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Research Paper

HEMATOLOGICAL ALTERATIONS IN INDIAN MAJOR CARP *Catla catla* (HAM.) UNDER THE STRESS OF ZINC METAL ION

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Abstract

Zinc containing products are widely used in daily lives. It is released into the aquatic environment as Zn^{2+}, especially affects the fish. The study was conducted to determine the acute/sub chronic toxicity of zinc metal ions on fingerlings. Under the stressed conditions, the toxicity may alter the normal blood constitution. For the experimental studies, two groups of fingerlings with similar length and weight were taken and exposed with zinc metal ions with 5ppm and 10ppm concentration. When observed after fixed intervals, RBC, WBC count, hemoglobin content and PCV were found to be distinctly low. This indicates the exposed fingerlings suffered from erythrocytopenia, leukocytopenia and hemolytic anemia. The value of MCV, MCH and MCHC were raised in all exposed fingerlings. Other changes have also been observed like structural damage of RBC membrane that could be due to hemolysis or impairment in hemoglobin synthesis. The observations clearly indicate that higher level of zinc metal ions exposure effects the blood constitution. The detailed study of hematological indices is considered as a crucial parameter to determine the response of fish at various environmental condition.

Key words: *Zinc metal ion, Hematological indices, Catla catla*.

INTRODUCTION

Heavy metal exposure proves to be useful for animal and human health at a limited concentration. Exposure of such metals in the environment result from soil-erosion, volcanic eruption or application in synthetic products *viz.* paints, mining, electroplating, dye etc. These heavy metals have been recognized as strong biological poisons and as hazardous pollutants because of their persistent nature, toxicity metals are considered due to their non-biodegradable nature and accumulation tendency in organisms and undergo food chain amplification; they also damage the aquatic fauna [1]. It is reported that exposure of heavy metals causes mutagenic, carcinogenic and cytotoxic effects [2]. As a result, they have deleterious effects on biota including growth of the fish [3].

Among these heavy metals, the present study was emphasized on exposure to zinc. It belongs to microelements and are metalloenzymes in nature [4]. Trace amount of zinc is essential for strengthening the immune system, protecting cell membrane as well as solubilization of blood sugar. Similarly, it is important for the health of fish. However, above certain levels, zinc exposure creates harmful effects. Detailed study of the zinc

chemistry showed that specific gravity of the zinc metal ions is 7135 kg/m^3, which is much higher than water [5]. That is the reason zinc metal cannot be metabolized in the fish body instead it accumulates there and cause long lasting toxic effects. It definitely alters the normal blood constitution. Elevated levels of zinc in aquatic systems can be due to leaching of domestic sewage, insecticides, galvanizing processes, liquid effluent discharge etc. [6,7]. The studies carried out suggest that nickel toxicity at low concentration may alter the physiological activities and the biochemical parameters both in tissues and in blood of fish [8].

Fish are used for the evaluation of the quality of aquatic environment and serve as a bioindicators of environmental pollution [9-11]. Experiments in the field of ecological toxicology are oriented towards studying the variations in the hematological changes [12]. Fishes can accumulate these heavy metals which may enter through four different routes (1) food ingestion (2) simple diffusion of metallic ions through gill pores (3) drinking water and (4) by skin adsorption [13]. Hematological techniques are gaining importance in toxicological research and environment monitoring as the stress created by the pollutants change the basic physiology. Zinc increase frequency of abnormal erythrocytes and compensatory reactions expressed by the appearance of immature erythrocytes in blood flow [14]. The morphological characteristics of fish blood cells show the possibility of development of compensatory adaptive processes under the noxious levels of content of heavy metals in water.

MATERIALS AND METHODS

Most of the studies of Zn^{2+} toxicity to aquatic vertebrates have been focused on zebra fish *(Danio rerio*) and little information is available on catla, which occupies a good position in Indian Major Carp. The main objective for this study is to find variations in hematological parameters such as RBC and WBC counting, hemoglobin content and PCV and other parameters such as MCV, MCH and MCHC value in fingerlings of *Catla catla.*

Study of fish and acclimatization

The fingerlings were obtained from a government fish farm, Surat (Gujarat). Mean length and weight of the fingerlings were 7.0±1cm and 5.5±1g respectively. Before the experiments, fingerlings were acclimatized in aerated, dechlorinated tap water at 28ºC temperature with the photoperiod of 12:12 h light and dark cycle and fed twice daily with commercial feed of 2% of their body weights. Two glass aquaria with capacity of 100L were used to acclimatize them to laboratory conditions prior to experiments for 15 days. 50 fingerlings were stocked in each aquarium. Fingerlings were not fed for 24 h prior to experiments.

