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Effect of Lead on Hematological Parameters and Serum Biochemistry of Bighead Carp (*Hypophthalmycthys nobilis*)

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Abstract:
The present study was planned to check acute toxicity of lead in bighead carp. Fish were exposed to two sub-lethal concentrations (5 and 10 mg Pb/L) for 96-hour. Blood was sampled after metals exposure to check the alterations in hematological as well as serum biochemical parameters. The analysis of hematological parameters and serum biochemical parameters showed that high concentration of Pb affects more the count of red blood cells, white blood cells, hemoglobin, MCV, MCHC, MCH, total Cholesterol, Glucose, total protein and albumin than low dose which, in turn, affect physiological condition of fish.

INTRODUCTION

Water is main natural resource which directly concern with human's welfare. Due to dispersion of urban and industrial waste products produced by social activities is the main cause of pollution in water and soil (Kumar, 2004). Some important applications of cultivated soils like uncontrolled dispersal of waste products, smelting and mining of metalliferous ores, process and accidental spillage is responsible for transferring the heavy metals into the non-contaminated sites (Ghosh and Singh, 2005). Heavy metals are the main cause of contaminations which includes organic and inorganic components like textile and phenol dyes, petroleum products, spoilable and explosive substances etc. (Gad et al., 2008, Jadhav et al., 2010). In seas, metal pollution is less visible but directly affect the marine organisms. The existence of metals is depending upon different fish features like developmental stage, fish age and also varies of fish species (Emami et al., 2005).

Metals can affect the fish body in two ways, one is directly depositing the metals into their body, second is indirectly transferring of metals by using their trophic level in food chain (Shah and Altindag, 2005). In aquatic bodies fish can be used as bio-indicator of metal's contamination (Javed, 2005). For the regulation of biochemical and physiological functioning in fish minute quantity of metals are important. However, when metals exceed from tolerance limits of fish, then affect physiological condition of fish (Bu-Olayan and Thomas, 2004). Presence of heavy metals is toxic for fish immune system, which leads towards declined in mortality, pathogenicity and its population (More et al., 2003). For the determination of fish toxicity, the acute methods are used because these methods give the rapid results of contaminated effects on aquatic organisms (Kai Sun et al., 1995).

Lead is a non-essential but toxic metal, which released from different sources like refining of ores, fertilizer and chemical industries in aquatic ecosystem (Handy, 1994). However, through leaching and erosion of soil lead enter into the aquatic ecosystem (Moore and Rainbow, 1987). Through gills lead enter into the fish body and attached with the mucus layer. Lead also enter in fish with water and food then absorbed into the intestine and other organs of fish (Kotze et al., 1999; Ay et al., 1999; Macdonald et al., 2002, Hensen et al., 2007). Likewise, accumulation of heavy metals

into the organs of fish depend upon different factors specially uptake rate of metals, storage and elimination (Roesijadi and Robinson, 1994; Longston, 1990).

Unsuitable ecological factors or the existence of stressing features with toxic chemicals alter the blood biochemistry of fish (Barcellos et al., 2004). When fish expose to the heavy metals, biochemical and physiologic parameters can change the blood sequence in fish (Cicik and Engin, 2005). When fish exposed to metals and their mixtures than fluctuations in the hematological values observed. Some hematological keys like red blood cells, white blood cells, hematocrit, hemoglobin and so on, used in aquatic environment as an indicator of metal contaminations. Due to the same factors erythrocytes cause stress in fishes (O' neal and Weirich, 2001).

Change in blood biochemistry may be symptomatic of inappropriate environmental factors like pH, temperature and oxygen concentration and also existence of toxic metals as stress factor (Barcellos *et al.,* 2004). When fish expose to metals they show biochemical and physiologic changing in tissues and blood of fish (Cicik and Engin, 2005). The level of serum glucose, cortisol, protein and cholesterol can increase or decrease due to presence of metals and also change enzyme activity of serum which depend upon metal type, time of experiment, water quality and types of fish (Vaglio and Landriscina, 1999; Monteiro et al., 2005). For the isolation of target tissues of toxicity and check the overall health conditions of organism's measurement of serum biochemical parameters can be particularly valuable (Folmar, 1993; Jacobson and Keller, 2001).

