The Protective Effect of Calcium Carbonate against Sublethal Copper Toxicity on Nile tilapia (Oreochromis niloticus)

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Abstract: This study was carried out to investigate the effect of copper toxicity on Tilapia fish (*Oreochromis niloticus*). After 15 days of acclimation, fish (45±4.8g) were exposed to 0.75 and 1.5 mg L^{-1} Cu^{+2} ($Cu_2SO_4.5H_2O$) with and without 100 mg $CaCO_3$ L^{-1} for 30 days followed by 15 days for recovery. The fish survival, hepatosomatic index, haematological parameters, residue (in fish muscles and gills) and histological changes (in fish liver and gills) were evaluated. There were no significant differences in hepatosomatic index during Cu exposure and recovery period. Significant differences ($p< 0.05$) in RBCs, WBCs and PCV were observed; however, these effects decreased after recovery period and in $CaCO_3$ treatment as well as in residues which reached its high (0.64 ±0.55 mg L^{-1}) at 1.5 Cu mg L^{-1} in muscles during exposure period and reached its low value (0.01 ±0.00 mg L^{-1}) at 0.75 Cu mg L^{-1} + $CaCO_3$ in muscles during recovery period. The histological sections supported the haematological effects and residual analysis. In summary, this work reported that copper has toxicity effects on fish and these effects decreased with the use of $CaCO_3$ as a protective agent and after recovery period.

Keywords: Heavy metals, Protective agent, Copper, Tilapia, Haematology, Residues, Histology.

INTRODUCTION

The development of aquaculture projects are being initiated in many parts of the world, especially in the developing countries as one partial solution for meeting the world's increasing demand for animal protein. Higher concentration of heavy metals beyond the tolerance limit of fish could affect fish population, reducing their growth, reproduction and/or survival and many even kill fishes (Abbas, 1994). Fish are particularly sensitive to water contamination and pollutants may impair many physiological and biochemical processes when assimilated by fish tissue (Durmaz *et al.*, 2006). Some metals are important and used as micronutrients for both plants and animals where they are an integral part of enzymes, hormones and other biologically significant compounds. Besides, these metals play essential roles in various biochemical processes including metabolic regulation, growth, reproduction and erythropoesis. Due to industrialization, the entrance of toxic metals into aquatic systems is on the increase and this creates an important environmental problem ecologically (Cicik *et al.*, 2004).

Copper (Cu) is an essential micronutrient for vertebrate animals and has vital roles in cellular respiration and as co-factor for over 30 different enzymes (Shaw and Handy, 2006). Copper sulfate (Cu_2SO_4) is one of the oldest chemicals used as Algaesides, Fungicides and Parasiticide in fish culture, A part from its help to pisculturists, it has serious disadvantage of being toxic to fish (Shalaby, 1997).

Calcium supplied through liming can reduce the uptake of heavy metals (Raddum, *et al.*, 1986 and Andersson and Borg, 1988). One mean to increase the uptake of calcium by aquatic organisms is to increase the level of environmental calcium through the application of liming agents.

The present study was plane to investigate the effect of copper on the physiological performance of Nile tilapia, and the possibility to use of $CaCO_3$ as a protective agent against Cu toxicity.

MATERIALS AND METHODS

Experimental design:

This study was designed to investigate the effect of Copper with and without $CaCO_3$ on the physiological performance of Nile tilapia fish via Five treatments; the first group was the untreated (control), the second and third groups were exposed to 0.75 and 1.5 Cu mg L^{-1} ($Cu_2SO_4.5H_2O$) respectively, while the fourth and fifth groups exposed to 0.75 and 1.5 Cu mg L^{-1} +100 $CaCO_3$mg L^{-1}, three replicates have been used for each treatment. Fish were exposed Cu with or without $CaCO_3$ for thirty days after which fish were transferred to clean water for 15 days for recovery.

Experimental fish:

One hundred and fifty apparently healthy Nile tilapia *Oreochromis niloticus* (L.) were collected from ponds of Fish Research Centre, Suez Canal University, Ismailia, Egypt. Fish (45±4.8g) were adapted in indoor tanks for twc weeks to laboratory conditions. Fish of mixed sex were distributed randomly at rate of 10 fish per 60 L glass aquaria (80x30x40 cm^3) that contained aerated water, fish were fed about 3% of its weight on (25% crude protein) diet, twice daily. Dead fish were removed and were recorded daily during experimental period.

Hepato-somatic Index (H.S.I):

H.S.I was determined as liver percentage to whole wet body weight.

H.S.I = [Liver weight (g) / Body weight (g)] *100

Survival rate (S.R):

The survival rate was determined from the equation

$$S.R = (N_i * 100) / N_0$$

According to (Harrell *et al.*, 1990)

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Where: N_i = Number of fish at the end, N_0 = Number of fish initially stoked.

Copper bioaccumulation

Copper residues were detected in fish liver and gills according to APHA (2000). Accumulation factor (AF) was calculated according to Aboul Ezz and Abdel-Razek.(1991), using the following equation:

AF=Pollutant concentration in fish organ (ug/g)/ Pollutant in water (mg / L).

