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**Research Article**

# EFFECT OF ZINC ON ENZYME ACTIVITY IN FRESHWATER FISH GONOPROKTOPTERUS KOLUS (SYKES)

**Karanjkar D. M. and V. Y. Deshpande**

Department of Zoology and Fisheries, Yashavantrao Chavan Institute of Science, Satara

**A R T I C L E I N F O**

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Zinc, Gonoproktopterus kolus, Intestine and intestinal bulb.

**A B S T R A C T**

The present study is aimed to investigate the effect of Zinc on protease, amylase and lipase activity in the intestinal bulb and intestine of freshwater fish Gonoproktopterus kolus (Sykes) after acute exposure. During experiment fishes were exposed to predetermined LC0 (2.430 ppm) and LC50 (4.860 ppm) concentrations of the Zinc sulphate for 96 hours. The effect of heavy metal showed decrease in protease, amylase and lipase activity in the intestine of fish. The significant alternations showed toxic effect of heavy metal at enzymatic level. The results are discussed with available literature.



## INTRODUCTION

In agricultural revolution there is increased use of metal based fertilizers that could result in the rise in the concentration of metal pollution in freshwater due to run-off. (Ibrahim *et al*., 2016). The effect of heavy metal on aquatic organism is currently widespread attention, particularly in studies related to pollution. With an early use of metals, there was little concern about environmental contamination. However, salts of the metal began to find their way into commercial and industrial applications, then it became evident that metallic salts posses certain biocidal properties. Though, many metals play vital role in the physiological processes of plants and animals and humans, yet excess concentration of metals is harmful. Pollution implies deleterious effects and is usually assessed in relation to biological system.

Heavy metals are the most harmful pollutants owing to their diverse effects. Living organisms readily absorb some metals which are soluble in water. High toxicity of metal ions are known to cause deleterious impact on organs and blood level in fish (Akahori *et al*, 1999 and Karan, *et al*, 1998 ). Heavy metals have been recognized as serious pollutants of the aquatic environment. It has been reported that heavy metals are extremely toxic to fish and other aquatic organisms (Shewta *et al*., 2012).Study of heavy metal toxicity to fish, showing changes in enzymatic components such as protease, amylase and lipase helps in understanding their correlation with ability to overcome toxic effects and their changes induced by heavy metal toxicity. Hence, in the present study, attempts have been made to find out the toxic effects of Zinc at acute exposure on the enzymatic changes in the intestinal bulb and intestine of freshwater fish *Gonoproktopterus kolus.*

**Corresponding author:* **Karanjkar D. M**
Department of Zoology and Fisheries, Yashavantrao Chavan Institute of Science, Satara

## MATERIALS AND METHODS

Live fingerlings of 7-8 cm in length and 10 - 12 gm in weight were collected from the Krishna river near Satara. Fishes were brought to the laboratory and stocked in rectangular glass aquaria of dimension 45 cm x 22cm x30cm and capacity of 25 liters for acclimatization. Feeding was stopped 24 hr. prior to the exposure of the fish to the metals. The well acclimatized fishes to the laboratory condition were used for experimentation. Pilot experiments were carried out to find out the toxic range (ppm) of the metal. Group of 10 fingerlings were held separately in plastic container of 20 litres capacity containing metals of different concentrations making total volume of 10 litres. Zinc sulphate of analytical grade was used as a source of heavy metal. The experiment were conducted for 96 hr while studying the toxicity of the metal. A control group without toxicant was run simultaneously. The experiments were conducted in the natural day light rhythm. The test concentrations from the experimental containers were renewed after every 24 hour. The mortality rate and behaviour of fishes were recorded before each change of the water from the plastic container. The toxicity tests were repeated thrice and values for 96 hr $LC_0$ and $LC_{50}$ were determined. After acute toxicity (96 hr.) experiments, alive fishes were immediately sacrificed (5from each group) from control, $LC_0$ and $LC_{50}$ group separately to

obtain intestinal bulb and intestine. The pooled samples of these organs were properly cleaned, blotted, weighed and used for estimation of protease by Eguchi and Iwamoto (1982), amylase by Bernfield (1955) and lipase by Hayashi and Tapple (1970) methods. The enzymatic analysis was repeated for five times and mean values of five readings were expressed in terms of specific gravity in mg/ gm protein / hour. The values were recorded and compared with control and percent changes were calculated for presentation of data. Results of the study were statistically analyzed and level of significance were determined by applying Students "t" test.

## RESULTS AND DISCUSSION

Physiochemical parameters of water used for holding the fish were – Temperature (25.8 – 27.8 °C, pH – (7.3 – 8.2), Dissolved Oxygen (4.5 5.5 mg/Lit.) and hardness (64 – 82 mg/Lit.). The observed $LC_0$ and $LC_{50}$ values for zinc sulphate were 2.430 and 4.860 ppm respectively.

### *Protease*

### *Control*

The specific protease activity in intestinal bulb and intestine was 0.326 and 0.762 mg/Tyrosine / gm protein /hour respectively.

