



Remediation of toxic effect of Mercuric Chloride on the Nile Tilapia, *Oreochromis niloticus* using Selenium

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ABSTRACT

The present results showed that heavy metals (HgCl_2) have affected the biological parameters of *Oreochromis niloticus*. These effects depended on the duration of the exposure and the concentration of the heavy metal used. The effect of various doses level of HgCl_2 on the erythrocyte count (Red blood cells) decreased under the effect of the high doses of HgCl_2 (0.08 mg/l or more). A significance difference was recorded in comparison with the control fish ($p < 0.05$). The total white blood cells counts significantly increased as the concentration of HgCl_2 increase when compared to the control fish. In contrast, hemoglobin (Hb) levels significantly ($p < 0.05$) decreased after the fish exposed to high level doses of HgCl_2 . Addition of selenium (Na_2SeO_3) at doses of 1.0 and 2.0 mg/kg to fish food for a period of 20 days of treatment with HgCl_2 improved blood constituent. Treated gills with high doses of HgCl_2 showed alteration on both of primary and secondary lamellae and an increase in the mucous secretion when compared with the control tissue. No abnormalities occurred in the liver of control fish. The main alterations in the liver of fish were observed after treatment with high doses level of HgCl_2 (0.06-0.08mg/l). The hepatocytes were hypertrophied and the nuclei showed necrosis.

INTRODUCTION

Mercury is considered as one of the most dangerous toxic heavy metals, especially in the aquatic environment (Asefi and Zamani, 2015). Mercuric chloride increases Hg deposition in the aquatic environments and results from the increased anthropogenic activities, enhancing bioaccumulation of Hg in the food chain and higher Hg concentration in fish (Jones *et al.*, 2013). The typical concentration of Hg in edible tissues of various species of fish ranges from 50 to 1,400 $\mu\text{g}/\text{kg}$ (IPCS, 2003). Nijmi and Kisson (1994) reported that the level could be reached to 20 mg/kg which may cause toxicity in fish. Kruzikova *et al.*, (2008) confirmed that Hg can be predominantly deposited as MeHg in fish tissue. However, Ikingura and Akagi (2003) postulated that a substantive level of Hg (0 to 44 %) can also be present as inorganic Hg in fish especially in seafood (Moon *et al.*, 2011). Mercury exposure to fish at high level inorganic or organic from can permanently damage the brain, kidney (ATSDR, 2003).

However, most researchers do not give enough attention to nutritional factors that might influence organisms in response to heavy metal intoxication (Shapman and Chan, 2000).

Fish considered as a well – balanced diet because of its nutritional benefits such as high quality of protein, vitamins, high energy and minerals (Pieniak *et al.*, 2010). In addition, fish are rich sources of omega-3 and they contain a very low level of cholesterol (Groth, 2010; Vieira, 2011). In human, fish help in neural tissue development of children and have an active role in coronary heart disease (Swanson *et al.*, 2012). In contrast, fish can also be a potential source of toxic metals at a higher trophic level through their consumption (Wang *et al.*, 2012).

The Nile tilapia was selected as the test organism in the present study for its great aquaculture and commercial value. Tilapia is the third most important cultured fish group in the world. In Egypt, the aquaculture tilapia production (38 %) is the highest (GAFRD, 2001; Beheary *et al.*, 2015). In the last few decades, fish exposure to heavy metals presents unique problems from both toxicological and nutritional perspectives (Clarkson, 1998; Moniruzzaman *et al.*, 2017; Beheary and El-Matary, 2018).

To reduce the problem that results from the effect of heavy metal HgCl_2 , Selenium (Na_2SeO_3) is a necessary micronutrient has been shown to interact with Hg in a variety of organisms (Truong *et al.*, 2014). Hg- Se interaction has commonly been described as antagonistic due to the protective effect of Se on Hg toxicity (Yang *et al.*, 2008; Bjork Lund *et al.*, 2017). However, synergistic effects have also been reported by Lemire *et al.*, (2010) and Penglase *et al.*, (2014). This inconsistent phenomenon may be caused by the differences in species, dosing concentration, and administration methods (Wyatt *et al.*, 2016; Wang and Wang, 2017). The aim of the present work is to study the effect of sub-lethal doses of mercuric chloride on haematology parameters and Histopathological changes on liver and gills. As well as using selenium (sodium selenite, Na_2SeO_3) to assess the detoxify of HgCl_2 impacts.