Haematological studies:

After anesthetizing fish by using MS222 (0.05g per L), blood samples were withdrawn from the heart. The collected blood transferred to eppendorff tubes contain EDTA used as an anticoagulation agent (Selma and Hatice, 2004). Blood samples were divided into two sets of eppendorf tubes, one set for haematology (haemoglobin, haematocrit and blood cell counting), the second set was centrifuged at 5000 r.p.m for 5 min. and the collected plasma was stored at -20°C for further biochemical analysis.

Three fish were dissected to get gills and liver and kept in formalin 10% for histological examination. The rest of fish stored frozen in refrigerator at 5°C for copper residual analysis. Total number of erythrocytes (RBCs) and leucocytes (WBCs) were counted using *Neubauer haemocytometer* and *Natt* and *Herrick's* solution as diluting fluid according to the method described by Natt and Herrick (1952) and Campbell (1995). Haemoglobin was estimated in blood by method described by Nilton *et al.* (2007). Packed cell volume (P.C.V.) was determined using the microhaematocrite method Schalm, (1965).

Mean corpuscular volume (M.C.V.μm^3/cell)

P.C.V.= [P.C.V. (%) / R.B.C.s (million / $mm^{3)}$] * 10

Mean corpuscular haemoglobine (M.C.H Pg/cell)

M.C.H=[Hb (gm/100 ml blood)/R.B.C.s (million/mm^3)]

Mean corpuscular haemoglobine conc. (M.C.H.C g/100ml)

M.C.H.C.=(Hb (gm/100 ml blood)/P.C.V.) * 100

Plasma alanine amino transferase (ALT) and aspartate amino transferase (AST) were determined colorimetrically by transaminases kites (Boehringer Mannheim kit). Plasma glucose was determined according to Zaki *et al.* (2008).

Histological studies:

Specimens from fish liver and gills were fixed in 10% neutral buffered formalin. then, they were washed in 70% ethanol, dehydrated, cleared in xylene, embedded in paraffin wax, sectioned, stained with haematoxylin and eosin, cleared in xylene and mounted in Canada balsam. Three sections of each tissue from each fish were examined by light microscope for histological alterations according to Drury and Wallington (1980) and Mohamed, (2009). Sections of liver and gills of controls and treated fish were photographed using Leitz microscope fitted with a camera and connected to a computer.

Statistical analysis

The obtained data were statistically analyzed by using one-way analysis of variance (ANOVA) procedure. Analysis system were done using SPSS program version $SPSS_{(PASW)18}$ Statistics ver. 18.0 (SPSS, Richmond,USA) as described by Dytham,(1999). Means were compared using Duncan's test (1955). All data were expressed as means±Standard Error. The significance level was set at the probability level of $P<0.05$.

RESULTS

Survival rate and Hepatosomatic index:

The results of survival rate and hepatosomatice index are summarized in table (1). Fish mortality was observed in all treatments accept control group during the experiment period as shown in table (1), the survival rate calculated as percentages after 45 days, hepatosomatic index showed that there were no significant differences between treatments during the exposure and recovery period.

Bioaccumulation results

The highest Cu concentration in fish muscles as shown in fig. (1) was (0.64±0.55 mg L^{-1}) compared with control (0.01±0.00 mg L^{-1}) at exposure period and the lowest value was reported at 0.75 Cu^{+2}+$CaCO_3$ (0.01±0.00 mg L^{-1}) compared with control in which Cu was undetectable after recovery period (Fig. 1), regarding the Cu residue in fish gills, the highest value (0.48±0.01 mg L^{-1}) was detected at 1.5 mg Cu^{+2} at exposure period while the lowest value (0.02±0.00) was detected in recovery period at 0.75 Cu^{+2} +$CaCO_3$ compared with control which is undetectable.

Table (1): Changes in S.R and H.S.I of Nile tilapia exposed to (0.75 and 1.5 mg Cu L^{-1}) with and without $CaCO_3$ for 30 days followed by recovery period in unpolluted water for 15 days.

Treatments	S.R.[1]	H.S.I[2]	
	At end of experiment 45d	Exp. 30d	Rec. 15d
Control	100 %	1.73±0.13	1.56±0.09
0.75 Cu^{+2}	45 %	2.23±0.49	1.50±0.40
Cu^{+2}+$CaCO_3$ 0.75	55 %	2.70±0.80	°1.80±0.3
1.5 Cu^{+2}	35 %	2.33±0.23	2.16±0.58
1.5 Cu^{+2}+$CaCO_3$	55 %	2.41±1.07	1.86±0.43

No significant different according to Duncan's multiple range test ($P < 0.05$)

