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SHORT TERM EFFECTS OF ZINC ON SOME SERA BIOCHEMICAL PARAMETERS AND ON TISSUE ACCUMULATION OF *Clarias gariepinus*

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ABSTRACT

Effects of zinc on sera aspartate aminotransferase (AST), alanine aminotransferase (ALT) activities and glucose, total protein and cholesterol levels together with liver, gill, kidney, spleen and muscle accumulation of the metal were studied after exposing *Clarias gariepinus* to 1.0 and 5.0 ppm Zn over 24, 48 and 96 hours.

Highest accumulation of Zn was in liver and spleen tissues in fish exposed to 1.0 ppm and 5.0 ppm Zn respectively after 96 hours of exposure.

Sera AST and ALT activities, cholesterol, total protein and glucose levels changed depending on zinc concentration and exposure period. Sera glucose levels increased with increasing exposure periods at both concentrations of zinc. There was also an increase in sera cholesterol and total protein levels at the beginning of the experiment which decreased with increasing exposure. The increase in sera ALT and AST activities were higher in 5.0 ppm Zn compared with 1.0 ppm Zn depending on exposure period.

KEYWORDS:
Clarias gariepinus, Sera biochemical parameters, Accumulation, Zinc

INTRODUCTION

Heavy metals are natural constituents of aquatic environments mainly through volcanic activities, floods and erosion. However their concentrations increased significantly by anthropogenic activities leading to important health and environmental problems [1]. High levels of heavy metals can cause mass mortalities in aquatic environments whereas they accumulate in various tissues at lower concentrations if detoxification mechanisms cannot compensate uptake levels [2].

Zinc is an element that is needed in trace amounts by organisms and act as a cofactor in about 300 enzymes [3-4]. In addition to its biological functions it is widely used in metallurgy, plastic and glazing industries and in agriculture. Unprocessed wastes of these activities increase the levels of this metal significantly in aquatic environments. Hence a number of studies were carried out both under laboratory conditions and in field concerning the accumulation of zinc in aquatic organisms [5-6]. Metals taken up by fish are carried to various tissues through blood stream where they cause tissue damages, changes in blood parameters and effecting endocrine system and metabolic activities.

Glucose is the main high energy compound in vertebrates and its excess amounts are stored in liver and muscle tissues in the form of glycogen and its level in sera is controlled by endocrine system [7]. Stress factors, such as hunger, hypoxic conditions, dense stocking and heavy metals, results in changes in carbohydrate metabolism, hence in sera glucose levels [8-9].

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are liver originated enzymes. They play a role in amino acid metabolism and their levels in blood is low under normal conditions [10].

Cholesterol is a structural component of lipoproteins, bile acids and steroid hormones and its level in sera changes through stimulation of lipolysis under the effect of pollutants such as metals [11].

Clarias gariepinus is a widely distributed species in inland waters and drainage channels of Mediterranean region and is consumed commonly as a protein source. Due to its wide tolerance against pollutants and its habitat being under direct influence of agricultural and industrial activities and urban discharges, the species was chosen as an experimental animal.

Long term effects of low doses of heavy metals result in accumulation. Significant variations in blood parameters occur, however, at the beginning of exposure. Hence AST and ALT activities and sera glucose, cholesterol and total protein levels were determined after exposing

C. gariepinus to 1.0 and 5.0 ppm Zn over 24, 48 and 96 hours.

MATERIALS AND METHODS

C. gariepinus was obtained from a private fish farm in Silifke-Mersin-Turkey. The mean length and weight of the animals were 27.5 ± 2.25 cm and 102 ± 1.21g respectively. Fish were adapted to laboratory conditions for one month in glass aquaria 40x120x40 cm in height. The same sized three aquaria were used in the experiments taking the control 0.0, 1.0 and 5.0 ppm zinc concentrations tested. The first two aquaria was filled with 120 L of 1.0 and 5.0 ppm zinc while the third one was filled with the same amount of zinc free tap water and used as control. Zinc sulphate ($ZnSO_4$:$5H_2O$) was used in the preparation of experimental solutions and trisodium citrate ($C_6H_5Na_3O_7.5H_2O$) was used to prevent precipitation and adsorption of the metal. Mean zinc levels in experimental water were determined as 1.0 ± 0.05 mg/L Zn and 5.0 ± 0.05 mg/L Zn. Experimental solutions were replaced daily, by serial dilutions of freshly prepared 1000 ppm Zn stock solution. Some physical and chemical properties of experimental water are given in Table 1.