MATERIALS AND METHODS

Fingerlings of bighead carp (*Hypophthalmycthys nobilis*) were used for the experiment. The animals were transported from fish hatchery to laboratory in University of Agriculture Faisalabad. They were acclimatized to water for 14 days in four experimental tanks (PVC, 17 liters). During the exposure period, the physicochemical parameters were checked on daily basis. Continuous aeration was delivered to each glass aquaria through a pump fitted with capillary system. Chemically pure compounds of lead were dissolved in ionized water and stock solutions were prepared. Blood was taken by caudal vein using plastic disposable syringe fitted with 26-gauge needles. The syringe was already moisture with heparin. The whole blood was used for the estimation of hemoglobin, erythrocytes and leucocytes count, remaining blood sample were centrifuged for 20 mints at 10,000 rpm and plasma was separated for the estimation of serum biochemical parameters.

Hematological Parameters

The red blood cells count was complete by Neubauer Hemocytometer. Blood was diluted 1:2 with Hayem's solution. Entire quantities were described as10^6 mm^{-3} (Dharam et al., 2008). The white blood cells count was also prepared by Neubauer Hemocytometer. Blood was diluted in 1:20 with Turk's diluting liquid and located in hemocytometer. The total number of WBC was quantified as10^3 mm^{-3} (Emere et al., 2013). Hematological parameters were tested in triplicates. RBC: 106 cells μl^{-1} and WBC: 104 cells μl^{-1} were calculated by hemocytometer technique (Sadness et al., 2002). Hematocrit (Ht v/v ratio or %) was measured by micro hematocrit method and hemoglobin conc. (Hb gl^{-1}), red cell indices, MCV (μm^3 $cells^{-1}$), MCH (pg $cell^{-1}$), MCHC (%), were calculated by cyanometer hemoglobin method (Lee et al., 2010).

Serum Biochemical Parameters

The blood in the Eppendorf tubes was permitted to coagulate at room temperature, almost 25° °C, for 30 min. Serum was separate from the coagulated sample later centrifugation at 3,000 rpm for

5 mints and freezing at 20 °C until chemical analysis. In Eppendorf tubes the serum was collected then analyzed for different serum parameters. The following parameters were calculated and stated in the given units: total cholesterol, glucose, total protein, albumin.

STATISTICAL ANALYSIS

Statistical analysis was applied by Mintab software. Analysis of variance was used to check statistical differences among variables.

RESULTS

Erythrocyte and leukocyte numbers are presented in Table 1. In the control group, overall erythrocyte number was predictable as 18.86±1.82 $10^6 mm^3$. In the lead exposure groups, the erythrocyte no. increased more after high dose exposure than low dose. Leukocyte no. predictable about 546.13±17.3 in normal, but in the investigational groups, excepting in the 5 mg l^{-1} Pb group, this no. improved about 558.48±28.22 and in the 10 mg/l Pb group, the leukocyte no. Increased more upto 689.49±28.22. Hemoglobin value was resolute as 26.85 g/100ml in the control group, hemoglobin values of fish expose to lead were higher than the control, even though, this alteration is not statistically significant after exposure to low dose of lead ($p > 0.05$). The MCV, MCHC and MCH values was also increased in lead exposure groups than the control group (Table1).

The fish was exposed to acute treatment to determine the serum biochemical parameters exposed to lead. The value of cholesterol, glucose and total proteins increased and albumin contents decreased in lead exposure groups than control group but change is not statistically significant. The presence of toxicants in the aquatic ecosystem exerts toxic effects at cellular and molecular levels which results in significant changes in biochemical composition of the aquatic biota. Physico-chemistry of test medium during acute toxicity tests were maintained throughout the exposure duration. Dissolved oxygen, carbon dioxide, sodium, total ammonia, potassium concentrations were maintained to a certain limit as requirements of trial. During these trials, mean water temperature, pH and total hardness were fixed at 32°C, 7 and 225 mg/L, respectively (Pandey et al., 2005). All the assessment ways presented substantial alterations for dissolved oxygen contents, total ammonia, sodium, potassium and carbon dioxide. Control medium had significantly higher dissolved oxygen contents of 6.16 ± 0.18 mg/L than those used for 3 metals throughout toxicity trials (Giguere et al.,2004)

Table 1. Hematological parameters of *H. nobilis* exposed to low and high concentration of Lead.