1. S.R: survival rate, 2. H.S.I.: hepatosomatic index.

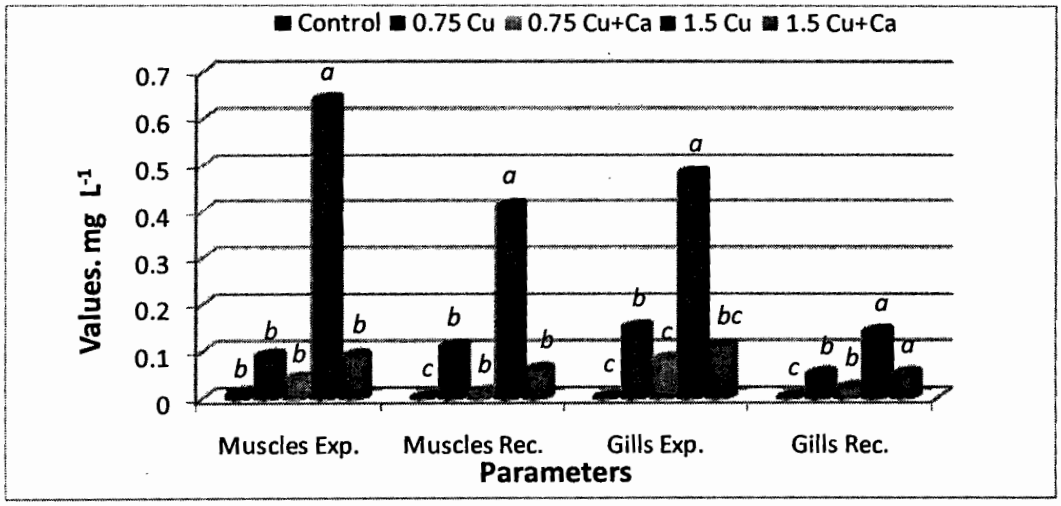

Figure (1): Changes in Cu residues in Muscles and Gills of Nile tilapia exposed (0.75 and 1.5 mg Cu L^{-1}) with and without $CaCO_3$ for 30 days followed by recovery period in unpolluted water for 15 days.

Haematological parameters: The data of haematological parameters are represented at table (2). There were no significant differences in haemoglobin content between treated groups and control group in both exposure and recovery period.

The packed cell volume showed significant differences between treated groups and control at exposure period (control 26.48 ±1.16%,1.5 mg L^{-1} Cu^{+2} 10.93 ±1.74 %) and there were significant difference too between control and treated groups at recovery period (control 28.26±2.03%, the concentration 1.5 mg L^{-1} Cu^{+2} 12.66±1.39%).

The results of M.C.V, M.C.H and M.C.H.C were represented at table (2). There are significant differences in values of M.C.V, M.C.H and M.C.H.C. The values of M.C.V reach its high at control group (195.58±7.81 μm^3/cell) and it was (95.29±5.19 μm^3/cell) at concentration 0.75 mg L^{-1} Cu^{+2}+$CaCO_3$ at exposure period and reach 137.18±6.89 μm^3/cell in control group and reported 106.29±5.17 μm^3/cell in concentration 0.75 mg L^{-1} Cu^{+2}+$CaCO_3$ at recovery period.

Regarding the M.C.H, the highest value appeared at concentration 1.5mg L^{-1} Cu^{+2} 45.58±3.33 pg/cell and the lowest value appeared at concentration 0.75 mg L^{-1} Cu^{+2}+$CaCO_3$ 32.29±2.46 pg/cell at exposure period and the highest value appeared at concentration 1.5 mg L^{-1} Cu^{+2} 54.71±5.15 pg/cell and the lowest value appeared at control group 29.17±3.38 pg/cell at recovery period.

The data of M.C.H.C showed that, at the concentration 0.75 mg L^{-1} Cu^{+2} the highest value was 45.09±7.31 g/100 ml while the control reported the lowest value 24.55±1.97 g/100 ml at exposure period whereas the highest value appeared at recovery period belong to the concentration 1.5 mg L^{-1} Cu^{+2} 45.97±3.32 g/100 ml and the lowest value was 21.27±1.40 g/100 ml at control group. The results found to be significantly different in values of M.C.V, M.C.H and M.C.H.C.

Biochemical results: The results of Glucose, AST and ALT are showedat fig. (2). The highest value (70.43±2.22 mg/dl) was obtained at 1.5 mg Cu^{+2} L^{-1} and 45.37±2.63 mg/dl at control at exposure period and were 47.51±1.39 mg/dl at 1.5 mg L^{-1} Cu^{+2} and 26.68±1.13 mg/dl at 0.75 mg L^{-1} Cu^{+2} +$CaCO_3$ at recovery period.

The ALT values were 6.67 ±0.37 IU/L, 20.37±1.41 IU/L, 16.88±0.87 IU/L, 27.68±1.88 IU/L and 23.21 ±1.43 IU/L for control, 0.75 mg L^{1-} Cu^{+2}, 0.75 mg L^{-1} Cu^{+2}+$CaCO_3$, 1.5 mg L^{-1} Cu^{+2}, and 1.5 mg L^{-1} Cu^{+2}+$CaCO_3$ respectively in treatment period and were 5.60±0.97 IU/L, 11.10±0.13 IU/L, 9.33±1.41 IU/L, 13.33±3.35 IU/L and 10.33±1.16 IU/L for control, 0.75 mg L^{-1} Cu^{+2}, 0.75 mg L^{-1} Cu^{+2}+$CaCO_3$, 1.5 mg L^{-1} Cu^{+2}, and 1.5 mg L^{-1} Cu^{+2}+$CaCO_3$ respectively in a recovery period.