MATERIALS AND METHODS

Fish collection:

The experiment was carried out at El-Serw station for fish researches, Damietta governorate, which belong to National Institute of Oceanography and Fisheries, Egypt. The fish were collected carefully to avoid any injury occurs and treated with 0.1 % KMnO_4 solution for about few minutes for removal any fungal infection.

In August 2017, a total number of 300 fish collected in a live state with total body weights ranged from 60-80 gm were grouped together in a holding tank of 1000 L capacity filled with de-chlorinated water for a period of 14 days. The water quality was adjusted for PH (7.0–7.5), dissolved oxygen (6.5–7.5 mg/l) and water temperature ranged from 25–27 °C.

Determination of LC_{50} .

Standard of stock solutions of HgCl_2 with the molecular weight (325.29) was 1000 mg/l. The working solution was freshly prepared by diluting mercuric chloride according to the level of required doses used. These doses were 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 and 0.8 mg/l of mercuric chloride were applied as follow and replicated:

The first glass aquarium was used as control without any treatment and used as control.

The second glass aquarium, the lethal dose used was 0.1 mg/l of mercuric chloride.

The third glass aquarium, the lethal dose used was 0.2 mg/l of mercuric chloride. The fourth and fifth glass aquariums, the lethal dose used were 0.3 and 0.4 mg/l of mercuric chloride respectively.

The sixth and seven glass aquariums, the lethal dose used were 0.5 and 0.6 mg/l of mercuric chloride respectively.

The eighth glass aquarium, the lethal dose used was 0.8 mg/l of mercuric chloride.

The total number of fish in each glass aquarium was 12 fish. The experiment of LC₅₀ was extended for 4 days (about 96 hours) after treatment.

Based on the 96 hours of LC₅₀ values, four groups of tilapia fish were then exposed to four sub-lethal concentration of HgCl₂. Four glass aquaria were used for this purpose. Each glass aquarium containing 60 l. of de-chlorinated water and gently aerated. The number of fish in each glass aquarium was 12 fish in addition to the control group. The experiment extended for 60 days and the doses used were applied as follow:

The first glass aquarium, the dose used was 0.02 mg/l of mercuric chloride.

The second glass aquarium, the dose used was 0.04 mg/l of mercuric chloride.

The third glass aquarium, the dose used was 0.06 mg/l of mercuric chloride.

The fourth glass aquarium the dose used was 0.08 mg/l of mercuric chloride.

The fifth glass aquarium was used as a control without any treatment.

About half of the water in each aquarium was renewed every 2 days and the concentrated HgCl₂ in each aquarium was adjusted according to required dose. Animals were fed with commercial fish food with 25% protein. The experiment was replicated three times. Addition of selenium compounds (Sodium Selenite, Na₂SeO₃) in the concentration of 1 and 2 mg of selenium mixed with 1 kg of fish food respectively to the toxic effect of HgCl₂ for a period of 20 days for fish that exposed to sub-lethal concentration of HgCl₂ (0.1mg/l).

Sample collection:

Samples were collected after 60 days of treatment. The mortality rates were recorded during the period of experimental study. The mortality rate was calculated according the following equation:

$$\text{Mortality rate} = \frac{\text{total number of mortality fish}}{\text{total number of fish}} \times 100$$

Haematological Parameters:

The blood from control and treated fish from each treatment was obtained in eppendorf tube containing EDTA anticoagulants according to MgBenka *et al.*, (2003). These treated and control blood samples were used to estimate the haematological parameters of different types of blood cells.

The total red blood cells were counted using Neubaux haemocytometer (Shah and Altindag, 2004). The total number of Red blood cells (RBCs) were recorded as 10^6 mm^2 (Wintrobe, 1967).

The total number of white blood cells (WBCs) was reported as $\text{mm}^3 \times 10^3$ according to (Wintrobe, 1967).

Estimation of hemoglobin (Hb) was determined with hemoglobin test kits (DIAGNOVA, Ranbaxy, India) using Cyanmethemoglobin method.

Histopathological Observation:

Parts of gills and liver tissue were removed and preserved in Bouin's fixation for about 24 hours, after that the tissues were processed, sectioned at 6 microns in thickness and then stained by using haematoxylin and eosin, then examined as usual manner under stereo microscope.

Statistical Analysis:

One-way analysis of variance (ANOVA) was used to determine the effect of the different concentrations of HgCl₂ on haematological parameters (WBCs, RBC, Hb, Ht, MCV, MCH, and MCHC). The statistical analyses were carried out using the Statistical (SPSS version 20). P-value of <0.05 was considered significant.

