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RESEARCH ARTICLE

IMPACT OF ZINC ON HAEMATOLOGICAL PARAMETERS OF FRESHWATER FISH, *CHANNA PUNCTATUS* (BLOCH.)

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ABSTRACT

The effects of sublethal concentration (4.42 mg L-l) of zinc on haematological alterations were investigated in freshwater fish *Channapunctatus* for 20, 40 and 60 days respectively. Result of the treated groups showed significant decrease in RBC count, Hb content, packed cell volume and mean cell haemoglobin concentration when compared to the control with increase in exposure period. On the contrary, the WBC count, mean corpuscular volume and mean corpuscular haemoglobin concentration were significantly increased. Both decreasing and increasing haematological characteristics of blood cells of Zn toxicity are mainly with time dependent effects. The alterations of haematological profile in zinc exposed fish might be due to the pathological consequences like haemolysis, anaemia and haemodilution.

INTRODUCTION

Haemotological investigation is important in toxicological research because; haematological alternations are used for rapid evaluation of chronic toxicity of a compound. The constant exposure of fish to several types of heavy metals may induce changes to various haematological parameters (Heath, 1995). Which are frequently used to assess the metabolism and health status of fish (Adhikari *et al.,* 2004). Blood is a good bio-indicator; the diagnosis of Haematological profiles in fishes has been extensively used to predict the physiopathlogical alternations in different conditions of metallic stress (Nussey *et al.,* 1995). Haematological indices such as red blood cells count, white blood cells count, haematocrit, haemoglobin, so on are used as indicator of metabolic status of fish under heavy metal toxicity (Sancho *et al.,* 2000). Haematological parameters fluctuate with eco-physiological factors such as age, sex, body length, weight, temperature, salinity, maturity, oxygen tension, pH of water, respiratory metabolism, blood constituents and seasonal variations. Subathra and Karuppasamy (2006) have reported red blood corpuscles, haemoglobin concentration and packed cell volume values of *Mystusvittatus*in relation of length and weight.

According to Hrubee *et al.* (2001) haematological parameters of striped Bass also vary in relation to weight and seasons. Karuppasamy *et al.* (2005) suggested that the air breathing fishes (*Channapunctatus*) possess more amounts of RBC and Hb than the non-breathing fishes. Fresh water cat fish, *Clariasgariepinus* exposed to sublethal cone of Zn showed decreased in Hb, Hct, MCH and MCV and increased blood cell count (Oti and Avoaja, 2005).

Summarwar (2012) observed decline in Hb, RBC and PCV during initial phase of exposure compared to later phase in *Labeorohita* after the chronic expose to Zinc. The chronic exposure of Zinc to fish *Labeoboga* reported the alternations in the haematological parameters have ultimately become the causative for affecting the health status of the fish (Raina and Anupriya, 2014). However, not much work has been done on haematological parameters with respect to chronic exposure of zinc on fishes. Hence the present study was undertaken to analyses the impact of sublethal concentration of zinc on haematological parameters of *C. punctatus* under long-term exposure.

MATERIALS AND METHODS

Healthy adult fish *C. punctatu s* (13 ± 2 cm length and 22 ± 2 gm weight) were collected from freshwater bodies in and around Annamalainagar and then acclimated in laboratory condition for a period of 20 days. During captivity, the fish were fed with boiled egg and the water was renewed daily. The heavy metal Zinc in the form of Zinc Sulphate ($ZnSO_4$ – AR grade) was used in the present study. A sublethal concentration (4.42 mg L^{-l}) of zinc was found out according to the method of Litchifield and Wilcoxon (1949). The obtained

