Influence of Sublethal Lead Concentrations on Glucose, Serum Enzymes and Ion Levels in Tilapia (Oreochromis mossambicus)

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Abstract. In this study, alterations in glucose, blood enzymes (alkaline phosphatase (ALP), lactate dehydrogenase (LDH), alanine transaminase (ALT), aspartate aminotransferase (AST)) and serum ion (P^{+++}, Mg^{+}, Cl^{-}, Ca^{++}, Fe^{++}) levels were investigated in Tilapia (*Oreochromis mossambicus*), which were semi-statically exposed to different lead concentrations *in vivo*. The fish were exposed to low (0.5 mg/L), medium (2.5 mg/L) and high (5 mg/L) concentrations of lead during 14 days. At the end of the experiment, biochemical blood parameters such as glucose, ALP, LDH, AST, chloride and magnesium increased ($p<0.05$). While, LDL and calcium levels decreased ($p<0.05$); ALT, cholesterol, albumin, iron and phosphor were fluctuated ($p<0.05$). Consequently, it was found that exposure of *O. mossambicus* to lead concentrations affected serum biochemical parameters negatively.

Keywords: lead, toxicity, glucose, serum enzymes, ion levels, *Oreochromis mossambicus*

1 Introduction

Lead is a persistent contaminant in the natural environment that can enter the water column through geologic weathering and volcanic action, or by various anthropogenic activities including mining and smelting of lead-ores, burning of coal, effluents from storage battery industries, automobile exhausts, metal coating and finishing operations, fertilizers, pesticides and from additives in pigments and gasoline (WHO, 1995).

Contamination of water through anthropogenic practices is the primary cause of lead poisoning in fish (Sorensen, 1991). Due to its nondegredable nature, it's get into the environment and eventually enters to fish and human body system. When it can enter to the body lead can accumulate to soft tissues such as liver, kidney, nervous system and brain of fish (Berman, 1980). It is well documented that lead can impair

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the health of humans and other organisms by neurotoxicity, renal toxicity, and deleterious effects on the hematological and cardiovascular systems (ATSDR, 2006). In studies examining the toxicity of lead on fish, it was determined that lead inhibited to Na^+, K^+-ATPase enzyme activity and caused to oxidative stress in tilapia (Kaya and Akbulut, 2015), it damage Ca^{2+} and Na^+ homeostasis in trout fish at concentrations found in ecosystems (Rogers et al. 2003; Patel et al. 2006), and caused hematological (Kaya et al. 2013) and neurological (Davies et al. 1976) effects in fish under chronic conditions. However, no studies evaluating the effects of sublethal lead concentrations on the biochemical parameters of fish could be found.

The present study aimed to investigate the effects of water-borne lead on fish with special reference to the blood glucose, serum enzymes and ions.

2 Material and Methods

2.1 Experimental design

Tilapia fish used in this study (n=144) were obtained from Çanakkale Onsekiz Mart University (Marine Sciences and Technology Faculty, Aquaculture Department), Çanakkale, Turkey, and were adapted to ambient conditions in 12 stock aquariums, each with dimensions of 45x28x80 cm and containing 80 L of rested, Çanakkale city tap water, for 4 weeks. Fish weighting 45.2±5 g (mean±SD) were divided into 12 experimental aquariums, each containing 12 fish, and an experimental design with three replicates was established. Feeding was interrupted 24 h before the start of the experiments to help maintain water quality. During the experiment, the fish were fed twice a day with feed at about 2% of their body weight and their behavior was observed during each feeding. Care was also taken to ensure that all of the feed added to the tanks was eaten and that fecal waste was quickly removed from the tanks at every water change. In the experiment, fish were exposed to the following sublethal concentrations of lead: low, 0.5 mg/L; medium, 2.5 mg/L; and high, 5 mg/L. The control group was maintained in freshwater only. Concentrations were determined by considering in Ay et al. (1999). The experiment had a semi-static regime, and water was changed every day: a 75% change in the morning and a 25% change in the evening (modified from Smith et al. 2007). After each water parameters were as follows: temperature, 25.4±0.3°C (mean±SE); dissolved oxygen, 6.31±0.11 mg/L; pH, 7.15±0.04; hardness, 125.0±6.2 mg/L $CaCO_3$; total ammonia, 0.151±0.02 mg/L. The electrolyte composition of the dechlorinated Çanakkale tap water was measured as 0.310±0.005, 0.049±0.001, 0.534±0.001, and 0.828±0.006 mmol/L for Na^+, K^+, Mg^+, and Ca^{2+}, respectively. Fish were randomly sampled on days 0, 7, and 14 for blood biochemistry analysis. The experiments were performed in accordance with the guidelines for fish research established by the Animal Ethics Committee at Çanakkale Onsekiz Mart University.

2.2 Preparation of the $Pb(NO_3)_2$ Solution and Application

The heavy metal salt, $Pb(NO_3)_2$ (99.5% purity; Sigma-Aldrich, Steinheim, Germany), was used in the experiment. To obtain the needed concentrations, the main stock solution was prepared in ultra-distilled water and appropriate dilutions made from it.

