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ESTIMATION OF CHROMIUM BIOACCUMULATION IN LYMPHOID ORGANS AND ITS TOXIC EFFECTS ON BLOOD PROFILES OF A FRESH WATER CATFISH, *SACCOBRANCHUS FOSSILIS* (BLOCH)

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ABSTRACT

The fresh water catfish,*Saccobranchus fossilis* (Bloch), 10- 15cm long and 20-25 g in weight, were experimentally exposed to sub lethal chromium concentrations of 0.56, 1.0 and 3.2 mg/L for 28 days. The bioaccumulation of chromium metal ions in various lymphoid organs viz., liver, spleen and kidney, was determined and selected hematological parameters were studied. In liver and kidney, highly significant quantities of chromium accumulated ($p < 0.001$) at all the exposed concentrations of 0.56, 1.0 and 3.2 mg/L on comparison to control group, however, in spleen at 0.56 mg/L concentration, it was accumulated less significantly as compared to higher concentrations. A significant concentration dependent decrease in the number of total erythrocytes and leucocytes occurred while the hemoglobin content was reduced at all concentrations with a more significant decrease at higher concentration. The results indicate detrimental effects of sub lethal concentrations of chromium metal ions on lymphoid tissues and blood profiles of fish and may pose serious adverse effects on fish health by predisposing them to vulnerable infections and diseases.

Key words

Saccobranchus fossilis, bioaccumulation in lymphoid organs, chromium hematotoxicity, hemotological profiles.

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Introduction

Fishes are living under serious threats of water pollution facing the panic for their survival. Population explosion, rapid industrialization and increased uses of chemicals and fertilizers, for enhanced agro-production, further intensify the problems of water pollution. The consistency of water pollution is enhancing the levels of toxic metals in water bodies and posing adverse effects on fish health. Fishes, being the source of delicious protein rich food, become more valuable for availing disease free and healthy life. Interestingly, the inherent nutritional and clinical benefits of fishes, as source of edibles, have exorbitantly increased their consumption in the modern world. Several detrimental effects of consuming heavy metal intoxicated fish as food, on human health, have been reported since long time (Castro-Gonzalez and Mendez-Armenta, 2008). It has precipitated in the form of serious threats including renal failure, liver damage, cardiovascular diseases and death (Al-Busaidi et al., 2011). Various international monitoring programs have been undertaken regularly for determining the quality of fish for human consumption and thus the health of aquatic ecosystem is monitored (Meche et al., 2010). The studies on fishes are more relevant for depicting the harmful effects of aquatic metal pollution for human health. Fish, besides many significant aspects, is also selected for study because of easy to maintain in lab, easy handling and common availability in the fresh water reservoirs of India. From the last few decades, fishes have been extensively studied mainly with regard to the concentrations of heavy metals across the world. Even low non lethal concentrations of heavy metals are more harmful to fishes because of causing immunosuppression and making fishes prone to a wide array of infections and diseases. Due to usual roaming nature and being relatively on top position in aquatic food chain, fishes normally get heavy metals accumulated from food, water and sediments (Yilmaz et al., 2007) and serve as bio-indicator of metallic pollution in aquatic habitats. The structural and functional aspects of fish health under stress conditions, including physiological changes in a number of fish species, are assessed by studying the blood profiles (Adhikari et al., 2004; Suvetha et al., 2010). The fish blood is indicative of heavy metals' pollution induced stresses and changes in hemoglobin content and erythrocytes' number may be used to monitor the stresses caused by pollutants (Romani et al., 2003; Barcellos et al., 2004). The easy availability of blood and lymphoid tissues etc. make such studies more relevant. The accumulation of metals in fish damages the structures of organs (Giari, et al., 2007) and alters negatively the red blood cells (Vosyliene, 1999). The levels of metal accumulation in different organs differentially depend upon the uptake and elimination rates (Lange et al., 2002).

