal of Natural Toxins* 4, 12-25.
32. Miranda, M. & Ilangovan, K. (1996). Uptake of lead by *Lemna gibba L.*: Influence on specific growth rate and basic biochemical changes. *Bulletin of Environmental Contamination and Toxicology* 56, 1000-1007.
33. Moussa, M.A. (1999). Biological and physiological studies on the effect of the gramoxon and stomp herbicides on Nile tilapia (*Oreochromis niloticus*). Ph.D. Thesis, Department of Zoology, Faculty of Science, Cairo University, 200p.
34. Narayan, A.S. & Singh, B.B. (1991). Histopathological lesions in *Heteropneustes fossilis* subjected to acute thiodan toxicity. *Hydrobiologica* 19, 235-243.
35. Oliveira, R., Filipak, C.A. & Neto, F.l. (2006). Haematological findings in neotropical fish *Hoplias malabaricus* exposed to subchronic and dietry doses of methylmercury, inorganic lead, tributyltin chloride. *Environmental Research* 101, 74-80.
36. Passow, H., Rothstein, A. & Clarkson, T.W. (1961). The general pharmacology of the heavy metals. *Pharmacological Reviews* 13,185-224.
37. Rabitto, I.S., Alves Costa, J.R.M., Silva de Assis, H.C., Pelletier, E., Akaishi, F.M., Anjos, A., Randi, M.A.F. & Oliveira, R. (2005). Effects of dietary Pb(II) and tributylin an neotroptical fish *Hoplias malabarius*: Histopathological and biochemical findings. *Ecotoxicology and Environmental Safety* 60, 147-156.
38. Rashed, M.N. (2001). Cadmium and lead levels in fish (*Tilapia niloticus*) tissues as
39. biological indicator for lake water pollution. *Environmental Monitoring and Assessment.* 68, 75-89.
40. Reitman, S. & Frankel, S. (1957). Colorimetric determination of glutamic oxaloacetic and glutamic pyruvic transaminases. *American Journal of Clinical Pathology* 28, 53-56.
41. Santschi, P.H. (1988). Factors controlling the biogeochemical cycle of trace elements in fresh and coastal waters as revealed by artificial radioisotopes. *Limnology and oceanography* 33,848- 886.
42. Sastri, K.V. & Gupta, P.K. (1978). Chronic mercuric chloride intoxication in digestive system of *Channa punctatus*. *Journal of Toxicology and Environmental Health* 4, 777.
43. Shaker, I.M., Ez-EI-Rigal, A. & El Ghobashy, H. (2000). Effects of EDTA on reducing of copper toxicity in water and *Oreochromis niloticus*. *Egyptian Journal of Applied Science* 15, 119-131.
44. Shalaby, A.M. (1997). Biochemical and physiological studies on metal contamination in the common carp (*Cyprinus carpio L*). Ph. D. Thesis, Zagazig University, Faculty of Science, Benha branch, 268p.
45. Seebaugh, D.R. & Wallace, W.G. (2005). Absorption of dietry cadmium by grass shrimp Palaemonetes spp. Collected along an environmental impact ingredients. In: Waldman, JR and Nieder WC (eds). Final report of the Tibor T. Polgar Fellowship Program, 2004, section VIII. Hudson River Foundation, New York P1.
46. Sparks, T. (2000). Statistics in Ecotoxicology. John Wiley and Sons, Ltd., West Sussex, England, 320 p.
47. Stehr, C.M., Myers, M.S., Johnson, L.L., Spencer, S. & Stein, J. E. (2004). Toxicopathic liver lesions in English sole and chemical contaminant exposure in *Vancouver harbour*, Canadian *Marine Environmental Research* 57, 55-74.
48. Stentiford, G.D., Evans, M.G., Bateman, K. & Feist, S.W. (2003). Co-infection by a yeast-like

organism in Hematodinium-infected European edible crabs Cancer pagurus and velvet swimming crabs *Necora puber* from the English Channel. *Diseases of Aquatic Organisms* 54,195-202.
49. Svoboda, M. (2001). Stress in fishes (a review*). Bulletin VÚRH Vodňany* 4, 169- 191.
50. Stromberg, P.C., Ferrante, J.G. & Carter, S. (1983). Pathology of lethal and sublethal exposure of fathead minnows, *Pimephales promelas*, to cadmium: a model for aquatic toxicity assessment. *Journal of Toxicology and Environmental Health* 11, 247–259.
51. Snedecor, O.W. & Cochran, W.O. (1982). Statistical Methods. 7th Ed,, Iowa University Press, Ames, Iowa.
52. Van Kampen, E.J. & Zijlstra, W.G. (1961). Standardization of hemoglobin- ometry. II. The hemiglobincyanide method. *Clinica Chimica Acta* 6, 583.
53. Voyer, R.A. (1975). Effect of dissolved oxygen concentration on the acute toxicity of cadmium to mummichog *Fundulus heteroclitus* (L.) at various salinities. *Transactions of the American Fisheries Society* 104, 129.
54. Wafaa, A.E., Ismail, G., Farid, A.E., Tarek, T., & Hammad, D. (2007). Assessment of the Efficiency of Duckweed (*Lemna gibba)* in Wastewater Treatment. *International Journal of Agriculure and Biology 9*, 681-687.
55. Wintrobe, M.M. (1978). In: Clinical hematology. Henry Kimpton, London, pp. 448.
56. Yamawaki, K., Hashimoto, W., Fujii, K., Koyama, J., Ikeda,Y. & Ozaki, H. (1986). Hemochemical changes in carp (*Cyprinus carpio*) exposed to low cadmium concentrations. *Nippon Suisan Gakkaishi Bulletin* 52, 459- 466.

Early Detection of Breast Cancer among Females at Fakous District, Sharqia Governorate, Egypt

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Abstract: Background: Breast cancer is the most important cancer, with women in an increasing numbers in incidence developing countries. It is by far the commonest cancer among Egyptian women and represents 37% of all female cancers. Objectives: Early detection of breast cancer, and determining the most frequent barriers of delay in breast cancer diagnosis among females at Fakous district, Sharqia Governorate, in addition to identification of the risk factors and prevalence of breast cancer in the year 2010. Subjects & Methods: Community based survey study at Fakous district, Sharqia Governorate for 6 months period (from 1 January 2010 –to 30 June 2010). A multistage cluster random sampling was adopted for this work on a sample of 390 females. The study tools included :a- a questionnaire which was designed and pretested in pilot testing before the study. It was prepared to ask women about some socio-demographic characteristics, and risk factors of breast cancer. In addition to any suspected symptoms of breast cancer as lump, pain or tenderness, or nipple discharge barriers of delay in breast cancer diagnosis. b-Weight and height were measured to calculate Body mass index (BMI). c-Clinical Breast examination. d-Referral of the suspected cases to Fakous Cancer Center for doing mammogram, ultrasound, and fine needle biopsy to ensure the diagnosis. Results: The most frequent interviewed age groups were 30-39 ys, 20-29 ys, and 40-49 years (27.9%, 24.4%, and 22.1% respectively) with mean age (38.7) years and median age 36.5 years. About 52% of the studied females had early menarche (<12 years), and Null parity constituted 4.1% of the studied sample. The age of women at 1st full term pregnancy (at ≥35years) represented more than one quarter (26.6%) of the studied females and no breast feeding which constituted 59% among the multipara women. More than 54% of the studied sample was overweight and obese with positive family history of breast cancer constituted 3.5%. About 86.7% of the menopausal women had delayed menopause (≥50 years). Illiteracy took the upper hand among the studied females (34.6%). 23.1% of the studied sample was oral contraceptive users. After doing clinical breast examination (CBE) of women and mammography; 23 cases were presented by breast lesions (5.9%). 18 cases were diagnosed as benign breast lesions (4.6%), and 5 cases were confirmed as breast cancer 'BC' (1.3%) by ultrasonography & fine needle aspiration biopsy. Histopathological reports for the discovered BC cases, revealed that 2 females presented with infiltrating duct carcinoma (IDC) and 2 cases presented with ILC, 3 cases (60%) had lymph node metastasis. Mean tumor size was 3.9 cm. By reviewing the BC stages among the females with breast cancer, it was found that 2 cases were in stage II (40%), 2 cases were in stage III (40%) . It was found that lack of doing mammography, annual CBE, & monthly BSE were the main limitations for early diagnosis of breast cancer. In addition to illiteracy, reluctance in seeking medical care, far distance from health services, negligence of the complaint, and fear from BC diagnosis constituted the most frequent barriers for early detection of breast cancer among the studied females. Recommendations: taking any women's breast complaints seriously, Proper training programs for women about monthly breast self examination for early detection of any breast lesion. Health education programs on a wide scale on the studied places to improve not only awareness, or knowledge but also changing the faulty attitudes & practices about breast cancer especially among illiterate women. In addition to training programs for health care providers at primary health care units about the importance of annual CBE in early detection of BC cases especially in low resources settings.

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Key words: Breast cancer, early detection, clinical breast examination, prevalence, risk factors, barriers for early diagnosis.

1. Introduction:

Breast cancer represents 10% of all cancers diagnosed worldwide annually and constituted 22% of all new cancers in women in 2008, making it by far the most common cancer in women. The rate of increasing incidence is higher in developing countries [(1)].

In the Eastern Mediterranean region, breast cancer is by far the most common cancer even when considering men and women together, with 2 time

more cases (N=57 000 new cases per year) than lung cancer (N=25 000) or bladder cancer (N=25 500)[2].

More than 60-80% of breast cancers present at advanced stage. Treatment of advanced cancer is more difficult and costly. In Egypt, Breast cancer can cost up to LE 250,000 to cure, and a mastectomy (removal of the breast) is sometimes the only solution. This can be very difficult for the patient to deal with. If the disease is detected at an early stage, however, the surgery won't cost more than LE 15,000 and offers a greater chance of removing the tumor without the trauma of a mastectomy[3].

The survival rate from breast cancer in developing countries is generally poorer than in developed countries, primarily as a result of delayed diagnosis of cases. Breast cancer is a dangerous disease but it could be very simple [to treat] if discovered in an early stage. The only way to decrease mortality and morbidity from breast cancer is to detect the disease before the patient presents with symptoms[4].

Early detection is the identification of breast cancer at a stage in its natural history where the impact of therapy has the greatest chance of producing a cure. It may include programmes by which breast cancer that is causing symptoms can be diagnosed at an earlier stage [5].

From the available cancer control measures for breast cancer, primary prevention, screening and improved therapy, only screening has the potential for a rapid and major effect though this will be restricted to a reduction in mortality rather than a reduction in incidence. From the available screening tests, mammography, physical examination of the breasts and breast self-examination, only mammography is established as effective in reducing mortality from breast cancer[6].

However, mammography requires expensive technology, highly trained radiologists and radiographers, and is out of reach for most developing countries [7,8]. Further, in women under age 50 there is little evidence for a benefit, and if a benefit exists, it is less than in older women [6].

Preliminary results about down staging programs based on clinical breast examination (CBE), breast self examination (BSE), public awareness campaigns and training of primary heath staff have shown very encouraging results in different low-income countries settings, for example urban Egypt [5] and Borneo Island [9].

Most doctors feel that tests for finding breast cancer early save many thousands of lives each year. Diagnosis of breast cancer during the early stages of disease has been positively linked to a decrease in the mortality and morbidity of the illness. A positive correlation also exists between breast cancer awareness & screening practice [10].

Data from the regional population-based cancer registry at Gharbia governorate 2000-2002 (Egypt) as well as data from the National Cancer Institute hospital based registry (Cairo) show that breast cancer is the first cancer in Egypt (19% of all cases, male and female considered together). It is by far the commonest cancer among Egyptian women and represents 37% of all female cancers. Incidence in term of crude Incidence and age standardized rate are relatively high for a low income country (37.6 / 100,000 and 49.6 / 100 000 respectively).

Objectives of the study: were to promote early detection of breast cancer among females at Fakous District, Sharqia Governorate, 2010 and to identify some risk factors and the prevalence of breast cancer among the studied females. In addition to determining the most frequent barriers of delay in breast cancer diagnosis.

Research question: How is cancer breast can be detected early at Fakous district, Sharqia Governorate, 2010?

2. Material and Methods:

Research design:

A large community based survey study at Fakous district, Sharqia Governorate. The data were collected twice weekly for 6 months (from 1 January 2010 –to 30 June 2010). Fakous district was selected because it has Fakous Cancer Center to be easily available and accessible to females who will be referred for further investigation when needed.

Sampling technique:

A multistage cluster random sampling was adopted for this work. Fakous District was divided into urban part (2 cities; Fakous and Elsalhia cities) and rural part (48 villages & 728 Ezbas); one city (Fakous city), 3 villages and 5 Ezbas were selected randomly as the 1st sampling stage. The selected city was divided into four sectors then one sector was selected randomly (2nd stage sampling of urban part). On the other hand, the selected villages & Ezbas were divided into main streets; so three of these streets were randomly selected as the 2nd sampling stage of rural place. The study sample included all the houses within the selected streets. Subjects were selected from these households according to the certain inclusion criteria: all females who had menarche and permanently residing at the studied place. Exclusion criteria: females who had previous history of breast cancer, surgical or chemotherapeutic interventions and those who refused to participate in this work.

The sample size was calculated by EPI Info Version 6.04 with expected frequency of breast

cancer among females of 35%, population size of 570340 (Central Agency for Public Mobilization & Statistics CAPMAS 2006), at 95% level of confidence (α error = 5%), using the equation for a single proportion. Accordingly, the estimated sample size is 349 females. After adjustment for a dropout rate of 10%, the sample size was increased to 390 females.

The research study tools:

A- a questionnaire was designed and pretested in a pilot study before use. It was prepared to ask women about some socio-demographic characteristics, and risk factors of breast cancer (BC) as; age, residence (urban, village or Ezba), education level, marital status, age of menarche, age of 1st full term pregnancy, number of children, history of breast feeding, age of menopause if present, family history of breast cancer, and any history of breast complaint. In addition to any suspected symptoms of breast cancer as lump, pain, tenderness, or nipple discharge barriers of delay in breast cancer diagnosis.

B- Weight and height were measured by a validated weighing scale & a stadiometer. Body mass index (BMI) was calculated for every interviewed female using the formula [weight (kg)/height (meters)^2] [7].

C- Clinical Breast Examination (CBE) was done by the research team to all the studied females.

D- Referral of the suspected cases by CBE to Fakous Cancer Center for doing mammogram, ultrasound, and fine needle biopsy to ensure the diagnosis.

Ethical issues were taken into consideration as free and informed consent from each woman before the beginning of the study and assuring them for keeping the integrity and security of data. Approval from the related health authorities at the research place was also taken.

Data manipulation:

Data entry was carried out by using SPSS software version 13, that was proceeded by revision, coding, & checking of the data in order to minimize errors during its entry. Data analysis; tabulation, and graphic presentations as well as simple statistical analysis were carried out. The results of the pilot study were not included in the analysis. Interpretation and commenting were done, in addition to discussion of the findings.

3. Results:

As regards to some socio-demographic characteristics and risk factors of BC, the most frequent interviewed age groups were 30-39 ys, 20-29 ys, and 40-49 years (27.9%, 24.4%, and 22.1% respectively) with mean age (38.7) years and median age of 36.5 years. The most frequent risk factors of breast cancer in a descending manner were; delayed menopause, no breast feeding among multipara women, overweight& obesity, early menarche, 1st full term pregnancy at ≥35years, oral contraceptive users, and positive family history of breast cancer. Illiteracy took the upper hand among the studied females (34.6%) as shown in (table 1).

After doing clinical breast examination (CBE), mammography, and ultrasonography for the studied women; the prevalence rate of breast lesions cases =23⁄390 X 100= 5.9%. 18 cases were confirmed by fine needle aspiration biopsy as inflammatory and benign breast lesions (18⁄390X100=4.6%), however 5 cases were confirmed as breast cancer (5⁄390X100=1.3%) as illustrated in (figure 1).

After doing histopathological reports for the discovered BC cases, it was noticed that 2females presented with infiltrating duct carcinoma (IDC), 2 cases ILC (Infiltrating lobular carcinoma), and 1 case with DCIS (Intraductal carcinoma in situ). Mean tumor size was 3.9 cm, however, 2 cases were presented withT2, another 2 cases were presented with T3 tumor sizes and the last case was T1. 3 cases of BC had lymph node metastasis (table2).

By reviewing the BC stages among the females discovered with breast cancer, it was found that 2 cases were in stage II, 2 cases were in stage III, followed by 1 case was in stage I as in (table3).

By analyzing the social, psychological and economical barriers to early diagnosis and treatment of breast cancer, it was found that the most important determinants of late presentation were lack of doing mammography (99.5%), not practicing regular breast self examination (BSE) (97.4%), not doing regular clinical breast examination (95.6%), illiteracy (65.9%), reluctance in seeking medical care (56.9%), far distance from health services (56.2%), negligence of the complaints (47.4%)from the health care providers, and fear from BC diagnosis (44.1%) constituted the most frequent barriers for early detection of breast cancer among the studied females (table 4).

4. Discussions:

Breast cancer is an urgent public health problem in high-resource regions and is becoming an increasingly urgent problem in low resource regions, where incidence rates have been increasing by up to 5% per year [12,6].

Table (1) Percentage distribution of some breast cancer risk factors among the interviewed females during survey (No=390).

Risk Factors	Number	Percentage (%)
• Mean Age (years) ± SD (38.7 ± 12.0) • Median age: 36.5 years		
• Age groups: ▪ <20 years ▪ 20-29 ys ▪ 30-39 ys ▪ 40-49 ys ▪ 50-59ys ▪ ≥ 60 ys	 5 95 109 86 50 45	 1.3 24.4 27.9 22.1 12.8 11.5
• Early menarche (<12 years)	203	52.1
• Age at 1st full term pregnancy: At ≥35 years	104	26.6
• No breast feeding	230	59.0
• Oral contraceptives	90	23.1
• Null parity	16	4.1
• Positive family history of breast cancer	14	3.5
• Delayed menopause (≥50 years).	338	86.7
• Illiteracy	135	34.6
• Overweight and obesity	213	54.6

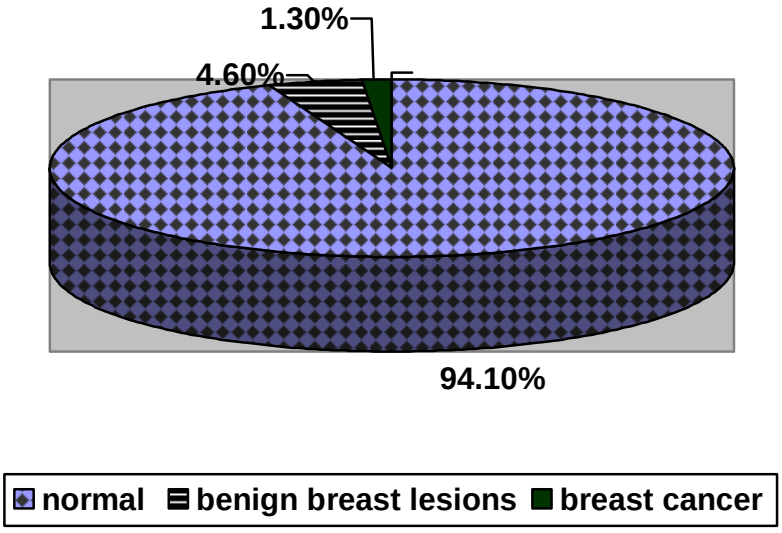

Figure (1) Pie diagram; The results of breast lesions among the examined females during house to house survey (No=390).

Table (2) Pathological type of the tumor, its size & the involved lymph node enlargement among the studied females with breast cancer (No=5).

Variable	Number	Percentage (%)
Pathological Type*:		
• IDC	2	40.0
• ILC	2	40.0
DCIS	1	20.0
Tumor size:**		
Mean tumor size ± SD	3.9 ± 1.1	
• T1	1	20.0
• T2	2	40.0
• T3	2	40.0
• T4	0	0.0
Lymph Node:		
• 1-3	**2**	40.0
• 4-9	**1**	20.0
• ≥ 10	**0**	0.0
N0 (no palpable axillary lymph nodes)	**2**	40.0

* **Pathological type:** IDC; Infiltrating duct carcinoma. ILC; Infiltrating lobular carcinoma. DCIS; Intraductal carcinoma in situ[(24)].

Tumor size according to international TNM staging: T1; 2cm diameter or less. T2; 2-5 cm diameter. T3; Tumor larger than 5 cm. T4; Any size with direct extension to chest wall or to skin [(24)].

Table (3) Percentage distribution of tumor stage among the studied females with breast cancer (no=5).

Tumor stage *	Number	Percentage (%)
Stage I	1	20.0
Stage II	2	40.0
Stage III	2	40.0
Stage IV	0	0.0
Total	**5**	**100.0**

***Stage 0** Ductal carcinoma in situ or lobular carcinoma in situ.

Stage I Invasive carcinoma 2 cm or less in size (including carcinoma in situ with micro invasion) without nodal involvement and no distance metastasis .

Stage II Invasive carcinoma < 5 cm without nodal involvement but with movable axillary nodes and no distance metastasis.

Stage III Invasive carcinoma < 5 cm in size with nodal involvement and fixed axillary nodes.

Stage IV Any form of breast cancer with distance metastasis[(24)].

Table (4) social, psychological, or economical barriers about delay in early detection of breast cancer among the studied females (No=390).

Barrier	Number	Percentage (%)
• Not doing regular mammogram	388	99.5
• Not practice monthly breast self examination	380	97.4
• Not doing annual clinical breast examination	373	95.6
• Illiteracy	257	65.9
• Reluctance in seeking medical advice	222	56.9
• Far distance from health services	219	56.2
• Negligence of the patient complaints	185	47.4
• Fear from BC diagnosis	172	44.1
• Patient poverty & High cost	109	27.9
• Lack of time	92	23.6

A key part of the fight against breast cancer is early detection, if treated in time the patient's life can be saved. Exploration of the most important risk factors of breast cancer, its prevalence, the tumor size and its stage at presentation are essential to all women.

This work described that there were high percentages of multiple risk factors for breast cancer among the interviewed women during survey as (early menarche, delayed menopause, late age at first birth, little or no breast feeding, overweight, oral contraceptive users & obesity, in addition to family history of breast cancer). These findings agreed with that reported by Albrektsen et al[13], & Lipworth et al[14] who explored the interaction between these risk factors and breast cancer.

Illiteracy constituted important precipitating factors of lack of awareness and knowledge of these women about breast cancer especially if complaining from breast conditions.

One in eight women and one in four women develop breast cancer in the US and the UK respectively, and Egypt follows closely behind the UK's prevalence rate [15].

Prevalence of breast cancer at this work was 1.3%. Although this prevalence was high but still underestimated as lack of trained personnel discover to this fatal disease. Boulos et al[3] revealed that about eight breast cancers per 1,000 women were found after the screening program in the first year, and when half the women were contacted again in the second year, two cancers per 1000 women were detected.

The incidence of breast cancer is lower in developing countries than in developed countries, but the stage at presentation is much later. Unfortunately, it was noticed that 4 cases (80%) of the discovered cases with breast cancer had tumor sizes T2 & T3 with lymph node metastasis, which indicated late diagnosis and denial of the women to be discovered early that would be reflected on their survival rate later on. In addition to the lack of mass screening programs in these studied areas.

But our results may differ with that of Maalej *et al.* 2008 (Tunisia)[17], CH Yip 1996 (Malaysia)[18], and university hospital, kuala lumpur who reported that T2 & T4 were the frequent tumor sizes in their studies.

In many developed countries, organized mammography screening is available at the population level while developing countries lack such facilities. An ideal screening test for developing countries needs to be simple, inexpensive and effective. Mammography is far from reaching these criteria. Hence, breast self-examination and clinical breast examination (CBE) to detect any abnormalities have been envisaged as alternatives. There are indications that good clinical breast examinations by specially trained health care workers could have an important role especially in women under 50 years [16].

Stage II & stage III of the tumor were the most frequent among the studied cases with breast cancer (80%), which were consistent with that found by Ezzat et al[19] Saudi Arabia & Bedwani *et al*[20] in Alexandria. While these were different in from that reported by Cairo project (all Egypt), which revealed that stage II, IV, & III were the most frequent tumor stages.

The importance of tumor size in improving survival is increasingly evident, and recent evidence by Elkin et al[21] has shown that measuring the impact of an early detection program by stage alone would fail to observe tumor downsizing benefits within stage groups.

Considering some barriers for early detection of breast cancer among the studied females, it was found that lack of healthy practices as doing regular mammography, annual breast examination, and breast self examination were the main limitations of early diagnosis of BC. The previous limitations may be attributed to culture and traditions of the studied females and their families.

Illiteracy was another barrier which reflects their ignorance and deficient awareness or knowledge about the breast cancer.

In addition to reluctance in seeking early medical care, far distance from health services, negligence of the complaint, and fear from BC diagnosis constituted other precipitating factors for delay in diagnosis. So this would lead to high morbidities & mortalities from this terrible disease and consequently bad prognosis as described by female (it is considered a death sentence). Poor survival rates were another impact.

It is estimated that 99% of Egyptian women are unaware of the dangers of breast cancer. Because of this lack of awareness, incidents of death from breast cancer are higher in Egypt than in other parts of the world. This is exacerbated by the fact that many people will not talk about cancer, nor are women educated to perform self-breast examinations and take mammogram tests. Unfortunately only highly educated women or those who have been overseas are aware that breast cancer if caught in the early stages, can be cured. Consequently women are coming to doctors when the cancer has reached an advanced stage, necessitating aggressive treatment [22].

There is also a common misconception in Egypt that cancer is contagious, a notion that has caused the husbands of many diagnosed women to seek a divorce. If a young woman is diagnosed with

breast cancer, she is considered unmarriageable. Egyptian women do not usually come forward until the late stages of the disease, when it is often too late to assist [23].

Recommendations:

Our results recommended the need for urgent health education programs on a wide scale on the studied places to improve not only awareness, or knowledge but also changing the faulty attitudes & practices about early detection of breast cancer especially among illiterate women. Proper training of women about monthly breast self examination for early detection of any breast lesion. In addition to training programs for health care providers at primary health care units about the importance of clinical breast examination in early detection of breast cancer cases especially in low resources settings, beside taking the women's complaints seriously. "Don't worry" attitude by PHC doctors should be changed. Primary health care professionals should be encouraged to refer patients to cancer specialists whenever there is a suspicion that breast cancer may be present.

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5. References:

1. Porter P: 'Westernizing' women's risks? Breast cancer in lower-income countries. N Engl J Med 2008; 358:213–216.
2. Ferlay J, Bray F, Pisani P, Parkin DM. GLOBOCAN 2002: cancer incidence, mortality and prevalence worldwide. IARC Cancerbase no. 5, version 2.0. Lyon, France: IARC Press, 2004; avaliable at http:// www-dep.iarc.fr/.
3. Boulos S, Godallah M, Neguib S. Breast Screening in the emerging world. High prevalence of Breast Cancer in Cairo, Egypt. Breast 2005; 14(5):340-6.
4. Motawy, M.; Hattab, O.E.; Fayaz, S.; Oteifa, M.; Ali, J.; George, T.; Barghash, I.; Abuzallouf, S.; Jarallah, M.E. Multidisciplinary Approach To Breast Cancer Management in Kuwait, 1993–1998. J. Egyptian Nat. Cancer Inst. 2004, 16, 85–91.
5. WHO 2006, Guidelines for Early Detection and screening of breast cancer EMRO Technical Publications Series 30.
6. IARC Working Group on the Evaluation of Cancer-Preventive Strategies. IARC Handbooks of Cancer Prevention. Vol. 7, Breast Cancer Screening. Lyon, France: Oxford University Press, 2002.
7. Miller AB, Chamberlain J,Tsechkovski M. Self-examination in the early detection of breast cancer. A review of theevidence, with recommendations for further research. J Chronic Dis. 1985; 38(6):527-40.
8. Vahabi M. Breast cancer screening methods: a review of the evidence. Health Care Women Int. 2003 Nov;24(9):773-93.
9. Devi B, Tang TS, Corbex M. Reducing by half the percentage of late stage presentation for breast and cervix cancer over 4 years: A pilot study of clinical downstaging in Sarawak, Malaysia. 2006 in press.
10. ACS Breast Cancer Screening Guidelines Memorial Sloan-Kettering Cancer centre. 2006
11. Ogden C, Kuczmarski R, Flegal K, et al. Centers for Diseases Control and Prevention 2000 growth charts for the United States: improvements to the 1997 National Center for health Statistics version. Pediatrics.2002;109(1):45–60 [Abstract/Free Full Text].
12. -Stewart B, Kleihues PE. World cancer report. Lyon, France: IARC Press, 2003.
13. Albrektsen G, Heuch I, Hansen S, Kvale G. Breast cancer risk by age at birth, time since birth and time intervals between births: exploring interaction effects. Br J Cancer 2005;92:167-75.
14. Lipworth L, Bailey LR, Trichopoulos D. History of breast-feeding in relation to breast cancer risk: a review of the epidemiologic literature. J Natl Cancer Inst 2000;92:302-12.
15. May El Meleigy FIGHTING BREAST CANCER WITH FIBER AND DIET First Published: March 9, 2007"
16. Shapiro S. Periodic screening for breast cancer. The HIP randomized control trial. J Natl Cancer Inst Monogr ((1997)) 22:27–30.[Abstract/Free Full Text]
17. Maalej M, Hentati D, Messai T, et al: Breast cancer in Tunisia in2004: a comparative clinical and epidemiological study Bull Cancer. 2008 Feb;95(2):E5.
18. C H Yip. E H Ng: Breast Cancer – A Comparative study Between Malaysian And Singaporean Women, SINGAPOR MED J 1996;Vol 37:264-267

19. Ezzat AA, Ibrahim EM, Raja MA, Al-Sobhi S, Rostom A,Stuart RK. Locally advanced breast cancer in Saudi Arabia: high frequency of stage III in a young population. Med Oncol1999; 16:95e103.
20. Bedwani R, Abdel-Fattah M, El Shazly M, Bassili A, Zaki A, Seif HA, et al. Profile of familial breast cancer in Alexandria, Egypt. Anticancer Res 2001; 21:3011-4.
21. Elkin EB, Hudis C, Begg CB, Schrag D. The effect of changes in tumor size on breast carcinoma survival in the U.S.: 1975–1999. Cancer 2005; 104:1149–57.
22. Jemal, A, Siegel, R, Ward, E, et al. Cancer statistics, 2007. CA Cancer J Clin 2007; 57:43.
23. World Health Organization. Executive summary. In: National Cancer Control Programmes: Policies and Managerial Guidelines. Geneva. Switzerland: World Health Organization, 2002:i–xxiv.
24. Singletary, S. Eva, M.D.; FACS; James, L.; Connolly, M.D.; Breast Cancer Staging: Working With the Sixth Edition of the AJCC Cancer Staging Manual.CA Cancer J. Clin. 2006, 56, 37–47.

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Effects of Renal Ischemia Reperfusion on Brain, Liver & Kidney Tissues in Adult Male Rats.

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Abstract: Several studies suggest that renal ischemia reperfusion (RIR) can induce acute kidney injury (AKI). However, remote effects of RIR injury need further investigations. Renal injury associated with liver disease or neurological manifestations is a common clinical problem.

The aim of this study was to examine the effects of RIR on brain and liver tissues in rats by inducing bilateral renal ischemia for 30, 45 and 60 minutes followed by one hour reperfusion and measurement of renal functions, liver functions & tumor necrosis factor alpha (TNF-α) in addition to histological examinations of kidney, liver and brain tissues. 40 rats were subjected to either sham operated (control group-1) or 30 min RIR (group-2), 45 minRIR (group-3), 60min RIR (group-4).

The results demonstrated that compared to sham rats, serum creatinine, blood urea nitrogen (BUN), alanine aminotransferase (ALT) and aspartate aminotransferase (AST), increased significantly 45 min & 60 min of RIR($P < 0.05$). There was a significant increase ($P<0.05$) in TNF-α in kidney, liver and brain tissues after 30 min, 45min, and 60min RIR compared to shame rats ($p<0.01$) and the rise of TNF-α after 60 min RIR is significantly higher ($p<0.05$) than that after 30min RIR. Histological examination of brain tissues showed mild pyknosis after 45 min RIR and patches of vacuolization after 60 min RIR. In liver tissues there were congestion & hydropic degeneration after 45 min RIR and there was leucocyte infiltration in addition to congestion after 60 min RIR. Stained kidney tissues showed mild glomerular collapse after 30 min RIR, mild necrosis of tubular cells after 45 min RIR and periarteriolar neutrophilic infiltration in addition to mild tubular necrosis after 60 min RIR. It is concluded that RIR causes inflammation in liver & brain tissues which was much more after 60 min. The effects of RIR on remote organs need to be investigated for a long period after RIR

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Key words: Renal ischemia reperfusion, brain, liver, tumor necrosis factor-alpha (TNF-α), rats

1. Introduction:

Ischemia reperfusion is a frequently encountered phenomenon in organisms. Prolonged ischemia followed then by reperfusion results in severe oxidative injury in tissues and organs[1]. Renal ischemia reperfusion (RIR) is a common cause of acute kidney injury (AKI).[2] RIR injury occurs in many clinical situations, such as transplantation, partial nephrectomy, sepsis, hydronephrosis, or elective urological operations.[3] Mortality during AKI is largely due to extrarenal manifestations.[4, 5] Several studies have begun to investigate mechanisms that underlie distant organ effects of RIR injury and found a significant inflammatory effect of RIR on the lung[6] and the heart.[7]

Liver and kidneys are both involved in the regulation of body homeostatic responses, metabolism and excretion of drugs and toxic products.[8] Many studies have suggested cross-talk between the liver and kidneys. [3] Central nervous system changes, the signs of which range from decreased mental status to obtundation and seizures, are one of the classic indications to begin dialysis during AKI.[9]

An increasing body of evidence suggests that the deleterious effects of AKI on remote organ function could, at least in part, be due to loss of the normal balance of immune, inflammatory, and soluble mediator metabolism that attends injury of the tubular epithelium. Such dysregulation, acting at least in part on endothelium, leads to compromise of remote organ.[10]

Kielar et al[11] have evaluated the extrarenal regulation of AKI. This regulation may be as a result of increased production of cytokines such as tumor necrosis factor-alpha (TNF-α) and growth factors such as hepatocyte growth factor produced by extrarenal organs.

The aim of the present study was to assess the changes in brain, liver and kidney tissues after different periods of renal ischemia followed by reperfusion.

2. Material and Methods:

Experimental Animals

Forty male Albino rats belonging to local strain weighing between 180-220 gm were obtained from the Animal House of Faculty of Medicine, Cairo University and included in this study. The animals were housed in wire mesh cages at room temperature with 12:12h light-dark cycles and maintained on standard rat chow and tap water. Veterinary care was provided by Animal House Unit of Cairo University. The animals were divided into 4 groups of 10 animals each.

Group-1: Control group (sham-operated rats)

The other animals were subjected to renal ischemia followed by 1 hour reperfusion. According to periods of ischemia they were divided into

Group-2: 30 min ischemia followed by 1 hour reperfusion

Group 3: 45 min ischemia followed by 1 hour reperfusion

Group 4: 60 min ischemia followed by 1 hour reperfusion

Surgical procedure

Rats were anaesthetized with pentobarbital sodium 60 mg /kg. A midline laparotomy was performed and the renal arteries were carefully separated from around the tissues. In the RIR groups, renal arteries were occluded by a non traumatic microvascular clips for 30, 45, 60 min followed by 1 hr reperfusion . Occlusion was approved visually by color change of the kidneys to a paler shade and reperfusion by blushing .[3] Sham-operated animals underwent identical surgical treatment, including isolation of both renal arteries. However, artery occlusion was not performed. At the end of the experimental procedure, blood samples were collected retro-orbitally for determination of alanine aminotransferase (ALT) , aspartate aminotransferase (AST), blood urea nitrogen (BUN) and creatinine. All rats were scarified and brain, liver and kidney tissues were removed and prepared for histological examination and measurement of TNF-α.

Assessment of renal and liver function

Determination of serum AST and ALT was carried out by colorimetric method[12]

Kidney function tests (BUN & creatinine) were assessed by conventional available kits

Histological procedures

Paraffin –embedded brain, liver & kidney sections after formalin fixation (10% phosphate-buffered) & dehydration were stained by Hematoxylin & Eosin. Histological examination of all tissues was evaluated per section in at least 10 randomly selected non-overlapping fields at x100, x 200 and x 400 magnification.

Measurement of TNF- α

A portion of the brain , liver and kidney were homogenized after the tissue samples had been diluted in 5 vol of homogenate buffer [10 mM HEPES (pH 7.9), 10 mM KCL, 0.1 mM EGTA, 1 mM DTT, and 0.5 mM phenylmethanesulfonyl fluoride] using a vertishear tissue homogenizer. The homogenates were centrifuged at 3,000 *g* for 15 min at 4°C. The supernatants were subsequently stored at –80°C until the ELISA technique could be performed TNF- α *was* determined in tissue homogenate using an ELISA. The ELISA was performed by adding 100 µl of each sample to wells in a 96-well plate of a commercially available rat ELISA kit (R&D system quantakine USA). The samples were tested in duplicate. The ELISA was performed according to the manufacturer's instructions and final results were expressed as picograms per mg tissues[13]

Statistical analysis

The data was encoded and entered using the statistical package SPSS Version 15. The results are given as mean ± standard error (M±SE). Statistical analysis was performed by ANOVA (analysis of variance) and multiple comparison Post-Hoc Tests to determine significant differences between groups. Correlations were done to test for linear relations between variables using Pearson correlation test. Statistical significance of a difference was defined when $p \leq 0.05$

3. Results:

The effect of RIR on the brain, liver and kidneys was investigated 30, 45 and 60 minutes of renal ischemia followed by 1 hour reperfusion

The effect of RIR on biochemical parameters

Effect of clamping of both renal arteries was confirmed by a significant increase in serum creatinine and BUN after 45 min and 60 min RIR compared to 30 min RIR and control group (Table 1).

RIR resulted in a significant increase in BUN after 45 min (70.04 ± 2.34 mg/dl) & 60 min RIR (79.06 ± 4.53 mg/dl) compared to control (37.70 ± 2.39 mg/dl $p< 0.05$) & 30 min RIR groups (47.39 ± 3.81 mg/dl $p<0.05$) and there was a significant rise in serum creatinine after 45 min ($p<0.05$) & 60 min RIR ($p< 0.05$) compared to sham control rats (sham versus 30 min RIR versus 45 min RIR versus 60 min RIR: 0.11 ± 0.01 vs. 0.27 ± .06 vs .0.44 ± .08 vs 0.89 ± .05 mg/dl) . Serum creatinine

was significantly higher after 60 min RIR than that after 30min & 45 min RIR ($p<0.05$)

The effect of RIR on liver function was demonstrated by a significant increase in liver enzymes ALT and AST (Table-1)

ALT was increased significantly ($p<0.05$) after 30 min, 45 min and 60 min RIR compared to control group (43.79±2.10, 68.04±4.67, 77.51±3.39 vs 23.37±1.71 U/L.) respectively. The levels of ALT after 60 min RIR & 45 min RIR was significantly higher than that after 30 min RIR ($p< 0.05$).

AST was found to be significantly increased ($p < 0.05$) after 45min and 60 min RIR compared to control group (82.16 ±2.23 & 89.73 ±2.90 vs 69.39 ±3.61U/L) respectively. AST was significantly higher ($p < 0.05$) after 60 min RIR than that after 30 min. RIR.

Effect of RIR on TNF-α in brain, liver and kidney tissues.

TNF-α was measured as one of pro inflammatory mediators to determine whether RIR would lead to inflammatory changes in brain, liver or kidney tissues.

In brain tissues, RIR produced a significant increase ($p < 0.05$) in TNF-α after 30min, 45min, and 60 min of RIR compared to control group (23.52± 1.28 , 30.94 ± 1.79 & 33.27± 3.39 pg/mg tissue vs 12.69 ± 0.89 pg/mg tissue, respectively (Table-2, Fig-1).

In liver tissues, there was a significant increase ($p < 0.05$) in TNF-α after 30 min (50.10 ± 4.10), 45 min (58.44 ± 2.49) and 60 min (70.47 ± 3.51 pg/mg tissue) of RIR compared to control group (17.15 ± 1.29 pg/mg tissue)

In brain and liver tissues, the increase of TNF-α after 60 min was significantly higher ($p < 0.05$) than that after 30 min of RIR (Table-2, Fig-1).

In kidney tissues the levels of TNF-α was increased significantly ($p<0.05$) after 30 min of RIR (39.86 ± 4.26), 45 min of RIR (65.16±1.97) and 60 min of RIR (71.42± 3.34) compared to control group (17.13 ± 1.04 pg/mg tissue). The increase after 45min & 60 min was significantly higher than that after 30 min of RIR ($p<0.05$). Although the rise of serum creatinine and BUN was not significant after 30min RIR, the TNF- α increased significantly after 30 min denoting presence of inflammatory changes after 30 min RIR (Table-2, Fig-1).

Statistical analysis showed positive correlation ($p < 0.05$) between TNF-α in brain & kidney tissues (Fig 2 -A) , between TNF-α in liver & serum creatinine (Fig 2-B) and between TNF-α in kidney tissues and serum creatinine (Fig 2 – C).

The effect of RIR on brain, liver and kidney histopathological structures.

To determine whether RIR resulted in adverse effects on structure of the brain, liver and kidney tissues, Hematoxylin & Eosin–stained sections from RIR groups & control rats were examined. Changes were very mild after 30 min RIR in the three types of tissues.

Histological examination of brain tissues (Fig-3) showed normal brain tissues of sham operated rats (A), mild pyknosis 45 min RIR (B) and patches of vacuolization 60 min RIR(C). In liver tissues (Fig-4), there was congestion & hydropic degeneration 45 min RIR (E) & there was leucocyte infiltration in addition to congestion 60 min RIR(F) when compared to normal liver tissues (D). Stained kidney tissues (Fig-5) showed normal kidney tissues (G), mild glomerular collapse 30 min RIR(H) , mild necrosis of tubular cells 45 min RIR (I) & periarteriolar neutrophilic infiltration in addition to mild tubular necrosis 60 min RIR (J).

Table-1: Effects of different periods of renal ischemia (30, 45 and 60 min) followed by one hour reperfusion (RIR) on renal and liver function tests

Groups (n=10)	BUN (mg/dl)	Creatinine (mg/dl)	ALT(U/L)	AST(U/L)
Control	37.70 ± 2.39	.11 ± .01	23.37 ± 1.71	69.39 ±3.61
30 min RIR	47.39 ± 3.81	0.27 ± .06	43.79 ± 2.10*	74.32 ±3.40
45 min RIR	70.04 ± 2.34* ▲	0.44 ± .08*	68.04 ± 4.67* ▲	82.16 ±2.23*
60 min RIR	79.06 ± 4.53* ▲	0.89 ± .05*▲□	77.51 ± 3.39* ▲	89.73 ±2.90* ▲

Results are mean ±SE

n: number of male rats in each group

* Significant compared to control value ($p < 0.05$)

▲ Significant compared to 30 min RIR($p < 0.05$)

□ Significant compared to 45 min RIR ($p <0.05$)

Table-2 : Effects of different periods of renal ischemia (30,45 and 60 min) followed by one hour reperfusion on tumor necrosis factor- alpha (TNF-α pg/mg tissues) in kidney, liver and brain tissues in male rats

Groups (n=10)	TNF-α in brain (pg/mg tissue)	TNF-α in liver (pg/mg tissue)	TNF-α in kidney (pg/mg tissue)
Control	12.69 ± .89	17.15 ± 1.29	17.13 ± 1.04
30 min RIR	23.52± 1.28 *	50.10 ± 4.10 *	39.86 ± 4.26 *
45 min RIR	30.94 ± 1.79 *	58.44 ± 2.49 *	65.16± 1.97 * ▲
60 min RIR	33.27± 3.39 *▲	70.47 ± 3.51*▲	71.42± 3.34 * ▲

Results are mean ±SE

n: number of male rats in each group

* Significant compared to control value ($p < 0.01$)

▲ Significant compared to 30 min RIR ($p< 0.05$)

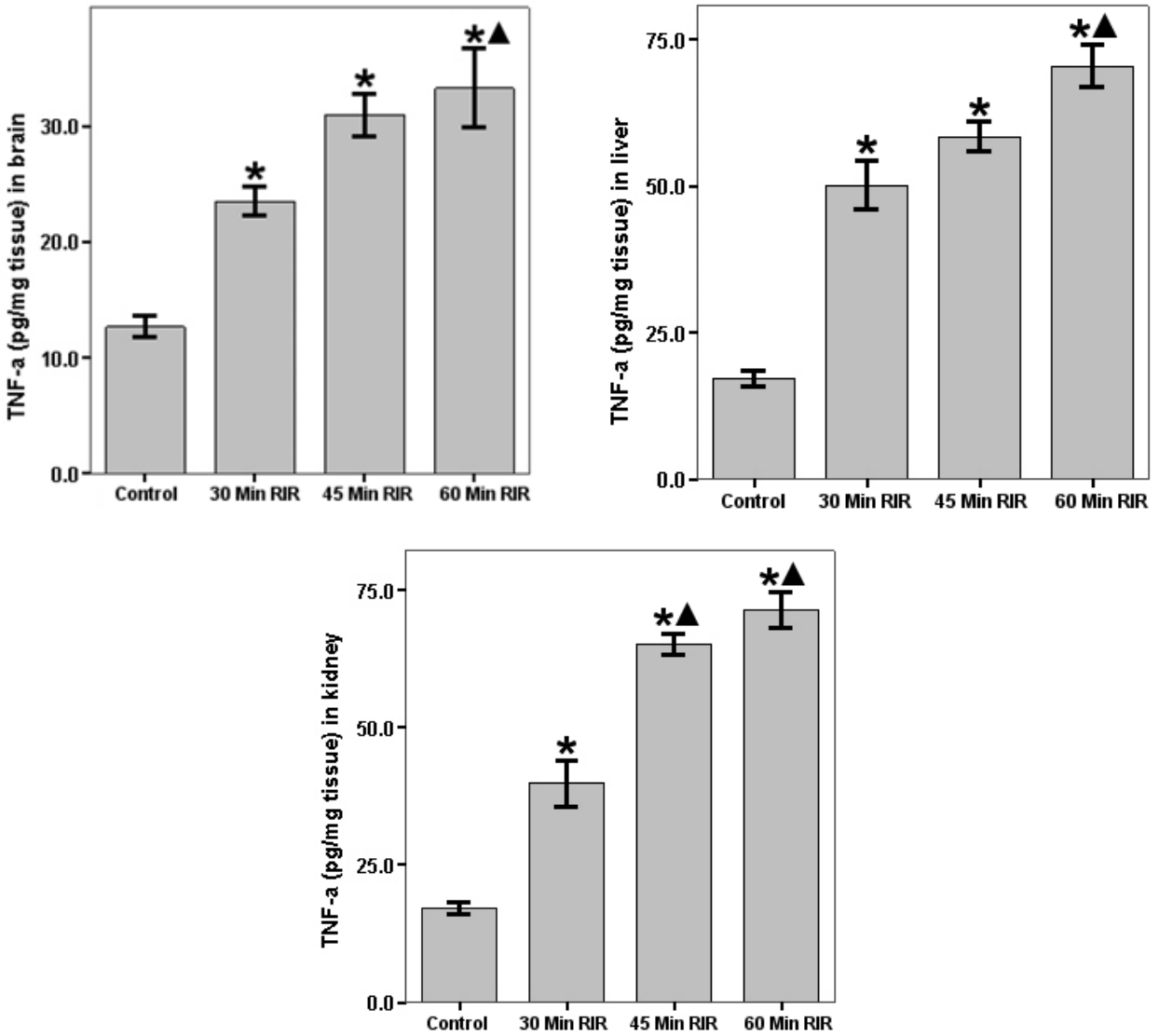

Fig-1 : Tumor necrosis factor -alpha(TNF-α) in brain , liver and kidney tissues after different periods of renal ischemia (30 , 45 & 60 min) followed by one hour reperfusion (RIR)

Results are mean ± SE

* Significant compared to control group ($p< 0.01$)

▲ Significant compared to 30 min RIR ($P< 0.05$)

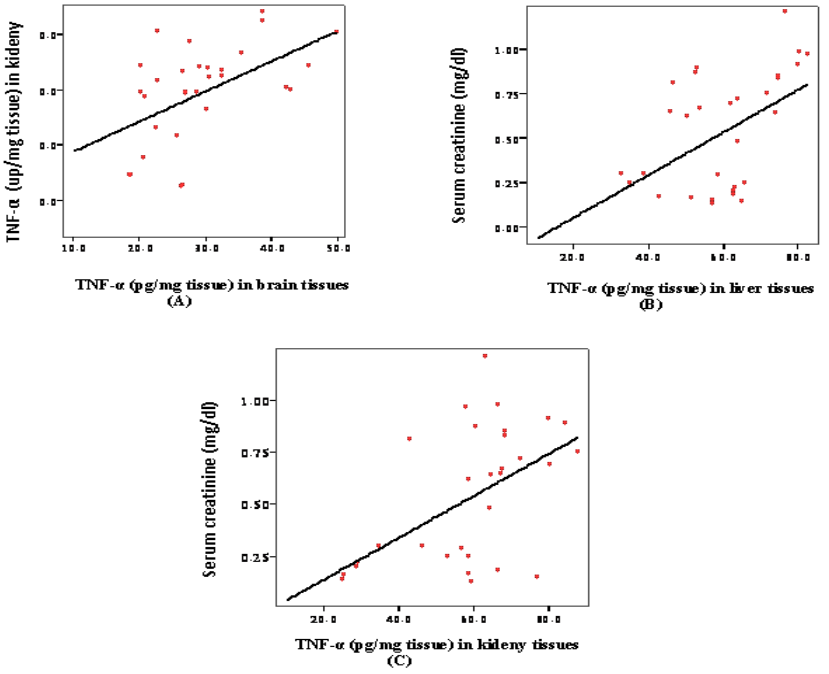

Fig 2: Positive correlation between tumor necrosis factor alpha TNF-α in brain & kidney (A), between TNF-α in liver & serum creatinine (B) , between TNF-α in kidney & serum creatinine (C)

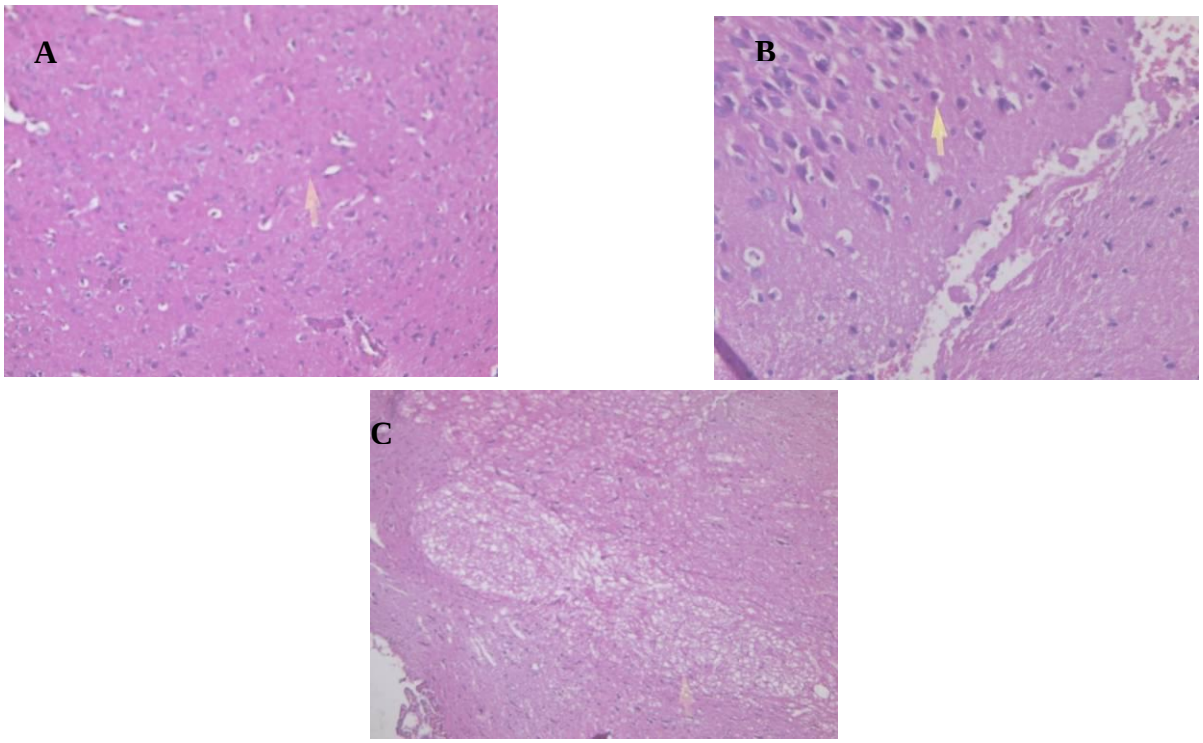

Figure 3. Hematoxylin & Eosin stained sections of rat brain. (A) Normal brain of sham operated rats(x200). Sections B&C from renal ischemia reperfusion groups(RIR) showed pyknosis & shrinkage of cytoplasm (B)45 min RIR (x200) & patches of vacuolization (C) 60 min RIR (x100).

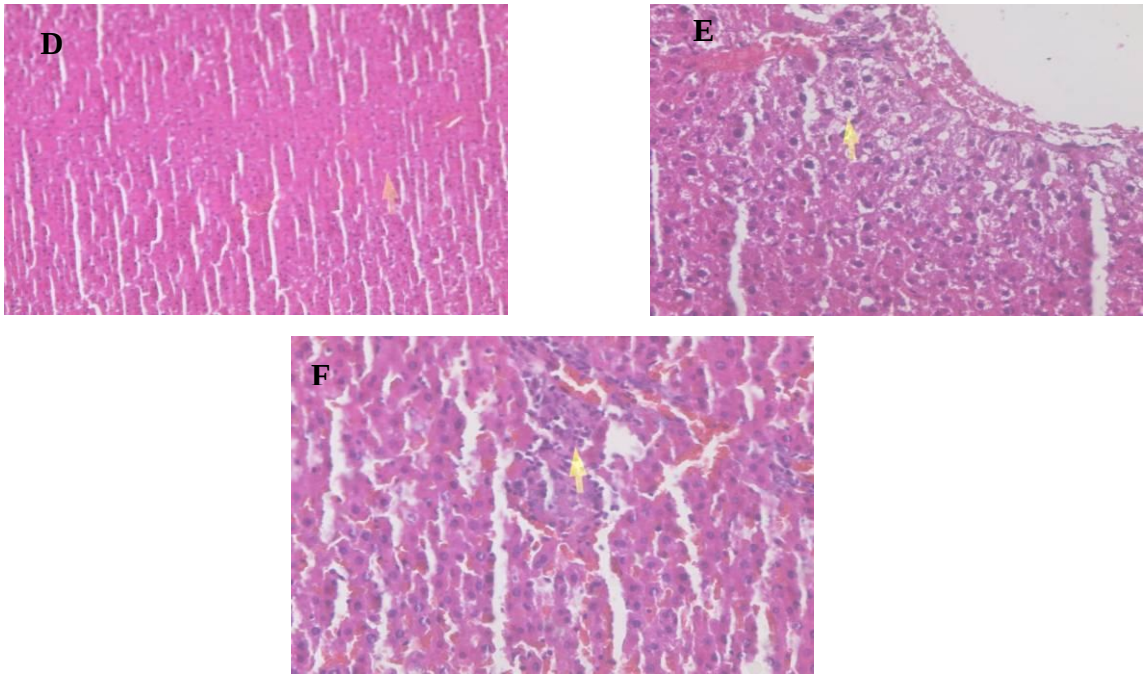

Figure 4. Hematoxylin & Eosin stained sections of rat liver. (D) Normal liver of sham operated rats (x200). Sections (E&F) from renal ischemia reperfusion groups(RIR) showed congestion with hydropic degeneration (E) 45 min RIR & congestion with leucocyte infiltration (F) 60 min RIR (x 400).