Water composition

The water quality parameters were carried out by standard method [15]

Table 1: Water quality parameter.

Parameter	Values
pH	7 ±0.5
Temperature	28 ±1ºC
Dissolved oxygen	7.44±0.3mg/L
Hardness	140±7mg/L
Alkalinity	111±2mg/L
Free CO_2	Absent
Inorganic phosphorus	0.1±0.01 mg/L

Experimental set up and Exposure

Experiments were conducted under semi-static conditions. Approximately 30 fingerlings were stocked in10L capacity aquarium each. $ZnCl_2$ was used to perform experiments. It was obtained from Qualigens Fine Chemicals with AnalaR grade and used without any further purification. Experimental suspensions of the desired concentration were prepared by mixing preweighed $ZnCl_2$ in distilled water. The Standard Bioassay methods were adopted in the present investigation to study of LC_{50} value [16] by plotting graphs. It was plotted on mortality versus metal ion concentrations. Based on the obtained 50 ppm LC_{50} values, two sub chronic concentrations of different metal ions were selected. Fingerlings were exposed to 5ppm zinc (as lower concentration) and 10 ppm zinc (as higher concentration). Experiment was performed after 5 days, 10 days and 15 days intervals. Triplicates were performed for each concentration. At the same time, group of 10 fingerlings were taken out as a control in fresh tap water. All aquaria were aerated during the experiments except for the period of dosing.

Hematological analysis

After interval of 5,10 and 15 days exposure to zinc, fingerling was sacrificed from each tank and blood samples were collected directly by puncturing the heart, stored in monovet units with EDTA (anticoagulant)and used for determination of hematological parameters (RBC and WBC counts, hemoglobin content, PCV, MCV, MCH, MCHC).

Erythrocyte (RBC) and leukocyte (WBC) counts were made utilizing a Thoma hemocytometer, using Hayem's solution and Turk's solution as the diluents [17]. Hemoglobin estimation was done by cyanmethemoglobin method [18]. PCV values (PCV %) were determined by using capillary hematocrit tubes. The parameters such as Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC) were calculated by using standard formulae [19].

RESULT

Water quality parameters studied during the experimental period are presented in Table - 1, no such fluctuations were found as compared to their desirable limits. In the present investigation marked changes in level of RBC and WBC count were observed. In both the concentrations of Zn^{2+}exposure there was a gradual decrease in RBC count. Slight fluctuation was observed in WBC count; after 5 days it decreased and after 10 days it increased again, but did not exceed up to control value. There was a remarkable decrease in WBC count after 15 days exposure in both the concentrations. Hemoglobin content decreased with enhancement of time interval. The obtained results were summarized in the Table - 2 with mean and standard error (SE) value and was found to be statistically significant *($p<0.05$)*.

PCV values showed decreasing trend in the blood sample taken from fingerlings exposed to zinc, at the end of all exposures in both the concentrations. The MCV showed marked increase low concentration, which decreased after 10 days slightly but not below normal. MCH values were remarkably increased in both concentrations after all exposure periods. MCHC values increased in both the concentrations but with slight variations. The alterations in these indices are enlisted in the Table - 3.

Table 2: Effects of Zinc on hematological parameters RBC, WBC and Hb

Blood Parameters	Control	Zn^{2+}Metal ion concentrations and exposure days					
		5 ppm			10 ppm		
		5 days	10 days	15 days	5 days	10 days	15 days
RBC count (10^6cells/mm^3)	1.89±0.01	1.21±0.01	0.69±0.01	0.32±0.02	1.09±0.09	0.75±0.01	0.22±0.01
WBC count (10^6cells/mm^3)	0.19±0.01	0.14±0.01	0.17±0.01	0.08±0.01	0.13±0.01	0.14±0.01	0.06±0.01
Hb (g/ dl)	7.0±0.09	6.2±0.02	3.6±0.04	3.2±0.02	6.2±0.04	4.5±0.04	2.9±0.05

[Each value is mean ± SE of three individual observations]

Table 3: Effect of Zinc on hematological parameters PCV, MCV, MCH and MCHC

Blood Parameters	Control	Zn^{2+}Metal ion concentrations and exposure days					
		5 ppm			10 ppm		
		5 days	10 days	15 days	5 days	10 days	15 days
PCV (%)	42.92±0.22	31.00±0.40	17.2±0.18	14.82±0.08	30.74±0.15	21.07±0.25	13.89±0.15
MCV(/10^6)(fl)	227.08±0.4	256.19±2.4	249.26±2.73	463.19±2.77	282.08±1.40	280.87±3.32	631.35±6.87
MCH(/10^6)(pg)	37.03±0.23	51.23±0.60	52.17±1.2	100±3.0	56.88±3.4	60±0.84	131.81±4.29
MCHC(%)	16.30±0.16	20.00±0.26	21.07±0.14	22.15±0.41	20.48±0.05	21.85±0.34	20.87±0.47