Chromium, a predominant toxicant in the effluents of many industries specially the nickel chrome plating, leather chrome tanning, bicycle manufacturing, paint and woolen hosiery etc., is drained into the aquatic habitats and fishes get frequently exposed. It is a common pollutant of surface and groundwater; however, its high levels of

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exposure, due to natural resources, have not been usually recorded (Robles-Camacho and Armienta, 2000). The chromium concentration, duration, exposure time, fish species type and specific body systems and even parameters play significant roles in posing differential adverse effects on fish health. The process of metal uptake is influenced by various factors like sex, age, size, reproductive cycle, swimming patterns, feeding behavior and living environment (Mustafa and Guluzar, 2003).

Besides, several beneficial aspects deliberated, the unavailability of sufficient scientific data on effects of heavy metals, mainly of chromium on fish health, necessitated significantly this study for ensuring a better health and quality life to fishes directly and also the human beings indirectly. Further, fish blood profiles suggest the deteriorated water quality, due to the noxious effects of metals, and aim at providing the people healthy fishes as safe and better quality food. The blood of fish, being closely related to the aquatic environment, will show the signs of illness more promptly than any other sign in fish body. The good quality of fish health can be an effective and relevant parameter for assessing the health of aquatic ecosystem and the human beings because of consuming fish as food (Meche et al., 2010). Therefore, the freshwater catfish- *Saccobranchus fossilis*, (Bloch) were experimentally exposed to several sub lethal chromium concentrations for 28 days and heavy metal accumulation in lymphoid organs was determined. Further, some selected hematological parameters were also studied.

Materials and Methods

Fish acclimatization and chromium exposure

The fresh water catfish, *Saccobranchus fossilis* , 10- 15cm long and 20-25 g in weight, were housed 25 in each all glass aquaria separately two weeks prior to experimentation and fed pelleted feed, minced goat liver and daphnids etc. at a rate of 2% body weight. The reagent grade potassium dichromate ($K_2Cr_2O_7.7H_2O$) was used at sub lethal concentrations like 0.56, 1.0 and 3.2 mg/L for 28 days.

Determination of metal bioaccumulation in lymphoid organs

The acclimatized fish were divided into four groups, each comprising of 10 fish and housed in smaller all glass aquaria. The natural water at 24 hrs regular interval and minced goat liver were provided. One group, from these was treated as control and remaining three were exposed to 0.56 mg/L, 1.0 mg/L and 3.2 mg /L. chromium concentrations for 28 days. After anesthetizing the fish, lymphoid organs like liver, spleen and kidney were dissected out, washed thrice in fresh water and briefly rinsed in double de-ionized water. Then, these organs were blotted dry with filter paper, weighed separately and transferred into 100 ml separate flasks containing 4 ml of concentrated nitric acid (AR-Grade) and 1 ml of perchloric acid. Afterward, these flasks were allowed to react with tissues till 30 minutes at room temperature. These tissues were then dried on hot plate by the process of evaporation. On cooling, 10 ml of 0.1N- HNO_3 was added in each flask. This procedure is known as hot digestion. These

prepared samples were then analyzed for chromium accumulation using AAS 5000 (Atomic Absorption Spectrophotometer-5000 Perkin, U.S.A.) and values were calculated and expressed in µg/ gm wet tissue weight.

Hematological Study

Blood was collected by puncturing the caudal vein of anaesthetized fish, using 1 ml disposable syringe, and put in heparinized and non heparinized glass tubes separately from each fish for hematological study. The blood samples were stored at 4 °C till the entire duration of study. Blood parameters like total erythrocytes' and leucocytes' counts and hemoglobin content were determined by standard routine hematological procedures (Wintrobe, 1974).

Statistical Analysis

The differences between control and treated groups were compared by applying the Student t test. The P value calculated and $P < 0.05$ was considered statistically significant.