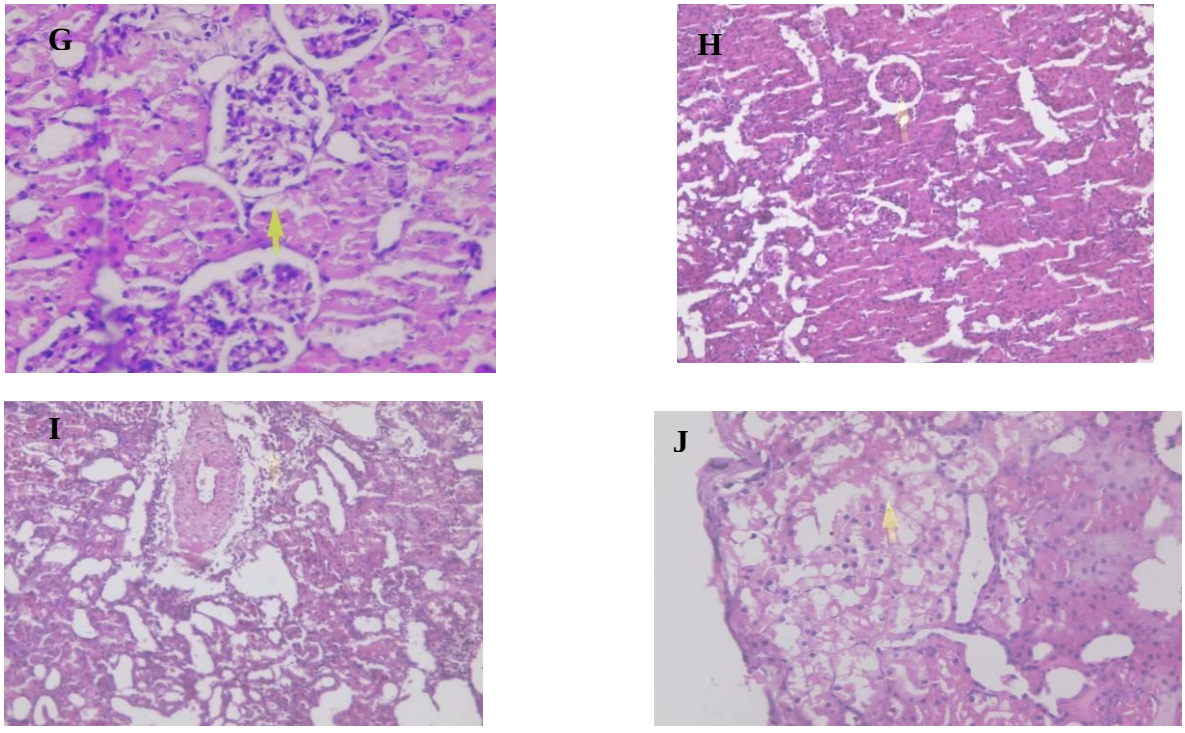

Figure 5. Hematoxylin & Eosin stained sections of rat kidney. (G) Normal kidney of sham operated rats (x400). Sections (H,I&J) from renal ischemia reperfusion groups(RIR) showed mild glomerular collapse (H)30 min RIR (x200), mild necrosis of tubular cells (I) 45 min RIR(x400) & moderate periarteriolar neutrophilic infiltrate(J) 60 min RIR (x 400).

4. Discussions:

Acute renal ischemia is associated with a high mortality rate. Most of mortality during RIR injury despite dialysis is from extrarenal organ dysfunction. This initiated investigation into the underlying mechanisms.[2]

The present study was conducted to determine whether an experimental RIR would lead to any measurable short term changes in liver or brain tissues as remote organs. The changes in the brain, the liver and the kidneys were examined after induction of various periods of renal ischemia (30, 45 & 60 min) followed by reperfusion for one hour in male rats.

The present study have demonstrated that 30min, 45min, & 60 min RIR resulted in a significant increase of TNF-α in brain tissues compared to sham operated group. The histological examination showed pyknosis & vacuolization of nerve cells. The changes were very mild at 30 min and increased after 45 & 60 min RIR

Liu et al [2] examined brain histology in mice that underwent 60 min of bilateral renal ischemia followed by 24 h of reperfusion. Compared with sham-operated control mice, they found that mice with ischemic AKI developed marked brain changes evidenced by increased soluble inflammatory proteins, increased cellular inflammation and increased microglial cells and pyknotic neuronal cells in the hippocampus. However there was a trend toward an increase in TNF-α in kidney but in contrast to our results, there was no change in TNF- α in the brain. This might be due to measurement of TNF-α in mice 24 hr after ischemia (TNF-α was measured one hour after ischemia in the present study) or may be due to differences between mice & rats.

Because previous studies have demonstrated that ischemic AKI leads to inflammatory response in the blood and lung.[6–7] Luis et al [2] hypothesized that AKI would also lead to brain inflammatory changes.

In the present study, the changes in liver functions (serum ALT and AST), histology and TNF-α in liver tissue were examined after induction of various periods of rat renal ischemic injury

Liver functions were reduced 30 min and 45 min after renal ischemia but showed a maximum reduction in the 60-min ischemia group. Liver histology showed congestion and hydropic degeneration 45 min RIR and there was leucocyte infiltration 60 min RIR. The TNF-α in the liver tissue was significantly increased 30 min, 45 min and 60 min RIR compared to control group and the increase after 60 min was significantly higher than that after 30 min of RIR.

The results of the present study are consistent with other investigators[3, 14] who reported that renal ischemia caused changes in liver histology, function, oxidative stress and inflammatory status, which led to a reduction in hepatic antioxidant capacity. With 30 min ischemia, the magnitude of these changes was less than those with 45 or 60 min ischemia. Their results showed a significant decrease in liver glutathione GSH, as well as a significant increase in TNF-α and IL-10 concentrations

Serteser et al [15] demonstrated some changes in hepatic TNF-α levels and oxidation products after RIR injury in mice. They have suggested that 30 min ischemia and 60 min reperfusion is sufficient to elicit remote effects of RIR injury

Gurley et al[16] found that RIR injury reduced hepatic oxidative drug metabolism, as determined by reduction of antipyrine clearance, 4 and 24 hours post IR injury & the peak level of TNF-α occurred one hour post ischemia reperfusion

In a more recent study, Vaghasiya et al[17] demonstrated that serum concentrations of ALT & AST were significantly increased after renal ischemia for 30 min followed by reperfusion for 24 hours in normal & diabetic rats but the changes were much more in diabetic than in normal rats

Fadillioglu et al[18] concluded that RIR may affect distant organs such as liver and oxidative stress may play role on this injury.

In the present study, as expected, RIR caused a reduction in renal functions, an increase in TNF-α and structural alteration in kidney tissues (necrosis of tubular cells & periarteriolar neutrophilic infiltration) in an ischemia-time-dependent manner.

These results are consistent with several studies that reported decreased renal functions in RIR in rats & mice[2,3]Rodent studies have demonstrated that the inflammatory response to hypoxia contributes to the resultant tissue injury.[19] RIR initiated changes in vascular endothelial cells, tubular epithelial cells and leukocytes that resulted in the loss of immune system homeostasis in the kidney.[20] One of the hallmarks of RIR, in mouse models, was neutrophilic accumulation in the post-ischemic kidney and depletion of neutrophils prevented AKI .[21]

In addition to the direct cytotoxic effects of hypoxia, RIR induces an inflammatory reaction within the renal parenchyma .[19] RIR causes renal synthesis of pro-inflammatory cytokines such as IL-1, IL-6, and TNF-α .[22,23] ATP depletion causes tubular epithelial cells to undergo apoptosis or necrosis in vitro [24], and both apoptotic and necrotic tubular epithelial cells may be seen in ischemic acute renal failure .[25] Necrosis of cells causes the release of a number of factors . High mobility group 1 protein, for example, is a nuclear factor that is released by necrotic cells and promotes inflammation .[26] When

released, it stimulates TNF-α production and leukocyte infiltration

Macrophages infiltrate the injured kidney shortly after neutrophils (within 1 h of reperfusion). Intracellular cytokine staining of kidney infiltrating macrophages by flow cytometry demonstrated that these leukocytes are significant producers of the cytokines IL-1α, IL-6, IL-and TNF-α.[20]

Dong et al. [27] demonstrated that after RIR, renal dendritic cells produce the pro-inflammatory cytokines/chemokines TNF-α and IL-6 and that depletion of dendritic cells prior to RIR significantly reduced the kidney levels of TNF-α produced after RIR.

Critical early roles for neutrophils, macrophages & lymphocytes have been established in mouse models of AKI.[20]

Laboratory and clinical evidence suggests that the inflammatory milieu associated with RIR leads to dysfunction of renal cells and this may be the key factor leading to acute kidney injury. Cells in injured tissues release immunological signals which communicate with remote organs including the kidney.[28]

The results of the present work showed that renal ischemia followed by reperfusion caused detrimental changes in the brain, liver & kidney histology & function. After 60 min, the magnitude of these changes is much more than after 45 or 30 min. The effect of RIR on remote organs may need to be investigated for a long period after RIR.

Further studies are needed to explore the mechanisms and pathophysiological pathways that mediate these changes in brain, liver and kidneys after RIR. Care should be taken to protect organs remote from sites of ischemia reperfusion especially during renal surgery.

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References:

1. Tan DX, Manchester LC, Sainz RM, Mayo JC, León J, Reiter RJ. Physiological ischemia/reperfusion phenomena and their relation to endogenous melatonin production: a hypothesis. Endocrine. 2005 Jul;27(2):149-58
2. Liu M, Liang Y, Chigurupati S, Lathia JD, Pletnikov M, SunZ , Crow M, Ross CA, Mattson MP, and Rabb H · Acute Kidney Injury Leads to Inflammation and Functional Changes in the Brain J Am Soc Nephrol. 2008 July; 19(7): 1360–1370.
3. Kadkhodaee M, Golab F, Zahmatkesh M, Ghaznavi R. Effects of different periods of renal ischemia on liver as a remote organ. World J Gastroenterol. 2009 March 7; 15(9): 1113–1118.
4. Rabb H, Chamoun F, Hotchkiss J: Molecular mechanisms underlying combined kidney-lung dysfunction during acute renal failure. *Contrib Nephrol* 2001,41–52.
5. Kelly KJ: Acute renal failure: much more than a kidney disease. *Semin Nephrol* 2006,26: 105–113,
6. Kramer AA, Postler G, Salhab KF, Mendez C, Carey LC, Rabb H: Renal ischemia/reperfusion leads to macrophage-mediated increase in pulmonary vascular permeability. *Kidney Int* 1999, 55: 2362–2367.
7. Grigoryev DN, Liu M, Hassoun HT, Cheadle C, Barnes KC, Rabb H: The local and systemic inflammatory transcriptome after acute kidney injury. *J Am Soc Nephrol* 2008, 19: 547–558.
8. Sural S, Sharma RK, Gupta A, Sharma AP, Gulati S. Acute renal failure associated with liver disease in India: Etiology and outcome. Ren Fail 2000; 22:623-34.
9. Star RA: Treatment of acute renal failure. *Kidney Int* 1998, 54: 1817–1831.
10. Paladino JD, Hotchkiss JR, Rabb H. Acute kidney injury and lung dysfunction: a paradigm for remote organ effects of kidney disease?. Microvasc Res. 2009 Jan;77(1):8-12.
11. Kielar ML, Rohan Jeyarajah D, Lu CY. The regulation of ischemic acute renal failure by extrarenal organs. Curr Opin Nephrol Hypertens. 2002; 11:451–457.
12. Reitman S, Frankel S. A colormetric method for the determination of serum glutamic oxalocetic and glutamic pyruvic transaminases. *Am J Clin Path.* 1957;28(1):56–63
13. Smith M. R. Direct evidence for an intracellular role for tumor necrosis factor- alpha 1. Microinjection of tumor necrosis factor kills target cells. J Immunol 1990, 144, 162-169
14. Golab F, Kadkhodaee M, Zahmatkesh M, Ghaznavi R, Arab HA, Seifi B, Soleimani M. Hepatic changes during various periods of reperfusion after induction of renal ischemia in rats. Transplant Proc. 2009 Sep;41(7):2749-50
15. Serteser M, Koken T, Kahraman A, Yilmaz K, Akbulut G, Dilek ON. Changes in hepatic TNF-alpha levels, antioxidant status, and oxidation products after renal ischemia/reperfusion injury in mice. J Surg Res. 2002;107:234–240
16. Gurley BJ, Barone GW, Yamashita K, Polston S, Estes M, Harden A. Extrahepatic ischemia-reperfusion injury reduces hepatic oxidative drug metabolism as determined by serial antipyrine clearance. Pharm Res. 1997 Jan;14(1):67-72

17. Vaghasiya J D , Sheth N R , Bhalodia Y S , Jivani N P . Exaggerated liver injury induced by renal ischemia reperfusion in diabetes: Effect of exenatide. Saudi J Gastroenterol 2010;16:174-80
18. Fadillioglu E, Kurcer Z, Parlakpinar H, Iraz M, Gursul C. Melatonin treatment against remote organ injury induced by renal ischemia reperfusion injury in diabetes mellitus. Arch Pharm Res. 2008 Jun;31(6):705-12. Epub 2008 Jun 19.
19. Joshua M. Thurman. Triggers of Inflammation after Renal Ischemia/Reperfusion. Clin Immunol.2007-4 123 (1) 7-3
20. Gilbert R. Kinsey, Li Li, and Mark D. Okusa. Inflammation in Acute Kidney Injury. Nephron Exp Nephrol. 2008; 109(4): e102–e107.
21. Kelly KJ, Williams WW, Jr, Colvin RB, Meehan SM, Springer TA, Gutierrez-Ramos JC, Bonventre JV. Intercellular adhesion molecule-1-deficient mice are protected against ischemic renal injury. *J Clin Invest.* 1996; 97:1056–1063.
22. Donnahoo KK, Meng X, Ayala A, Cain MP, Harken AH, Meldrum DR. Early kidney TNF-alpha expression mediates neutrophil infiltration and injury after renal ischemia-reperfusion. *Am J Physiol.* 1999;277:R922–9
23. Burne-Taney MJ, Kofler J, Yokota N, Weisfeldt M, Traystman RJ, Rabb H. Acute renal failure after whole body ischemia is characterized by inflammation and T cell-mediated injury. *Am J Physiol Renal Physiol.* 2003;285:F87–94
24. Lieberthal W, Menza SA, Levine JS. Graded ATP depletion can cause necrosis or apoptosis of cultured mouse proximal tubular cells. *Am J Physiol.* 1998;274:F315–27
25. Kashgarian M, editor. *Heptinstall's Pathology of the Kidney.* Lippincott-Raven Publishers; Philadelphia: 1998. Acute Tubular Necrosis and Ishcmeic Renal Injury; pp. 863–889
26. Scaffidi P, Misteli T, Bianchi ME. Release of chromatin protein HMGB1 by necrotic cells triggers inflammation. *Nature.* 2002; 418:191–5.
27. Dong X, Swaminathan S, Bachman LA, Croatt AJ, Nath KA, Griffin MD. Resident dendritic cells are the predominant TNF-secreting cell in early renal ischemia-reperfusion injury. *Kidney Int.* 2007; 71:619–628.
28. Wen X, Murugan R, Peng Z, Kellum JA. Pathophysiology of acute kidney injury: a new perspective. Contrib Nephrol. 2010; 165:39-45. Epub 2010 Apr 20.

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Application of Constructivist Educational Theory in providing Tacit Knowledge and Pedagogical Efficacy in Architectural Design Education: A Case Study of an Architecture school in Iran

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Abstract: Beginning with general debates about the character of pedagogy in architectural design studios, and the role of constructivist educational theory and tacit knowledge in efficacious learning and teaching, this article makes some predictions about the interrelationship between these processes. These predictions are then tested by conducting an empirical research at one of the early design studios of architecture education devoted to residential design, in the department of art and architecture at Kerman Azad University in Iran. These tests have shown significant differences between the design performance of students who have been subjected to constructivist educational theory in their design process, and those who encountered other pedagogical approaches. This paper uses these findings to confirm that applying constructivist educational theory in the design studio leads to an increase in tacit knowledge, the kind of knowledge that is required among designers, as design is related to a skill-based domain dealing more with knowing how to complete tasks than of mere reliance on knowing facts. In this article, it is argued that if such educational strategy gains currency in schools of architecture, the outcome will be a positive experience of pedagogical efficacy through supporting lifelong learning. This efficacy, when woven with tacit knowledge, can create architects that are more dependent on their own critical thinking abilities, more interrelated to communities of people, and more responsive to their feedbacks, which in turn allows them to be more realistic in their endeavors.

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Keywords: Constructivism; Architectural Design Education; Computer-Aided Design; Tacit Knowledge; Iran

1. Introduction

Debates about appropriate methods for architectural design education that have recently surfaced in architecture schools suggest new ideas regarding the nature of architectural design education which deserve to be explored theoretically and empirically.

This study uses experiences from the design studio and constructivist educational theory to propose a new perspective for architectural design education. It claims that the instructor's constructivist approach in the design studio encourages the acquisition of tacit knowledge rather than explicit knowledge. This is supported by an empirical research conducted at the end of the paper. Thus, the article consists of two main parts: Part one deals with theoretical debates about architecture design education, constructivist educational theory and the role of tacit knowledge in learning. Part two concerns an empirical research conducted in a real design studio in order to examine the relationship between the application of constructivist educational theory and the formation of tacit knowledge for architecture students. Based on this, the aim of the paper is to illustrate how one might integrate constructivist educational theory with the acquisition of tacit knowledge in design pedagogy.

1.1 Theoretical Perspective

A review of literature dealing with the architectural design process and education will be presented first, succeeded by a discussion of the tacit domain of knowledge. The review will continue with the theoretical basis of constructivism theory, which is our pedagogical framework. After these two stages, the article's instructional framework for constructivist design education will be introduced.

1.1.1 Architectural design education

Until recently, design was defined merely as an uncontrolled creative turn into the unconscious. In the past, architecture dealt with creating specific works of art on specific sites, where the method of design was intuitive and relied heavily upon the talent of the individual designer (Salama 1995). In more recent years, design has come to be considered

a more conscious activity, as 'the first step taken by researchers was to look into the process of design and build up control mechanisms over the design process' (Uluog-lu 2000, 34). Hence, systematic design methods were discussed and applied by designers. In this respect, debates on design seemed 'to change from product-orientedness to process-orientedness, and finally to cognitive processes of the designer based on design knowledge' (Uluog-lu 2000, 34).

In response to this approach, many studies in the last three decades of 20th century were devoted to the role of knowledge in the architectural design process (Uluog-lu 2000). The common factor observed in most of these studies was that the process of design requires the skillful activity of the body (hands) and intuitive feelings of the soul, as well as the rational activity of the mind (Schon 1981). In a study of architecture design, Uluoglu (2000) claims that, as designing is not simply an act of doing, it requires a controlled conduct of a general course made up of knowledge. Designing is not merely an activity based on certain skills, to be taught by instruction, and thus the inclination toward knowledge-based design processes has caught the attention of design education as well (Uluoglu 2000).

Despite all considerations given to knowledge-based process of design in architectural literature and education, the inadequate amount of knowledge applied in the practice of professional and educational design processes has been the focus of many criticisms in the last three decades (Salama 1995, Uluog-lu 2000). That is because, as Robinson (1986) cites, 'Educational institutions are apparently turning away from the pragmatic, rational approach, towards an approach based more in art than science' (67). Due to this current situation, a growing dissatisfaction with design education appears to be the main concern of researchers and academicians who have voiced the notion that the education of future architects should be knowledge-based (Banham 1981; Boyle 1977; Wolfe 1981; Juhasz 1981; Bowser 1983; Mayo 1985 & 1991; Ozkan 1986; Cuff 1991; Schon 1983, 1985 & 1988; Gutman 1968, 1987 & 1988; Gerlenter 1988; Dagenhart 1993; Watson 1993; Weber 1994 and many others). A review of the above studies demonstrates that most criticisms of design education are concerned with forming analogies between the artistic and instructor-centered approach to architecture in the current design studios, with the actual way of developing architecture in professional practice. The criticism claims that in the current educational system, instructors are not required to position themselves on what Stevens (1998) calls the continuum from workaday practice to activity in a sphere of symbolism. Instruction is therefore based on the preferred behavior of instructors and those who select them for the job (Stevens 1998). Hence upon entering the workforce, the graduates will be armed with their instructors' architectural interests and tastes, but unable to create by themselves. This criticism accentuates the point that, although architectural design is a broadly knowledge-based activity regarding all aspects of human life, the essence of architectural design education follows ready-made principles and rules developed in the past, and not equipped to confront future architects with the environmental needs of contemporary societies.

Analyzing this criticism, which considers design studio instruction, confirms that the current design studio is greatly influenced by the *Beaux-Arts* (Salama 1995) According to Balfour (1981), Beinart (1981), Bostick *& Pettena* (1985) and McCommons (1989 & 1994), studio instruction has essentially remained unchanged since the establishment of the *Beaux-Arts* approach. This approach has placed emphasis on the formal aspects of architecture with little concern for socio-cultural issues (Salama 1995). According to Papanek (1971), designers following this conventional approach are likely to distance themselves from the real world and real human problems, and so do not touch the depth of human experience and needs.

The strength and assurance of the *Beaux-Arts* approach was in leading toward the creation of architecture and design programs in many developing countries in the early 20th century (Van Zanten 1977, 115). Hence, all the numerous criticism that has questioned this educational model is relevant to all the programs that adopted the same system of design education. Iran, the focus of this article's empirical study, has also followed the *Beaux-Arts* pattern in architectural education. The years between the two world wars were crucial for Iran's architecture profession, as it witnessed a complete transformation in the organization of the profession. At that time many Iranian students went abroad to study architecture, and many to Paris. As a result, the *Beaux-Arts* model of architectural education came to be favored. The graduates who had experienced the *Beaux-Arts* system became the founders of academic education of architecture in Iran. As a result, the first schools of architecture in Tehran, like other Middle Eastern countries, focused on *Beaux-Arts* based curricula. (Etesam 2004, Bavar 2006)

The traditional master/apprentice *Beaux-Arts* model of studio instruction involves a heavy dependence on the instructor for decision-making and mere reliance on manipulation of formal configurations (Salama 1995). This traditional model encourages a studio environment that inhibits students' thinking capabilities and declines the

general level of design. It actually establishes a lack of knowledge, promoting students to look to the instructor for design ideas and wait for his or her approval before making design decisions—a custom found in most studio teaching practices worldwide (Salama 1995). This lack of knowledge, which was defined by Salama (1995) as one of the factors that affect and limit the capabilities of architects should be abolished from design education.

Ochner (2000) describes the traditional model of studio instruction as a setting through which some studio instructors pass along design solutions to their students in order to enable them to produce projects at a high degree of completeness that meet their expectations. Indeed, this is likely inevitable in any design studio to some extent, but too much can be destructive for the students's later professional career. Continuing this debate, Ochner (2000) asks a fundamental question that challenges the educational goal of the traditional model of studio instruction: 'The results in one studio may look good, but what of the students' development over time?' (Ochner 2000, 198). In such instruction, the students will always depend on an external source for making design decisions. Psychoanalyst Fred Pine (1990) has written:

> If one were stranded on a desert isle, it would probably be better to find there a set of tools than, say, a finished house. The house would indeed provide shelter …, but the tools could each be used flexibly in innumerable ways—including the building of a house. *(*20)

Design studios should provide students with tools to solve problems, not with products and solutions to specific challenges that are likely to change over the course of the students' careers. Reviewing different studies on architecture education indicates that the current model of teaching and learning is not used because it is the 'right way,' but because the method has worked for such a long time (Farrington 1999). This dissatisfaction has led many researchers to study the deeper aspects of design education (Ochner 2000). Hence, as a reaction to this situation, several revolutionary concepts have been developed by different design instructors in an attempt to respond to the new demands of the profession and the changing role of the architect in society. But as such struggles are still in the early stages, and because there are few studies on the instructional framework appropriate for design education, the *Beaux-Arts* approach is still current in design education all over the world (Salama 1995).
Many researchers and academicians have expressed a concern that the education of future architects must reinforce an independent ability of design decision-making (Schon 1983). The ability to support such decisions throughout the student's professional career could be acquired through conversion of the superficial type of learning to an in-depth learning that comes with endogenous knowledge construction (Etesam, 2008)). While the literature of architectural education may be silent on how such lifelong learning can be created, lessons can be drawn from the experience of educationalists in developing instructional models. This article is devoted to such an approach.

1.1.2. Lifelong learning through tacit knowledge acquisition

Professional knowledge acquisition and promoting lifelong professional learning are some of the most important aims defined for education in various studies (Facione & Facione 1994, OCED 1996, Tiwari, Lai, So & Yuen 2006). As Facione & Facione (1994) declare, such emphasis on lifelong learning is due to the 21st century's 'global, social, economic, educational and environmental challenges, which does not demand the teaching of soon-to-be obsolete facts' (1). Education has been faced with new challenges in the 21st century, including the knowledge concept, its management and the development of a workforce with the skills necessary to support professional careers (Grant, 2000). One such approach to this has been the development of lifelong learning policies (OCED, 1996). Lifelong learning, which is defined as 'continuous professional development' or CPD by Chisholm and Holifield (2004, 242), facilitates the efficacy of the working population by continuously up-skilling them forever. Such attitudes toward education can generate self-reliance among students, which is desirable in many fields of education, including architecture. Based on such studies one can argue that pedagogical efficacy is related to acquisition and promoting lifelong professional learning at all levels of education, and in architectural design education as well. Thus, how education reacts to and develops knowledge for lifelong learning is now a critical dimension to be addressed. Hence, the critical to ask is how professional knowledge acquisition can lead to lifelong learning. To answer this question, one should enter the domain of different types of ideas regarding knowledge acquisition.

Treatises and textbooks on knowledge management distinguish explicit knowledge from tacit knowledge as two major types (Polanyi 1996; Rumizen 2002). While explicit knowledge includes that knowledge which can be stated and is in that sense objective, tacit knowledge can not necessarily be explicitly stated or written down, and tends to be

more subjective. The distinction between tacit knowledge and explicit knowledge has been described in terms of `knowing-how' and 'knowing-that,' respectively (Ryle 1949/1984). Tacit knowledge includes judgment, experience, insights, rules of thumb, and intuition, and its retrieval depends upon motivation, attitudes, values, and the social context. Professionals and other experts generally perform their practice primarily on the basis of tacit knowledge. Thoughtful writing, group work on complex problems and interactions with multifaceted applications depends heavily on tacit knowledge. Chisholm and Holifield (2004) review the role of tacit knowledge in organizations with an emphasis on its role and importance for lifelong learning. The reason why tacit knowledge best enables lifelong learning lies in its nature, the personal knowledge resident within the mind, behavior and perceptions of individuals (Casonato & Harris 1999, Rumizen 2002) Based on all these facts, one could argue that if the knowledge acquired through the learning process becomes tacit knowledge, it may be retained in the learner's mind for life. Hence a preliminary answer to the question of the way through which professional knowledge acquisition may lead to lifelong learning would deal with the type of acquired knowledge. Thus, while tacit knowledge may be needed to facilitate the acquisition of skills, it can be argued that it no longer becomes necessary for the practice of those skills once the person becomes an expert in exercising them (Polanyi 1958 & 1974).

Current research in tacit knowledge is motivated by the acceptance that much of what underpins a successful career in an organization is directly associated with implicit knowledge and learning (Chisholm & Holifield 2004). Thus, tacit knowledge plays a major role in learning and teaching when complex problem solving is involved. Architectural design education is one of the complex domains of problem solving. As architecture is a science with roots in the field of art to a great extent, the kind of knowledge that its design process deals with is not entirely explicit. Architecture's relation to the world of art along with the skill-based character of design, reveal its relevance to the `knowing-how' domain of knowledge, thus architectural designing skills could be defined as tacit knowledge. In this respect, architectural design, which belongs to the `knowing how' domain of knowledge, is associated with an artist who designs and makes judgments without always explicitly reflecting on the principles of the rules involved. Of course as architectural design also relates to the field of science as well as art, the skills used by designers are based on consciously-accessible knowledge that can be articulated. It can be argued, however, that while this knowledge may be needed to facilitate the acquisition of skills, it no longer becomes necessary for the practice of those skills once the person becomes an expert in exercising them (Polanyi 1958 & 1974). Based on this discussion, the skills needed in the process of architectural design have their roots in the tacit domain of knowledge. Thus, the acquisition of tacit knowledge in architectural design education can be defined as pedagogical efficacy.

The question then arises as to which educational approaches are be able to promote the acquisition of tacit knowledge and lifelong learning. In order to move toward the answer, this paper will review the studies, although few, that have been conducted on this topic in the following section.

1.1.3. Constructivist Education as a key to Lifelong Learning and Tacit Knowledge Acquisition

Since little research has been completed on tacit knowledge production (Koskinen 2003), it is difficult to propose an exact response to the question seeking a proper educational approach that would be able to promote tacit knowledge and lifelong acquisition. This problem with tacit knowledge is essentially due to its implicit nature making it difficult to scientifically discuss and explain explicit knowledge. While the articulate manner of explicit knowledge makes it possible to be acquired through formal education, writings and books, tacit knowledge when transferred by sight, 'is either acquired through an "intimate" relationship between a "master" and an "apprentice," or through learned experience over time' (Busch 2004, 17).

Despite the dilemmas that exist over the acquisition of tacit knowledge, and despite the silence of educational literature, a profound scrutiny of the available text exposes some points about the implicitly recommended approaches to creating tacit knowledge. One such suggested approach is the study by Chisholm and Holifield (2004) regarding tacit knowledge and professional development. The authors, after reviewing the role of tacit knowledge in companies and its importance for lifelong learning, conclude by demonstrating that a work-based learning model is the most effective way to deliver lifelong learning supported by the emphasis on tacit knowledge acquisition. The work-based learning model can be associated with lifelong learning policies and knowledge construction by individuals. One of the educational approaches supporting the work-based learning model as defined in their study is constructivist educational theory.

Constructivism is based on the doctrine that learning takes place in contexts, and that learners form or construct much of their learning as a function

of their experiences in various situations (Schunk 2000). More recently, researchers (e.g. Lave 1990; Saxe, Guberman & Gearheart 1987) have presented more qualitative documentation of learning in context. Hence, this approach can be simulated to the work-based model of learning, which was previously introduced as a key to tacit knowledge and lifelong learning. This tendency of work-based learning and constructivist educational theory to provide tacit knowledge and lifelong learning has been expressed implicitly in other studies as well (von Krogh & Roos 1996, von Krogh, Ichijo & Nonaka 2000, Atherton 2002, Burns 2001, Chisholm 2002, Saint-Onge 1996). A review of the above literature on tacit knowledge may lead to the conclusion that constructivist educational theory is one of the key approaches to acquiring tacit knowledge and lifelong learning, as it is consequent in both educational and professional environments. Before coming to the specific focus of the paper, which addresses architecture education and acquisition of tacit design knowledge, an analytical discussion on constructivist educational theory in general is presented.

1.1.4. An Overview of Constructivism Theory

Previous discussions presented on tacit knowledge acquisition and lifelong learning imply that constructivist views of learning and education could help to achieve such knowledge. Constructivism, derived mainly from the works of Piaget (1970), Bruner (1962, 1979), Vygotsky (1962, 1978), and Papert (1980, 1983), is both a philosophical and psychological approach (Schunk 2000). Although the roots of constructivism are most often attributed to the work of Jean Piaget, constructivist doctrines emerged much earlier in history, as seen in the writings of Giambattista Vico, who declared in 1710 that 'the human mind can know only what the human mind has made' (von Glasersfeld 1995, 21). The meaning of constructivism varies according to perspective and position. Within educational contexts there are philosophical meanings of constructivism (Mathews 1998), as well as personal constructivism as described by Piaget (1967), social constructivism outlined by Vygtosky (1978), and radical constructivism advocated by von Glasersfeld (1995).
Educational constructivism is clearly defined through Piaget's focus on the active role of the individual in learning. Based on this focus, constructivist views assert that learning is the active process of constructing rather than passively acquiring knowledge. Hence, instruction is the process of supporting the knowledge constructed by the learners rather than the mere communication of previously-learned knowledge (Duffy & Cunningham 1996; Honebein, Duffy & Fishman 1993; Jonassen 1999). The Main features of constructivism as derived from the constructive-interpretive literature are presented in Table 1.

von Glasersfeld (1995) emphasizes the outcome of constructivism education and defines knowledge as having no alternative except construction in the mind of learners. Through this way of teaching, students gain a stronger grasp of complex ideas (Applefield, Huber & Moallem 2001). Hence, one of the merits of constructivism is that it will accentuate the goal of achieving depth of learning rather than breath of learning (Brooks & Brooks, 1993), which may reduce the probability of forgetting the learnt material. This is by itself an important educational goal. This learning approach, which 'involves learning with depth' (Applefield, Huber & Moallem 2001, 29), is also defined by Lock (1947) as the ability of mind to 'put together those ideas it has, and make new complex one' (65). Considering this and the fact that the information received by individuals is not convertible to knowledge until they understand what it is, one could argue that the new complex made through the constructivist view relates to the field of knowledge, not to the field of raw data and information (Busch 2004). Therefore, as heavy emphasis of traditional education on information and its recall causes inevitable results of quickly forgetting (Applefield, Huber & Moallem 2001), the constructivist focus on knowledge instead of information can be defined as another educational merit.

The great contribution of constructivism to education may be through the shift in emphasis from knowledge as a product to knowing as a process (Jonassen 1991). This property of constructivism is capable of causing a lasting and meaningful change in the structure of formal education.
In the final analysis, it becomes of great significance that the application of constructivism to instructional design has certain advantages, including acquisition of deeper levels of understanding, providing more meaningful learning outcomes through more meaningful learning contexts, more independent problem-solving capabilities, more flexibility in both design and instruction activities, and supporting the learners with an ability to apply their learning in non-academic contexts (Karagiorgi & Symeou 2005, Russell & Schneiderheinze 2005). Despite the merits cited for constructivism, the translation of constructivism into practice constitutes an important challenge for instructional designers (Karagiorgi & Symeou 2005). An overview of some efforts conducted to move towards pragmatic constructivism is presented below.

Table 1. Family Characteristics of Constructivist Instructional Design

1	**The process Is Recursive, Non-linear, and Sometimes Chaotic:** Development is recursive, iterative and also non-linear. There is no required beginning task that must be completed before all others. Some problems, improvements, or changes will only be discovered in the context of use. It Plan for false starts and redesigns as-well as revisions.
2	**Planning Is Organic, Developmental, Reflective, and Collaborative:** Begin with a vague plan and fill in the details as you progress. "Vision and strategic planning come later. Premature visions and planning can blind" (Fullan, 1993). Development should be collaborative. Vision may emerge over the process of development. It cannot be "established" at the beginning. "Today, ... If people don't have their own vision, all they can do is 'sign up' for someone else's." (Senge, 1990, 206-211)
3	**Objectives Emerge from Design and Development Work:** Objectives do not guide development. Instead, during the process of development, objectives emerge and gradually become clearer.
4	**General ID Experts Don't Exist:** General ID specialists, who can work with subject matter experts, are a myth. You must understand the "game' being played before you can help develop instruction.
5	**Instruction Emphasizes Learning in Meaningful Contexts:** The Goal Is Personal Understanding Within Meaningful Contexts. Standard direct-instruction approaches that focus on teaching content outside a meaningful context often result in "inert' knowledge. The instructional emphasis should be on developing understanding in context. This approach favors instructional approaches that pose problems and provide students with access to knowledge needed to solve the problems.
6	**Formative Evaluation Is Critical:** Invest the most assessment effort in the formative evaluations because they are the ones that provide feedback you can use to improve the product. Summative evaluation are not useful.
7	**Subjective Data May Be the Most Valuable** : Many important goals and objectives cannot be adequately assessed with multiple choice exams, and exclusive reliance on such measures often limits the vision and value of instruction. during the instructional design process, there are many points where informal or qualitative approaches, such as interviews, observations, user logs, focus groups, expert critiques, and verbal student feedback, can be much more valuable than a data from a 10 item, questionnaire.

1.1.5. Instructional Models for Constructivist Education

Despite the theories presented by constructivist experts such as Duffy, Lowyck & Jonassen (1993), Jonassen (1999), and Wilson (1996), few studies have implemented constructivist strategies and confirmed their effectiveness. The reason for this is that most instructional designers do not unconditionally embrace this new epistemology, as there are many areas of conflict (Karagiorgi & Symeou 2005). Educational models subscribing to constructivism have described its impact on instructional design in general (Lebow 1993; Ertmer & Newby 1993). Most such generally proposed constructivist instructional models do not prescribe a design procedure or elaborate on the precise teaching style of each task. Instead they describe the design process in terms of general principles and guidelines. Some of these models include chaos theory (You 1994); recursive and reflective design and development, or R2D2 (Willis 1995), layers of negotiation (Cennamo & Chung 1996); the 5E model—engage, explore, explain, elaborate, and evaluate (Bybee 1997), initiating – constructing – utilizing (Stephens & Brown 2000); and the 7E Model—elicit, engage, explore, explain, elaborate, evaluate and extend (Eisenkraft 2003).

In addition to these general models, some important attempts toward defining the relationship between theory and practice have been made as well. As the constructivist approach is entirely reliable on an investigative approach, its main beliefs are expressed through investigative activities, cooperative learning and a variety of hands-on experiments (Applefield, Huber & Moallem 2001). In addition to positive outcomes of constructivism in science (Neale, Smith, & Johnson 1990), similar successes have been reported for this approach in reading, writing and language arts instruction (Duffy & Roehler 1986, Bereiter & Scardamalia 1987). Russell and Schneiderheinze (2005) conduct a study on the theoretical and practical applications of constructivist learning principles in the United States. The process was an online collaboration aiming to develop higher-order thinking responses among students. For the purposes of this study, the researchers identify important concepts based on a constructivist theoretical framework that contributes to the design of a constructivist instruction. The concepts used in this study include ‘scaffolding and mediation (Vygotsky, 1986; Wertsch, 1998), goal-directed, meaningful context and inquiry (Lave & Wenger, 1991), and collaboration (Salomon, 1993)’ (Russell and Schneiderheinze 2005, 9). The potential

and limitations of translating constructivism into instructional design are discussed in another study by Karagiorgi and Symeou (2005). In this study, use of cognitive and technology tools is recommended to develop constructivist methods.

An example of how tools can develop practical constructivism is presented in a model by Jonassen (1999) aimed at designing constructivist learning environments. Through the model, learners are aided in understanding the problems and finding solutions through related information resources. Recommended tools in this model include 'task representation tools (visualisation tools), static and dynamic knowledge modeling tools, performance support tools and information-gathering tools. In addition, conversation and collaboration tools are helpful to construct meaning of the problem' (Karagiorgi & Symeou 2005, 23). Hence, it can be argued in general that technology-related environments, in addition to virtual reality and real world simulations, are alternatives to making constructivist approaches more practical by providing more learner-centered opportunities and by offering multiple representations of reality (Wilson 1997, Mergel 1998, Cey 2001, Karagiorgi and Symeou 2005). Despite the general recommendations cited in the reviewed literature regarding constructivist practical instruction, no specific constructivist instructional model exists that is able to fit all fields of education. Therefore it is necessary for specialist educators in each individual field of education to develop specific proper models of their own, according to general guidelines of constructivist educational theory. To achieve such a goal in architectural design education, the few accomplished studies relevant to this field will be reviewed in the following section.

1.1.6. Implications for Constructivist Architectural Design Education

As discussed in the previous section, a number of theorists have approached the ways in which constructivist values influence instructional design, and have proposed several generalized principles of the constructivist instructional model (see for example Lebow, 1993; Jonassen, 1994; Willis, 1995). However a review of the relevant literature reveals little research on how general constructivist principles impact particular educational fields such as architectural design. However, in recent years, some efforts have been conducted in the field of design education based on the constructivist learning approach. Most of the studies in this field relate to the recent developments in information and communication technologies. In a study by Al-Ali (2007), some VDS (Virtual Design Studio) pedagogical techniques, with debates on their relevance to constructivism, are proposed. Some of these techniques cited by Al-Ali (2007) include project-based learning, group discussion, critical thinking and simulated real-world imitation. In a study by Gul, Gu and Williams (2008), 3D virtual world technologies, which support synchronized design communication and real-time 3D modeling' (1), are introduced to make a significant contribution to design education as a constructivist learning environment. This study discusses how some virtual computer-based programs facilitate constructivist learning by providing the affordances of modeling, communication and computational features of 3D virtual worlds. In another study regarding constructivist architecture education, Wang (2009) seeks to explain how the expanded use of computer-aided design in the professional education of architects is related to constructivist education. Although the study holds the promise of introducing a constructivist model of architecture education, it was unable to fill the large gap between constructivism theoretical potential for education and its actual performance in a design studio, primarily due to its focuses on ICT techniques as the only methods of constructivist education.

In addition to the reviewed studies that support technology-related tools as a potential to facilitate constructivist learning activities, a review of the basis of computer-based education, which appears through Anderson's cognitive theory (Anderson 1983 & 1976), reveals similar relationships between computer-based education and constructivism. Anderson's theory for computer-based education includes the following principals:

'1. Identifying the goal structure of the problem space,
2. Providing instruction in the context of problem-solving,
3. Providing immediate feedback on errors,
4. Minimizing working memory load,
5. Adjusting the "grain size" of instruction with learning to account for the knowledge compilation process, and
6. Enabling the student to approach the target skill by successive approximation' (Anderson 1983 & 1976, in Gül et al. 2008, 580).

While the first three principles defined by Anderson (1983, 1976) express the merits of computer-based education, the last three stand precisely on the principles cited for constructivist learning and education. In the fourth approach, which relies on an understanding of how students interact with knowledge, the assumption is that knowledge is constructed by the students themselves,

not delivered by the instructor (Winn, 1993). This approach is the same as accepted constructivism theory, as in the constructivist view, knowledge is constructed, not transmitted, and the students actively learn (Jonassen, 1999). In the fifth and sixth approach, it is expected that students are given the opportunity for exploration and manipulation within the environment, as well as opportunities for discourse between them to enhance learning (Dickey, 2007). In learning as a constructivist activity, as well, the role of the teacher is only 'to help and guide the student in the conceptual organization of certain areas of experience' (Glasersfeld 1983, 23).

Based on the above discussion, it can be argued that virtual models offer constructivist learning environments and can enhance learning by providing opportunities for exploration, facing students with the consequences of their design decisions in a real context, and providing opportunities for more critical discourse between students and teachers based on the designed spaces now visible thanks to 3D modeling tools. Hence, the use of 3D modeling and computer-aided design can be employed as a design teaching approach, which includes the facilitation of constructivist learning and tacit knowledge acquisition as its subsequent.

1.1.7. Constructivist Design Process and its Relevance to Computer-Aided Design

Before arriving at the empirical section of the study, it is necessary to achieve a practical framework for architectural design education based on constructivist educational theory. For the purposes of this article, reference is made to the relevance of computer-aided design in constructivism in terms of the three major stages of architecture design process, as defined by Lang (1987)—the information gathering stage (preparation for design), the design development stage (establishing the design solutions), and the evaluation stage (choosing). These three dimensions are used here as poles for further discussion.

1.1.7.1. Stage 1: Information Gathering (preparation for design)

Throughout this phase of the design process, students gather an abundance of information relevant to different aspects of the design problem. In the constructivist approach and in the content of required information, this stage cannot be pre-specified (Karagiorgi & Symeou 2005, Gül et al. 2008). Constructivist instruction of design avoids the breakdown of context into component parts, as traditional design instruction does, and is instead in favor of environments in which design knowledge and solution can emerge naturally. Designers (here, students) in this stage distinguish between various needs and requirements of the given design problem, which their proposing design solution aims to fulfill. As design problems have no absolute solution, the task in this stage is one of providing a rich context within which specific objectives of understanding the environment, for proposing the best design solution, can emerge (Al-Ali 2007). The goal, for instance, is not to gather information on different forms of traditional architecture to be imitated, but to make students understand the context and environment in which a specific form of architecture has emerged, and the requirements it must have fulfilled.

To achieve such goals, designers (here, students) refer to different sources in order to understand concepts important to the design of the problem. This in-depth research enables students to identify design tasks, clients, and legal constraints. As students work to develop the requirements of a design problem, the teacher helps the students by providing them with the opportunity to adapt the acquired information to their needs, to make choices with which to direct their learning, and to construct their own understanding of the information. This constructivist approach aims to help students develop useful knowledge rather than inert knowledge (Russell & Schneiderheinze 2005, Al-Ali 2007). In this stage of the design process, students require access to information such as text documents, videos, sound files and graphics to begin formulating meaning about the problem, as well as related cases to represent the complexity of the problem from multiple perspectives. The teacher can help to establish the meaningful context by providing students with opportunities to gather information and question the relevance of that information to their community and the problem. Consequently, the teacher can provide opportunities for students to analyze case studies (Shulman 1992) about other projects related to design problem-solving in order to enrich the context for students to apply expertise and identify interrelationships among those areas of expertise.

The application of computer-aided technologies into this stage of the design process offers significant potential for design schools, through their capacity of advancing research and development, to prepare students for designing in the next stage of the design process. Computer-aided design tools also support the so-called library-based design method which comprises a set of objects, materials, textures and light sources provided by the object library of the design platform (Gül et al. 2008). Based on the above discussion, technology application can extract more meaning from the design problem and can be helpful in supporting the research

in the design studio. It is further able to foster the development of a design solution in the following step of the design process.

1.1.7.2. Stage 2 + Stage 3: Design Development + Evaluation

Based on literature reviewed above regarding architectural design education (see for example Salama 1995), it could be cited that in the traditional instruction of design, which is teacher-centered and teacher-directed, the stage of design development stands completely apart from the stage of design evaluation. This is primarily due to the fact that these two tasks are expected to be carried out by separate individuals—the role of te students is only to propose alternatives and design solutions, while that of the instructor is as the main center of instruction, with the role in evaluating and judging the students' designs. But these two stages, in the constructivist approach of design education, are interwoven. Since constructivists points to student-centered, student-directed and collaborative environments based on inter*active* learning, both stages should be accomplished through students' self-relied activities. Students may evaluate their design solutions in terms of whether they do what they claim to do (Spiro et al. 1991b). The students' ability to promote insight into alternative perspectives is an important element of evaluation, and is related to the development of their critical thinking skills and self-reflective processes (Karagiorgi & Symeou 2005). Such a learning environment requires an abundance of tools to confront students with opportunities to experience the critical thinking inherent in design education. Computer-aided design tools and 3D-modeling tools can be helpful in providing such an environment (Al-Ali 2007). As computer-aided design tools can support different viewpoints, such as first-person and third-person, they offer many possibilities for understanding the spatial arrangement of the objects and developing the student's spatial abilities (Gül et al. 2008). Thus, to modify a design, the students are able to rely heavily on their own judgment of the finished proposed design, which is visible now through the aids of technology.

From these studies it is clear that designing with the help of 3D virtual worlds encourages immediate and detailed design decisions for students. While they decide on a particular concept or design alternative, both its construction and testing occurs simultaneously. Hence this method has the potential to facilitate self-reliance among students in the design process, due to the faact that computer-aided design tools allow learners to develop, compare, and understand multiple perspectives of an issue with the goal of achieving the rigorous process of reflective thinking, multiple perspectives, developing and evaluating the arguments by self-mentoring to guide learning (Bednar et al., 1992, Gül et al. 2008). As a result, students are capable of experiencing the evaluation stage of the design process along with the development of design. By applying computer-aided design tools, teachers can plan a constructivist instruction in architectural design education that goes beyond routine learning toward meaningful learning that is more likely to lead to deeper and longer-lasting understandings.

1.2. Empirical Study

According to the discourses presented in the theoretical section of this article, it is assumed that the application of constructivist educational theory in architectural design instruction, which deals with skills and knowledge of the 'how' questions (Busch 2004), can lead to tacit knowledge acquisition. If so, we can, and should, expect the studio to be an environment in which such knowledge is transferred to students in various ways.

As discussed above, lifelong learning is one of the merits of focusing on tacit knowledge acquisition. Based on this property of tacit knowledge, the length of time that the acquired knowledge is retained in the mind is considered one of the indicators of tacit knowledge existence

2. Material and Methods

This section examines the students' tacit knowledge acquisition and the effect of applying constructivism educational theory in architectural design instruction. As discussed above, tacit knowledge is closely related to the learner's potential for retaining the learnt knowledge over a long period of time. The more tacit knowledge one acquires through the learning process, the longer he or she is likely to retain it. The project was an experimental research study based on an analysis of students' design performance in accordance with the studio instruction in 'priori and posteriori' stages (Groat & Wang 2002). The goal was to gain a multilayered outcome of the students' designs, as they were treated with constructivist instruction in the design studio.

In this study the experimental design with pre- and post-tests is applied. The sample was comprised of second-year architecture students from the Department of Architecture at Islamic Azad University - Kerman Branch, in the second semester of the 2009-2010 academic year. They had taken the architectural design course, dedicated to the design of a one-family residential house in a pre-determined site. The size of the sample group was 32 students; 9 male and 23 female. Sixteen students were considered the control group, and 16 students were

considered the experimental group. In order to reduce the impact of different variants in the instruction model, the two groups were instructed by the same teacher. In addition, to reduce the impact of varied learning potentials among the students, the students of each group were selected in equal ratios based on their previous grades. The experimental group encountered the constructivist approach during the instructional design process, while the control group was instructed using the traditional method of direct instruction. Ultimately, acquisition of tacit knowledge in both groups was measured by the students' capability to retain the learnt knowledge of design after nine months. The second semester of the academic year in Iran begins in February and finishes in June, and the following semester begins after a three-month summer vacation. The project spanned a nine-month period. The long break between the two semesters provided an opportunity to check the students' capability of retaining knowledge. In order to maintain the natural condition of the two design studios, and to avoid role playing, the students were not aware of the goals behind this difference in their instruction. At the beginning of the semester, the basic design knowledge of all 32 students was determined by asking them to take part in designing a one-family residential flat for 4 family members in a suburban site of about 100 square meters in the span of eight hours. The site presented to the students was a real-world existing site that students can walk through to explore the site's real limitations and potential. One of the problems of the site was that the carpentry workshop was located on the north side with an abundance of disturbing sounds. Each student's sketch was kept for further comparison and analysis. This exercise is called "sketch number one" in the following discussions. After this stage, the students were divided into experimental and control groups. The 16 students who comprised the experimental group encountered the constructivism method during their instructional process through the three-month semester. The other group, as the control, was instructed via methods other than constructivism. At the end of the term, both groups had arrived to satisfactory results; hence all students passed the architectural design course one. An example of the way through which the students in each group were instructed regarding one special aspect of residential design is presented below.

Example 1- Unsuitable Space Adjacency: not considering sound disturbing zones in a site

It was obvious that because of the noise condition, the north side of the site was not a proper location for resting zones, for example, bedrooms, of a residential unit. Some of the students in both the control group and the experimental group, however, did not pay attention to this aspect at the beginning of the design process, and placed the bedrooms in this zone. In the instructional process, the instructor behaved in two different ways regarding the students of the experimental and control group. The designs of the control group members, confronted with the traditional direct instruction, were corrected directly by the instructor; they were told that the design was not adequate, and that "the bedrooms should be placed in this quiet zone, on the south side." But the experimental group students were just asked to go to the site and try to rest, study or spend time in the specific location they had proposed for the bedrooms. They were asked to record the sounds and put the recorded sound to the 3D modeling simulation they had created of the proposed bedroom space of their designs. They were then asked to show this simulation with the relevant sound on, to other students, friends, relatives and others to observe their responses to such an environment.

In this example, while the control group was confronted with explicit, codified knowledge by being told that 'the bedrooms should be placed in a quiet zone,' the experimental group was confronted with another method.

The experimental students succeeded in experiencing the impact of placing the bedrooms in an unsuitable location and had constructed the above rule by themselves. They did not receive an explicit kind of knowledge about this subject, but instead acquired the experience and skill from reliance on their own abilities and observations. Three months later, after the summer vacation, all 32 students began the following semester and took the second architectural design course. During the first week of the new semester, the 32 students were asked to take part in "sketch number two," an eight-hour design struggle with the same subject as "sketch number one." In the second sketch exercise, however, the site location of the desired residential house and its number of family members differed. In this sketch a carpentry workshop was located at the eastern side of the new site, and a freeway was located at the southern edge. The natural lightening potential of the site was also limited to the east and south, preventing windows in the west and north. They were asked to use natural light for all spaces. Each student's sketch was collected to be compared with his or her first sketch. An example of this stage is presented below.

Example 2- Unsuitable Space Adjacency: not considering sound disturbing zones in a site

The students of the control group did not place the bedrooms next to the carpentry, but instead placed them on the southern edge next to the freeway in

order to use the light from the south. Because of their prior experience in being prohibited from placing bedrooms next to the carpentry workshop, they reclined the disturbing sound of the freeway instead (for example Student C.8). Some of them also placed the bedrooms in the north or west sides of the site ignoring the criterion of benefiting from light. Students of the experimental group avoided disturbing sounds from both the freeway and the carpentry workshop, as they had already experienced the simulated space with harmful sounds. As they were obliged to use natural light under these conditions, most came to new solutions. Student E.11, for example, placed an internal yard in the residential unit to solve the light problem, and then placed the bedrooms on the west and north of the site.

After preparing this data, a comparative analysis between each student's two sketches, over a nine-month period, was conducted in order to detect the effect of using constructivism educational theory in design education for creating tacit knowledge. Emerging improvement between the two sketches for those who encountered the constructivist educational method demonstrates that the student was able to retain the learnt knowledge for a long period of time, and created a kind of tacit knowledge. If the location of the student's bedrooms remained unchanged in their design capability, it would prove that the constructivist educational method had no effect on students' tacit knowledge acquisition.

3. Results

The analysis of the results began with an in-depth analysis of each student's sketches. At this stage, an initial list of the most repeated mistakes was compiled. After reviewing the initial list of mistakes and their relationships, connections, similarities and differences, 14 categories of major mistakes emerged. Tables 2, 3 and 4 contain a summary of the categories of mistakes. The most important mistakes that the students committed while designing sketch number one are listed in Table 2. The data provided here shows that the majority of students made basic mistakes in designing the sketch.

