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Study of effect of Zn exposure on some biochemical parameters in air breathing fish, *Heteropneustes fossilis.*

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ABSTRACT

The present investigation has been designed to study the effect of heavy metal Zn on the blood glucose, plasma protein, cholesterol and enzyme activity in *Heteropneustes fossilis* after exposure of 24hrs, 48hrs, 72hrs and 92hrs. The present study shows that serum metabolites such as glucose, protein and cholesterol were increased while liver cholesterol and serum alkaline were increased. Thus, this study gives an overview of the manipulation of fish, *Heteropneustes fossilis* as a biomarker of heavy metals through alternation in biochemical parameters.

Key Words - Zinc, heavy metal, Blood glucose, Protein, Cholesterol, Liver

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INTRODUCTION

Zinc is an important element and is also considered one of the most frequent contaminants from heavy metals. an important element. Although it is an essential element acting as a structural component and having specific properties indispensable for life. Zn in larger quantities adversely affects the fish populations (Benoit & Holcombe, 1978; Saxena *et al*., 1993; Lloyd, 1960; Skidmore, 1964; Chapman & Stevens, 1978; Hughes & Flos, 1978; Banerjee & Mukherjee, 1994; Gupta & Chakraborti, 1993;). It enters the aquatic ecosystem through a variety of businesses, including those producing rubber, paint, ceramics, cosmetics, fertilizer, and textiles (Luckey & Venugopal, 1977). The quantity of heavy metals in the environment has further increased as a result of extensive urbanization and industry. Although the primary sites for direct contact stress are skin and gills, little is known about how Zn interacts with the gills of fish that breathe air. Similarly, to this, there is a lack of information regarding $ZnCl_2$ toxicity to air-breathing fish (Hemalatha and Banerjee, 1977). Zinc accumulates in the gills of fish and this indicates a depressive effect on tissue respiration leading to death by hypoxia. It can be said that the accumulation of it has attained a serious condition. Zn in certain concentrations is desirable for the growth of freshwater animals but its over-accumulation is hazardous to exposed fish as well as to those who consume them directly or indirectly through food chain. Aquatic organisms are exposed to unnaturally high levels of these metals. Fish are relatively sensitive to changes in their surrounding environment. Therefore, the aim of the present study it to investigate the different biochemical changes occurs in blood of *Heteropneustes fossilis* due to exposure of Zn.

MATERIALS AND METHODS

FISH AND THEIR MAINTENANCE

Healthy *H. fossilis* fishes with body weights of 35–40 g and lengths of 18–20 cm was collected from local pond. Fish were maintained in large glass aquaria for 14 days (d) for acclimation. They were fed daily with small pieces of fish and chicken liver.

EXPERIMENTAL PROTOCOL

50 fishes were divided into 5 groups were exposed to $ZnCl_2$. Five groups of 10 fish each were exposed to $ZnCl_2$ (7.5 ppm, 10% of 96 h LC50 value) for the estimation of sublethal toxicity after Hemalatha and Banerjee, 1977.

BIOCHEMICAL ESTIMATION

Blood glucose test was done by o-Toulidine method (Plumer, 1971). Plasma protein and Cholesterol were done by Lowry, 1951 method and Zak method (Zak, 1953) respectively.