The AST results showed high activity at concentration 1.5mg L^{-1} Cu^{+2} 46.27±1.33 IU/L and the activity in control were 12.39±1.41 IU/L at treatment period, and were 23.33±1.63 IU/L in concentration 1.5 mg L^{-1} Cu^{+2} and 11.78±1.2 IU/L in control at a recovery period. It is clear that there were significant differences in Glucose, ALT and AST activity of studied fish among the different studied treatments.

Histological results: The previous biochemical and physiological investigations were confirmed by the results of histological examination, where histopathological alterations and clear damage of gills and liver were observed. No histological changes were observed in the gills and liver of the control fish, while fish exposed to copper exhibited histopathological changes in both organs. Histological changes of control and copper treated *O. niloticus* were represented in fig. (3 and 4).

DISCUSSION

Fish, as bio-indicator species, play an increasingly important role in the monitoring of water pollution because they respond with great sensitivity to changes in aquatic environment (Siroka and Drastichov, 2004).

Mishra and Srivastava (1980) and Seong-Gil and Ju-Chan(2004) reported that hepatosomatic index of fresh water teleost , *Colisa fasciatus* was not significantly differed after copper nitrate exposure.

It is well known that fish have the ability to concentrate heavy metals and different pollutants in

their muscles and different organs (Katalay and Parlak, 2004). Metal accumulation in fish tissues depends on exposure dose and time as well as other factors such as temperature, age of fish, interaction with other metals, water chemistry and metabolic activity of the fish (Heath, 1995). Copper residue in gills was significantly elevated with time of exposure and concentration. Our data showed that the highest Cu concentrations were found in fish gills. The $CaCO_3$ play an important role against Cu accumulation (Abdel-Tawwab, 2007) and the result showed that, there was a decrease in Cu concentrations after recovery period in $CaCO_3$ treatments more than non $CaCO_3$ treatments.

In haematological and biochemical studies, treatments showed significant differences in relation to control. The reduction in P.C.V values is due to a decrease in circulating RBCs number, as reflected in the M.C.V values, occurring primarily at the highest concentration examined. The values for total P.C.V varied between 11.53% and 17.20%, with differences at all treatments concentrations with or without $CaCO_3$, compared to control at exposure and recovery periods. In determining Hb content, (M.H.C) and concentration of (M.C.H.C) in erythrocytes, a slight rise in mean values was observed during the course of the experiment, suggesting a small increase in erythrocytes volume and of the haemoglobin content inside.

O'Connor and Fromm (1975) reported a decrease in RBCs number and Ht, due to hemolysis as a consequence of Hg toxicity. On the other hand, some authors (Sampaio *et al.*, 2007) suggested that in experiment of toxicity a lowered Ht level could be related to the conditions of confinement or stress induced by the lack of food. Handy *et al.* (1999) examined blood chemistry in rainbow trout exposed to dietary Cu and found no significant change in haematocrit, haemoglobin and RBCs. The decrease in blood parameters is accompanied by an increase in M.C.V and M.C.H and M.C.H.C which may be due to haemolytic action which led to fluid loss to the tissue with subsequent decrease in plasma volume.

In the erythrocytes of trout apoptotic reaction was observed when it was exposed to the tributyltin toxicity caused by water pollution (Tiano *et al.*, 2003). Significant increases in the haemoglobin concentration and the number of the haematocrit were found in *Carassius auratus gibelio* and they were attributed to the toxic effects of textile dyes (Al-Sabti, 2000). M.C.V and M.C.H.C levels increased in tilapia, *Orecohromis mosambicus* exposed to cadmium (Ruperalia *et al.*, 1990). The increase of WBCs of fish was suggested to indicate an action on the immunological defence (Zaghloul, 1997). Generally, metal exposure can result in gill damage, which in turn can affect blood parameters (Pelgrom, et al., 1995), and waterborne exposure is more toxic than dietary exposure in fish (Handy, 1996). Blood glucose appeared to be sensitive, reliable indicator of environmental stress in fish (Nemcsok and Boross, 1982). Environmental pollution may produce stress in fish enhancing glycogen breakdown in liver and consequently raise blood glucose lever (Diwan *et al.*, 1979)

The increase in glucose level may be attributed to accumulation of heavy metals in pancreatic islets and damage the insulin production ß cells are reported by Havu (1969) and Khanna and Gill (1975). Clinical enzymology applies the science of enzymes to the diagnosis and treatment of disease processes AST and ALT belong to the non plasma specific enzymes which are located within tissue cells and have no known physiological function on plasma (Sandnes *et al.*, 1988).

