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# Mercuric Chloride's Effect on Oxidative Stress and Haemato-Biochemical Markers in Catla catla Fingerlings

Bernhard, Bischoff

Department of **Zoological Research**

## Article Info

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**Abstract:** Aquatic animals can be used to simulate toxicological tests in the lab. Heavy metals include lead, mercury, cadmium, chromium, copper, zinc, manganese, nickel, and silver. The effects of mercuric chloride on the oxidative stress and hemato-biochemical processes of Catla catla fingerlings have been assessed in this study. Catla catla species' bioaccumulation of mercury has detrimental effects and upsets ecosystems. Carbohydrate, protein, and fat levels were significantly lower in Catla catla fingerlings exposed to high environmental contamination. Mercury chloride changed a number of haematological markers. Catla catla treated to mercuric chloride showed hematological changes, indicating the toxicity of mercury. MDA, GSH, and antioxidant enzymes such as CAT, SOD, and GPx were used to assess oxidative stress in mercuric chloride exposed to Catla catla. Changes in oxidative stress markers brought on by mercury chloride toxicants demonstrated physiological stress. Mercury chloride is extremely harmful to catla catla. According to biochemical and oxidative stress markers, fish in acidified soft waters are more susceptible to mercuric chloride damage. Changes in blood, oxidative state, and biochemistry are possible indicators of pollution.

**Keywords:** Mercury, oxidative stress, fish toxicity, heavy metals, and metal pollution

Introduction: Heavy metals include things like copper, zinc, selenium, silver, antimony, cadmium, mercury, lead, and thallium. Arsenic, cadmium, chromium, lead, and mercury are necessary for human survival while being toxic. Such toxins are harmful to both humans and the environment. Heavy metals can result from both natural and artificial processes (Fergusson, 1990). Significant environmental problems are the result of rapid industrialization and urbanization. Rivers are the main way that metals are transported, rendering developing countries especially vulnerable to the global issue of heavy metal contamination brought on by growing urbanization and industry (Mohiuddin et al., 2010; Silambarasan et al., 2012).

Heavy metals are still present everywhere we look (Vashishat and Kler, 2014). The main sink for heavy metals released into the environment by human activity is soil; however, most metals do not undergo chemical or microbial degradation and remain in soils for a long time, in contrast to organic contaminants that are oxidized to carbon (IV) oxide by microbial activity.

Mercury is situated adjacent to zinc and cadmium on the periodic table. Mercury's chemical and biological properties lead to its molecular toxicity (Valko et al., 2005). Sulfhydryl groups in mercury are reactive when exposed to oxidative stress. During eukaryotic metabolism, mitochondria produce reactive oxygen species (ROS). Inorganic mercury produces reactive oxygen species that harm ubiquinone-cytochrome b5 during

oxidative phosphorylation and electron transport (Palmeira and Madeira, 1997). Rats given HgCl2 have elevated MDA levels in their liver, kidney, lungs, and testicles (Lash et al., 2007). Reactive oxygen species (ROS) produced by radiation damage DNA and cause cancer (Valko et al., 2005). However, research on toxicants and human behavior is highly valued in contemporary aquatic toxicology. A regime's fish diversity is a useful indicator of ecosystem health and pollution for fisheries management. Numerous fish species may act as bioindicators of pollution levels in their specific environments. Fish are exposed to a variety of environmental pollutants, including heavy metals, thus protecting them is essential to the health of fish populations. Researchers have looked into this matter. In this work, the biochemistry, haematology, and oxidative stress of Catla catla fingerlings exposed to mercury chloride were investigated.

## Materials and Methods

*Experimental fish collection and acclimation*: *Catla catla* fingerlings (average weight- 6.39 g) were collected by cast net in March 2022 from a fish farm in Thittai, Thanjavur District, Tamil Nadu, India. They were then brought to the lab, where they were housed in a glass aquarium tank and acclimated to the tap water t for two weeks. In this time span, fish were given a measured quantity of fish food.

*Experimental design*:

Fish were selected for this study based on their size and weight (7.44 cm in length and 6.39 g in weight), and then acclimated for 3 days to the laboratory's temperature (28.2 ℃), pH range (7.5-7.8), and photoperiod (12:12 h light:dark). The fish, in two sets of ten (control and experimental), were released into the trough filled with dechlorinated tap water. All of the fish in the experiments were given their medication by mouth.