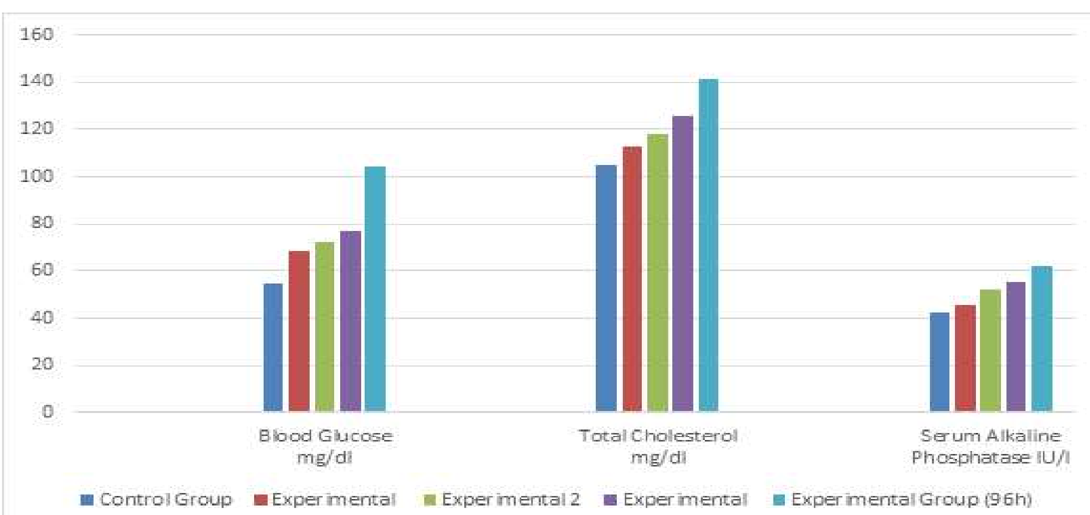
RESULT AND DISCUSSION

After 24h, 48h, 72h and 96h exposure, fish from the respective experimental as well as control groups were sacrificed and blood was collected from the ventricle of heart. Liver tissue was also collected for the estimation of liver cholesterol. 500mg tissue of liver was collected and homogenized in 5ml distilled water. Biochemical tests were done and results of experimental group fish were compared with controlled group.

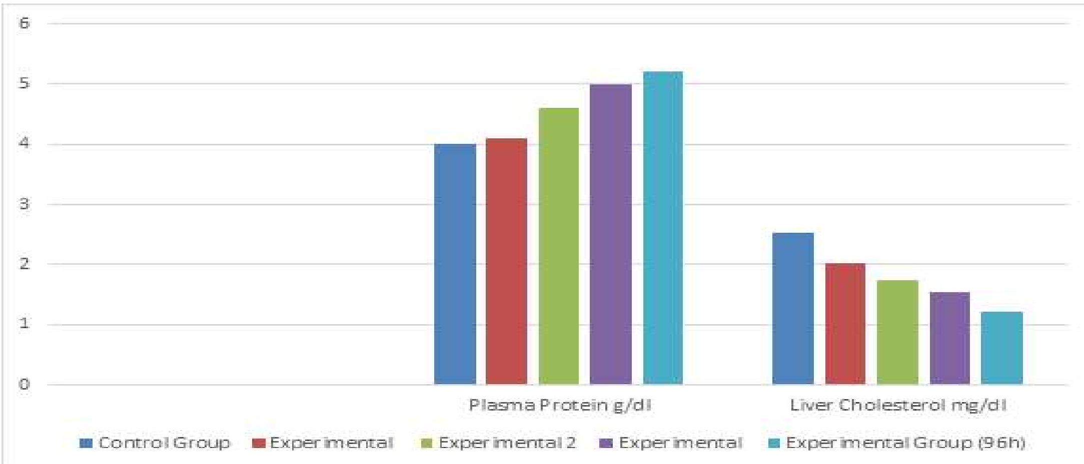
Table A: Change in values in the different blood parameters and Liver cholesterol in *Heteropneustes fossilis* exposed to different Zn concentrations for different durations.

Parameters	Control Group	Experimental Group (24hrs)	Experimental Group (48hrs)	Experimental Group (72hrs)	Experimental Group (96h)
Blood Glucose mg/dl	54.6±0.05	68.2±0.04	72.5±0.05	76.8±0.03	104.3±0.04
Plasma Protein g/dl	4.0 ± 0.02	4.1 ± 0.01	4.6 ± 0.02	5.0 ± 0.03	5.2 ± 0.01
Total Cholesterol mg/dl	105.2± 1.3	112.5 ± 1.5	118.3 ± 1.8	125.6 ± 1.9	141.2 ± 2.1
Liver Cholesterol mg/dl	2.53±0.13	2.02±0.19	1.74±0.13	1.53±0.09	1.21±0.06
Serum Alkaline Phosphatase IU/l	42.5 ±1.9	45.7 ±2.3	52.2 ±2.2	55.7 ±2.5	61.9 ±3.1

Values are expressed as mean ± SD (n=10 fish per treatment).