2.3 Blood Sampling

In the experiment, total 12 fish on the first day (from the stock aquarium), 6 fish from each aquarium on the 7^{th} and 14^{th} day were used for blood analysis. For blood sampling, fish were anaesthetized with MS222 (Smith et al. 2007). They were well wiped and cleaned in order to avoid mucus mixing into the blood, and then, blood was taken from the fish through the caudal vein by a 5 ml plastic syringe, without harming the fish (Val et al. 1998). Then, a sample of blood was transferred to EDTA tubes, BD Microtainer®, UK for hematological analysis. Plastic biochemistry tubes (Kima-vacutest®, Italy) were used for biochemical analysis. Blood serum was isolated by centrifugation (4000xg, 10 min) and it was stored below -20°C.

2.4 Biochemical Analysis

For biochemical analysis, the blood collected was centrifuged at 4000 rpm for 10 minutes and blood serum was separated (Bricknell et al. 1999). Then, the serum extracted was analyzed on the spectrophotometer (T80+UV/VIS) using an analyzer (Bioanalytic Diagnostic Industry, Co). The biochemical parameters that were detected during the test included glucose (GLU), alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), calcium (Ca^{2+}), magnesium (Mg^{+}), iron (Fe^{++}), phosphorus (P^{+++}) and chlorine (Cl^{-}).

2.5 Statistical analysis

ANOVA with Dunnett post-test (one-way ANOVA for comparison between exposure groups and control group) was used. The statistical analysis was made by using SPSS 17.0, and the significance level was considered to be 0.05 (Logan, 2010).

3 Results

Glucose and serum enzymes obtained from the study were given in Table 1.While the glucose levels registered an increase in medium and high concentrations on 7^{th} and 14^{th} days compared to the control group, they did not show any differences with the control group in low concentrations. While the ALP increased across all groups on day 7 compared to the control group, on day 14, the low and medium

concentrations registered an increase. The enzyme AST was revealed to be high across all groups on day 7 and on day 14, it was found out to be higher in low and medium concentrations compared to control group. While the ALT activity was revealed to be lower on day 7 compared to control group, on day 14, a decrease in low and medium dose was determined compared to the control group as an increase in high group was experienced. While the enzyme LDH showed similarities with control group in every group, ($p>0.05$), it increased across all groups on day 14.

Serum electrolytes during the study are given in Table 1. While the Ca^{2+}, one of the serum ions of the study decreased on days 7 and 14 across all groups compared to control group, the Mg^{+} increased in medium and high concentrations on day 7. On the other hand, while Fe^{++} showed a decrease in low and high doses on day 7 compared to control group, the medium dose increased. On day 14; a decrease compared to the control group in low and medium doses was determined while an increase was registered in high doses. The Cl^{-} electrolyte showed an increase on days 7 and 14 across all groups compared to the control group. P^{+++} on the other hand, showed an increase in low and high concentrations on day 7 and showed a decrease on day 14 in high group compared to the control group.

Table 1. Effects of different concentrations of lead on glucose, serum enzymes and some serum minerals. Exposure groups are represented as follows control: 0 (Control), low (0.5 mg/L), medium (2.5 mg/L), high (5 mg/L); ALP, alkaline phosphatase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase, Ca^{2+}, calcium; Mg^{+}, magnesium; Cl^{-}, chloride; Fe^{++}, iron; P^{+++}, phosphor. The differences among the times shown with the small letters for each ion are significant ($p<0.05$).

		GLU (mg/dL)	**ALP (U/L)**	**AST (U/L)**	**ALT (U/L)**	**LDH (U/L)**	**Ca^{2+} (mmol/L)**	**Mg^{+} (mmol/L)**	**Fe^{++} (µg/dL)**	**Cl^{-} (mmol/L)**	**P^{+++} (mmol/L)**
	Initial	172.044±0.78	5.13±0.26	6.33±0.87	16.71±2.01	58.22±2.93	10.621±0.50	3.026±0.05	79.681±0.8	162.035±4.67	5.281±0.43
Control	**7th day**	167.199±4.10^{c}	5.73±0.77^{d}	6.52±0.44^{c}	16.58±0.66^{a}	61.17±4.19	10.599±0.39^{a}	3.006±0.18^{b}	77.134±1.58^{b}	163.107±6.62^{c}	5.371±0.20^{b}
Low		182.484±4.98^{c}	9.52±0.47^{c}	6.56±0.12^{c}	13.05±0.77^{ab}	73.17±2.70	9.149±0.37^{b}	3.130±0.03^{b}	61.087±1.36^{c}	345.095±4.68^{b}	7.607±0.52^{a}
Medium		215.884±7.68^{b}	18.76±1.7^{a}	18.21±0.99^{b}	13.26±1.39^{ab}	74.38±3.02	7.391±0.16^{c}	3.583±0.11^{a}	129.717±2.8^{a}	379.362±2.58^{a}	6.044±0.19^{b}
High		271.954±2.80^{a}	13.41±0.87^{b}	38.15±1.6^{a}	9.34±0.75^{b}	85.29±2.77	8.186±0.27^{bc}	3.707±0.06^{a}	59.148±1.31^{c}	390.79±6.63^{a}	8.366±0.43^{a}
Control	**14th day**	172.900±2.60^{c}	5.89±0.72^{b}	6.80±0.60^{d}	17.88±1.46^{b}	54.27±3.03^{b}	10.373±0.28^{a}	3.258±0.07^{ab}	78.374±2.66^{b}	170.763±8.04^{c}	5.642±0.58^{a}
Low		182.424±5.91^{c}	10.19±1.12^{a}	21.81±2.86^{b}	2.12±0.15^{c}	145.03±4.21^{a}	9.616±0.20^{a}	3.473±0.05^{a}	58.289±0.86^{c}	161.338±4.68^{c}	6.459±0.37^{a}
Medium		206.338±7.67^{b}	8.90±0.77^{a}	14.86±1.05^{c}	7.50±0.61^{c}	163.30±5.60^{a}	6.080±0.007^{c}	2.962±0.12^{b}	61.463±0.94^{c}	392.107±6.68^{a}	5.874±0.13^{a}
High		229.235±5.94^{a}	3.13±0.19^{b}	85.48±2.78^{a}	27.77±1.61^{a}	142.41±3.24^{a}	7.638±0.007^{b}	2.924±0.02^{b}	97.392±3.31^{a}	346.97±1.26^{b}	3.961±0.10^{b}