Table 2. The most important design mistakes committed by students in sketch number one

students' mistakes in sketch No.1	Number Total 32	percent	C:16 N:16	Number	percent
Unsuitable orientation	31	96.8%	E	16	100%
			C	15	93.75%
Wrong circulation	28	87.5%	C	15	93.75%
			N	13	81.25%
Unacceptable lightening	26	81.2%	C	14	87. 5%
			N	12	75%
Unsuitable space adjacency	32	100%	C	16	100%
			N	16	100%
Poor organization of spaces	32	100%	C	16	100%
			N	16	100%
Poor Approach to the building	32	100%	C	16	100%
			N	16	100%
Functional problems	29	90.6%	C	15	93.75%
			N	14	87. 5%
Aesthetic ignorance	32	100%	C	16	50%
			N	16	100%
Poor composition of form	31	96.8%	C	16	100%
			N	15	93.75%
Unsuitable standards	32	100%	C	16	100%
			N	16	100%
Unsuitable spatial relations	32	100%	C	16	100%
			N	16	100%
Unsuitable separation of Private and public zones	28	87.5%	C	14	87. 5%
			N	14	87. 5%
Poor interior design	32	100%	C	16	100%
			N	16	100%
Poor providing of views	30	93.7%	C	16	100%
			N	14	87. 5%

Table 3. The most important design mistakes committed by students through their final project

students' mistakes in final project	Number Total 32	percent	C:16 N:16	Number	percent
Unsuitable orientation	1	3.12%	C	0	0
			N	1	6.25%
Wrong circulation	0	0	C	0	0
			N	0	0
Unacceptable lightening	2	6.25%	C	1	6.25%
			N	1	6.25%
Unsuitable space adjacency	1	3.12%	C	0	0
			N	1	6.25%
Poor organization of spaces	3	9.36%	C	1	6.25%
			N	2	12.5%
Poor Approach to the building	6	18.75%	C	2	12.5%
			N	4	25%
Functional problems	2	6.25%	C	0	0
			N	2	12.5%
Aesthetic ignorance	17	53.1%	C	8	50%
			N	9	56.25%
Poor composition of form	10	31.2%	C	4	25%
			N	6	37.5%
Unsuitable standards	0	0	C	0	0
			N	0	0
Unsuitable spatial relations	1	3.12%	C	1	6.25%
			N	0	0
Unsuitable separation of Private and public zones	2	6.25%	C	0	0
			N	2	12.5%
Poor interior design	23	71.8%	C	11	68.75%
			N	12	75%
Poor providing of views	8	25%	C	3	18.75%
			N	5	31.25%

Table 4. The most important design mistakes committed by students in sketch number one

Code	students' mistakes in sketch No.2	Number Total 32	Percent In whole	C:16 N:16	Number	Percent In each group
A	Unsuitable orientation	15	46.87%	C	2	12.5%
				N	13	81.25%
B	Wrong circulation	14	43.75%	C	3	18.75%
				N	11	68.75%
C	Unacceptable lightening	14	43.75%	C	4	25%
				N	10	62.5%
D	Unsuitable space adjacency	15	46.87%	C	2	12.5%
				N	13	81.25%
E	Poor organization of spaces	19	59.37%	C	5	31.25%
				N	14	87.5%
F	Poor Approach to the building	13	40.62%	C	2	12.5%
				N	11	68.75%
G	Functional problems	10	31.25%	C	0	0%
				N	10	62.5%
H	Aesthetic ignorance	20	62.5%	C	6	37.5%
				N	14	87.5%
I	Poor composition of form	17	53.12%	C	4	25%
				N	13	81.25%
J	Unsuitable standards	13	40.62%	C	1	6.25%

				N	12	75%
K	Unsuitable spatial relations	16	50%	C	2	12.5%
				N	14	87.5%
L	Unsuitable separation of Private and public zones	13	40.62%	C	0	0%
				N	13	81.25%
M	Poor interior design	20	62.5%	C	5	31.25%
				N	15	93.75%
N	Poor providing of views	19	59.37%	C	4	25%
				N	**15**	**93.75%**

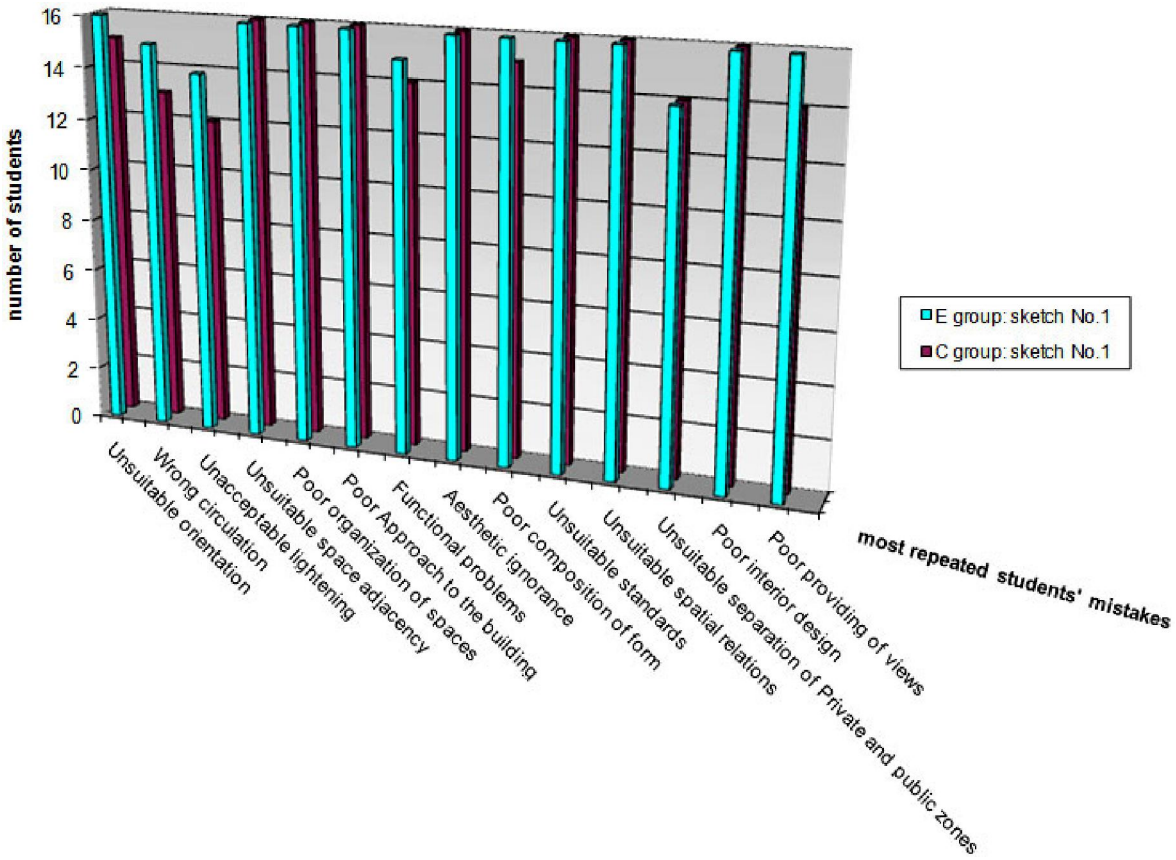

Figure 1. Comparison between experimental and control group in sketch number one

4. Discussions

The findings indicate that the application of constructivist educational theory in architectural design instruction can lead to acquisition of tacit knowledge. The rate of students' design mistakes in the experimental group decreased meaningfully between two similar designing exams taken at the beginning and at the end of a nine-month period.

The students' knowledge that was required through constructivist educational instruction was retained for a longer time than the knowledge that was obtained through other educational strategies.

The experimental group's mistakes decreased from 93.75 percent in sketch number one, to 17.85 percent in sketch number two. Such a high reduction in similar mistakes indicates that these students retained knowledge for a longer period of time. The length of time that a learner can retain learnt knowledge is one of the exterior indicators of tacit knowledge acquisition that can be inspected.

Meanwhile the control group's mistakes decreased only from 93.37 percent in sketch number one, to 79.4 percent in sketch number two. Such a low reduction in repeating mistakes among the control group indicates that the students did not retain knowledge for long, meaning that the acquisition of tacit knowledge through instructional methods other than constructivist methods did not occur meaningfully.

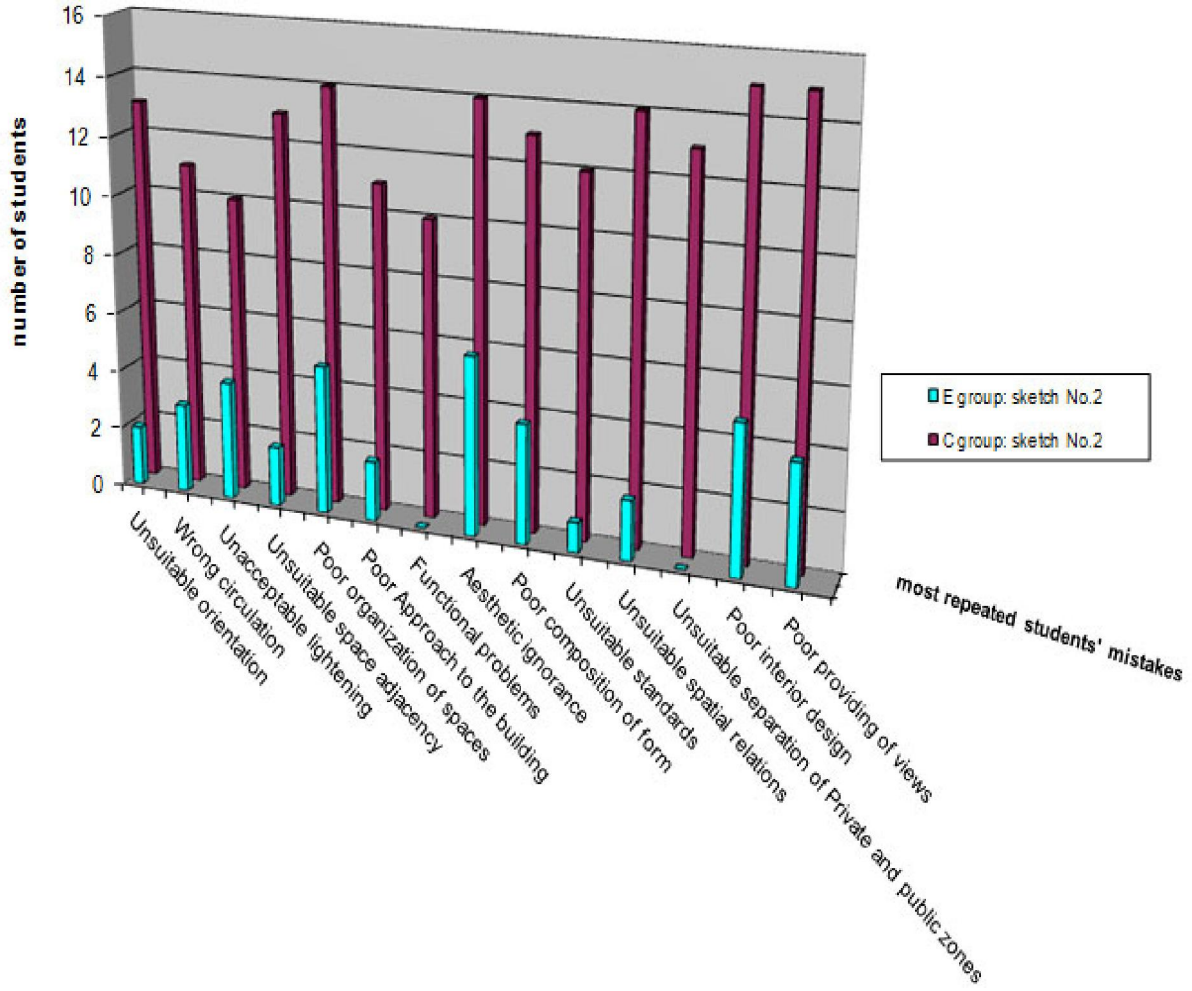

Figure 2. Comparison between experimental and control group in sketch number two

Figure 4 shows that the control group made little progress and the rate of mistakes decreased by only about 14 percent. This reduction is due to the result of instructional strategies other than constructivist theory. This effect is negligible when compared to the effect of constructivist strategy, for which mistakes declined about 76 percent. The findings indicate that the effect of constructivist instruction in design education is 5.4 times stronger than more traditional strategies.

In the present study, a significant relationship was found between application of constructivism educational theory and students' tacit knowledge acquisition, which ensured the ability to retain knowledge over a long period of time. The capability of architecture students to retain academic knowledge in their future professional careers as architects is an educational desire, confirmed by many researchers. Salama, in a number of studies on architecture education (1995, 1998 & 2005), argues about the inefficiency of the mechanistic orientation of pedagogy typically used in architecture schools today.

The student is evaluated with respect to his/her ability to reproduce what he/she has been told or shown. In turn, examinations are tests of the ability to reproduce material previously presented (Salama 2005, 3).

Students do not retain or internalize the knowledge made by mechanical orientation in their future professional careers. Because this orientation in pedagogy does not serve students with opportunities to construct and explore the required knowledge of design for their own work, instead of receiving it as readymade 'body of knowledge.' (Salama 2005, 9). The systemic pedagogical orientation, recommended by Salama (2005) for shaping students' future professional careers, resembles the results achieved from the constructivist instruction with the experimental group.

Based on an analysis of a number of studies (Anthony 1991; Boyer and Mitgang 1996; Cuff 1991; Koch et al 2002; Sanoff 2003; Schon 1981, 1983, 1985, & 1988; Stamp 1994; Teymur 1996), contemporary methods of design education suggest that gaps exist between knowing 'that' and knowing 'why and how' during the act of designing. While the students are only the receivers of knowledge, not constructors or explorers, they know the 'that' of the cases, but if they take part in knowledge construction and exploration, they may arrive the realm of knowing "why and how," which is more long-lasting. Carroll et al. (2010) cite a design instructor's point of view about the design studio's instruction philosophy, which is reminiscent of constructivist instruction.

So any kinds of projects that come in and talk about, 'We're not just going to think about a problem, but we're going to think about how to think about a problem,' is huge. (Carroll et al. 2010, 47).

On the same line, Peter Rowe (1987) refers to professional design education by introducing the concept of actionable knowledge, which is defined as knowing'that' and 'how' intertwined. Actionable knowledge cannot be simply a matter of theory and practice, but a different kind of knowledge upon which professional design education must be fundamentally focused (Schon 1983). Such pedagogy, which results in long retention of the acquired knowledge, is also in accordance with the results achieved from the present empirical study with an application of constructivist educational strategy in design education.

4.2. Limitations

This study featured a small design instructor to student ratio that does not reflect all design studios. This was due to the special character of constructivist instructional strategy which takes a much longer time than traditional direct studio instruction typically used. The definition of tacit knowledge in the present study was based on one of the exterior properties of this kind of knowledge, which is its permanence in time. This property had the capability of being examined after passing a considerable amount of time. This was due to the fact that it is not easy to monitor transfer of knowledge, which may be explicitly recognized only *post facto* at a later stage in one's development. Thus, students' ability to retain the learnt knowledge over a length of time was chosen as an indicator to show that the knowledge had been transformed to tacit knowledge.

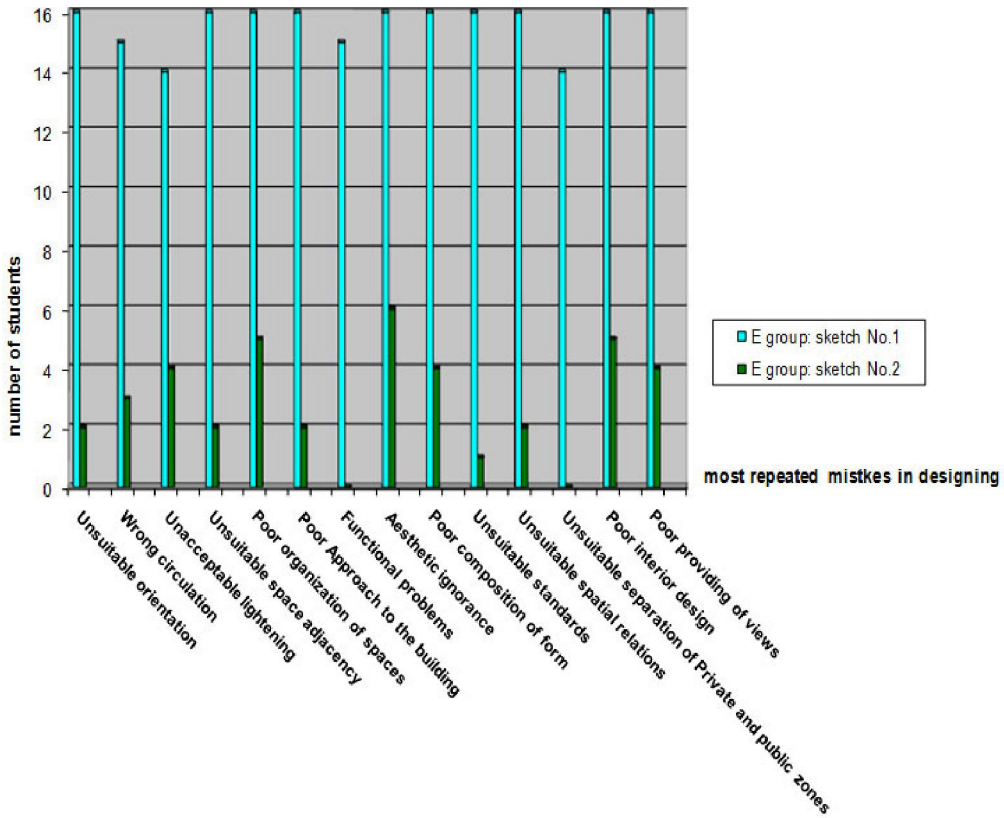

Figure 3: Comparison of students' mistake repeats between sketch number one and number two in experimental group.

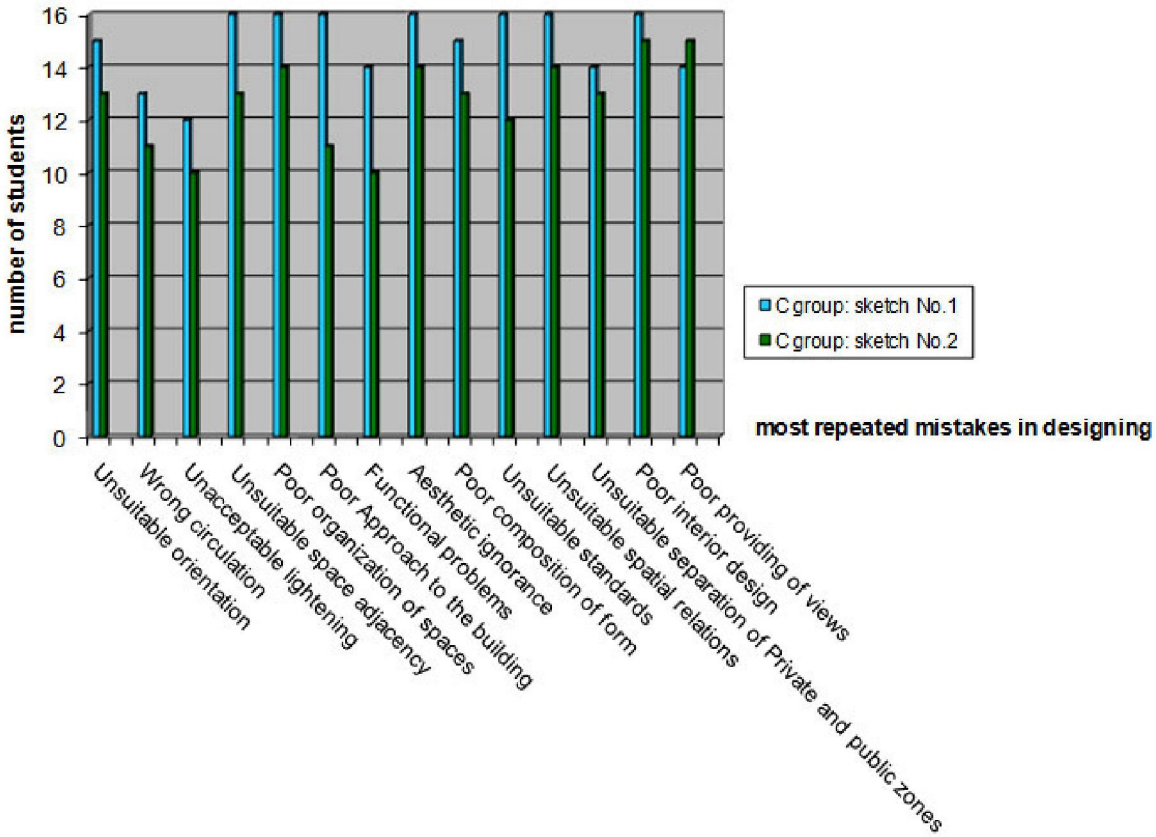

Figure 4. Comparison of students' mistake repeats between sketch number one and number two in control group

4.3. Conclusions

In this study, constructivist education became part of the design studio learning environment in diverse ways. The findings indicated that the application of constructivist educational theory in architectural design instruction can lead to tacit knowledge acquisition. A direct relationship was noticed between this educational theory and students' ability to retain knowledge over time. This capability, defined as tacit knowledge acquisition in this study, leads to the ability of future architects to make independent decisions. In the final analysis, the summary of constructivist methodology for design education in the light of the reviewed studies in this article, which could be accomplished in favor of a computer-aided design studio, is reviewed below.

Step 1:

1- Define real-world problems and support the essential research to redefine the design problem
2- Guide students to gather the required information relevant to the design problem
3- Establish critical arguments regarding the design problem
4- Familiarize students with the different aspects of the design context and environment

Step 2:

1- Allow learners to develop, compare, and understand multiple perspectives on an issue
2- Emphasize knowledge construction and not reproduction during the design process
3- Emphasize problem-solving, exploration, critical thinking skills and deep understanding in knowledge construction
4- Display 3D modeling of the proposed design

Step 3:

1- Direct students to self-criticize and self-mentor their design
2- Synchronize the design phase with the evaluation phase of the design process, as assessment is authentic and interwoven with teaching in the constructivist view.

The application of constructivist educational methodology in design education transfers the responsibility for design decisions from the instructors to the students, thus improving the critical

thinking skills of the students and enabling them to gain confidence in their decision-making capacity. Constructivist instruction may help students become empowered agents in their own learning who possess both the tools and the confidence to change the world.

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References

1. AL-ALI, A.I., (2007) Readiness for the use of Technology for effective learning via the VDS: Case of the United Arab Emirates. 3rd Int'l ASCAAD Conference on Embodying Virtual Architecture: ASCAAD-07, Alexandria, Egypt, SECTION VIII: CAAD Curriculum
2. Anderson J. (1976) Language, memory and thought, Hillsdale, NJ, Erlbaum Associates.
3. Anderson J. (1983) The architecture of cognition, Cambridge, MA, Harvard University Press.
4. Anthony, K. (1991) Design Juries on Trial: The Renaissance of the Design Studio. New York: Van Nostrand Reinhold.
5. Applefield, J.M., Huber, R. & Moallem, M. (2001) Constructivism in Theory and Practice: toward a better understanding, The High School Journal, Vol.84: No.2, pp.35-53.
6. Atherton J. S., (2002) Learning and Teaching: Tacit Knowledge and Implicit Learning, Available at: http://www.dmu.ac.uk/"-jamesa/learning/tacit.htm (accessed 11 March 2008)
7. Balfour, L. (1981), Education and Practice in Architecture, Architecture Education Study, Consortium of East Coast Schools of Architecture.
8. Banham, R. (1980) Theory and Design in the First Machine Age. Cambridge, MA: MIT Press.
9. Bavar, C. (2009) The Advent of New Architecture in IRAN, FAZA Publication: IRAN, Tehran.
10. Bednar, A. K., Cunningham, D., Duffy, T. M., & Perry, J. D. (1992). Theory into practice: How do we link? In Duffy, T. M. & Jonassen, D. H. (Eds.), Constructivism and the technology of instruction: a conversation, Hillsdale: Lawrence Erlbaum Associates, pp. 17-34.
11. Beinart, J. (1981) Structure of the Content of Design, Architecture Education Study, Consortium of East Coast Schools of Architecture.
12. Belkis, U. (2000) Design Knowledge Communicated in Studio Critiques, Design Studies, Vol.21, No.1, pp 33-58.
13. Bereiter, C. & Scardamilia, M. (1987) Cognitive coping strategies and problems of "inert" knowledge. In S. Chipman, J. Segal & R. Glaser (Eds.), Thinking and learning skills. Hillsdale, NY: Erlbaum.
14. Bose, M., Pennypacker,E. & Yahner,T. (2006) ENHANCING CRITICAL THINKING THROUGH 'INDEPENDENT DESIGN DECISION MAKING' IN THE STUDIO, Open House International – Design Studio Teaching Practices: Between Traditional, Revolutionary, and Virtual, Vol.31, No.3, pp. 33-42.
15. Bostick, J. & Pettena, G. (1985) Architectural Teaching in USA, Firenze: La Casa Esherc.
16. Bowser, W. (1983) Reforming Design Education, Journal of Architectural Education, Vol.37, No.2.
17. Boyer, E. & Mitgang, L. (1996) Building Community: A New Future for Architecture Education and Practice. Princeton NJ: Carnegie Foundation for the Advancement of Teaching.
18. Boyle, B. M. (1977) Architectural Practice in America, 1865 – 1965: Ideal and Reality. In S. Kostof (Ed.), The Architect, New York: Oxford University Press.
19. Brooks, J. G. & Brooks, M. G. (1993) In Search of Understanding: The Case for Constructivist Classrooms. Alexandria: Association for Supervision of Curriculum and Development.
20. Broadbent, G. (1973) Design in architecture, London: John Wiley & Sons.
21. Bruner, J. S. (1962) On knowing: essays for the left hand, Cambridge, MA: Belknap Press of Harvard University Press.
22. Bruner, J. S. (1979) On knowing: essays for the left hand (Expanded ed.), Cambridge, MA: Belknap Press of Harvard University Press.
23. Burns G. R. (2001) Work-based Learning and Manufacturing Management, Harold Armstrong Memorial Lecture, Monash University, Melbourne, Australia

24. Busch, P.A. (2004) Knowledge Management Implications of Articulable Tacit Knowledge: Case Studies on its Diffusion, unpublished dissertation submitted to Macquarie University for the degree of Doctor of Philosophy in the Department of Computing, Division of Information and Communication Sciences.
25. Bybee, R. W. (1997) Achieving Scientific Literacy, Portsmouth, NH: Heinemann.
26. Carroll, M., Goldman, S., Britos, L., Koh, J., Royalty, A. & Hornstein, M. (2010) Destination, Imagination and the Fires Within: Design Thinking in a Middle School Classroom, International Journal of Art & Design Education, Vol. 29, No. 1, pp. 37–53
27. Casonato R., Harris K., (1999) Can an enterprise really capture 'Tacit Knowledge': We answer two top questions on knowledge management from the Electronic Workplace 1999 Conference, Gartner Group Research Note Select Q&A
28. Cennamo, K. S., Abell, S. K. & Chung, M. (1996) A 'Layers of Negotiation' model for designing constructivist learning materials, Educational Technology, No.36, pp. 39-48.
29. Chisholm C. U. (2002) Analysis of why work-based learning is a better model for creating the engineers that society needs. 3rd Global Congress on Eng. Edu., Glasgow, Scotland, p 380-383.
30. Chisholm C. & Holifield, D. (2002). THE ROLE OF TACIT KNOWLEDGE IN CONTINUOUS PROFESSIONAL DEVELOPMENT OF WORK-BASED PRACTITIONERS. 3rd Global Congress on Eng. Edu., Glasgow, Scotland, pp. 242-250
31. Cuff, D. (1991) Architecture: The Story of Practice, Cambridge, MA: MIT Press.
32. Dagenhart, R. (1993) Design Research: The Studio and the Laboratory, Conference on Knowledge Based Architectural Education: Reconfiguration the Studio. Architectural Research Centers Consortium: St. Antonio, Tx.
33. Demirbaş, O. & Demirkan, H. (2003) Focus on architectural design process through learning styles, Design Studies, Vol. 24, No. 5, pp 437-456.
34. Dickey M.D. (2007) Teaching in 3D: Pedagogical affordances and constraints of 3D virtual worlds for synchronous distance education, Distance Education, Vol. 24, pp. 105-121.
35. Doblin, J. (1987) A Short Grandiose Theory of Design (online). Available at: http://www.noisebetweenstations.com/personal/third_party/Jay_Doblin_A_Short_Grandiose_Theory_of_Design.pdf (accessed 28 March 2010).
36. Duffy, T.M., Lowyck, J. & Jonassen, D. (Eds.) (1993) Designing environments for constructivist learning, Heidelberg: Springer-Verlag.
37. Duffy, G., Roehler, L. & Radcliff, G. (1986) How teachers' instructional talk influences students' understanding of lesson content. Elementary School Journal, Vol. 87, pp. 3-16.
38. Duit, R. & Treagust, D. (1998) Learning in science: From behaviorism towards social constructivism and beyond. In B. J. Fraser & K. G. Tobin [eds] International Handbook of Science Education. Dordrecht: Kluwer Academic Publishers, Part 1.
39. Eisenkraft, A. (2003) Expanding the 5E Model, The Science Teacher: National Science Teacher Association (NSTA), Vol. 70, No. 6, pp. 57-59.
40. Ertmer, P. A. & Newby, T. J. (1993) Behaviorism, cognitivism, constructivism: comparing critical features from an instructional design perspective, Performance Improvement Quarterly, Vol.6, No.4, pp.50-72.
41. Etesam, I. (2008) Technology Alongside Traditional Education, Journal of Architecture and Culture, Vol.10, No. 32, pp 18-22.
42. Facione, N.C. & Facione, P.A. (1994) The California Critical Thinking Skills Test and the National League for Nursing Accreditation Requirement in Critical Thinking, Millbrae, California: California Academic Press.
43. Farrington, G.C. (1999) July/August). The new technologies and the future of residential undergraduate education, Educom Review, Vol.34, No.4, (online). Available at: http://www.educause.edu/LibraryDetailPage/666?ID=ERM9949 (accessed 28 May 2005).
44. Fox, R. (2001) Constructivism Examined, Oxford Review of Education, Vol.27, No.1, pp. 23-35.
45. Fullan, M. (1993) Change Forces: Probing the Depths of Educational Reform, London: Falmer Press.
46. Gerlenter, M. (1988) Reconciling Lectures and Studios, Journal of Architectural Education, Vol.41, No.2.
47. Glasersfeld E.V. (1983) Learning as constructive activity, (Bergeron J.C. and Herscovics N. editors), Proceedings of the 5th Annual Meeting of the North American Group of PME, Montréal: PME-NA.
48. Goldschmidt, G. (2002) One-on-One: A Pedagogic Base for Design Instruction in the Studio, in Durling, D. and Shackleton, J. [Eds] Proceedings of "Common Ground" Design Research Society International Conference, Brunel University, Stoke-on-Trent: Staffordshire University Press, pp 430-437.

49. Grant, R.M. (2000) The New Knowledge Economy. Knowledge Horizons ed. Despres, C. and Chauval, D.
50. Groat, L. & Wang, D. (2002) Architectural research methods. London: John Wiley & Sons.
51. Gül, L.F,. Gu, N. & Williams, A. (2008) Virtual World as a Constructivist learning platform: Evaluations of 3D virtual worlds on design teaching and learning, ITcon, Vol. 13, pp. 578-593.
52. Gutman, R. (1968) What schools of Architecture Expect from Sociology, Journal of Architectural Education, Vol.24, No.2.
53. Gutman, R. (1987) Education and the World of Practice, Journal of Architectural Education, Vol.40, No.2.
54. Gutman, R. (1988) Architectural Practice: A Critical View, New York: Princeton Architectural Press.
55. Jenkins, E. W. (2000) Constructivism in school science education: Powerful model or the most dangerous intellectual tendency?, Science & Education, No. 9, pp. 599-610.
56. Johnson, J. (2005) Art in contentious spaces, in D. Atkinson & P. Dash [Eds] Social and Critical Practices in Art Education. Stoke-on-Trent: Trentham Books, pp. 124-7.
57. Jonassen, D.H. (1991) Objectivism versus constructivism: Do we need a philosophical paradigm? Educational Technology Research and Development, Vol. 39, No.3, pp. 5-14.
58. Jonassen, D.H. (1999) Designing constructivist learning environments. In C. Reigeluth [Eds] Instructional design theories and models: A new paradigm of instructional theory. Mahwah, NJ: Lawrence Erlbaum Associates, Vol. II, pp. 215-239.
59. Jones, M. G. & Brader-Araje, L. (2002) The Impact of Constructivism on Education: Language, Discourse, and Meaning, American Communication journal, Vol. 5, No. 3.
60. Juhasz, J. (1981) The Place of Social Sciences in Architectural Education, Journal of Architectural Education, Vol.35, No.3.
61. Karagiorgi, Y. & Symeou, L. (2005) Translating Constructivism into Instructional Design: Potential and Limitations, Educational Technology & Society, Vol. 8, No. 1, pp. 17-27.
62. Koch, A., Schwennsen, K., Dutton, T. & Smith, D. Eds. (2002) The Redesign of Studio Culture. DC: The American Institute of Architecture Students.
63. Koskinen, K.U. (2003) Evaluation of tacit knowledge utilization in work units, Journal of Knowledge Management, VOL. 7: NO. 5, pp. 67-81.
64. Lang, J. (1987) Creating Architectural Theory: The Role of the Behavioral Sciences in Environmental Design, New York: van Rostrand Reinhold.
65. Lave, J. (1990) The culture of acquisition and the practice of learning, in J. W. Stigler, R. A. Shweder & G. Herdt [Eds] Cultural psychology: Essays on comparative human development. Cambridge, UK: Cambridge Univeristy Press, pp. 259-286.
66. Lave, J., & Wenger, E. (1991) Situated learning: Legitimate peripheral participation, Cambridge, UK: Cambridge University Press.
67. Lebow, D. (1993) Constructivist values for systems design: five principles toward a new mindset, Educational Technology Research and Development, No. 41, pp. 4-16.
68. Lock, J. (1947) An essay concerning human understanding. Dent: London.
69. McCommons, R. (1989) Guide To Architecture Schools, Washington D.C.: Association Collegiate Schools of Architecture.
70. McCommons, R. (1994) Guide To Architecture Schools, Washington D.C.: Association Collegiate Schools of Architecture.
71. Mathews, M. (1998) Constructivism in science education. Dordrecht, The Netherlands: Kluwer.
72. Mayo, J. (1985) Political Avoidance in Architecture, Journal of Architectural Education, Vol.38, No.2.
73. Mayo, J. (1991) Dilemmas of Architecture Education in the Academic Political Economy, Journal of Architectural Education, Vol.44, No.2.
74. Naylor, S. & Keogh, B. (1999) Constructivism in classroom: Theory into practice, Journal of Science Teacher Education, No. 10, pp. 93-106.
75. Neale, D., Smith, D. & Johnson, V. (1990) Implementing conceptional change teaching in primary science. Elementary School Journal, Vol. 91, pp. 109-132.
76. Noddings, N. (1990) Constructivism in mathematics education. In R. Davis, C. Maher, & N. Noddings [Eds] Constructivist views on the teaching and learning of mathematics. Reston, Va: National Council of Teachers of Mathematics, pp. 7-18.
77. OCED (1996) Learning for All. Paris OCED
78. Ochsner, J.K. (2000) Behind the Mask: A Psychoanalytic Perspective on Interaction in the Design Studio, Journal of Architectural Education, Vol. 53, No.4, pp. 194–206.
79. Ozkan, S. (1986) An Overview of Architectural Education in Islamic Countries. Architectural Education in the Islamic World, Geneva: Aga Khan Award for Architecture.

80. Öztürk, M. N. & Türkkan, E. E. (2006) The Design Studio as Teaching/Learning Medium — A Process- Based Approach, International Journal of Art & Design Education, Vol. 25, No. 1, PP. 96–104.
81. Papanek, V. (1971) Design for the Real World: Human Ecology and Social Change, New York: Pantheon Books.
82. Papert, S. (1980) Computer-based microworlds as incuboators for powerful ideas. In R. Taylor [Eds] The computer in the school: Tutor, tool, tutee, New York, NY: Teachers College, pp. 203-210.
83. Papert, S. (1983) The children's machine: Rethinking school in the age of the computer. New York: Basic Books.
84. Piaget, J. (1967) Biologie et connaissance (Biology and knowledge). Paris: Gallimard.
85. Piaget, J. (1970) Piaget's theory. In P. H. Mussen [Eds] Carmichael's manual of child psychology. New York: Wiley, Vol. 1, 3rd ed., pp. 703-732.
86. Pine, F. (1990) Drive, Ego, Object, and Self: A Synthesis for Clinical Work. New York: Basic Books.
87. Polyanyi M., (1958/1974) Personal knowledge: Towards a Post-Critical Philosophy. Chicago, University of Chicago & Press.
88. Russell, D. & Schneiderheinze, A. (2005) Implementing an Innovation Cluster in Educational Settings In Order to Develop Constructivist-Based Learning Environments. Educational Technology & Society, Vol. 8 (2), pp. 7-15.
89. Robinson, J.W. (1986) Design as exploration, DESIGN STUDIES, Vol. 7, No.2, pp. 67-80.
90. Rowe, P. (1982) A Priori Knowledge and Heuristic Reasoning in Architectural Design, Journal of Architectural Education, Vol.36, No.1, pp. 18-23.
91. Rowe, P. (1987) Design Thinking, Cambridge: The MIT Press.
92. Ryle, G. (1949/1984) The Concept of Mind. Chicago: The University of Chicago Press.
93. Rumizen, M. C. (2002) The Complete Idiot's Guide to Knowledge Management, Indianapolis, IN: Alpha Books.
94. Saint-Onge, H. (1996) Tacit knowledge: The key to the strategic alignment of intellectual capital, Strategy & Leadership, V 24: No.2, Mar/April, pp. 10 -14.
95. Salama, A. (1995) New Trends in Architectural Education: Designing the Design Studio, Raleigh, NC, USA: Tailored Text and Unlimited Potentials.
96. Salama, A. (1998). A New Paradigm in Architectural Pedagogy: Integrating Environment-Behavior Studies into Architectural Education teaching Practices, In J. Teklenburg, J. Van Andel, J. Smeets & A. Seidel [Eds] Shifting Balances: Changing Roles in Policy, Research, and Design. Eindhoven, Netherlands: EIRSS Publishers. PP. 128-139.
97. Salama, A. (2005) SKILL-BASED / KNOWLEDGE -BASED ARCHITECTURAL PEDAGOGIES: AN ARGUMENT FOR CREATING HUMANE ENVIRONMENTS, 7th Intl Conference on Humane Habitat-ICHH-05 – The International Association of Humane Habitat IAHH, Rizvi College of Architecture, Mumbai, India, January 29-31.
98. Sanoff, H. (2003) Three Decades of Design and Community. Raleigh, NC: College of Design, North Carolina State University.
99. Saxe, G. B., Guberman, S. R. & Gearheart, M. (1987) Social processes in early number development. Monographs of the Society for Research in Child Development, Vol.52, No. 216.
100. Schon, D. (1981) Learning a Language, Learning to Design: Architectural Education Study. Cambridge, MA: Consortium of East Coast Schools of Architecture.
101. Schon, D. (1983) The Reflective Practitioner: How Professionals Think in Action. New York, NY: Basic Books.
102. Schon, D. (1985). The Design Studio: An Exploration of Its Tradition and Potentials. London: RIBA Publications.
103. Schon, D. (1988) Toward a Marriage of Artistry and Applied Science in the Architectural Design Studio, Journal of Architectural Education, Vol. 41, No.4, PP. 16-24.
104. Schunk, D. H. (2000) Learning theories: an educational perspective. New Jersey: Prentice-Hall.
105. Senge, P. (1990) The Fifth Discipline: The Art and Practice of the Learning Organization. New York: Doubleday Currency.
106. Shulman, L. (1992) Toward a pedagogy of cases. In J. H. Shulman (Ed.), Case methods in teacher education,NY: Teachers College Press.
107. Spiro, R. J. Feltovich, P. J., Jacobson, M. J., & Coulson, R. L. (1991b) Knowledge representation, content specification, and the development of skill in situation-specific knowledge assembly: Some constructivist issues as they relate to Cognitive Flexibility theory and hypertext. Educational Technology, Vol. 31 (9), pp. 22-25.
108. Stamp, A. (1994) Jungian Epistemological Balance: A Framework for Conceptualizing Architectural Education. Journal of Architectural Education. Vol. 48, No.2, PP. 105-112.

109. Stephens, E.C & J. E. Brown (2000) A Handbook of Content Literacy Strategies: 75 Practical Reading and Writing Ideas. Inc. MA, USA: Cristopher-Gordon Publishers.
110. Stevens, G. (1998) The Favored Circle: The Social Foundations of Architectural Distinction, Cambridge, MA: MIT Press.
111. Teymur, N. (1996) City as Education: Habitat II Edition. London: Question Press.
112. Tiwari, A., Lai, P., So, M. & Yuen, K. (2006) A comparison of the effects of problem-based learning and lecturing on the development of students' critical thinking, MEDICAL EDUCATION, No. 40, pp. 547–554.
113. Tobin, K. & Tippins, D. (1993) Constructivism as a referent for teaching and learning, In K.Tobin [Eds] The practice of constructivism in education, New Jersy: Lawrence-Erlbaum, Hillsdale, pp. 3-21.
114. Uluo_lu, B. (2000) Design Knowledge Communicated in Studio Critique, Design Studies, Vol. 21, No. 1, pp 33-58. Tables, p 44.
115. Johnson, J. (2005) Art in contentious spaces, in D. Atkinson & P. Dash [Eds] Social and Critical Practices in Art Education. Stoke-on-Trent: Trentham Books, pp. 124-7.
116. Van Zanten, D. (1977) The Architecture of the Ecole des Beaux-Arts, Architectural Composition at the Ecole des Beaux-Arts from Charles Percier to Charles Garnier, in Arthur Drexler, New York: Museum of Modern Art.
117. Von Glasersfeld, E. (1995) Radical constructivism: A way of knowing and learning. Washington, DC: Falmer.
118. von Krogh, G. and Roos, J. (1996), Five claims of knowing, European Management Journal, Vol. 14, pp. 423-6.
119. von Krogh, G., Ichijo, K. and Nonaka, I. (2000), Enabling Knowledge Creation. How to Unlock the Mystery of Tacit Knowledge and Release the Power of Innovation, Oxford University Press, New York, NY.
120. Vygotsky, L. S. (1962) Thought and Language. Cambridge, MA: MIT Press
121. Vygotsky, L. (1986) Thought and language, Cambridge: The MIT Press.
122. Vygotsky, L. S. (1978) Mind in society: The development of higher psychological processes. Cambridge, MA: Harvard University Press.
123. Wang, T. (2009) The Transformational Promise of Information and Communications Technologies (ICTs) for the Professional Education of Architects. Educational Technology & Society, Vol. 12 (3), pp. 206–213.
124. Watson, D. (1993) Towards A New paradigm in Education, Conference on Knowledge Based Architectural Education: Reconfiguration the Studio. Architectural Research Centers Consortium: St. Antonio, Tx.
125. Weber, R. (1994) The Integrated Design Studio, Design Studies, Vol. 15, No. 1.
126. Wendler, V.W. & Rogers, J.S. (1995) The Design Life Space: Verbal Communication in the Architectural Design Studio, Journal of Architectural and Planning Research, Vol. 12, No.4, pp. 319-335.
127. Wertsch, J. V. (1998) Mind as action, NY: Oxford University Press.
128. Willis, J. (1995) Recursive, reflective instructional design model based on constructivist-interpretist theory. Educational Technology, Vol. 35, No. 6, pp. 5-23.
129. Wilson, B.G. (Ed.) (1996). Constructivist learning environments: Case studies in instructional design. Englewood Cliffs, NJ: Educational Technology Publications.
130. Wolfe, T, (1981) From Bauhaus To Our House. Toronto: McGraw Hill Rycrson Ltd.
131. Winn W. (1993) A conceptual basis for educational applications of virtual reality, Human interface technology laboratory, Washington Technology Center, University of Washington.
132. You,Y. (1994) What can we learn from Chaos Theory? An alternative approach to instructional design, ETR & D, Vol. 41, No. 3, pp. 17-32..

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Role of Selenium in Attenuating Cardiac and Hepatic Damages Induced By the Antitumor Agent, Doxorubicin

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Abstract: Background and Objectives: The clinical use of doxorubicin, one of the most effective antitumor agents, soon proved to be hampered by such serious problems as the development of cardiomyopathy and liver damage. The current study aims at evaluating the role of trace element, selenium, in attenuating cardiac and hepatic damages induced by the antitumor agent, doxorubicin. **Materials and Methods:** Animals were divided into normal control group and doxorubicin -treated group injecting doxorubicin i.p. as 6 equal doses of 2.5 mg/kg, twice weekly/ 3 weeks. The doxorubicin - treated animals were divided into 2 groups, one kept without further treatment (doxorubicin -group), second group, (doxorubicin + selenium) received selenium (Na Selenite) 0.5 mg/kg orally, 3 times/week/4 weeks including one week before the doxorubicin 1st dose. Serum creatine phosphokinase, lactate dehydrogenase, as cardiac damage markers, and alanine aminotransferase, as indicator of hepatic damage, were measured. Malondialdehyde and nitric oxide levels, as cardiac oxidative status indices, cardiac glutathione content, glutathione peroxidase, glutathione-S–transferase and superoxide dismutase activities, as measures for cardiac antioxidant capacity, were also investigated. Histopathological changes in cardiac and liver tissues were examined. The results were analyzed statistically by one-way analysis of variance with subsequent multiple comparisons using Tukey test. **Results:** doxorubicin induced significant increase in serum lactate dehydrogenase; creatine phosphokinase; alanine aminotransferase activities, cardiac nitric oxide, malondialdehyde levels, superoxide dismutase, glutathione peroxidase, glutathione-S–transferase activities, and reduction in glutathione content. Selenium co-administration caused significant decrease in serum lactate dehydrogenase and creatine phosphokinase levels; normalization of serum alanine aminotransferase; significant decrease in cardiac malondialdehyde, nitric oxide levels, glutathione peroxidase, glutathione-S–transferase, superoxide dismutase activities and significant elevation in cardiac glutathione content, compared to doxorubicin -treated group values. Histopathological examination of cardiac and liver tissues supported the previous biochemical results. **Conclusions:** Chronic doxorubicin administration caused cardiomyopathy and hepatic damage. Selenium co-administration produced partial, but significant, protection against cardiomyocyte damage; however, it alleviated hepatic damage-induced by the antitumor agent, doxorubicin.

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Key words: Doxorubicin, cardiomyopathy, liver damage, selenium

Introduction:

Anthracyclines rank among the most effective anticancer drugs ever developed [1]. The first anthracycline was isolated early in the 1960s from the pigment producing Streptomyces Peucetius, a species of actinobacteria [2] and was named doxorubicin (Dox). Doxorubicin is an essential component of treatment of breast cancer [3], soft tissue sarcomas [4] and many other cancers [5]**.** Because Dox has been shown to produce free radicals, it was suggested earlier that free radical injury might be a mechanism of Dox antitumor activity [6]. There now appears to be general agreement that oxidative stress is unlikely to be a significant contributor to the antitumor activity of Dox [7]**.** Liver is the main site of Dox metabolism, reduction of side chain carbonyl group by NADPH-Cyto P450 yields a more polar and toxic metabolite, doxorubicinol. Such metabolite accumulates in the heart and contributes significantly to chronic cumulative cardiotoxicity of Dox [8]. The enormous value of Dox in treating a variety of solid and

hematologic malignant conditions is unquestioned. However, as with any other anticancer agent, the clinical use of Dox soon proved to be hampered by such serious problems as the development of resistance in tumor cells [9] or toxicity in healthy tissues, most notably in the form of chronic cardiomyopathy and congestive heart failure[1]. These adverse effects of the drug can preclude its use in some patients and limit the duration of its use in many others [10].

Selenium (Se) plays an important biological role in living organisms, mostly through its incorporation in a family of proteins, selenoproteins. The main biological form of Se is selenocysteine, a cysteine analog that is synthesized from a serine bound to tRNA. The biological roles ascribed to Se include the prevention of cardiovascular disease [11] and cancer [12]. In the heart, Se supplementation caused increase in the cardiomyocyte glutathione peroxidase (GPx) activity, the total antioxidant activity, glutathione (GSH) concentration and catalase activity, leading to decreased generation of reactive oxygen species (ROS)[13]. The present study aims at evaluating the attenuating effect of Se, as an adjuvant therapy, on Dox- induced cardiac and hepatic damages.

Materials and Methods:

A. Animals:

Total numbers of 32 male albino rats of the Wister strain, weighing 170-200 g, were used in the present study. The animals were obtained from the central animal facility at the Faculty of Pharmacy, Cairo University, Cairo, Egypt. All rats were housed in a room with a controlled environment, at a constant temperature of 23 ± 1^0C, humidity of 60% ± 10%, and a 12 hrs light/dark cycle. The animals were housed in groups and kept at constant nutritional conditions throughout the experimental period. The experimental protocols were approved by the Ethical Committee of Cairo University.

B- Drugs and chemicals:

Doxorubicin HCL was obtained from Pharmacia & Upjohn, Milan, Italy. Sodium selenite was obtained from Sigma Chemical Company, USA. Other chemicals in the experiments were of analytical pure grade and supplied by British Drug House (BDH, UK), Merk (Germany) and Sigma Chemical Company (USA).

C- Experimental design:

Animals were divided into a normal control group (10 rats), receiving the appropriate volume of saline i.p, and Dox-treated group. Doxorubicin was dissolved in saline and injected i.p., as a total cumulative dose equal to 15 mg/kg, divided into 6 equal doses, 2.5 mg/kg each. They were injected twice weekly/ 3 weeks [14]. Dox-treated animals were divided into tow groups, one kept without further treatment (Dox-group), and a second group (Dox + Se) received Se, as sodium selenite, 0.5 mg/kg, orally, 3 times/week/4 weeks including one week before the 1st Dox dose[15].

D- Serum and Tissue sampling:

24 hours following the last Dox injection, rates were sacrificed by decapitation. Blood sample of each animal was collected into a dry centrifuge tube. Serum was separated by centrifugation at 3000 r.p.m. /15 minutes and used to determine creatine phosphokinase (CPK), lactate dehydrogenase (LDH) and alanine aminotransferase (ALT). Serum CPK activity was determined using a kit provided by STANBIO, USA. CPK catalyses the transphosphorylation of ADP to ATP through a series of coupled enzymatic reactions. NADH is provided at a rate directly proportional to CPK activity. The method determines NADH absorbance increase per minute at 340 nm [16]. Serum LDH activity was determined using a kit provided, also, by STANBIO, USA. LDH specifically catalyzes the oxidation of lactate into pyruvate with subsequent reduction of NAD to NADH. Rate of NADH formation is proportional to LDH activity. The method described determines NADH absorbance increase per minute at 340 nm [17]. Serum ALT activity was determined, using a kit provided by Quimica Clinica Aplicada, Spain[18].

Histopathological study:

The hearts and livers were removed by dissection, washed by ice-cold isotonic saline and blotted between two filter papers. Autopsy samples were taken from heart and liver in different groups of rats and fixed in 10% formol saline for 24 hrs. Washing was done, then, serial dilutions of alcohol were used for dehydration. Paraffin bees wax tissue blocks were prepared for sectioning the studied tissues. The obtained sections were stained by hematoxylin and eosin stains [19] for histopathological examinations through the light microscope.

Biochemical parameters:

10% w/v homogenate was prepared in ice-cold deionized water for the remainder of the heart tissues of the different groups.

Measurement of cardiac oxidative status indices: A portion of the homogenate was mixed with ice-cold 2.3% KCL (in ratio of 1:1) and centrifuged at 3000 r.p.m./15 minutes. Thiobarbituric acid (TBA) – reactive substance, malodialdehyde (MDA), content was determined in the supernatant [20], depending on measuring the coloured complex formed between TBA and MDA in acidic medium. Another aliquot of homogenate is centrifuged at 17.000 r.p.m./4ºC/20 minutes. The resulted supernatant was used for the determination of nitric oxide (NO), as nitrite ($N0_2^-$) and nitrate ($N0_3^-$) concentrations [21], using Griess reagent after the enzymatic reduction of nitrate to nitrite. The Griess reaction involves the reaction of nitrite with sulfanilamide in an acidic solution to yield a diazonium salt, followed by coupling with N-(1-naphthyl) ethylenediamine to yield a colored azo dye that can be measured colourimetrically at 540 nm.

Measurement of some cardiac antioxidant systems: A portion of homogenate was mixed with ice-cold 7.5% sulfosalicylic acid (in a ratio 1:1) and centrifuged at 3000 r.p.m/ 15 minutes. The resulted supernatant was used for determination of GSH [22]. Another part of homogenate was mixed with equal volume of ice-cold Tris- EDTA buffer (pH =7.6), centrifuged at 39.000 r.p.m./ 4º C/ 20 minutes. The supernatant was used for determination of superoxide dismutase SOD; glutathione peroxidise (GPx) and glutathione S-transferase (GST). Determination of GST activity [23, 24] depends on the ability of GST to catalyze the formation of glutathione adduct with 1-chloro,2,4 dinitrobenzene(CDNB). This adduct was measured by noting the net increase in absorbance at 340nm. Determination of GPx [25] depends on measuring the rate of oxidized GSH formation, by following up the decrease in absorbance of the reaction at 340 nm as NADPH was converted to NADP. Superoxide dismutase activity was determined [26], depending on the fact that the spontaneous autoxidation of pyrogallol, at alkaline pH less than 9.5, produces superoxide anion, which in turn enhances further oxidation of pyrogallol with a resultant increase in absorbance at 420 nm. The presence of SOD in the reaction medium retards pyrogallol autoxidation by scavenging the formed superoxide anion.

Statistical analysis:

The results were analyzed statistically by one-way analysis of variance (ANOVA test) with subsequent multiple comparisons using Tukey test. Differences were considered statistically significant at p less than 0.05. The results were presented as the mean ± standard error of the mean (SEM). Data obtained were submitted to a computerized statistical treatment using SPSS statistical package, version 17. Graphs were represented by Harvard graphics version 4 computer program.

Results:

Results revealed that Dox caused significant increase in serum levels of LDH and CPK, amounting to 182.4% and 183.6% respectively, as compared to the normal values (Fig. 1). Selenium co-administration caused significant decrease in the activities of LDH and CPK reaching to 139.5% and 153.6%, respectively of the control values. Figure (2) illustrated that, Dox caused a significant increase in cardiac MDA and NO contents, amounting to 183.36% and 177.7%, respectively, compared to the control values. Concomitant administration with Se caused significant decrease in MDA and NO levels reaching to 126% and 120%, compared to the control values.

As shown in figure (3), Dox administration caused a significant decrease in cardiac GSH level reaching to 64% of the normal values. Co-administration of Se significantly elevated GSH content to about 77.9% of the control values. Figure (4) showed significant increases in cardiac activities of GPx and GST in the Dox-treated rats, amounting to 410% and 184% respectively, compared to the normal values. Meanwhile, the co-administration of Se caused significant decrease in the levels of GPx and GST to about 166% and 136% of the normal values. Figure (5) showed significant increases in cardiac activity of SOD in the Dox-treated rats amounting to 225% compared to the normal value. Co-administration of Se caused significant decrease in the level of SOD to about 172% of the normal values. Results of figure (6) revealed that Dox administration caused significant elevation in the serum ALT level to reach 118% of the normal control level. Selenium co-administration caused normalization of the elevated ALT level.

Cardiac histopathological results showed that; in the control sections, the cardiac muscle fibers were grouped in bundles with connective tissue in between. The single muscle fiber had acidophilic cytoplasm and a central nucleus (Figure 7). In the cardiac sections obtained from rats administrated Dox, hyalinization was observed in the myocardial bundles associated with either inflammatory cells infiltration only or inflammatory cells and edema in focal manner in

between the bundles. Edema was also noticed in the subendocardial layer. The subendocardial adipose tissue was infiltrated by inflammatory cells (Figures 8, 9, 10). In the cardiac sections obtained from rats administrated Dox + Se, there was mild hyalinization in the myocardial muscle bundles (Figure 11).

Examination of liver sections of the different groups illustrated that: Liver tissue of the normal group showed hepatic lobules with normal architecture (Figure 12). In case of liver sections of rats administrated Dox, congestion was observed in the central vein, in addition to kupffer cells proliferation in diffuse manner between the fatty degenerated hepatocytes (Figures 13). In case of liver sections of rats administrated Dox + Se, least liver damage was shown, just kupffer cells proliferation was observed in between hepatocytes (Figure 14).

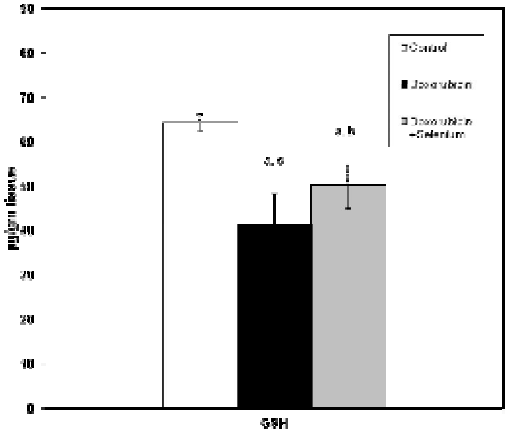


Fig (3):Effect of Selenium on doxorubicin- induced alterations in cardiac glutathione (GSH) content in rats.

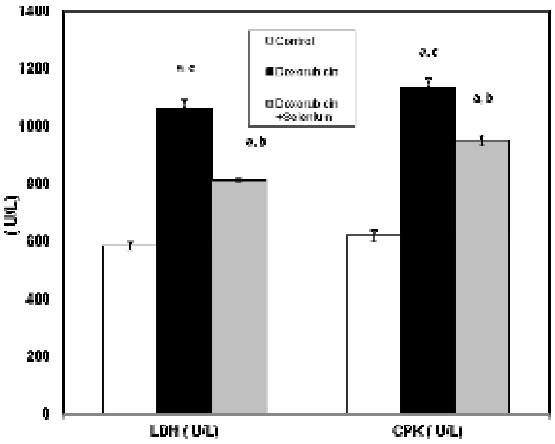


Fig (1): Effect of selenium on doxorubicin- induced alterations in serum lactate dehydrogenase (LDH) and creatine phosphokinase (CPK) activities in rats.

