Effect Of Zinc Toxicity On Some Haematological Parameters Of Fish, *Ophiocephalus Punctatus*

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Abstract

The present study aimed to evaluate some haematological changes resulting from the exposure of the freshwater fish, Ophiocephalus punctatus to the sublethal concentration of 6 mg/l of zinc sulphate in water for a period of 7, 14, 21 and 28 days. The studied haematological parameters were haemoglobin content, haematocrit percentage, erythrocyte count, total leucocyte count and mean corpuscular haemoglobin. Zinc sulphate caused reduction in haemoglobin content and increase in haematocrit values. Tremendous increase in erythrocyte count after 7 days zinc sulphate treatment was recorded in the experimental fish but later on there was significant decrease in the erythrocytes count after 28 days. Rise in leucocyte count was observed after 7 days zinc sulphate treatment but later on the number decrease. Fish also showed significant drop in mean corpuscular haemoglobin after 7 and 14 days, followed by slight increase. These haematological parameters may be used as an indicator of stress in fish induced by zinc toxicity.
Keywords: *Ophiocephalus punctatus, Zinc sulphate, haematological parameters, toxicity.*

Introduction

Factories discharge large amount of waste product into the river or nearest streams, lakes etc., which may join the river and thereby the ocean. Therefore, life in the water system is affected and biopurification system of water gets disturbed. Zinc is essential in small quantities for normal development of the organism but if consumed in excess amount, it may become toxic. The excessive zinc from the environment may enter into the fish body through nutrients, general body surfaces and gills. Toxic concentration of zinc compound cause adverse effect in the morphology, physiology and haematology of the fish. Srivastava and Punia (2011) studied the haematological and biochemical changes resulting from the exposure of common carp *Cyprinus carpio* to sublethal concentration of zinc in water at different time interval. Celik *et al*. (2013) investigated the changes in haematological and innate immune parameters and accumulation in the liver, gills and muscle tissues of *Oreochromis mossambicus* exposed to sublethal concentrations of zinc. Haematological alterations are used for rapid evaluation of chronic toxicity of compound. Hence, haematological investigation is important in toxicological research. Ganesan and Karuppasamy (2015) analyzed the impact of sublethal concentration of zinc on some hematological parameters of *Channa punctatus* under long term exposure. Aim of current study was to evaluate some haematological parameters like haemoglobin content, haematocrit value, erythrocyte count, total leucocyte count and mean corpuscular haemoglobin in freshwater fish *Ophiocephalus punctatus* exposed to sublethal dose at different time interval.

Material and Methods

The fish, *Ophiocephalus punctatus*, common air breathing freshwater teleost, were used in the present study. Fish weighing 20-25 gm and up to 12 cm in length were purchased from local fish market. Then they were treated with 0.1 % $KMnO_4$ solution for 2 minutes to clear dermal infection. Fish were maintained under laboratory condition in aquarium for one week. They were fed with commercial feed. The water in the aquarium was changed daily to remove detritus.

a) Water used - Aged tap water was used throughout the experiment. The physiochemical parameters of water was determined periodically as per standard method for examination of water and waste water (APHA, 1998). Water having pH $= 7.4 \pm 0.5$; Temperature $= 25^0C \pm 2^0C$; Dissolved oxygen = 6.3 mg/l ; and Total hardness = 65 – 90 mg/l was used to keep fish. The same water also served a control medium throughout the experiment.

b) Test Toxicant - Zinc sulphate, a salt of zinc was used as toxicant for present study.

c) Bio assay study - To study effect of toxicant $ZnSO_4$ on haematological parameters LC_{50} was determined for 24 hours, it was found to be 20.5 mg / l. The sublethal concentration of 6 mg of $ZnSO_4$ /l of water was selected. For haematological study fish were taken at 7 days, 14 days, 21 days and 28 days.

d) Haematological Studies -

1. Estimation of haemoglobin content: Haemoglobin content in the blood was estimated using Sahli's haemometer with permanent glass standards and expressed in gm/100 ml of blood.
2. Estimation of haematocrit: Haematocrit value of blood was estimated by centrifuging blood in heparinized haematocrit tubes at 7000 rev./min. for 30 minutes. From the volume of blood taken and packed cell volume after centrifugating, haematocrit percent was calculated.
3. Erythrocyte and leucocyte count: For enumeration of erythrocyte and leucocytes, haemocytometer with improved ruling was used. The number of erythrocytes and leucocytes was counted and represented in million cells per cubic millimetre of blood and thousand cells per cubic millimetre of blood respectively.
4. Estimation of mean corpuscular haemoglobin (MCH): The mean corpuscular hemoglobin content in µgm, was computed from the values of haemoglobin content and erythrocyte count using the formula-

$$MCH = \frac{Haemoglobin\ (gm/100ml)\ x\ 10}{Erythrocyte\ count\ (million\ cells\ per\ cubic\ mm\ of\ blood)}$$

