RESEARCH ARTICLE

Zinc induced histopathological and biochemical anomalies in the liver of fish *Ophiocephalus punctatus*

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ABSTRACT

The continuous discharge of effluent containing heavy metals and their compounds at an unprecedented and constantly increasing rate, even below permissible level from various industries into aquatic bodies may result in accumulation and subsequent magnification up to dangerous level because of their toxicity, water solubility and non- degradable qualities. Their tendency to accumulate in the organism and in undergoing food chain amplification, causing disorder in the aquatic ecosystem with deterious effect on biolife in unnaturally high concentration. The present study deals with the toxicity of zinc ($ZnSO_4$), as a component of industrial waste and its effect on liver of fish *Ophiocephalus punctatus*. The toxicity of zinc even at sublethal level causes histopathological disorganization of hepatocytes. The estimated protein concentration increased, whereas, glycogen and lipid content were found to be reduced in the liver during the exposure periods. These adverse effects of zinc toxicity reduce functional capacity of liver and caused reduction in nutritive value of fish significantly.

Key words: *Ophiocephalus punctatus*, zinc, liver, protein, glycogen, lipid.

INTRODUCTION

Zinc in traces is essential to sustain biological processes such as optimum body growth, development, reproduction and as immune stimulant. Its presence is essential for smooth working of various important enzymes like DNA and RNA polymerase, reverse transcriptase, alcohol dehydrogenase, sarbitol dehydrogenase, glucose -6- dehydrogenase etc. Its deficiency leads to retardation of growth, chronic renal disease, oligospermia, cessation of estrous and menstrual cycle in mammals. Zinc is an essential and beneficial element in human metabolism.

Despite being an essential trace element, Zn is toxic to most organisms above certain concentrations. (Ho, 2004). When fishes are exposed to great elevate level of metal in polluted aquatic ecosystem, they tends to take these metals up from their direct environment (Hoo *et al.*, 2004;

Charjan and Kulkarni, 2013). These heavy metal toxicants are accumulated in the fish through general body surface which affect severally their life support system at molecular biochemical levels. Once these toxic substance enters into body, they damage and weaken the mechanism concerned leading to physiological, pathological and biochemical disorders (Arasta *et al.*, 1999). Liver has been recommended by many authors as the best environmental indicator of both the water pollution and chronic exposure to heavy metals (Dural *et al.*, 2006; Agah *et al.*, 2009). Liver is an important organ of detoxification where break down of toxic substances is carried out by the endoplasmic reticulum of hepatocytes due to which the hepatic cells are damaged severely and became disorganized (Bhatkar, 2011).

MATERIALS AND METHODS

The fish, *Ophiocephalus punctatus* (Bloch), a common air breathing fresh water teleost, which are locally priced as food fish and abundant in various lakes near Amravati (Maharashtra state) were used in the present study. Fish weighing 20-25 gm and between 10-12 cm in length were purchased from local fish market. The fish were treated with 0.1 % $KMnO_4$ solution for 1 to 2 minutes to clear any dermal infection. They were maintained under laboratory condition in aquarium for acclimatizing them for seven days. They were fed with commercial feed. The water in the aquarium was changed daily to remove detritus.

a. *Water used* - Water used through out experiment was aged tap water. The physiochemical parameters of aged tap water were determined periodically (Table 1) as per standard method for examination of water and waste water (APHA, 1998). The same water also served a control medium throughout the experiment.

Table 1: Physiochemical properties of water used to keep fish, *Ophiocephalus punctatus*.

Sr. No.	Parameter	Range
1	PH	7.4 ± 0.5
2	Temperature	25°C ±2°C
3	Dissolved oxygen	6.3 mg/l
4	Total hardness	65 – 90 mg/l

b. Test Toxicant - Zinc sulphate ($ZnSO_4$), a salt of zinc was a toxicant for present study.

c. *Bio assay Study* - To study effect of toxicant $ZnSO_4$ on liver, 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 histopathological and biochemical study fish were taken at 7 days, 14 days, 21 days and 28 days.

d. *Histopathological Studies* - For histopathological study, tissue (liver) were fixed in aqueous Bouin's fluid. After proper fixation tissue were washed with running tap water, then dehydrated in different grades of alcohol, cleaned in xylene and finally paraffin blocks were prepared. Sections cut at 5µ were stained with haematoxylin, eosin stain.

e. *Biochemical Studies* - Protein, glycogen and lipids contents of liver were estimated in 7, 14, 21 and 28 days exposed fishes.

RESULTS AND DISCUSSION

Histological study appears to be a very sensitive parameter in determining cellular changes occurs in target organ like liver. Liver of *Ophiocephalus punctatus* consists of polygonal hepatic cells (Fig. 1).