RESULTS

LC₅₀ value of mercuric chloride for *O. niloticus* was 0.3 mg/l following probit analysis method described by Finney (1971). In determination of LC₅₀ of Hg for a period of 96 hours, 6 out of 12 fish were died after 96 hr of treatment. The mortality rate reached 50%, however, at high concentration of Hg doses from 0.4 mg/l reaching to 0.8 mg/l, the mortality rate increased and ranged from 60 to 100% at 96 hr (table 1). So, sub-lethal doses was estimated under this doses starting from 0.02 to 0.08 mg/l of Hg.

Table 1: Mortality rate of different doses of HgCl₂ (mg/l) for of *O. niloticus*.

Parameters	concentrations of HgCl ₂ (mg/l)							
	30	35	45	50	60	70	80	96
Time (hr)	30	35	45	50	60	70	80	96
doses of HgCl ₂ (mg/l)	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8
Mortality rate %	30	40	50	60	70	75	90	100

Erythrocyte cells count:

The erythrocyte count of the control fish showed a mean value of 1.56×10^6 . The fish exposed to sub-lethal dose of mercuric chloride showed significant decrease in the red blood cells mean values as (1.5, 1.3, 1.06 and 1.0×10^6) after two months of treatment.

Total white blood cells count:

The results indicated that the total white blood cells after one and two months of treatment showed significant increases ($p < 0.05$) as (8.0, 12.27, 13.0 and 14.0×10^3) when compared with the control value (7.16×10^3).

Hemoglobin content:

The mean value of hemoglobin content of the control fish was 72.3 g/dl. Fish exposed to the sub-lethal concentrations of mercuric chloride showed that the mean values of Hb were 64.5, 48.25, 43.0 and 34.66 g/dl under exposure of 0.02, 0.04, 0.06 and 0.08 ppm doses, respectively. These values showed a significance decrease as compared to the control ($P < 0.05$) as shown in Table (2).

Table 2: Effect of HgCl₂ in sub-lethal doses on haematological parameters after two months of treatment on *O. niloticus*.

Variables	control	Hg dose (mg/l)			
		0.02	0.04	0.06	0.08
WBC (10^3 /mL)	7.16±0.05 ^c	8±0.18 ^d	12.27±0.27 ^c	13±0.17 ^b	14±0.35 ^a
RBC (10^6 /mL)	1.56±0.10 ^a	1.5±0.03 ^a	1.3±0.04 ^b	1.06±0.05 ^c	1±0.06 ^c
Hb (g/dL)	72.3±0.23 ^a	64.5±0.35 ^b	48.25±0.35 ^c	43±0.46 ^d	34.66±0.14 ^e
Ht (%)	38.25±0.18 ^a	34.16±0.16 ^b	26.5±0.23 ^c	22.8±0.07 ^d	22±0.48 ^d
MCV (Fi)	140.7±1.04 ^a	135±0.69 ^b	119±0.98 ^c	121±0.69 ^c	120.66±0.67 ^c
MCH (Pg)	27.83±0.87 ^a	26.16±0.16 ^a	23±0.64 ^b	22±0.46 ^b	21±0.64 ^c
MCHC (g/dL)	37±0.64 ^a	31±0.81 ^b	25.2±0.75 ^c	23.5±0.80 ^c	23.16±0.21 ^c

Different letters = significance difference occurred between the sample treated with selenium and the others without treated ($P < 0.05$).

Effect of Selenium on fish-treated with HgCl₂:

The effect of selenium (1.0 and 2.0 mg/kg fish food) on the fish exposed to lethal dose of HgCl₂ (0.1 mg/l) for a period of 20 days of treatment were studied. In

fish treated with HgCl_2 , the white blood cells count was $11.4 \times 10^3/\text{ml}$, the Red blood cells count was $1.1 \times 10^6/\text{ml}$, hemoglobin value was 34.66 g/dl. After treatment with selenium which mixed with fish food to detoxify the toxin impact for a period 20 days of treatment, Red blood cells and Hb were increased, however the white blood cells were decreased as shown in Table (3). Moreover, the blood indices were increased after treatment with selenium.

Table 3: Effect of HgCl_2 in high doses (0.1 mg/l) on haematological parameters and treatment with selenium for 20 days of treatment on *O. niloticus*.

