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Mercuric Chloride Toxicity and Its Influence on Enzymatic Antioxidants – A Study on Zebra Fish Model (*Danio rerio*)

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Authors' contributions

This work was carried out in collaboration between all authors. Authors Jyotsna and AKM designed the study. All the authors performed the statistical analysis, wrote the protocol, and author BP wrote first draft of the manuscript and managed literature searches. Authors Jyotsna, AKM, BP managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

Short Research Article

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ABSTRACT

Enzymatic antioxidant was determined to establish a possible environmental impact on toxic metal in fish *Danio rerio*. Alterations in the antioxidant cellular systems have often been proposed as biomarkers of pollutant-mediated toxicity. This prompted us to investigate the activities of catalase, superoxide dismutase, Reduce Glutathione in the liver of mercuric chloride treated zebra fish. The fishes were exposed to Mercuric chloride for a period of 96 hours. The LC50 value was obtained by using probit software based on 50% of fish that died at each concentration. Mortality was recorded at 24th, 48th, 72nd and 96th hours. Various concentrations of Mercuric chloride (70µg/ml and 250µg/ml as 1st stage, 20mg/ml, 30mg/ml, 50mg/ml and 60mg/ml as 2nd stage, and 5mg/ml, 2.5mg/ml, 2mg/ml, 1mg/ml and 0.5mg/ml as 3rd stage) were injected into the tanks containing the fishes for 96 hours with a photoperiod of 12hours. After the 96 hours, Liver of the fishes was collected in an Eppendorf and stored at -20ºC for further analysis. In the present study, increase in SOD activity as well as Catalase activity and a decrease in glutathione activity were registered.

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1. INTRODUCTION

Heavy metals enter aquatic habitats by a number of routes and cause hazardous effects on their morphology and physiology. Water pollution by heavy- metal is a major environmental problem facing the modern world [1]. Heavy-metals are often present at elevated concentrations in aquatic ecosystems due to the rapid growth in population [2]. Mercury is a potent toxic agent as it is released into the environment, mainly through anthropogenic action [3]. Numerous studies have shown that the amount of mercury being deposited from the atmosphere has increased from the onset of the industrial age [4,5,6]. Some mercury deposits arise from natural sources while others are derived from anthropogenic activities. Mercuric chloride is a cumulative poison and is considered as a direct-acting toxicant. Fishes are sensitive to contaminants of the water, and pollutants may damage certain physiological and biochemical processes when they enter the organs of the fish [7].

Fish act an indicator for monitoring land- based pollution as they get concentrated in their tissue, directly from water though respiration and also through their diet. Fishes are frequently subjected to pro-oxidant effects of different pollutants present in the aquatic environment [16].

Oxidative stress plays a vital role in heavy -metal toxicity. Much experimental data provides evidence to the metal interaction with nuclear proteins and DNA that cause oxidative deterioration of biological macromolecules. The antioxidative defense system (ADS) is necessary for the maintenance of redox homeostasis in organisms. Alterations in the antioxidant cellular systems have often been proposed as biomarker of pollutant-mediated toxicity. The reactive oxygen species (ROS) cause serious pathology in humans and animals at an early stage of the disease [8]. As oxygen was released into the atmosphere by photosynthetic processes, its toxicity has posed a great threat to life [9]. Many mammalian species, including aquatic animals such as fish posses defensive mechanisms to counteract the impact on ROS. These systems include various antioxidant defense enzymes such as superoxide dismutase that catalyze the dismutation of superoxide radical to hydrogen peroxide, glutathione S-transferase that detoxifies lipid hydroperoxides generated by heavy metals [10]. The liver was chosen for the present study as it plays a vital role in regulating the overall body metabolism and thereby intense involvement in the detoxification of xenobiotics. Thus, evaluating antioxidant responses to liver is highly relevant, since toxic chemicals that cause temporary or permanent disturbance of homeostasis, can disrupt their functions [11]. Hence the present study focused to investigate the activities of catalase, superoxide dismutase, Reduce Glutathione on the liver of mercuric chloride treated zebra fish.