TABLE 1
Some physical and chemical properties of experimental water.

Illumination regime: 12h/12h light/dark	Temperature: 21 ± 1^0C
Total alkalinity: 305 ± 0.5 mg $CaCO_3$/L	Dissolved oxygen: 7.01 ± 0.6 mg/L
pH: 8.0 ± 0.5	

Experiments were run in triplicate being 3 fish in each replicate, hence 9 fish were placed in each aquarium. Fish were fed once a day with readymade fish feed (Pınar, Pellet No: 2) at amounts of 2% of total biomass. Three fish were removed from each aquaria at the end of each exposure period. Fish were anesthetized with MS222 to prevent changes in studied parameters. They were then washed with tap water and dried with Whatman filter papers.

Blood samples to be used in determining sera parameters were obtained by cutting caudal peduncle vertically. They were transferred to anticoagulant free centrifuge tubes and centrifuged at 4000 rpm for 10 minutes. Sera samples were then transferred to sera tubes and analyzed using a Beckman Coulter LH 750 auto-analyzer.

Fish were dissected for their gill, liver, kidney, spleen and muscle tissues to be used in metal analysis. Tissues were brought to a constant weight at 150^0C for 48 hours. They were then transferred to volumetric flasks and wet burned in 2:1 (v/v) nitric acid (Merck, 0.65 %, SG, 1.40) perchloric acid (Merck, 60 %, SG, 1.53) mixture at 150^0C for three hours [12]. Burned tissues were transferred to polyethylene tubes and their volumes were made up to 5 ml with distilled water. Metal levels in tissues were determined using an ICP-AES spectrophotometer.

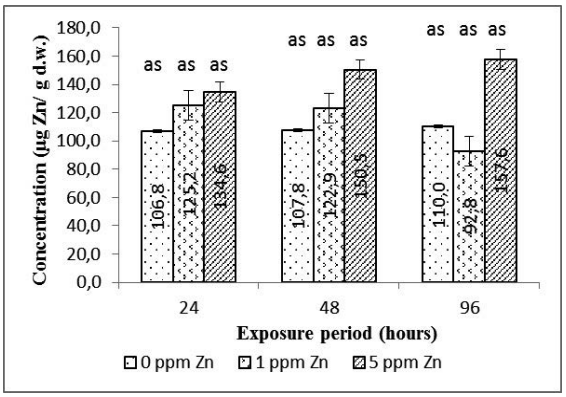
Statistical analysis of the data were carried out by Analysis of Variance and Student Newman's Procedure (SNK) using SPSS-16 statistical package program [13].

RESULTS

Highest accumulation of Zn was in liver followed by spleen, kidney, gill and muscle tissues in fish exposed to 1.0 ppm Zn, whereas this order was spleen, kidney, gill, liver and muscle tissues in fish exposed to 5.0 ppm Zn over 96 hours (Fig. 1). Liver accumulation of Zn increased significantly when exposed to 5 ppm Zn after 24 hours compared with control fish ($P<0.05$). Zinc levels in this tissue, however turned to normal levels after 48 and 96 hours of exposure to this concentration (Fig. 1b). No significant difference was observed in Zn levels of gill (Fig. 1a), kidney (Fig. 1c) and muscle (Fig. 1e) tissues of *C. gariepinus* compared with control at the exposure periods tested ($P>0.05$). There was no significant increase in spleen accumulation of Zn compared with control at 24 and 96 hours of exposure, while Zn accumulation was higher at 5 ppm Zn compared with 1 ppm Zn and control (Fig. 1d; $P<0.05$).