Results and Discussion

The patterns of change in the blood parameter levels of zinc sulphate treated fish were found to be interesting (Table-1). Zinc sulphate caused an uniform reduction in haemoglobin content following all the period of exposure of the fish. The tremendous increase in erythrocyte count in the blood of 7 days zinc sulphate treated fish is noteworthy. It is 3.30 ± 0.29 million/mm^3in control fish and after 7 days of treatment it increased to 5.39 ± 0.12, but later on there was significant decrease in the erythrocyte count after 28 days. Heavy metals might alter the properties of haemoglobin by decreasing their affinity towards oxygen binding capacity (Witeska and Kosciuk, 2003). This process could deform cell by swelling and damage to erythrocytes. In addition, the perturbation RBCs may be attributed to a defense reaction against toxicity through the stimulation of erythropoiesis (Maheswaran *et al*., 2008). The fall in the data of haemoglobin content and erythrocyte count and occurrence of erythopenia in *Ophiocephalus punctatus* after the treatment with sublethal concentration of zinc sulphate has been in agreement with the observations of Ganesan and Karuppasamy (2015). The mean total number of leucocyte is estimated to be 8.95 ±2.5 thousand/mm^3. A significant rise in leucocyte count was observed after 7 days of zinc sulphate treatment but later on the number decreased but could not attend the number as in control fish. Increase WBC count in fish exposed to sublethal doses indicating leucocytosis which matches with the findings of Kori-Siakpere and Ubogu (2008). Leucocytosis has been considered to be an adaptation to meet stressful condition by the animal. During the present study, increase in number of leucocytes during the exposure of fish at 6 mg/l of zinc sulphate along with reduction in number of erythrocytes and haemoglobin percentage indicates dyshaemopoiesis mostly caused by bone marrow depression. Leucocytosis as evidenced in present investigation might be due to immunological reaction to produce more antibodies to cope with the stress induced by the toxicant. Such leucocytosis has also been reported by Raina and Sachar (2014).

Significant increase in haematocrit values could be observed in the blood of fish exposed to zinc sulphate during different periods of exposure. Haematocrit and haemoglobin are directly influenced by fluctuation of RBC (Soltani *et al.,* 2016). Zinc sulfate altered the shape and size of red blood cells by effecting the structure and function of cell membrane. The fish also showed significant drop in mean corpuscular haemoglobin (MCH) content after 7 and 14 days of zinc sulphate exposure followed by slight increase in the level in blood. Ganesan and Karuppasamy (2015) observed a low increase in the mean cell haemoglobin (MCH) which clearly indicates that the concentration of haemoglobin in the red blood cells were much lower in the exposed fish than in the control fish, thereby, depicting an anaemic condition.

Table-1: Haematological observations in *Ophiocephalus punctatus* after sublethal exposure to zinc sulphate.

Sr. No.	Blood Parameter	Control	Experimental			
			7 Days	14 Days	21 Days	28 Days
1	Hb content (gm/100ml)	15.50± 0.54	14.80±0.35	14.65± 0.37	14.58±0.12	14.10± 0.18

2	Erythrocyte count (million/mm^3)	3.30±0.29	5.39±0.12	2.08±0.11	2.02±0.08	1.04±0.09
3	Leucocyte count (thousand/mm^3)	8.95±2.5	15.16±3.6	12.98±1.8	10.52±0.90	9.72±2.6
4	Haematocrit (%)	6.68±0.39	11.62±1.89	11.83±0.56	13.58±0.40	15.62±0.33
5	Mean Corpuscular Haemoglobin (MCH)	34.03±2.51	8.92±0.70	23.67±2.13	36.05±5.2	42.05±2.6

Each value is the mean of 5 observations.

Conclusion

From the present result and about discussion it is clear that alterations in the haematological parameters are due to defense against toxic effect of zinc in fish *Ophiocephalus punctatus.* These haematological parameters can be used as indicators of zinc related stress in fish on exposure to elevated zinc levels in the water.

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