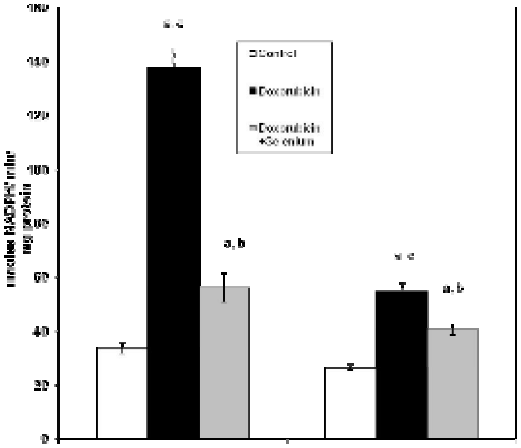


Fig (4):Effect of Selenium on doxorubicin- induced alterations in cardiac glutathione peroxidase (GPx) and glutathione-S-tranferase (GST) activities in rats.

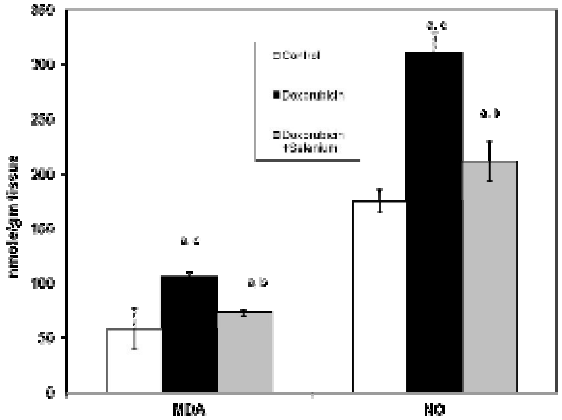


Fig (2):Effect of Selenium on doxorubicin- induced alterations in cardiac malondialdehyde (MDA) and nitric oxide (NO) levels in rats.

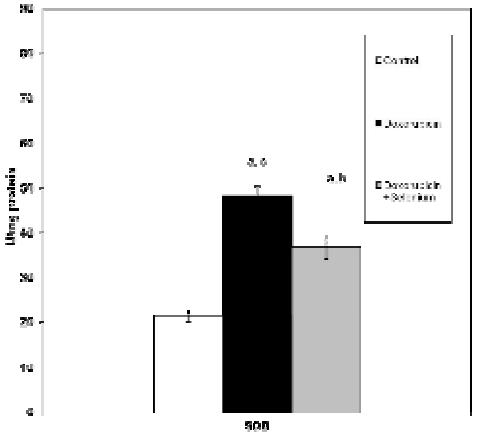
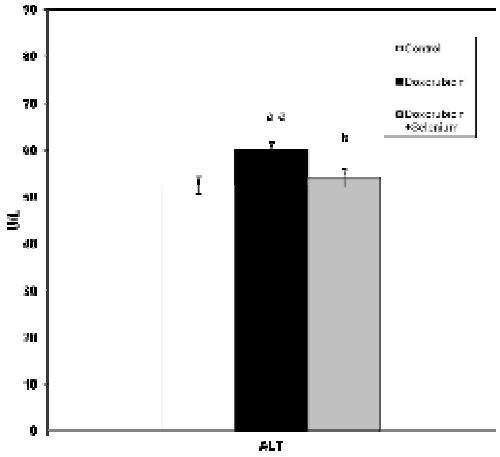
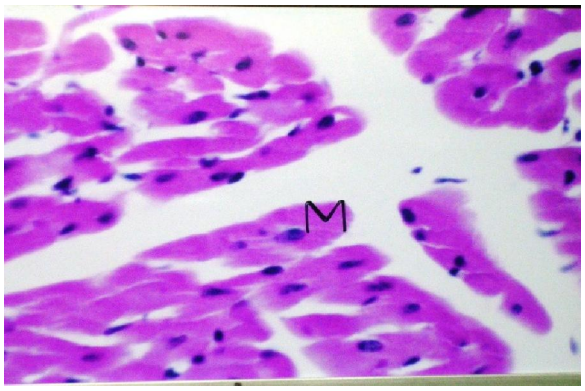


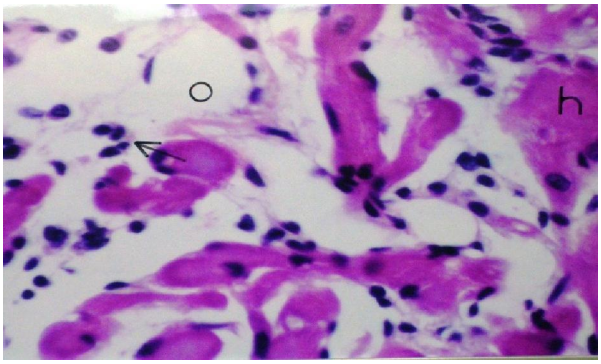
Fig (5): Effect of Selenium on doxorubicin- induced alterations in cardiac superoxide dismutase (SOD) activity in rats.



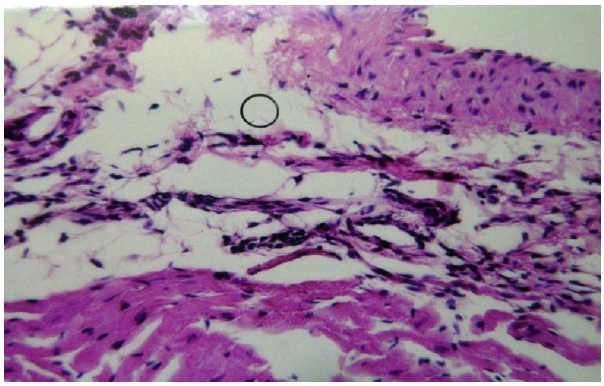
Fig(6):Effect of Selenium on doxorubicin- induced alteration in serum alanine aminotransferase (ALT) activity in rats.



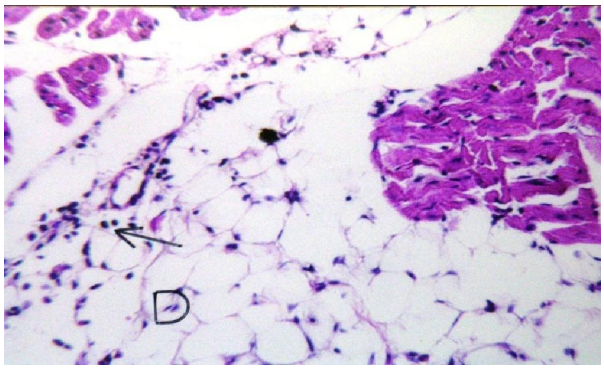
Figure(7): A photomicrograph of cardiac muscle fibers of control group showing normal histological strucrure of myocardium(M) (H&E x160)



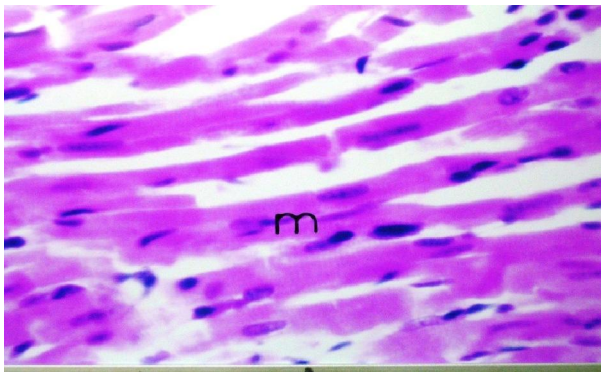
Figure(8): A photomicrograph of cardiac muscle fibers of Dxo group showing Oedema(o) with inflammatory cells infiltration(arrow) in focal manner between the myocardial bundles. (H&E x160)



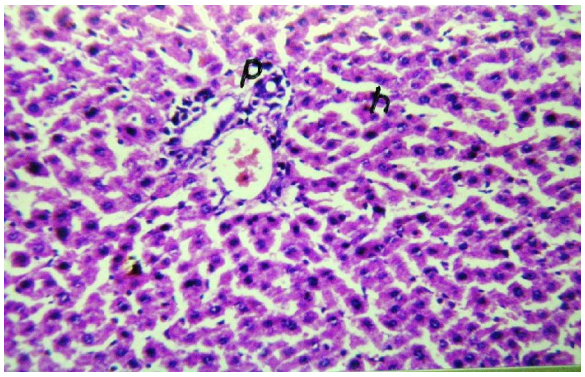
Figure(9): A photomicrograph of cardiac muscle fibers of Dox group showing Subendocardial oedema(o). (H&E x64)



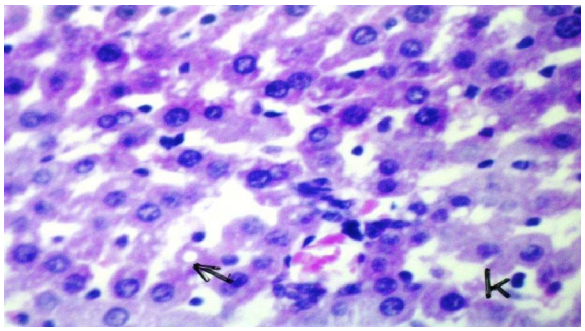
Figure(10): A photomicrograph of cardiac muscle fibers of Dox group showing inflammatory cells infiltration(arrow) in the subendocardial adipose tissue(D) (H&E x64)



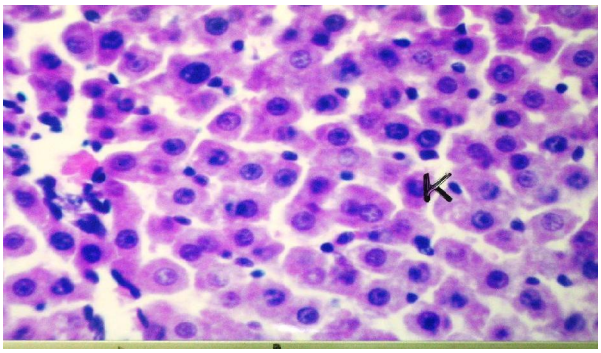
Figure(11): A photomicrograph of cardiac muscle fibers of Dox+Se group showing mild hyalinization in myocardial bundles(m) (H&E x160)



Figure(12): Photomicrograph of liver of normal group showed hepatic lobules (h) and portal vein (p) with normal architecture *(H&E x64)*



Figure(13): Photomicrograph of liver of Dox group showing diffuse kuffer cells proliferation(k) inbetween the fatty degenrated hepatocytes (arrow) (H&E x160)



Figure(14): Photomicrograph of liver of Dox + Se group showing diffuse kuffer cells proliferation(k) inbetween the fatty hepatocytes (H&E x160)

Discussion:

Doxorubicin-induced cardiomyopathy has long been a serious side effect in treating human cancers, which limits the clinical dosage of Dox [27]**.** The mechanism of Dox-induced cardiotoxicity is attributed to the formation of ROS and subsequent changes of membrane fluidity and integrity. Oxidative stress is generally held as the mediating mechanism in the multiple biological processes leading to Dox cardiotoxicity [28]**.** Nutritional strategies designed to augment cellular defense systems have been identified as a promising approach to combat oxidative stress-associated disease conditions. In this respect, dietary supplementation with Se, potentially adjusting antioxidant enzymatic status, could offer protection in preventing free radical-induced cardiac injury. In the present study, role of trace element, selenium, in attenuating cardiac and hepatic damages induced by antitumor agent, doxorubicin was studied.

Results of the present study revealed that 15 mg/kg total cumulative dose of Dox induced cardiac and hepatic damages, manifested biochemically by significant increase in serum activities of LDH; CPK and ALT. Additionally, Dox caused elevation in cardiac NO, MDA levels, SOD, GPx, GST activities, and reduction in GSH content. Histopathological examination of heart and liver sections of Dox-treated animals supported these biochemical results. Selenium administration, concomitant to Dox therapy, caused significant decrease in the serum activities of LDH and CPK; cardiac MDA, NO levels, GPx, GST and SOD activities and significant elevation in cardiac GSH content, compared to Dox-treated group values, as well as normalization of serum ALT level.

The present results showed significant increase in serum levels of LDH and CPK in Dox-treated group. These enzymes are considered important markers of cardiac injury. Many previous studies have demonstrated similar results in rats following Dox administration [29, 30]**.** Different types of Dox cardiotoxicity can be recognized [31]**:** "Acute" cardiotoxicity occurs during Dox administration**, h**owever, these effects are never of major concern because these are generally reversible and/or clinically manageable. "Early chronic" cardiotoxicity develops later in the Dox treatment course and characterized by dilated cardiomyopathy, with subsequent development of congestive heart failure [32]**.** It is now well established that Dox cardiotoxicity may manifest even decades after the completion of anticancer treatment [33]**.** Co-administration of Se with Dox therapy resulted in decrease in the elevated activities of serum LDH and CPK. This finding is in harmony with this stated by Simoni *et al.*[34], who reported that the elevation in serum LDH activity, as a result of hemoglobin cardiotoxicity, is significantly decreased by Se dietary supplementation. Our biochemical results are supported by the histopathological examination of

the cardiac tissue, since, the marked morphological changes shown in the hearts of Dox- treated animals have been partially preserved by Se administration.

Dox therapy caused significant increase in MDA level. Previous studies reported similar results [35, 36]. This elevation might be attributed to Dox mediated oxidative stress. Heart tissue is rich in mitochondria, which occupy about forty percent of the total intracellular volume of cardiomyocytes [37]. Dox has high affinity for cardiolipin, a negatively charged phospholipid abundant in the mitochondrial inner membrane, leading to mitochondrial accumulation of Dox [38]. Under clinically relevant plasma Dox concentrations, the heart becomes a site of redox reactivity. The quinone functionality of Dox is transformed, in the presence of NADH, into a semiquinone via one-electron reduction by complex I of the electron transport chain [39]. The semiquinone form reacts with O_2 to produce a superoxide radical (O •-), whereby Dox returns to the quinone form. The cycling of Dox between quinone and semiquinone generates large amounts of O_2, which further give rise to a variety of ROS/RNS species [40]. ROS can damage membrane lipids and other cellular components and consequently lead to cardiomyocyte apoptosis or death [41]. Our results showed that lipid peroxidation induced by Dox is significantly decreased in the pretreatment of Se, as manifested by significant reduction in the elevated level of cardiac MDA, which is consistent with previous studies [34, 42]. Previously, it was reported that Se supplementation can protect against free radical damages by increasing myocardial Se content and improving the expression and activity of GPx [43].

The present study revealed a marked increase in cardiac NO level in those Dox-treated rats. This finding is in agreement with the results reported by Saad *et al* [36] who used a model of doxorubicin chronic cardiotoxicity similar to that used in our study. The increase in NO level can be explained on the basis of the ability of Dox to mediate the induction of NOS expression and NO release in heart [44]. Several reports indicate that exposure of endothelial cells to H_2O_2 promotes eNOS expression [45]. Previous studies also suggested that stimulation of endothelial cells with calcium-mobilizing agents could activate eNOS [46]. Because Dox-induced toxicity is mediated by intracellular H_2O_2 as well as the calcium influx, Dox treatment causes an increase in eNOS transcription and protein activity in aortic endothelial cells and thus NO synthesis. On the same line, recent study provides evidence of upregulation of iNOS gene and protein expressions in Dox-induced cardiomyopathy[47]. The concomitant overproduction of NO and ROS is known to yield highly reactive nitrogen species, peroxinitrite, which may attack and destroy important cellular biomolecules [48]. Selenium, in the present study, caused significant decrease in the elevated cardiac NO level shown in the Dox-treated group, which is in agreement with that reported by Ayaz and Turan [49]. The exact mechanism by which Se influence cardiac NO synthases expression is unknown. Of interest in this context is the report that treatment of nuclear extracts of lipopolysaccharide-activated human T cells with relatively high concentrations of selenite inhibited nuclear factor -κB binding and thus decreased NO production [49]. This is because nuclear factor -κB is a transcription factor that regulates a number of cellular genes, such as those encoding iNOS [50].

Doxorubicin administration, as shown in our results, caused a significant decrease in cardiac GSH content, which is quiet compatible with previous studies [35]. The overproduction of ROS, caused by Dox administration, can account for this decrease in GSH content, as these species are detoxified by endogenous antioxidants mainly GSH causing their cellular stores to be depleted [51]. The decrease of cardiac GSH content may also be attributed to the enhanced activities of GSH metabolizing enzymes by Dox administration, as shown in the present study. One is GPx which reduces H_2O_2 and various peroxides using GSH as reducing agent.The other is GST which consumes GSH in the conjugation of Dox toxic metabolites [52]. The present study showed that cardiac GSH concentration is higher in (Se+ Dox) -treated rats rather than those administered Dox alone, which is in agreement with recent results [53]. Such effect might be attributed to the antioxidant properties of Se and its ability to reduce Dox- induced oxidative stress. Selenium reduces the consumption of GSH by ROS [53]. Because GSH is one of the essential compounds for maintaining cell integrity [54] and the GSH redox cycle is one of the most important intracellular antioxidant systems, the increase in GSH content could be one of the mechanisms for cardiac protection by Se supplementation [53]. In addition, we assumed that the observed increase in cardiac GSH content might be related to the decreased activities of GSH-utilizing enzymes, GPx and GST, shown in the (Dox + Se) -treated group, leading to preservation of their substrate, GSH.

Our results showed significant increase in cardiac activity of SOD in the Dox-treated rats, which is consistent with some studies [35, 55]. The increase in SOD activity can be explained on the basis that the redox cycling of Dox between quinone and

semiquinone forms generates large amounts of O_2 [40], which in turn stimulate SOD as an adaptive response to counteract oxidative stress. The increased activity of SOD could lead to overproduction of hydroperoxides, in consequence, GPx might be stimulated in response to the accumulated peroxides. This assumption was supported by our results, which showed a significant enhancement in cardiac GPx activity in the Dox-treated group, and also by some authors [36]. On the same line, GPx have been reported to be over expressed in Dox-treated cells, especially those tumor resistant ones [56]. The current study revealed that Se administration caused significant decrease in Dox-induced elevation in SOD cardiac activity, which still higher than that of the normal group value. This result is in harmony with several previous studies. Selenium has been reported to decrease the elevated activity of SOD in heart as a result to cadmium toxicity [57] and hemoglobin mediated cardiotoxicity [34], which used Se dose similar to that used in our study. It is now well established that Se, through its incorporation in selenoproteins, could actively protect against free radicals generation, and hence, ROS- induced damage [58]. As a result of this protective effect, Se consumption could attenuate superoxide radical production, and consequently, decreased the activity of such antioxidant enzyme. Also, our study showed that pretreatment with Se relieved Dox induced hyperactivity of cardiac GPx, which is in harmony with Ayaz and Turan [49]. Glutathione peroxidase is one of the most active antioxidant enzymes in the myocardium [59], and selenium, present in its active site, is essential for its activity. One of the major roles of this essential trace element in the body is to act as cofactor of this key antioxidant enzyme in which it contributes to both catalytic activity and spatial conformation [60]. Therefore, any significant modification of Se status would lead to changes in the activity of GPx and have important consequences on the susceptibly of the tissues to oxidative stress [13, 61].Selenium has prophylactic action, when it is administered before doxorubicin, it increases myocardial selenium content and improves both expression and activity of GPx. This may account for the increased cardiac GPx activity in (Se+ Dox)-treated group, compared to the normal control value, as shown in our results. Upon pretreatment with Se, myocardial tissues became already protected, and therefore, when exposed to Dox, we assume that there is no need for farther dramatic increase in cardiac GPx activity as an adaptive mechanism. This might be an explanation for the decrease in GPx cardiac activity in (Se+Dox) group value, compared to that result shown in the group treated with Dox alone. Additionally, the obtained biochemical results were supported by each other, since, as we mentioned later, Se supplementation caused decrease in the SOD activity, which consequently, leads to decreased production of H_2O_2 and hence, decreased activity of GPx enzyme.

Results of the present study revealed significant increase in cardiac GST activity in rats treated with Dox, which is in agreement with many studies [62]. GSTs are family of dimeric proteins that posses a multitude of functions including the enzymatic conjugation of GSH to electrophilic xenobiotics [63]. It has been reported that cellular exposure to xenobiotics and antioxidants leads to coordinated induction of a battery of genes encoding detoxifying enzymes including GST [64]. Indeed, GSTs belong to phase II enzymes that in contrast to phase I, who can participate in both metabolic activation and inactivation, predominately participate in the detoxification of xenobiotics [65]. It has been known that Dox is metabolized via alkedoreductases yielding C13 hydroxyl derivative, doxorubicinol. This metabolite is actually more polar and toxic than Dox itself. Doxorubicinol accumulates in the heart and contributes significantly to chronic cumulative cardiotoxicity induced by Dox [8]. It has been reported that Dox toxic metabolites are efficiently conjugated with reduced GSH, a reaction that is catalyzed by GST [52]. Moreover, GST, due to its peroxidase activity, can serve to reduce Dox-induced peroxides [52]. In brief, GST has showed elevation after Dox injection to detoxify Dox and its metabolites and to attenuate the elevated oxidative stress [66]. The current study revealed that Se caused significant decrease in Dox-induced elevation in GST cardiac activity, which still higher than that of the normal group value. This result is in agreement with previous studies stated that Se afforded reduction in cadmium- induced elevation in GST cardiac activity [57]. The protective effects of Se are mainly related to its physiological antioxidant properties, and hence, decreased generation of ROS and RNS. Thus, Se supplementation could decrease the activity of GST enzyme responsible for the detoxification of such free radicals.

Our results showed an elevation in serum ALT upon Dox administration which agrees with many previous studies [67, 68] and supported by the present histopathological examinations. Doxorubicin -induced hepatotoxicity might be less severe than its cardiotoxicity, which can be related to the fact that liver mitochondria, unlike cardiac mitochondria, lack the NADH-related pathway of reducing equivalents from the cytosol to the respiratory chain. As a result, liver mitochondria do not generate significant amounts of Dox semiquinones [69]. Selenium co-administration was shown to decrease the elevated serum ALT when

administered with Dox, as reported previously [34]. Selenium supplementation could reduce hepatotoxicity by rendering hepatic tissues less susceptible to lipoperoxidative attack by the drug [70]. Selenium prevents hepatocyte oxidative damage and thus leakage of liver enzymes into serum as in the cases of cadmium hepatotoxicity [71]. This biochemical result is supported by the histopathological examination of liver sections of the different groups which illustrated that, in the liver sections of rats administrated Dox, congestion and kupffer cells proliferation were observed, while sections from rats administered Dox+Se showed least liver damage.

In conclusion, Se supplementation produced partial, but significant, protection against Dox-induced cardiomyocyte damage; however, such trace element could alleviate the Dox- induced hepatic damage, as evidenced by the biochemical measurements and histopathological examinations of the cardiac and hepatic tissues.

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References:

1. **Weiss RB (1992):** The anthracyclines: will we ever find a better doxorubicin? *Semin Oncol*, 19, 670–686.
2. **Arcamone, F., Cassineli, G., Fantini, G., Grein, A., Orezzi, P., Pol,C., and Spalla, C. (1969):** Adriamycin, 14-ydroxydaunomycin,anew antitumor antibiotic from S. peucetius var. caesius. *Biogechnol.Bioengin. 9,* 1101-1110.
3. **Bonadonna, G.;M. and Valagussa, P.(1996):**Sequential or alternating doxorubicin and CMF regimens in breast cancer with more than three positive nodes.JAMA,237,542-547.
4. **Bacci, G;Ferrari,S. and Delepine, N.(1998):**predictive factors of histologic response to primary chemotherapy in osteosarcoma of extremity.j.Clin.Oncol.,16,658-663.
5. **Kufe DW, Holland JF, Frei E, (2003):** American Cancer Society: Cancer medicine 6., BC Decker, Hamilton.
6. **Muindi JR, Sinha BK, Gianni L, Myers CE (1984):** Hydroxyl radical production and DNA damage induced by anthracycline iron complex. *FEBS Lett*, 172, 226–230.
7. **Gewirtz DA (1999):** A critical evaluation of the mechanisms of action proposed for the antitumor effects of the anthracycline antibiotics adriamycin and daunorubicin. *Biochem Pharmacol* **57:** 727–741.
8. **Olson, RD, Mushlin, PS, Brenner, DE, Fleischer, C, Change, BK and Baucek, RJ (1988):** Doxorubicin cardiotixicity may be caused by its metabolite,doxorubicinol. *Proc.Natl.Acad.Sci.USA.*, 85: 3585-3589.
9. **Lage, M, Helbach H, Dietel M, and Schadendorf, D (2000):** Modulation of DNA topoisomerase II activity and expression in melanoma cells with acquired drug resistance. *Br J Cancer* **82:** 488 – 491.
10. **Bryant J, Picot J, Levitt G, Sullivan I, Baxter L, Clegg A. (2007):** Cardioprotection against the toxic effects of anthracyclines given to children with cancer: a systematic review. *Health Technol Assess* 11: 1-84.
11. **Rayman MP (2002):** The argument for increasing selenium intake. *Proceeding of the Nutrition Society*. **61:** 203–215.
12. **Combs GF and Lu J (2001):** Selenium as a cancer preventative agent. In *Selenium. Its Molecular Biology and Role in Human Health*, pp 205–219. Ed DL Hatfield. Boston: Kluwer Academic Publishers.

13. **Tanguy S, Boucher F, Besse S, Ducros V, Favier A, de Leiris J. (1998):** Trace elements and cardioprotection: increasing endogenous glutathione peroxidase activity by oral selenium supplementation in rats limits reperfusion-induced arrhythmias. *J. Trace Elem. Med. Biol.*; 12: 28–38.
14. **Matsui H, Morishima I,Numaguchi Y, Toki Y, Okumura K, Haayakawa T. (1999):** Protective effect of carvedilol against doxorubicin-induced cardiomyopathy in rats. *Life Sci.*; 65(12): 1265-74.
15. **Eun-sung Park,Sun-dong Kim,Min-hye Lee,Heung-shik S. Lee, In-se LEE,Je-Kyung Sung (2003):** Protective effects of N-acetylcysteine and selenium against doxorubicin toxicity in rats.*J. Vet. Sci.*,4(2),129-136**.**
16. **Rosalki SB. (1967):** An improved procedure for serum creatine phosphokinase determination. *J Lab Clin Med.* 69(4):696-705.
17. **Buhl SN, Jackson KY. (1978):** Optimal conditions and comparison of lactate dehydrogenase catalysis of the lactate-to-pyruvate and pyruvate-to-lactate reactions in human serum at 25, 30, and 37 degrees C. *Clin Chem.* 24(5):828-31.
18. **Reitman, S and Frankel, S (1957):** a colormetric method for the determination of serum glutamic oxaloacetic acid and glutamic pyruvic transaminases. *Am. J. Clin. Pathol.*, 28, 56.
19. **Banchroft, J.D; Stevens, A.and Turner, D.R. (1996):** theory and practice of histopathological techniques.fourth Ed. Chuchil Livingstone, New York, London, Sanfrancisco, Tokyo
20. **Uchiyama M, Mihara M. (1978):** Determination of malondialdehude precursor in tissues by thiobrbituric acid test. *Anal Biochem*; 86: 271-288.
21. **Tracey WR, Tse J, Carter G. (1995):** Lipopolysaddharide-induced changes in plasma nitrite and nitrate concentrations in rats and mice:pharmacological evaluation of nitric oxide synthase inhibitors. *J Pharmacol Exp Ther*; 272: 1011-1015.
22. **Beutler E, Duron O, Kelly BM. (1963):** Improved method for the determination of blood glutathione.*J Lab Clin Med* 61:882-888.
23. **Habig, WH, Pabst, MJ and Jakoby, WB (1974):** Glutathione S-transferase: the first enzymatic step in mercapturic acid formation J.Biol. Chem., 249, 7130-7139.
24. **Gawai, KR and Pawar, SS (1984):** Purification and characterization of glutathione S-transferase froom liver cytosol of Phenobarbital treated rabbits *Xenobiotica*, 4, 605-607.
25. **Paglia DE, Vatentine WN. (1967):** Studies on the quantative and qualitative characterizationof erythrocyte glutathione peroxidase. *J Lab Clin Med;* 70: 158-161.
26. **Marklund S, Marklund G. (1974):** Involement of superoxide anion radical in autooxidation of pyro gallol and convenient assay for superoxide dismutase. Eur J Biochem; 47: 469-474.
27. **Horan PG, McMullin MF, McKeown PP. (2006):** Anthracycline cardiotoxicity. *Eur Heart J.*; 27: 1137–1138.
28. **Kotamraju S, Chitambar CR, Kalivendi SV, Joseph J, Kalyanaraman B. (2002):** Transferrin receptor-dependent iron uptake is responsible for doxorubicin-mediated apoptosis in endothelial cells - role of oxidant-induced iron signaling in apoptosis. *Journal of Biological Chemistry.*; 277: 17179–17187.
29. **Al-majed A, Gado A, Al-shabanah O, Mansour MA. (2002):** Alpha-lipoic acid ameliorates myocardial toxicity Induced by doxorubicin. *Pharmacol Res.*; 46(6): 499-503.
30. **Yagmurca M, Fadillioglu E, Erdogan H, Ucar M, Sogut S, Irmak MK. (2003):** Erdosteine prevents doxorubicininduced cardiotoxicity in rats. *Pharmacol Res.*; 48: 377-82.
31. **Wouters KA, Kremer LC, Miller TL, Herman EH, Lipshultz SE (2005):** Protecting against anthracycline-induced myocardial damage: a review of the most promising strategies. *Br J Haematol*, 131, 561–578.
32. **Jones RL, Swanton C, Ewer MS (2006):** Anthracycline cardiotoxicity. *Expert Opin Drug Saf*, 5, 791–809.
33. **Scully RE, Lipshultz SE (2007):** Anthracycline cardiotoxicity in long-term survivors of childhood cancer. *Cardiovasc Toxicol*, 7, 122–128.
34. **Simoni J, Simoni G, Garcia EL, Prien SD, Tran RM, Feola M, Shires GT. (1995):** Protective effect of selenium on hemoglobin mediated lipid peroxidation in vivo. *Artif Cells Blood Substit Immobil Biotechnol.*; 23(4): 469-86.
35. **Balachandar AV, Malarkodi KP, Varalakshmi P. (2003):** Protective role of DLalpha-lipoic acid against adriamycin-induced cardiac lipid peroxidation. *Hum Exp Toxicol.* May; 22(5): 249-54.

36. **Saad SY, Najjar TA, Alashari M. (2004):** Cardiotoxicity of doxorubicin/paclitaxel combination in rats: effect of sequence and timing of administration. *J Biochem Mol Toxicol.* 18(2):78-86.
37. **Goffart, S., von Kleist-Retzow, J.C., Wiesner, R.J. (2004):** Regulation of mitochondrial proliferation in the heart: Power-plant failure contributes to cardiac failure in hypertrophy. *Cardiovasc. Res.* **64,** 198–207.
38. **Sarvazyan, N. (1996):** Visualization of doxorubicin-induced oxidative stress in isolated cardiac myocytes. *Am. J. Physiol. Heart Circ. Physiol.* **271,** H2079–2085.
39. **Marcillat, O, Zhang, Y, and Davies, KJ (1989):** Oxidative and non-oxida- tive mechanisms in the inactivation of cardiac mitochondrial electron transport chain components by doxorubicin. *Biochem. J.* **259,** 181–189.
40. **Takemura G, Fujiwara H (2007):** Doxorubicin-induced cardiomyopathy from the cardiotoxic mechanisms to management. *Prog Cardiovasc Dis*; 49: 330-352.
41. **Menna P, Minotti G, Salvatorelli E. (2007):** *In vitro* modeling of the structure-activity determinants of anthracycline cardiotoxicity. *Cell Biol Toxicol*; 23: 49-62.
42. **Ates B, Orun I, Talas ZS, Durmaz G, Yilmaz I. (2008):** Effects of sodium selenite on some biochemical and hematological parameters of rainbow trout (Oncorhynchus mykiss Walbaum, 1792) exposed to Pb2+ and Cu^{2+}. *Fish Physiol Biochem.*; 34(1): 53-9.
43. **Huang Y, Bai H, Zhang Z. (1999):** Mechanism of selenium protecting against free radical damages during myocardial ischemia/reperfusion in rats. *Zhonghua Yi Xue Za Zhi.*; 79(11):852-6.
44. **Fogli S, Nieri P, Breschi MC. (2004):** The role of nitric oxide in anthracycline toxicity and prospects for pharmacologic prevention of cardiac damage. FASEB J.; 18 (6): 664-75.
45. **Drummond GR, Cai H, Davis ME, Ramasamy S, Harrison DG. (2000):** Transcriptional and Posttranscriptional Regulation of Endothelial Nitric Oxide Synthase Expression by Hydrogen Peroxide. *Circ. Res.* 86: 347–354.
46. **Goetz RM, Thatte HS, Prabhakar P, Cho MR, Michel T, Golan DE (1999):** Estradiol induces the calcium-dependent translocation of endothelial nitric oxide synthase; *Proc. Natl. Acad. Sci. U. S. A.* 96:2788–2793.
47. **Liu B, Li H, Qu H, Sun B. (2006):** Nitric oxide synthase expressions in ADR-induced cardiomyopathy in rats. *J Biochem Mol Biol.* 30; 39(6): 759-65.
48. **Weinstein DM, Mihm MJ, Bauer JA (2000):** Cardiac peroxynitrite formation and left ventricular dysfunction following doxorubicin treatment in mice. *J Pharmacol Exp Ther*, 294, 396–401.
49. **Ayaz M, Turan B. (2006):** Selenium prevents diabetes-induced alterations in [Zn2+]i and metallothionein level of rat heart via restoration of cell redox cycle. *Am J Physiol Heart Circ Physiol.*; 290(3): H1071-80.
50. **Griscavage JM, Wilk S, Ignarro LJ. (1996):** Inhibitors of the proteasome pathway interfere with induction of nitric oxide synthase in macrophages by blocking activation of transcription factor NF-kappa B. *Proc Natl Acad Sci USA.* 16; 93(8): 3308-12.
51. **Thomas JA. (1994):** Oxidative stress: Oxidant defense and dietary constituents. In: Maurice E, Shils ME, Olson JA,Shike M, editors. Modern Nutrition in Health and Disease.Challotesville, University of Virginia: Awaverly Company; pp. 501-512.
52. **O'Brien ML, Tew KD (1996):** Glutathione and related enzymes in multidrug resistance. *Eur J Cancer*; 32A: 967–78.
53. **Danesi F, Malaguti M, Nunzio MD, Maranesi M, Biagi PL, Bordoni A. (2006):** Counteraction of adriamycin-induced oxidative damage in rat heart by selenium dietary supplementation. *J Agric Food Chem.* 22; 54(4): 1203-8.
54. **Conklin, KA (2000):** Dietary antioxidants during cancer chemotherapy: Impact on chemotherapeutic effectiveness and development of side effects. *Nutr. Cancer* 37, 1-18.
55. **Signal PK; Siveski-Iiiskovic; Hill, M; Thomas, TP and Li, T (1995):** Combination therapy with probucol prevents adriamycin-induced cardiomyopathy. *J Mol. Cell Cardiol.*, 27: 1055-1063.
56. **Doroshow, JH, Akman, S, Esworthy, S, Chu, FF & Burke, T (1991):** Doxorubicin resistance conferred by selective enhancement of intracellular glutathione peroxidase or superoxide dismutase content, *Free Radical Res. Commun.* 12 – 13 Pt 2:779-81.
57. **Zikić RV, Stajn AS, Ognjanović BI, Saicić ZS, Kostić MM, Pavlović SZ (1998):** The effect of cadmium and selenium on the antioxidant enzyme activities in rat heart. *J Environ Pathol Toxicol Oncol.*; 17(3-4): 259-64.

58. **Holben DH, Smith AM. (1999):** The diverse role of selenium within selenoproteins: a review. *J. Am. Diet. Assoc.*; 99: 836–843.
59. **Dhalla NS, Elmoselhi AB, Hata T, Makino N. (2000):** Status of myocardial antioxidants in ischemia-reperfusion injury. *Cardiovasc Res.* 18; 47(3): 446-56.
60. **Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, and Hoekstra WG. (1973):** Selenium: biochemical role as a component of glutathione per-oxidase. *Science* 179: 588–590.
61. **Pucheu S, Coudray C, Tresallet N, Favier A, de Leiris J. (1995):** Effect of dietary antioxidant trace element supply on cardiac tolerance to ischemia-reperfusion in the rat. *J Mol Cell Cardiol.*; 27(10): 2303-14.
62. **Paranka NS, Dorr RT. (1994):** Effect of doxorubicin on glutathione and glutathione-dependent enzymes in cultured rat heart cells. *Anticancer Res.*; 14(5A): 2047-52.
63. **Hayes, JD, and Pulford DJ (1995):** The glutathione 5-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. *Rev. Biochem. Mol. Biol*. 30: 445-600.
64. **Venugopal, R; Joseph, P and Jaiswal, AK (1997):** Gene expression of DT-diadhorase in cancer cells. In: Oxidative stress and signal induction. (Forman, H.j. and Cadenas, E. eds.), P. 441. Chapman & Hall, New York.
65. **Wang, CJ; Wang, SW; Shiah, HS and Lin, JK (1990):** Effect of ethanol on hepaypyoxicity and hepatic DNA-binding of aflatoxin B1 in rats. Biochem. Pharmacol., 40(4), 715-712.
66. **L'Ecuyer T, Allebban Z, Thomas R, Heide R Vander. (2004):** Glutathione S-transferase overexpression protects against anthracycline-induced H9C2 cell death. Am J Physiol Heart Circ Physiol.; **286:** 2057–2064.
67. **Deepa PR, Varalakshmi P. (2003):** Protective effect of low molecular weight heparin on oxidative injury and cellular abnormalities in adriamycin-induced cardiac and hepatic toxicity. *Chem Biol Interact.* 25; 146(2): 201-10.
68. **Jagetia GC, Reddy TK, Malagi KJ, Nayak BS, Naidu MB, Ravikiran PB, Kamath SU, Shetty PC, Reddy DS. (2005):** Antarth, a polyherbal preparation protects against the doxorubicin-induced toxicity without compromising its Antineoplastic activity. *Phytother Res.*; 19(9): 772-8.
69. **Nohl H, Gille L, Staniek K (1998):** The exogenous NADH dehydrogenase of heart mitochondria is the key enzyme responsible for selective cardiotoxicity of anthracyclines. Z Naturforsch [C], 53, 279–285.
70. **Bulucu F, Ocal R, Karadurmus N, Sahin M, Kenar L, Aydin A, Oktenli C, Koc B, Inal V, Yamanel L, Yaman H.(2009):** Effects of N-Acetylcysteine, Deferoxamine and Selenium on Doxorubicin-Induced Hepatotoxicity. *Biol Trace Elem Res.* 132:184-196.
71. **Newairy AA, El-Sharaky AS, Badreldeen MM, Eweda SM, Sheweita SA. (2007):** The hepatoprotective effects of selenium against cadmium toxicity in rats. Toxicology. 5;242(1-3):23-30.

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Influence of Growth Media Composition on the Emulsifying Activity of Bioemulsifiers Produced by Four Bacterial Isolates with Wide Substrate Specificity

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Abstract: The influence of growth media composition on the emulsifying activity of bioemulsifiers produced by four bacterial isolates were monitored by using three standard growth media formulations of Rosenberg *et al*, 1979, Monticallo *et al*, 1985 and Mills *et al*, 1978 with a common carbon source, Acetate but with varied Nitrogen, Phosphate and Trace element constituents. Three of the four bacterial isolates namely; *Pseudomonas mallei, Pseudomonas pseudomallei* and *Pseudomonas* sp. recorded their highest emulsifying activity of 30.80, 26.30 and 29.10 µ/ml respectively when grown on Rosenberg *et al*, 1979 growth medium with respective pH optimums of 7.09, 7.60 and 7.40 while the last isolate, *Pseudomonas aeruginosa* recorded its highest emulsifying activity of 28.40 µ/ml when grown on Mills *et al*, 1978 growth medium with an optimum pH of 8.11. Monticello *et al*, 1985 growth medium which lack $MgS0_4$ salts recorded lower emulsifying activity in all the four bacterial isolates tested. The results indicate that the three bacterial isolates that grew better on Rosenberg *et al*, 1979 growth medium showed preference for $(NH_4)_2S0_4$ as opposed to $NaN0_3$ as the ideal source of Nitrogen, however the reverse was the case with the isolate that grew better on Mills *et al*, 1978 growth medium which showed preference for $NaN0_3$ as its best Nitrogen source. Magnesium ions and other trace elements constituents of Rosenberg *et al*, 1979 growth medium are suspected to stimulate higher emulsifying activity as the other growth media formulations that lacked them showed lower emulsifying activity.
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1. Introduction

Bioemulsifiers are amphipathic molecules which can be divided into two major groups; (i) Low molecular weight compounds such as glycolipids and phospholipids which lower interfacial tension between hydrophobic liquids and water and thus reduce the energy required for emulsions. (ii) Polymers which stabilise emulsions (Rosenberg, 1986). In the recent years, bioemulsifiers have received increasing attention because of their role in the growth of microorganisms on water insoluble hydrophobic materials such as hydrocarbons and also because of their commercial potential in the cosmetics, food and agricultural industries (Rosenberg, 1986).

The effect of variations of media components such as carbon, nitrogen, phosphate and metal ions on bioemulsifier production have been investigated by several authors. These investigations revealed that different types of microorganisms have preference for different and specific media components for optimum production of bioemulsifiers.

Carbon is a very essential component of media for microbial growth and different microorganisms that produce bioemulsifier have preference for specific sources of Carbon for optimum production of their bioemulsifier. Navon-Venezia *et al*, 1995 demonstrated that the biological activity of a bioemulsifier, Alasan produced by *Acinetobacter radioresistens* was higher when Citrate was used as carbon source than when Acetate or Tris-Hcl buffer was used. With different bacterial isolates, *Pseudomonas mallei* and *Pseudomonas pseudomallei*, Okoro *et al*, 2002 advanced that combination of acetate and diesel seem to be the preferred carbon sources when compared with other carbon sources such as Crude oil, Olive oil, Kerosine and diesel for optimum bioemulcifier production by these organisms.

Nitrogen sources also effect the production of bioemulcifier by microorganisms. Among the inorganic salts tested by Desai and Banat, 1997, Amonium salts and Urea were the preferred nitrogen sources for optimum bioemulsifier production by *Athrobacter paraffineus.* Nechemania and Rosenberg (1983) have also demonstrated the preference of Amonium ions as nitrogen sources in the production of bioemulsifier by *Acinetobacter calcoaceticus* strains. Other authors such as Okoro *et al*, 2002, Moussa *et al*, 2006, Namir *et al*, 2009 and Batista *et al*, 2010 have equally demonstrated maximum production of bioemulsifier when ammonium ions were used as nitrogen sources. On the contrary, other

investigations carried out by Navon-Venezia *et al*, 1995 showed that Urea was the best nitrogen source for the production of a bioemulsifier, Alasan by *Acinetobacter radioresistens*. Similarly, Sifour *et al*, 2005 also demonstrated that Bacillus species showed preference for Urea as the ideal nitrogen source for bioemulsifier production. On the contrary, some other researchers have implicated nitrate as the best nitrogen source for bioemulsifier production. Graziella *et al*, 2010 demonstrated that nitrate was the best nitrogen source for production of bioemulsifier by *Rhodococcus erythropolis*. Guera-Santos *et al*, 1984 also demonstrated the preference of nitrate as an ideal nitrogen source for bioemulsifier production by *Pseudomonas aeruginosa*, same goes with Ramana and Karanth (1989) who advanced that *Pseudomonas aeruginosa* showed more preference for $NaN0_3$ as the best nitrogen source in the production of glycolipid under submerged conditions.

Other media constituents like Phosphate and metal ions also influence the production of bioemulsifier by microorganisms. For instance, in the production of glycolipid bioemulsifier by *Pseudomonas aeruginosa*, it was discovered that using K_2HP0_4 gave 3 fold yield of glycolipid than what was obtained when KH_2P0_4 was used (Ramana and Karanth, 1989). Magnesium ions have also been shown to positively affect the process of emulsification (Sifour *et al*, 2005). Navon-Venezia *et al*, 1995 also demonstrated that Magnesium ions stimulated the activity of Alasan produced by *Acinetobacter radioresistens* over a wide range of pH.

In summary, both past and present investigations relating to the influence of media constituents on bioemulsifier production showed that different microorganisms have preference for different and specific sources of carbon, nitrogen, phosphate and metal ions for optimum production of bioemulsifier. The emulsifying activity of the bioemulsifier produced is very important because it determines to a great extent the strength and quality of the bioemulsifier produced.

In the present study, we investigated the influence of growth media composition on the emulsifying activity of the bioemulsifier produced by four bacterial isolates with wide substrate specificity. Three standard media formulations of Rosenberg *et al*, 1979, Monticallo *et al*, 1985 and Mills *et al*, 1978 were used in this study for the purpose of comparison. The common carbon source used in all the three media was Acetate but sources of nitrogen, phosphate and metal ions varied. We conducted preliminary characterisation of the bioemulcifier produced by these isolates and their various hydrocarbon substrate specificities which ranged from normal alkanes to aromatics and complex hydrocarbon mixtures.

2. Materials and Methods:

Bacterial Isolates:

The four bacterial isolates used in the study namely *Pseudomonas mallei*, *Pseudomonas pseudomallei*, *Pseudomonas aeruginosa and Pseudomonas* sp. were isolated from produced water from Chevron's Escravos tank farm using minimal salts medium of Mills *et al*, 1978 and partial characterisation was done with the aid of the BBL enterotube computerised identification systems as previously described (Okoro, 1999). These isolates were maintained in nutrient agar slants at low temperature (5^0C) and sub-cultured weekly.

Growth Media Composition:

(i) Rosenberg *et al;* (1979): Composition (g/l): NaCl(5), Na_2HP0_4,(13.7), KH_2PO_4(7.26), $(NH_4)_2SO_4$(3), $MgSO_4$(0.4). Trace elements (mg/10ml); $CaCl_2.2H_20$(3.68), $CuS0_4.5H_20$(6.24), $FeS0_4.7H_20$(5.94), $MnS0_4.2H_20$(4.22), $ZnS0_4.7H_20$(7.88), $CoCl_2.6H_20$(6.96).

(ii) Monticello *et al;* (1985): Composition (g/l): KH_2P0_4(4), Na_2HP0_4(4), $(NH_4)_2S0_4$(2), $CaCl_2.2H_20$(0.001), $FeS0_4.7H_20$(0.001).

(iii) Mills *et al*; (1978): Composition (g/l): NaCl(10), KCL(0.29), $MgS0_4.7H_20$(0.42), KH_2P0_4(0.83), Na_2HP0_4(1.25), $NaN0_3$(0.42).

Hydrocarbon substrate specificity of the four bacterial isolates:

The ability of the four bacterial isolates to grow on pure hydrocarbon substrates as sole carbon source were tested on a liquid minimal salts media of Mills *et al*, 1978. All the substrates except the highly flammable ones were autoclaved before use, the flammable ones such as n-alkanes, and kerosene were sterilised by filtration before use. 100mls of the minimal salt media was prepared in a 250ml Erlenmeyer flask and 0.1% hydrocarbon substrate was inoculated followed by the addition of 1ml of the bacterial inoculum from the already prepared nutrient broth and incubation for 48hrs at room temperature. Emulsion turbidity was measures as described in Rosenberg *et al*, 1979.

Determination of Emulsification activity:

The standard emulsification assay of Rosenberg *et al*, 1979 was used in the determination of emulsification activity of the four bacterial cultures used for the studies. The samples to be tested (0.5-0.1ml) were introduced into a 125ml flask containing TM buffer (20Nm Tris-HCL) pH (7.0), 10nM, $MgS0_4$ to a final volume of 7.5ml and then 0.1ml of a 1:1 (v/v) mixture hexadecane and 2-

methylnaphthalene was added. The samples were incubated at 30^0C with reciprocal shaking (160 strokes/min) for 1hr. Turbidity was then determined in a Klett-Summerson photometer (fitted with green filter). One unit of emulsifying activity per millilitre is defined as the amount of biopolymer that yielded 100 Klett units in the assay mixture. Emulsion turbidity was directly proportional to the amount of biopolymer produced.

Partial Biochemical characterisation of Bioemulsifiers;

Lipid Analysis: Thin layer chromatography was carried out on a 20 by 20 cm precoated silica gel plates with petroleum ether, diethyether and acetic acid (90:10:1) as developing solvents. After air drying, the silica gel plates were stained with 5% sulphuric acid in 95% ethanol followed by heating at 15^00C for 30mins. RF values of developed spots were calculated and compared with values of standard compounds in similar solvents as described by Kates (1972).

Protein Analysis: The protein content of the cell extracts was determined by using the method of Bradford (1976). The reagent contained Coomassie blue, 9250 (0.16ml), Percloric acid (5.15ml), and distilled water added to make up to 200ml. The reagent was stirred in a dark bottle overnight and filtered with a Whatman No. 1 filter paper. Protein solution (0.5ml) was added to a 1ml cuvette and 0.5ml of the reagent was added. The absorbance at 620nm was red immediately against a reagent blank made up of 0.5ml of water and Coomassie reagent. The concentration of the protein was extrapolated from the standard curve prepared with bovine serum albumen as a standard.

Carbohydrate Analysis; The carbohydrate content of the bioemulsifier was estimated by using Anthrone method as described by Spirro (1966). 720ml of concentrated sulphuric acid was added to 280ml of distilled water. This was followed by the addition of 500mg of anthrone and 10g of thiourea and mixing till the contents were properly dissolved. The reagent was cooled by storing in a refrigerator 24hrs. before use. A standard curve was prepared by adding 20-200µg of glucose in 1ml of water in pyrex tubes. The test reaction was carried out by adding 1ml of the bioemulsifier extract to 5mls of cold anthrone reagent in a 10ml testube. The tubes were shaken vigorously to ensure complete mixing and this was followed by capping and heating the tubes in a boiling water bath for 15mins and cooling thereafter. The absorbance was red at 620nm against a reagent blank.

SDS-Polyacrylamide Gel Electrophoresis: Polyacrylamide gel (12%) electrophoresis was carried out to determine the molecular weight of proteins as described in Bradford (1976). The following protein makers purchased from sigma chemicals (Sweden) were used as standard makers in the electrophoresis. They include Lysosyme, egg white (14,000Da), β-Lactoglobulin ,Bovine milk(18,400Da), Trypsinogen, Bovine pancreas (24,000DA), Pepsin, Porcine stomach mucosa (34,700Da), Egg albumin (45,000Da), Bovine plasma albumin (66,000Da)

3. Results:

Hydrocarbon substrate specificity of the bioemulsifier produced by four bacterial isolates

Various hydrocarbon substrates were tested on the bioemulsifier produced by the four bacterial isolates to determine their emulsion turbidity. All the four bioemulsifiers tested exhibited very wide substrate specificity ranging from n-Alkanes to aromatics and some complex hydrocarbon mixtures such as Hexadecane+ Methylnaphthalene (1:1), Benzene + Cyclohexane (1:1), Toluene + Cyclohexane (1:1), Olive oil, Kerosine, Diesel oil and Crude oil. Among all the hydrocarbon substrates tested, the highest emulsion turbidity was recorded with crude oil, and closely followed by diesel oil. Lower molecular weight hydrocarbons (n-Alkanes) recorded lower emulsion turbidities than mixtures of complex hydrocarbons. The results are shown in Table 1.

Effects of media constituents on the growth and activity of the bioemulsifier producing bacterial isolates;

The three growth media used in the study include;

1. Rosenberg *et al*, 1979 growth media with the following composition; NaCl (5), Na_2HP0_4,(13.7), KH_2PO_4 (7.26),($NH_4)_2SO_4$ (3), $MgSO_4$ (0.4), Trace elements (mg/10ml); $CaCl_2.2H_20$(3.68), $CuS0_4.5H_20$(6.24), $FeS0_4.7H_20$(5.94), $MnS0_4.2H_20$(4.22), $ZnS0_4.7H_20$(7.88), $CoCl_2.6H_20$(6.96).
2. Monticello *et al*; (1985) growth medium with the following composition; KH_2P0_4(4), Na_2HP0_4(4), $(NH_4)_2S0_4$(2), $CaCl_2.2H_20$(0.001), $FeS0_4.7H_20$(0.001).
3. Mills et al; (1978) growth medium with the following composition; NaCl(10), KCL(0.29), $MgS0_4.7H_20$(0.42), KH_2P0_4(0.83), Na_2HP0_4(1.25), $NaN0_3$(0.42).

Table 1: Hydrocarbon Substrate Specificity of the Bioemulsifier produced by the bacterial isolates used in the present study

Hydrocarbon Substrates	**Emulsion Turbidity (KU) of the Bacterial Cultures**			
ALKANES	***Pseudomonas mallei***	***Pseudomonas pseudomallei***	***Pseudomonas aeruginosa***	***Pseudomonas sp.***
n-Pentane	36	44	56	22
n-Hexane	28	14	32	43
Cyclohexane	12	32	44	32
Decane	22	46	13	38
Pentadecane	110	86	65	120
Hexadecane	88	25	63	65
Octadecane	147	102	132	160
AROMATICS				
Benzene	144	186	88	67
Toluene	22	12	67	88
Xylene	36	82	96	120
Butyl benzene	46	48	75	87
Octyl benzene	76	81	66	43
HYDROCARBON MIXTURES				
Hexadecane+ Methylnaphthalene (1:1)	220	160	180	107
Benzene + Cyclohexane (1:1)	140	260	87	170
Toluene + Cyclohexane (1:1)	33	48	13	66
Olive OIL	320	420	330	440
Kerosine	110	46	260	28
Diesel Oil	440	360	380	280
Crude Oil	760	650	580	460

All the three growth media used for this study have a combination of KH_2P0_4 and Na_2HP0_4 as the source of phosphate. The Nitrogen source however differed, while Rosenberg *et al,* 1979 and Monticello *et al* 1985 media had ($NH_4)_2SO_4$ as their nitrogen source, Mills et al; had $NaN0_3$ as its nitrogen source. Magnesium sulphate salts which is essential for enhancement of emulsification was present in both Rosenberg *et al,* 1979 and Mills et al;1978 growth media but absent in Monticello *et al*; (1985) growth medium. Rosenberg *et al,* 1979 and Mills et al; (1978) growth media used NaCl salts while Monticello *et al*; (1985) growth medium used $CaCl_2$ salts. Rosenberg *et al,* 1979 growth media in addition fortified its growth media with some trace elements such as (mg/10ml); $CaCl_2.2H_20$(3.68), $CuS0_4.5H_20$(6.24),$FeS0_4.7H_20$(5.94),$MnS0_4.2H_20$(4.22), $ZnS0_4.7H_20$(7.88), $CoCl_2.6H_20$(6.96). All these media components in one way or the other influenced the emulsification activity of the bioemulsifier produced by the four bacterial isolates.

The emulsification activity of the bioemulsifier produced by the four bacterial isolates showed that *Pseudomonas mallei, Pseudomonas pseudomallei and Pseudomonas sp.* all grew better in Rosenberg *et al,* 1979 growth medium and the emulsification activity of the bioemulsifier produced were 30.80, 26.30 and 29.10 µ/ml respectively with respective final pH values of 7.09, 7.11 and 7.40. The fourth organism *Pseudomonas aeruginosa* however grew better in Mills *et al*, 1978 growth medium and the bioemulsifier produced had an emulsification activity of 28.40 µ/ml at a final pH of 8.11. The detailed results are shown in Table 2.

Partial biochemical characterisation of the bioemulsifiers produced by the four bacterial isolates

Partial biochemical characterisation of the bioemulsifiers produced by the four bacterial isolates showed that *Pseudomonas mallei* and *Pseudomonas pseudomallei* showed presence of carbohydrate and protein moieties with no trace of lipids. The respective concentrations of proteins and carbohydrates of the bioemulsifier produced by *Pseudomonas mallei* were 20.77 and 413g/L while that of *Pseudomonas pseudomallei* were 27.23 and 242g/L. The protein moieties of the two bacterial isolates had a molecular weight of about 34,700Da suggesting that the two bacterial isolates might be closely related. They were tentatively classified as glycoproteins.

