

Research Article

Sublethal Toxicity of Zinc Chloride on Antioxidant Enzyme activity of *Catla catla* (Hamilton)

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Abstract: Fishes are extensively used for the assessment of the health of the aquatic ecosystem as their enzymes serve as pollution biomarkers. Heavy metals are one of the major pollutants discharged to the aquatic ecosystem as industrial effluents. The present study determined the alteration in enzyme activities in different tissue of *Catla catla* exposed to sublethal concentrations of zinc chloride. *Catla catla* were exposed to different concentration of zinc chloride and control were maintained without the zinc chloride. After 96 hour exposure, brain and muscle tissue from both control and experimental group were selected for enzyme assay. The biochemical studies show that acetylcholinesterase and catalase activity in both brain and muscle tissue were reduced after exposure, whereas glutathione S-transferase activity in both tissues were increased. Heavy metal pollution causes detrimental effects on fishes and constitutes potential risks for human health through consumption of contaminated fishes.

Keywords: Heavy metal, Zinc chloride, Antioxidant enzymes, *Catla catla*.

1. Introduction

Heavy metals are the major pollutant in the aquatic ecosystem because of their persistence, toxicity and tendency to accumulate in food chains. The previous studies show that the contamination of metals is a serious pollution problem for aquatic organisms. These accumulated heavy metals in food chain are absorbed by all aquatic organisms from lower to higher trophic level [1]. Though many metals play an important role in plants, animals and human physiological processes, yet the excess concentration of metals is harmful. Zinc is an important micronutrient for aquatic life and zinc compounds have relatively high solubility. But it can be a potential toxicant by interfering metabolism when its concentration becomes higher than the required amount. Zinc may be transported to aquatic ecosystem as a result of both natural (weathering and erosion) and anthropogenic (industrial and agricultural) activities. Upon entrance in body, metals change the genetic makeup and biochemistry of the living organisms. These changes include oxidative stress, DNA damage and anti-oxidative defense system indicating the level of pollution in the aquatic environment [2,3]. The direct toxic effect of zinc on fish was to precipitate the mucus layer on the gill surface, causing suffocation [4,5].

Fishes are extensively used for the assessment of the health of aquatic ecosystem and physiological changes serve as biomarkers of environmental pollution [6]. Fishes as bioindicators of toxicant effects are being used because of their sensitivity to changes in the environment and to assess potential risk associated with contamination of chemicals in aquatic environment [7]. The uses of fish biomarkers as catalogues of the properties of pollution are of cumulative importance and can permit initial recognition of environmental glitches in marine or freshwater ecosystem [8]. Catalase enzyme located in peroxisomes that catalyze the conversion of hydrogen peroxide into water and molecular oxygen and glutathione S-transferase catalyzes the conjugation of reactive electrophilic groups. The physiological function of acetylcholinesterase is the rapid elimination of acetylcholine released at cholinergic synaptic clefts, thus allowing precise temporal control of muscle contraction.

2. Materials and Methods

The experiment was performed on *Catla catla* which were collected from Rosen fisheries Marathakkara, Thrissur. They were brought to the

laboratory and acclimatized for two weeks. Dechlorinated water was used throughout the experimental period. Experiment were carried out in plastic containers containing ten liters of water. Chemically pure chloride compounds of the zinc were dissolved in the distilled water for the preparation of stock solutions. The ranges of test concentrations used were 1, 5, 10 and 15 ppm. A group of fishes were maintained without the zinc chloride served as control and control fishes were held under acclimatized condition and monitored. Fishes average length and weight was about 8-10cm and 10-12gm respectively. At the end of 96 hours, fishes were sacrificed from both control and treated groups and tissues such as brain and muscle were collected immediately. Brain and muscle washed with distilled water and 0.8% saline, blotted and weighed before homogenization.

2.1 Acetylcholinesterase assay

Acetylcholinesterase (AChE) activity was estimated as per the method described by Ellman *et al.*, (1961) [9]. AChE activity was determined using the Ellman's reagent DTNB [5,5'-Dithiobis-(2-nitrobenzoic acid), 0.5mM] and acetylthiocholine iodide as substrate. The rate of change in absorbance was measured at 412nm. Blank samples were taken to make sure that there was no non-specific esterase or other background activity. Protein was estimated as described by Lowry *et al.*, (1951) [10] and AChE activity expressed as $\mu\text{mol}/\text{min}/\text{mg}$ protein.