Exposure to Zn increased sera total protein levels which decreased with prolonged exposure. Sera total protein increased significantly compared with control at all exposure periods (Fig. 2c; $P<0.05$). There was a significant increase in sera AST and ALT activities of *C. gariepinus* at both concentrations of Zn, being higher at 5.0 ppm than at 1.0 ppm Zn (Fig. 2d; $P<0.05$). Sera glucose level increased at both Zn concentrations with increasing exposure periods except at 24 hours. This increase was also significant when compared with control at 48 and 96 hours (Fig. 2a; $P<0.05$). There was a

significant increase in sera cholesterol levels at the beginning of experiment under the effect of Zn which gradually decreased with prolonged exposure. Sera cholesterol levels increased significantly compared with control at all Zn concentrations and exposure periods tested. This increase was higher at low then at high concentration of Zn tested (Fig. 2b: P<0.05).



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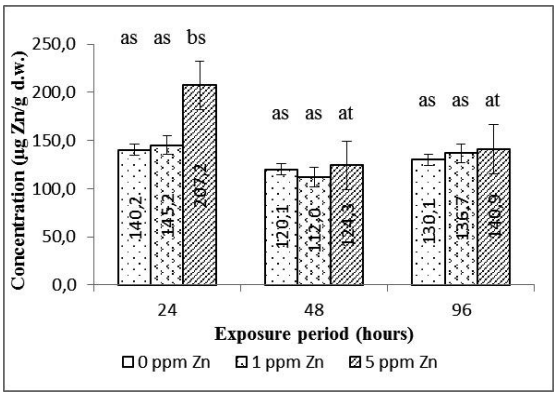

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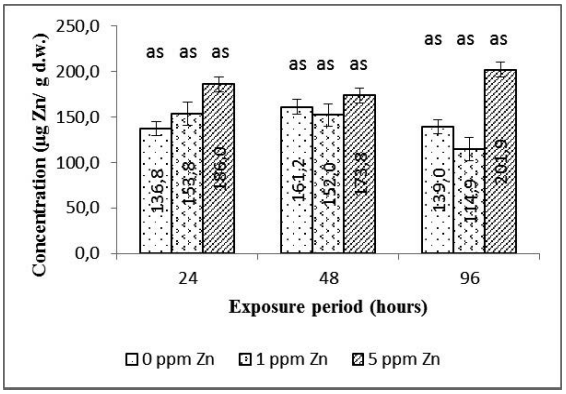

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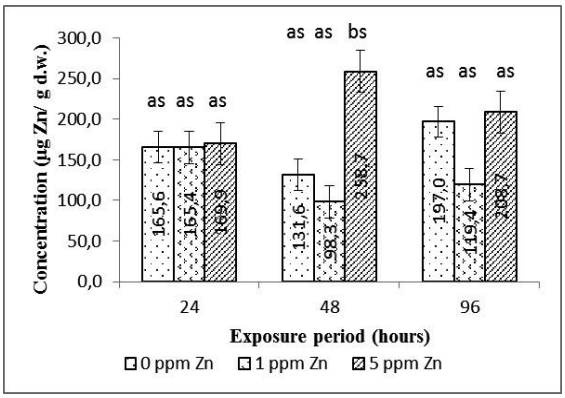

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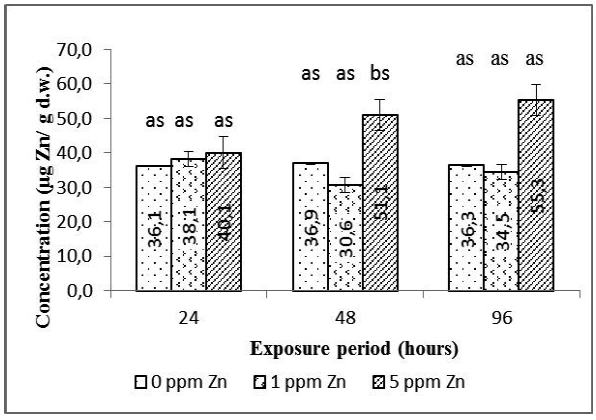