2. METHODOLOGY

The zebra fish (*Danio rerio*) was taken as the sample organism because the developmental behavior can be observed easily and tested. Fishes of size ranging from 2.5 - 3cm were randomly introduced into the respective tanks mentioned as control and test. At the first stage, five fishes were exposed to the tank containing 70µg/ml and 250µg/ml mercuric chloride. Five fishes (20mg/ml, 30mg/ml, 50mg/ml and 60 mg/ml mercuric chloride) were introduced into the respective tank at the 2nd stage, and 5mg/ml, 2.5mg/ml, 2mg/ml, 1mg/ml and 0.5mg/ml at the 3rd stage for the period of 96 hours. Mortalities were

recorded at 24th, 48th, 72nd 0f 96 hours and the experiments was repeated twice. LC 50 was calculated using probit software. Five fishes were introduced into the tanks containing 5%, 10%and 20% of LC50 mercuric chloride (0.0662 mg, 01324 mg, 0. 2648mg). The fishes were observed for 96 hours mortality and physical changes. At the 96th hr, equal numbers of fishes were collected from the test and from the control, and their liver was dissected. Biochemical assays such as superoxide dismutase, catalase and reduced glutathione were carried out in the liver sample. Superoxide dismutase was estimated at the tissue lysate of liver samples by Marklund and Marklund method [12]. An increase in absorbance was recorded at 420nm for 3 minutes in the spectrophotometer. The rate of auto oxidation of pyrogallol was determined by change in absorbance/minute at 420 nm. Catalase was assayed by Beers and Sizer method [13]. Reduced glutathione were estimated by Ellman's method [14]. Reduced glutathione is a substrate for the glutathione peroxidases, which provide a mechanism for the detoxification of peroxides. This assay is based on the reduction of glutathione (GSSG) by NADPH in the presence of glutathione reductase. The Glutathione Reductase Assay enables the spectrophotometric measurement of glutathione reductase activity. The activity can be measured by the increase in absorbance caused by the reduction of DTNB [5; 5dithio bis (2-nitrobenzoic acid)] at 412 nm. Feeding response to mercuric chloride such as pigmentation were made by observing the color change (brown color). Respiration measured by counting the number of times the fish opens and closes its mouth in a minute and locomotion by visual observation. Data was analyzed by using one-sample t-test Mercuric chloride.

3. RESULTS AND DISCUSSION

Bioassay is a necessary to determine the concentration of a toxicant, which could be allowed in water without adverse effects on the living organisms. The estimation of the lethal concentration values (LC 50) was carried out using the linear regression of Probit program and according to Guedenon P. et al. [15] the LC50 value was determined to be 0.200mg/L in mercury chloride treated Poecilia reticulate. However in the present study, the median lethal concentration was the highest recorded (1.324mg/l) compared to those reported in the fore mentioned investigations. The chemical product being the same, the difference in the results could be attributed to the variety of species used. Here, *Danio rerio* proved to be more resistant to mercuric chloride than the various species involved in the studies already mentioned.

The Table 1 represents the feeding response of the zebra fish to mercuric chloride. The color change was not observed at lower concentration and at increased concentration the color of the fish changed to light brown. The Normal respiration is 60-66 breaths/minute, and at higher concentration, the fishes were found to respire more than the normal. The movements of the fishes were found to be faster than usual at increased concentration.

The Table 2 represents the Antioxidant status in the liver of Zebra fish exposed to different concentrations of mercuric chloride in comparison to the control.

Increase level of catalase and decrease level of superoxide dismutase were observed in different concentration of mercuric chloride exposure. There are no significant changes only in 5 % of LC50 of catalase activity. This is accordance with the findings of L. Velkova-Jordanoska et al. [16]. The response to environmental pollution and toxic impact of the pollutant in the aquatic environment represents one of the possible reasons. Many scientists have reported that antioxidant enzyme's level depends on age, nutrition and spawning of the fish samples. M. St. Dimitrova [17] in his study on the combined effect of zinc and lead on

the hepatic superoxide dismutase-catalase system in carp (*Cyprinus carpio*) showed an increase in superoxide dismutase and the catalase activity on 24-hr exposure and a decrease activity after 5-day exposure. Mercuric chloride was found to suppress the antioxidant activity of these enzymes during oxidative stress.