4 Discussion

The glucose is the primal source of the energy that is required for the vital actions and its level in serum is regulated through the endocrine system (Dange, 1986). In fish, in addition to the stress induce such as hunger, dense stocking etc; the pollutants such as metals also increase the secretion of the cortisol, epinephrine and glucocorticoid thus leading to the changes in carbohydrate metabolism (Sastry and Subhadra, 1985). Under the influence of lead metal and the environmental concentrations, it was revealed that the glucose levels in serum increased compared to the control group. It is thought that such an increase forms hyperglycemia and in addition, leads to damages in liver and hormonal irregularities (insulin deficiency).

The enzymes of dehydrogenize and phosphatase are important and critical enzymes in terms of biological processes and thus they are responsible for the detoxification and biosynthesis of macro molecules (Yousef et al. 2007). In tilapia; increases in LDH and ALP activities (both are blood serum enzymes) indicate that liver damage due to the presence of lead metal. The increases are thought to be occurring due to the fact that as the result of the liver damage, the liver cytoplasm leaks into the blood stream (Wang and Zhai, 1988). Rahman et al. (2000) reported that in fish that were exposed to the pollutants, the increase results from the LDH enzyme, mixing into blood due to the necrosis in liver. The transaminase enzymes such as ALT and AST play an important role in the metabolism of protein and amino acids. In this study, while the AST activity was found out to be increasing in all concentrations, time and concentration varied increases and decreases were registered in ALT enzyme activity. It is thought that the increases in such enzyme activities are resulted after the enzymes are introduced to the circulatory system due to the damages in liver tissue. ALT, AST, ALP and LDH serum enzymes that were assessed within the scope of this study are prone to be used as sensitive biomarkers in ecotoxicological studies because of their characteristic of being an early warning mechanism against the heavy metal based pollution in aquatic ecosystems (Vaglio and Landriscina, 1999).

The aquatic organisms have to preserve the osmotic pressure of the plasma in order to survive the ever changing environmental conditions and to maintain the water and ion homeostasis. In bony fish, there are advanced structures to provide the aforementioned regulations and such structures keep the inorganic ion concentrations of the fish in close levels. The changes that may occur in electrolyte levels may induce stress and thus may lead to the damages (Sjöbeck and Larsson, 1979). Calcium is an ion that has various roles in ion regulation, membrane permeability, muscle and neuron cell functions and skeletal bone metabolism and in the blood clotting. The most important serum electrolyte for the lead toxicity is calcium. In this study, the calcium levels of tilapia fish that were subjected to lead concentrations were observed to be lower compared to the control group. Such decreases are found out to be consistent with the literature and the decreases in blood calcium levels (hypocalcaemia) was observed. In this study, unlike the calcium ions, increases were revealed in magnesium electrolyte under the impact of lead. The previous study

indicates an inverse relationship between calcium and magnesium ions (Marshall, 2002). Chloride plays an important role in osmotic pressure and ion balance as well as in acid–base equilibrium. In this study, under the effect of water-borne lead concentrations, Cl^- ion levels of experimental groups registered increases compared to the control group. Na^+ and Cl^- levels in fish are responsible for the osmolarity. Changes in such ions may cause increase in gill permeability and damages to osmoregulation. It is known that in some studies, under the effect of the pollutants, the inhibitions that occur in the gill Na^+, K^+-ATPase enzyme activity lead to the disarray in ion regulation (Haux and Larsson, 1979).
The changes in parameters that were examined within the scope of this study such as glucose, LDH, ALP, ALT and AST showed that sublethal lead concentrations inflict damage to the liver as the changes in magnesium; calcium and chloride indicate gill damages.

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