Table 2: Emulsification Activity of the Bioemulsifier Producing Bacterial Isolates Using Different Growth Media

BACTERIAL CULTURES	GROWTH MEDIA	Final pH	Turbidity (A_{600})	Activity (µ/ml)
Pseudomonas mallei	*Rosenberg et al; (1979)*	7.09	1.42	30.80
	Monticello et al; (1985)	7.25	0.88	27
	Mills et al; (1978)	8.71	0.63	7.20
Pseudomonas pseudomallei	*Rosenberg et al; (1979)*	7.11	0.99	26.30
	Monticello et al; (1985)	7.60	0.68	14.10
	Mills et al; (1978)	8.54	0.64	1.90
Pseudomonas aeruginosa.	*Rosenberg et al; (1979)*	7.11	1.32	23.60
	Monticello et al; (1985)	7.20	1.40	26.80
	Mills et al; (1978)	8.11	1.60	28.40
Pseudomonas sp.	*Rosenberg et al; (1979)*	7.40	1.38	29.10
	Monticello et al; (1985)	7.80	1.23	16.60
	Mills et al; (1978)	8.12	1.30	21.10

The two other bacterial isolates; *Pseudomonas aeruginosa* and *Pseudomonas* sp. produced bioemulsifiers with considerable concentrations of carbohydrate and lipid moieties but no trace of protein. The respective concentrations of carbohydrates and lipids in the two bacterial isolates were 312:12.6g/L and 286:16.8g/L. The bioemulsifier produced by the two bacterial isolates were tentatively classified as glycolipids.

4. Discussion

The data presented in this paper showed that media constituents can influence the emulsification activity of the bioemulsifier producing bacterial isolates used in the study. With Rosenberg *et al*, 1979 growth media, *P. mallei*, *P. pseudomallei* and *Pseudomonas* sp. grew better in it than the other two and the bioemulsifier produced had an emulsification activity of 30.8, 26.30 and 29.10 µ/ml respectively. Comparatively, the same three group of bacterial isolates grew poorly on Monticello *et al*, 1985 growth medium with respective emulsification activity values of 27, 14.10 and 16.60. It should be noted that both Rosenberg *et al*, 1979 growth medium and Monticello *et al;* (1985) growth medium had a common nitrogen source $(NH_4)_2S0_4$ but differed in the presence of magnesium salts and other essential trace elements which were present in Rosenberg *et al*, 1979 growth medium but lacking in Monticello *et al*; (1985)growth medium. The presence of magnesium salts and other essential trace elements in Rosenberg *et al*, 1979 growth medium must have contributed in the enhancement of the emulsification activity of the bioemulsifier produced. Some investigators like Sifour *et al*, 2005 and Batista *et al*, 2010 have demonstrated that magnesium salts and other trace elements positively affect the emulsification activity process.

On the contrary, *Pseudomonas aeruginosa* grew better on Mills *et al*, 1978 medium than in Rosenberg *et al*, 1979 and Monticello *et al*; (1985)growth media. It should be noted that the basic difference between the three growth media under investigation was the source of nitrogen. Whereas Mills *et al*, 1978 growth medium uses nitrate as its nitrogen source, Rosenberg *et al*, 1979 and Monticello *et al*; (1985) growth media uses $(NH_4)_2S0_4$ as their preferred nitrogen source. Despite the fortification of Rosenberg media with trace elements, *Pseudomonas aeruginosa* still preferred nitrate as its ideal nitrogen source for optimal production of its bioemulsifier. Many investigators have reported that *Pseudomonas aeruginosa* have always shown preference for nitrate as its best nitrogen source (Ramana and Karanth, 1989, MacElwee and Treros, 1990, Guero-Santos *et al*, 1984, and Robert *et a*l, 1989). All the other bacterial isolates that grew better on Rosernberg *et al*, 1989 growth medium however showed preference for $(NH_4)_2S0_4$ salts as opposed to $NaN0_3$ as the preferred nitrogen source.

Partial biochemical characterisation of the bioemulsifier produced by the four bacterial isolates showed that *P. mallei* and *P. pseudomallei* produced the glycoprotein type of bioemulsifier while *P. aeruginosa* and *Pseudomonas* sp. produced the glycolipid type. The glycoprotein bioemulsifier produced by *P. mallei* and *P. pseudomallei* though have not been reported widely in literature are very potent and have been used in the past to enhance the remediation of hydrocarbon contaminated mangrove swamp in the Nigerian oil rich Niger Delta (Okoro, 2009). The glycolopids produced by *P. aeruginosa* and *Pseudomonas* sp. are one of the commonest type of bioemulsifier and have been reported widely in literature (Javis and Johnson, 1949, Hisatsukka *et al*,

1971, Itoh and Suzuki, 1972, Ramana and Karanth, 1989 and Guerra Santos *et al*, 1984.

5. Conclusion

In conclusion, the present study have clearly demonstrated that some essential components of growth media have specific influence on the emulsification activity of the bioemulsifier produced by the four bacterial isolates used in the present study, while some components of the media depending on the type of bacterial isolate influenced bioemulsifier production and its emulsification activity positively, the reverse was the case with other media components. The present study have clearly established that magnesium salts and other essential trace elements in Rosenberg *et al*, 1979 growth media influenced the emulsification activity positively. *Pseudomonas aeruginosa* also proved its consistency on the use of nitrate as its best nitrogen source.

In summary, all the bioemulsifier produced by the four bacterial isolates used in the present study had very wide substrate specificity. They emulsified considerably a variety of water in oil emulsions, including n-Alkanes, aromatics, complex hydrocarbon mixtures, crude oil, olive oil, diesel oil and kerosene. With the ideal media components, the emulsification activity of the produced bioemulsifier can be further enhanced for commercial application.

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References:

1. **Batista, R. M., Rufino, R. O., Luna, J. M., de-Souza , J. E and Sarubbo, L. A.** 2010. Effect of medium components on the production of a biosurfactant from *Candida tropicalis* applied for the removal of hydrophobic contaminants in soil. Water Environ. Research. 85(5):418-425.
2. **Bradford, M. M**. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principles of dye binding. Anal. Biochem. 72: 248-254.
3. **Desai, J.D. and Banat, I. M. 1997.** Microbial production of biosurfactants and their commercial potential. Microbiol. Mol. Biol. Rev. 61(1):47-64.
4. **Graziela, J. P., Elisa, M. P. C, Edelvio, B.G. and Nei, P.G**. 2010. Biosurfactant production by *Rhodococcus erythropolis* and its application in oil removal. Braz. J. Microbiol. 41(3):110-115.
5. **Guerra-Santos, L H., Kappeli, O and Fiechter, A**. 1984. *Pseudomonas aeruginosa* biosurfactant production in continious culture with glucose as the carbon source. Appli. Environ. Microbiol. 48:301-305.
6. H**isatsuka, K. T., Nakahara, T., Sano, N and Yamada, K**. 1971. Formation of rhamnolipids by *Pseudomonas aeruginosa*; Its function and hydrocarbon fermentation. Agric. Biol. Chem. 35: 686-692.
7. **Itoh, S., Honda, H., Tomita, F and Suzuki, T. 1971.** Rhamnolipids produced by *Pseudomonas aeruginosa* grown on n-paraffin. J. Antibiotic. 24: 855-859.
8. **Itoh, S and Suzuki, T**. 1972. Effect of Rhamnolipid on growth of *Pseudomonas aeruginosa* mutant deficient in n-paraffin utilizing ability. Agric. Biol. Chem. 36: 2233-2235.
9. J**arvis, F. G and Johnson, M. J.** 1949. A glycolipid produced by *Pseudomonas aeruginosa*. J. Am. Chem. Soc. 71: 4124-4126.
10. Kates, M. 1972. Techniques in Lipidology. Nothern Holland Publishing Co. Newyork.610pp.
11. M**acElwee, C.G.H and Trevos, J.T.** 1990. Production of extracellular emulsifying agent by *Pseudomonas aeruginosa.* UG-1. J. Ind. Microbiol. 5: 25-52.
12. **Mills, A. L., Breuil C and Colwell, R. R.** 1978. Enumeration of petroleum degrading marine and estuarine microorganisms by the most probable number method. Can. J. Microbiol. 24: 552-557.
13. **Monticello, D. J. And Finnerty, W. R** 1985. Plasmid degradation of Dibenzothiophene by *Pseudomonas sp*. Appl. Environ. Microbiol. 49: 756-760.
14. **Moussa, A., Ahmed, G.M., Abdel-Hamid**, S and Sheren, M. 2006. Optimisation of cultural conditions for biosurfactant production by *Nocardia amarae.*
15. **Namir, I. A., Wang, J.I and Bozhong, M. U**. 2009. Identification of Biosurfactant producing strain. *Bacillus Subtilis*. HOB2. Protein and Peptide letters. 16: 7-13.
16. **Navon-Venezia S., Zosim, Z., Gottlieb, A., Legmann, R., Carmeli, S., Ron, E.Z and Rosenberg E.** 1995. Alasan, a new bioemulsifier

from *Acinetobacter radioresistens. Appl. Environ. Microbiol. 61(9):3240-3244.*

17. **Nechmia S and Rosenberg, E.** 1983. Emulsifier production by *Acinetobacter calcoaceticus* strains. Current Microbiol. 9:309-314.
18. **Okoro, C. C**. 1999. Microbial degradation of hydrocarbons in produced water from crude oil production operations in Escravos tank farm. Ph.D. Thesis. University of Lagos, Nigeria. 269pp.
19. **Okoro, C.C., Amund, O. O and Rosenberg E.** 2002. The effect of Nitrogen and Carbon sources on the ability of two microbial isolates to produce bioemulsifiers. J. Sci. Tech. Environ. 2(1): 1-8.
20. **Okoro, C .C. 2009.** Biosurfactant enhanced remediation of hydrocarbon contaminated mangrove swamp. Int. J. Biol. Chem. Sci. 3(1):63-74.
21. **Ramana, K. V and Karanth, N.G.** 1989. Factors affecting biosurfactant production using *Pseudomonas aeruginosa* under submerged conditions. J. Chem. Tech. Biotech. 45: 249-257.
22. **Robert, M., Mercarde, M.E., Bosch, M.P., Para, J. L, Espunny, M.J., Mansera, M.A and Guinea, J**. 1989. Effect of Carbon source on biosurfactant production by *Pseudomonas aeruginosa* 44T. Biotechnol. Lett. 11:871-874.
23. **Rosenberg, E**. 1986. Microbial surfactants. Critical Review. Biotechnol. 3:109-132
24. **Rosenberg, E., Zuckerberg, A., Rubinovitis C and Gutnick, D.L.** 1979. Emulsifier of Arthrobacter RAG-1. Isolation and Emulsifying properties. Appl. Environ. Microbiol. 37:407-413.
25. **Sifour, M., Qulen-Hadder, H and Aziz, G.M.** 2005. Production of biosurfactants from two bacillus species. Egypt. J. Aqua. Res. 31: 142-148.
26. **Spiro, R.G.** (1966). Analysis of Sugars found in glycoproteins. Methods in Enzymology. 8:7-9

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Study of the Cardiovascular Effects of Exposure to Electromagnetic Field

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Abstract: This study was conducted to throw light on electromagnetic radiofrequency (EMR) emitted from cell phones which was accused of causing a number of negative health effects in the form of influencing on the heart and circulatory system.

110 adult albino rats, of both sexes, weighing 180- 200 gms were used in the present study. Animals were allocated into two main groups: group I, including rats exposed to cell phone EMF for 4 weeks; and group II, including rats exposed to EMF for 8 weeks. Each group was further subdivided into four subgroups, a control group and three subgroups exposed to EMF for either 1h/day, 2hrs/day or 3hrs/day, exposure being carried out six days/ week, at fixed time of the day. All rats were subjected to measurement of the systolic blood pressure on the day prior to the day of sacrifice, ECG recording, assessment of cardiac weights, absolute& relative, and MDA level in cardiac tissue, as well as determination of plasma renin activity, plasma total antioxidant capacity and plasma calcium level. Specimens from the apex of the heart were subjected to histopathological examinations.

Obtained results revealed that systolic blood pressure was significantly increased in all EMF-exposed rats compared to their respective controls. The heart rate, deduced from the ECG tracings, was non-significantly altered in all groups exposed to EMF for 4 weeks and in the 8 weeks-1hr/day exposure group, but was significantly reduced in rats exposed to EMF for 2hrs or 3hrs/day for 8 weeks. The ECG recording of rats exposed to EMF for 4 weeks revealed a significantly higher R voltage in the group exposed for 3hrs/day, a significant increase in QRS duration in the groups exposed for 2hrs and 3hrs/day and significant prolongation of QT-c interval in the group exposed for 3hrs/day. On the other hand, the ECG recording of rats exposed to EMF for 8 weeks revealed significantly higher R and T voltages, and significantly prolonged P-R and QT-c intervals in the groups exposed for 2hrs or 3hrs/day, the QRS duration being significantly increased in all the 8 weeks- exposed groups. In addition, a significant increase in the absolute and relative weights of the whole heart and of the left ventricle in rats exposed to EMF 2hrs or 3hrs/day for either 4 or 8 weeks was obtained. Plasma renin activity was increased in all exposed rats, the increase being statistically significant in rats exposed to EMF 3hrs/day for 4 weeks, and in all the groups exposed to EMF for 8 weeks. Plasma calcium level was significantly decreased in all the exposed groups except for the group exposed for 1hr/day for 4 weeks. The plasma total anti-oxidant capacity was significantly decreased in all exposed groups, for either 4 or 8 weeks, while the MDA level in the cardiac tissue was only significantly elevated in the 8 weeks-3hrs/day exposed group compared to the matched control group. The histopathological examination revealed hypertrophy, fragmentation and vacuolation of the myocardium, which were directly proportional to the exposure time.

On conclusion, long-term exposure to cell phone EMF increases the liability for hypertension reflected on the ECG and cardiac weights which is accompanied by histopathological changes in the myocardium. In addition, an interaction of EMF with biological functions was achieved in the form of increased PRA, decreased plasma total antioxidant capacity and hypocalcemia.

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Key words: electromagnetic field- cell phone- cardiac effects- oxidative stress.

1. Introduction:

Electromagnetic fields (EMFs) permeate our environment everywhere: in our homes, at work, in schools, and elsewhere wherever there are electric wires, electric motors and electronic equipments [(1)].

The radiofrequency (RF) emitted from the recently introduced digital global system mobile communications (GSM) is around 900-1800 MHZ. [(2,3)], the emission of radiofrequency waves from the GSM phones being continuous and not in pulses as the old analog mobile phones [(4)].

Although the amount of electromagnetic energy due to cell phones is quite small in comparison to other radiofrequency sources, the increased use of

wireless mobile phones worldwide (3.8 billion mobile users) has focused interest on its possible side effects, and the potential health impacts [5].

The biological effects of exposure to EMF from mobile phones were reported to be variable, depending on many factors including duration of exposure, distance from the various sources, species and tissues as well as the conditions of exposure [6,1,7].

A variety of negative health effects have been attributed to exposure to radiofrequency electromagnetic field (RF-EMF) from mobile phones, such as cold and flu-like symptoms and electromagnetic hypersensitivity [8,9], reduced sperm quality and therefore male infertility [10,11,12], memory and sleep problems [13], behavioral changes in children who had been exposed prenatally to RF [14], the development of brain tumors [15,16] as well as inner ear damage with long-term use [17]. Also, autonomic control of the heart was altered [18,19] and the carcinogenic potential of RF radiation was achieved [20].

Recently, it was reported that RF radiation from mobile phones could alter intracellular signaling pathways through changes in Ca^{2+} permeability across cell membranes and cellular calcium levels [21].

With regard to the cardiovascular effects of EMF emitted by cell phones, EMF might interfere with work of cardiac pacemakers and other implantable medical devices like cardioverter defibrillators [22,23,24]. Mobile phones were reported to cause a rise of blood pressure of 5-10 mm Hg each time of exposure, and it was suggested that cell phone-EMR could induce constrictive effect on blood vessels [25]. Also, cell phones could increase the blood pressure (BP) and heart rate (HR) among healthy adults [26]. In addition, an increase in blood pressure in rats upon exposure to mobile phone EMF was detected [27]. Moreover, an increase in foetal and neonatal heart rate and decrease in cardiac output were found during subjecting pregnant women to cell phones [28]. Furthermore, it was suggested that EMF emitted by cell phones would influence the autonomic tone, thus modifying the function of the circulatory system [29]. However, other study mentioned that changes in heart rate and in arterial blood pressure were independent of the EMF resulting from the use of 900 MHz mobile phones [30].

This study aimed to throw more light on the cardiovascular impact of RF-EMF emitted from mobile phones, and to probe the effect of changes in duration of exposure on the resulting effects.

2. Materials and Methods

Animals:

The current study was carried out on 110 albino rats, of both sexes, weighing 180-200 gms. Rats were purchased from the Research Institute of Ophthalmology (El-Giza), and maintained in the Physiology Department Animal House under standard conditions of boarding and feeding, with free access to water.

Animals were allocated into 2 main groups of equal rat number, based on the duration of exposure to the electromagnetic field (EMF): group I, including rats exposed to EMF for 4 weeks, and group II, including rats exposed to EMF for 8 weeks.

Each group was further subdivided into 4 subgroups:

***Control group** {group I_C (n=13) and group II_C (n=13)}, including rats not exposed to the cell phone EMF, and kept in the animal house until the day of sacrifice.

***One hour/day-exposed group** {group I_1 (n=14) and group II_1 (n=14)}, including rats exposed to the cell phone EMF 1hr /day, 6 days / week for either 4 weeks or 8 weeks. [Total exposure time for group I_1=24 hours, and for group II_1=48 hours].

***Two hours/day-exposed group** {group I_2 (n=14) and group II_2 (n=14)}, including rats exposed to the cell phone EMF 2hrs /day, 6 days / week for either 4 weeks or 8 weeks. [Total exposure time for group I_2=48 hours, and for group II_2=96 hours].

***Three hours/day-exposed group** {group I_3 (n=14) and group II_3 (n=14)}, including rats exposed to the cell phone EMF 3hrs /day, 6 days / week for either 4 weeks or 8 weeks. [Total exposure time for group I_3=72 hours, and for group II_3=144 hours].

Exposure Technique:

Test rat groups were exposed to radiofrequency electromagnetic field (RF-EMF) of 1800 MHz frequency band mobile phone (Nokia 1208 model), which, according to the GSM, operates with microwave carrier frequencies in the range 900-1800 MHZ [31,32]. The exposure was done in special plastic cages, the cell phone being placed under the cage at a distance 0.5 cm below the undersurface of the cage [33,34], and the cell phone was kept in the ringing position, receiving calls from another phone during hours of EMF exposure, but in silent mode, during the whole time of exposure. The intensity of the EMF radiated from the cell phone was 2.2 milli Gauss (10^{-7} Tesla) at the center of the exposure cage, as measured by Gauss/Teslameter, 4048, USA *(Courtesy of the Biophysics Department, Faculty of Science, Ain Shams University)*.

RF-EMF exposure was carried out at a fixed time of the day.

Methods:

On the day prior to the day of sacrifice, the systolic blood pressure of all the control and test

animals was measured, using rat tail blood pressure monitor (Harvard apparatus) *(Courtesy of the Physiology Department, Faculty of Medicine, Cairo University).*

On the day of sacrifice, overnight fasted rats were weighed and anaesthetized with i.p. injection of pentobarbitone sodium, in a dose of 40 mg/kg B.W. Rats were subjected to ECG recording, using the ECG recorder Cardimax FX-2111(Fukuda Denshi Co., Ltd., Japan). All leads were established by subcutaneous needle electrodes. From lead II - ECG tracing, the heart rate, the voltages of P, R and T waves, as well as the QRS duration and the durations of P-R and Q-T intervals were calculated. Corrected QT interval (QT-c) was calculated according to Goldschlager and Goldman [35]**:**

$$QT\text{-}c = \frac{\text{Q-T interval (in seconds)}}{\sqrt{\text{R- R interval (in seconds)}}}$$

After the ECG monitoring, an incision was made in the anterior abdominal wall, and the abdominal aorta was exposed and cannulated. A blood sample was collected in a tube containing EDTA, that was centrifuged at 10000 rpm for 10 minutes, and the supernatant plasma used for determination of plasma renin activity. A second blood sample was collected in a heparinized tube, centrifuged at 3000 rpm for 15 minutes, and plasma obtained was used for measurement of plasma calcium level and total antioxidant capacity.

Handling of the Hearts:

After blood collection, all hearts, from both control and test groups, were isolated, washed in saline, dried by filter paper, cleaned of fat and fibrous tissue, and then weighed as a whole in 5-Digit-Metler balance (Sartorious AG, BL-210S), and the weight expressed as absolute value (in mg). Then, the atria were separated together, the right ventricular wall was peeled evenly and the remaining was the left ventricle plus the septum, and each of these cardiac chambers was weighed and their absolute weights recorded (in mg.). The relative heart (or chamber) weight / body weight (mg/gm) was calculated for each specimen. A cut section of left ventricle was weighed and stored frozen at -80°C for subsequent MDA determination in the cardiac tissue. Specimens of the apices of the hearts were subjected to histopathological examination.

Determination of Plasma Renin Activity (PRA) was performed by radioimmunoassay according to the method described by Malvano et al. [36]**,** using kits supplied by DiaSorin, USA. PRA was calculated as ng angiotensin I generated /ml/hour.

Determination of Plasma Calcium level was determined according to the method described by Cali et al. [37], using a colorimeter (Unico, 7200 series, Shanghi, China) at wave length 570 nm. The colorimetric kit was supplied by Teco Diagnostics, Anaheim with.

Determination of Total Antioxidant Capacity in Plasma was performed according to the method described by Koracevic et al. [38], using kits supplied by Biodiagnostic- Egypt, and depending on colorimetric technique (by using spectrophotometer of Unico, 7200 series, Shanghi, China) at wave length 505 nm.

MDA Determination in the Cardiac Tissue

Cardiac tissues, stored frozen at -80°C till the day of MDA determination, were homogenized according to Eissa et al. [39], using the homogenizer Karl Kolb (scientific technical supplies D−6072, Dreieich, West Germany). The homogenization buffer (pH 7.2) consisted of 0.32 mmol/L Sucrose, 20 mmol/L N−2 hydroxyethyl piperzine N−2 ethan sulfonic acid (HEPES), 0.5 mmol/L Ethylene diamine tetra-acetic acid (EDTA), 1 mmol/L 1, 4 Dithio DL-threitol (DTT), 1 mmol/L Phenyl methane sulfonyl floride (PMSF)(Sigma). One ml buffer was added for each 0.1 gm tissue. After homogenization, samples were centrifuged at 3000 rpm for 10 min., and MDA in the supernatant was determined according to the technique of Esterbauer and Cheeseman [40]**,** in which MDA in the sample reacts with thiobarbituric acid in the reagent, and the produced color read at wave length 535 nm. The obtained concentrations of MDA were then divided by 1000, the results being expressed in µmol/ gm wet tissue.

Histopathological Examination:

Specimens from the apices of the heart were fixed in 10% buffered neutral paraformaldehyde solution. Tissues were sectioned at 5µm, stained with H&E and examined using light microscope [41].

Statistical analysis:

Student's "t" test for unpaired data was used to assess the statistical significant differences between groups. All statistical data and statistical significance were performed by using SPSS statistical package (SPSS Inc.) version 16.0.0. A probability of $P < 0.05$ was considered as significant.

3. Results:

Changes in Heart rate and Systolic Blood Pressure:

As shown in Table 1 and Fig. 1-A; the heart rate, deduced from the ECG recording, was significantly reduced in the 8 weeks-2hrs and 3hrs/day EMF-exposed groups (groups II_2&II_3) compared to their matched control group ($P < 0.005$ and < 0.05 respectively).

On the other hand, in table 1 and fig.1-B, the systolic blood pressure was significantly increased in all EMF-exposed groups compared to their respective controls ($P < 0.001$ for all). The systolic blood pressure was significantly higher ($P < 0.005$) in the 8 weeks-3hrs/day exposed rats (group II_3) compared to the corresponding 4 weeks-3 hrs /day exposed group (group I_3).

ECG Changes:

As shown in table 2 and Figs 2-4, rats exposed to EMF for 4 weeks, the ECG revealed significant ($P < 0.02$) increase in R voltage in the 3 hrs/day exposure group as well as significant ($P < 0.05$ for both) increase in QRS duration in the 2 hrs and 3 hrs/day exposure groups and significant ($P < 0.05$) prolongation of $QT\text{-}_C$ interval in the 3 hrs/day exposure group compared to their matched control values. On the other hand, the ECG tracing of rats exposed to EMF for 8 weeks revealed significant increase ($P < 0.005$, < 0.002, < 0.01 and < 0.01 respectively) in R wave and T wave voltages in both the 2 hrs and 3 hrs /day exposure groups, together with significant increase in QRS duration in all exposed groups ($P < 0.005$ for group II_1 , < 0.005 for group II_2 and < 0.001 for group II_3) and significant prolongation of P-R and $QT\text{-}_C$ intervals in the 2 hrs and 3 hrs/day exposure groups ($P < 0.01$, < 0.001, < 0.05 and < 0.02 respectively).

Compared to the 4 weeks exposure groups, the ECG of the corresponding 8 weeks exposure groups revealed significant ($P < 0.05$ for both) increase in the P wave and T wave voltages in the 2 hrs/day exposure group, as well as significant ($P < 0.001$ and < 0.002 respectively) prolongation of P-R interval and increase of T voltage in the 3 hrs/day exposure group.

Changes in Absolute and Relative Cardiac Weights:

In table 3, rats exposed to EMF for 4 weeks, the absolute weight of the whole heart and its relative weight to body weight were both increased significantly ($P < 0.001$ and < 0.05 for group I_2 and < 0.001 for both for group I_3) in the 2 hrs and 3 hrs /day exposure groups compared to the matched control values. Likewise, the absolute left ventricular weight and its relative weight to body weight were both significantly ($P < 0.001$ and < 0.01 for group I_2 and < 0.001 for both for group I_3) increased in the 2 hrs and 3 hrs/day exposure groups compared to the control group.

In rats exposed to EMF for 8 weeks, the relative whole heart to body weight as well as absolute and relative left ventricular weight were all significantly increased whereas the absolute and relative right ventricular weight was significantly reduced in the 2 hrs/day exposure group compared to the control values ($P < 0.001$ for WH/BW, < 0.01 for the absolute LV weight, < 0.001 for LV/BW, < 0.001 for absolute RV weight and < 0.01 for RV/BW). The absolute and relative whole heart weight, and the absolute and relative left ventricular weight were significantly increased and the absolute right ventricular weight was significantly decreased in the 3 hrs/day exposure group compared to the control group ($P < 0.05$ for whole heart weight, < 0.001 for WH/BW, < 0.005 for left ventricular weight, < 0.001 for LV/BW and < 0.05 for the absolute right ventricular weight).

Compared to the corresponding 4 weeks exposure groups, the relative whole heart and left ventricular weights were both significantly higher and absolute right ventricular weight was significantly reduced in the 2 hrs/day exposure group ($P < 0.01$ for WH/BW, < 0.01 for LV/BW and < 0.02 for absolute right ventricular weight).

Changes in Plasma Renin Activity (PRA):

In table 1 and fig.1-C, compared to the respective control values, PRA was significantly ($P < 0.01$) increased in the 4 weeks-3 hrs/day EMF-exposed rats and in all groups exposed to EMF for 8 weeks ($P < 0.02$ for groups II_1 & II_2 and < 0.002 for group II_3). PRA was significantly ($P<0.005$) higher in the 8 weeks- 1 hr/day exposed rats compared to the corresponding 4 weeks-1 hr/day exposed group.

Changes in Plasma Calcium:

In table 1 and Fig.1-D, compared to the matched controls, plasma calcium level was significantly reduced in all EMF-exposed groups except for the 4 weeks-1hr/day exposed group ($P < 0.05$ for group I_2, < 0.02 for group I_3 , < 0.05 for group II_1, < 0.005 for group II_2 and < 0.002 for group II_3).

Changes in Plasma Total Antioxidant Capacity and Cardiac Tissue MDA:

As shown in table 1 and fig.1-E, the plasma total anti-oxidant capacity was significantly decreased in all exposed rats compared to their respective control groups ($P < 0.005$ for groups I_1 & I_2 and < 0.001 for all the other groups).

In the 8 weeks exposed groups, cardiac tissue MDA level showed an increase, which was only statistically significant in the 8 weeks-3hrs /day

exposed group, compared to the matched control group (P < 0.01) (Table 1; Fig. 1-F).

Histopathological Changes:

As shown in fig.5-a, b & c, the wall of the apical region of the left ventricle of the control groups revealed regularly arranged cardiac muscle fibers, appearing branching, anastomosing and running in various directions. The myocardial cells were attached end to end. The nuclei appeared central and vesicular, and the sarcoplasm appeared acidophilic and striated. In transverse section, the cardiac myocytes appeared more or less comparable in size with noticeable myofibrillar content.

The cardiac muscle specimens of rats exposed to EMF for 1hr and 2 hrs/day for 4 weeks exhibited congestion of blood vessels without apparant affection of cardiac myocytes (Fig.6-a). In rats exposed for 3hrs/day for 4 weeks as well as in all the 8 weeks exposure groups, apparent hypertrophy of many of the cardiac myocytes, with deeply acidophilic sarcoplasm and vesicular nuclei, (Fig.6-b&c), was detected, accompanied by mononuclear cellular infiltration of the cardiac muscle (Fig. 7-a). However, distortion of some cardiac myocytes, together with some areas of complete degeneration and fragmentation of the cells was observed (Fig.7-b). Loss of the regular arrangement of the cardiac myocytes was observed in the 8 weeks-2 hrs and 3 hrs /day exposure groups (Fig.8-a), together with marked cell vacuolation, particularly in the 8 weeks-3hrs/day EMF-exposed group (Fig.8-b).

Table (1): Changes in heart rate (beats/min), systolic blood pressure (mmHg), plasma renin activity (ng/ml/hr), plasma calcium level (mg/dL) total antioxidant capacity in plasma (mM/L) and cardiac tissue MDA level (µmol /gm wet tissue) in the different studied groups.

Group		HR (beats/min)	Systolic Blood Pressure (mmHg)	Plasma Renin Activity (ng/ml/hr)	Plasma Calcium Level (mg/dL)	Plasma Total Antioxidant Capacity (mM/L)	MDA in Cardiac Tissue (µmol /gm wet tissue)
4 week Exposure Groups	Control gr.	396 ±7.58 (14)	122.46 ±2.48 (13)	102.79 ±15.48 (11)	10.42 ±0.2 (11)	2.66 ±0.02 (13)	0.71 ±0.07 (12)
	1hr exposure gr.	408 ±15.05 (14)	153.71 ±2.41 (14)	117.22 ±18.7 (12)	10.04 ±0.17 (12)	2.45 ±0.03 (14)	0.65 ±0.06 (11)
	P	NS	<0.001	NS	NS	<0. 005	NS
	2hr exposure gr.	394 ±22.02 (14)	168.07 ±0.99 (14)	147.85 ±17.88 (13)	9.91 ±0.17 (14)	2.44 ±0.03 (14)	0.66 ±0.07 (8)
	P	NS	<0.001	NS	<0.05	<0. 005	NS
	3hr exposure gr.	404 ±15.12 (14)	176.36 ±1.89 (14)	168.36 ±23.58 (13)	9.87 ±0.15 (14)	2.09 ± 0.1 (14)	0.67 ±0.03 (10)
	P	NS	<0.001	<0.01	<0.02	<0. 001	NS
8 week Exposure Groups	Control gr.	446 ±16.31 (14)	118 ±4.32 (13)	128.22 ±15.42 (13)	10.31 ±0.17 (13)	2.67 ±0.01 (13)	0.43 ±0.08 (9)
	1hr exposure gr.	455 ±15.88 (14)	152.79 ±3.21 (14)	187.26 ±12.52 (13)	9.87 ±0.12 (14)	2.38 ±0.02 (14)	0.65 ±0.09 (10)
	P	NS	<0.001	<0.02	<0.05	<0. 001	NS
	P*	<0. 05	NS	<0.005	NS	NS	NS
	2hr exposure gr.	376 ±15.37 (14)	171.29 ±3.18 (14)	189.4 ±14.11 (13)	9.69 ±0.14 (14)	2.36 ±0.03 (14)	0.68 ±0.13 (9)
	P	<0. 005	<0.001	<0.02	<0.005	<0. 001	NS
	P*	NS	NS	NS	NS	NS	NS
	3hr exposure gr.	395 ±17.84 (14)	187.79 ±2.88 (14)	209.3 ±13.89 (13)	9.56 ±0.11 (12)	2.06 ±0.07 (14)	0.81 ±0.15 (9)
	P	<0. 05	<0.001	<0.002	<0.002	<0. 001	<0. 01
	P*	NS	<0.005	NS	NS	NS	NS

In parenthesis is the number of rats studied in each group. Values are expressed as means ±SEM.
P: Significance by LSD at P <0.05 from control group.
P*: Significance by LSD at P <0.05 of 8 week groups from respective 4 week groups.
NS: Not significant. gr.: group.

Table (2): Changes in ECG waves and segments in the different studied groups.

Group		P wave Voltage (µV)	PR interval (msec.)	R voltage (µV)	QRS duration (msec.)	T wave voltage (µV)	QT-c Interval (msec.)
4 week Exposure Groups	Control gr. (13)	107.69 ±5.21	46.15 ±2.67	584.62 ±38.97	30.77 ±2.88	207.69 ±12.46	217.15 ±8.57
	1hr exposure gr. (14)	114.29 ±6.27	44.29 ±2.28	607.14 ±50.78	34.29 ±2.51	214.29 ±12.21	240.79 ±11.53
	P	NS	NS	NS	NS	NS	NS
	2hr exposure gr. (14)	107.14 ±10.29	47.14 ±2.66	635.71 ±35.71	37.86 ±1.14	217.86 ±17.86	237.93 ±9.81
	P	NS	NS	NS	<0. 05	NS	NS
	3hr exposure gr. (14)	103.57 ±6.34	44.29 ±2.28	728.57 ±46.21	37.86 ±3	192.86 ±4.85	246.29 ±8.68
	P	NS	NS	<0. 02	<0. 05	NS	<0. 05
8 week Exposure Groups	Control gr. (13)	115.38 ±6.66	43.08 ±2.08	546.15 ±24.33	23.85 ±1.4	207.69 ±11.1	226.31 ±10.06
	1hr exposure gr. (14)	132.14 ±8.46	50 ±2.77	592.86 ±19.51	32.86 ±1.63	221.43 ±13.58	247.07 ±8.23
	P	NS	NS	NS	<0. 005	NS	NS
	P*	NS	NS	NS	NS	NS	NS
	2hr exposure gr. (14)	132.14 ±11.25	52.86 ±2.66	664.29 ±41.41	33.57 ±2.25	260.71 ±15.88	255.71 ±7.63
	P	NS	<0. 01	<0. 05	<0. 005	<0. 01	<0. 05
	P*	<0. 05	NS	NS	NS	<0. 05	NS
	3hr exposure gr. (14)	121.43 ±8.64	57.14 ±2.86	742.86 ±45.35	37.14 ±1.25	260.71 ±15.88	259.29 ±7.81
	P	NS	<0. 001	<0. 002	<0. 001	<0. 01	<0. 02
	P*	NS	<0. 001	NS	NS	<0. 002	NS

In parenthesis is the number of rats studied in each group.
Values are expressed as means ±SEM.
P: Significance by LSD at P <0.05 from control group.
P*: Significance by LSD at P <0.05 of 8 week groups from respective 4 week groups.
NS: Not significant.
gr.: group.

Table (3): Changes in body weight, absolute cardiac weights (mg) and relative weights (mg/g) of whole heart (WH), atria (At), right ventricle (RV) and left ventricle (LV) and in the different studied groups.

Group		BW (g)	WH (mg)	WH/BW (mg/g)	At (mg)	At/BW (mg/g)	RV (mg)	RV/BW (mg/g)	LV (mg)	LV/BW (mg/g)
4 weeks Exposure Groups	Control gr. (12)	196.92 ±3.34	511.95 ±8.95	2.607 ±0.047	33.43 ±4	0.171 ±0.022	66.09 ±2.7	0.336 ±0.014	411.93 ±8.92	2.095 ±0.042
	1hr exposure gr. (10)	209.5 ±3.53	558.57 ±12.01	2.674 ±0.075	40.18 ±6.12	0.205 ±0.031	63.55 ±3.91	0.304 ±0.182	456.84 ±11.76	2.185 ±0.063
	P	NS	NS	NS	NS	NS	NS	NS	NS	NS
	2hr exposure gr. (8)	216.25 ±3.37	653.24 ±38.1	3.017 ±0.164	32.31 ±5.07	0.148 ±0.022	65.94 ±5.1	0.304 ±0.022	554.99 ±33.52	2.565 ±0.148
	P	NS	<0.001	<0.05	NS	NS	NS	NS	<0.001	<0.01
	3hr exposure gr. (10)	191.3 ±6.27	627.5 ±26.91	3.281 ±0.083	29.37 ±7.03	0.153 ±0.036	61.57 ±3.96	0.324 ±0.022	536.56 ±23.61	2.804 ±0.071
	P	NS	<0.001	<0.001	NS	NS	NS	NS	<0.001	<0.001
8 weeks Exposure Groups	Control gr. (9)	203.33 ±9.75	548.94 ±12.03	2.739 ±0.116	39.67 ±3.4	0.197 ±0.017	75.51 ±4.56	0.376 ±0.025	437.52 ±9.32	2.183 ±0.09
	1hr exposure gr. (10)	208.5 ±10.11	600.64 ±19.62	2.935 ±0.155	33.18 ±3.51	0.164 ±0.021	71.62 ±5.57	0.342 ±0.023	495.84 ±18.69	2.43 ±0.151
	P	NS	NS	NS	NS	NS	NS	NS	<0.05	NS
	P*	NS	NS	NS	NS	NS	NS	NS	NS	NS
	2hr exposure gr. (9)	168.33 ±11.18	593.23 ±20.17	3.598 ±0.176	34.32 ±4.42	0.207 ±0.027	47.96 ±6.8	0.285 ±0.032	509.96 ±14.87	3.106 ±0.168
	P	<0.002	NS	<0.001	NS	NS	<0.001	<0.01	<0.01	<0.001
	P*	<0.001	NS	<0.01	NS	NS	<0.02	NS	NS	<0.01
	3hr exposure gr. (9)	175.56 ±5.86	611.29 ±17.83	3.531 ±0.196	32.6 ±4.17	0.186 ±0.024	58.74 ±6.16	0.331 ±0.029	519.94 ±22.51	3.014 ±0.211
	P	<0.02	<0.05	<0.001	NS	NS	<0.05	NS	<0.005	<0.001
	P*	NS	NS	NS	NS	NS	NS	NS	NS	NS

In parenthesis is the number of rats studied in each group. Values are expressed as means ±SEM. NS: Not significant. gr.: group.

P: Significance by LSD at P <0.05 from control group. P*: Significance by LSD at P <0.05 of 8 week groups from respective 4 week groups.

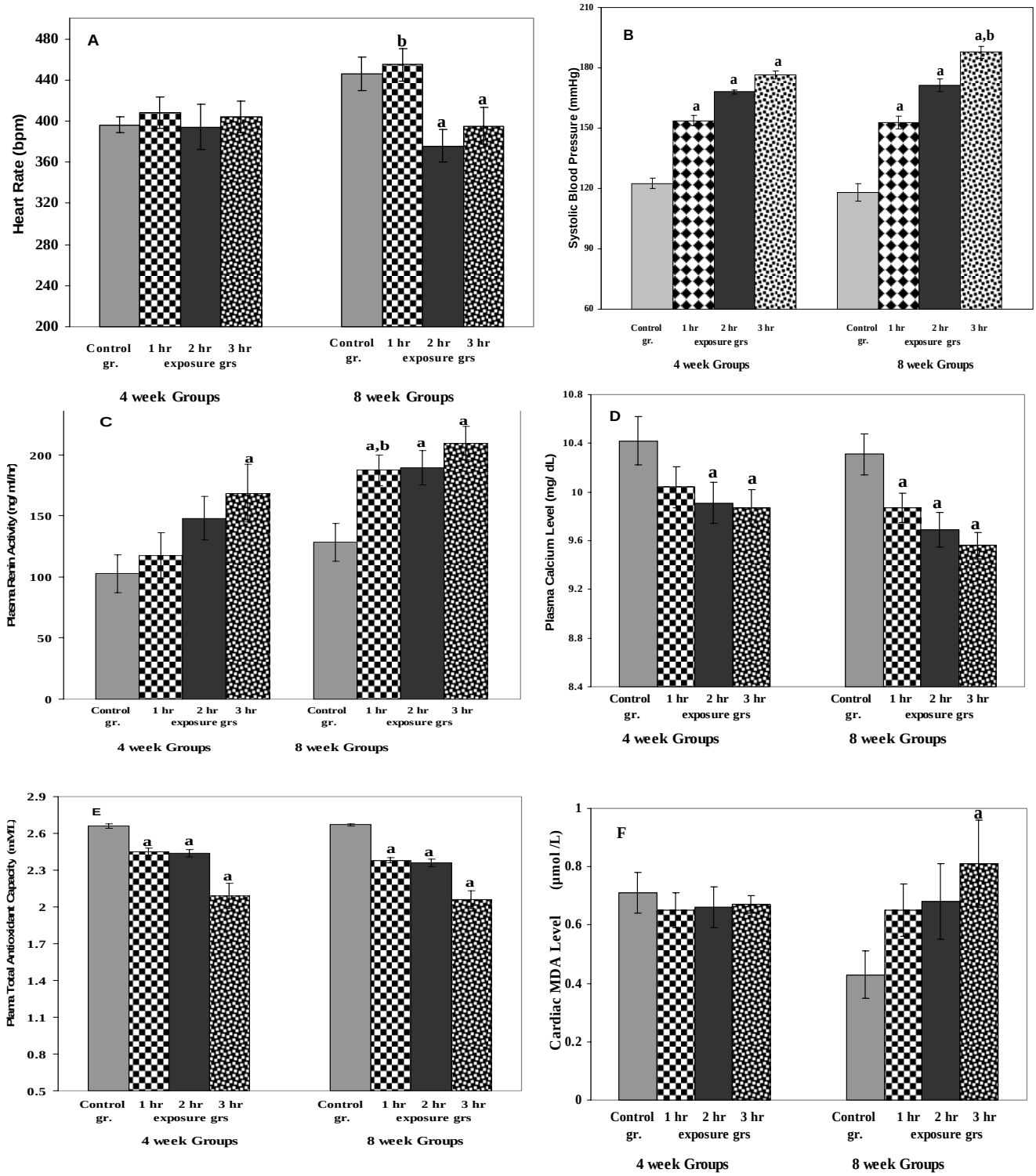


Fig.(1): Heart rate (1_A), systolic blood pressure (1_B) , plasma renin activity (1_C), plasma calcium level (1_D), plasma total antioxidant activity (1_E) and cardiac MDA level (1_F) in the different studied groups.
a: Significance by LSD at P<0.05 from respective control group.
b: Significance by LSD at P<0.05 of 8 week groups from respective 4 week groups.

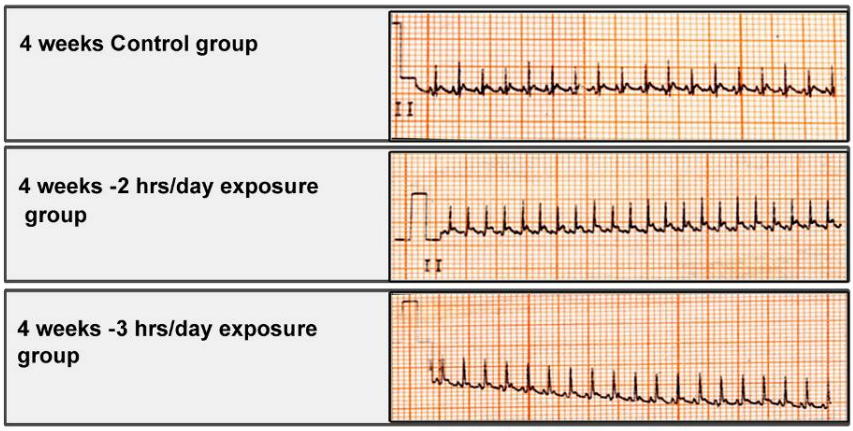


Fig.(2): ECG tracings of rats exposed to EMF for 4 weeks (prolonged QRS duration in 2 hrs/day group and higher R voltage & prolonged QRS duration in 3 hrs/day groups relative to control group).

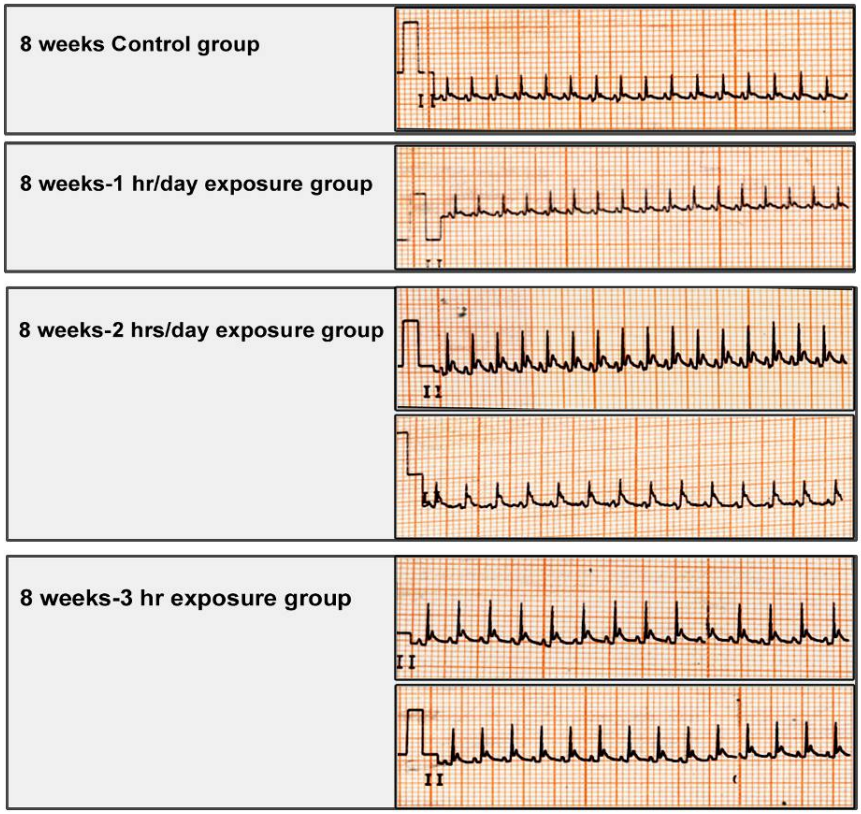
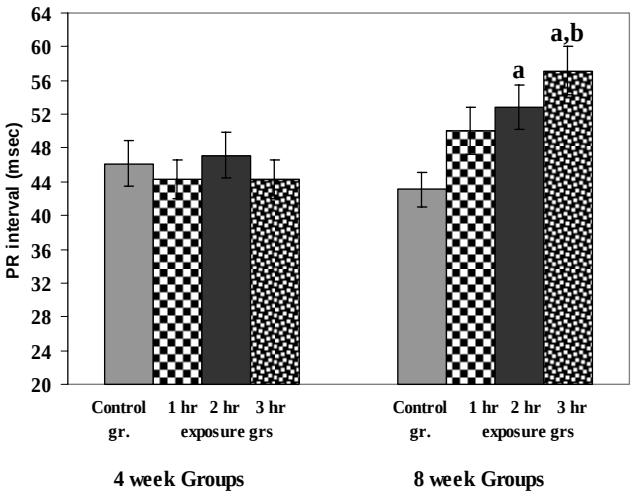
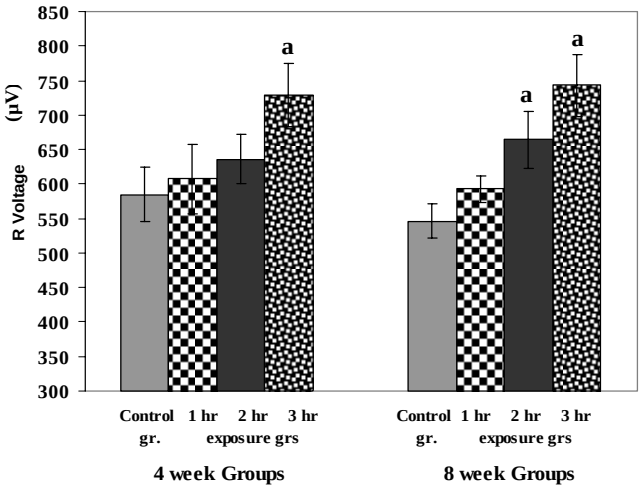


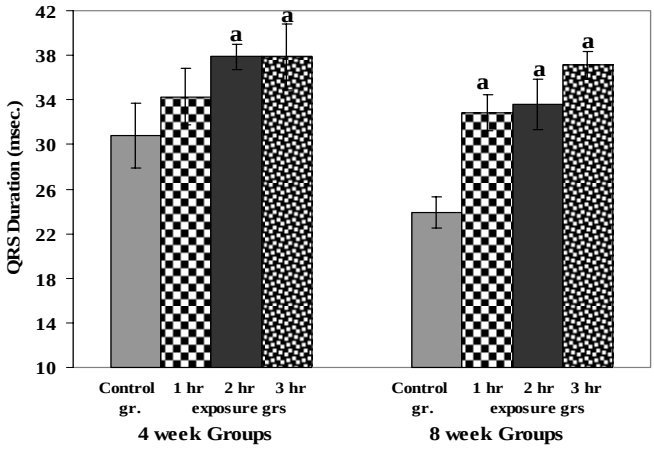
Fig.(3): ECG tracings of rats exposed to EMF for 8 weeks (prolonged QRS duration in 1 hr/ day group, decreased heart rate, prolonged PR interval, prolonged QRS duration and higher R and T voltage in 2 hrs and 3 hrs / day groups).



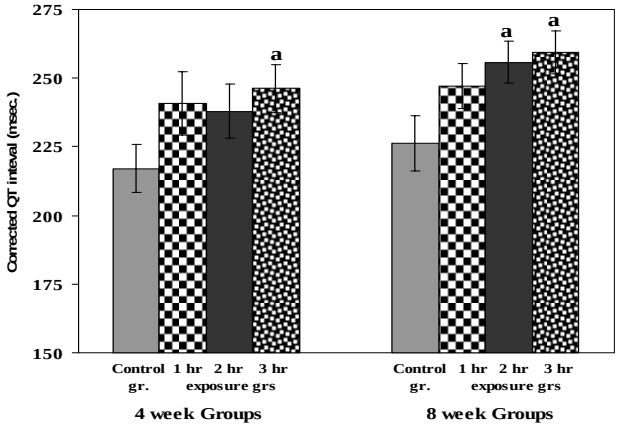
PR interval



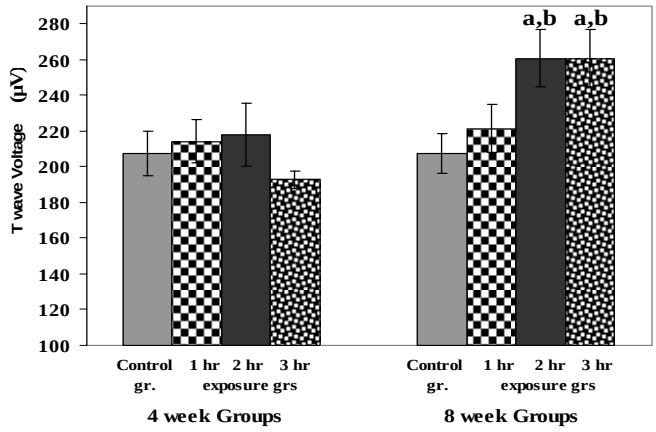
R wave Voltage



QRS Complex Duration



T wave Voltage



QT-$_C$ interval

Fig.(4): ECG parameters in the different studied groups.

a: Significance by LSD at P<0.05 from respective control group.
b: Significance by LSD at P<0.05 of 8 week groups from respective 4 week groups.

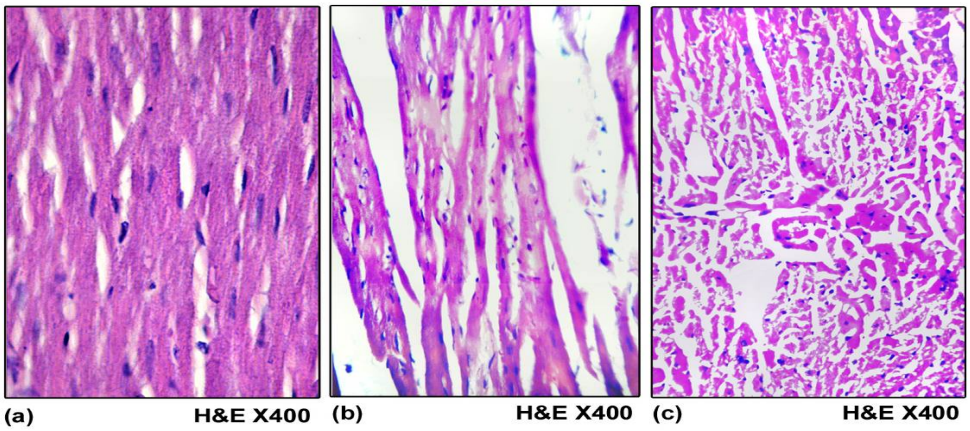


Fig. (5): Photomicrograph of control rat cardiac muscle showing regularly arranged cardiac muscle fibers **(5-a)**, nuclei appeared central and vesicular and their sarcoplasm appeared acidophilic **(5-b).** The cardiac myocytes in transverse section appeared more or less comparable in size with noticeable myofibrillar content **(5-c).**

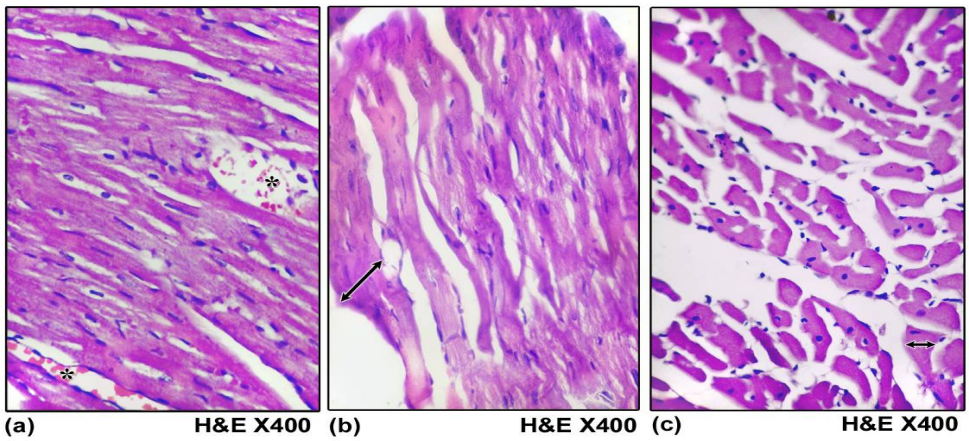


Fig.(6): photomicrograph ,in 1hr &2hr.4week exposed rats **,** showing congestion of blood vessels and extravasation of RBCs * **(6-a)**, in 3hr .4w & all exposed subgroups in 8 w group showing hypertrophy of many of the cardiac myocytes with deeply acidophilic sarcoplasm and vesicular nuclei (L.S) **(6-b)** &(T.S**) (6-c).**

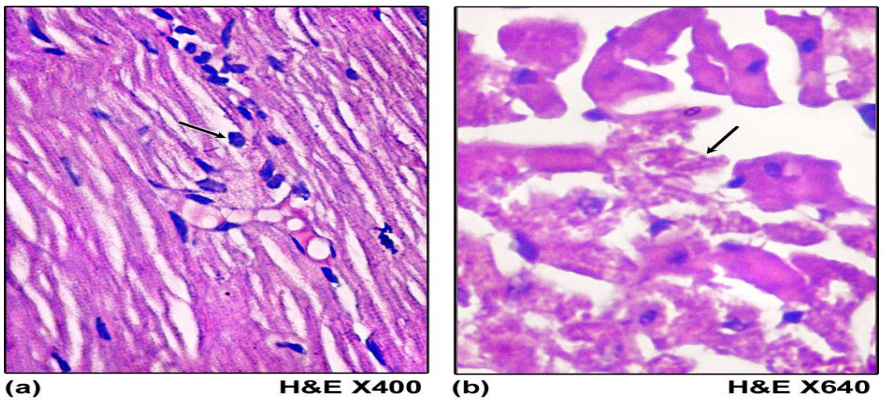


Fig.(7): photomicrograph in 3hr .4w & all exposed subgroups in 8 w group showing mononuclear cellular infiltration **(7-a)** and distortion of some cardiac myocytes, together with some areas of complete degeneration and fragmentation **(7-b)**.