2.2 Catalase assay

Catalase activity measured by the method of Sinha (1972) [11]. Weighed amount of tissue was homogenized in phosphate buffer and centrifuged at 5000 rpm for 10 minutes. The reaction mixture includes 0.1ml of hydrogen peroxide, 1.95ml phosphate buffer, and 0.05ml tissue extract. Change in absorbance was recorded at 240nm and one unit of enzyme activity is defined as the amount of enzyme consuming $1\mu\text{mol}$ of substrate per minute. The enzyme activity was expressed as $\mu\text{mol}^{-1} \text{H}_2\text{O}_2$ decomposed $\text{min}^{-1} \text{mg}^{-1}$ protein.

2.3 Glutathione S-transferase assay

Activity of glutathione S-transferase was measured by the methodology of Habig *et al.*, (1974) [12]. Tissue were homogenized in 0.5M phosphate buffer and centrifuged at 5000 rpm for 15 minutes at 40°C and use supernatant as enzyme source. The reaction mixture was combination of 100mM CDNB (1-chloro-2,4-dinitrobenzene), 100mM 2ml buffer of potassium phosphate. Both, of the reagents, were then mixed with 100mM GSH (glutathione) and then sample. Change in absorbance was measured at 340nm. One unit of enzyme activity defined as the amount of reduced

glutathione and CDNB conjugate formed per minute. The enzyme activity expressed as $\mu\text{mol}^{-1} \text{min}^{-1} \text{mg}$ protein.

3. Results and Discussion

Acetylcholinesterase, glutathione S-transferase and catalase activity in both brain and muscle tissue of *Catla catla* exposed to zinc chloride for 96 hours were provided in Fig. 1, 2 and 3 respectively. Lowest activity of acetylcholine esterase, $2.498 \pm 0.0146 \mu\text{mol}^{-1} \text{min}^{-1} \text{mg}$ protein has been observed in muscle tissue of fishes exposed to zinc chloride of 5 ppm concentration of zinc chloride.

Acetylcholinesterase enzyme is vital for prey location, food orientation and predator escaping [13]. As it plays an important role in neurotransmission at cholinergic synapses by rapid hydrolyzing the neurotransmitter acetylcholine to choline and acetate. Though inhibition of acetylcholinesterase activity by any pollutant poses a serious threat to fish survival. The enzyme activity of acetylcholinesterase is inhibited by a variety of different contaminants including heavy metals, both under *in vivo* and *in vitro* conditions. The present study observations, decreased activity of AChE in both muscle and brain of *Catla catla* exposed to different sublethal concentrations of zinc chloride, were in agreement with the others, such as significant suppression in acetylcholinesterase activity was recorded in gill, kidney, intestine, brain, liver and muscle tissue of *Cyprinus carpio* exposed to zinc (6mg/l) for different duration, 1, 15 and 30 days [14]. Inhibitory effect of zinc chloride also found on brain acetylcholinesterase of a marine teleost, *Arius nenga* [15].

Most of the pollutants and their metabolites induce toxicity via oxidative stress arising from the increased production of free oxygen radicals. The antioxidant enzymes involved in removing free oxygen radicals are the principal candidates for biomarkers of oxidative stress. However, the antioxidant defense enzymes are highly variable and depend on the nature of the pollutants involved, organisms and organ tissues [16]. Zn exposure ($500\mu\text{g L}^{-1}$) in freshwater killifish induced an increase in reactive oxygen species (ROS) and inhibited antioxidant defense mechanisms such as catalase enzyme in liver and other tissues [17]. *In vitro* effects of individual heavy metal ions and their combinations on *Sarotherodon mossambicus* catalase activity were studied and observed that Zn has inhibitory effects on catalase activity [18]. The present study also observed that the activity of antioxidant enzyme catalase inhibited in both brain and muscle tissue by zinc chloride.

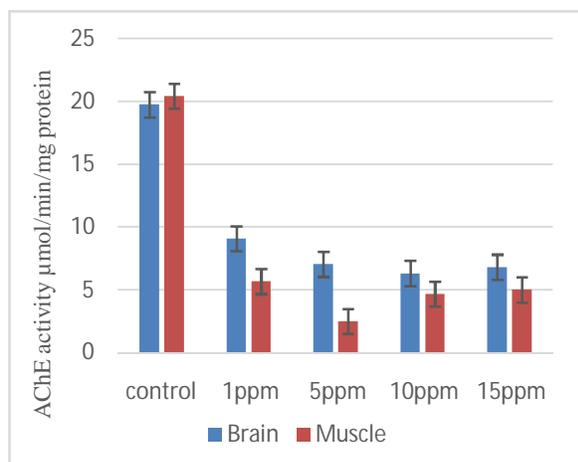


Fig. 1: Acetylcholinesterase activity (AChE) $\mu\text{mol}/\text{min}/\text{mg}$ protein in brain and muscle of *Catla catla* exposed to different concentrations of zinc chloride.

