



Effect of Sub-lethal Doses of Zinc Chloride on Antioxidant Enzyme Activity of *Cirrhina mrigala*

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ABSTRACT

Three groups of *Cirrhina mrigala* (one year old) were exposed, separately, to sub-lethal concentrations (96-hr LC₅₀, 2/3rd of LC₅₀, 1/4th of LC₅₀ and 1/5th of LC₅₀) of ZnCl₂ in glass aquaria along with the control group. After 30 days exposure, the fish were sacrificed and peroxidase activity in liver and kidney were determined. The peroxidase activity in both organs of ZnCl₂ exposed fish were compared with control. The physico-chemical parameters of the experimental media viz. pH, dissolved oxygen, carbon dioxide, total hardness, calcium, magnesium and total ammonia were also monitored twice a day. The results showed that the peroxidase activity was significantly increased in both the organs as a result of ZnCl₂ exposure in all the treatments than control. The peroxidase activities in all treated and control fish groups were found lower in kidney than in the liver.

Keyword: *Cirrhina mrigala*, Zinc, Sub-lethal, Peroxidase activity, Organs.

INTRODUCTION

The natural aquatic environments are being polluted by the disposal of untreated effluents released from sewage, agricultural and industrial sources. Among aquatic pollutants, there existed organic and inorganic compounds such as combustible substances, petroleum products, phenols, textile dyes and heavy metals. Metals are important ecological pollutants that may cause mutagenic and cytotoxic effects in the aquatic organisms [1]. Fish are more vulnerable to the harmful impacts of pollutants as they can uptake heavy metals from the surrounding environment and accumulate them in various organs and tissues resulting into biomagnification in the food chain making the fish an important indicator of metallic ions pollution [2].

Essential heavy metals like zinc, chromium, copper, nickel, molybdenum, iron and cobalt play an important role in several biological processes. However, the essential metals, when found at higher concentrations, can cause toxic effects on the organisms [3]. Zinc is widely used in solar panels, paints, semiconductors and waste water treatment plants [4]. It is an essential trace element and a structural component of many enzymes. For metabolism and normal development, small quantities of zinc are required upto permissible limits. But when the concentration of zinc exceeds beyond the physiological requirement of the fish body, it becomes toxic [5]. At higher concentrations, ZnCl₂ can induce oxidative damage and antioxidant disturbances in the tissues of fish which ultimately lead towards inhibition of glutathione reductase along with an increase in antioxidant defense, as an adaptive response [6].

The organisms have developed antioxidant defense system to protect their cells against oxidative damage caused by heavy metals [7]. Oxidative stress is an imbalance between the production of reactive oxygen species (ROS) and antioxidant defense system of the cell. Highly reactive ions, molecules or compounds are formed by incomplete reduction of one electron of oxygen that induces alterations in the physiological responses of the living organism. As a consequence of metallic ions exposure, formation of reactive oxygen species is enhanced that may cause peroxidation of lipids, proteins, DNA and even apoptotic cell death [8]. Elevated levels of heavy metals, like

zinc, in the tissues of fish can produce reactive oxygen species (ROS) such as hydrogen peroxide, superoxide and hydroxyl radicals [9]. Among the antioxidant enzymes, peroxidase protects the tissues from damage caused by harmful effects of H₂O₂ as well as lipid peroxidation [10]. The liver plays an important role in regulating the metabolism of the body, involving detoxification of xenobiotics and is the first target of ingested oxidants. It serves as an important tissue in studying the role of GSH-Px in the protection from lipid peroxidation [11]. In addition to this, fish kidney is pivotal organ, involved in osmoregulation, detoxification and excretion of xenobiotics [12]. Metallic ions released from various industries are discharged untreated in the riverine systems of Pakistan causing adverse effects on the indigenous fish fauna, especially major carps and their populations that are continuously declining in the natural aquatic habitats [13]. In the field of ecotoxicology, the use of oxidative stress biomarkers is gaining importance because they provide an early warning to the potentially hazardous substances in the aquatic environments. Therefore, the present study was conducted to determine the effect of sub-lethal doses of ZnCl₂ on antioxidant enzyme activity in the liver and kidney of *Cirrhina mrigala* under controlled laboratory conditions.

MATERIALS AND METHODS

The proposed research work was conducted in the laboratories of Fisheries Research Farms, Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad. One year old *Cirrhina mrigala* were procured from the ponds and brought to the laboratory for two weeks acclimatization in cemented tanks. Fish were fed with pelleted feed (30 % DP and 3.00 Kcalg⁻¹ DE) twice daily. After acclimation period, healthy fish of similar weights and lengths, were selected for these experiments. Pure chloride compound of zinc (ZnCl₂) was dissolved in 1000 mL deionized water for the preparation of metal stock solution. Three fish groups (n = 10) were transferred to the glass aquaria of 50-L water capacity to check the effect of ZnCl₂ on peroxidase activity in the selected tissues viz. liver and kidney of *Cirrhina mrigala*. Fish were exposed to 96-hr LC₅₀ of zinc chloride (56.67±3.41 mgL⁻¹) as determined by [14] and 2/3rd, 1/4th and 1/5th of 96-hr

LC₅₀ values, separately, for 30 days in glass aquaria. Each test was conducted with three replications for each test dose along with a control group (un-stressed). Water pH and dissolved oxygen were monitored on 12-hr basis by using digital meters viz. HANNA HI-8424 and HI-9146 while carbon dioxide, total hardness, calcium, magnesium and total ammonia contents of water were determined by following the methods of [15]. After 30 days exposure period, fish were sacrificed and their tissues viz. liver and kidney were isolated for the enzyme assay.