In the present study the evaluation of these enzymes could be attributed to damaged damage of liver cells by the action of heavy metals (Bell, 1968; Zaghloul, 1997; Abdel-Tawwab *et al.*, 2007), or to the changes in blood ammonia levels in fish exposed to hepatotoxicants (D'Appollonia and Anderson, 1980), which may be responsible for the observed increase in plasma transaminases activities than the control.

Gills, which participate in many important functions in fish, such as respiration, osmoregulation and excretion, remain in close contact with the external environment, and particularly sensitive to changes in the quality of the water, are considered the primary target of the contaminants (Mazon *et al.*, 2002 and Fernandes and Mazon, 2003). Alterations like epithelial lifting, hyperplasia and hypertrophy of the epithelial cells, besides partial fusion of some secondary lamellae are examples of defence mechanisms, since, in general, these result in the increase of the distance between the external environment and the blood and thus serve as a barrier to the entrance of contaminants (Hinton and Laurén, 1990 a&b; Poleksic and Mitrovic, 1994 and Fernandes and Mazon, 2003). Several other studies showed necrosis and desquamation of secondary lamellar epithelium, lifting up of epithelium, intraepithelial oedema, fusion of adjacent secondary lamellae, haemorrhage at primary lamellae, hypertrophy and hyperplasia of epithelial cells (Cengiz and Unlu, 2002, 2003). Increasing the water hardness by adding $CaCO_3$ after the recovery period showed regenerative changes in the epithelium of the secondary lamella of fish gills and in the hepatocyte cells of fish liver exposed to copper. The obtained results here in agreement with Zaghloul (1997). Lliver is the organ that suffers serious morphological alterations in fish exposed to contaminants. The liver is the main organ for detoxification (Dutta *et al.*, 1993). Alterations in the liver may be useful as markers that indicate prior exposure to environmental stressors. Gill and liver histopathological alterations, such as those observed in this study and findings from previous studies, may result in severe physiological problems, ultimately leading to the death of fish. Damaged to the liver is the most frequently reported histopathological response to organic and non organic compounds. The importance of the liver as a marker for pathological change reflects the central role of teleost hepatic tissues in nutrition, lipid and carbohydrate storage, synthesis of protein and enzymes, fatty acid metabolism and the biotrans formation and elimination of xenobiotics. The liver accumulates xenobiotic and hepatocytes bitransform these compounds and transport them to the bile for elimination (Hinton *et al.*, 1992).

Table (2): Changes in RBCs and WBCs count, Hb, P.C.V, M.C.V, M.C.H and M.C.H.C of Nile tilapia exposed (0.75 and 1.5 Cu mg L^{-1}h and without $CaCO_3$ for 30 days followed by recovery period in unpolluted water for 15 days.

		Sampling day	Control	0.75	0.75+$CaCO_3$	1.5	1.5 +$CaCO_3$
RBCs[1](×10^6/µL)	Cu^{+2}exposure	30	1.60 ^{a}±0.18	1.21^{ab}±0.12	1.35 ^{ab}±0.18	0.95 ^{b}±0.	1.28 ^{ab}±0.16
	Recovery	45	2.06 ^{a}±0.15	1.61^{ab}±0.19	1.59 ^{ab}±1.30	0.98 ^{c}±0.	1.39 ^{bc}±0.15
WBCs[2](×10^3/µL)	Cu^{+2}exposure	30	4.39 ^{c}±0.86	37.90^{c}±1.92	23.10 ^{d}±1.41	53.50 ^{a}±1	49.20 ^{b}±1.01
	Recovery	45	4.49 ^{d}±0.57	40.40 ^{a}±1.60	21.70 ^{c}±1.16	42.00 ^{b}±1.	29.90 ^{b}±1.34
Hb[3] g/dl	Cu^{+2}exposure	30	6.50±0.57	5.20±0.50	4.36±1.39	4.33±0.3	5.70±0.56
	Recovery	45	6.01±0.63	6.83±0.95	6.96±1.06	5.82±1.3	5.07±1.47
P.C.V.[4] %	Cu^{+2}exposure	30	26.48 ^{a}±1.16	11.53 ^{b}±1.13	13.30 ^{b}±1.37	10.93 ^{b}±1.'	13.86 ^{b}±1.52
	Recovery	45	28.26 ^{a}±2.03	17.73 ^{b}±1.48	16.90 ^{b}±2.06	12.66 ^{b}±1.3	15.36 ^{b}±2.19
M.C.V.[5] µm^3/cell	Cu^{+2}exposure	30	195.58 ^{a}±7.81	95.29 ^{b}±5.19	98.51 ^{b}±6.43	115.05 ^{b}±12.	108.28 ^{b}±8.11
	Recovery	45	137.18 ^{a}±6.89	110.12 ^{b}±7.95	106.29 ^{b}±5.17	129.18 ^{a}±4.4	110.50 ^{b}±5.61
M.C.H.[6] pg/cell	Cu^{+2}exposure	30	40.63 ^{ab}±2.55	42.97 ^{a}±2.14	32.29 ^{b}±2.46	45.58 ^{a}±3.3.	44.53 ^{a}±2.64
	Recovery	45	29.17 ^{c}±3.38	42.42 ^{ab}±3.84	43.77 ^{ab}±2.78	54.71 ^{a}±5.1:	36.47 ^{bc}±2.67
M.C.H.C[7]g/100ml	Cu^{+2}exposure	30	24.55 ^{b}±1.97	45.09 ^{a}±7.31	32.78 ^{ab}±2.92	39.62 ^{a}±2.81	41.13 ^{a}±3.28
	Recovery	45	21.27 ^{c}±1.40	38.52 ^{ab}±6.54	41.18 ^{ab}±1.88	45.97 ^{a}±3.32	33.00 ^{b}±1.83

