

Full Length Research Paper

Hematological and enzymatic responses of Nile tilapia *Oreochromis niloticus* during short and long term sublethal exposure to zinc

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Freshwater fish *Oreochromis niloticus* were exposed to sublethal concentrations of zinc for short and long terms. Responses of hematological parameters which include red blood cells count (RBC) and hemoglobin content (Hb) were investigated. Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were also determined. Two hundred and forty fish (24.30 ± 2.85 g) were distributed randomly in twenty four glass aquaria (80 L) with stocking density of 10 fish for each. The aquaria were divided into two groups: the first group was exposed for one week to 2, 4 and 6 mg/L of ZnCl₂ which represent 0.25, 0.50 and 0.75 of Zn LC₅₀, respectively with three replicates for each concentration, in addition to 0.00 LC₅₀ as the control; while the second group was exposed to the same concentrations of ZnCl₂ with the same replicates for 4 weeks. The results of hematological parameters indicated that RBC and HB were significantly increased with increasing the concentration of ZnCl₂ sequentially for both short and long exposure periods. The enzymatic estimation showed that the activity of AST and ALT also significantly increased sequentially in zinc treated fish at both exposure periods. Results of the present study suggest that sublethal concentrations of zinc affect the hematological changes and impaired liver functions. These parameters can be useful in environmental biomonitoring of zinc contamination.

Key words: Nile tilapia, *Oreochromis niloticus*, liver enzymes, hematological parameters, LC₅₀ of zinc.

INTRODUCTION

The aquatic environment is continuously being contaminated with chemicals from agriculture and urban activities. In many aquatic systems, metal concentrations are elevated over natural background levels due to a continuous release of metals from industrial and agricultural sources. Frequently, metals are present as mixtures in the environment because of their concomitant release from mining activities or industrial uses. Copper and zinc play an important role in cellular metabolism acting as

co-factors in a number of important enzymes. However, they can become toxic when elevated concentrations are introduced into the environment (Marr et al., 1996; Karan et al., 1998). Studies on the toxicity of metals for fish have been focused on the effects of short term exposures to single metal at relatively high concentrations; rather than investigating the toxic impact of long-term exposures to metal mixtures at environmentally realistic concentrations. Under conditions of acute, high-dose metal exposure, the maintenance of branchial osmoregulation and gas exchange is of prime importance for the survival of the fish; whereas under conditions of sublethal, chronic metal intoxication, the adaptive capacity of internal metal-accumulating organs such as the liver may gain importance (McDonald and Wood, 1993; Schlenk et al., 1999; Stubblefield et al., 1999). Several biochemical and

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Abbreviations: RBC, Red blood cell; Hb, hemoglobin; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

physiological responses can occur when a toxicant is absorbed by aquatic organisms which may be a compensatory response or a toxicity mechanism (Begum, 2004). Chronic exposure of fish to ions, Cu, Cd or Zn, has been shown to cause a variety of behavioral, biochemical and physiological changes including loss of appetite, reduced growth, decreased aerobic scope and mortality (McGeer et al., 2000; Sloman et al., 2003).

Hematological parameters are used as an index to detect physiological changes and to assess structural and functional status of health during stress conditions in a number of fish species (Adhikari et al., 2004; Suvetha et al., 2010). Fish blood is sensitive to pollution-induced stress, and changes on the hematological parameters, such as hemoglobin content, hematocrit and number of erythrocytes can be used to monitor stress caused by pollutants such as heavy metals (Romani et al., 2003; Barcellos et al., 2004). Enzymes activity are considered as sensitive biochemical indicators before hazardous effects occur in fish and are important parameters for testing water for the presence of toxicants (El-Demerdash and Elagamy, 1999; Gül et al., 2004). Estimation of enzymes likes aspartate and alanine aminotransferase (AST, ALT) and lactate dehydrogenase (LDH) are considered useful biomarkers to determine pollution level during chronic exposure (Asztalos et al., 1990; Basaglia, 2000; Ozman et al., 2006).

MATERIALS AND METHODS

Experimental fish

Fingerlings of *Oreochromis niloticus* (mean body weight 24.3 ± 2.85 g) were collected from the fish seed hatchery of King Abdulaziz City for Sciences and Technology (KACST) Deerab, Riyadh, Saudi Arabia. Fish were acclimated to laboratory conditions for two weeks.