### *Experimental*

Changes in protease activity in the intestinal bulb and intestine of fish *Gonoproktopterus kolus* due to zinc sulphate for 96 hours are shown in Table No. 1. & Fig. No. 1

**Table No 1** Effect of Zinc Sulphate on Protease activity in intestinal bulb and intestine of fish Gonoproktopterus kolus after acute exposure (Enzyme activity in mg Tyrosine/gm protein / hr).

| Organ | Control | Zinc Sulphate | |
|---|---|---|---|
| | | $LC_0$ (2.430 ppm) | $LC_{50}$ (4.860 ppm) |
| **Intestinal bulb** | 0.326 ±0.0769 | 0.305 ±0.0586 -6.44 N S | 0.288 ± 0.0936 (-11.65) * |
| **Intestine** | 0.762 ±0.0860 | 0.707 ±0.1387 (-7.21) N S | 0.658 ± 0.0964 (-13.64) * |

* P<0.05, **P<0.001, NS – Non Significant, Values in parenthesis are percentages,± - Standard deviation of five animals.

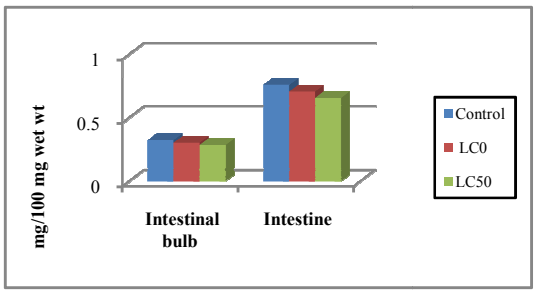


**Fig. No 1** Effect of Zinc Sulphate on Protease activity in intestinal bulb and intestine of fish *Gonoproktopterus kolus* after acute exposure(Enzyme activity in mg Tyrosine/gm protein / hr).

The specific enzyme activity in the intestinal bulb was non-significantly decreased in $LC_0$ and significantly (P< 0.05) decreased in $LC_{50}$ concentrations of zinc sulphate. The percent depletion was 6.44 and 11.65 for $LC_0$ and $LC_{50}$ respectively. There was non-significant decrease in the enzyme activity of intestine 0.707 in $LC_0$ group and significant (P<0.05) decrease in $LC_{50}$ concentration. The percent depletion was 7.21 and 13.64 respectively. In general there was increase in protease activity of intestinal bulb in $LC_0$ concentration and decrease in $LC_{50}$ group. But there was decrease in the enzyme activity of intestine in both $LC_0$ and $LC_{50}$ group as compared to control.

### *Amylase*

***Control:*** Specific amylase activity in the intestinal bulb and intestine of freshwater fish *Gonoproktopterus kolus* was 3.808 and 3.760 mg maltose / gm protein / hour respectively.

### *Experimental*

Changes in amylase activity in the intestinal bulb in fish *Gonoproktopterus kolus* due to Zinc Sulphate for 96 hours are shown in Table No. 2 & Fig. No. 2.

**Table No 2** Effect of Zinc Sulphate on Amylase activity in intestinal bulb of fish *Gonoproktopterus kolus* after acute exposure (Enzyme activity in mg Maltose /gm protein / hr).

| Organ | Control | Zinc Sulphate | |
|---|---|---|---|
| | | $LC_0$ (2.430 ppm) | $LC_{50}$ (4.860 ppm) |
| **Intestinal bulb** | 3.808 ± 0.1275 | 3.016 ± 0.1937 (-20.79) * | 2.464 ± 0.1986 (-35.29) * * |
| **Intestine** | 3.76 ± 0.1943 | 2.384 ± 0.1488 (-36.59) * * | 2.344 ± 0.2946 (-37.65) * * |

* P<0.05, **P<0.001, NS – Non Significant, Values in parenthesis are percentages,± - Standard deviation of five animals.

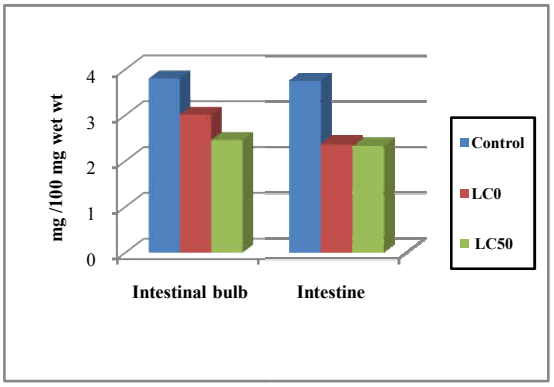


**Fig. No 2** Effect of Zinc Sulphate on Amylase activity in intestinal bulb of fish *Gonoproktopterus kolus* after acute exposure. (Enzyme activity in mg Maltose /gm protein / hr).