^{a,b,c,d,e} Means followed by the same superscript in the same row are not significantly different according to Duncan's multiple range test ($P<0.05$).

1. RBCs: Erythrocytes, 2. WBCs: Leucocytes, 3. Hb: haemoglobin content, 4. P.C.V.: packed cell volume. 5. M.C.V: Mean corpuscular volume.
6. M.C.H.: Mean corpuscular haemoglobin, 7. M.C.H.C.: Mean corpuscular haemoglobin concentration.

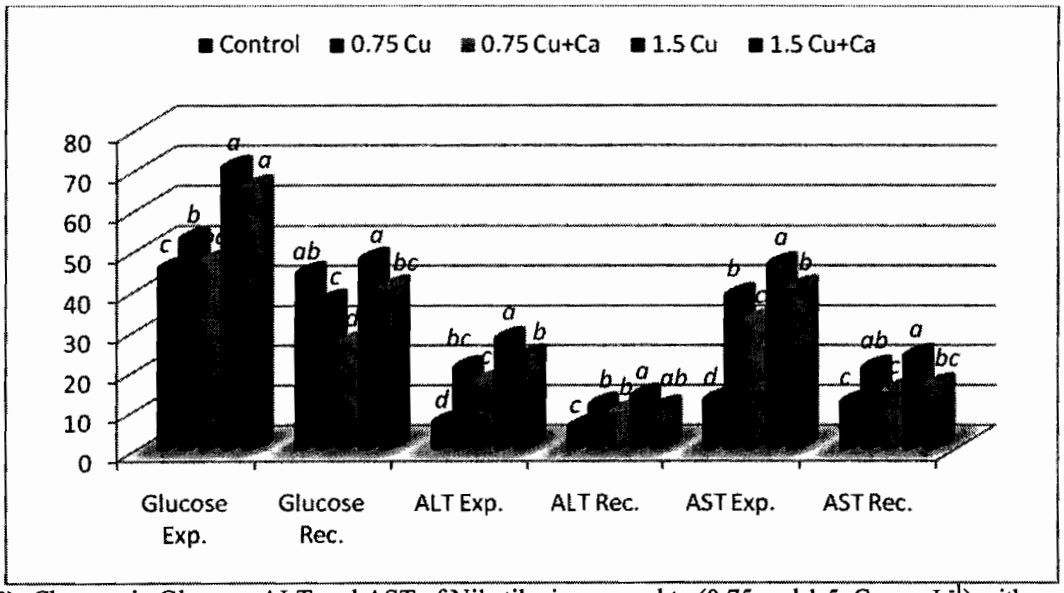

Figure (2): Changes in Glucose, ALT and AST of Nile tilapia exposed to (0.75 and 1.5 Cu mg L^{-1}) with and without $CaCO_3$ for 30 days followed by a recovery period in unpolluted water for 15 days.