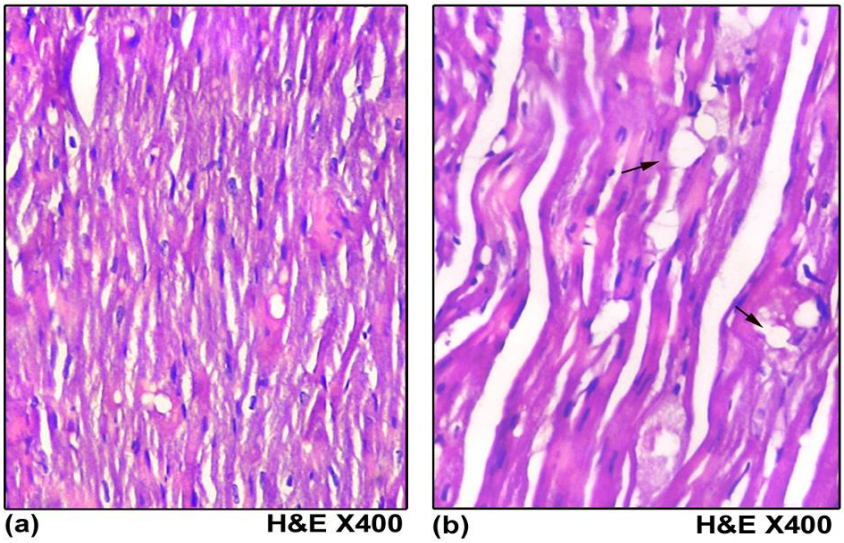


Fig.(8): Photomicrograph showing loss of the regular arrangement of the cardiac myocytes being most prominent in 2 hrs & 3 hrs subgroups in the 8 weeks group **(8-a)** ,and marked cell vacuolation in the different groups in particular, in 8 weeks -3hrs EMF-exposed groups **(8-b)**.

4. Discussion:

Exposure to cell phone EMF caused significant increase in the systolic blood pressure in all test groups, the pressure being higher with prolonged exposure, together with decreased heart rate only in the groups exposed for longer periods. The observed increase in blood pressure, in the present study, could be the result of increased plasma renin activity (PRA), which was increased in all the exposed groups, though the increase was only statistically significant in the 4 weeks-3hrs/ day exposed group as well as all the groups exposed to the cell phone EMF for 8 weeks.

Also, the absolute and relative whole heart and left ventricular weights were increased, particularly in the groups exposed to EMF for longer periods indicating hypertrophic changes. The increased blood pressure encountered in this study could explain the changes in absolute and relative cardiac weights. Earlier, it was stated that one of the early and most common consequences of chronic hypertension is left ventricular hypertrophy (LVH), and that the renin-angiotensin system contributes to the development of LVH in hypertension [42,43].

The observed increase in blood pressure agrees with the earlier study which reported that mobile phones caused a rise of blood pressure of 5-10 mmHg each time of exposure [25]. Also, a recent study demonstrated that blood pressure in rats was increased through hours after exposure to mobile phone EMF [27]. The present findings

disagree, however, with another study which stated that exposure to mobile phone EMF did not affect

significantly the blood pressure, heart rate or cardiac electrical activity [44].

In addition, the R wave and T wave voltages as well as the P-R and QT-$_C$ intervals were all increased in the groups exposed for longer periods while the QRS duration was prolonged in almost all groups. The increased R wave voltage observed in the 4 weeks-3hrs/day exposed group and in the 8 weeks-2hrs &3hrs /day exposed groups could be due to the increased thickness of the left ventricular wall. This left ventricular hypertrophy, evident also from the histopathological examination, could explain the higher T wave voltage observed in the 8 weeks-2hrs & 3hrs /day exposure groups.

The present study revealed, also, reduction in the heart rate in the 8 weeks-2hrs &3hrs/day exposed groups (groups II_2&II_3), i.e. occurring in rats subjected to longer durations of exposure to EMF. This reduced heart rate could possibly be explained by an increase in parasympathetic tone in these rats. In a previous study, the parasympathetic tone was suggested to be increased, while the sympathetic tone was lowered in humans during cell phone call, thus modifying the functioning of circulatory system [29]. Further, the reduced heart rate could be due to the associated increase in plasma renin activity. High concentration of renin-angiotensin II was reported to lead to significant baroreceptor- mediated bradycardia [45].

The prolonged P-R interval observed in the present study in groups II_2 and II_3 could be explained by the increased tone of the parasympathetic system, or possibly as a result of the encountered hypocalcaemia which cause diminished conduction velocity in the heart [46].

The significantly prolonged QT-$_C$ interval in the groups with prolonged exposure to EMF could be attributed to the associated increased renin activity that was reported to remodel the cardiac ion channels, resulting in prolongation of ventricular repolarization and thus prolongation of QT-$_C$ interval [47].

The observed significant hypocalcaemia associated with exposure to EMF occured as a result of alteration of intracellular signaling pathways resulted from RF radiation exposure through changes in Ca^{2+} permeability across cell membranes [21]. It has been reported that calcium positive ions strengthen cell membranes because they bind together the negatively-charged phospholipid molecules, and that electromagnetic radiation could lead to the replacement of calcium with monovalent ions that weakens the membrane and makes it more likely to tear and form pores [48, 49]. Thus, the observed hypocalcemia, in this study, might be one of the mechanisms by which EMF interacts with biological tissues that RF radiation from cell phone could alter.

The present findings also revealed significant decrease in plasma total antioxidant capacity in all exposed groups. As, acute exposure to RF fields of cell phones could modulate the oxidative stress and free radical generation by enhancing lipid peroxidation and reducing the activation of SOD and GSH-Px, free radical scavengers [50]. Further, RF-EMF exposure was reported to cause production of extracellular superoxide [51]. So the decrease in plasma total antioxidant capacity encountered in this study might be the result of its exhaustion in defending the free radical believed to be generated with RF-EMF.

The significantly increased cardiac MDA content encountered in the present study in the 8 weeks-3hrs/day exposed group, with the longest duration of exposure (144hr), points to the limits of the cardiac antioxidants to cope with the excessive MDA generation due to RF-EMF exposure.

The significant increase in cardiac tissue MDA level with prolonged EMF exposure to from cell phone, in this study, was in agreement with other studies [52,53]. It has been suggested that increased total oxidant status levels due to RF radiation emitted from GSM cell phones might play a role in inducing oxidative damage by increasing lipid peroxidation and oxidative stress [54, 55].

The forementioned effect of RF-radiation in generation of free radicals, increased lipid peroxidation and tissue damage could possibly explain the vascular congestion with the shorter duration of exposure, namely the 4 weeks- 1hr & 2hrs/day exposed rats (groups I_1&I_2). A recent study on rats exposed to cell phone 1hr/day for 4 weeks demonstrated congestion of blood vessels and extravasation of RBCs in the myocardium, together with disruption of few cardiac fibers. These findings were suggested to be due to free radical generation with EMF [56].

The hypertrophic myocardial changes observed in the present study, which were especially related to the longer duration of exposure, could be explained by the associated increased blood pressure in these groups.

5. Conclusion:

The results encountered in the present study revealed that long-term exposure to cell phone EMF increases the liability for hypertension reflected on the ECG and cardiac weights, accompanied by histological changes in the myocardium. Also, the associated increased PRA, decreased plasma total antioxidant capacity and hypocalcemia could be suggested as contributing mechanisms reflecting interaction of EMF with biological functions.

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6. References:

1- Valberg PA, van Deventer TE and Repacholi MH. (2007): Workgroup Report: Base Stations and Wireless Networks- Radiofrequency (RF): Exposures and Health Consequences. Environmental Health Perspectives; 115 (3):416-424.

2- Hardell L, Carlberg M and Mild HK (2005): Use of cellular telephones and brain tumour risk in urban and rural areas. Occup. Environ. Med.; 62: 390-4.

3- Schuz J, Bohler E, Berg G, Schlehofer B, Hettinger I, Schlaefer K, Wahrendorf J, Kunna-Grass K and Blettner M (2006): Cellular phones, cordless phones, and the risks of glioma and meningioma (INTERPHONE study group, Germany). Am. J. Epidemiol.; 163(6):512–520.

4- Moulder JE, Foster KR, Erdreich LS and McNamee JP (2005): Mobile phones, mobile phone base stations and cancer: a review. Int J. Radiat. Biol.; 81:189-203.

5- Hoskote SS, Kapdi M and Joshi SR (2008): An Epidemiological Review of Mobile Telephones and Cancer. JAPI; 56: 980-984.

6- Ozguner F, Oktem F, Armagan A, Yilmaz R, Koyu A, Demirel R and Vural H, Uz E (2005): Comparative analysis of the protective effects of melatonin and caffeic acid phenethyl ester (CAPE) on mobile phone induced renal impairment in rat. Mol. Cell Biochem.; 276(12):317.

7- Ahlbom A, Bridges J, de Seze R, Hillert L, Juutilainen J, Mattsson MO, Neubauer G, Schüz J, Simko M and Bromen K (2008): Possible effects of electromagnetic fields (EMF) on human health--opinion of the scientific committee on emerging and newly identified health risks (SCENIHR).Toxicology; 246(2-3):248-50.

8- Regel SJ, Negovetic S, Röösle M, Berdiñas V, Schuderer J, Huss A, Lott U, Kuster N and Achermann P (2006): UMTS base station-like exposure, well being, and cognitive performance. Environ. Health Perspect.; 114:1270–1275.

9- Eltiti S, Wallace D, Zougkou K, Russo R, Joseph S, Rasor P and Fox E (2007): Development and evaluation of the electromagnetic hypersensitivity questionnaire. Bioelectromagnetics; 28:137–151.

10- Yan JG, Agresti M, Bruce T, Yan YH, Granlund A and Matloub HS (2007): Effects of cellular phone emissions on sperm motility in rats. Fertil. Steril.; 88(4):957-64.

11- Agarwal A, Deepinder F, Sharma RK, Ranga G and Li J (2008): Effect of cell phone usage on semen analysis in men attending infertility clinic, Fertil. Steril.; 89(1):124-8.

12- Desai NR, Kesari KK and Agarwal A (2009): Pathophysiology of cell phone radiation: oxidative stress and carcinogenesis with focus on male reproductive system. Reprod. Biol. Endocrinol.; 7: 114.

13- Hung CS, Anderson C, Horne JA and McEvoy P (2007): Mobile phone 'talk-mode' signal delays EEG-determined sleep onset. Neurosci. Lett.; 421(1):82-6.

14- Divan HA, Kheifets L, Obel C and Olsen J (2008): Prenatal and postnatal exposure to cell phone use and behavioral problems in children. Epidemiology; 19(4):523-9.

15- Hardell L, Carlberg M, Söderqvist F and Mild HK (2008): Meta-analysis of long-term mobile phone use and the association with brain tumours. Int. J. Oncol.; 32(5):1097-103.

16- Sage C and Carpenter DO (2009): Public health implications of wireless technologies. Pathophysiology.; 16(2-3):233-46.

17- Panda NK, Jain R, Bakshi J and Munjal S (2010): Audiologic disturbances in long-term mobile phone users. J. Otolaryngol. Head Neck Surg.; 39(1):5-11.

18- Carpenter DO and Sage C (2008): Setting prudent public health policy for electromagnetic field exposures. Rev. Environ. Health; 23(2):91-117.

19- Comba P and Fazzo L (2009): Health effects of magnetic fields generated from power lines: new clues for an old puzzle. Ann. 1st Super Sanità; 45(3): 233-237.

20- Kohli DR, Sachdev A and Vats HS (2009): Cell phones and tumor: Still in no man's land. Indian J. Cancer; 46(18):5-12.

21- Maskey D, Kim M, Aryal B, Pradhan J, Choi IY, Park KS, Son T, Hong SY, Kim SB, Kim HG and Kim MJ (2010): Effect of 835 MHz radiofrequency radiation exposure on calcium binding proteins in the hippocampus of the mouse brain. Brain Res.; 1313:232-41.

22- Altamura G, Toscano S, Gentilucci G and Ammirati F. (1997): Influence of digital and analogue cellular telephones on implanted pacemakers. Eur. Heart; 18:1632–41.

23- Barbaro V, Bartolini P, Donato A and Militello C (1999): Electromagnetic interference of analog cellular telephones with pacemakers: in vitro and in vivo studies. Pace; 22: 626–34.

24- Gimbel JR and Cox JW (2007): Electronic article surveillance systems and interactions with implantable cardiac devices: risk of adverse interactions in public and commercial spaces. Mayo Clin. Proc.; 82(3):276-8.

25- Braune S, Wrocklage C, Raczek J, Gailus T and Lücking CH (1998): Resting blood pressure increase

during exposure to a radio-frequency electromagnetic field. Lancet; 351(9119):1857-8.
26- Hietanen M, Hämäläinen AM and Husman T (2002): Hypersensitivity symptoms associated with exposure to cellular telephones: no causal link. Bioelectromagnetics; 23: 264-70.
27- Li BF, Guo GZ, Ren DQ, Jing-Li and Zhang RB (2007): Electromagnetic pulses induce fluctuations in blood pressure in rats. Int. J. Radiat. Biol.; 83(6):421-9.
28- Rezk AY, Abdulqawi K, Mustafa RM, Abo El-Azm TM and Al-Inany H (2008): Fetal and neonatal responses following maternal exposure to mobile phones. Saudi Med. J.; 29: 218-23.
29- Andrzejak R, Poreba R, Poreba M, Derkacz A, Skalik R, Gac P, Beck B, Steinmetz-Beck A and Pilecki W (2008): The Influence of the Call with a Mobile Phone on Heart Rate Variability Parameters in Healthy Volunteers. Ind. Health; 46(4):409-17.
30- Braune S, Riedel A, Schulte-Monting J and Raczek J (2002): Influence of a RF EMF on Cardiovascular and Hormonal Parameters of the Autonomic Nervous System. Radiat. Res.; 158: 352-356.
31- Forgács Z, Somosy Z, Kubinyi G, Bakos J, Hudák A, Surján A and Thuróczy G (2006): Effect of whole-body 1800MHz GSM-like microwave exposure on testicular steroidogenesis and histology in mice. Reproductive Toxicology; 22:111–117.
32- Peter AVT, Emilie VD and Michael HR (2007): Workgroup Report: Base Stations and Wireless Networks—Radiofrequency (RF) Exposures and Health Consequences. Environmental Health Perspectives; 115:416–424.
33- Aksen F, Dasdag S, Akdag MZ, Askin and Dasdag MM (2004): The Effects of Whole Body Cell Phone Exposure on the T1 Relaxation Times and Trace Elements in the Serum of Rats. Electromagnetic Biology and Medicine; 23(1): 7 – 17.
34- Dasdag S, Akdag MZ, Aksen F, Bashan M and Buyukbayram H (2004): Does 900 MHZ GSM Mobile Phone Exposure Affect Rat Brain? Electromagnetic Biology and Medicine; 23 (3):201-214.
35- Goldschlager N and Goldman MJ (1984): Electrocardiography: Essantials of interpretation. Middle East Edition, ch.3, Pp.13-18. Lang Medical Publications, Los Anglos, California.
36- Malvano R, Zucchelli GC, Rosa U and Salvetti A (1972): Measurement of plasma renin activity by angiotensin I radioimmunoassay: (I) an assessment of some methodological aspects. J. Nucl. Biol. Med.; 16(1):24-31.
37- Cali JP, Bowers GN Jr and Young DS (1973): A referee method for the determination of total calcium in serum. Clin. Chem.; 19(10):1208-13.
38- Koracevic D, Koracevic G, Djordjevic V, Andrejevic S and Cosic V (2001): Method for the measurement of antioxidant activity in human fluids. J. Clin. Pathol.; 54(5):356-61.
39- Eissa S, Khalifa A and El-Ahmady O (1990): Tumour markers in malignant and benign breast tissue CA15.3, CEA and ferritin in cytosol and membrane enriched fractions. J. Tumour Marker Oncol.; 5:220.
40- Esterbauer H and Cheeseman KH (1990): Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxynonenal. Methods. Enzymol.; 186:407-421.
41- Bancroft JD and Gamble M (2002): Theory and Practice of Histological Techniques., fifth ed., London (UK), Churchill Livingstone.
42- Devereux RB, Wachtell K, Gerdts E, Boman K, Nieminen MS, Papademetriou V, Rokkedal J, Harris K, Aurup P and Dahlöf B (2004): Prognostic significance of left ventricular mass change during treatment of hypertension. JAMA; 292(19):2350-6.
43- Crowley SD, Gurley SB, Herrera MJ, Ruiz P, Griffiths R, Kumar AP, Kim HS, Smithies O, Le TH and Coffman TM (2006): Angiotensin II causes hypertension and cardiac hypertrophy through its receptors in the kidney Proc Natl. Acad. Sci USA; 103(47):17985-9.
44- Tamer A, Gündüz H and Ozyildirim S (2009): The cardiac effects of a mobile phone positioned closest to the heart. Anadolu. Kardiyol. Derg.; 9(5):380-4.
45- Yeung MY and Smyth JP (2000): Hypertension and bradycardia associated with high dose of dexamethasone in premature infant with border line hyponatremia. Australian. Journal of hospital pharmacy; 30(5): 205-7.
46- Cascio WE, Yan GX and Kléber AG (1990): Passive electrical properties, mechanical activity, and extracellular potassium in arterially perfused and ischemic rabbit ventricular muscle. Effects of calcium entry blockade or hypocalcemia. Circ. Res.; 66(6):1461-73.
47- Raizada V, Skipper B, Luo W, Garza L, Hines CW, Harford AA, Zager PG, Griffith J, Raj D and Spalding CT (2005): Renin-angiotensin polymorphisms and QTc interval prolongation in end-stage renal disease. Kidney International; 68: 1186–1189.
48- Ha BY (2001): Stabilization and destabilization of cell membranes by multivalent ions. Phys. Rev. E.; 64: 051902- 051907.
49- Melikov KC, Frolov VA, Shcherbakov A, Samsonov AV, Chizmadzhev YA and Chernomordik LV (2001): Voltage-induced nonconductive pre-pores and metastable single pores in unmodified planar lipid bilayer. Biophys. J.; 80(4):1829-36.
50- Moustafa YM, Moustafa RM, Belacy A, Abou-El-Ela SH and Ali FM (2001): Effects of acute exposure to the radiofrequency fields of cellular phones on plasma lipid peroxide and antioxidase activities in human erythrocytes. J. Pharm. Biomed. Anal.; 26(4):605-608.

51- Friedman J, Kraus S, Hauptman Y, Schiff Y and Seger R (2007): Mechanism of short-term ERK activation by electromagnetic fields at mobile phone frequencies. Biochem. J.; 405(3):559-568.
52- Ozguner F, Altinbas A, Ozaydin M, Dogan A, Vural H, Kisioglu AN, Cesur G and Yildirim NG (2005): Mobile phone induced myocardial oxidative stress: protection by a novel antioxidant agent caffeic acid phenethyl ester. Toxicol. Ind. Health; 21(9):22330.
53- Devrim E, Ergüder IB, Kılıçoğlu B, Yaykaşlı E, Cetin R and Durak I (2008): Effects of Electromagnetic Radiation Use on Oxidant/Antioxidant Status and DNA Turn-over Enzyme Activities in Erythrocytes and Heart, Kidney, Liver, and Ovary Tissues From Rats: Possible Protective Role of Vitamin C. Toxicol. Mech. Methods; 18(9):679-683.
54- Dasdag S, Bilgin HM , Akdag MZ , Celik H and Aksen F (2008): Effect of long term mobile phone exposure on oxidative-antioxidative processes and nitric oxide in rats. Biotechnology & Biotechnological Equipment. Diagnosis Press; 22:4.
55- Ongel K, Gumral N and Ozguner F (2009): The Potential Effects of Electromagnetic Field: A Review. Cell Membranes and Free Radical. Research; 1(3):85-89.
56- Hanafy LK, Karam SH and Saleh A (2010): The Adverse Effects of Mobile Phone Radiation on Some Visceral Organs. Research Journal of Medicine and Medical Sciences; 5(1): 95-99.

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The Level of Managerial Functions Practiced by the Head of household and Family Economic Status in Kerman, Iran

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ABSTRACT: Household management is a process of using the required resources to attain the families' goals through planning and taking the necessary steps to meet these goals. The aim of this article is to determine, the level of managerial functions practiced by the families, and the relationship between the levels of managerial functions practiced and family economic status. Management functions include five dimensions (planning, coordinating, organizing, directing and controlling). Family economic status included three dimensions (income, expenditure, and ownership of physical asset). The instrument used for this study is a questionnaire survey; the researcher selected 390 households, out of a total of 127,892 families in Kerman City. Data collection was through face-to-face interviews to obtain information from the heads of households. The relationship between household expenditure, income, ownership of physical asset, and management functions was investigated using Pearson product-moment correlation coefficients. Findings indicated that elements of management functions have effect on family economic status, but the affected is not considerable, and there is a weak relationship between management functions and family economic status. It may be interesting for future studies to look at the effect of other elements on family economic status.

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Keywords: Management functions, Family economic status, Income, Expenditure, Ownership of physical Asset.

INTRODUCTION

Household management is much more than just "paying bills." It plays an overarching role in all household production. The critical role of household management as part of household production has received significant attention in family economics, including the publication of the seminal text by Deacon and Firebaugh (Anne & Thomas, 2009). Researchers have traced the assessment of the study on resource management from an importance on economy and efficiency in the household in 1900s to the growth of systems-based research models obtains an ecosystems approach to management. Households take input, convert it in the throughput phase into output, and at that time use the output internally or replace it with a different system. Family resource management has a fundamental role in helping meet and alter the increasing complexities faced by the families. Household management is the process of using the resources to attain the families' goals through planning and taking the steps necessary to meet these goals. A crucial part of the management process is the allocation of resources for the appropriate goals (Deacon & Firebaugh, 1988). In other words, management is the process of using what one has to get what one wants. The management process involves thinking, action, and results. Although household management is practical, it is not necessarily simple. It becomes complex because the choices of the individual and the family are constrained by limited resources. Each individual has his or her own resource, attitudes, talents, and skills that are brought to bear on situations. Management, therefore, has to be viewed within the context of the greater life environment, which is constantly changing (Goldsmith, 1996). Household management consists of more than merely the economic management of resources to produce a high standard of living through consumption.

The management process begins with a problem, need, want, or goal, which has to be identified.

Once identified, the individual or family moves to the second step, which is the clarification of values. The third step involves identifying the available resources. Deciding, planning, and implementing are the fourth step of the process. In the fifth step, the goals are accomplished or fulfilled and the process as a whole is evaluated. Then, the information returns to the system and enables the individual's overall management knowledge and ability to grow, (Figure 1) (Goldsmith, 1996).

Each person has his or her own management style, or way of making decisions and acting. Various factors including history, biology, culture, personality, and technology influence the individual's management style. It can be either an individual or a group activity. Life management encompasses all the decisions a person or family will make, and the way their values, goals, and resources affects their decision-making. It includes all the goals, events, situations, and decisions that make up their lifestyle. Thus, life management is a holistic approach that looks at management as a process that evolves over an entire lifespan (Goldsmith, 1996).

The study of household management is a combination of theory, concepts, techniques, research, and practice. There is not just one management theory or framework; instead, management is an interdisciplinary field that borrows concepts and theories from related disciplines (Goldsmith, 1996,). Much of a family's decision-making is shaped by the environmental settings in which the family functions. These environments either constrain this decision-making or offer opportunities for the family. Because the physiological and the psychological makeup of the family members differ, so does the environment in which they interact, it becomes essential to view decision-making from an ecological perspective (Paolucci, Hall, & Axinn, 1977).

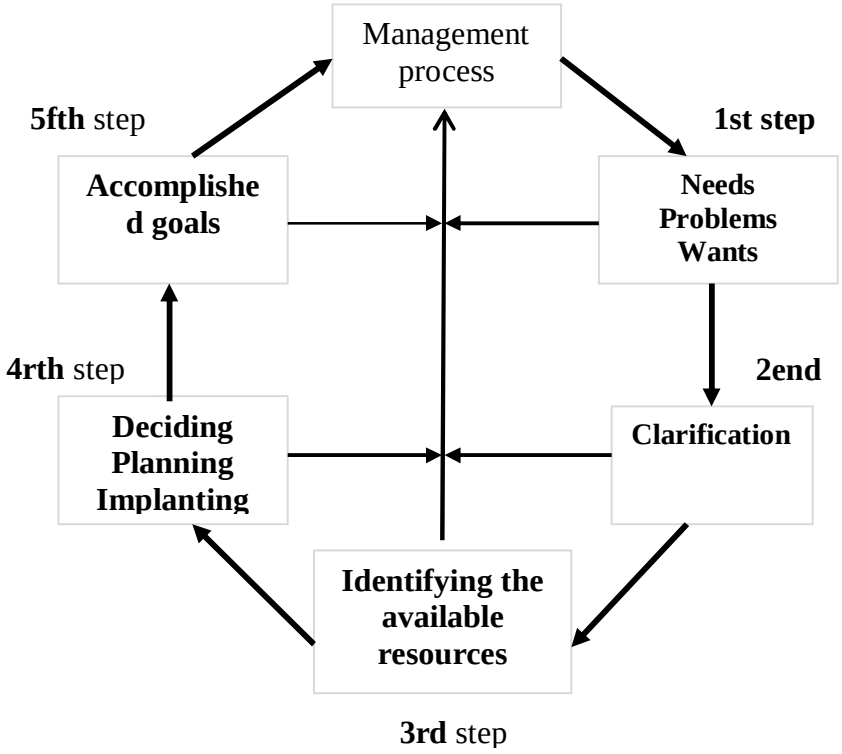

Figure 1: Management process (Goldsmith 1996).

Systems Theory

In the mid-1970s, family resource management researchers studying the family unit began to use paradigms evolving from other social science disciplines, including psychology and sociology (Doherty et al., 1993; Key & Firebaugh, 1989a, 1989b). Today the prevalent paradigm is systems theory. Using systems theory, hypotheses have been developed and tested with quantitative methods (Godwin, 1990a). Most studies have used net worth as the predicted outcome (Beutler & Mason, 1987; Godwin, 1990a; Hira, 1987; Sumarwan & Hira, 1993; Titus et al., 1989).

Financial management has integrated systems theory with a set of recommended financial management practices, the 'normative practices,' to assess whether families are managing their financial affairs properly (Rettig & Mortenson, 1986).

However, family financial management researchers using the recommended practices and systems theory for explanation and discovery have expressed uneasiness with the existing outcomes (Beutler & Mason, 1987; Godwin, 1990a; Key & Firebaugh; 1989; Winter, 1986b; Varcoe, 1990). They are frustrated by a lack of understanding about the exact management practices occurring within the family as a microeconomic unit. Godwin (1990a) stated that "much of the literature on family financial management is prescriptive, including extensive discussions of what families should do in managing their financial resources. Davis and Carr (1992) and Godwin (1990a, 1990b) stated that "the incentives that actually lead people to embrace (or reject) the process… remain unclear" (Davis & Carr, 1992, p.14). Thompson, Sharpe, and Hamilton (1998) is an example of research that attempts to fill this gap of how planning is actually being done. They studied the retirement planning process of single, midlife women.

Management functions Theory

Henri Fayol was an eminently successful practitioner, who promoted the theory of administration (Fayol, 1949). Fayol was perhaps the first to note the need for management education (Brodie, 1967). Fayol (1949) used the term "administration" in the title, perhaps unfortunately, as it would have been better termed as "management". Fayol's work was clearly about management but the foreword argues that no such word exists in the French language. He argued that all industrial undertakings precipitate activities that can be categorized into six groups: technical, commercial, financial, security, accounting and management. Fayol's work focused on the latter category, management, and categorizes management into five major functions: planning, organizing, directing, coordinating, and controlling (Figure 2).

Planning, "means both to assess the future and make provision for it". Fayol views the action plan as the most useful output of the planning process. He notes that this plan must consider the firm's resources, work-in-progress, and future trends in the eternal environment. Fayol discusses the features of a good action plan and highlights unity, continuity, flexibility and precision.

Organizing: Fayol enumerates the managerial duties of organizations that must be realized through personnel. He identifies many key objectives of organizing, including: ensuring proper plan preparation and execution; aligning objectives with resources; establishing a single guiding authority; harmonizing and coordinating of activities; maximizing personnel deployment; clear delineation of duties; encouraging initiative and responsibility; maintaining discipline; ensuring the subordination of individual interests to corporate interests; supervision of both material and human order; and maintaining full control (Fayol, 1949).

Commanding: is the responsibility of every manager. The purpose is achieving the maximum contribution to the interests of the business from all personnel within the manager's unit. Fayol (1949) discusses several maxims: Have a thorough knowledge of personnel – Fayol notes that in large organizations this knowledge could only reasonably apply to direct reports as per the manager's span of control. Eliminate the incompetent. Be well versed in the agreements binding the business and its employees. Set a good example. Conduct periodic audits of the organization and use summarized charts to further this. Bring together chief assistants by means of conferences, at which unity of direction and focusing of effort is provided for. Do not become engrossed in detail. Aim at making unity; energy, initiative and loyalty prevail among the personnel.

Coordinating: He suggests that this is the harmonization of resources in their optimum proportions in order to achieve results. Fayol identifies some of the characteristics of being well coordinated.

Controlling: consists of the ongoing, routine verification of plan implementation, instructions issued, and principles. Controlling applies to all processes. Its purpose is to identify weaknesses and problems such that they can be rectified and recurrences prevented. Fayol notes that to be effective, control must be timely and be supported by penalties. Fayol stresses the need for independent, objective and impartial inspection.

Research Methodology

Design of the Study

The aim of this study is on the level of managerial functions practiced by the families.

And also, to determine the relationship between the levels of managerial functions practiced and family economic status in Kerman city.

This is a quantitative study that investigates a social or human problem based on testing a theory composed of variables measured with numbers and analyzed with statistical measures, in order to conclude whether the forecasting generalizations of the theory hold accurate (Creswell, 1994). The survey design can present a quantitative or numeric description of some portion of the population (sample) by asking questions. This data collection technique allows a researcher to generalize the results from a sample of respondents to a population (Fowler, 1988). Therefore, the survey

methodology was deemed appropriate for this research as this study intends to use the survey responses from the Kerman City families to explore management functions and family economic status.

The research design employed was correlation research. Correlation research investigates the degree to which variables are related and the direction of the relationship. This study is also descriptive, and, therefore, will provide a description of population, the instruments, the data collection procedures, and the data analysis utilized in this study.

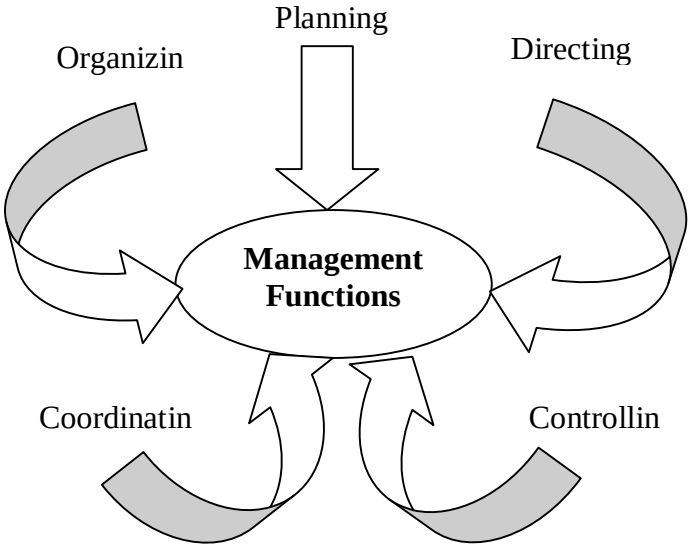

Figure 1: Management functions, Henry Fayol (1949).

Population and Sampling

The target population of the study is all 127,891 families in Kerman City. The list and map areas were obtained from Kerman Planning and Management Organization. Therefore, the list of areas is presented as the sampling frame for the study. The samples are households selected randomly from the five areas. The unit of analysis is the head of households that were selected as respondents, so for selecting sample size. For this survey, the researcher selected 390 households, out of a total of 127,892 families in Kerman City, based on Kerjcie and Morgan's Table and the sample size schedule (Saifollahi, 2008).

Due to the large research area, the researcher used a cluster or area for random sampling. First, the population is divided into clusters. In the proportionate cluster sampling, the population members are usually grouped in units that can be used conveniently as clusters. Based on the Kerman municipality breakdown, this city has five regions, which were numbered, respectively. It is not necessary that all clusters have the same number of population members (Wiersma, 2000). Second, the researcher referred to the Kerman planning and management organization map and selected five regions. Third, the researcher selected clusters in areas to measure all units within the sample cluster in the regions. Normally, each cluster comprised around eight to nineteen families to be interviewed.

Instrumentation

This study examines the effects of the managerial functions practiced on the economic status of families in Kerman City, Iran. For the purpose, instrument used for this study is a questionnaire consisting of three sections. The first section focuses on information concerning the demographic characteristics such as age, gender, level of education, marital status, and occupation. The second section consists of managerial functions which are reflected by five measured variables, namely: a) planning, b) organizing, c) directing, d) coordinating, and e) controlling. The last part of the questionnaire concerns family economic status, including income, ownership of physical assets and expenditure. Researcher used Delphi technique in different research steps, such as research framework, design of the research questionnaire; determine spectrum of choices scales (array of choices scales), set of indicators, data analysis, and conclusion.

Data Collection

After the three sections of the questionnaires were pilot tested to ensure the high reliability of the items, the final version of the questionnaire sets were distributed to the respondents with the help of five

representatives from Kerman Planning and Management Organization.

All essential and suitable precautionary measures were taken by the researcher in order to improve the response rates. These measures were as follows: initially, each questionnaire had a letter from the researcher attached to introduce the researcher and to develop a rapport between the researcher and the heads of the households. Second, the research purposes and the procedures in answering the questionnaire were explained in the letter.

In this study, data collection was through face-to-face interviews to obtain information from the heads of households. Face-to-face interviews were deemed the most appropriate method for data collection for a number of reasons. First, if the researcher sent the questionnaire to people's homes they would not necessarily answer the questions. Second, in Kerman a mail survey technique would be absolutely inappropriate. Finally, the most important reason outweighing all other reasons was that the respondents include illiterate people who would be unable to respond to mail surveys. Also, it is difficult to gather information concerning expenditure, income and assets, as people usually prefer to keep such information a secret. The interviews began with an introduction to the purpose of the study, and an explanation to the respondents that being a scientific study the reporting will be done without mentioning any names. During the interview, the interviewer interacted with the participants using a relaxed, friendly and informal tone. In cases of misunderstanding, outright incomprehension or topic avoidance, the interviewer repeated the statements for clarification to fulfil the purpose of the interview

The researcher decided to use the city map, which was detailed enough to facilitate the identification of each state, sector, block, and house. Kerman City comprises five municipality states (Jalal Maab, 2008). Using the map to randomly select households has its own merits; first, this map is updated regularly and, second, it helped minimize the selection bias by including all houses in Kerman and by equalizing the probability of each house and household for selection. The selection process was absolutely random since no information concerning the demographic or socioeconomic characteristics of the households was available at the time, and the selection process was not based on any stratum or other characteristics. The final sample size was 390 households and it was used to analyze the data. The interviews were carried out from November 2008 until the end of January 2009.

Data Analysis

Subsequently, the collected data was coded before being entered into the computer for analysis. The collected data was summarized, analyzed, and interpreted to answer the research questions and the research objectives. For descriptive purposes, the demographic variables of the head of households were categorized into a range of different categories and levels for comfortable analysis and interpretation.

The objective is to determine the relationship between the managerial functions practiced and family economic status. The level of managerial functions is the independent variable (x) and family economic status is the dependent variable (y). The relationship between the managerial function practice and FES was investigated using Pearson product-moment correlation coefficients. Pearson product moment correlation is the most commonly used method of computing a correlation coefficient between variables that are linearly related. Correlation is a measure of the strength of the relationship between two variables (Bryman & Bell, 2003; Hair, Black, Babin, Anderson, & Tatham, 2006). Furthermore Pearson correlation is suitable for interval and ratio scale (Sekaran, 2001). The direction of the relationship is indicated by + and – signs. The value can range from -1 indicating a perfect negative relationship, 0 indicating no relationship, and +1 indicating perfect positive relationship.

Findings and Discussion

Respondents Profile

The respondents' profile is described in terms of demographic characteristics, which comprised of gender, age, level of education, and employment status. Most of the respondents were male (92.3%) compared to female respondents (7.7%). The respondent's age ranged from 22 to 87 with a mean of 45.40 years old and a standard deviation of 13.40 indicating variability in age among the respondents. A total of 17.7% of the respondents are young with 30 years old and younger, 32.3% was between 31 to 45 years old, while 37.9% were between 46 to 60 years old and only 12.1% were more than 60 years old.

Looking at the marital status, majority of the respondents were married (89.5%), while the remaining 3.1% were divorced, 6.9% were widowed, and only .5% heads of households were single.

Comparing the research data about the level of education of respondents with the information from the Statistics Center of Iran (SCI), we can see that, there is significant difference. National data indicated that 18.3% of the illiterate population as compared to only 4.9% of the respondents studied, 21% population had primary and secondary level of education as compared to 26.6% of the respondents; 7.4% population had high school education as compared to 17.3% of the respondents; 38.6% of the population had diploma and associate degree as compared to bachelor degree of the respondents (7.3%); in Iran 7.3% of the population had

bachelor degree, while respondents was 14.6%; mean master's and above degree population was 2.3%, and respondents was 7.2%. Generally, the average level of education among respondents was higher compared to the Iranian population. According to Ministry of Statistics Small Industries & Industrial Parks Organization, Iran's Statistics Center and Planning, Development and Technology Planning Office in Iran (2009), Kerman categorised in the first level of industrial developed provinces. And this indicated that Kerman province is more developed in various aspects such as industry, agriculture, level of education and so on as compared to other provinces. Fidings revealed that 72.% of the heads of the households in Kerman were self employed (35.1%), full time employed (25.4%) and part time employed (11.5%).In addition, 27.7% were pensioners and retirees. The percentage of head of the households who were homemakers is lower (0.3%).

Economic Status of the Families in Kerman City

There are three dimensions of economic status used in this study: income, expenditure, and ownership of physical asset.

First, income is the most important element of economic status which indicates the financial situation of families. In other words, financial situation of families are strongly and positively influenced by income. Other factor that plays an important role in economic status is expenditure which largely depends on income and property. Household expenditure is usually depends on household size or family size; some families tend to spend more than others, even with the same size. The family expenditure, therefore, will be used to examine the inequality in the distribution of expenses.

The last factor to measure the economic status was ownership of physical asset. In Iran history, property and wealth carry an interesting implication, since they are mostly immovable as land and buildings and are transferred through generations. In evaluating socioeconomic status, and additional especially family economic status, measuring variables other than household income could be useful, for instance assets such as inherited wealth, savings, employment benefits, or ownership of homes (Berkman & Macintyre, 1997). Although income represents a flow of resources over some period of time, wealth captures the stock of assets at a given point in time, and thus economic reserves. Wealth is a foundation of economic security given that indicators of a household's capacity to get together emergencies or absorb economic shocks like unemployment. Income and wealth are wholly interrelated, but they are not exchangeable, as showed by the instance of an elderly person with a modest fixed income but substantial accumulated wealth (John, Catherine, & MacArthur, 2002). In United States the correlation between income and wealth is about 0.5, which is improved by the inclusion of asset income (generated by wealth) in the assess of total income (Keister & Moller, 2000).

Some researchers measuring family economic status based on expenditures (Xu et al., 2003). While, other researchers believed that the amount of food expenditures is the basis of a measurement of a family economy status (Deaton & Muellbauer, 1980). The most important category which are mentioned in the world through the expenditures on the measurement of a family economy are; food, clothes and shoes (foot wear), rental expenses, healthcares, training & learning, transports and communications.

Also, researcher studied the amount of individual's ownership of physical asset for measuring family economic status. In particular, the factors such as the ownership of house, factory, company, private garden or villas and investment in the stock exchange market were accounted as the indexes for measuring the economic status of a family. One of the most important factors in Iran is buying the house which allocated to the economic status of individuals, so in this study, the researcher tried to investigate the situation of individuals' ownership, specifically the ownership of house.

Many American researchers consider that there are straightforward three class model that integrated the better off, the middle class, and poor in economic status of family or society (Eichar, 1989). In this study, the income and expenditure were compared to the Iran Statistics Centre data. If the mean family income and expenditure were lower than the mean of Iran population, this family would be in the poverty status and if the family status was in higher level than this mean, the family would be in a better off situation. And finally if the status of family was the same or near the same mean of Iran population, the family would be in middle class.

Family Monthly Income

Income is perhaps the most important indicator of family economic status. Household income has been widely used as an indicator of economic status in US studies (Greg et al., 2002). Income has been employed broadly as a measure of SES, with the majority typical income-based measure being a household's total cash income, measured over various time periods like a month, or the 12-months period. Income and the money management or income management have significant effect on families goals, for the reason that the lack of family financial management may result in intra-family conflict due to diverse and uncoordinated financial

strategies by family members (Stephen, 2000). Anyway, accurate measurement of family income is also difficult. Since family income is personal, people tend to understate or inaccurately state their family income due to previous high taxation levels or due to subsidy offered to lower income groups by the government.

Based on the urban families income and expenditure plan in Iran (2007-2008) by Statistics Centre of Iran (Madad, 2008), the mean income in Iran in 2007 was USD 7800 yearly and USD 650 monthly and also the mean income for Kerman province was USD 6600 yearly, and USD 550 monthly. The mean of USD 1190 of family income in Kerman City (sample) indicated a high income level compared to Iran population.

Table 1 indicated that, the head of household high income percentage indicated that in Kerman City, majority of income obtained by head of household and the second highest income percentage obtained by spouse's income. Likewise, there are other sources of incomes which are indicated in Table 1.

Table 1: Household Income Sources & Percentage (n=390)

Sources of Income	Mean (USD)	%
Main Income	789	66.3
Supplementary	46.4	3.9
Spouse Income	76.5	6.4
Spouse Supplementary	3.8	.32
Rent Land	26.2	2.2
Rent House	22.6	1.9
Daughter Income	6.5	.55
Son income	7.1	.6
Grandchild Income	0	0
Relative Income	5.2	.44
Agriculture Activities	30.3	2.55
Pensioner and Retired	73.8	6.2
Social Welfare Benefit	14.9	1.25
Business	71.4	6
Profit, Interest and Dividend	12	1
Other Sources	4	.34
Total	1190	100

Household Expenditure

Household's ability to pay is defined as sufficient income remaining after basic survival needs have been met. sufficient income is taken to be the whole consumption expenditure of the household, which in a lot of countries is a more perfect reflection of purchasing power than income reported in household survey (Xu et al., 2003). Investigating household expenditure is essential, because in term of policy viewpoint, one needs to obviously recognize the determinants of housing expenditures and the relative importance of every determinant. Determinants of household expenditure, especially on fundamental products similar to food and clothing, have been of regular interest to economists for countries. New work starts from Engel (1985) and this centre of attention on relationship between expenditure on food and income. The nature and patterns of food bought and expenditure reflected and in diverse ways continue to reveal wealth, income and life style (Jacobson et al., 2009).

Gan and Vernon (2003) decision that food is, in actuality more shared than is supposed to be the case. For some researchers food is not the best pattern of a private good, they thought clothing is a better one (Gan & Vernon, 2003). It is possible, researchers respond, that economies of scale in food production are possible, but their theoretical argument even now leads them to make decision (Deaton & Paxson, 2003).

Expenditure as one of the dependent variable, has caught the attention in most literature. The researcher measured it by sixteen indicators, including: Food and raw materials purchased and consumed at home and elsewhere, maintenance of assets and furniture, children education, celebration, transportation service, tax, travelling, holiday, replacing of home furniture, education and training expenses, health care, leisure activities, house rental, utilities, clothing and other disposable household items. Any expenditure made by the members of the selected household for business purposes were not considered in this study.

Table 2 indicated monthly household expenditure in Kerman City.

Table 2: Monthly Household Expenditure (n=390)

Household Expenditure	Frequency	Percentage
Lowest 250	97	24.9
251 to 500	131	33.6
501 to 750	78	20
751 to 1,000	42	10.8
1,001 and Above	42	10.8

Ownership of Physical Asset

Beside income and expenditure the researcher used ownership of physical assets to measure the family economic status. Researchers found family economic status categorization is different for rural and urban based on asset ownership (Chuma & Molyneux, 2009). Ownership of physical assets is an apparent indicator for situation in the social construction, and almost definitely a better indicator to income-based measure (Podder & Kakwani, 1976). To measure ownership of physical asset, the researcher used several questions related to ownership of home, factory, villa or vacation home, dividend and investment in stock exchange. In sample, 66.2% of families were home owners, 21.5% of families staying in rental houses, 2.6% of families lived in government quarters, and 9.7% of them lived with others such as relatives.

Looking into all the economic status of the heads of households the value of their home ownership was found to be the largest component of wealth or asset. This is an indication of the importance of owning a home as a source to measure the economic status.

Management functions

Management functions include five dimensions (planning, coordinating, organizing, directing and controlling). Table 3 indicated that Kerman people use from management functions in their life.44.3% of the respondents had moderate management, and majority of head of households (51%) believed to strong management practiced in their life.

Table 3. Management Functions (n=390)

	Frequency	Percent
Weak	11	2.8
Moderate	173	44.3
Strong	199	51
Very strong	7	1.8
Total	390	100

Table 4 indicates the heads of households' ideas about the management functions. About 39% of heads of households had moderate planning in the family affairs, about 45.6% of them had strong planning, i.e. these number of heads of households had an acceptable knowledge about goals and life planning, or this kind of people had very good knowledge about future strategies, life goals in short and long term, making the future visible and how to achieve the goals. About 39% of respondents had moderate coordinating, i.e. they had knowledge and information about coordinating methods among family members, about 43.6% had strong coordinating, i.e. these number of heads of households had good knowledge about coordinating methods among family members, about 10.8% had very strong coordinating in his/her life, which indicates that this category of people had very good knowledge about collaboration for family problems solving, coordination for family costs reduction, optimum use of resources and family facilities and ability to achieve information.

Majority of the head of the households (74.9%) believed that their organizations in family affair is weak, they cannot do this function very well i.e. looking ahead, collaboration between family members, division of labour between family members, to determine functions of family members, authority entrusted to the family members, to give responsibility to family members. 23.1% of respondents had moderate organizing in family affairs; it means that this number of heads of households had knowledge about family organizing. This indicates that this category of heads of households had knowledge about mention functions in organizing. around 36.7 % of the heads of households believed that they had moderate directing between family members, about 54.4 % of them had strong directing, i.e. this number of heads of households had a good knowledge about family directing methods, and about 5.4 % of heads of households had very strong directing in his/her life. It indicates that this category had very high knowledge about decision making, establishing friendly relationship between family members, usage of violence and reward between family members, and the kind of attention to family members' need? Based on continuous data, the mean was 30.83, the median was 31, the mode was 27, the range was 36, and the minimum and maximum were 9 and 45 (Table 4). About 47.7% of heads of households had moderate controlling in their life's, this number of head households had an acceptable knowledge about controlling family affairs, 42.1% of the respondents had strong controlling in his/her life which shows that this category of people had very good knowledge about controlling, they had special attention to the family affairs, they tried to investigate the causes of the problems, they had control on entrusting functions in family and they compared existing and optimum situation. However in management functions, based on five elements, the highest percentage was in organizing with 74.9 % (weak organizing), and in other aspects or functions, most of the respondents were believed to use these functions moderate and strong in their life. This indicated that people with consideration of their level of education, their age, gender, and occupations, had almost similar views about management functions.

Table 4: Management Functions (n=390)

	Planning		**Coordinating**		**Organizing**		**Directing**		**Controlling**	
	n	%	n	%	n	%	n	%	n	%
Very weak	0	0	0	0	8	2	1	.25	2	.5
Weak	14	3.6	26	6.7	292	74.9	13	3.3	12	3.1
Moderate	152	39	152	39	90	23.1	143	36.7	186	47.7
Strong	178	45.6	170	43.6	0	0	212	54.3	164	42
Very strong	46	11.8	42	10.7	0	0	21	5.4	26	6.7
Total	390	100	390	100	390	100	390	100	390	100

Table 0-1: Management Functions

	Management	Planning	Coordinating	Organizing	Directing	Controlling
Mean	145.92	37.56	24.32	16.69	30.8	36.5
Median	146.5	37	24	16	31	35
Mode	129	33	21	15	27	33
Range	155	38	27	20	36	44
Minimum	60	17	8	5	9	11
Maximum	215	55	35	25	45	55
Skewness	-0.197	-0.005	-0.196	-0.047	-0.273	0.05
Kurtosis	-0.282	-0.637	-0.436	0.216	-0.001	0.518

For confirm this findings, in the model Deacon and Firebaugh (1988), the managerial subsystem is explained at the same time as comprising both planning and implementing behaviours. Planning is comprised of setting regular and sequencing behaviours. Standard or regular setting included two activities descriptive the demand(s) that is (are) to be met and evaluation the resources accessible to meet the demand(s). The standards or regulars and sequences comprise a program families apply plans throughout the behaviours of actuating or doing the plan and controlling the plan. Controlling includes inspection how the plan is succeeding and adjusting or making changes as required to the plan as it progresses. In the managerial subsystem, demands and resources are converted to the outputs of responses to the demand and alter to the composition of the resources stock. Managing these managerial actions well is consideration to guide to improved management. Improved management is supposed to guide to a higher quality of life.

Relationship between Managerial Functions Practiced and Family Economic Status in Kerman City

Money management is the most commonly prescribed technique for the household. Money management is advocated in order that plan and control spend, to identify where immoderate expenditure has occurred, to “make ends meet”, to dishearten impulse buys, to attentive the head of household to the likelihood of reducing into debt, to expose the range for savings and investment, to foster the management abilities of family members, and to make sure that short-term income and expenditure patterns are matching with the achievement of long-term goals (Bremner, 1988; Crary & Donaldson, 1980; Dibben, 1984; Gundrey, 1975; Hancock, 1979; McGlone & Metland, 1984; Munnion, 1969; Nickell, Rice, & Tucker, 1976; Potter, 1972). The following hypotheses will be tested in order to get the approximate measures of management functions and family economic status dimensions (expenditure, income, and ownership of physical asset).

The relationship between household expenditure, and management functions was investigated using Pearson product-moment correlation coefficients. Preliminary analyses were performed to ensure no violation of the assumptions of normality and linearity. Since there were five (5) bivariate pairs, Bonferroni adjusted alpha of 0.01 (0.05/5) was used to test null hypothesis of the bivariate pairs.

As depicted in Table 5, the linear positive relationships were found between household expenditure, and planning ($r = .28$, $p = .0001$), coordinating ($r = .20$, $p = .0001$), organizing ($r = .243$, $p = 0.0001$), directing ($r = .251$, $p = 0.0001$), and controlling ($r = .29$, $p = 0.0001$). All correlation coefficients indicate weak and positive linear relationship between household expenditure and elements of management functions.

Although this study was not designed to determine, whether, an increase in one variable caused an increase in the value of a second variables, it would seem logical to say that the household expenditure is more likely to increase when management functions increase. Also confidently we can say that these relationships are genuine and not happen by chance. Previous studies have stressed on the importance of life cycle stage in establishing expenditure patterns (Abdel-Ghany & Sharpe, 1997; Bloom & Koreanman, 1986; Chung & Magrabi, 1990; Edmondson, 1999; Gallo & Boehm, 1987; Robey & Russell, 1983; Rubin & Nieswiadomy, 1994; Sexauer, 1997). These findings confirmed with the earlier household expenditure researchers views, they believed that different elements have effect on Household expenditure (HE) one of these elements was level of education and it was indirectly related to management knowledge of head of households (Abdel-Ghany & Foster, 1982; Dardis et al., 1981; Horton & Hafstrom, 1985). Western sociological study above the last few years has create that patterns of monetary management

and expenditure decision making be different among diverse cultures, beyond social classes and over time (Simon, 2002). The management of households' day-to-day expenditure has been conventionally seen as a subject of household decision- making influence (Ray-may et al., 2006). The management of household expenditure is a significant family economic action. Household sociologists in Taiwan have discovered that growth of an economy, resource differences between husband and wife, cultural conditions and family life cycle might all illustrate the management rule of household expenditure (Chen et al., 2000).

The relationship between family income, and management functions was investigated using Pearson product-moment correlation coefficients. Preliminary analyses were performed to ensure no violation of the assumptions of normality and linearity. Since there were five (5) bivariate pairs, Bonferroni adjusted alpha of 0.01 (0.05/5) was used to test null hypothesis of the bivariate pairs.

As depicted in Table 5, the linear positive relationships were found to exist between family income, and planning (r = .25, p = .0001), coordinating (r = .219, p = .0001), organizing (r = .205, p = 0.0001), directing (r = .243, p = 0.0001), controlling (r = .29, p = 0.0001). All correlation coefficients indicate a weak and positive linear relationship between family income elements of management functions.

To confirm this findings, the peoples controlling the finances is capable to make expenditure decisions, other than as well as responsible for ensuring that he desires of family members are met. In a circumstance where the money attainable is scarcely enough to meet those needs, the role of control the household money is further likely to be a burden than a source of ability (Simon, 2002). Also can see other study that confirm study findings, it is specific noticeable that, in over 80% of families, the budget is said to be controlled cooperatively; although in merely one in eight households it is managed by one person; (Simon, 2002). Researchers believed that reasonable planning, management and accounting for family moneys are apparent in perspective literature as virtuous practice which decrease uncertainly and take the sentiment out of household money matters (Allen, 1977; McGlone & Metland, 1984; Norling et al., 1989). Money management systems are frequently complex for persons and families as "part of the art of living" (Allen, 1973). As Firebaugh identified, and Haskins before him, personal accounting has an extensive social and behavioural importance in everyday life. Its training serves "to strengthen economic morality, self-reliance and regulation (Haskins, 1903).

The relationship between ownership of physical asset, and management functions was investigated using Pearson product-moment correlation coefficients. Preliminary analyses were performed to ensure no violation of the assumptions of normality and linearity. Since there were five (5) bivariate pairs, Bonferroni adjusted alpha of 0.01 (0.05/5) was used to test null hypothesis of the bivariate pairs.

As depicted in Table 5, the linear relationship was found to exist between ownership of physical asset, and planning (r = .184, p = .0001), coordinating (r = .097, p = .055), organizing (r = .161, p = 0.0001), directing (r = .165, p = 0.0001), and controlling (r = .195, p = 0.0001). All correlation coefficients indicate weak and positive linear relationship between ownership of physical asset and elements of management functions. With a glance to the above results, only coordinating didn't have significant relationship with ownership of physical asset, and other management functions had significant relationship with asset. The results indicate that respondents do the planning, organizing, directing, and controlling in life affairs, and they can increase the rate of ownership of physical asset, but this increase cannot be very significant.

Some researchers supported the relationship between asset and management functions. Financial and individual tangible assets are to be said in the net worth statement at realisable values and while their whole is added to expected life span earnings a determine is gained of "the total sum of economic value available to that personal for individual monetary planning" (Crary & Donaldson, 1980). Annual or more frequent statements of net worth are recommended in order to carry out intermittent "wealth checks" (Jennings, 1996).

Conclusion & Recommendation

Family resources management fulfils a fundamental role in addressing and raising the awareness of the increasing complexities faced by families. Household management is a process of using the required resources to attain the families' goals through planning and taking the necessary steps to meet these goals. Financial management has integrated systems theory with a set of recommended financial management practices, the 'normative practices,' to assess whether families are managing their financial affairs properly.

The aim of this study is to determine the level of managerial functions practiced by the families, as well as the relationship between the levels of managerial functions practiced and family economic status in Kerman city. This is a quantitative study that investigates a social or human problem based on testing a theory comprising variables measured with numbers and analyzed using statistical measures. The research design employed was correlation research, which

examines the degree to which variables are related and the direction of the relationship. For this survey, based on Kerjcie and Morgan's Table and the sample size schedule, the researcher selected 390 households out of a total of 127,892 families residing in Kerman City. The instrument used for this study was a questionnaire consisting of three sections, with data collection through face-to-face interviews to obtain information from the heads of households.