Results

Bioaccumulation study of lymphoid organs

Table 1 showing the bioaccumulation of chromium metal ions in various lymphoid organs of *S. fossilis* following 28 days exposure.

S. No.	Lymphoid organs	Quantity of chromium accumulated (µg/ gm wet wt.)			
		Control	0.56 mg/L	1.0 mg/L	3.2 mg/L
1.	Liver	21.09 ±0.56	33.03 ^{c} ±0.69	42.43 ^{c} ±0.53	84.48 ^{c} ±0.84
2.	Spleen	25.18 ±0.46	28.55 ^{b} ±0.62	30.40 ^{c} ±0.57	34.05 ^{c} ±0.59
3.	Kidney	24.39 ±0.56	35.69 ^{c} ± 0.51	40.51 ^{c} ± 0.58	45.42 ^{c} ± 0.56

a. Values are expressed as mean ± SE of five observations.

b. $p < 0.01$. c. $p < 0.001$. d. five to six fish per group.

The results clearly demonstrate (Table 1) bioaccumulation of chromium metal ions in various lymphoid organs, in a dose dependent manner with the exposures of increasing concentrations, in *S. fossilis* for 28 days. The highly significant quantity of chromium metal accumulated at relatively higher concentrations on comparison to the control groups. The maximum quantity of chromium accumulated in liver with the increasing concentration of chromium exposures. Comparing the respective control groups, at 0.56 mg/L of chromium exposure, the quantity accumulated in liver and kidney increased 63.85% and 68.33% respectively, however, it was not pronounced in spleen. At higher concentrations i.e. 1.0 mg/L and 3.2 mg/L of exposure, the consistency of increasing trend of accumulation, in the lymphoid organs, was highly significant ($p < 0.001$) comparing to the respective control groups. This analysis has reflected the idea

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that adverse effects of chromium metal are because of its more accumulation in lymphoid and other target organs.

Chromium toxicity and blood parameters

Table 2 showing 28 days sub lethal chromium exposure caused hematological alterations in *S. fossilis.*

S. No.	Blood parameters	Control	Test concentrations of chromium (mg/L)		
			0.56	1.0	3.2
1.	Erythrocytes (x 10^6/mm^3)	4.15 ±0.15	4.03 ±0.12	3.57 ^{b} ± 0.12	2.93 ^{c} ±0.085
2.	Leucocytes (x 10^3/mm^3)	6.68 ±0.16	6.48 ±0.15	5.47 ^{d} ±0.12	5.08 ^{d} ±0.13
3.	Hemoglobin (gm/100ml)	12.59 ±0.14	12.38 ±0.16	11.58 ^{e} ±0.16	10.80 ^{d} ± 0.19

a. Mean Values represented ± SE of five observations.

b. $p < 0.05$. c. $p < 0.02$. d. $p < 0.001$ e. $p < 0.01$.

The results of Table 2 demonstrate a concentration dependent decrease in the number of erythrocytes, leucocytes and hemoglobin content in chromium treated *S. fossilis* for 28 days as compared to untreated groups. This decrease may be interpreted as bad consequences of metal toxicity on fish hematological profiles by mounting the hematotoxic effects of experimental chromium intoxication to *S. fossilis*. These alterations are dose dependent. At higher concentration of chromium exposure (3.2 mg/L), the number of erythrocytes, leucocytes and hemoglobin content drastically decreased comparing with the control groups. However, this depletion was not so marked at 0.56mg/L chromium exposure. RBCs, WBCs and Hb depleted in fish exposed to all concentrations of chromium, being, in each case, lower than the respective control groups ($p< 0.05$).