Estimation of Zn LC₅₀

The 96 h LC₅₀ values for various fishes depending on the environmental conditions, in case of zinc, as a rule, vary from 0.07 to 21.5 mg/L (Scott and Sloman, 2004). To determine the 96 h LC₅₀ of exposure, seventy fish were randomly distributed in seven small aquaria (40 L). The fish were exposed to various concentrations (2, 4, 6, 8, 10, 12 and 14 mg/L) of zinc chloride (ZnCl₂) which were previously dissolved in distilled water and added to the aquaria. Fish were exposed to the aforementioned concentrations for 96 h. Mortality in each aquarium were removed and recorded daily. To find out the survival time in each concentration of ZnCl₂, observations were recorded and then the LC₅₀ value was calculated from the regression line drawn according to Finney (1964). The 96 h LC₅₀ was 8 mg /L.

Experimental design

Two hundred and forty acclimated fish were divided into two groups; the first group was exposed to 2, 4 and 6 mg/L of ZnCl₂

which represent 0.25, 0.50 and 0.75 of 96 h Zn LC₅₀, respectively for one week (short exposure period) in addition to the aquaria at the same conditions without ZnCl₂ (0.00 LC₅₀) as control. Eighty liter glass aquaria (100 × 50 × 40 cm) were used with three replicates for each concentration. The second group was exposed to the same concentrations of ZnCl₂ with the same replicates for 4 weeks (long exposure period). Fish were fed twice daily at a rate of 2% of the body weight with 32% crude protein diet. Temperature was maintained at 26 ± 1 °C, and the water quality parameters were monitored biweekly and were kept within the optimal ranges as described earlier by Ali et al. (2008). At the end of the period for the two groups, fish blood samples were collected from the caudal vein by sterile syringe containing EDTA solution as anticoagulant. These blood samples were divided into two parts, the first part was used for determining the count of red blood cells (RBC) method of Dacie and Lewis (1984), and hemoglobin content (Hb) method described by Van Kampen and Zijlstra (1961). The second part of the blood samples was used to estimate the plasma AST and ALT activity using a colorimetric method of Reitmen and Frankel (1957).

RESULTS

Short term exposure

Hematological parameters of the fish exposed to Zn for one week were significantly different compared to the control group (Table 1). There were significant increase ($p < 0.05$) in both red blood cells count (RBC) and hemoglobin content (Hb) for fish exposed to 0.25, 0.50 and 0.75 of LC₅₀, respectively. According to the results of the enzymes activity estimation, AST and ALT activities of the control *Oreochromis niloticus* were 18.62 ± 0.87 and 14.36 ± 0.51 IU/L, respectively. Exposure of fish to Zn for one week resulted to increased AST and ALT activity (Table 1). The average levels of AST activities of Zn treated fish, 0.25, 0.50 and 0.75, were 28.14 ± 0.56 , 28.43 ± 0.67 , and 32.95 ± 0.71 IU/L, respectively which were significantly higher in relation to the average level of the control group. Similarly, the ALT activity was significantly increased compared to untreated fish, and the average levels of ALT activities of individuals exposed to Zn 0.25, 0.50 and 0.75 of LC₅₀ were 21.70 ± 0.42 , 22.16 ± 0.39 , and 26.81 ± 0.54 IU/L, respectively. However, there were significant differences among treatments of AST and ALT for fish exposed to 0.25 and 0.50 compared to fish exposed to 0.75 of LC₅₀ (Table 1).

Long term exposure

Hematological and liver enzymatic parameters for Nile tilapia exposed to Zn for four weeks are shown in Table 2. There was significant increase ($p < 0.05$) in RBC compared with the control group. The results obtained were 1.72 ± 0.06 , 2.37 ± 0.08 , 2.50 ± 0.10 and 2.93 ± 0.11 cell/ μ l for the control group, and fish exposed to 0.25, 0.50 and 0.75 of LC₅₀, respectively. In the same trend, hemoglobin content also increased significantly ($p < 0.05$) after fish exposed to long term zinc chloride

Table 1. Change in the hematological parameters red blood cell (RBC), hemoglobin (Hb) and serum enzyme aspartate aminotransferase (AST), alanine aminotransferase (ALT) of *Oreochromis niloticus* exposed to sublethal concentration of zinc chloride for short term (one week).