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concentration was used to experimental fish for 20, 40 and 60 days of exposure and simultaneously, the control was set side by side. The blood was collected by severing the caudal peduncle using a sharp knife for haemotological studies. The red blood corpuscles (RBC) and white blood corpuscles (WBC) were counted by Neubauer's improved haemocytometer using Hayem's and Tuerk's solution as a diluting fluid, respectively. The packed cell volume (PCV) or hematocrit values were measured by Wintrobe's method. For estimating hemoglobin (Hb), blood sample was treated with N/10 HCl and the colour of the acid hematin was matched with given standards using Sahli's, hemoglobinometer. The mean carpuscularhemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular volume (MCV) were calculated by following standard formulae (Dacie and Lewis, 1991).

$$MCV = \frac{PCV \times 10}{RBC / mm^3}\ fl$$

$$MCH = \frac{Hb(g/100\ ml^{-1}) \times 100}{RBC / mm^3}\ pg$$

$$MCHC = \frac{Hb(g/100\ ml^{-1}) \times 100}{PCV / 100 ml^{-1}}\ g/dl.$$

Statistical significance of differences between the control and treated groups of different exposure periods were tested by using ANOVA.

RESULTS

The results of the haematological parameters analyzed for the fish exposed to Zn are presented in Table 1. A significant decrease was observed in the RBC count, Hb, PCV and MCHC values of treated fish in all the exposure periods. There was also a significant increase in the total number of leucocytes (WBC), MCH and MCV values after 60 days of exposure. Statistical analysis indicated that all the values were significant at 5% level ($P < 0.05$).

DISCUSSION

Haematological investigations are used as an index of fish health status in various fish species to direct different stress conditions like diseases and exposure to heavy metal and pollutants (Blaxhall, 1975). The fish exposed to sublethal concentration of zinc in the present study showed remarkable Haematological variations. The result of the present investigation revealed that the Red Blood Corpuscles (RBC), Haemoglobin (Hb), Hematocrit (PCV) and MCHC values were significantly decreased after the exposure periods of 20, 40 and 60days when compared to the control with statistically significant difference ($P<0.05$). On contrast to this, the White Blood Cells (WBC); MCH and MCV levels were found to be increased significantly in experimental groups (20, 40 and 60 days) as compared to control groups.The significant reduction in the RBC count and Hb% of *C. punctatus* to sublethal concentration of Zn leads to anaemia. The low RBC count coupled with low haemoglobin content might be attributed to the destructive action of heavy metal Zinc on peripheral erythrocyte as a result of which the viability of the RBC may be affected (Karuppasamy, 2000). Anaemia under Zinc induced stress, might be due to erythrocytic injury and disrupted haemoglobin synthesis (Goel *et al.,* 1985). Similar results with significant reduction in RBC and Hb content in fishes exposed to metal are in good agreement with the earlier reports by Goel *et al.* (1985) and Goel and Sharma (1987).

The present investigations showed a significant decrease in the level of RBC count, Hb count and PCV% in experimental fish *C. punctatus* throughout the exposure periods (Table 1). The results recorded in the present study are in close agreement with the previous findings in vairous species. *O.mossambicus* (Sampath *et al.,* 1998); *Clariasgariepinus* and *Heteroclarias* (Oti and Avoaja, 2005); *Labeorohita* (Sammarwar, 2012); *Labeoboga* (Raina *et al.,* 2014) tested for chronic exposure of zinc. The significant reduction of erythrocytes might be secondary action of the toxicant, as the resulting from a primary action on erythropoietic tissues causing failure in RBC production and / or due to increase in the rate of erythrocyte destruction (Agarwal and Srivastava, 1980).

Table 1. Haematological parameters of *Channapunctatus*under sublethal concentration (4.42 mg L^{-1}) of Zinc