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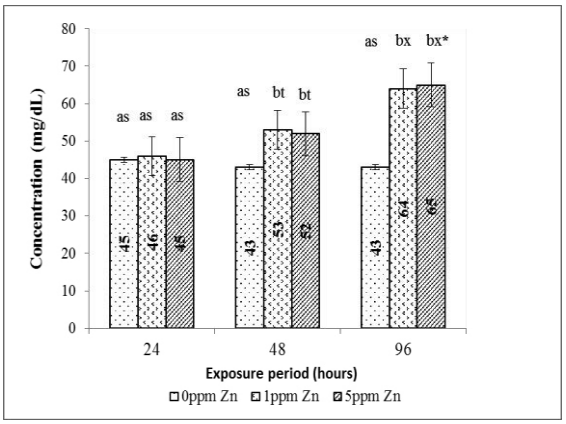
FIGURE 1
Accumulation of zinc in a. Gill, b. Liver, c. Kidney, d. Spleen, e. Muscle tissues of *C. gariepinus* (µg Zn/g d.w.).
***=SNK; Letters a, b and s, t show differences among exposure periods and concentrations at a given tissue respectively. Data shown with different letters are significant at the P<0.05 level.**



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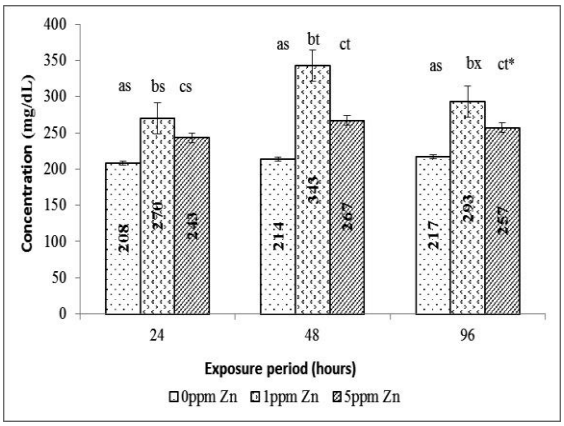

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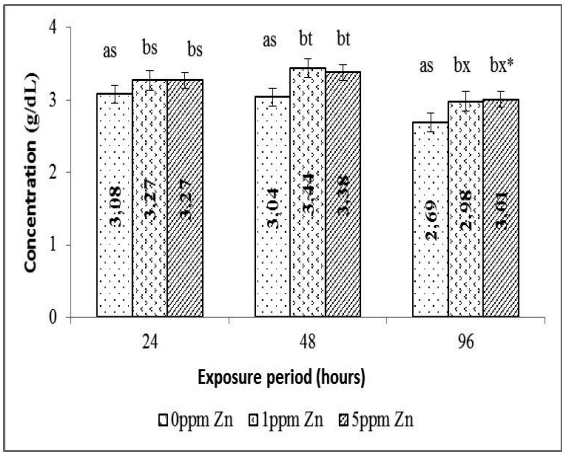

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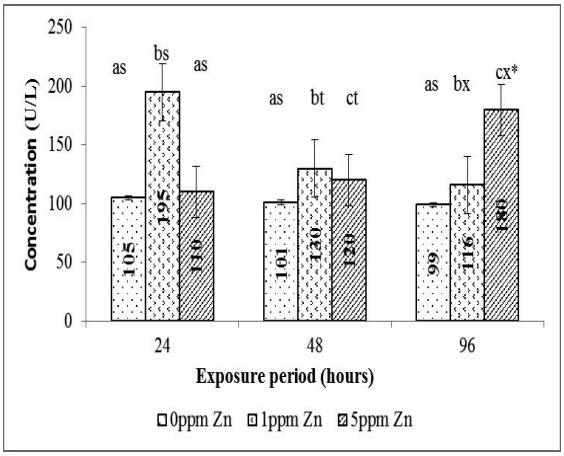