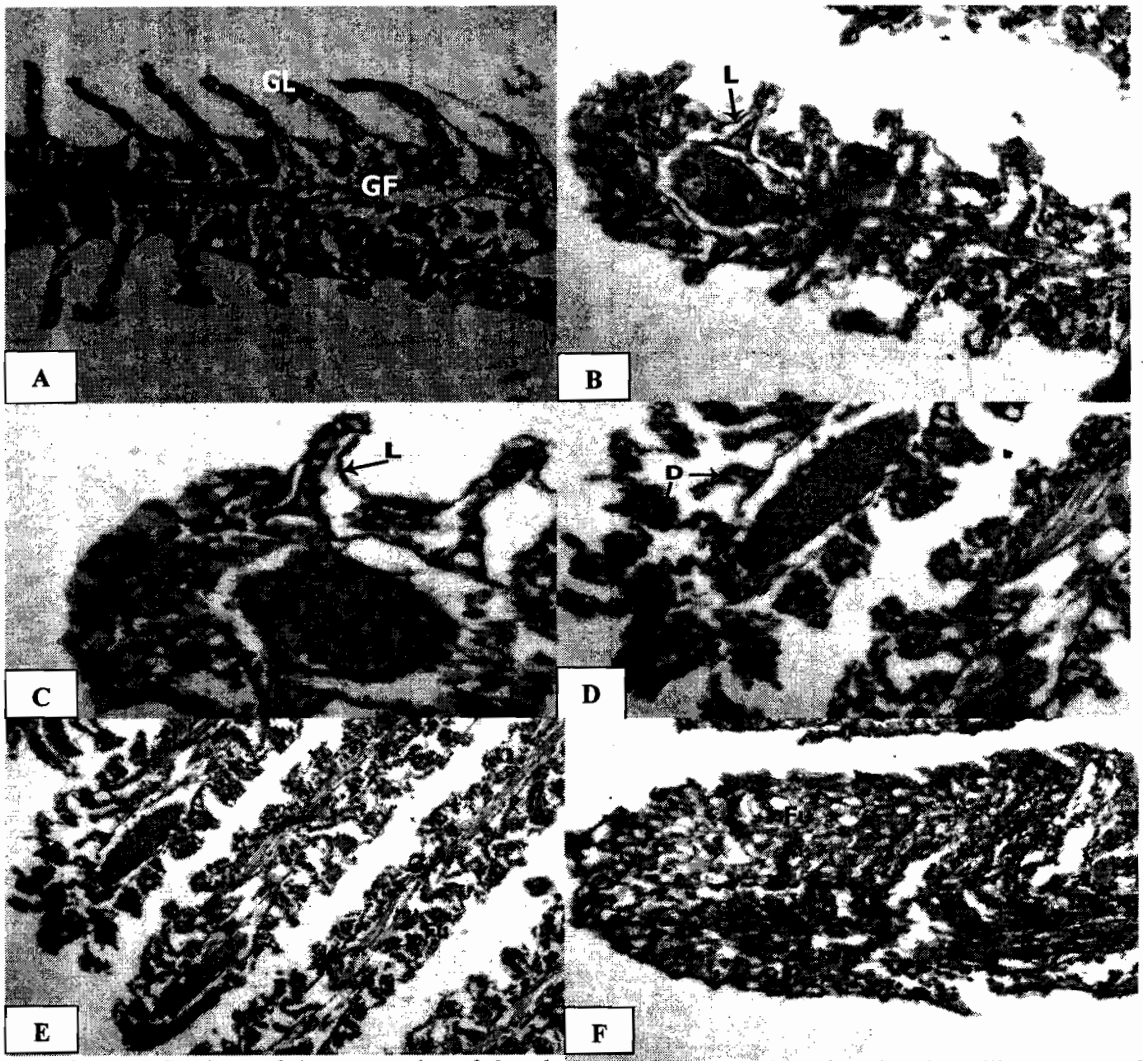

Figure (3): (A- F) : Sections of tissue samples of *O. niloticus* (A) A control section showing gill filaments (GF) and gill lamellae(GL) (X400). (B and C) 0.75 mg l^{-1} Cu exposed fish showing hemorrhage (Ha) and epithelial lifting (L) (X200 and X400 respectively). (D) 1.5 mg l^{-1} Cu exposed fish showing hemorrhage (Ha) and gill deformation (D) (X200). (E and) 1.5 mg l^{-1} Cu exposed fish showing hemorrhage (Ha) and lamellar fusion (Fu) (X100 and X200 respectively).