[Each value is mean ± SE of three individual observations]

DISCUSSION

Our results are in good agreement with earlier work that reported decrease in RBC count, hemoglobin content and PCV of fresh water fish exposed to heavy metals [20, 21]. WBC count, and erythrocyte count and hemoglobin content decreased, while other indices like MCV, MCH and MCHC value were increased in all exposures in this study. Similar results were obtained during exposure of Azo dye [22]. The decrease in RBC and Hb content indicates acute anemia in exposed fingerlings. The anemia could be due to the destruction of RBC. The anemia may also be of hemolytic type. In the present investigation, hemolysis might have been one of the causes for reduction in Hb, RBC and PCV values. The fall in hematological parameters might be due to decreased rate of production or increased loss of destruction of RBC [23]. Another reason for decrease in RBC count due to damage to the hemopoietic tissue. PCV appears to be positively correlated with RBC counts, hence, a decrease in PCV was also observed. White blood cells in fish respond to various stressors including infections and chemical irritants [24]. Thus, increasing or decreasing numbers of white blood cells are a normal reaction to a toxicant, which demonstrate the effect of immune system under toxic conditions [25]. The decreased number of WBC may be the result of bio concentration of the test metal in the kidney and liver [26]. The erythrocyte constants MCV, MCH, and MCHC allow the determination of morphological anemia i.e. normocyte, macrocyte or microcytic anemia. The alterations in the hematological indices i.e. increase in MCV, MCH and MCHC in the present study may be due to a defense against the toxic effect of zinc metal ion and in

turn due to decrease in RBCs, Hb and PCV and the disturbances occurred both in metabolic and haemopoietic activities in fish. Increase in WBC count suggests that the anemia is macrocytic type [27]. The increase in MCH and MCHC in the present study clearly indicates the reduction in cellular blood iron, resulting in reduced oxygen carrying capacity of blood and eventually stimulating erythropoiesis MCH is a good indicator of RBC swelling [28]. The significant increase in the MCHC values in the present study may be due to direct or feedback responses of structural damage to red blood cells membranes resulting in hemolysis and impairment in hemoglobin synthesis and stress-related release of red blood cells from the spleen and hypoxia, induced by exposure to toxicant.

CONCLUSION

The hematological parameters were found to decrease from control in all exposure periods. From the above investigation it can be inferred that fingerlings were affected by the zinc metal ion. Significant changes are observed both at the end of 10 and 15days exposure. It caused severe anemia and alterations in hematological indices in the fresh water fingerlings *Catla catla*. The decreased level of hemoglobin, hematocrit, WBC and RBC revealed the hematotoxic effect of zinc. Catla was found to be the most vulnerable to the stress caused by zinc metal ion. Hematological parameters could be effectively used as potential biomarkers of metal toxicity to the freshwater fish in the field of environmental biomonitoring.

REFERENCES

1. Kamble, G., and Muley, D. 2000 Effect of acute exposure of endosulfan and chlorpyriphos on the biochemical composition of the fresh water fish *Sarotherodon mossambicus*, Indian J. Environ. Sci.4, 97-102.
2. More, T., Rajput, R., and Bandela, N. 2003 Impact of Heavy metals on DNA Contents in the whole body of fresh water Bivalve, *Lamellidens marginalis*, Pollut. Res. 22, 605-611.
3. Hayat, S., Javed, M., and Razzaq, S. 2007 Growth performance of metal stressed major carps viz. *Catla catla, Labeo rohita* and *Cirrhina mrigala* reared under semi-intensive culture system, Pakistan Vet. J. 27, 8-12.
4. Ololade, I., and Ogini, O. 2009 Behavioural and hematological effects of zinc on African catfish, *Clarias gariepinus*, Int. J. Fish. Aquac 1, 22-27.
5. Lide, D. R. 1992 Handbook of Chemistry & Physics 73rd Edition, Vol. 73, CRC.
6. Holmes, S. 1995 South African Water Quality Guidelines, Department of Water Affairs and Forestry, Aquat. Ecos.
7. Nussey, G. 1998 Metal ecotoxicology of the upper olifants river at selected localities and the effect of copper and zinc on fish blood physiology, Rand Afrikaans University.
8. Ololade, I., and Oginni, O. 2010 Toxic stress and hematological effects of nickel on African catfish, *Clarias gariepinus*, fingerlings, J. Environ. Chem. Ecotoxicol 2, 14-19.
9. Dautremepuits, C., Paris-Palacios, S., Betoulle, S., and Vernet, G. 2004 Modulation in hepatic and head kidney parameters of carp (*Cyprinus carpio* L.) induced by copper and chitosan, Com. Biochem. Physiol C., Toxicol. Pharmacol. 137, 325-333.
10. Lopes, P. A., Pinheiro, T., Santos, M. C., da Luz Mathias, M., Collares-Pereira, M. J., and Viegas-Crespo, A. M. 2001 Response of antioxidant enzymes in freshwater

fish populations (Leuciscus alburnoides complex) to inorganic pollutants exposure, Sci. Total. Environ. 280, 153-163.