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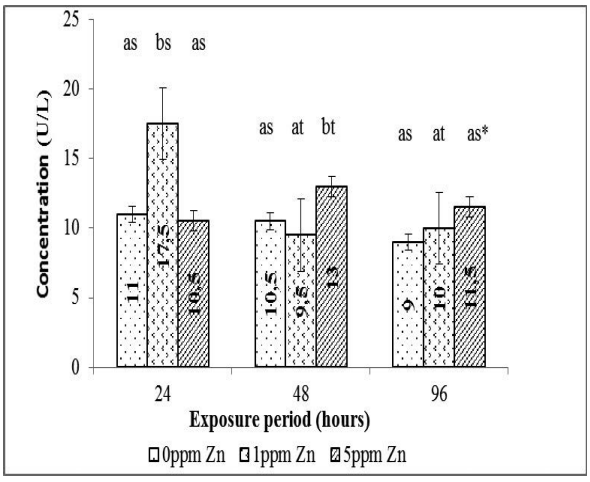

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FIGURE 2
Effects of zinc on sera a. Glucose (mg/dL), b. Cholesterol (mg/dL), c. Total protein (g/dL), d. Aspartat aminotransferaz (AST) (U/L), e. Alanin aminotransferaz (ALT) (U/L) levels of *C.* gariepinus. *=SNK; Letters a, b, c and s, t show differences among exposure periods and concentrations at a given tissue respectively. Data shown with different letters are significant at the P<0.05 level.

DISCUSSION

There was no mortality *C. gariepinus* exposed to 5.0 ppm Zn over 96 hours which was probably due to short exposure periods and low metal concentrations. Previously observed behavioral differences were also true for *C. gariepinus* exposed to zinc at the beginning of experiments [14-15].

Highest accumulation of Zn was in liver tissue of *C. gariepinus* followed by spleen, kidney, gill and muscle tissues in fish exposed to 1.0 ppm Zn whereas this order was spleen, kidney, gill, liver and muscle tissues in 5.0 ppm Zn exposed fish. Accumulation of Zn and Cu was higher in the liver tissue of *Scyliorhinus canicula* whereas the lowest accumulation was in muscle tissue [16]. These difference among the tissues in accumulating metals can be explained by discrepancies of metabolic activities of these tissues. Although Zn is a trace element excess levels of this metal are also toxic and are carried to liver for detoxification and to kidneys for excretion.

Gills are the target organs in accumulating metals since they are in direct contact with the external media [10]. Short exposure to high concentrations of Zn resulted in higher gill and liver accumulation in *C. lazera* and *Tilapia zillii* [17] and in *Channa punctatus* [18]. No significant difference was observed in Zn levels of gill, which might be due to the low concentration and short exposure periods.

Metals entering from gills are firstly transferred to liver by the circulatory system, and when the carrying capacity of liver is exceeded, they are transferred and stored in metabolically active tissues, especially in kidney and spleen [19]. Cicik [20] reported liver accumulation of Cu and Zn accumulation was higher compared to gill and muscle tissues in *C. carpio*. *Puntius parrah* exposed to 0.9.mg/L Zn over 1, 3, 7, 14, and 28 days accumulated higher levels of Zn in liver, followed by kidney, gill and muscle tissues [21]. Zinc accumulation in liver increased on days 1 and 3, remained unchanged on day 7 and increased again on the following days. Liver accumulation of zinc in 5.0 ppm Zn exposed *C. gariepinus* increased compared with control after 24 hours of exposure and returned to control levels on prolonged exposure probably due to activation of detoxification mechanisms.