Peroxidase Assay

Red blood cells were removed from the liver and kidney by rinsing these organs with phosphate buffer of pH 6.5 (0.2 M) and homogenized in cold buffer (1:4 W/V) using a blender. After homogenization, both the organ's homogenate was centrifuged for 15 minutes at 10,000 rpm at 4 °C, separately. After centrifugation process, clear supernatant was preserved at -4 °C for enzyme assay while residue was discarded. For the determination of peroxidase activity, the sample was subjected to enzyme assay [16]. Activity of peroxidase was assessed by measuring the conversion of guaiacol to tetraguaiacol, spectrophotometrically, at a wavelength of 470 nm. The required reagents for enzyme assay were 0.2 M phosphate buffer (pH 6.5), guaiacol and hydrogen peroxide. Guaiacol (750 µL) was added to phosphate buffer (47 mL) and mixed well on vortex agitator. After agitation H₂O₂ (0.3 mL) was added to buffer solution. The reaction mixture contained buffered substrate solution (3 mL) and enzyme extract (0.06 mL).

A cuvette containing 3 mL of blank (phosphate buffer) solution was inserted into the spectrophotometer and set it to zero. Then a cuvette containing buffered substrate solution was placed in the spectrophotometer and initiation of reaction was occurred by adding enzyme extract. The reaction time was 3 minutes and the absorbance was observed after the specified time.

The peroxidase activity was calculated by using following formula:

$$\text{Activity (Unit / mL)} = \frac{\Delta A / 3}{26.60 \times 60 / 3000}$$

Statistical Analyses

The data were subjected to statistical analyses by following

Table 1: Peroxidase activity (UmL⁻¹) in the liver and kidney of *Cirrhina mrigala* after chronic exposure of ZnCl₂

Organs	96-hr LC ₅₀	2/3 rd of LC ₅₀	1/4 th of LC ₅₀	1/5 th of LC ₅₀	Control	Overall Means±SD
Liver	0.564±0.009 a	0.424±0.005 b	0.396±0.006 c	0.244±0.002 d	0.126±0.002 e	0.305±0.004 a
Kidney	0.146±0.002 a	0.088±0.002 b	0.062±0.002 c	0.043±0.001 d	0.021±0.003 e	0.072±0.002 b
Overall Means±SD	0.355±0.005 a	0.256±0.003 b	0.229±0.003 c	0.143±0.001 d	0.073±0.002 e	

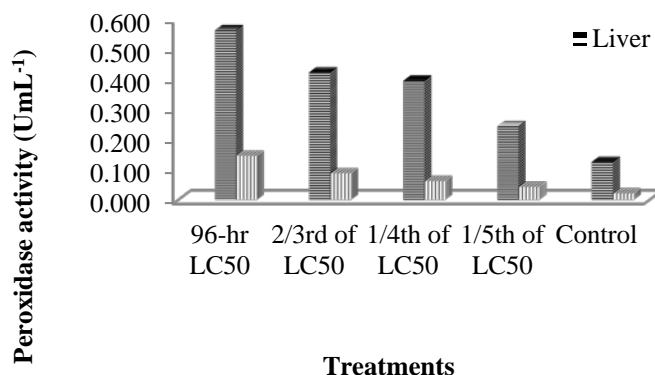


Figure 1: Peroxidase Activity (UmL⁻¹) of Liver and Kidney of *Cirrhina mrigala* at Different Treatments of ZnCl₂

Factorial experiments, with three replications for each test concentration, in order to evaluate the variations in the enzyme activity due to different exposure concentrations of zinc chloride. The means were compared by using Duncan's Multiple Range test.

RESULTS AND DISCUSSION

During present research work, statistically significant differences at P<0.05 were found among all the exposure treatments and the organs. Comparison of means revealed that activity of enzyme "peroxidase" in both liver and kidney of *Cirrhina mrigala* increased significantly due to zinc chloride treatments than that of the control group (Table 1; Figure 1). In the liver of *Cirrhina mrigala*, significantly highest activity of peroxidase was observed at 96-hr LC₅₀ as 0.564±0.009 UmL⁻¹, followed by 2/3rd of LC₅₀, 1/4th of LC₅₀, 1/5th of LC₅₀ and control, for which the peroxidase activities were found as 0.424±0.005, 0.396±0.006, 0.244±0.002 and 0.126±0.002 UmL⁻¹, respectively. The peroxidase activities in the kidney of *Cirrhina mrigala* were found in the order: 96-hr LC₅₀ > 2/3rd of LC₅₀ > 1/4th of LC₅₀ > 1/5th of LC₅₀ > control. The sub-lethal exposure of Zn to African catfish (*Clarias gariepinus*) and Nile tilapia (*Oreochromis niloticus*) significantly caused an elevation in the level of enzyme peroxidase [17]. The present results are in conformity to the findings of [18]. They reported that activity of peroxidase increased in the liver and kidney of African catfish (*Clarias gariepinus*) with increasing concentration of Zn as compared to the control fish. It was also observed during present experiment that, after exposure of ZnCl₂, the peroxidase activity was more pronounced in fish liver as compared to the kidney indicating quick response of the liver peroxidase in preventing the cells against oxidative stress caused by zinc chloride (Figure 1). Alkaladi *et al.* [19] reported that Zn can induce significant variations in the activity of enzyme peroxidase in the liver of Nile tilapia (*Oreochromis niloticus*). The present results are also in accordance with the findings of [20]. They found that exposure of Zn resulted into increased production of ROS in the liver of *Oreochromis niloticus* and ultimately the enzyme activity was also increased in order to overcome the oxidative stress.

CONCLUSION

In the liver and kidney of *Cirrhina mrigala*, the activity of antioxidant enzyme “peroxidase” was found significantly increased after exposure to various concentrations of ZnCl₂. Among both the selected organs, statistically significant and higher peroxidase activity was observed in the fish liver as compared to kidney.

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