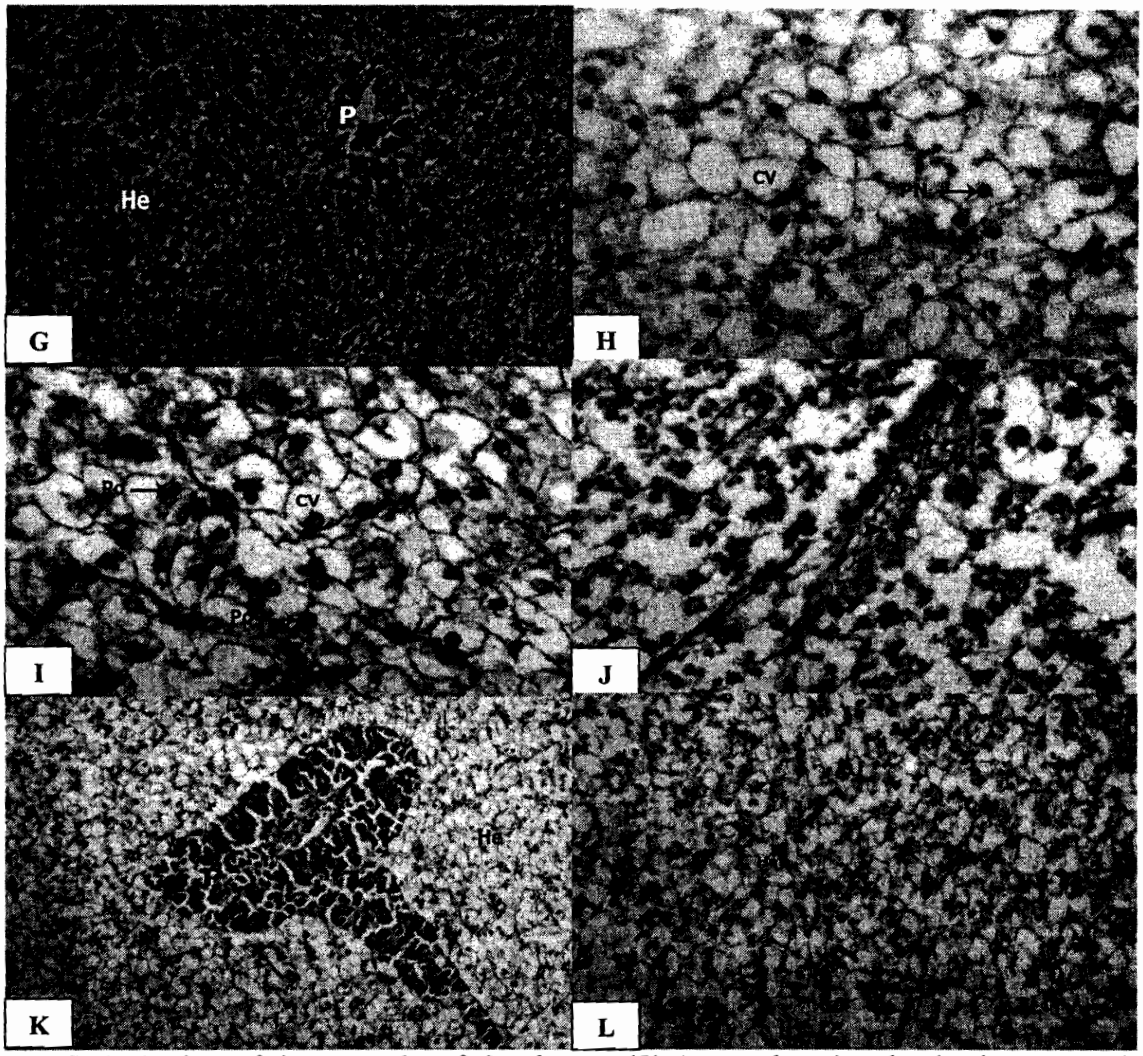

Figure 4. (G-L): Sections of tissue samples of *O. niloticus* (G) A control section showing hepatocytes (He) and pancreas (P) (X200). (H) 1.5 mg l^{-1} Cu exposed fish showing cytoplasmic vocalization (CV) and piknotic nuclear (PN) (X200). (J) 1.5 mg l^{-1} Cu exposed fish showing polymaro-nuclei (PO) and cytoplasmic vocalization (CV) (X400). (J) 0.75 mg l^{-1} Cu exposed fish showing fibrous connective tissue (arrow head) (X400). (K and L) 1.5 mg l^{-1} Cu exposed fish after recovery showing normal hepatocytes (He) and pancreas (P) (X100 and X200 respectively).

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التأثير الوقائي لكربونات الكالسيوم ضد سمية تركيزات النحاس الشبه مميتة في أسماك البلطي النيلي

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أجريت هذه الدراسة بهدف بحث تأثير سمية النحاس على أسماك البلطي النيلي (٤٥±٤,٨ جم)، تم تعريض الأسماك إلى ٠,٧٥ و ١,٥ ملجم نحاس / لتر مع وبدون ١٠٠ ملجم كربونات الكالسيوم / لتر بعد ١٥ يوم من الأقلمة لمدة ٣٠ يوم كفترة تعريض تبعتها فترة ١٥ يوما كفترة إستشفاء.تم تقدير كل من معدل الإعاشة، دليل الكبد بالنسبة للجسم، قياسات الدم (كرات الدم، كرات الدم البيضاء، الهيموجلوبين، نسبة مكداس الدم، متوسط حجم كريات الدم الحمراء، متوسط وزن الهيموجلوبين في الدم و متوسط تركيز وزن الهيموجلوبين في كرات الدم الحمراء) ، المتبقيات (في العضلات والخياشيم) والقطاعات النسيجية (في الكبد والخياشيم).

لم يكن هناك أي اختلافات معنوية في دليل الكبد بالنسبة للجسم خلال فترتي التعريض والإستشفاء. ظهر عدد من الإختلافات في مقاييس الدم بعد فترة التعريض وكانت هناك اختلافات معنوية في كل من كرات الدم الحمراء وكات الدم البيضاء ونسبة مكداس الدم وانخفضت هذه التأثيرات بعد فترة الإستشفاء وفي المعاملات الخاصة بكربونات الكالسيوم وحدث نفس الأمر مع المتبقيات التي وصلت لأعلى قيمة لها خلال التركيز المرتفع من النحاس إلى(٠,٦٤±٠,٥٥ ملجم/لتر) خلال فترة التعريض في العضلات وانخفضت إلى (٠,٠١±٠,٠٠ ملجم/لتر) في التركيز ٠,٧٥ ملجم/لتر + كربونات الكالسيوم في العضلات خلال فترة الإستشفاء. ودعمت القطاعات النسيجية كلا من الإختلافات في قياسات الدم والمتبقيات. الخلاصة: أن هذا العمل سجل تأثير سمية النحاس على الأسماك و انخفضت هذه التأثيرات مع استخدام كربونات الكالسيوم مادة الوقائية وكذلك بعد فترة الإستشفاء.