Parameter	Control	0.25 LC ₅₀	0.50 LC ₅₀	0.75 LC ₅₀
RBC (cell/ μ l)	1.67 \pm 0.05 ^b	2.30 \pm 0.08 ^a	2.45 \pm 0.09 ^a	2.89 \pm 0.11 ^a
Hb (g/L)	82.13 \pm 0.68 ^b	97.95 \pm 0.79 ^a	100.30 \pm 0.84 ^a	103.41 \pm 0.83 ^a
AST(IU/L)	18.62 \pm 0.87 ^c	28.14 \pm 0.56 ^b	28.43 \pm 0.67 ^b	32.95 \pm 0.71 ^a
ALT(IU/L)	14.36 \pm 0.51 ^c	21.70 \pm 0.42 ^b	22.16 \pm 0.39 ^b	26.81 \pm 0.54 ^a

Means with the same superscript in the same row are not significantly different at ($p < 0.05$).

Table 2. Change in the hematological parameters red blood cell (RBC), hemoglobin (Hb) and serum enzyme aspartate aminotransferase (AST), alanine aminotransferase (ALT) of *Oreochromis niloticus* exposed to sublethal concentration of zinc chloride for long term (four weeks).

Parameter	Control	0.25 LC ₅₀	0.50 LC ₅₀	0.75 LC ₅₀
RBC (cell/ μ l)	1.72 \pm 0.06 ^b	2.37 \pm 0.08 ^a	2.50 \pm 0.10 ^a	2.93 \pm 0.11 ^a
Hb (g/L)	83.21 \pm 0.69 ^b	98.97 \pm 0.76 ^a	101.38 \pm 0.77 ^a	105.45 \pm 0.89 ^a
AST(IU/L)	18.55 \pm 0.91 ^d	31.64 \pm 0.49 ^c	35.22 \pm 0.81 ^b	40.19 \pm 0.71 ^a
ALT(IU/L)	14.43 \pm 0.51 ^d	25.32 \pm 0.57 ^c	29.67 \pm 0.38 ^b	33.95 \pm 0.73 ^a

Means with the same superscript in the same row are not significantly different at ($p < 0.05$).

concentrations.

The results of the enzyme activity determinations, AST activity of control, *O. niloticus*, were 18.55 ± 0.91 ; whereas, the average levels of Zn treated fish, 0.25, 0.50 and 0.75 of LC₅₀, were 31.64 ± 0.49 , 35.22 ± 0.81 , and 40.19 ± 0.71 IU/L, respectively which is significantly higher compared to the control group. Similarly, the average levels of ALT activities of individuals exposed to Zn 0.25, 0.50 and 0.75 of LC₅₀ were 25.32 ± 0.57 , 29.67 ± 0.38 , and 33.95 ± 0.73 IU/L, respectively which also showed significant increase compared to untreated fish (14.43 ± 0.51 IU/L).

DISCUSSION

The current study reported that there was significant increase in RBC count and Hb content with increase in the concentration of zinc in both short and long term exposure. These results are in agreement with the findings of Lavanya et al. (2011) who stated that there was significant increase in Hb and hematocrit (Hct) content of arsenic trioxide treated Indian major carp *Catla catla* compared to the control group. The significant increase in these hematological parameters indicated impaired respiratory capacity of the fish due to damage in the gill caused by the toxicant (Lavanya et al., 2011).

The increases in the number of red blood cells, due to the short and long term sublethal exposure to zinc can be attributed to several factors. During the exposure of *O. niloticus* to zinc, it seemed that the fish developed an oxygen deficiency. This decrease in the availability of

dissolved oxygen causes the build-up of oxygen debt, hypoxia, in the fish. Fish also compensate for a poor oxygen intake in prevailing hypoxic conditions, which may be caused by epithelial lifting of the gill lamellae, by an increase in the number of red blood cells (Wepener et al., 1992a, b). O'Connor and Fromm (1975) concluded that metal ions are known to stimulate erythropoiesis. Short and long term sublethal exposure to zinc induced increases in red blood cells, hemoglobin and haematocrit values, and reflects an attempt by *O. niloticus* to survive in an environment with an increased demand for oxygen, resulting from the destruction of gill membranes, causing an impaired gaseous exchange (Buckley, 1976).