Parameters (Units)	Fish Group	Duration of exposure (day) 20	40	60	Significance of difference P < 0.05 within groups F-value
Total RBC count	Control	2.75 ± 0.231	2.72 ± 0.182	2.78 ± 0.273	a
(×10⁶/mm³)	Treated	2.24 ± 0.372	1.78 ± 0.813	1.54 ± 0.572	b
Total WBC count	Control	6.40 ± 0.581	6.46 ± 0.412	6.43 ± 0.712	a
(×10³/mm³)	Treated	7.19 ± 0.723	7.79 ± 0.813	8.64 ± 0.253	b
Haemoglobin (Hb)	Control	8.78 ± 0.194	8.82 ± 0.237	8.80 ± 0.375	a
(g/100 ml)	Treated	7.43 ± 0.274	6.12 ± 0.335	5.27 ± 0.672	b
Packed Cell Volume	Control	36.10 ± 1.142	36.02 ± 1.123	36.38 ± 1.214	a
(PCV%)	Treated	32.61 ± 1.127	28.45 ± 1.321	25.85 ± 1.021	b
(ml/100 ml)					
MCH (ρg)	Control	31.92 ± 0.235	32.42 ± 0.145	31.65 ± 0.527	a
	Treated	33.16 ± 0.438	34.38 ± 0.416	34.22 ± 0.325	b
MCHC	Control	24.32 ± 0.652	24.48 ± 0.282	24.18 ± 0.372	a
(g/dl)	Treated	22.78 ± 0.325	21.51 ± 0.324	20.38 ± 0.215	b
MCV (fl)	Control	131.27 ± 1.862	132.42 ± 2.721	130.86 ± 1.121	a
	Treated	145.80 ± 1.125	159.83 ± 2.562	167.87 ± 2.215	b

Mean ± SD (n = 6)
a – changes in period non-significant within group
b – changes in period significant within group

According to Goel and Kalpana (1987) the significant decrease in RBC count and Hb level resulting macrolytic anaemia in *H.fossilis* exposed to zinc, which supports the present findings. Pamila *et al.* (1991) pointed that the significant reduction in haemoglobin concentration in fish exposed to heavy metal could also be due to its inhibitory effects on enzyme system responsible for synthesis of Hb. Many haematological investigations reported that the sublethal exposure of heavy metal produced haemolytic anaemia could be attributed to the lysis of erythrocytes with concomitant decrease in Hb content and RBC count (Srivastava and Shashikala, 1979; Das *et al.,* 1987; Banerjee and Banerjee, 1988). The observed depictions in the haemoglobin level coupled with decreased RBC are obvious signs of anemia (Maheswaran *et al.,* 2008). Total WBC count (TLC) of the experimental fish *C. punctatus* showed a significant increase after of 20 days followed by further increment on the subsequent period (40 and 60 days) of exposure under Zn toxicity (Table1). A high WBC count indicates the increased production of WBC (Leukemia) followed by the damage of body tissues due to Zinc toxicity and severe physical stress. Similar findings were documented the increased WBC content in fish *Labeoboga* (Raina, 2014); *clariasgariepinus* (Oti and Avoaja, 2008); *Labeorohita* (Summarwar, 2012) exposed to zinc concentration. These similar results were also obtained from the different heavy metal tested for *C. punctatus* with lead (Hymavathi and Rao, 2000); copper and chromium by Singh *et al.* (2008) and cadmium (Karuppasamy *et al.,* 2005).

WBC count can also be affected by variety of physiological and environmental factors and the responses were found when fish are subjected to toxicant. According to Mahajan and Dheer (1979), the leuocyte showed greatest sensitivity to changes in the environment and the most important of WBC were lymphocytes Leucocytosis may be directly proportional to the severity of the causative stress condition may attributed to an increased leucoytes mobilization (Srivastava and Narain, 1998). According to Sampath *et al.* (1998) and Nair *et al.* (2000) Leucocytosis was considered as a immunological defense attributed to the presence of toxic substance or associated tissue damage. Hence in the present study Leucocytosis is evidenced by the increased WBC count and adaptive value of fish under Zn intoxication.