Graph 1- Graphical representation of change in values in the different blood parameters in *Heteropneustes fossilis* exposed to different Zn concentrations for different durations.



Graph 2- Graphical representation of change in values in the plasma protein and Liver cholesterol in *Heteropneustes fossilis* exposed to different Zn concentrations for different durations.

In control group the blood glucose was recorded 54.654.6±0.05 mg/dl but glucose level was gradually increases in controlled group, 68.2±0.04mg/dl after 24hrs treatment, 72.5±0.05 mg/dl, 76.8±0.03 and 104.3±0.04 mg/dl after 48hrs, 72hrs and 96hrs of treatment respectively. It is found that the blood glucose level rises after treatment with Zn. The plasma protein level and total cholesterol level of *Heteropneustes fossilis.* were also increases with the treatment of Zn whereas, liver cholesterol decreased with the increase of Zn exposure period. Serum Alkaline phosphatase activity also increases with the increase of the Zn exposure period.

It is well known that animals and plants both require zinc. However, in high concentrations, it has negative consequences by causing structural damage, which interferes with the fish's ability to grow, develop, and survive (Tuurala and Soivio, 1982). Zinc alters tissue respiration, which causes hypoxia-related mortality. Additionally, it alters the physiology of the heart and veins. The haematocrit and haemoglobin levels of *Heteroclarias* sp. significantly dropped after exposure to sublethal zinc doses. Annune *et al.* (1994b) have reported a comparable decrease.

With Zn exposure, plasma proteins were shown to decline in the current investigation. This may be related to liver disease, decreased protein synthesis, or renal excretion (Kori-Siakpere, 1995). On the other hand, the observed reduction in plasma protein levels may also be due to the protein's first breakdown into amino acids and subsequently into nitrogen and other simple molecules. Following exposure to chlordane, a similar decrease in protein has also been noted in *Saccobranchus fossils* (Verma *et al*., 1979). In present study blood glucose level was decreased but in the study of Kori-Siakpere *et al*., 2008 reduction of plasma glucose was observed. It was found to be significant at low concentrations of zinc. Suggested changes in carbohydrate metabolism occur in fish exposed to various sublethal concentrations of pollutants. Blood glucose has been employed as an indicator to environmental stress (Silbergeld, 1974).

Increased blood glucose level may be due to the mobilization of glycogen and the onset of hyperglycemia is typically linked to the rise in blood sugar. The hyperglycaemia response varies with the nutritional status of the fish (McLeay, 1977). It is not known whether zinc exposure affects glucose reserve directly or indirectly via other internal factors. The kidneys are the most likely location for glucose loss, which may mean that the energy-dependent glucose retention in kidney tubules has been suppressed. *Pontius conchnius* exposed to mercury nitrate (Gill and Pant, 1981) and *C.*

isheriensis (Sydenham) exposed to water borne lead both showed decreased glucose absorption (Kori-Siakpere, 1995).

In ecotoxicology, serum enzymes are sensitive biomarkers because they can detect potentially harmful changes in contaminated freshwater ecosystems early on. In the present study increase in Serum alkaline phosphatases level were observed in zinc exposed *Heteropneustes fossilis* as compared to control groups.

Hydrolytic enzymes like Alkaline phosphatase (ALP) plays an active role in the biosynthesis of fibrous protein; stimulation or inhibition of these enzymes will cause metabolic disturbance (Sanisa *et al*., 1982). Jiraungkoorskul *et al*., (2003) showed that the change in ALP activity was a result of physiological and functional alteration in metal-exposed fish. Hadi *et al*., (2009) also advocated alteration in enzymatic activities of fish under stress of xenobiotics.

CONCLUSION

A potential threat to aquatic organisms is the presence of significant quantities of heavy metals in the aquatic environment as a result of industrial, agricultural, and human activities. It is possible to draw the conclusion that the heavy metal Zinc caused significant biochemical changes in the *H. fossilis* based on the findings and analysis presented here. Aquatic ecosystems with high Zn concentrations changes in the metabolism of biomolecules like lipid, protein, and carbohydrates in *H. fossilis.* The hepatic tissue is damaged by metal toxicity, which releases these enzymes (phosphatases) into the bloodstream and may cause their concentration to rise.

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