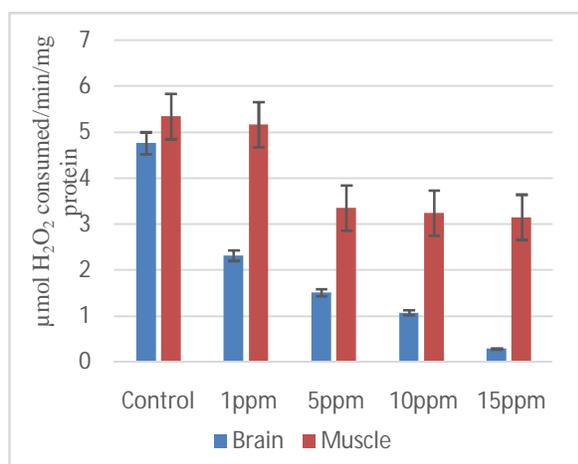


Fig. 2: The activity of catalase enzyme in brain and muscle of *Catla catla* after exposure to different concentrations of zinc chloride. Catalase activity expressed as $\mu\text{mol H}_2\text{O}_2$ consumed/min/mg protein.

Catalase (CAT) is an antioxidant enzyme that catalyzes the breakdown of hydrogen peroxide to oxygen and water, thus protecting the cells from oxidative stress. When compared to the control, catalase activity considerably decreased in both brain and muscle tissue exposed to zinc chloride. Catalase activity observed in brain and muscle tissue of fishes exposed to highest concentration of zinc chloride were 0.286 ± 0.079 , 3.153 ± 0.199 and control fishes were 4.766 ± 0.024 , 5.344 ± 0.095 . Maximum inhibition of catalase activity were observed in brain exposed to highest concentration of zinc chloride (15 ppm). Glutathione S-transferase (GST) activity increased during sublethal exposure to zinc chloride. Glutathione S-transferase activity observed in brain and muscle tissue of fishes exposed to highest concentration of zinc chloride were 20.19 ± 0.165 , 22.54 ± 0.068 and control fishes were 10.07 ± 0.075 , 11.66 ± 0.068 .

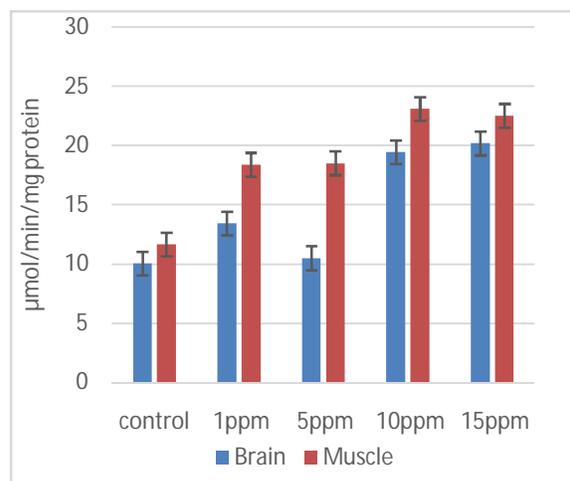


Fig. 3: The activity of glutathione S-transferase ($\mu\text{mol}/\text{min}/\text{mg}$ protein) in *Catla catla* exposed to different concentrations of zinc chloride.

Changes in glutathione S-transferase activity reflect detoxification process in fish exposed to different toxic compounds [19,20]. In the present study, glutathione S-transferase activity increased in both brain and muscle tissues of fish exposed to different sublethal concentrations of zinc chloride. The activity of GSH is increased, after the 7 days of treatment with ZnO nanoparticles both in liver and gills of different fishes. Activity of both GSH and superoxide dismutase increased in *Clarias gariepinus* exposed to lead nitrate and zinc chloride [22]. Thus, induction in GST activity could indicate a defense of fish against oxidative stress damage produced by adverse conditions.

4. Conclusion

This study reports a significant decrease in acetylcholinesterase and catalase activity, whereas an increase in glutathione S-transferase activity in brain and muscle exposed to different sublethal concentrations of zinc chloride. Toxic effect depends on the concentration of the toxicant and duration of the exposure. Heavy metals may adversely have an effect on the physiological functions of fish resulting in increased susceptibility to mortality and diseases. Thereby enzymes can serve as biomarkers for early detection of environmental pollution during biomonitoring programs.

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