The enzyme activity was significantly (P<0.05) decreased in intestinal bulb of fish exposed to $LC_0$ group of Zinc sulphate. In $LC_{50}$ group their was more significant decrease (P<0.001) in enzyme activity. The percent depletion in $LC_0$ and $LC_{50}$ concentrations was 20.79 and 35.29 respectively. In intestine their was more significant decrease (P<0.001) in the enzyme activity in $LC_0$ and $LC_{50}$ group. The percent depletion $LC_0$ and $LC_{50}$ was 36.59 and 37.65 respectively. In general the decrease in amylase activity was more in $LC_{50}$ group than in $LC_0$ group in both the organs as compared to control.

### *Lipase*

***Control:*** The specific lipase activity in intestinal bulb and intestine of *Gonoproktopterus kolus* was 1.728 and 0.922 mg palmatic acid /gm protein / hour respectively.

***Experimental***

Changes in lipase activity in intestinal bulb of *Gonoproktopterus kolus* after acute exposure (96 hours) to zinc sulphate are shown in Table No. 3. & Fig. No. 3.

**Table No 3** Effect of Zinc Sulphate on Lipase activity in intestinal bulb of fish *Gonoproktopterus kolus* after acute exposure (Enzyme activity in mg Palmatic acid /gm protein / hr).

| Organ | Control | Zinc Sulphate | |
|---|---|---|---|
| | | $LC_0$ (2.430 ppm) | $LC_{50}$ (4.860 ppm) |
| Intestinal bulb | 1.728 ± 0.3643 | 1.684 ± 0.0395 (-2.54) N S | 1.694 ± 0.0706 (-1.96) N S |
| Intestine | 0.922 ±0.3924 | 0.795 ±0.0703 (-13.77) * | 0.76 ± 0.0545 (-17.57) * |

* P<0.05, **P<0.001, NS – Non Significant, Values in parenthesis are percentages,

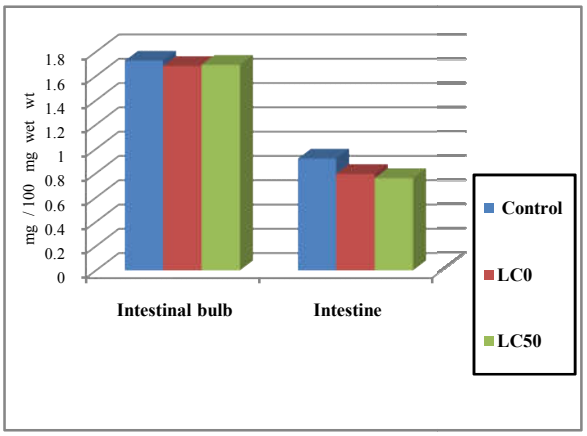


**Fig. No. 3** Effect of Zinc Sulphate on Lipase activity in intestinal bulb of fish *Gonoproktopterus kolus* after acute exposure. (Enzyme activity in mg Palmatic acid /gm protein / hr).

Their was non significant decrease in the lipase activity of intestinal bulb in $LC_0$ and $LC_{50}$ concentration of zinc sulphate. The percent depletion was 2.54 and 1.96 respectively. In intestine there was significant (P<0.05) decrease in lipase activity in $LC_0$ and $LC_{50}$ concentration. The percent depletion was 13.77 and 17.57 respectively. In general, their was non significant decrease in lipase activity in intestinal bulb and significant decrease (P<0.05) in intestine at $LC_0$ and $LC_{50}$ group respectively.

## DISCUSSION

Kotorman (2000) studied the effect of heavy metals (Cd2+, Cu2+, Pb2+ and Zn2+) on activities of carp trypsin, alpha-chymotrypsin, carboxypeptidase A and lipase. Maximum reduction was registered for both the enzymes, when the fishes were treated with single heavy metal, Cd (0.06 ppm), , in accordance with our findings. Combination of heavy metals were found to have severe toxic effects than single heavy metal treatment. Likewise, in our study also, Zn showed maximum reduction of protease and amylase enzyme activity. Even high concentrations of heavy metals can result in enzyme inhibition (Gould and Karolus, 1974). Depending upon the concentration of the heavy metals, they can produce both beneficial and adverse effects by binding with sites other than the active sites of the enzyme molecule, (Eichhorrn 1975) .

Inhibitory action of the heavy metals on enzyme is due to the binding of metals with enzyme protein was reported by (Hodson, 1988). Heavy metal ions can displace metals situated at the active site of the enzyme and inhibit the enzyme activity. Dinodia *et al*. (2003) also investigated the effects of Cd toxicity on proteolytic enzyme activity in fresh water carps *Cirrhinus mrigala* and it was reported that proteolytic enzyme activity declined maximally from 26% at 2.5 ppm to 55.60% at 5.0 ppm.

The present study thus revealed that even allowable concentrations of Zinc in aquatic environments could considerably reduce enzyme activity in the aquatic fauna; and the metal acted, in consonance with the findings of Samanta *et al*. (2010). This may, in turn, disturb the electrolyte balance in fish body, resulting in difficulties in osmoregulation and thus challenging survival in the polluted water bodies. It is hence recommended to prevent the water bodies from getting polluted and ban the waste discharge so as to provide a healthy environment for aquatic fauna to flourish.

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