Hematological parameters	Control	Low values	High values
RBC (10^6 mm^3)	18.86±1.82	20.91±1.02	37.75±3.40
WBC (10^3 mm^3)	546.133±17.473	558.482±28.227	689.492±30.338
HB (g/100ml)	26.85±1.64	28.05±1.02	43.74±5.01
MCV (10^{-4} mm^3)	302.08±3.86	345.67±4.45	387.55±4.75
MCHC (%)	25.77±1.36	27.34±1.40	34.75±2.20
MCH (10^{-5} pg)	47.9±0.31	50.05±2.05	69.75±3.05

Values are means ± standard error.

Table 2. Serum biochemistry of *H. nobilis* exposed to low and high concentration of Lead.

Serum Parameters	Metals	Control values	Low values	High values
Total cholesterol	Lead	72.95±1.30	73.10±0.03	73.22±0.15
Glucose	Lead	6.30±0.80	5.56±0.29	5.30*±6.07
Total protein	Lead	17.81±0.58	16.51±0.58	14.02±0.47
Albumin	Lead	2.72±0.10	2.58±0.08	2.60±0.02

Values are means ± standard error.

DISCUSSION

Hematology of blood can provide information about the anatomy of fish, according to recent research work the exposure of lead concentrations showed significant changes in different hematological parameters. The present research work caused significant changes in hematological parameters as compared to control medium when exposed to different concentrations of lead. During lead exposure, increase and decrease in different parameters of hematology and serum biochemistry in the same way as observed by Witeska and Kosciuk (2003).

As compared to control medium, the present outcomes described that the hematological parameters were increased in number when exposed to different toxic metals. The number of WBC's was increased during lead exposure. Same results were observed by Mousa and Khattab, 2003 and increased level of WBC's observed as compared to control level. Change in hematocrit, red blood cells counts and hemoglobin conc. were observed due to leukocytosis actions in erythrocytic anemia with erythroblastosis (Vosyliene and Kazlauskiene, 2004). Change in blood biochemistry may be symptomatic of inappropriate environmental factors like pH, temperature and oxygen concentration and also existence of toxic metals as stress factor (Barcellos et al., 2004). When fish exposed to metals, they show biochemical and physiologic changing in tissues and blood of fish (Cicik and Engin, 2005). The level of serum glucose, cortisol, protein and cholesterol can increase or decrease due to presence of metals that can change enzyme activity of serum which depend upon metal type, time of experiment, water quality and types of fish (Vaglio and Landriscina, 1999 and Monteiro et al., 2005).

RBC's also increase, after high level metal concentration slight increase in hemoglobin was observed. The scientist also observed that reduction in blood cells explain the anemic condition due to low level of red blood cells. Failure in RBC's production, internal bleeding under stress condition and impaired osmoregulation may lead to reduce the total number of red blood cells (Joshi et al., 2002). The maximum decrease in total leucocyte count, hemoglobin concentration hematocrit value and in total erythrocyte was observed in exposed fish bighead carp during 48 hrs. Same results were obtained previously that reduce in hematocrit and hemoglobin concentration due to anaemia (Vinodhini et al., 2009).

During acute exposure of metals more mean corpuscular hemoglobin concentration, mean corpuscular hemoglobin and mean corpuscular volume were determined than control group. In contrast to this, Shah (2006), Talas and Gulhan, (2009); Kavitha et al. (2010) a observed decreased number of MCV, MCHC and MCH due to destruction in RBC's membrane as results in toxic stress of metals, during hemolysis. According to recent research work the level of glucose decreased during lead exposure in blood of fish from the control value towards on high level of metals exposure. The level of total protein primarily decreases in blood from low to high level. The previous results were also supported (Haney et al., 1992) due to reduction in red blood cells and subsequent release of cell substances into the blood, total protein level reduced to a certain limit,

and did not have significant (P>0.05) values from control to infected fish. In blood the level of glucose increases due to contamination of heavy metals because of intensive glycogenolysis supported by Bouck and Ball (1967).

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