Variables	Hg (0.1 mg/l)	Selenium	
		1 mg/kg fish food	2 mg/kg fish food
WBC ($10^3/\text{mL}$)	11.4±0.16 ^a	6.5±0.07 ^c	7.03±0.09 ^b
RBC ($10^6/\text{mL}$)	1.1±0.07 ^c	1.5±0.05 ^b	1.8±0.06 ^a
Hb (g/dL)	34.66±0.39 ^c	52±0.69 ^b	60.33±0.27 ^a
Ht (%)	27.33±0.13 ^c	30±1.27 ^b	34.16±0.17 ^a
MCV (Fi)	108.5±0.35 ^c	148±0.06 ^b	150±0.92 ^a
MCH (Pg)	35±0.52 ^b	42.33±0.25 ^a	42.66±0.76 ^a
MCHC (g/dL)	26±0.46 ^b	39.66±0.32 ^a	38.33±0.21 ^c

Different letters = significant difference (P<0.05).

Heavy metal (Hg) and fish behavior:

The control fish was observed in normal swimming state. The fish exposed to heavy metal (0.1 mg/l HgCl_2) was observed in stress state and hypersensitivity. The movement and swimming of fish were observed in the abnormal condition. The morphological appearance showed that the most scales over skin were removed and the fish could not be protected from fungi and bacteria. The color of fish changed from normal condition to pale white color and the skin covered with much amount of mucus. The fish lost its ability to eat and decreased in body weight, and then died.

Histopathology of treated liver:

The liver of the control *Oreochromis niloticus* exhibited normal structure. The hepatocytes contain homogenous cytoplasm and centrally located nuclei, small blood sinusoids distributed between the hepatocyte and its function to collect the aggregated blood from these cells to pour into the central vein. The hepatopancrease was also observed and contained in the center vacuole red blood cells. No lesions occurred in the liver of the control fish (Fig. 1A). The slight alternations occurred inside the hepatocyte of the liver of treated fish with doses ranged between 0.02 to 0.04 mg/l of HgCl_2 for a period 60 days of treatment. The main alteration occurred either in the cytoplasm of hepatic cells or inside the nucleus. The cytoplasm appeared in abnormal shape and some of them appeared as vacuole. Small blood vessels were slightly dilated and contained red blood cells (Fig. 1B). Abnormalities were observed in the treated liver with 0.04 mg/l of HgCl_2 for a period of 60 days. The main alteration occurred in the cytoplasm and the nucleus. The cytoplasmes were hypertrophied and sever degradation of liver parenchyma and the most nuclei were disintegrated (Fig. 1C). The treated liver, *Oreochromis niloticus* with 0.06 mg/l HgCl_2 for 60 days showed that the hepatocytes were more hypertrophied and some nuclei become necrosis. The blood vessels appeared between the hepatocytes were dilated and branched vessels contained red blood cells (Fig. 1D). The main alteration occurred in the liver of *Oreochromis niloticus* after 60 days of treatment with 0.08 mg/l HgCl_2 . The hepatocyte were more hypertrophied and most of these cells appeared as vacuoles without nuclei. The blood sinusoid was congestion (Fig. 2A). The treated liver of *Oreochromis niloticus* with acute dose 0.1 mg/l of HgCl_2 for 20 days showed the main alteration occurred in

cytoplasm and nucleus. The cytoplasm were disorganized and nuclei showed necrosis (Fig. 2B).

The treated liver with 0.1 mg/l HgCl_2 and 1.0 mg selenium (Na_2SeO_3) /kg of fish food which used as recovery for 20 days, the hepatocyte appeared with few lesions and the nuclei located towards the center of cells. The parenchyma of liver appeared similar to that observed in the normal state (Fig. 2C).

Histopathology of gills

The gills in *Oreochromis niloticus* consists of filaments and secondary lamellae. In control fish, the gill had a normal structure. There is no lesions occurred in both of primary and secondary lamellae (Fig-3A). Gills of *Oreochromis niloticus* treated with 0.04 mg/l of HgCl_2 for a period of 60 days showed that the secondary lamellae were shorted and some of these lamellae were lifting up and fused at the base of filaments (Fig. 3B). Moreover these were alternation occurred in the treated gills of *Oreochromis niloticus* with 0.06 mg/l of HgCl_2 for a period of 60 days. The lesions in both secondary and primary lamella were observed. The primary lamellae increased in thickness and secondary lamellae become more shortening. The mucous cells scattered along the epithelial of the secondary lamellae (Fig. 3C)

The treated gills with 0.08 mg/l of HgCl_2 for 60 days showed that the secondary lamellae were fused and the cells of the primary lamellae were more proliferated (Fig. 3D).