Table 0: Pearson's Correlation of Household Expenditure, Family Income, Ownership of Physical Asset and Management Functions

	Variables	Y	X_1	X_2	X_3	X_4	X5
Y	Household Expenditure						
X_1	Planning (2)	.278**					
X_2	Coordinating (3)	.200**	.785**				
X_3	Organizing (4)	.243**	.612**	.659**			
X_4	Directing (5)	.251**	.616**	.672**	.628**		
X_5	Controlling (6)	.290**	.623**	.631**	.681**	.698**	
Y	Family Income (1)						
X_1	Planning (2)	.247**					
X_2	Coordinating (3)	.219**	.785**				
X_3	Organizing (4)	.205**	.612**	.659**			
X_4	Directing (5)	.243**	.616**	.672**	.628**		
X5	Controlling (6)	.292**	.623**	.631**	.681**	.698**	
Y	Asset (1)						
X_1	Planning (2)	.184**					
X_2	Coordinating (3)	.097	.785**				
X_3	Organizing (4)	.161**	.612**	.659**			
X_4	Directing (5)	.165**	.616**	.672**	.628**		
X_5	Controlling (6)	.195**	.623**	.631**	.681**	.698**	

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

In this study, we investigated the relationship between management functions and family economic status. The effect of different functions of management was tested on the dimensions of FES (income, expenditure, and ownership of assets). The relationship between household expenditure, and management functions (planning, coordinating, organizing, directing and controlling) was investigated using Pearson product-moment correlation coefficient.

The findings indicate that linear positive relationships were found to exist between family income, household expenditure, and ownership of physical assets with management functions. All correlation coefficients indicate a weak and positive linear relationship between family income, household expenditure, and ownership of physical assets with elements of management functions.

It may be interesting for future studies to look at the effect of other elements on family economic status, for example, the effect of leadership approaches on family economic status, the effect of different leadership styles on family economic status, and the effect of the surrounding environment on family economic status. Future studies can look at the effect of family economic status on management functions. Furthermore, it is recommended that policy makers give attention to future plans for such research.

References

1. Abdel-Ghany, M., & Foster, A. (1982). Impact of income and wife's education on family consumption expenditure. *J Consumer Studies & Home Economics, 6*, 21-28.
2. Abdel-Ghany, M., & Sharpe, D. L. (1997). Consumer pattern among the young-old and old-old. *Journal of Consumer Affairs, 31*(1), 90-112.
3. Allen, M. (1977). *The Money Book. Your Money and Your Life*. London: Pan Books.

4. Allen, S. (1973). *Your Money's Worth*. London: Aldus Books.
5. Anne, E. W., & Thomas, R. I. (2009). Time Spent in Household Management: Evidence and Implications. *J Fam Econ Iss 2009*(30), 293-304.
6. Berkman, L. F., & Macintyre, S. (1997). *The measurement of social class in health studies: old measures and new formulations. In: Kogevinas, M., Pearce, N., Susser, M. & offetta, P. (eds) Social Inequalities and Cancer. Lyo*: IARC Scientific Publications No. 138, International Agency for Research on Cancer.
7. Bloom, D. E., & Koreanman, S. D. (1986). Spending habits of American consumers. *American Demographics, 8*(3), 22-25.
8. Bremner, M. (1988). *Enquire within upon Everything*. London: Century.
9. Brodie, M. B. (1967). *Fayol on Administration*. London: Lyon, Grant and Green
10. Bryman, A., & Bell, E. (2003). *Bussiness research methods*: Oxford: Oxford University Press.
11. Chen, Y. H., Yi, C. C., & Lu, Y. H. (2000). Married Women's Status in the Family: An example of Decision Making Pattern. *Taiwanese Journal of Sociology 24*, 1-58.
12. Chuma, J., & Molyneux, C. (2009). Estimating inequalities in ownership of insecticide treated nets: does the choice of socio-economic status measure matter? *Health Policy & Planning, 24*(2), 83-83.
13. Chung, Y. S., & Magrabi, F. M. (1990). *Age related changes in expenditure patterns.* Paper presented at the Proceedings of 36th Annual Conference of the American Council on Consumer Interests, Columbia.
14. Crary, D. T., & Donaldson, E. F. (1980). *Personal Finance*. New York, NY: J. Wiley & Sons.
15. Creswell, J. W. (1994). *Research Design: Qualitative and Quantitative Approaches*. London: Sage.
16. Dardis, R., Derrick, F., & Lehfeld, A. (1981). *How Have Families with Children Been Faring? Discussion paper No 801-86.* Madison, Wl: Institute for Research on Poverty.
17. Deacon, R. E., & Firebaugh, F. M. (1988). *Family Resource Management. Principles and Applications*. Boston: Allyn and Bacon, inc.
18. Deaton, A., & Muellbauer, J. (1980). *Economics and consumer behavior*. Cambridge: Cambridge University Press.
19. Deaton, A. S., & Paxson, C. (2003). Engel's what? A response to Gan and Vernon. *Journal of Political Economy, 111*(2003), 1378–1381.
20. Dibben, M. (1984). *The Guardian Money Guide*. London: Collins.
21. Doherty, W. J., Boss, P. G., LaRossa, R. L., Schumm, W. R., & Steinmetz, S. K. (1993). *Family theories and methods: A contextual approach. In P. G. Boss, W. J. Doherty, R. L. LaRossa, W. R. Schumm & S. K. Steinmetz (Eds.), Sourcebook of family theories and methods: A contextual approach (pp. 3-30).* New York: Plenum Press.
22. Edmondson, B. (1999). Do the math. *American Demographics, 21*(10), 50-56.
23. Eichar, D. (1989). *Occupation and Class Consciousness in America*: Westport, Connecticut: Greenwood Press. ISBN 0-313-26111-3.
24. Fayol, H. (1949). *General and Industrial Management*: Constance Storrs Sir Isaac Pitman & Sons Ltd. London.
25. Fowler, F. J. (1988). *Survey Research Methods*. Newbury Park: CA:Sage.
26. Gallo, A., & Boehm, W. T. (1987). Food purchasing patterns of senior citizens. *National food Review, 75*(3), 42-45.
27. Gan, L., & Vernon, L. (2003). Testing the Barten model of economies of scale in household consumption: toward resolving a paradox of Deaton Paxson. *Journal of Political Economy, 111*(2003), 1361–1377.
28. Goldsmith, E. (1996). *Resource Management for Individuals and Families.* Florida State university: West Publishing Company. USA. St. Paul.
29. Greg, J., Mary, C., Peggy, M., David, R., & Williams. (2002). Optimal Indicators of Socioeconomic Status for Health Research. *American Journal of Public Health, 92*(7), 1151-1158.
30. Gundrey, E. (1975). *Making Ends Meet. A Practical Guide to Family Finance*. London: Arrow Books.
31. Hair, J. F., Black, W. C., Babin, B. J., Anderson, R. E., & Tatham, R. L. (2006). *Multivariate data analysis*: Upper Saddle River, NJ:Prentice Hall.
32. Hancock, G. (1979). *Home Budgeting*. London: Oyez Publishing Ltd.
33. Haskins, C. W. (1903). *How to Keep Household Accounts*. New York, NY: Harper & Brothers.
34. Horton, S., & Hafstrom, J. (1985). Income elasticity's for selected consumption categories: Comparison of single female headed and two-parent families. *Home Economic Research Journal, 13*, 292-303.
35. Jacobson, D., Mavrikiou, P. M., & Minas, C. (2009). Household size, income and expenditure on food: The case of Cyprus. *Journal of Socio-Economics, In Press, Corrected Proof*.
36. Jalal Maab, M. (2008). *Kerman Statistical year book* (1st ed.): Kerman Planning and Management Organization

37. Jennings, M. (1996). *Good Housekeeping Consumer Guide. Your Family and Money*. London: Ebury Press.
38. John, D., Catherine, T., & MacArthur. (2002). Research Network on Socioeconomic Status and Health. Retrieved 13 April 2009, from http://www.macses.ucsf.edu/Research/Social%20Environment/notebook/economic.html
39. Keister, L. A., & Moller, S. (2000). Wealth Inequality in the United States. *Annual Review of Sociology, 26*, 23–81.
40. Key, R. J., & Firebaugh, F. M. (1989a). Family resource management: Preparing for the 21st century. *Journal of Home Economics (Fall), 81*(1), 13-17.
41. Key, R. J., & Firebaugh, F. M. (1989b). Family resource management: Preparing for the 21st century. *Journal of Home Economics, 81*(1), 13-17.
42. Madad, M. (2008). Urban Families income and expenditure plan: Statistic centre Iran.
43. McGlone, J., & Metland, D. (1984). *1000 Housekeeping Hints*. London: Octopus Books.
44. Morris, L. (1995). *Social Divisions: Economic Decline and social Structural Change*. London: UCL Press.
45. Munnion, C. (1969). *Budget and Buy*. London: Nelson.
46. Nickell, P., Rice, A. S., & Tucker, S. P. (1976). *Management in Family Living*. New York, NY: John Wiley & Sons.
47. Norling, D. N. J., Hess, K. M., & Nystrom, K. K. (1989). *Your Financial Planning*. New York, NY: Kit, J. Wiley &Sons.
48. Pahl, J. (1983). The Allocation of Money and the structuring of Inequality within Marriage. *Sociological Review, 31*(2), 237-262.
49. Paolucci, B., Hall, O. A., & Axinn, N. (1977). *Family Decision Making: An Ecosystem Approach*. New York: John Wiley & Sons.
50. Podder, N., & Kakwani, N. C. (1976). Distribution of Wealth in Australia. *Review of Income and Wealth 22*(1), 75–92.
51. Potter, D. (1972). *Understanding Money*. London: Batsford.
52. Ray-may, H., Chin-chun, Y., & Yang, c. F. (2006). Overlapping Social Networks: How Couples Manage Family Expenditure in Taiwan. *Current Sociology, 54*(2), 187-208.
53. Rettig, K. D., & Mortenson, M. (1986). *Household production of financial management competence. In R. E. Deacon & W. E. Huffman (Eds.), Human Resources Research (pp. 137-145)*: Ames, IA: College of Home Economics.
54. Robey, B., & Russell, C. (1983). How consumers spend. *American Demographics, 5*(10), 16-21.
55. Rubin, R. N., & Nieswiadomy, M. (1994). Expenditure patterns of retired and non retired persons. *Monthly Labor Review, 117*(4), 10-21.
56. Saifollahi, M. (2008). *Kerman municipality statistic and information*: Kerman News.
57. Sekaran, U. (2001). *Research method of business: a skill-building approach* New York: John Wiley and Son, Inc.
58. Sexauer, B. (1997). The effect of demographic shift and changes in the income distribution on food-away-from-home expenditure. *American Jounal of Agricultural Economics, 61*, 1046-1057.
59. Simon, C. (2002). Budgetary Management in Russian Households *sociology, 36*(3), 539-557.
60. Stephen, P. W. (2000). Accounting at home: some interdisciplinary perspectives. *Accounting, Auditing &Accountability Journal, 13*(4, 2000), 425-449.
61. Vogler, C. (1994). *Money in the Household*. Oxford: Oxford University Press.
62. Wiersma, W. (2000). *Research Methods in Education: An introdauction* (7th ed.): Allyn and Bacon.
63. Xu, K., Klavus, J., & Kawabata, K. (2003). Household health system contributions and capacity to pay: definitional, empirical and technical challenges. In: Murray CJL, Evans DB, ds.Health systems performance assessment: debate, new methods and new empiricism. Geneva: World Health Organization (in press). *Health Systems Performance Assessment*.

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Bioactivities and Biochemical Effects of Marjoram Essential Oil used against Potato Tuber Moth *Phthorimaea operculella* Zeller (Lepidoptera: Gelechiidae)

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Abstract: The bioactivities of marjoram essential oil against immature stages and adults of potato tuber moth *Phthorimaea operculella* Zeller were evaluated. The essential oil showed significant contact and fumigation insecticidal activities against different stages. The oil revealed strong contact toxicity and moderate fumigant activity against immature stages. Both adult males and females showed high susceptibility to the fumigation. Oviposition deterrent effects were found to be insignificant. Furthermore, the results showed that treatment of immature stages with the essential oil produced adult deformations. The essential oil tested had some biochemical effects on the last larval instar treated by the contact method, based on LC_{50} during metamorphosis to the adult. The results showed increases in the total protein and triacylglycerol content of most post-treatment days. Insignificant increases were found in the activities of acetylcholinesterase and chitinase**.** These results suggested that marjoram essential oil could be used as a potential control agent for potato tuber moth in storage facilities.
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Key words: Essential oils- marjoram- *Phthorimaea operculella*- insecticidal activity- biochemical effects.

1. Introduction:

The potato tuberworm, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae), is an important and ubiquitous pest of potato, *Solanum tuberosum* L. (Solanaceae), in both field and stores in the subtropical and tropical zones (Golizadeh & Razmjou 2010). It is wide spread in Egypt, especially in the northern areas of Lower Egypt (Sharaby *et al.,* 2009).

An integrated pest management (IPM) strategy for potato tuber pests has been developed and promoted by various institutions. The main component of this IPM package is a biopesticide that is applied to the surface of the tubers in farm storage (Zeddam *et al.*, 2008). Among biopesticides, botanical pesticides have received a great deal of attention because of their favourable eco-toxicological properties, e.g., low human toxicity, rapid degradation and reduced environmental impact. These properties make them suitable insecticides for organic agriculture. Aromatic plants are among the most effective insecticides of botanical origin. Essential oils often constitute the bioactive fraction of plant extracts (Shaaya *et al,*. 1991; 1997; Regnault-Roger 1997). Their have lipophilic nature facilitates their interference with basic metabolic, biochemical, physiological and behavioural functions of insects (Nishimura, 2001). They have potential as ovicides, fumigants, insect growth regulators and insecticides against various insect species (Regnault-Roger, 1997 and Shaaya *et al,*. 1997). In addition, most of these substances are volatile and can act as fumigants, thus offering the option of use against stored-product insects (Stamopoulos *et al*, 2007). Sweet marjoram *Majorana hortensis* Moench (family: Lamiaceae) is an Old World perennial aromatic herb that was cultivated and used as flavouring in foods. The leaves and stems yield an essential oil. Its volatile constituents have previously been found to have a broad spectrum of biological activities, including antifeedant, repellent and insecticidal properties. It is used against a number of agricultural and stored-product pests (Pavela, 2004; Mohamed and Abdelgaleil, 2008). Numerous studies have addressed the general use of essential oils against *P. operculella* (Guerra *et al.*, 2007; Sharaby *et al.*, 2009).

Compounds extracted from plants, or the derivatives of such compounds may affect insect physiology in various ways (Shekari *et al.,* 2008). This investigation aimed to investigate the repellency, toxicity and some biochemical effects of crude oil extracted from *M. hortensis* for use against *P. operculella*.

2. Material and Methods:

2.1. Insects:

A culture of *P. operculella* was maintained in our laboratory over 3 years without exposure to insecticides. Larvae were kept in wire cages and reared on potato tubers. The bottoms of cages were furnished with a thin layer of clean sand (previously exposed to a high temperature in an oven) for pupation (El-Sinary, 1995). Culture and experiments

were maintained at 29±1º C, and 12L: 12D photoperiod.

2.2. Essential oil:

Sweet marjoram *(Majorana hortensis)* essential oil (EO) was purchased from El-captain Company (CAP. PHARM., Egypt) for extracting natural oils, herbs and cosmetics, Cairo, Egypt.

2.3. Contact bioassay:

The insecticidal activities of the essential oil against larval (4^{th} last larval instar), prepupal and pupal stages were evaluated using the contact method in a sandy soil. Ten grams of clean sand was placed in 250 ml glass jars and treated with different doses (0.2, 0.1, 0.05, 0.025 and 0.012 ml) of oil solutions diluted in 1 ml of acetone. The sand was stirred continuously for 1 min to ensure the even spread of the oil over the surface. The solvent was allowed to evaporate for 10 min. Twenty individuals of each test stage were placed in the jars and then covered with a thin layer of the treated sand. In the control group, the sand was treated only with acetone. Each jar was covered with nylon mesh held in place with rubber bands. Mortality percentage was recorded 24 hours later. The experiments were observed until the emergence of the adults to assess total inhibition of metamorphosis and adult malformations.

2.4. Fumigant bioassay:

The fumigant activity of tested oil was determined according to the method described by Prates *et al.,* (1998). Twenty test insects (last larval instars, prepupae, pupae and adult males or females) were put into separate 250 ml glass jars. Marjoram essential oil at doses of (0.2, 0.1, 0.05, 0.025 and 0.012 ml) was diluted in 1 ml of acetone and applied to 5 cm diameter filter paper. The filter papers were attached to the underside surface of the screw caps of the glass jars after solvent evaporation (10 min). The jars were first covered with nylon mesh. The caps were then attached. This measure was taken in order to prevent a direct contact between insects and the bioinsecticide. Another group of filter papers was treated only with acetone and used for the control group. Mortality percentage was recorded 24 h later. Experiments with the immature stages (larvae, prepupae and pupae) were followed until adult emergence to assess total inhibition of metamorphosis and adult malformations.

2.5. Ovicidal bioassay:

The toxicity of marjoram essential oil to eggs was examined with contact and fumigant bioassays. Adult insects (males and females) were collected from the stock culture after emergence and put together in galss jars covered with muslin (5 cm diameter) for oviposistion. Muslin-egg batches of 1 day-old were collected, numbered and divided into two groups. In order to test the contact toxicity of essential oil, the first group of eggs was dipped in different concentrations of test oil (0.2, 0.1, 0.05, o.025 and 0.012 ml) diluted in 1 ml of acetone. Acetone solution was used only for control group. After drying for 20 minutes, egg batches were inserted in Petri dishes and subsequently covered. In order to test the toxicity of essential oil vapours, the second group of egg batches was inserted into 250 ml glass jars covered with filter papers attached to the under surface of the screw cap. The filter papers were treated previously with different concentrations of EO diluted in 1 ml of acetone (0.2, 0.1, 0.05, 0.025 and 0.012 ml) and allowed to dry. Another group of filter papers treated with acetone only were used for the control group. Hatchability percent was recorded after 3 days in all groups.

2.6. Oviposition repellency bioassay:

Muslin pieces (5 cm diameter) were treated with doses (0.008, 0.004, 0.002 and 0.001 ml) of EO diluted in 1 ml of acetone and dried for 20 minutes. Ten (one day old) sexed adults were placed in 250 ml glass jars. The muslin were attached to the under surface of the glass jar screw caps. The caps were screwed tightly on the jars. Another group of the muslin was treated with acetone only and used for the control group. The percent effective oviposistion repellency was recorded 24h later for three successive days.

2.7. Biochemical analysis:

Last instar larvae of *P. operculella* were treated with LD_{50} of the EO *M. hortensis* by the contact method previously described, in order to estimate the activities of chitinase and acetylcholinesterase (AChE), and total protein and triacylglycerol (TAG) content. The results were recorded during the metamorphosis of larvae to the adult stage at intervals of 0 and 1 day for larvae, 1, 3 and 6days for pupae and 1 day for adults.

Assays of AChE and chitinase activities were performed according to Waterhouse *et al.,* (1961) and Simpson *et al.,* (1964), respectively. The method described by Bradford (1976) was applied to measure total protein content. TAG was measured by using a kit produced by Randox Laboratories LTD. (United Kingdom BT294QY).

2.8. Statistical Analysis

LC_{50} value was determined according to Finney (1971) for the contact method. Means were tested for significance by the one way analysis of

variance (ANOVA). When the ANOVA statistics were significant ($P < 0.05$), means were compared using Duncan's multiple range test. Percent of insect mortality was calculated using the corrected Abbott's formula (Abbott, 1925).

The percent effective oviposition repellency for each dosage was calculated using the following formula: ER (%) = NC – NT / NC x 100,
where ER (%) = percent effective repellency, NC = number of eggs of control, and NT = number of eggs of treated group.

3. Results:

3.1. Contact toxicity:

Analysis of the toxicity data showed that marjoram essential oil exhibited strong toxic activity against larvae and prepupae after 24 h exposure (Table 1). All larvae and prepupae (100%) died at the highest dose (0.2 ml/10gm). The mortality percent decreased significantly with decreasing the dosage. The lowest dose induced insignificant mortality. On the other hand, the pupal stage was more tolerant than other stages. The highest dose induced a significantly higher mortality of 16.67% relative to the control. The other doses produced insignificant effects.

The data in Table (2) showed that *M. hortensis* affected the emergence of adult insects from treated larvae and prepupae. The highest dose (0.2 ml) induced significant reductions in the emergence of adults from all treated immature stages, relative to the control. Although insignificant effects of the contact bioassay on treated pupae were observed within 24 h of application, a significant reduction ($P< 0.05$) in adult emergence was observed at most dose levels. Some deformed adults emerged from immature stages treated by the contact method (Table 2). Some doses induced various degrees of adult deformation. Permanently dumpy fore-wings, expanded membranous wings, retained pupal skin, and failure of wing formation were observed.

3.2. Fumigant toxicity

These experiments were conducted in order to determine whether the insecticidal activity of marjoram oil against *P. operecculella* was attributable to fumigant action (Table 3). The oil exhibited strong insecticidal activity against larval and prepupal stages at the highest doses. The lowest doses (0.025 and 0.012 ml/250 ml) had insignificant effects against the same stages. As shown in the contact method, the pupal stage was more tolerant to fumigation than other stages. The mortality percentage of pupae was not significantly different from the control in all treatments. Fumigant efficacy tests of EO against adult males and females showed very high susceptibility in almost all treatments after 24 h exposure. The oil induced 100% mortality of in both males and females at s 0.2 and 0.1ml doses. The male was more susceptible to lower doses than was the female.

Some adults emerged from immature stages treated by the fumigant method (Table 4). Some doses induced various degrees of adult deformation. Permanently dumpy fore-wings, expanded membranous wings, retained pupal skins, and failure of wing formation were observed.

3.3. Ovicidal activity

In contact bioassay (Table 5), all doses had significant effects compared with the control. The strongest adverse effect on egg hatchability was observed at 0.2, 0.1 and 0.05 ml doses. Hatchability increased gradually as the dosage decreased. The same table shows that fumigation had significantly lower effects ($P< 0.05$) on egg hatchability. The lower doses seem to have moderate or insignificant effects on this biological parameter.

3.4. Oviposition repellency:

The data (Table 6) revealed that *M. hortensis* had insignificant oviposition deterrent activity. The data showed that even at the highest dose (0.008 ml), the effective oviposition repellency was only 11.5 %. This value does not differ significantly ($P< 0.05$) from those found in other treatments and control values. The hatchability percentage of eggs oviposited by the treated females did not differ significantly from the corresponding value for control females.

3.5. Biochemical analyses:

The effects of LC_{50} doses (0.037 ml / 10 gm) of EO applied by the contact method to the last larval instar were tested for biochemical changes during the metamorphosis to the adult stage.

Figure (1) shows that chitinase activity increased gradually with time in control and treated larvae. However, treated larvae had slightly increased enzyme activity. Chitinase could not be detected in adults emerged from both treated and control larvae.

AChE activity did not changed in treated larvae, relative to the control (Fig. 2). Insignificant inhibition of AChE activity was observed in treated larvae for all days during metamorphosis to the adult, relative to the control.

Triacylglycerol content (TAG) decreased gradually in both treated and control insects during the metamorphosis to the adult stage (Fig. 3). The amount of reduction in treated larvae was significantly higher ($P< 0.05$) than in untreated larvae. The highest reduction was observed after one day of treatment and 3 days of pupation. No

difference in TAG content was observed between the treated and control insects at the 1st day of pupation. TAG content increased suddenly on the 1st day for newly emerged adults, both in control and in treated insects. The degree of elevation was more marked in the treated insects.

Hyperproteinemia was observed in infected larvae during metamorphosis to the adult stage relative to the corresponding control (Fig. 4). The exposure of potato tuber larvae to the LD_{50} of *M. hortensis* resulted in elevation of the total protein content on the 1st day after treatment. The protein content of the day-one pupa decreased significantly ($P< 0.05$) and irreversibly. The total protein content of treated insects increased again at the 3rd and 6th days of the pupal stage relative to the values for the control. The day-one treated adult did not show a significant difference in total protein content, relative to the control. Generally, total protein content decreased in treated and control larvae.

Table (1): Contact toxicity of marjoram essential oil against different stages of *P. operculella* after 24h of exposure.

Dose (ml/10gm)	Mortality % ± SE		
	Larva	Prepupa	Pupa
0.2	100 ± 0.0[a]	100± 0.0[a]	16.67± 3.3[a]
0.1	90.0± 5.8[a]	66.67 ± 8.82[b]	6.67±3.3[b]
0. 05	61.7± 4.4[b]	50.0± 5.7[b]	6.67±3.3[b]
0.025	30.0± 5.8[c]	23.33± 3.3[c]	0.0±0.0[b]
0. 015	6.7± 3.3[d]	11. 67± 4.4[d]	0.0±0.0[b]
0	0.0±0.0[d]	0.0±0.0[d]	0.0±0.0[b]

Means within a column followed by the same lower case letter are not significantly different ($P< 0.05$).

Table (2): Percent reduction and deformation in adult emergency from immature stages of *P. operculella* treated with marjoram essential oil by contact method.

Dose (ml/10gm)	% Reduction in adult emergence ±SE and % Deformations					
	Larva		Prepupa		Pupa	
	% Reduction	% Deformation	% Reduction	% Deformation	% Reduction	% Deformation
0.2	100±0.0[a]	-	100± 0.0[a]	-	41.7± 4.4[a]	-
0.1	100±0.0[a]	-	95. 0 ±2.9[a]	-	25.0± 2.9[b]	7.6
0. 05	86.7± 1.6[b]	20	71.7±6.0[b]	25	16.7± 1.7[b]	10.5
0.025	55.0± 5.7[c]	6.9	36.0± 6.0[c]	-	10.0 ± 2.9[c]	-
0. 012	38.3± 1.6[d]	3.9	13.3± 6.7[d]	10	5.0± 0.0[c]	-
0	1.6± 1.7[e]	-	0.0±0.0[d]	-	0.0±0.00[c]	-

Means within a column followed by the same lower case letter are not significantly different ($P<0.05$).

Table (3): Fumigant toxicity of marjoram essential oil vapours against different stages of *P operculella* after 24 h of exposure.

	Mortality % ± SE				
	Larva	Prepupa	Pupa	Adult	
				male	female
0.2	100 ±0.0[a]	100±0.0[a]	11.3±1.7[a]	100±0.0[a]	100 ±0.0[a]
0.1	88.6± 3.7[a]	63.6±4.4[b]	5.0± 2.9[a]	100± 0.0[a]	100±0.0[a]
0. 05	45.7±5.1[b]	34.3±3.4[c]	0.0± 0.0[a]	93.3± 3.3[a]	85.0± 2.9[a]
0.025	11.4± 0.9[c]	9.3±4[d]	0.0±0.0[a]	70.0± 5.0[b]	64.0± 5.8[c]
0. 012	3.8±1.5[c]	2.3±1.3[d]	0.0±0.0[a]	25.7±6.0[c]	19.3±4.9[d]
0	0.0±0.0[c]	0.0±0.0[d]	0.0±0.0[a]	0.0±0.0[d]	0.0±0.0 [e]

Means within a column followed by the same lower case letter are not significantly different ($P< 0.05$).

Table (4): Percent reduction and deformation in adult emergency from larvae of *P. operculella* treated with marjoram essential oil by fumigation method.

Dose (ml/250ml)	% Reduction in adult emergence ±SE and % Deformation					
	Larva		Prepupa		Pupa	
	% Reduction	% Deformation	% Reduction	% Deformation	% Reduction	% Deformation
0.2	100.0 ± 0.0 [a]	-	100.0±0.0[a]	-	13.3± 1.7[a]	-
0.1	100.0±0.0[a]	-	100.0±0.0[a]	--	9.0± 0.0[a]	-
0. 05	71.7 ±3.6[b]	22.2	63.3± 1.7[b]	21.3	7.3± 1.7[a]	-
0.025	46.3± 7. 3[c]	-	28.6 ±1.7[c]	9.5	0.00±0[a]	-
0.012	18.3± 1.7[d]	6.8	12.5 ± 2.4[d]	-	1.7± 3.3[a]	-
0	0.0±0.0[d]	-	0.0±0.0[d]		0.0±0.0[a]	-

Means within a column followed by the same lower case letter are not significantly different (P< 0.05).

Table (5): Ovicidal effects of marjoram essential oil against egg stages of *P. operculella.*

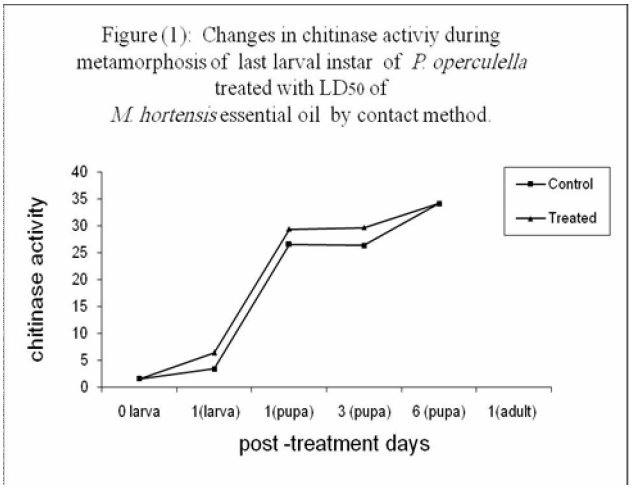
	Contact Bioassay		Fumigation Bioassay	
	Total No. of eggs	% hatched eggs ±S.E.	Total No. of eggs	% hatched eggs ±S.E.
0.2	146.8± 4.3	0 .0 ± 0.0[d]	147.8 ±4.3	67.3 ± 5.8[c]
0.1	158.6 ± 5.21	0 .0 ± 0.0[d]	159.2 ± 5.7	77.7 ± 6.1 [ac]
0. 05	155.2± 3.9	8.1 ± 1.8[d]	150 ± 4.2	88.3±6.4[ab]
0.025	153.4 ± 3.44	48.1± 2.1[c]	146.8 ±4.	95.2 ± 3.1[a]
0.012	165.4 ± 6.3	73 ± 7.6[b]	152 ± 4.2	94.7 ± 3.2[a]
0	145.4 ± 4.1	98.0 ± 1.5[a]	149.75± 3.8	99.0 ± 1.5[a]

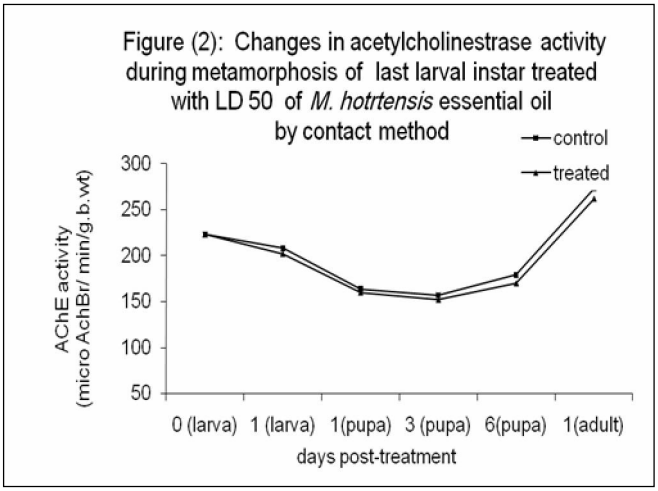
Means within a column followed by the same lower case letter are not significantly different (P< 0.05).

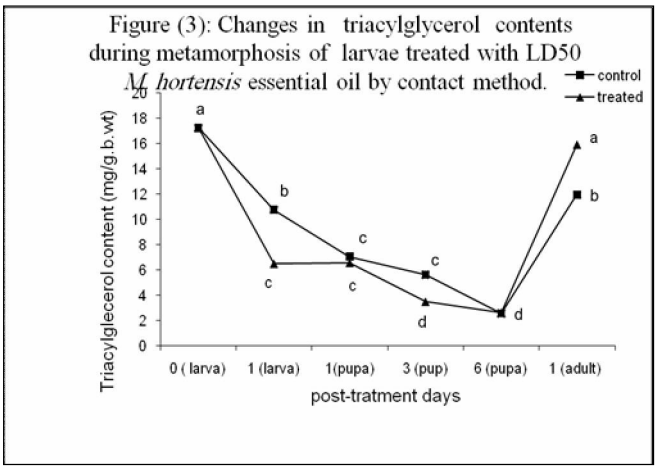
Table (6): Oviposition deterrent activity of marjoram essential against *P. operculella* adult and the percent of egg hatchability.

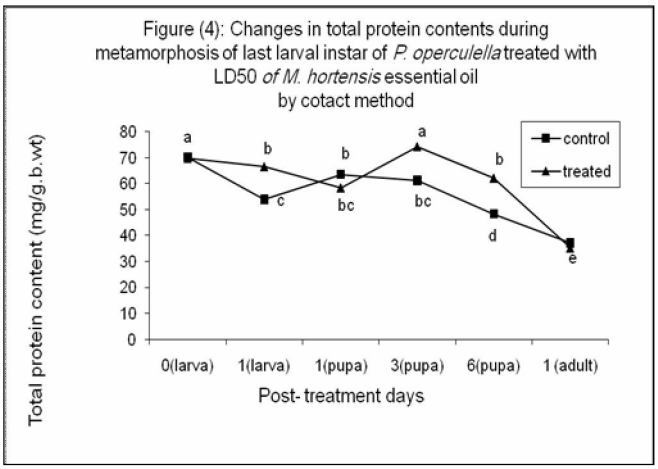
Dose (ml)	Mean eggs laid per female ± S.E.	Effective repellency (ER%)	% hatched eggs ± S.E.
0.008	77.3±3.2[a]	11.5	88.4 ±8.6[a]
0.004	83.7± 2.9[a]	2.9	91.7 ±4.8[a]
0.002	83.3 ± 2.2[a]	3.5	96.6 ±1.1[a]
0.001	84.6 ±6.4 [a]	1.9	95.3 ±7.6[a]
0	86.2 ± 3. 3[a]	-	99.3± 2.4[a]

Means within a column followed by the same lower case letter are not significantly different (P< 0.05).









Means bearing different subscripts are significantly different ($p < 0.05$)

Means bearing different subscripts are significantly different ($p < 0.05$)

4. Discussion:

The most effective botanical oils would be those offering a broad spectrum of activity against various life stages of the pest. The control agent should reduce the insect population at all stages, and it should decrease the incidence of the pest (Lamiri *et al.*, 2001). The present investigation showed that *M. hortensis* essential oil exhibited strong contact toxicity and moderate fumigant activity against test stages. Furthermore, adult males and females showed high susceptibility to the fumigation. In a related study, Mohamed & Abdelgaleil (2008) stated that *M. hortensis* displayed strong contact toxicity and did not cause fumigant toxicity in *Sitophilus oryzae* and *Tribolium castaneum.* Furthermore, Shaaya *et al.*, (1991) and Prates *et al,* (1998) showed that essential oils produced contact toxicity through the insect cuticle and produced fumigant toxicity through the respiratory and digestive systems.

The results of the current study demonstrated that the pupa was the most tolerant stage and that the adult was the most sensitive one. It is well known that for fumigants, the active stages (adults and non-diapausing larvae) of insects are more susceptible than the sedentary stages (eggs and pupae), owing to differences in their respiratory rates (Rajendran and Sriranjini 2008).

The major constituents of the essential oil of *M. hortensis* plant growing in Egypt are 4-terpineol (29.96%) and β-terpinene (11.34%) (Mohamed, Abdelgaleil 2008). Lee *et al,*. (2001) and Koschier *et al.*, (2002) stated that 4-terpineol and β-terpinene exhibited insecticidal effects. Moreover, Lamiri *et al.*, (2001) demonstrated that the insecticidal activity of an essential oil could be attributed either to the major compound present in the oil or to the synergistic and/or antagonistic effects of all the components of the oil.

In the ovicidal bioassay, the test oil exhibited weak fumigant toxicity and strong contact activity against egg hatchability. Ability of the monoterpenoid vapours, especially those of terpinen-4-ol and 1,8-cineole, to reduce fecundity and hatchability of the eggs laid, recalls analogous properties of IGRs (Semple, 1992). It is likely that oil vapours diffused into eggs and thereby affected the physiological and biochemical processes associated with embryonic development (Raja *et al.*, 2001). Hence, in the fumigation bioassay the very low vulnerability of the eggs to vapours at the beginning of embryogenesis results from the fact that the permeability of the egg's external surface is lower at the start of embryogenesis. This relatively impermeable surface opposes the diffusion of vapours into the young eggs (Maciel *et al.*, 2010). A second explanation offered by Emekci *et al.*, (2002) was that because respiration rates are much lower at the egg stage than at the active stages, the lower rate of air exchange results in less monoterpenoid diffusion into the egg.

In the present study, essential oil of marjoram showed insignificant oviposition deterrent ability. Non-oviposition deterrent toxicity of the insecticide is perhaps because of the absence of corresponding organs or tissues in relation to behavior of oviposition (Hu *et al.*, 2009). Another plausible suggestion is that low doses were not effective. High doses could not be used to test the oviposition repellency because, as previously described, the adult stage showed high susceptibility to high doses.

The contact and fumigation bioassays used against immature stages resulted in some malformed adults. The toxicity of the monoterpenoids has all the characteristics of juvenile hormone activity. The occurrence of deformed adults could be explained by assuming a direct effect on the insect hormonal system similar to that of the insect growth regulators (Schwarz *et al.,* 1970). Blass and Hunt (1980) suggested that the mutation dumpy wing may involve a defect in chitin. The deformations induced by essential oils in other pests have been described by Vardhini *et al.,* (2001); de Mendonc *et al.,* (2005); Shekari *et al.,* (2008).

Along with its larvicidal activity, the effects of EO on insect metamorphosis would decrease the reproductive efficiency of the adult insect and further reduce the population (de Mendonc *et al.,* 2005). Consequently, such IGR-like properties should not be ignored when evaluating a substance exhibiting low direct toxicity, because it could be used in concert with other toxic substances to enhance their insecticidal activity (Stamopoulos *et al.,* 2007).

Furthermore, Christopher *et al.,* (1995) and Turner & Adler (1995) found that increased chitinase activity resulted in dramatic adult morphogenesis. Chitinases are among a group of proteins that digest the structural polysaccharide chitin in exoskeletons and gut linings during the molting process (Fukamizo, 2000). Activity of integumental chitinases is restricted to periods of molt and pupation (Filho *et al.,* 2002). These results are similar to those reported in this paper. The chitinase activity increased in treated and control larvae during morphogenesis to the adult stage. But it was insignificantly higher in treated larvae than the control which resulted in evident adult deformations.

Another reason for the adult deformations observed may be the change in TAG content of treated larvae, relative to the change in untreated larvae, during metamorphosis to the adult stage. TAG is the main form of storage for fatty acids that originate mainly from dietary fats absorbed by midgut epithelium, or from de novo biosynthesis (Kofronova *et al.,* 2009). Lipid accumulation during the larval stages is primarily used to support metamorphosis during the pupal stage and, in many instances, to support the flight demands and reproductive activities of non-feeding adult stages (Canavoso, *et al.,* 2001). The present study found that TAG content decreased gradually in both treated and control insects during metamorphosis to the adult stage. However, the level of reduction in treated larvae was more significant than in untreated larvae. In the tobacco hornworm, *Manduca sextu,* widely used as an insect model, the maximum content of fat body TAG occurs at the end of larval development, as a consequence of the accumulation of reserves during larval feeding. The TAG stores start to decline as a result of lipolysis and of the fatty acid oxidation required to sustain energy metabolism during the subsequent non feeding periods (pupal and adult) (Warnakulasuriya *et al.,* 1988). Most fatty acids are released from the fat body as sn-1,2-diacylglycerols (DG). The DG is carried from the fat body to the sites of utilization, e.g., to flight muscle (Wheeler and Goldsworthy 1985) and ovary (Kawooya *et al.,* 1988). The reduction in TAG content in treated larvae could be due to the energy required by the insect. Gregoire *et al.,* (1998) stated that hydrolysis of TAG occurs in order to generate fatty acids to be used by other organs during periods of energy deprivation. The decrease in TAG content in treated larvae could be due to the energy demands. Such energy demands are associated with increased production of haemocytes following activation of the immune system (Nappi and Ottaviani 2000).

The obtained data showed that AChE activity decreased insignificantly during the metamorphosis to the adult stage. AChE plays an essential role in neurotransmission at cholinergic synapses by catalysing the hydrolysis of the neurotransmitter acetylcholine. It is well known that AChE alteration is one of the main resistance mechanisms in many insect pests (Wang *et al.,* 2004). Several essential oils from aromatic plants, monoterpenes, and natural products act as AChE inhibitors (Shaaya and Rafaeli 2007; L◌opez *et al.,* 2010). It is also known that AChE inhibition is not necessarily related to insect mortality levels. In fumigant toxicity tests with monoterpenes against *Sitophilus oryzae* adults, Lee *et al.,* (2001) did not found a direct correlation between insect toxicity and AChE inhibition. Menthone from *Mentha arvensis* L. was highly toxic to *S. oryzae*, but it had a relatively small inhibitory effect on AChE activity. However, less toxic b-pinene showed high-level inhibition. Therefore, it is suspected that, in addition to producing AChE inhibition, the monoterpenes may act on other vulnerable sites (e.g., cytochrome P450-dependent monooxygenases).

The present study also showed that the total protein content of treated larvae increased, compared with the control, during metamorphosis to the adult stage. However at the 1st pupal , protein content decreased relative to that of the control. The reduction in the amount of total protein at the 1st pupal day and in the control could result because aminotransferase activity prevents the release of free amino acids into the hemolymph (Khanikor *et al.,* 1998). Mukherjee *et al.,* (1998) showed that higher concentrations of azadirachtin increased the amount of protein in the hemolymph of *T. castaneum,*

probably owing to the increased activity of detoxifying enzymes. Shekari *et al.*, (2008) showed that the amount of protein at 24 h after treatment with *Artemisia annua* decreased significantly relative to the value for the control group and increased slightly at 48 h relative to the control.

The present study provides evidence that marjoram essential oil has toxic effects against different stages of *Phthorimaea operculella* and that it also produces considerable biochemical changes. Marjoram essential oil therefore has potential for use in sustainable management of potato tuber moth in storage facilities. This approach is likely to be advantageous, as it is environmentally safe and socially acceptable. However, further studies need to be conducted to evaluate the cost of this essential oil when used in commercial storage applications.

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5. References:

1. Abbott, W.S. (1925): A method of computing the effectiveness of an insecticide. J. Economic Entomology, 18, 265–267.
2. Blass, D.H. and Hunt, D.M. (1980): Pyrimidine biosynthesis in the dumpy mutants of Drosophila melanogaster. Molecular Genetics and Genomics, 178, 437-442.
3. Bradford, M.M. (1976): A rapid and sensitive method for the quotation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, 72, 248-254.
4. Canavoso, L.E., Jouni, Z.E., Karnas, K.J., Pennington, J.E. and Wells, M.A. (2001): Fat Metabolism in Insects. Annual Review of Nutrition, 21, 23-46.
5. Christopher, M., Turne, P. and Adler, N. (1995): Morphogenesis of Drosophila pupal wings in vitro. Mechanism of Development, 52, 247-255.
6. de Mendonc, F.A.C., da Silva, K.F.S., dos Santos, K.K., Ribeiro, K.A.L., and Sant'Ana, A.E.G. (2005): Activities of some Brazilian plants against larvae of the mosquito Aedes aegypti . Fitoterapia, 76, 629– 636
7. El-Sinary, N.H. (1995): Magnitude and applicability of gamma radiation and controlled atmospheres to minimize the hazards of potato tuber moth, P. operculella Zeller (Lepidoptera: Gelechiidae). Ph. D. Thesis, Cairo Univ, 187 pp.
8. Emekci, M., Navarro, S., Donahaye, E., Rindner, M. and Azrieli, A. (2002): Respiration of T. castaneum (Herbst) at reduced oxygen concentrations. Journal of Stourd Product Research, 38, 413-425.
9. Filho, B.P., Lemos, F.J., Secundino, N.F., Pascoa, V., Pereira, S.T. and Pimenta, P.F. (2002): Presence of chitinase and beta-Nacetylglucosaminidase in the Aedes aegypti: a chitinolytic system involving peritrophic matrix formation and degradation. Insect Biochemistry and Molecular Biology, 32, 1723-1729.
10. Finney, D.J. (1971): Probit Analysis, third ed. Cambridge University Press, London.
11. Fukamizo, T. (2000): Chitilinolytic enzymes: catalysis, substrate-binding and their application. Current Protein & Peptide Science, 1, 105-124.
12. Golizadeh, A., Razmjou, J.(2010): Life Table Parameters of Phthorimaea operculella (Lepidoptera: Gelechiidae), Feeding on Tubers of Six Potato Cultivars. Journal of Economic Entomology, 103(3), 966-972.
13. Gregoire, F.M., Smas, C.M. and Sul, H.S. (1998): Understanding adipocyte differentiation. Physiological Reviews, 78(3), 783–809.
14. Guerra, P.C., Molina, I.Y., Yabar , E. and Gianoli, E. (2007): Oviposition deterrence of shoots and essential oils of Minthostachys spp. (Lamiaceae) against the potato tuber moth. Journal of Applied Entomology. 131(2), 134-138.
15. Hu, Q.B., An, X.C., Jin, F.L., and Ren, S.X. (2009): Toxicities of destruxins against Bemisia tabaci and its natural enemy, Serangium japonicum. Toxicon 53, 115–121.
16. Kawooya, J.K., Osir, E.O., and Law, J.H. (1988): Uptake of the major hemolymph lipoprotein and its transformation in the insect egg. Journal of Biological Chemistry, 263, 8741-8747.
17. Khanikor, D., Unni, B.G., Rai, A.K. and Barauh, R. (1998): Biochemical aspects of protein biosynthesis in the fat body of mug silkworm Antheraea assama. Advanced Biosensors, 17, 89-98.
18. Kofronova, E., Cvacka, J., Vrkoslav, V., Robert, H., Pavel, J., Jiri K., Oldrich H., and Valterova, I.A. (2009): A comparison of HPLC/APCI-MS and MALDI-MS for characterising triacylglycerolsin insects: Species-specific composition of lipids in the fat bodies of bumblebee males. Journal of Chromatography B, 87, 3878-3884

19. Koschier, E.H., Sedya, K.A., and Novak, J. (2002): Influence of plant volatiles on feeding damage caused by the onion thrips Thrips tabaci. Crop Protection, 21, 419-425.
20. Lamiri, A., Lhaloui, S., Benjilali, B., and Berrada, M. (2001): Insecticidal effects of essential oils against Hessian fly, Mayetiola destructor (Say). Field Crop Research, 71, 9-15.
21. Lee, B.H., Choi, W.S., Lee, S.E., and Park, B.S. (2001): Fumigant toxicity of essential oils and their constituent compounds towards the rice weevil, S. oryzae (L.). Crop Protection, 20, 17-320.
22. Loֹpez, M.D., and Pascual-Villalobos, M.J. (2010): Mode of inhibition of acetylcholinesterase by monoterpenoids and implications for pest control. Industrial Crops and Prodution, 31, 284-288.
23. Maciel, M.V., Morais, S.M., Bevilaqua, C.M.L., Silva, R.S., Barros, R.N., Sousa, L.C., Sousa, E.S. and Brito, Souza-Neto, M.A., (2010): Chemical composition of Eucalyptus spp. essential oils and their insecticidal effects on Lutzomyia longipalpis. Veterinary Parasitology, 167, 1-7.
24. Mohamed, M.I.E., and Abdelgaleil, S.A.M., (2008): Chemical composition and insecticidal potential of essential oils from Egyptian plants against S. oryzae (L.) (Coleoptera: Curculionidae) and T. castaneum (Herbst) (Coleoptera: Tenebrionidae). Applied Entomology and Zoology, 43(4), 599-607.
25. Mukherjee, S.N., Rawal, S.K., Ghumare, S.S. and Sharma, P.N. (1993): Hormetic concentration of azadirachtin and isoesterase profiles in T. castaneum (Herbst) (Coleoptera: Tenebrionidae). Experientia, 94, 557–560.
26. Nappi, A.J., and Ottaviani, E. (2000): Cytotoxicity and cytotoxic molecules in invertebrates. Biography Essays, 22, 469-480.
27. Nishimura, H. (2001): Aroma constituents in plants and their repellent activities against mosquitoes. Aroma Research, 2, 257–267.
28. Pavela, R. (2004): Insecticidal activity of certain medicinal plants. Fitoterapia,
a. 75, 745-749.
29. Prates, H.T., Santos, J.P, Waquil, J.M., Fabris, J.D,, Oliveira, A.B. and Forster, J.E. (1998): Insecticidal activity of monoterpenes against Rhyzopertha dominica (F.) and T. castaneum (Herbst). Journal of Stored ProductResearch, 34, 243-249.
30. Raja, N., Albert, S., Ignacimuthu, S. and Dorn, S. (2001): Effect of plant volatile oils in protecting stored cowpea Vigna unguiculata (L.) Walpers against Callosobruchus maculatus (F.) (Coleoptera: Bruchidae) infestation. Journal of Stored Product Research, 37, 127-132.
31. Rajendran, S., and Sriranjini, V. (2008): Plant products as fumigants for stored-product insect control. Journal of Stored Product Research, 44, 126-135.
32. Regnault-Roger, C. (1997): The potential of botanical essential oils for insect pest control. Integrated Pest Management Reviews, 2, 25-34.
33. Schwarz, M., Sonnet, P.E. and Wakabayashi, N. (1970): Insect juvenile hormone activity of selected terpenoid compounds. Science, 167, 191-192.
34. Semple, R.L. (1992): Insect growth regulators. In: Semple, R.L., Hicks, P.A., Lozare, J.V., Castermans, A. (Eds.), Towards Integrated Commodity and Pest Management in Grain Storage. A Training Manual for Application in Humid Tropical Storage Systems. A REGNET (RAS/86/189) publication in collaboration with NAPHIRE, p. 526.
35. Shaaya, E. and Rafaeli, A. (2007): Essential oils as biorational insecticides potency. In: Ishaaya, I., Ralf, N., Rami, H.A. (Eds.), Insecticides Design Using Advanced Technologies. Springer, Berlin, Heidelberg, pp. 249-261
36. Shaaya, E. , Kostjukovski, M., Eilberg, J. and Sukprakarn, C. (1997): Plant oils as fumigants and contact insecticides for the control of stored-product insects. Journal of Stored Production Research, 33, 7-15.
37. Shaaya, E., Ravid, U., Paster, N., Juven, B., Zisman, U. and Pissarev, V. (1991): Fumigant toxicity of essential oils against four major stored product insects. Journal of Chemical Ecology, 17, 499-504.
38. Sharaby, A.M, Abdel-Rahman, H. and Moawad, S. (2009): Biological effects of some natural and chemical compounds on the potato tuber moth, P. operculella Zeller (Lepidoptera:Gelechiidae). Saudi Journal of Biological Sciences, 16,1-9.
39. Shekari, M., Jalali Sendi, J., Etebari, K and Shadparvar, A. (2008): Effects of Artemisia annua L. (Asteracea) on nutritional physiology and enzyme activities of elm leaf beetle, Xanthogaleruca luteola Mull. (Coleoptera: Chrysomellidae). Pesticide Biochemistry and Physiolog, 91, 66-74.
40. Simpson, D. R., Bull, D. L, and Linquist, D.A. (1964): A semi-micro technique for estimation of cholinesterase activity in boll weevils. Annals of the Entomological Society of America, 57, 367-371.
41. Stamopoulos, D.C., Damosb, P. and Karagianidoub, G. (2007): Bioactivity of five monoterpenoid vapours to T. confusum (du Val)

(Coleoptera: Tenebrionidae). Journal of Stored Product Research, 43, 571–577.
42. Turner, C.M, Adler and P.N. (1995): Morphogenesis of Drosophila pupal wings in vitro. Mechanisms of Development, 52,247-255.
43. Vardhini, D., Raja, S.S., Varalakshmi, K. and M. A. Quddus, (2001): Sujiol, a new potent insect growth regulator from Juniperus communis L. against last instar larvae of Spodoptera litura. Journal of Applied Entomology, 25(8), 425-488.
44. Wang, J.J., Cheng, W.X., Ding, W. and Zhao, Z.M. (2004): The effect of the insecticide dichlorvos on esterase activity extracted from the psocids, Liposcelis bostrychophila and L. entomophila. Journal of Insect Science, 4, 1-5.
45. Warnakulasuriya, F.G., Tsuchida, K. and Wells, M.A. (1988): Effect of dietary lipid content on lipid transport and storage during larval development of Manduca sexta. Insect Biochemistry, 18, 211-214.
46. Waterhouse, D.F., Hochman, R.H. and Mckellar, J.W. (1961): An investigation of chitinase activity in cockroach and termite extract. Journal of Insect Physiology, 6, 96-112.
47. Wheeler, C.H and Goldsworthy, G.J. (1985): Specificity and localization of lipoprotein lipase in the flight muscles of Locusta migratoria. Biological Chemistry Hoppe-Seyler, 366, 1071-1077.
48. Zeddam, J.L., Orbe, K., Léry, X., Dangles, O., Dupas, S. and Jean-François, S. (2008): An isometric virus of the potato tuber moth Tecia solanivora (Povolny) (Lepidoptera: Gelechiidae) has a trisegmented RNA genome. Journal of Invertebrate Pathology, 99, 204-211.

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Design and Hepatoprotective Evaluation of Biphenyl Dimethyl Dicarboxylate (DDB) and Silymarin Solid Dispersion and Self-Micro Emulsifying Drug Delivery Systems

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Abstract: Biphenyl Dimethyl Dicarboxylate (DDB) and Silymarin are widely used drugs for the treatment of hepatitis C virus, have poor bioavailability due to their low aqueous solubility that limits their dissolution rates. To overcome these limitations solid dispersions (SD_s) and self-microemulsifing drug delivery systems (SMEDDS) were prepared in an attempt to improve their release profile. SD_s were prepared using co-precipitation and melting methods at various drug-polymer ratios. Polyethylene glycol (PEG 6000), polyvinylpyrrolidone (PVP K30 and PVP K17) or sodium desoxycholate were used to prepare SD_s by co-precipitation method. PEG 4000, PEG 6000 or poloxamers (F68 and F127) were used to prepare SD_s by melting method. On the other hand, Ternary phase diagram was constructed using Miglyol® 812 (oil), Tween® 80 (surfactant), Transcutol® HP (co-surfactant) and water to identify the efficient self-microemulsification region. In-vitro release studies were studied for the prepared SD_s and SMEDDS. DDB release from all prepared SD_s did not show any significant improvement when compared to their corresponding commercial product except for the melts prepared by poloxamer F68 used in 1:5 drug to carrier weight ratio. Silymarin release from all SD_s was significantly improved when compared to their corresponding physical mixture, Silymarin powder or its commercial product. Silymarin:Sodium desoxycholate with 1:3 weight ratio, gave the highest drug dissolution behaviour. On the other hand, it was found that the optimal formulation with the best Self-microemulsifying and dissolution behaviour for DDB or Silymarin consisted of 10% Miglyol® 812, 40% Tween® 80, and 50% Transcutol® HP. This formulation showed higher extends of DBB or Silymarin release compared to their powder or commercial products. The optimized formulations of DDB or Silymarin SD_s and SMEDDS were evaluated regarding their hepatoprotective activity against carbon tetrachloride-induced oxidative stress in Albino rats when challenged with commercial products DBB pillules® and Mariagon® capsules. These develobed formulations might be useful in the prevention of used successfully hepatic fibrosis.

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1. Introduction:

It has been estimated that there are over 170 million carriers of hepatitis C virus (HCV) worldwide, with an increasing incidence of new infections [(1)]. Hepatitis C represents a public health problem in Egypt. Different studies done in Egypt showed high prevalence (on different populations in different regions, and different age groups) [(2)]. Dimethyl 4.4` – dimethoxy – 5,6,5`,6` dimethylene – dioxybiphenyl – 2,2` dicarboxylate [(3)](DDB) is synthetic analogue of schizandrin C, one of the active components isolated from *Fructus schizandra*, a traditional oriental medicinal plant. This compound (DDB) was shown to protect against liver injury induced by Carbon tetrachloride (CCL_4) [(4)]. In addition, DDB was used successfully for treatment of cases of chemically induced hepatitis [(5 & 6)] and has a beneficial effect on liver enzymes and the resulting histopathological changes [(7)]. However, its oral preparations have been known to have limited bioavailability due to its extremely low solubility in water, a problem that also limits its parenteral dosage forms preparation.