Table 1. Feeding response of mercuric chloride treatment

Concentration	Pigmentation	Respiratory function	Locomotion	Mortality in percentage
70µg/ml	No change	Normal	Normal	0%
250µg/ml	No change	Normal	Normal	0%
20mg/ml	Light brown	Normal	Normal	80%
30mg/ml	Light brown	Fast	Medium	100%
50mg/ml	Light brown	Fast	Fast	100%
60mg/ml	Light brown	Fast	Fast	100%
5mg/ml	Light brown	Medium	Fast	60%
2.5mg/ml	No change	Normal	Normal	40%
2mg/ml	No change	Slow	Slow	40%
1mg/ml	No change	Slow	Slow	60%
0.5mg/ml	No change	Slow	Slow	0%

Table 2. Antioxidant status in the liver of mercuric chloride treated zebra fish

Concentration	Catalase absorbance at 570 nm	SOD absorbance at 420nm	Reduced glutathione absorbance at 412nm
Control	0.105	0.286	0.738
5% of LC50	0.108	0.238*	0.640*
10% of LC50	0.222*	0.229*	0.581*
20% of LC50	0.319*	0.213*	0.497*

**p < 0.05 as compared to respective control*

Glutathione has been shown to be a significant factor of heavy- metal mobilization and excretion, specifically with application to mercury, cadmium, and arsenic. Glutathione depletions and glutathione supplementation have specific effects on mercury toxicity, both by altering antioxidant status in the body and by directly affecting excretion of mercury and other heavy metals in the bile [18]. Glutathione reductase catalyzes the reduction of oxidized glutathione (GSSG) to reduced glutathione (GSH). In the study investigated by Rengaswamy Gopal on nickel-induced toxicity in *Cirrhinus mrigala* the total protein content, reduced glutathione, glutathione peroxidase and lipid peroxidation were found to be decreased in the nickel chloride treated tissue [19]. In the present study, the effect of mercuric chloride of fish was evident in a decrease of glutathione activity and reduced glutathione activity during oxidative stress.

Farombi et al. [20] reported that heavy- metals on the oxidative stress act as surrogate bio - indicators of aquatic pollution in *Clarias gariepinus*. Sies (1993) [21] reported that heavy-metal depleted GSH and GPx and may be due to deleterious oxidative changes. The heavy metals led to induction of lipid peroxidation and alteration in the antioxidant enzymes in the organs of the fish. Metal catalyzed formation of ROS capable of damaging tissues such as DNA, proteins and lipids is well documented [22]. The accumulation of heavy metals might have led to the production of superoxide anions, which led to H_2O_2. The SOD catalytically scavenges superoxide radicals that appear to be an important agent of toxicity of oxygen,

and this provides a defense against this aspect of oxygen toxicity [23]. Glutathione, as both a carrier of mercury and an antioxidant, has three specific roles in protecting the body from mercury toxicity. Glutathione, specifically binding with methylmercury, forms a complex that prevents mercury from binding to cellular proteins and causing damage to both enzymes and tissue [24]. Glutathione-mercury complexes also reduce intracellular damage by preventing mercury froth entering tissue cells and becoming a toxin. The decrease in Glutathione level and SOD might be due to the toxic effect of the pollutants that may overwhelm the antioxidant defenses. The increase in CAT activity represents the first line of defense against oxidative stress [25].

4. CONCLUSION

The study concludes that the sub lethal dose of mercuric chloride has drastic effect on physiology and morphology of the fishes. Mercury cause severe liver damage and this may be due to the reactive oxygen species produced by mercury. Hence, mercuric chloride imparts a harmful effect on aquatic systems.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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