More modification occurred showed the treated gills with acute of 0.1 mg/l HgCl_2 for a period of 20 days. The secondary lamellae in disorganization and fusion in lamellae in the most portion of filament. The hemorrhage was also observed at the base of filament (Fig. 4A). The results declared that the treated gills with 0.1 mg/l of HgCl_2 and selenium (Na_2SeO_3) at doses of 1.0 mg/kg of fish food for a period of 20 days of treatment appeared similar to the gills of the control fish. The primary and secondary lamellae appeared with slight lesions.

DISCUSSION

Heavy metals such as (Cr, Pb, Zn, Cu, Ni, Cd and As) are the most important environmental problems today (Nariagu and Pacyma, 1988; Witeska, 2003). Mercuric chloride (HgCl_2) is one of the most important heavy metal representing the greatest hazards to human consumer of fish. So, the analysis of changes in some hematological and biochemical blood indices remain as variable tools in determining the effects of sub-lethal concentrations of pollutants. In the present study a decrease in the erythrocyte count indicated the worsening of an organism state and finally, the anemia occurred. Similar findings were observed in *Puntius conchoni* after mercury exposure for weeks (Gill and Pant, 1987). In this respect, Gill and Epple (1993) have attributed anemia to impaired erythrocyte due to the effect of heavy metals on hematopoietic centers (kidney and spleen). In our opinion, the deficiency in red blood cells to inability of blood to carry sufficient oxygen and distributed it to various organs in body fish. Similar observation were obtained by Shah and Altindag (2004) who stated that a number of hematological indices such as hematocrit, hemoglobin and red blood cells are used to assess the functional status of oxygen carrying capacity in the blood stream and used as indicator of metal pollution in the aquatic environment. In the present study, the percentage value of hemoglobin was markedly decreased after the fish exposed with the high doses of HgCl_2 (0.08–0.1mg/l). These results are similar to the data obtained by Gill and Epple (1993). McKim *et al.*, (1979) reported that mercury accumulated in fish blood. Similar results

were obtained in previous study by Sastry and Kamana sharma (1980) showing that hemoglobin and hematocrit were declined as *Channa punctatus* exposed to mercury.

It is known that the immune system considers as the first defense line against the bacteria, virus and pollutants. Therefore total white blood cells play an important role in defense mechanism In the present study. The total white cell in fish that exposed to high level dose of HgCl_2 was increased in compared to the control fish. The exposed fish with high level does of HgCl_2 (0.1 mg/l) and Selenium (Na_2SeO_3) of (1.0 and 2.0 mg/kg) for a period of 20 days of treatment improved Red and white blood cells count and blood indices. It appears that Selenium (Se) is a necessary as micronutrient to ameliorate the effect of Hg in several organisms as reported by Truong *et al.*, (2014).

Selenium interacted with Hg showed protective effect of toxicity as described by Yang *et al.*, (2008). The present results showed that erythrocyte, hemoglobin, and hematocrit value increased, however, white blood cells decreased to a normal value.

In the present study, the behavior of the treated fish was observed showing hypersensitivity. The swimming fish were observed in the abnormal state and the color change from natural normal color to white color (Srivastava, 1982). This change in the behavior of fish according to previous studies and the present study were attributed to hormonal imbalance caused by the toxic effect of HgCl_2 .

The effect of HgCl_2 on the liver cells was dependent on the duration of exposure and its concentration. As observed in the present study, the treated fish with HgCl_2 doses (0.02 -0.06 mg/l) revealed alterations as observed in the nuclei and cytoplasm of the hepatocyte. In this respect, cytoplasmic vacuolation was observed in hepatocyte and similar results were described by Fanta *et al.*, (2003) and EL-Greisy *et al.*, (2016) on *Oreochromis niloticus* and EL-Greisy and EL-gamal (2015) on *Cyprinus carpio*.

Another vital organ in the fish is gills which play on important part in respiratory, osmoregulation and excretion function, furthermore, the gills are regarded as the primary target of the contamination (Ahmed *et al.*, 2014). As observed in the present study, the normal structure of gill consists of primary lamellae, secondary lamellae that have pillar cells which separate the walls that composed of squamous epithelium. The fish gills treated with sub-lethal concentration (0.02 – 0.06 mg/l) have showed slight damage after 30 days of treatment. These results are inconsistent with EL-Greisy *et al.*, (2016). In the fish gill treated with doses level ranged from 0.08 to 0.1 mg/l, a damage occurred in the epithelial covering the secondary lamella and more fusions occurred in secondary lamellae with hemorrhage of blood collected at the base of the filament. The damage in the epithelial membrane is primary reaction, whereas mercury element picks up charge which is similar to many of the ions charges Ca^{+2} and Mg^{+2} and competing on the union and transit through the chloride cells (Change *et al.*, 2003; Wu *et al.*, 2008). In the present study, the tissues gills and liver treated which 0.1 mg/l of HgCl_2 and the Selenium (NaSeO_3) at dose of 1.0 mg/kg of fish food for a period of 30 days of treatment showed recovery of both tissues were observed. Truong *et al.*, (2014) stated that Selenium (NaSeO_3) is necessary micronutrient which interact with Hg in several organisms.