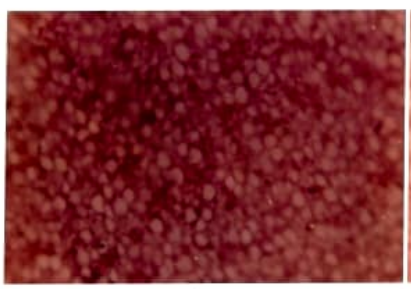

Fig. 1

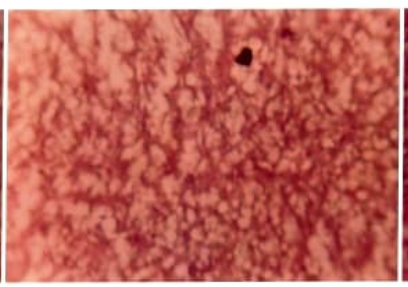

Fig. 2

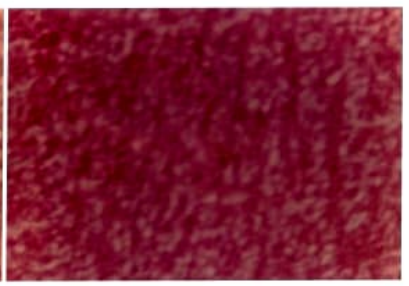

Fig. 3

Table 2 - The results of biochemical estimation of protein, glycogen and lipid in liver tissue of the *Ophiocephalus punctatus*.

Biochemical estimation	Control	Experimental			
		7 days	14 days	21 days	28 days
Protein	171.12 ± 2.5	183±3.5 (+7.03)	176.6± 5.2 (+ 3.22)	188.19 ± 4.1 (+ 9.97)	191.81 ± 1.6 (+ 12.09)
Glycogen	148.15± 1.6	116.18 ± 1.8 (−21.57)	90.10± 2.8 (−39.18)	62.24 ± 1.5 (−57.98)	58.72 ± 1.4 (−60.36)
lipid	0.18 ± 0.05	0.17 ± 0.01 (−5.55)	0.15 ± 0.02 (−16.66)	0.12 ± 0.02 (−33.33)	0.09 ± 0.01 (−50.00)

Each value (μg / mg wet tissue) is the mean of 5 estimations ($\pm SD$).
Values in parenthesis are percent change over control.

It also shows the sinusoids and sections of bile ducts and blood capillaries. Though the liver has no direct contact with the toxicant water, it is indirectly affected through its contact with blood.

After seven days of exposure, the nuclei of the hepatic cells appeared prominent and large (Fig. 2). Dark granular patches were observed in between the hepatocytes. Shrinkage of blood vessels, clumped erythrocytes and widely separated bile canaliculi were noticed. After twenty eight days of exposure, perilobular and centrilobular cirrhosis, cellular necrosis, proliferation of bile duct epithelial cells leading to the formation of new bile canaliculi were seen (Fig. 3).

Because of drastic changes in the liver histology after treatment of sublethal concentration of zinc sulphate, disorder and imbalance in the metabolic state of fish might have caused and hence that reflected in biochemical changes (Table 2).

In the present investigation, an increase in protein content was observed in liver of experimental fish. Liver exhibited 12.09% increase in protein after 28 days of exposure. It might be due to stimulation of protein synthesis to form detoxification enzymes. A significant decrease in glycogen and lipid content was noticed. After 28 days, the glycogen drastically reduced by 60.36% and lipid by 50.00%. This might be due to increased glycogenolysis to meet the excess energy demands imposed by the severe anaerobic stress of zinc intoxication. Among the fish organs, the liver and kidney appear to have a significantly higher tendency for the accumulation of most of the metals (Rauf *et al.*, 2009). Kumar *et al.* (2015) recorded a maximum of 151.12% elevation in zinc accumulation in liver of fish *Channa punctatus* exposed to 10mg/l concentration of $ZnSO_4$ for 28 days, over controls. Several degenerative changes in liver of fish, *Channa punctatus* due to acute toxicity of zinc sulphate were also observed by Avinashe and Farheen (2017).

Liver is the major metabolic center and any damage to this organ would subsequently do, so many physiological disturbances leading to subsequent mortality of fish (Saxena *et al.*, 2009). Through the food chain ultimately this metal-contaminated food reaches to the body of human beings. Long-term intake of Zn (150 – 2000 mg/day) induces sideroblastic anemia, leukopenia and hypochromic microcytic anaemia (Simon-Hettich *et al.*, 2001) and may face greater risk of health problems.

CONCLUSION

Thus, the toxicity of $ZnSO_4$ even at sublethal level may damage liver tissue to reduce functional capacity of liver and reduce nutritive value of the fish significantly.

Conflicts of interest: The authors stated that no conflicts of interest.

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