The significant increase in the hemoglobin content in zinc treated fish was accompanied by an increase in the number of red blood cells. Fish were subjected to an oxygen tension (due to internal haemorrhage) and the increase in hemoglobin could well have been to elevate the oxygen capacity of the blood in order to supply more oxygen to the tissues. This is, therefore, a mechanism by which the body attempts to absorb more oxygen from the surrounding medium to meet the increased oxygen demand (Cyriac et al., 1989).

During long term exposure, toxicants may accumulate in various tissues which lead to changes in the enzymatic activities. In fish, the liver act as an important organ for uptake, accumulation, biotransformation and excretion of toxicant during sublethal treatment (Maher et al., 1999; Pedlar and Klaverkamp, 2002). The liver function tests like analysis of serum, AST and ALT, are widely used to demonstrate liver function or toxicant-induced hepatotoxicity (Yang and Chen, 2003; Roy and Bhattacharya,

2005; Datta et al., 2007). In the present study, the significant increase in AST and ALT levels in zinc treated fish with short and long term sublethal exposure indicates hepatic damage due to zinc accumulation which in turn releases these enzymes into the bloodstream. The damage and severity of the organ is dependent on the type of toxicant and duration of exposure (Casillas et al., 1983; Jacobson-Kram and Keller, 2001). Roy and Bhattacharya (2005) noted significant changes in serum GOT (AST) and GPT (ALT) in *Channa punctatus* exposed to As₂O₃, and indicated that the changes may be due to histopathological lesions in liver. Therefore, Abdel-Warith et al. (2011) reported that Nile tilapia exposed to zinc cause histopathological injury in the liver.

Aspartate aminotransferase (AST) catalyzes an important reaction of the molecular rearrangement involving amino acids linked to the citric acid cycle at two points (oxaloacetic and ketoglutaric acids), being the most important mechanism for introducing reduction equivalents into mitochondria (Urich, 1994). Alanine aminotransferase (ALT) predominates in organs with intensive glycogenesis, such as the liver (Urich, 1994; Torre et al., 2000). Serum AST and ALT are important diagnostic tools in medicine and clinics, and are used to detect the toxic effects of various pollutants (Nelson and Cox, 2000).

The effects of heavy metals on serum or liver enzyme activities including AST and ALT activities of several teleost species have recently been studied (Vaglio and Landriscina, 1999; Torre et al., 2000; Kim and Kang, 2004). After sub-chronic dietary, Cu exposure for 40 days increased serum AST and ALT concentrations with increasing time and dose were observed in the rockfish, *Sebastes schlegeli* (Kim and Kang, 2004). The authors suggest that the liver is rich in AST and ALT, therefore damage to it can result in the liberation of large quantities of these enzymes into the blood. Therefore, increases in AST and ALT activities in the serum of heavy metal-treated fish are assumed to be a result of liver damage by the heavy metals. These findings were in agreement with the results in the current study which found that the activity of AST and ALT increased with the increase of zinc chloride concentration for both short and long term exposure of fish. In addition, it was also reported in another teleost (*Sparus aurata*) that Cd exposure may decrease AST and ALT activities in liver cells (Vaglio and Landriscina, 1999). It is very interesting that Cd and Zn showed different effects on the AST and ALT activities. Unlike Cd, a non-essential and xenobiotic element, Zn is an essential element for maintaining normal functions of most organisms (Wu et al., 2008).

Conclusion

The results of our study demonstrated that a short and long term exposure of *Oreochromis niloticus* to sublethal concentrations of zinc could have hematological changes

and impaired liver functions. It was evident that the increase in RBC, hemoglobin, AST and ALT related to the increase of exposure period and concentration of zinc chloride sequentially. It can therefore be concluded that hematological parameters and the activity of liver enzymes of exposed fish allow the blood and liver of *O. niloticus* to be used as a biomarker of prior exposure to zinc. Hematological and liver function changes were mainly observed in fish exposed over the short-term exposure periods while regenerative responses were noted in fish exposed over the long-term period.

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