CONCLUSION

Based on this study, it can be concluded that the heavy metal (mercury) is a dangerous to the Nile Tilapia (*O. niloticus*). The present study detected a method

that can be beneficial for the removal of mercuric chloride toxicity, that by using selenium (NaSeO_3). The present study give explanation for the toxic effect of HgCl_2 on liver and gill tissues as well as the hematological parameters after 60 days of exposure. These effects were ameliorated after addition of selenium (1.0-2.0 mg/kg fish food) for a period of 20 days.

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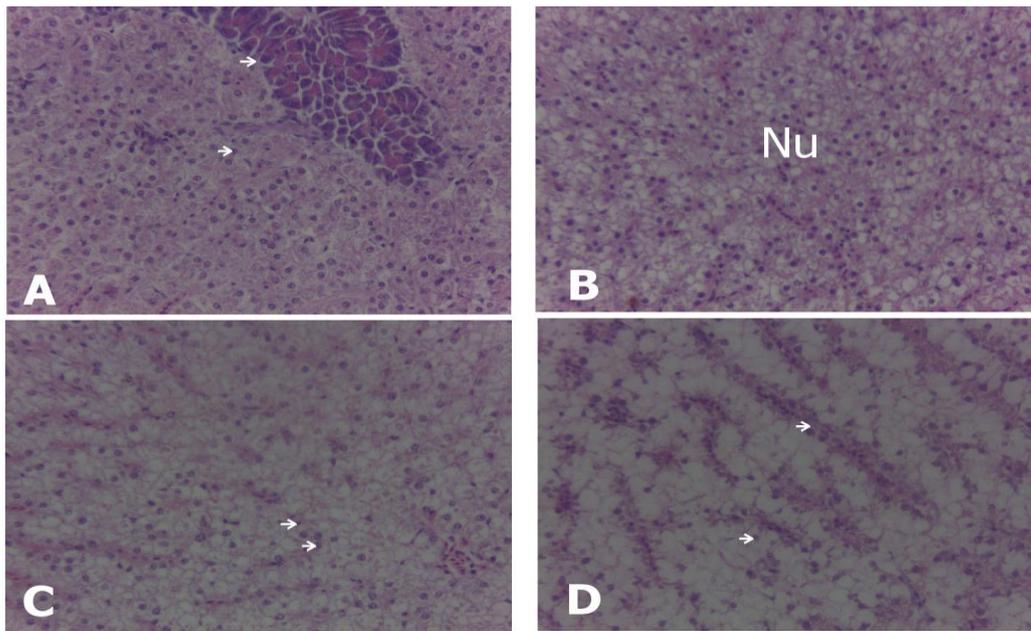


Figure 1. Photomicrograph sections of the control and treated liver of *O. niloticus*

- A- control liver of *Oreochromis niloticus* showing normal structure of liver cells with centrally located nuclei (Arrow head), hepatopancreas contained blood cells was also observed (Arrow).(H&E,X400).
- B- liver of *Oreochromis niloticus* treated with 0.02 mg/l of HgCl_2 for 60 days showing the hepatocytes with irregular nuclei (Nu), blood sinusoids (BS) (H&E, X400).
- C- liver of *Oreochromis niloticus* treated with 0.04 mg/l of HgCl_2 for 60 days showing hypertrophied hepatocytes, with degenerated nuclei, sever degradation of liver parenchyma (Arrow) and hepatic portal veim (Arrow heads) (H&E,Hx400).
- D- liver of *Oreochromis niloticus* treated with 0.06 mg/l of HgCl_2 for 60 days, showing hypertrophied hepatocytes with some necrotized and degenerated nuclei (Arrow), dilated and branched blood vessel containing red blood cells (Arrow head) (H&E,X400).