Spleen accumulation of Zn was highest compared with the other tissues studied in *C. gariepinus* exposed to 5.0 ppm Zn. Studies carried out with other fish species have also shown that spleen accumulate high levels of this metal [22]. Muscle, although not an active tissue in accumulating metals, plays an important role in transferring metals to higher trophic levels through food chain at more concentrated forms. Muscle accumulation of metals was generally low compared with liver, kidney and gill tissues as shown by previous studies which was also shown to be true in the present study [23-24]. Muscle is an important tissue for carrying the metals to upper trophic levels, although it is not an effective tissue in metal accumulation. Although muscle is not an effective tissue in binding metals it is important to know metal levels in this tissue as far as food chain and human health is concerned.

Heavy metals above given concentrations activate stress conditions in fish as other stress factors, such as temperature and anaerobic conditions, which in turn increases the need for energy. The increase in energy demand stimulates the release of glucocorticoids such as cortisol, epinephrine, and catecholamine causing mobilization of liver and muscle glycogen by glycogenolysis and result in hyperglycemia. Copper increases sera glucose and cortisol levels in *Salmo trutta* depending on exposure concentration and period [25]. McLeay [26] reported that sera glucose levels increased in *Oncorhynchus mykiss* exposed to zinc for 7 days. Sera glucose levels of *C. gariepinus* exposed to 1.0 and 5.0 ppm Zn increased with exposure period except for 24 hours. This might result from stimulation of glycogenolysis in liver and muscle depending upon the energy demand under the effect of zinc.

Cholesterol is the main structural component of lipoproteins, bile acids and steroid hormones. Effects of heavy metals on cholesterol levels in fish varies between fish species and metals tested. Sera cholesterol levels increased in *Oreochromis niloticus* exposed to Ag, Zn, Cr, Cu and Cd singly [27] while long term effect of methyl mercury and lead decreased sera cholesterol levels in *Lepomis macrochirus* [28] and in *Prochilodus lineatus* [29] respectively. Short term exposure to Zn increased sera cholesterol levels of *C. gariepinus* whereas its level decreased with increasing periods. Variations in sera cholesterol levels under the effect of Zn might be due to tissue damage caused by the metal, breakdown in cholesterol synthesis and/or the use of cholesterol in the synthesis of steroid hormones.

Trace elements are transported between various tissues by binding to proteins such as albumin, globulin and ceruloplasmin. Sera total protein levels of *O. mykiss* increased exposed to copper for 15 and 30 days [30]. Sera total protein levels of *Cyprinus carpio* increased under the effect of Cu and Zn [31] and in *T. zillii* and *C. lazera* exposed to Zn [16]. These increases, however, were not continuous. Sera total protein levels of

C. gariepinus increased on the 24th hour of exposure to Zn and decreased with increasing periods. The increase in synthesis of metal binding proteins and time depended increase in protein catabolism might explain these variations.

Heavy metals not only induces stress conditions in fish but also cause tissue damages. Stress conditions in fish increase energy requirements which is derived primarily from carbohydrates, such as glucose and from non-carbohydrate sources, such as proteins and lipids, through gluconeogenic enzymes, namely AST and ALT. The levels of gluconeogenic enzymes are low under normal conditions. Sera AST and ALT activities of *O. mykiss* increased on the 15 and 30 days of exposure to cadmium [32]. Zinc increased sera AST and ALT activities in *O. niloticus* at both short and long exposure periods [33]. Sera AST and ALT activities increased to higher levels at 5.0 ppm than at 1.0 ppm Zn concentration in *C. gariepinus*.

The results of the present study revealed that zinc even at low concentrations and exposure periods can result in tissue accumulation and cause variations in sera glucose, cholesterol, total protein levels, AST and ALT activities which all can be used in determining Zn toxicity to the species mentioned. It was concluded that the studied zinc concentrations caused significant alterations in the carbohydrate and protein metabolism by effecting sera parameters.

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