Discussion

Chromium bioaccumulation in lymphoid organs

The present study (Table 1) shows that chromium metal accumulates in various lymphoid organs like liver, spleen and kidney in a concentration dependent manner in *S. fossilis* following 28 days of experimental exposure. The significantly higher quantity of chromium accumulated in lymphoid organs at increased concentrations on comparison with the control groups. Maximum quantity of chromium accumulated in liver with its increasing concentrations of exposures**.** Studies carried out with different fish species have shown that liver is the prime organ for metal accumulation and also contributes significantly in storage, redistribution and detoxification of metals (Erdem and Kargin,

1992; Evans et al., 1993). Filipovic´ Marijic´ and Raspor (2006) argued that the metals are transferred to storage organs like liver or kidney following long term exposure. The observations of Gill and Epple (1993) on metals straightway affecting the hematopoietic stem cells in kidney and spleen, possibly due to abnormal membrane permeability and mechanical defects, strongly uphold the significance of my research studies. Further, comparing the respective control groups, at 0.56 mg/L of chromium exposure, the quantity accumulated in liver and kidney increased by 63.85% and 68.33% respectively. At higher chromium concentrations of 1.0 mg/L and 3.2 mg/L exposure, the consistency of increasing trend of accumulation, in all the lymphoid organs, was highly significant ($p < 0.001$) comparing with the respective control groups. Metal accumulation in fish is known to occur in a concentration dependent manner (Isani et al., 2009). My finding with liver, accumulating chromium several folds more at 3.2 mg/L exposure, suggests a direct relationship between bio-accumulation and the increasing chromium concentration.

Interestingly, Cattani et al. (1996) reported that more cadmium accumulated in kidneys of fishes (bass and flounder) than in the liver and it can be interpreted that pollutants get distributed uniformly throughout the fish body and may follow different mode of accumulation (Brown et al., 2006).The uptake and elimination rates determine the level of metal accumulation in different organs (Lange et al., 2002). However, my studies demonstrated that spleen could not accumulate chromium so immensely as liver. These findings are in good consonance with earlier study where cadmium and copper were found accumulated more in liver than in other tissues (Cogun et al., 2003). My observations have demonstrated that metal accumulation largely depends on the concentration, period of exposure and the type of organ. Similar findings were reported by Wu et al. (1999) in which differences in concentrations and exposure times affected the accumulation of metals in organs. Further**,** Kraal et al. (1995) stated that the metal accumulation is tissue specific and largely depends on the exposure route. The hepatic tissues normally act as an essential metal store (Zn and Cu) to fulfill enzymatic and other metabolic demands (Roesijadi, 1996; Amiard et al., 2006) thereby showing the prompt and positive effects on metal accumulation in liver- the vital lymphoid organ.

This study endorses the significance of my present investigations. Further, I observed that the metal accumulation largely depends on three factors; concentration of metal, period of exposure and the type of organ. Furthermore, fish are mostly migratory and hardly settle down at one place and hence, the metal accumulation in their body organs directly indicates the contaminated status of aquatic environment (Qadir and Malik, 2011). It could be used to sense the prevailing health condition of the inhabited area. The increase in experimental concentration and exposure time of various metals to fish body cause several detrimental impacts and increased mortality that can be interpreted as a serious consequence of metal intoxication destroying the ability of

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natural detoxifying systems. This analysis has reflected the idea that adverse effects of chromium metal are because of its more accumulation in lymphoid and other target organs.

Hematological parameters and chromium toxicity

The results of present investigation (Table 2) demonstrate that the number of erythrocytes, leucocytes and hemoglobin content decreased in fish, *Saccobranchus fossilis*, exposed to several concentrations of chromium for 28 days. This significant concentration dependent decrease occured in each case lower than the control group ($p< 0.05$). Further, the total number of erythrocytes depleted significantly ($p< 0.05$). These alterations may be interpreted as bad consequences of metal toxicity on fish hematological profiles due to hematotoxic effects of experimental chromium exposure. My findings are in congruence with reports for other fishes and metallic exposures (Gill and Epple, 1993; Karuppasamy et al., 2005) where significant lowering in red blood corpuscles and hemoglobin content occurred due to cadmium exposure in American eel *Anguilla rostrata* and air breathing fish, *Channa punctatus*. Furthermore, at higher concentration of chromium exposure (3.2 mg/L), the number of erythrocytes, leucocytes and hemoglobin content drastically decreased comparing with the respective untreated groups. However, it was not so marked at 0.56mg/L chromium exposure. Similar to my findings, Saravanan et al. (2011) observed a significant reduction in RBC count and Hb concentration in chromium exposed fish-*Platichthys stellatus* and interpreted as consequences of toxic effects in the form of hemophilia, red cell shrinkage, osmoregulation and gill injury.