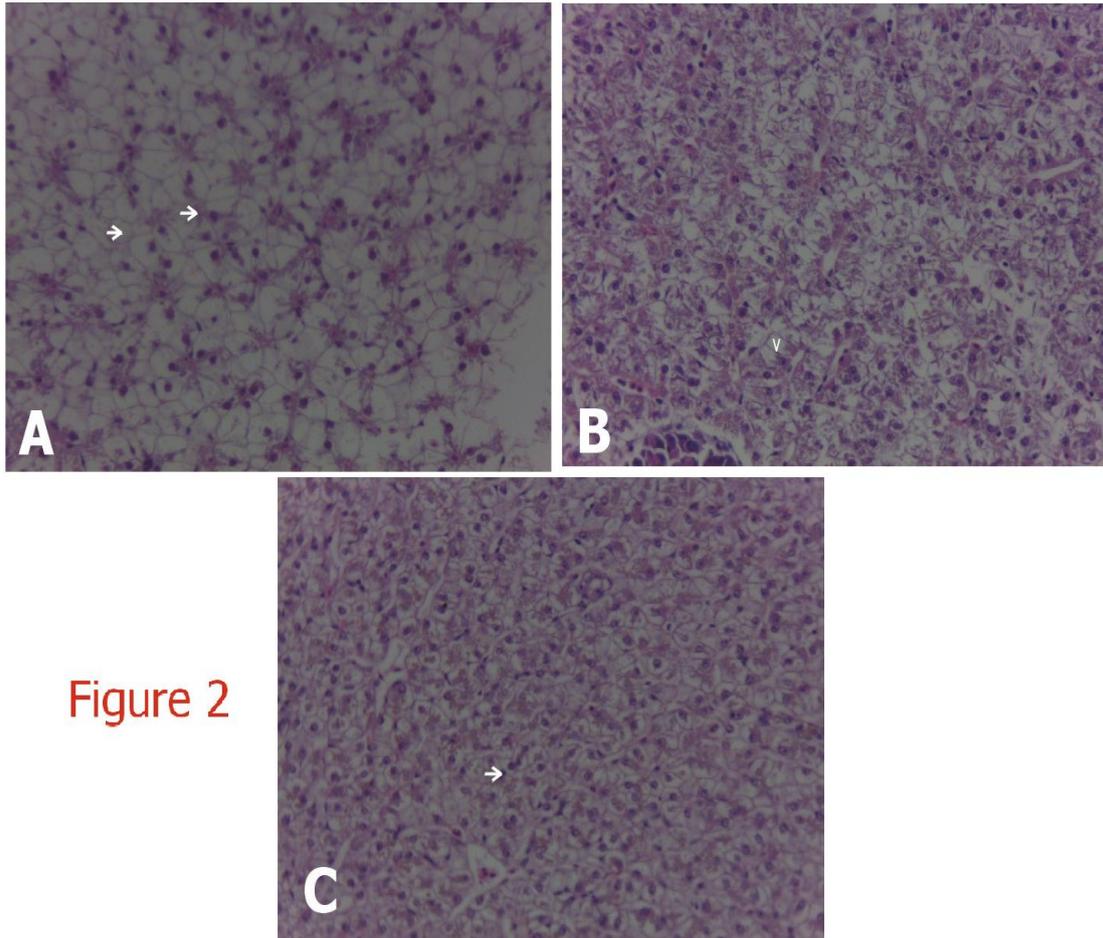


Figure 2

Figure 2. Photomicrograph sections of the treated liver of *Oreochromis niloticus* (A-C) showing:

- A- liver of *Oreochromis niloticus* treated with 0.08 mg/l of HgCl₂ for 60 days showing the hepatocytes more hypertrophied and most of them devoid from nuclei (Arrow) with the congestion of the blood sinusoids were (Arrow head) (H&E, X400)
- B- liver of *Oreochromis niloticus* treated with 0.1 mg/l of HgCl₂ showing the hepatocytes were disorganized with necrosis of nuclei (Arrow), vacuoles inside hepatocyte (V) (H&E, X400)
- C- liver of *Oreochromis niloticus* treated with 0.1 mg/l of HgCl₂ and 1.0 mg selenium (Na₂SeO₃) / kg for about 20 days showing slight lesions of hepatocyte (Arrow) and nuclei in the center of cells (Arrow head) (H&E, X400).