In the experimental fish the PCV values decreased significantly due to decline in Hb level and RBC count. Appreciable decline in such reduction in Hb, total RBC count and PCV parameters indicated the anaemic stage of fish manifested by the chronic introduction of heavy metal. PCV in the present study reflects the anaemic stage of fish (Karuppasamy *et al.,* 2005; Praveena *et al.,* 2013). The PCV values are the best indicator of the heavy metal stress on the health of fish and also used to determine the oxygen carrying capacity of blood (Larsson *et al.,* 1985). In the present study, the distinct decrease in the level of Haemoglobin and Haematocrit (PCV) in fish *C. punctatus* exposed to zinc clearly indicated a haemodilution mechanism possibly due to gill damage or impaired osmoregulation. In our results the decreased RBC count, Hb and PCV values are attributed to the impaired absorption of iron are in line with the Smit *et al.* (1979). The decreased RBC count, Hb and PCV values of the present results have been attributed to the anaemic state of fish under the stress of Zn may be inhibition of erthropoiesis of haemopoietic tissue and haemodillution.

The erythrocyte constants MCV, MCH and MCHC offer relationship on size, form and Hb constants of erythrocytes. They allow the determination of morphological anaemia that whether Normocyte, Macrocyte or Microcytic anaemia. The distinct decrease in the level of haemoglobin (Hb) and increase in the mean corpuscular volume (MCV) observed after the 20, 40 and 60 days of exposure clearly suggest that a haemodilution mechanism being operational. Increase in MCV and WBC counts also indicated that the anemia is of macrocytic type (Afaq, 2009). The mean corpuscular volume gives an indication of the status or size of the red blood cells and reflects abnormal or normal cell division during erythropoiesis. The increase in MCV may be attributed to the swelling of the erythrocytes. As a result of hypoxic condition or impaired water balance (Osmotic stress) or macrocytic anaemia in fishes exposed to zinc pollution. Haemodilution has been interpreted as a mechanism that reduces the concentration of an irritating factor in the circulatory system (Smit*et al.,* 1979). Tort *et al.* (1987) observed a process of erythrocyte swelling in the dog fish, *Scyliorhinuscanicula*exposed to copper. Such an increase of erythrocyte size is generally considered as a response against stress and would be consequences of several factors like high PCO_2 high lactate concentration or low PO_2 in the blood, leading to a low ATP concentration, which would increase the oxygen affinity of blood (Sovio and Nikinmaa, 1981). In this instance, it is difficult to ascribe the swollen red blood cells to one of these factors owing to the nature of the experiment and therefore, necessitates further elucidation.

In this study, a low increase in the mean cell haemoglobin (MCH) and decrease in mean cell haemoglobin concentration (MCHC), 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. Anaemia can be caused by a number of pathological conditions. In this instance, the change from a normochromic, microcytic condition after long term (60 days) exposure, leading to hypochromic, macrocytic type is recorded for the first time in Zinc exposure. These findings further support the hypothesis that haemodilution is a probable cause for decrease in Hb count in Zinc dosed fishes. The MCHC is a good indicator of red blood cell swelling followed by haemodilution (Wepener *et al.,* 1992). The MCHC which is the ratio of blood haemoglobin concentration as opposed to the haematocrit, is not influenced by the blood volume nor by the number of cells in the blood but can be interpreted incorrectly only when new cells, with a different haemoglobin concentration, are released into blood circulation. (Soivio and Nikinmaa, 1981). Buckley *et al.* (1976) reported that prolonged reduction in haemoglobin content is deleterious to oxygen transport and any blood dyscrasia and degeneration of the erythrocytes could be ascribed as pathological conditions in fishes exposed to toxicants. Moreover, the present study on Zn toxicant in fishes have revealed microscopic damage to gill tissues and ion regulatory disturbances of plasma characterized by plasma acidosis and concomitant hypoxia and

osmoregulatory, breakdown resulting in a net flux of water (Birchall *et al.,* 1989).

Hence, the alterations in the haematological indices i.e. increase in MCV, MCH and decrease of MCHC in the present study revealed the defense against toxic effect of zinc and in turn due to the decrease in RBC's, Hb and PCV and the disturbances occurred both in metabolic and haemopoitic activities in fish.

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