Farag et al. (2006) also reported that exposure of chromium increased its bioaccumulation in tissues and caused several alterations including abnormal behavior, decreased growth and increased mortality. The fish exposed to 4.64 mg/L cadmium concentration suffered a marked reduction in the number of erythrocytes and hemoglobin content (Mekkawy et al., 2011). Similarly, 10 ppm cadmium experimental exposure to *O. niloticus* for 15 and 45 days caused reduction in these blood parameters (Shalaby, 2007). This depletion in fish blood profiles, at sub lethal levels of cadmium exposure, may be interpreted as a possible cause of mature erythrocytes' destruction and inhibition in their formation causing acute hemolytic crisis. Our previous reports on copper toxicity to fish blood are in fair agreement with this finding (Khangarot and Tripathi, 1991). Similar findings were reported for cadmium reducing significantly the number of erythrocytes and hemoglobin content in fish, however, the counts of leucocytes and large lymphocytes significantly increased (Gill and Epple,1993) owing to their significant roles in immunological defense systems during exposure to toxicants like heavy metals. It might further be interpreted as an eventuality of lymphocytosis and immune response (Shah and Altindag, 2005).

The metals are reported to act directly on hematopoietic stem cells in kidney and

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spleen and cause severe anemia in most of the vertebrates, including fish species exposed to various environmental contaminants (Khadre, 1988; Gill and Epple, 1993). These observations suggest that toxic consequences are specific from metal to metal, fish to fish and even parameter to parameter. The present study clearly shows that chromium stressed alterations in hematological parameters are the consequential effects of more metal accumulation in lymphoid organs like liver, spleen and kidneys involved in blood cell formation. Hematological parameters are good indicators to assess fish metabolism in metal caused stressful conditions (Vinodhini and Narayanan, 2009). Further, these parameters are largely used in toxicological investigations and environmental monitoring as a promising indicator of physiological changes in toxicant stressed fish (Kavitha et al., 2010). These hematological disturbances can also be used as sensitive bio-indicators for evaluating the stresses caused by the metallic pollution (Romani et al., 2003; Barcellos et al., 2004). Fish blood components indicate the harmful effects of water quality and therefore, ensures safety food for people and act as the biological indicator of aquatic pollution. Hematological indices of fishes are closely related to the aquatic environment and the blood will expose conditions, within the body of the fish, earlier than any visible sign of disease.

Conclusion

It may be concluded that 28 days sub lethal chromium experimental exposure significantly increased its accumulation in the lymphoid organs of *S. fossilis* and adversely affected the hematological profiles. Liver accumulated chromium metal more prominently at higher concentration when compared with spleen and kidney. A concentration dependent decrease in the total number of erythrocytes, leucocytes and fall in the content of hemoglobin reflected chromium induced hematotoxic alterations in this fresh water catfish. My present research shows that metal accumulation largely depends on factors like concentration of metal, period of exposure and the type of organ. Further, this investigation also indicates the possibility of chromium working as a stressor and adversely affecting fish health and physiology. Importantly, the fish hematological parameters are used in toxicological investigations and environmental monitoring as a promising indicator of stressed physiology. Fish blood components indicate the harmful effects of water quality and therefore, ensures safety food for people and act as the biological indicator of aquatic pollution. Such studies are highly required for improving the health related aspects of fish and also the human beings.

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