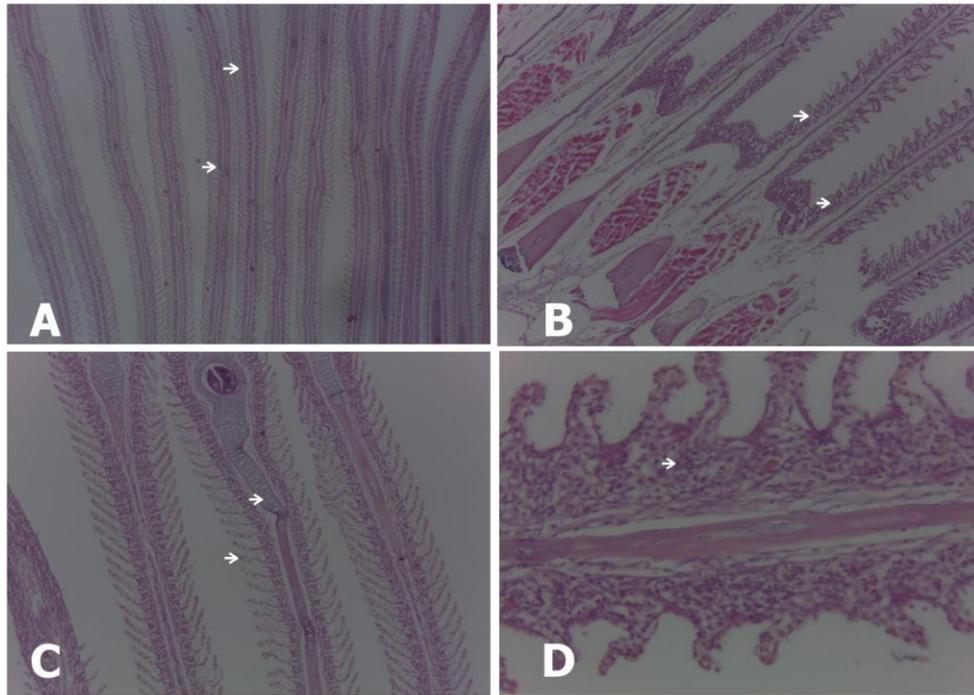


Figure 3. Photomicrograph sections in the gills of *O. niloticus* (A-D) showing:

- A- The normal structure of gills in control fish, primary lamellae and secondary lamellae were observed in normal state (Arrow) (H&E, X100)
- B- Gills of *Oreochromis niloticus* treated with 0.04 mg/l of HgCl_2 for 60 days, showing some secondary lamellae were shorted and some of them were lifting and fused at the base of filaments (Arrow) (H&E, X400)
- C- Gills of *Oreochromis niloticus* treated with 0.06 mg/l of HgCl_2 for 60 days, showing some secondary lamellae were more shorted (Arrows) and the primary lamellae increased in the thickness (Arrow head), (H&E, X100)
- D- Gills of *Oreochromis niloticus* treated with 0.08 mg/l of HgCl_2 for 60 days, showing secondary lamellae fused and primary lamellae more proliferated (Arrow) (H&E, X400).

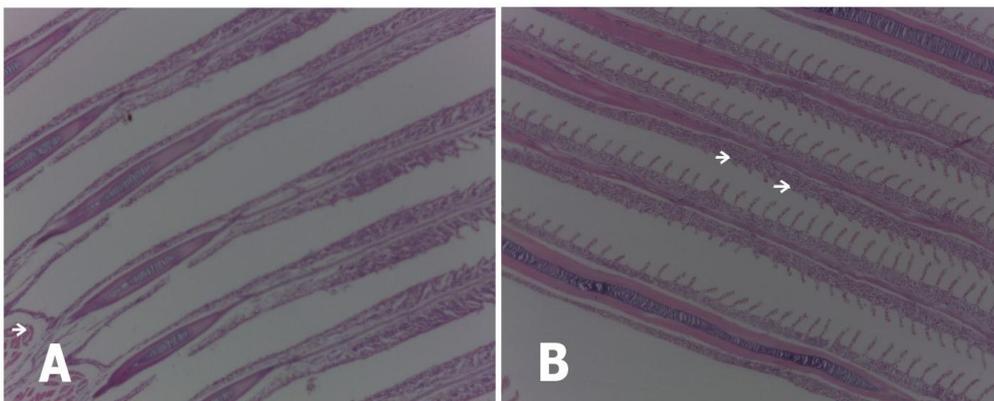


Figure 4. Photomicrograph sections in the gills of *O. niloticus* (A-B) for 30 days showing:

- A- Gills of *Oreochromis niloticus* treated with 0.1 mg/l of HgCl_2 showing disorganization and fusion in the secondary lamellae (Arrow), (H&E, X100).
- B- Gills of *Oreochromis niloticus* treated with 0.1 mg/l of HgCl_2 and 1.0 mg selenium (Na_2SeO_3) / kg for a period of 20 days showing the gills appeared similar to the gills of the control in both of primary and secondary lamellae (Arrow) (H&E, x100).