11. Whitfield, A., and Elliott, M. 2002 Fishes as indicators of environmental and ecological changes within estuaries: a review of progress and some suggestions for the future, J. Fish. Biol. 61, 229-250.
12. Storelli, M., and Marcotrigiano, G. 2001 Heavy metal monitoring in fish, bivalve molluscs, water, and sediments from Varano Lagoon, Italy,Bull. Environ. Contam. Toxicol. 66, 365-370.
13. Vilella, S., Ingrosso, L., Lionetto, M., Schettino, T., Zonno, V., and Storelli, C. 2000 Effect of cadmium and zinc on the Na^+/H^+ exchanger present on the brush border membrane vesicles isolated from eel kidney tubular cells, Aquat. Toxicol. 48, 25-36.
14. Sindayigaya, E., Van Cauwenbergh, R., Robberecht, H., and Deelstra, H. 1994 Copper, zinc, manganese, iron, lead, cadmium, mercury and arsenic in fish from Lake Tanganyika, Burundi, Sci Total Environ 144, 103-115.
15. A. P. H., Association, 1915 Standard methods for the examination of water and wastewater, American Public Health Association 2.
16. Doudoroff, P., Anderson, B., Burdick, G., Galtsoff, P., Hart, W., Patrick, R., Strong, E., Surber, E., and Van Horn, W. 1951 Bio-assay methods for the evaluation of acute toxicity of industrial wastes to fish, Sewage Ind. Wastes, 1380-1397.
17. Dacie, J., and Lewis, S. 1984 Practical Haematology, fifth edR Churchill Livingstone, Edinburgh.
18. Blaxhall, P., and Daisley, K. 1973 Routine haematological methods for use with fish blood, J. Fish Biol. 5, 771-781.
19. Wickham, L., Costa, D., and Elsner, R. 1990 Blood rheology of captive and free-ranging northern elephant seals and sea otters, Canadian J. Zool., 68, 375-380.
20. Vutukuru, S. 2005 Acute effects of hexavalent chromium on survival, oxygen consumption, hematological parameters and some biochemical profiles of the Indian major carp, *Labeo rohita*, Int. J. Environ. Res. 2, 456-462.
21. Shalaby, A. 2001 Protective effect of ascorbic acid against mercury intoxication in Nile tilapia (*Oreochromis niloticus*), J. Egypt. Acad. Soc. Environ. Develop.(D-Environmental studies) 2, 79-97.
22. Barot, J., and Bahadur, A. 2014 Toxic effect of azo dye (ci direct green 6) on blood parameters of freshwater fish *labeo rohita* (Ham.), J. Cell. Tissue Research 14, 4251-4254.
23. Roche, H., and Boge, G. 1993 Effects of Cu, Zn and Cr salts on antioxidant enzyme activities in vitro of red blood cells of a marine fish *Dicentrarchus labrax*, Toxicol. in vitro 7, 623-629.
24. Christensen, G., Fiandt, J. T., and Poeschl, B. A. 1978 Cells, proteins, and certain physical-chemical properties of brook trout (*Salvelinus fontinalis*) blood, J. Fish Biol.12, 51-60.
25. Kori-Siakpere, O., Ake, J., and Avworo, U. 2006 Sublethal effects of cadmium on some selected haematological parameters of Heteroclarias (a hybrid of *Heterobranchus bidorsalis* and *Clarias gariepinus*), Int. J. Zool. Res. 2, 77-83.
26. Agrawal, S., and Srivastava, A. K. 1980 Haematological responses in a fresh water fish to experimental manganese poisoning, Toxicol.17, 97-100.
27. Afaq, S., and Rana, K. 2009 Toxicological effects of leather dyes on total leukocyte count of fresh water teleost, *Cirrhinus mrigala* (Ham), Biol Med. 1, 134-138.

28. Wepener, V., Van Vuren, J., and Du Preez, H. 1992 The effect of hexavalent chromium at different pH values on the haematology of *Tilapia sparrmanii* (Cichlidae), Com. Biochem. Physiol. C.101, 375-381.