Silymarin, a mixture of three isomeric flavonolignans, was first isolated from milk thistle seeds in 1968. Silymarin consists primarily of three flavonolignans: silybine (Silibinin), silychristin (silichristin) and silidianin [(8)]. Silybine is the most biologically active component with regard to milk thistle's antioxidant and hepatoprotective properties. A standardized milk thistle extract composed of Silymarin and silybine was developed in Europe and is known commercially as Legalon® [(9)]. Silymarin therapy decreases complications, hastens recovery, and shortens hospitalization in patients with acute

viral hepatitis [10]. Silymarin prevent hepatic fibrosis through suppression of inflammation and hypoxia in the fibrotic liver. Silymarin was found to be poorly absorbed from the GI tract, with bioavailability estimated as 23-47%. Peak plasma concentrations occur two to four hours after an oral dose. The elimination half-life is approximately six hours. The major problem in the development of an oral solid dosage form of Silymarin is the extremely poor aqueous solubility [11].

Solid dispersion (SD) is defined as a dispersion of one or more active ingredients in an inert carrier or matrix at solid state prepared by either the melting (fusion), solvent or melting - solvent method [12]**.** Dispersions obtained through the fusion process are often called melts and those obtained by the solvent method are frequently referred to as co-precipitates or co-evaporates [13,14]. Formulation of solid dispersions is advantageous in enhancing the dissolution of poorly water-soluble drugs and is often accompanied by an increase in their relative bioavailability.

Self-microemulsifying drug delivery systems (SMEDDS) also represent a promising approach for formulating drugs with poor aqueous solubility. They are defined as isotropic mixtures of natural or synthetic oils, solid or liquid surfactants, together with hydrophilic cosolvents/cosurfactants these systems upon mild agitation followed by dilution in aqueous media, such as gastrointestinal fluids, these systems can form fine microemulsions [15 , 16].

These microemulsions empty rapidly from the stomach promoting wide distribution of the drug throughout the gastrointestinal tract, thereby minimizing irritation that frequently encountered with extended contact between bulk drug substances and the gut wall. Moreover, these self-microemulsifying formulations provide a large interfacial area for partitioning of the drug between oil and water, as well as a good interface for lipolytic enzymes to digest the oil, there by promoting rapid drug release between various phases of intestinal contents [17]. Furthermore, the break down products that result from the oils enzymatic hydrolysis are surface active products which stabilize the formed microemulsions as well as act as membrane permeation enhancers[18] .

Thus, for lipophilic drug compounds that exhibit dissolution rate limiting absorption, the SMEDDS may offer an improvement in the rate and extent of absorption and may result in more reproducible blood time profiles [19].

When comparing the SMEDDS to conventional emulsions; SMEDDS were proved to be superior as they are physically stable formulations and are easily manufactured. Moreover, when compared to microemulsions; SMEDDS showed improved physical stability upon long term storage due to the absence of water content, thus they can be filled directly into soft or hard gelatin capsules for convenient oral delivery [16].

In our study, solid dispersion (SD) and SEDDS were prepared with the intention of improving the solubility, dissolution rate and ultimately bioavailability of the poorly water soluble drugs, DDB and Silymarin. Solid dispersion was prepared by co-precipitation and melting method. Polyethylene glycols exemplified by PEG 6000 and 4000, polyvinylpyrrolidone namely PVP K30 and PVP K17, bile salts represented by sodium desoxycholate, poloxamers exemplified by poloxamer F68 and poloxamer F127 were used in different drug: polymer weight ratios as hydrophilic matrix. SMEDDS were also developed to increase the dissolution rate of DDB and Silymarin. Various types of Self-microemulsifying formulation were prepared using Miglyol® 812, Tween® 80, and Transcutol®HP as oil phase, surfactant and co-surfactant, respectively. In vitro dissolution studies of the prepared solid desperations and SEDDS of DDB and Silymarin were performed and compared to that of pure DDB and pure Silymarin powder to their commercial products. Then selected formulations were subjected to hepatotoxicity studies.

2. Materials and Methods:

2.1. Materials:

Dimethyl-4-4`-dimethoxy-5, 6, 5`, 6` dimethylenedioxy-biphenyl-2, 2,-dicarboxylate (DDB) and Silymarin pure powders were obtained from Arabic Company of Medicinal Plants (Mebaco Co.), Cairo, Egypt. DDB Pilules® (each pillule contains 1.5 mg of DDB) was obtained from Beijing Union Pharmaceutical Factory, China, and imported by AL Ahram Pharmaceuticals and Medical Equipment Company Cairo, Egypt. Mariagon® capsules containing 140 mg of Silymarin were obtained from Alpha Chemical Advanced Pharmaceutical Industries Co. (ACAPI), Cairo, Egypt. Sodium phosphate dibasic anhydrous was obtained from El- Gomhouria Company for Drugs, Cairo, Egypt. Potassium dihydrogen orthophosphate, pure grade of methanol, formalin 10% and ethyl alcohol were obtained from El-Nasr Pharmaceutical Chemical Company, Cairo, Egypt. Polyvinylpolypyrrolidone (PVP K30 and PVP K17) and Polyethylene glycols (PEG 4000 and PEG 6000) were obtained from Fluka, Switzerland. Poloxamer F127, poloxamer F68 and Hematoxylin-Eosin stain were obtained from Sigma-Aldrich chemie-GmbH Steinheim, Germany. Sodium

desoxycholate was obtained from Difco laboratories, Michigan, U.S.A. Tween® 80 (polyoxyethylene sorbitan monooleate, HLB=15) was purchased from Merck Schwchardt, Darmstadt, Germany. Transcutol® HP (diiethylene glycol monoethyl ether) was kindly donated by Gattefosse, Saint Priest, France. Miglyol® 812 (medium chain triglycerides) was kindly provided by Sasol, Germany. Carbon tetrachloride (CCL_4) was obtained from Egyptian Company for Chemicals and Pharmaceuticals (ADWIA), Cairo, Egypt.

2.2. Methods:

2.2.1. Preparation of DDB and Silymarin Solid Dispersions:

Both solvent evaporation method (co-precipitation method) and fusion method (melting method) were used to prepare DDB and Silymarin solid dispersions.

2.2.1.1. DDB and Silymarin Solid Dispersions prepared by Co-precipitation method:

Different polymeric carriers were used to prepare DDB or Silymarin co-precipitation solid dispersion namely: polyethylene glycols exemplified by PEG 6000 in weight ratios of 1:1, 1:2 and 1:3 drug to carrier, polyvinylpyrrolidone namely PVP K30 and PVP K17 in weight ratios of 1:1, 1:2, 1:3, 1:4 and 1:5 drug to carrier and bile salts represented by sodium desoxycholate in weight ratio of 1:2 and 1:3 drug to carrier. Each of the aforementioned carriers was dissolved in the least volume of methanol then added to drug methanol solution. The resultant solution was evaporated until dryness using thermostatically controlled magnetic stirrer. The obtained solid masses were kept in desiccators over anhydrous calcium chloride until complete dryness. Finally, the dried masses were pulverized and granules that passed throughout the sieve (USA standard testing sieve set) of 0.63 mm size were clarified for further investigation.

2.2.1.2. Preparation of DDB and Silymarin Solid Dispersions using Melting Method:

Different polymeric carriers were used to prepare DDB and Silymarin solid dispersions using melting method, namely: polyethylene glycols represented by PEG 4000 and PEG 6000 in weight ratio of 1:1, 1:2, and 1:3 drug to carrier and poloxamers exemplified by poloxamer F68 in weight ratio of 1:1, 1:2, 1:3, 1:4 and 1:5 drug to carrier and poloxamer F127 in weight ratio of 1:1, 1:2 and 1:3 drug to carrier. Each of the aforementioned carriers was melted over a heated water bath maintained at 70^{o}C. Then, the drug was added to the melted mass and stirred well till homogenous matrices were formed. The obtained masses were kept in desiccators over anhydrous calcium chloride until complete dryness. Finally, the dried masses were pulverized and the granules that passed through sieve (USA standard testing sieve set) of 0.63 mm in diameter were clarified for further investigation.

2.2.1.3. Preparation of DDB and Silymarin Physical Mixtures:

The physical mixtures of DDB and Silymarin with aforementioned carriers were prepared in the same molar ratios utilized previously for comparative purpose.

2.2.2. Construction of Ternary Phase Diagrams:

Ternary phase diagram was constructed with systems comprising of an oily phase, a surfactant and a cosurfactant. Briefly, mixtures of the oil, surfactant, and cosurfactant were accurately weighed at different ratios into well capped vials and were then shaken to ensure complete mixing. Following that, these mixtures were poured into 200 ml of distilled water containing a magnet adjusted to rotate at a speed of 60 rpm. The clarity of the formed aqueous dispersion and the emulsification time, time needed for the system to completely disperse upon dilution, were visually assessed to identify the microemulsifying regions.

2.2.2.1. Preparation of DDB and Silymarin SMEDDS:

After identification of the microemulsifying regions in the constructed phase diagrams, SMEDDS were selected at desired component ratios as presented in table (1) for further studies.

Oil, surfactant, and cosurfactant were accurately weighed into glass vials. Then, either DDB (10 mg/gm of SMEDDS) and Silymarin (70 mg/gm of SMEDDS) was added to the above mixture and the components were gently stirred and vortexed until the added drug was perfectly dissolved. These mixtures were visually analyzed for drug precipitation after storage for 72 hours at ambient temperature.

Table 1: Composition of the investigated SMEDDS.

System Number	Composition		
	Oil Miglyol® 812	Surfactant Tween® 80	Co-surfactant Transcutol®HP
S_1	10	40	50
S_2	10	50	40
S_3	10	60	30
S_4	10	70	20
S_5	10	80	10

2.2.3. In- Vitro Release Studies of DDB and Silymarin Solid Dispersions and SMEDDS:

The in- vitro release of DDB and Silymarin from the prepared solid dispersions and SMEDDS were performed in 900 ml phosphate buffer of pH 7.4 maintained at 37±0.5°C using USP Dissolution Tester apparatus II (Dissolution apparatus, Varian, Germany). Solid dispersion corresponding to 30 mg of DDB, 140 mg of Silymarin, as well as SMEDDS corresponding to 10 mg of DDB /gm SMEDDS., 70 mg of Silymarin /gm SMEDDS. Each preparation was separately placed in the dissolution vessel and the shaft was adjusted at rotation speed of 50 rpm. Samples of 5 ml were withdrawn at predetermined time intervals, filtered through Millipore® filter membrane of 0.45 µm pore size (Millipore, Massachusetts, USA.) and spectrophotometrically measured for DDB at 280 nm. [20] and for Silymarin at 324 nm. [21] using Shimadzu Spectrophotometer UV-1601 PC, Japan.

The removed samples were replenished with equal volumes of the medium to keep the dissolution volume constant. Cumulative amount of DDB or Silymarin dissolved after one hour was used to compare between different investigated formulations.

2.2.4. Hepatotoxicity test:

2.2.3.1. Design of the experiment:

Forty eight Sprague Dawley albino rats of mixed sex weighing 100 g each were obtained from the Animal House Unit of the National Research Center, Dokki, Giza, Cairo, Egypt. The animals were housed for 7 days before using in this study under constant environmental nutritional conditions according to "the principles of laboratory animals care", (NIH publication85-23, revised1985).Prior to the experiment the rats were fasted overnight with free access of water. Rats were divided into eight groups, each group comprising of six rats.

Group 1: Placebo group which ingested one ml saline daily.

The animals in the remaining seven groups received 0.25 ml of carbon tetrachloride (CCL_4) in liquid paraffin (1:1 v/v) per 100g body weight intraperitoneally once to induce hepatic damage [22]. The drug doses in the forthcoming groups were calculated according to Paget and Barnes table [23].

Group 2: Received only CCL_4 and blood samples were taken after 3 days according to the method reported by Janakat and Al Merie [22].

Group 3: Received commercial product (Marriagon®). Each rat received the equivalent of 2.52 mg of Silymarin and repeated for 7 days.

Group 4: Received powder of formula 1 composed of 1 gm Silymarin+3 gm sodium desoxycholate (co-precipitates) prepared by solvent method). Each rat received the equivalent of 2.52 mg of Silymarin and repeated for 7 days.

Group 5: Received SMEDDS solution of S1 composed of 70 mg Silymarin dissolved in 10% Miglyol®, 40% Tween® 80 oil and 50% Transcutol® HP. Each rat received the equivalent of 2.52 mg of Silymarin daily and repeated for 7 days.

Group 6: Received the commercial product DDB (pilules®). Each rat received the equivalent of 0.27 mg of DDB and repeated for 7 days.

Group 7: Received powder of formula 2 composed of 1 gm DDB+5 gm poloxamer (F68) prepared by fusion method. Each rat received the equivalent of 0.27 mg of DDB and repeated for 7 days.

Group 8: Received SMEDDS solution of S1 composed of 10 mg DDB dissolved in 10% Miglyol® 812, 40% Tween® 80 oil and 50% Transcutol® HP. Each rat received the equivalent of 0.27 mg of DDB daily and repeated for 7 days.

2.2.3.2. Histopathological investigations:

Histopathological survey was performed aiming at declaration of the changes occurred in liver tissues of rats. This was done for comparative purposes between newly reached preparations from pure materials of Silymarin and DDB to their commercial products. Tissue specimens from liver of treated and control rats were fixed in 10%neutral buffered formalin solution. The fixed specimens were trimmed, washed and dehydrated in ascending grades of alcohol, cleaned in xylene, embedded in paraffin then sectioned (4-6 micron) and stained with hematoxyline and eosin according to Bancroft et al. [24]. The sections were thereafter examined and photographed using a microscope at a magnification power of 200 X. The degree of hepatic injury was estimated using an ordinal scale modified from Palaa and Charbonneau [25] according to table (2).

Table 2: Histological Grading of Liver Injury.

Grade	Description
0	No apparent injury by light microscopy
I	Swelling of hepatocytes
II	Ballooning of hepatocytes
III	Lipid droplets in hepatocytes
IV	Necrosis of hepatocytes

2.2.4. Statistical analysis:

Data obtained were statistically analyzed using Fischer Exact Probability test at $P < 0.05$ (SPSS 14, 2006).

3. Results and Discussion:

3.1. In-Vitro Release Study of DDB and Silymarin Solid Dispersions

With the aim of improving the dissolution behaviour of DDB and Silymarin, the in-vitro release studies of their different solid dispersions as well as their commercial products were shown in figures (1-9).

It was found that pure DDB powder didn't dissolve in the release conditions adopted in the current investigation. However, about 19.38% of DDB was released from the commercial DDB Pilules® after 1 hour. Regarding the prepared physical mixture, co-precipitation and melts of DDB with the investigated carriers, they all showed high extent of drug dissolution when compared to DDB powder but showed less extent of drug dissolution when compared to the commercial DDB Pilules® (data not shown) except poloxamer F68 melt ($P<0.05$). In 1:5 (drug to carrier) ratio, the results revealed comparable drug dissolution when compared to commercial DDB pilules® (Figure 1).

It is worth mentioning that pure Silymarin powder and the commercial Silymarin capsules released nearly 39% of their Silymarin content after 1 hour of the dissolution run.

Generally, it was apparent from investigating the dissolution results of the prepared physical mixtures of Silymarin with the investigated carriers that its dissolution was low when compared to that of either pure Silymarin powder or commercial Silymarin capsules($P<0.05$) (Figs 2-8). This unexpected observation could be related to the salting out of Silymarin from the dissolution medium due to the preferential dissolution of the water soluble carriers.

On the other hand, upon increasing the carrier weight ratio in the prepared coprecipitates formulae, a simultaneous increase in the extent of Silymarin dissolved was detected (Figs 2-8). This finding is probably attributed to the crystallization of drug molecules in very minute crystals in the polymeric matrices [26], which results in rapid dissolution of the embedded drug. The co-precipitates of Silymarin with, polyethylene glycol 6000 (Fig. 2), polyvinyl pyrrolidone K30 (Fig.3) and sodium desoxycholate (Fig. 4) polyvinyl pyrrolidone K 17 (Fig.5), prepared at 1:3 (drug to carrier) weight ratios, released nearly 62.27%, 62.29%, 74.82% and 50.95% of their drug content after 1 hour respectively. It is worth mentioning that a twofold increment in the extent of Silymarin dissolution was calculated for sodium desoxycholate coprecipitates when compared to the commercial Marriagon® capsules and pure Silymarin.

Regarding the prepared melts of Silymarin, all carriers showed higher extent of drug dissolution ($P<0.05$), even when employed at low carrier ratio (Figs.2-8). Moreover, it was observed that increasing the weight ratio of the carrier caused a concurrent increase in the extent of Silymarin dissolved when polyethylene glycol 6000 (Fig. 2), polyethylene glycol 4000 (Fig. 6) and poloxamer F127 (Fig. 8) were employed as carriers ($P<0.05$). Controversy, there was no appreciated increase in drug dissolution when the weight ratio of poloxamer F68 (Fig.7) to Silymarin was increasing from 2:1 to 3:1 in the prepared melts ($P<0.05$).

Therefore solid dispersion of DDB with poloxamer F68 in weight ratio of 1:5 (drug to carrier) prepared by melting method (Fig.1) as well as Silymarin- sodium desoxycholate coprecipitates in weight ratio of 1:3 (Fig.9) were selected for further investigation.

3.2. Construction of Ternary Phase Diagrams:

Ternary phase diagram composed of Miglyol® 812, Tween® 80, and Transcutol®HP was constructed to identify microemulsifying regions and to select suitable concentration of each component required for SMEDDS formation.

Tested oil implicated in SMEDDS in the present investigation was Miglyol® 812, a medium chain triglyceride derived from coconut oil. It is reported that medium chain triglycerides improve the intestinal absorption of drugs [27, 19, 28]. And have been extensively used in SMEDDS due to their high fluidity at ambient temperature, better solubility properties, and superior self-emulsification ability compared to long chain triglycerides [29, 30]. Additionally, medium chain triglycerides showed better chemical stability due to the lack of double bonds that can catalyze the oxidation of the incorporated drugs of drug substance [31].

Tween® 80 was selected to prepare the SMEDDS as non-ionic SAA which tends to form stable microemulsions unaffected by pH and ionic strength changes [31]. Furthermore, Tween® 80 was proven to enhance the intestinal permeability of drugs [32].

Transcutol® HP (diethylene glycol monoethyl ether) was used in the present investigation as it has previously shown to possess high solubilization power for DDB and is known to enhance the permeability of drugs [33]

It is quite obvious from figure 10 that there are five distinct regions in the phase diagram.

Oil, surfactant, and co-surfactant mixtures present in region (a) are considered efficient SEDDS as they emulsify in nearly 2 minutes or less forming an emulsion with milky appearance. However, the ternary mixtures present in region (b) represent SEDDS that take more than 2 minutes to completely emulsify forming a milky emulsion. This long emulsification time is attributed to the presence of high surfactant concentration above 50% w/w that led to the formation of gel like masses that take long time to dissolve when added to aqueous media.

On the other hand, ternary mixtures present in region (c) represent efficient SMEDDS as they formed clear microemulsions when added to aqueous media in less than 2 minutes. Ternary mixtures present in region (d) represent SMEDDS that took long time to emulsify. Both region (c) and (d) had high ratio of Transcutol® HP that enabled the microemulsion region to be reached where Transcutol® HP molecules penetrate into the surfactant film at the oil globule interface by positioning themselves towards the aqueous phase, causing the contraction of the polyoxyethylene part of the surfactant molecules, which leads to stabilization of the interfacial surfactant film [34]. However, mixtures present in region (e) did not form any emulsion and the oil droplets were visualized on the surface due to the presence of high oil concentration associated with low concentrations of surfactant and co-surfactant.

3.3. Preparation of DDB and Silymarin SMEDDS:

The mixtures present in region (c) and (d) were loaded with 10 mg DDB or 70 mg Silymarin for further investigation. It is worth noting that none of the drug loaded systems showed any signs of drug precipitation after storage for 72 hours at ambient temperature.

3.4. In-Vitro Release Studies of DDB and Silymarin from the Prepared SMEDDS:

In-vitro release studies are often performed to predict how a delivery system might work in ideal situations, which might give some indication of its performance in vivo [35]. Hence drug release investigations were performed for the prepared SMEDDS containing either DDB or Silymarin in comparison to their commercial products in phosphate buffer of pH 7.4 for one hour. The results were graphically illustrated in Figures (11) and (12).

It was observed from DDB release profiles presented in Figure (11) that all of the investigated SMEDDS showed more than 85% of DDB released after one hour. This could be resorted to the complete solubility of DDB in the tested SMEDDS that formed minute oil droplets in the dissolution medium and gave higher extent of drug release [36]. On the other hand, the commercial DDB showed lower extent of drug dissolution which was expected due to its poor aqueous solubility.

It was remarkable that the higher the ratio of Transcutol® HP used, the higher was the extent of DDB released as exemplified by system S1 which contained 50% Transcutol® HP. On the contrary, the higher the ratios of Tween® 80 used, the lower was the extent of DDB released as illustrated in system S5 that contained 80% Tween® 80. The first could be attributed to the creation of void spaces among surfactant molecules owing to the high ratio of Transcutol® HP which facilitated the diffusion of DDB into the aqueous medium [37]. However, the surfactant, at high concentration, formed transparent viscous liquid crystalline gel at the surfactant-water interface [38, 39] that delayed DDB diffusion into the release media [40].

Regarding the prepared SMEDDS, containing Silymarin it is obvious that only system S1 had higher extent of drug release in comparison to Marigon® capsules (Fig 12). This may be due to the presence of large amount of Transcutol® HP in system S1 (50 %) w/w in which Silymarin is extremely soluble. unexpectedly, the rest of the prepared SMEDDS had lower extent of Silymarin release compared to both the raw material and commercial product. This might be attributed to the precipitation of Silymarin due to lowering the solvent capacity of the surfactant and or the co-surfactant as a result of SMEDDS dilution [41].

Conclusively, system S1 composed of 10 % Miglyol 812®, 50% Transcutol HP® and 40 % Tween 80® was further investigated in the histopathology study as it showed higher extent of dissolution when loaded with either DDB or Silymarin.

3.5. Liver toxicity Studies:

The gross appearance of liver specimens of the control group (group 1) was normal regarding their size and colour. Liver histological examination showed normal hepatic lobules as shown in figure 13, with grade (0). These results were in complete agreement with those reported by Dass et al. [42].

3.5.1. Carbon Tetrachloride Hepatitis Induction in Rats:

Rats liver specimens belonging to group (2) treated with CCL_4 were examined after 3 days. They showed necrobiotic changes of hepatocytes including vascular degeneration, nuclear pyknosis and necrosis. Narrowing of hepatic sinusoids and hyperplasia of Kupffer cells were also noticed (Fig. 14). The hepatic injury appeared as grade IV.This result was in accordance with Johnson et al. [43] and Recknagel et

al. [44] who mentioned that CCL_4 is one of the most commonly used hepatotoxic agents in experimental study of liver diseases. Furthermore, mentioned that CCL_4 is biotrasformed by cytochrom P-450 in liver to produce highly reactive trichloromethyl free radical. This radical, in presence of oxygen generated by metabolic leakage from mitochondria, cause lipid peroxidation of membrane lipids which led to loss of integrity of cell membranes and damage of hepatic tissue. Moreover, De Groot and Noll [45] and Azri et al. [46] reported that the changes in structures of the endoplasmic reticulum and other membrane cause loss of metabolic enzyme activation, reduction of protein synthesis and loss glucose-6-phosphatase activation which over all leads to liver damage.

3.5.2. Different Silymarin formulae for Treatment of Induced Liver Hepatitis in Rats:

Liver specimens of rats, belonging to group (3) which were exposed to CCL_4 followed by treatment with the commercial Silymarin capsules (Mariagon®) for 7 days, showed swelling of hepatocytes and narrowing of sinusoids. Moreover, focal areas of coagulative necrosis were also seen. The liver specimens appeared as grade (III) as clearly demonstrated in figure 15.

Liver specimens of rats belonging to group (4), exposed to CCL_4 and then treated by Silymarin co-precipitate prepared according to formula 1 using Silymarin and sodium-desoxycholate in 1:3 weight ratio respectively, revealed mild swelling of hepatocytes. The latter appeared granular with vesiculated nuclei. The hepatic sinusoids contained mononuclear cells mainly lymphocytes and macrophages. The liver specimens appeared as grade (I) as clearly demonstrated in figure 16.

Liver specimens of rats belonging to group (5), exposed to CCL_4 then treated by Silymarin loaded SMEDDS prepared according to system S1 for 7 days, showed necrobiotic changes of hepatocytes, with disorganisation of hepatic cords. The hepatocytes appeared foamy with focal necrotic areas. Accordingly, the liver specimens appeared to be Grade (IV) as shown Figure (17).

Therefore, histopathological studies showed that CCL_4 caused necrosis and fibrosis of liver tissue and administration of Silymarin after CCL_4 treatment, showed hepatocytes regeneration that varied in its grade according to the type of formula administrated. Silymarin co-precipitate prepared according to formula (1) was proved to be more effective than commercial capsules as it improves liver tissue injury to be appeared as grade (I). This is probably substantiated by the increase in drug dissolution from this formula when compared to commercial capsules. Therefore, it is rational to state that the improvement in drug dissolution led to a simultaneous increase in the proportion of drug absorbed which may have contributed to the observed enhancement in hepatocytes regeneration. Further studies are required to confirm the above mentioned mechanism and also establish other mechanisms involved in the observed hepato-regenerative effect.

3.5.3. Different DDB formulae for Treatment of Induced Liver Hepatitis in Rats:

Liver specimens of group (6) treated with DDB commercial product for 7 days showed mild swelling of hepatocytes and narrowing of sinusoids as depicted in figure 18. The liver specimens appeared as grade (II).

Regarding, liver specimens of rats of group (7), treated by DDB melt prepared according to formula 2 composed of DDB and poloxamer F68 in 1:5 weight ratio for 7 days, showed swelling of hepatocytes. Moreover, the hepatic sinusoids were infiltrated with mononuclear cells mainly lymphocytes and macrophages. The liver specimens appeared to be grade (II) as clearly demonstrated in Figure (19).

Interestingly, liver specimens of rats of group (8) treated by DDB loaded in SMEDDS prepared according to system S1 and composed of 10 % Miglyol 812®, 40% Tween 80®, 50% Transcutol® HP in 1:4:5 weight ratios respectively, for 7 days, showed mild swelling of hepatocytes with prominent central situated nuclei. In addition, narrowing of hepatic sinusoids was observed. The liver specimens appeared to be grade (I) as shown in figure 20.

This improvement was attributed to the increase in drug dissolution from this formula and supported by previous study that had been shown that the ingredients present in the SMEDDS play a vital role in improving the solubility and absorption of the DDB [33].

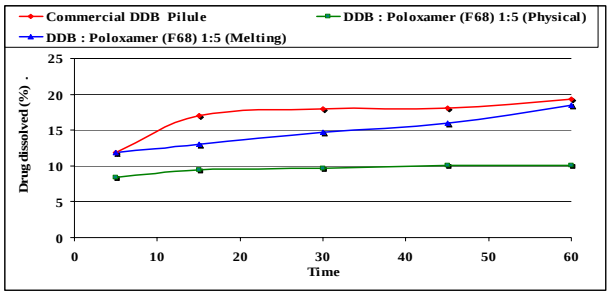

Fig. (1): Percentage Dissolution of DDB from its Solid Dispersions with poloxamer at 1:5 Weight Ratios Prepared by Physical Mixture and melting methods Compared with its Value from Commercial DDB Pilule.

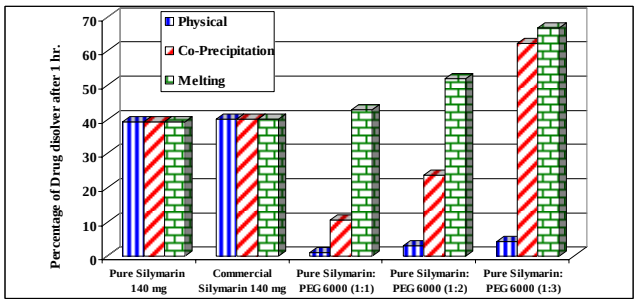

Fig. (2): Percent of Silymarin release after 1 hour for solid dispersions prepared by physical, co- precipitation and melting using PEG 6000.

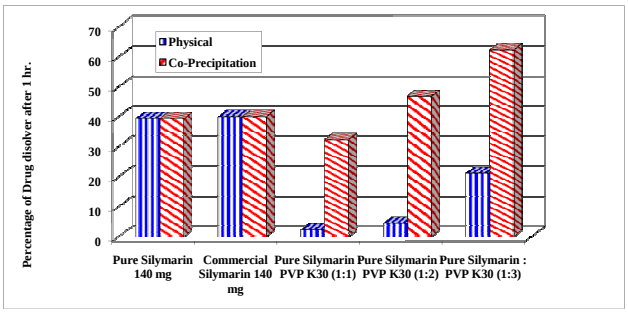

Fig. (3): Percent of Silymarin release after 1 hour for solid dispersions prepared by physical and co- precipitation using PVP K30.

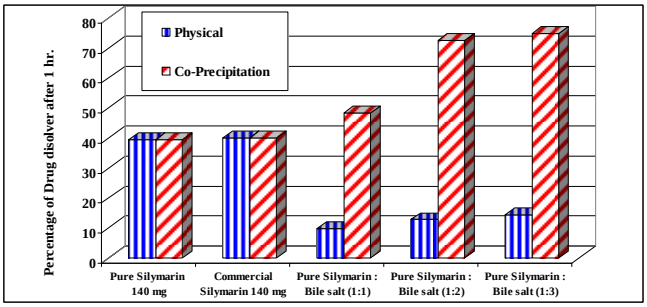

Fig. (4): Percent of Silymarin release after 1 hour for solid dispersions prepared by physical and co- precipitation using bile salt (sodium desoxycholat).

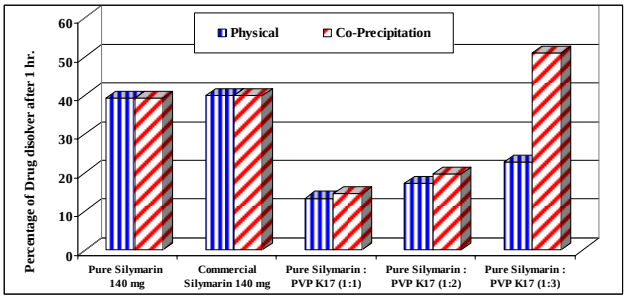

Fig. (5): Percent of Silymarin release after 1 hour for solid dispersions prepared by physical and co- precipitation using PVP K17.

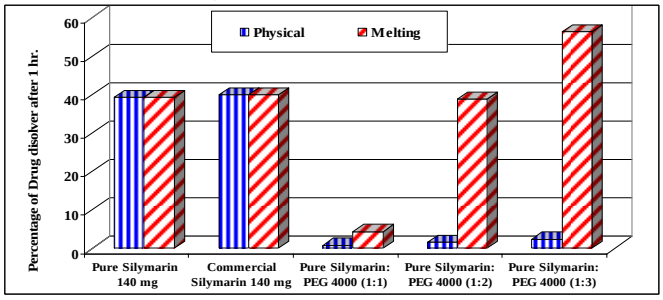

Fig. (6): Percent of Silymarin release after 1 hour for solid dispersions prepared by physical and melting using PEG 4000.

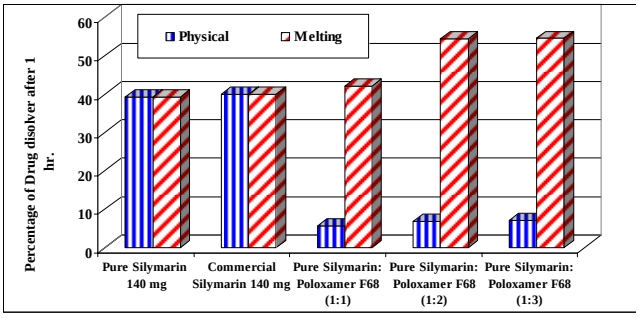

Fig. (7): Percent of Silymarin release after 1 hour for solid dispersions prepared by physical and melting using poloxamer F 68.

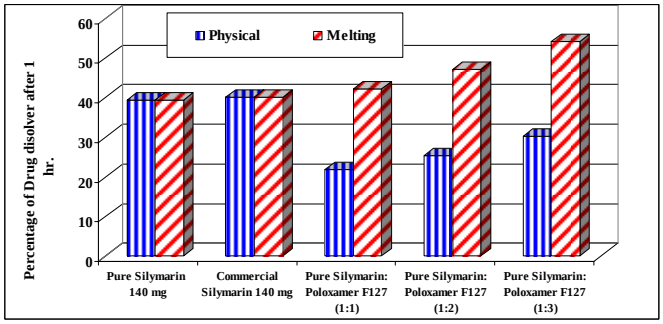

Fig. (8): Percent of Silymarin release after 1 hour for solid dispersions prepared by physical and melting using poloxamer F 127.

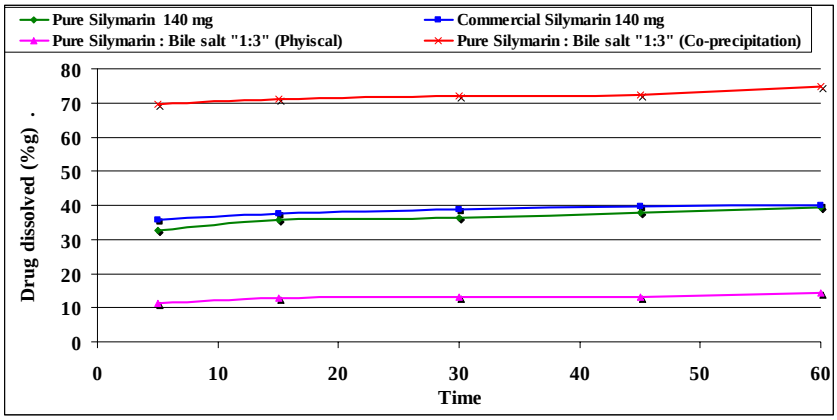

Fig. (9) Percentage Dissolution of Silymarin from its Solid Dispersions with Sodium Desoxycholate as Bile Salt at 1:3 Weight Ratios Prepared by Physical Mixture and coprecipitation Compared with its Value from Commercial Silymarin (Marriagon) in Phosphate Buffer of pH 7.4 at 37 ± 0.5 ºC.

Region (a) = SEDDS that forms milky emulsion when added to aqueous media in < than 2 minutes.
Region (b) = SEDDS that forms milky when added to aqueous media in > than 2 minutes.
Region (c) = Efficient SMEDDS that forms transparent microemulsion when added aqueous media in < than 2 minutes.
Region (d) = SMEDDS that forms transparent microemulsion when added to aqueous media in > than 2 minutes.
Region (e) = No emulsion is formed, oil droplets float on surface.

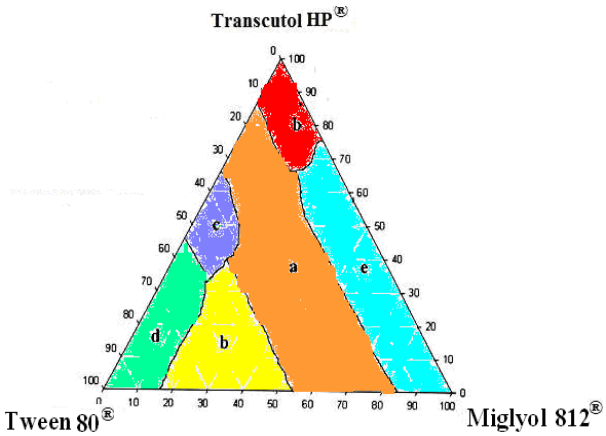

Fig. (10): Ternary phase diagram composed of Miglyol812®: Tween 80®: Transcutol HP® showing the SEDDS and SMEDDS.

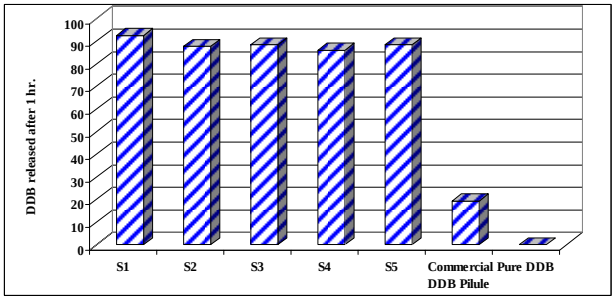

Fig.11.Percent of DDB Released from SMEDDS Composed of Different Weight Ratios of Miglyol812®, Tween 80® and TranscutolHP®.

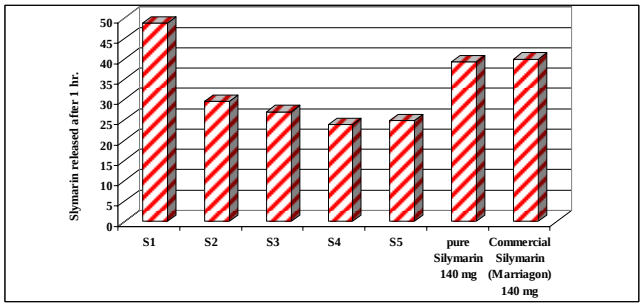

Fig.12.Percent of Silymarin Released from SMEDDS Composed of Different Weight Ratios of Miglyol812®, Tween 80® and TranscutolHP®.

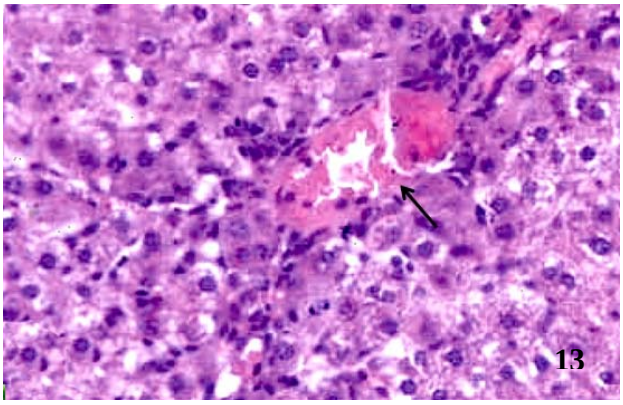

Figure 13: Liver of rats from control group (Group 1) showing normal histological structure of it is portal triads (H&E X200). Grade 0.

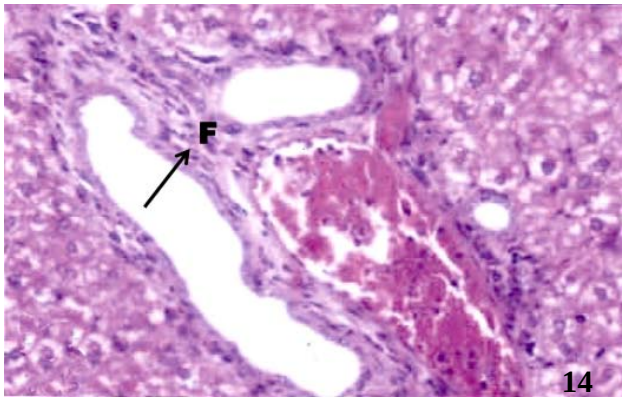

Figure 14: Liver of rats exposed to CCL_4 group (Group 2) and examined after 3days showing proliferation of fibrous connective tissue (F), and hyperplasia of bile duct (H&E X200). Garde IV.

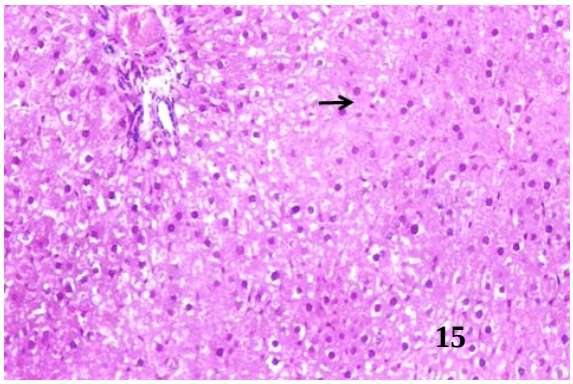

Figure 15: Liver of rats exposed to CCL_4 (Group 3) then treated with Silymarin (commercial product) for 7 days, showed swelling of hepatocytes and narrowing of sinusoids (H&E X200). Garde III.

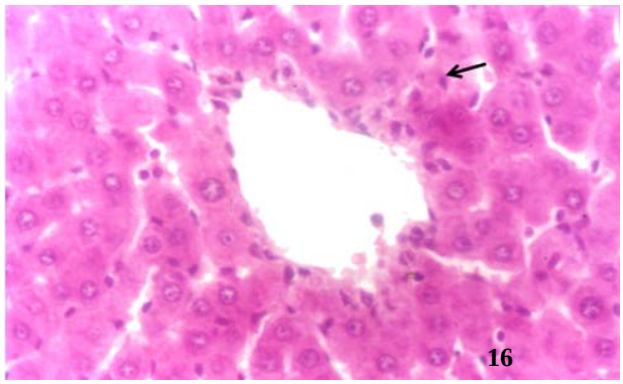

Figure 16: Liver of rats exposed to CCL4 (Group 4) then treated by Silymarin with Sodium desoxycholate prepared by co- precipitates methods (formula 1) for 7 days showing hepatocytes with eosinophilic granules, (arrow) (H&E X200) .Garde I.

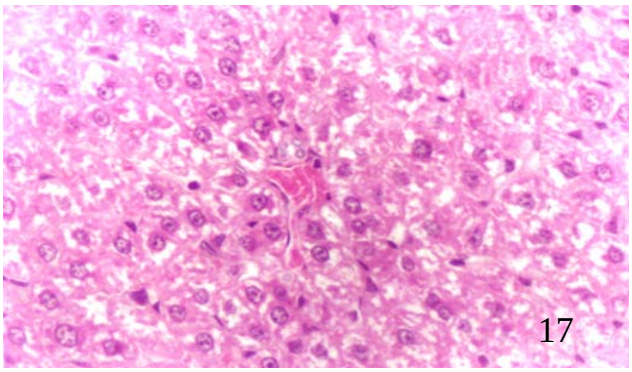

Figure 17: Liver of rats exposed to CCL4 (Group 5) then treated with Silymarin S1 for 7 days showing disorganization of the hepatic cords (H&E X200). Garde IV.

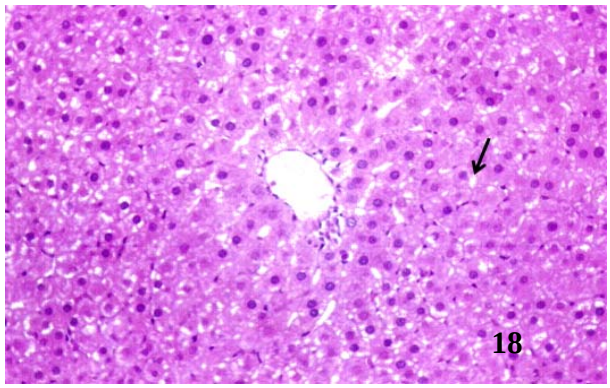

Figure 18: Liver of rats exposed to CCL4 (group 6) then treated with DDB, commercial product for 7 days, showed mild swelling of hepatocytes and narrowing of sinusoids (H&E X200). Garde II.

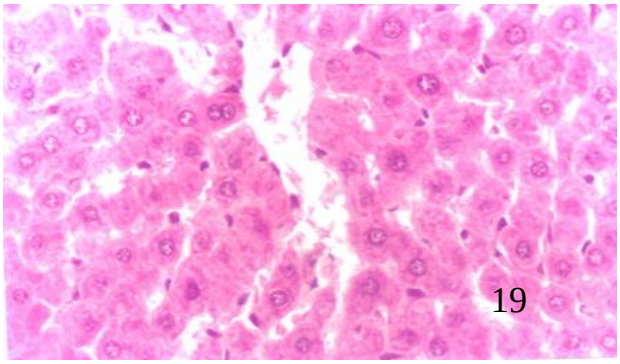

Figure 19: Liver of rats exposed to CCL4 (Group 7) then treated by DDB with poloxamer (F68) prepared by fusion method (formula 2) for 7 days showing swelling of with narrowing of hepatic sinusoids (H&E X200). Garde II.

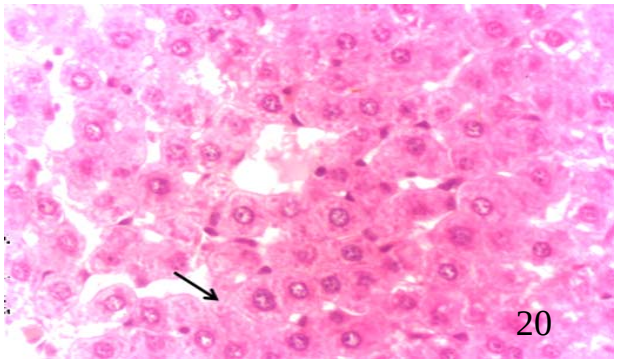

Figure 20: Liver of rats exposed to CCL4 (Group 8) then treated by DDB S1 showing swelled hepatocytes with central vesiculated nuclei (H&E X200). Garde I.

4. Conclusion:

This paper demonstrated that the poorly soluble drugs, Biphenyl Dimethyl Dicarboxylate (DDB) and Silymarin when prepared into solid dispersions (SD_s) and or self-microemulsifing drug delivery systems (SMEDDS) showed improved in their release behaviour thus avoiding the dissolution step which is the rate limiting step in the absorption process. The optimal formulation of DDB SD_s was prepared by melting method using poloxamer F68 used in 1:5 drug to carrier weight ratio and that for Silymarin was obtained by co-precipitation technique using Sodium desoxycholate in 1:3 weight ratio respectively. The DDB and Silymarin SMEDDS consisting of 10% Miglyol® 812, 40% Tween® 80, and 50% Transcutol® HP exhibited potential in vivo hepatoprotective activity against carbon tetrachloride-induced oxidative stress in Albino rats when challenged with commercial products DDB pillules® and Mariagon® capsules.These develobed formulations might be useful in the prevention of used successfully hepatic fibrosis.

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5. References:

1. World Health Organization (1997): Hepatitis C: Global prevalence. Weekly Epidemiology Rec., 72: 341 – 344.
2. MOHP (1999): The National prevalence of HCV antibody. Ministry of Health and Population Annual Report. Cairo, Egypt.
3. Xie J.X., Zhon J., Zhang C.Z., Jin J.H.Q. and Chen J.X. (1981): "Synthesis of Schizandrin C analogs." Acta Pharm. Sinica, 16: 306.
4. Oh S.Y., Lee C.H. and Ku Y.S. (2000): Pharmacokinetics and hepatoprotective effects of 2-methylaminoethyl-4,4'-dimethoxy-5,6,5', 6'dimethylenedioxybi-phenyl -2-carboxylic acid-2'-carboxylate monohydro-chloride in rats with CCl4-induced acute hepatic failure. J-Pharm-Pharmacol.; 52, 1099-1103.
5. Kim J.H., Mun Y.J., Chun H.J., Jeon K.S., Kim Y.O. and Woo W.H. (2000): Effect of biphenyl dimethyl dicarboxylate on the humoral immunosuppression by ethanol. Int-J-Immunopharmacol.; 22, 905-913.
6. El-Sawy S.A., El-Shafey A.M. and el-Bahrawy H.A. (2002): Effect of dimethyl diphenyl bicarboxylate on normal and chemically-injured liver . East-Mediterr-Health-J., 8: 95-104.
7. Xu Q., Lu J., Wang R., Cao J., and Chen X. (1997): Liver injury model induced in mice by a cellular immunologic mechanism-study for use in immunopharmcological evaluations. Pharmacol-Res., 35, 273-278.
8. Leung A. Y. and Foster S. (1996): Encyclopedia of Common Natural Ingredients Used in Food, Drugs, and Cosmetics, Second Edition, John Wiley & Sons, New York, NY.
9. Brown D. (1994): Milk thistle provides natural support for the liver. Drug Store News for the Pharmacist4: 58, 60.
10. Joanne Barnes, Linda A. Anderson and J. David Phillipson :(2007): Herbal Medicines, 3rd Edition, and Pharmaceutical Press Publication.

11. Weyhenmeyer, R, Mascher, H, and Birkmayer, J. (1992): Study on dose-linearity of the pharmacokinetics of Silibinin diastereomers using a new stereopecific assay, Int J clin pharmacal Ther Toxical. 30(4):134-138.
12. Goldberg A.N., Gibaldi M., and Kanig J.L. (1966): "Increasing dissolution rates and gastrointestinal absorption of drugs via solid solutions and eutectic mixtures III - experimental evaluation of griseofulvin- succinic acid solid solution", J.Pharm.Sci., 55: 482.
13. Ventura C.A., Trirendi S., Puglisi G., Bousquet E. and Panza L. (1997): Improvement of water solubility and dissolution rate of ursodeoxycholic acid and chenodeoxycholic by complexation with natural and modified b- cyclodextrins.Int. J. Pharm., 149: 1–13.
14. Ford J.L. (1986): The current status of solid dispersions. Pharm. Acta Helv., 6, 69-88.
15. Kang B.K., Lee J.S., Chon S.K., Jeong S.Y., Yuk S.H., Khang G., Lee H.B. and Cho S.H. (2004): Development of self-microemulsifying drug delivery systems (SMEDDS) for oral bioavailability enhancement of simvastatin in beagle dogs. Int. J. Pharm., 274: 65-73.
16. Li P., Ghosh A., Wagner R.F., Krill S., Joshi Y.M. and Serajuddin (2005): Effect of combined use of nonionic surfactants on formation of oil-in-water microemulsions. Int. J. Pharm., 288: 27-34.
17. Alvarez F.J, and Stella V.J. (1989): Pancreatic lipase-catalysed hydrolysis of esters of hydroxymethyl phenytoin dissolved in various metabolizable vehicles dispersed in micellar systems and in aqueous suspensions. Pharm. Res., 6: 555-563.
18. Yeh P.Y., Smith P.L., and Ellens H. (1994): Effect of medium chain glycerides on the physiological properties of rabbit intestinal epithelium in vitro. Pharm. Res., 11: 1148-1154.
19. Charman S.A., Charman W., Rogge M.C., Wilson T.D., Dutko F.J., and Pouton C.W. (1992): Self-emulsifying drug delivery systems: formulation and biopharmaceutical evaluation of an investigational lipophilic compound. Pharm. Res., 9: 87-93.
20. Suk Kyu Han, Gap Yul Kim, and Yong Hun Park (1999): Solubilization of Biphenyl Dimethyl Dicarboxylate by Cosolvency. Drug Development and Industrial Pharmacy. 25(11).1193-1197.
21. Feng-Qian, Li, and Jin–Hong, Hu.(2004) ; Improvement of the Dissolution Rate of Silymarin by Means of Solid Dispersions.Department of Pharmaceutical Sciences, Changhai Hospital, Second Military Medical University; 200433 Shanghai, China.
22. Janakat S. and Al-Merie H. (2002): Optimization of the dose and route of injection, and characterization of the time course of carbon tetrachloride-induced hepatotoxicity in the rat. J. phrmacol. Toxicol. Methods, 48, 41-44.
23. Paget G.E. and Barnes J.M. (1964): toxicity tests. Chapt. 6, p.135-166. Evaluation of drug activities: Pharmacometrics, vol. I. edited by Laurence, D.R. and Bacharach, A.L. academic press, London and New York.
24. Bancroft J.D., Stevans A. and Turnes, DR. (1996): Theory and Practice of Histological Techniques. 4th Ed. Churchil livingstone, Edinburgh, London, Melbourne, New York.
25. Plaa G.L. and Charbonneau M. (1994): Detection and evaluation of chemically induced liver injury:InHayes,A,W.(ed.) Principles and methods of toxicology.3rdEd.,Raven press,New York.
26. York P. (1983): Solid-state properties of powders in the formulation and processing of solid dosage forms. Int. J. Pharm., 14, 1-28.
27. Shah N.H., Carvajal M.T., Patel C.I., Infeld M.H. and Malick A.W. (1994): Self emulsifying drug delivery systems (SEDDS) with polyglycolized glycerides for improving in vitro dissolution and oral absorption of lipophilic drugs. Int. J. Pharm., 106: 15-23.
28. Constantinides P.P. (1995): Lipid microemulsions for improving drug dissolution and oral absorption: physical and biopharmaceutical aspects. Pharm. Res., 12: 1561-1572.
29. Wei L., Sun P., Nie S. and Pan W. (2005): Preparation and evaluation of SEDDS and SMEDDS containing carvedilol. Drug Dev. Ind.Pharm., 31:785-794.
30. Grove M., Müllertz A., Nielsenc J.L. and Pommergaard G. (2006): Bioavailability of seocalcitol II: Development and characterization of self-microemulsifying drug delivery systems (SMEDDS) for oral administration containing medium and long chain triglycerides. Eur.J.Pharm. Sci., 28; 233-242.
31. Gao Z.G., Choi H.G., Shin H.J., Park K.M., Lim S.J., Hwang K.J., and Kim, CK. (1998): Physicochemical characterization and evaluation of a microemulsion system for oral delivery of cyclosporine A. Int. J. Pharm., 161: 75-86.
32. Nerurkar M.M., Burton P.S., and Borchardt R.T. (1996): The use of surfactants to enhance the permeability of peptides through Caco-2 cells by inhibition of an apically polarized efflux system. Pharm. Res., 13: 528-534.
33. Hanan M .EL-Laithy.(2008):Self-Nanoemulsifying Drug Delivery system for Enhanced Bioavailability and Improved Hepatoprotective

Activity of Biphenyl Dimethyl Dicarboxlate. Current Drug Delivery,5,170-176.
34. Agatonovic-Kustrin S., Glass B.D. and Wisch M.H. (2004): Strategy for the development of a thermodynamically stable oral microemulsion. Curr. Drug Disc. Techno. 1: 165-171.
35. Srinath P., Vyas S.P, and Prakash V. (2000): Preparation and pharmacodynamic evaluation of liposomes of indomethacin. Drug Dev. Ind. Pharmacy. 26: 313-321.
36. Tarr B.D., and Yalkowsky S.H. (1989): Enhanced intestinal absorption of cyclosporine in rats through the reduction of emulsion droplet size. Pharm. Res. 6, pp. 40-43.
37. Gershanik T., Benzeno S., and Benita S. (1998): Interaction of the self-emulsifying lipid drug delivery system with mucosa of averted rat intestine as a function of surface charge and droplet size. Pharm. Res. 15: 863-869.
38. Gursoy N., Garrigue J.S., Razafindratsita A., Lambert G. and Benita S. (2003):Excipient effects on in vitro cytotoxicity of a novel paclitaxel selfemulsifying drug delivery system. J. Pharm. Sci. 92: 2420-2427.
39. Iranloye T.A., Pilpel N. and Groves M.J. (1983): Some factors affecting the droplet size and charge of dilute oil-in-water emulsions prepared by self-emulsification. J. Disp. Sci. Technol. 4: 109-121.
40. Anitha P., Mahmoud S., Indra K. and Khan M. (2004): Preparation and in vitro characterization of self-nanoemulsified drug delivery systems of coenzyme Q10 using chiral essential oil components. www.pharmatechnlogy.com.
41. Shaji J. and Joshi V. (2005): Self-microemulsifying drug delivery system (SMEDDS) for improving bioavailability of hydrophobic drugs and its potential to give sustain release dosage form. Indian J. Pharm. Educ. 39 130-135.
42. Das G., Sarma G.and Barman S. (2008): Hepatoprotective Activity Of Aqueous Extract Of Fruit Pulp Of Cassia Fistula (AFCF) Against Carbon Tetrachloride (CCL4) Induced Liver Damage In Albino Rats,MD (Pharmacology). Journal of Clinical and Diagnostic Research
43. Johnson D.E. and Kroening C. (1998): Mechanism of early carbontetrachlorid toxicity in cultured rat hepatocytes pharmacol Toxicol; 83: 231-39.
44. Recknagel R.O. (1983): A new direction in the study of carbon tetrachloride hepatotoxicity.Life Sciences 33,401-408.
45. DeGroot H. and Noll T. (1986): The crucial role of low steady state oxygen partial pressures in haloalkane free-radical-mediated lipid peroxidation. Biochemical Pharmacology 35: 15-19.
46. Azri S., Mata H.P., Reid L.L., Gandlofi A.J. and Brendel K. (1992): Further examination of the selective toxicity of CCL4 rat liver slices. Toxicology and Applied Pharmacology 112: 81-86.

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