Group I received a control solution of 0.9% saline, while Group II received 50 mg/l of mercuric chloride in a single dosage. Before being given to the fish, mercury chloride was dissolved in water at 70 ℃ and then cooled to 37 ℃. At 72 h, the fish were decapitated to ensure their deaths. The muscles were dissected and processed for biochemical examination. All estimations were performed in duplicate.

*Analytical biochemistry*:

We used a Teflon homogenizer to mix the 1 g of tissue and then weighed the results. To determine biochemical values, a homogenate of tissue was made in 0.1 M Tris HCl buffer (pH 7.4). Protein content was estimated by Lowry *et al*. (1951). According to Folch *et al*. (1957) technique, total lipids were calculated in the tissues. We used the Anthrone technique to determine the exact amount of carbohydrate in fish tissues (Plummer and David, 1988). Beuge and Aust's (1978) thiobarbituric acid test technique was used to calculate malondialdehyde levels. Measurements of reduced glutathione were performed by using the technique developed by Moron *et al*. (1979). The technique developed by Rotruck *et al*. (1973) to measure glutathione peroxidase activity in mitochondria was used in this study. Kakkar *et al*. (1984) method was employed to measure copper-zinc superoxide dismutase activity in plasma. Beers and Sizer's (1952) technique was used to measure catalase activity.

*Analysis of blood*:

Fish in the experiment and the controls were given 10 ppm Benzocaine and allowed to rest for 3 min before being exposed to metals. Under general anaesthesia, a heparinized disposable syringe was

used to draw blood from the caudal circulation. Haematological characteristics were analysed by testing blood samples. Cyanmethaemoglobin technique (Dacie and Lewis, 1968) was used to estimate haemoglobin (Beacon Diagnostic Kit). White blood cell (WBC) and red blood cell (RBC) counts were performed according to the Ochei-Kolhatkar (2000) technique.

*Data analysis*:

Three tests were conducted, with 10 fishes per group being exposed; mean± SD values were calculated. SPSS version 20 was used to conduct the statistical analysis, and a significance level was accepted at $p < 0.05$.

## Results and Discussion

Industrial and anthropogenic activities and contemporary industrialization have increased exposure to heavy metals, which have detrimental impacts on human health. Millions of people all over the globe are impacted by the environmental problem of water and air contamination by harmful metals. Heavy metal contamination of food is another issue that has implications for human and animal health. As such, we can evaluate the levels of toxicity in our drinking water, air, and food (Mousavi *et al*., 2013; Ghorani-Azam *et al*., 2016; Luo *et al*., 2020). It's possible that metals, along with other contaminants, arise naturally and persist in the environment. As a result, people will inevitably be exposed to metals. Few studies have shown that certain metals are more or less harmful for different sexes (Vahter *et al*., 2007; Tchounwou *et al*., 2012).

Some of the most frequent heavy metals that cause human toxicity include mercury, lead, chromium, cadmium, and arsenic. Exposure by water, air, or food may result in either acute or chronic toxicity. The buildup of these metals in the body leads to toxicity in many different organs and tissues. Changes in proximal composition and oxidative stress indicators were measured in this investigation after mercuric chloride exposure to *Catla catla* fingerlings (Fig. 1).

Toxic effects on organisms after just a brief time of exposure is called acute toxicity. Knowing the safe threshold concentration (STC) of a toxicant in the environment is important (Ay *et al*., 1999). Toxicity tests like this are crucial for determining what kind of water conditions fish need to thrive. Because of its simplicity to assess and clear biological and ecological relevance, mortality is the most essential component in aquatic toxicology. Fish poisoning from mercury chloride was discovered due to a change in close- by chemical composition, namely nitrate and phosphate levels. *Catla catla* fingerlings exposed to 50 mg/l of mercuric chloride had muscle protein levels of 83.98± 4.21 mg/g of tissue in group I, and 65.79± 3.95 mg/g of tissue in group
II. Group II exposed fish had a considerable drop compared to the control group (group I). Muscle carbohydrate in group I (2.45±0.23 mg/g tissues) and group II (2.09±0.19 mg/g tissues) were recorded in *Catla catla* fingerlings exposed to 50 mg/l of mercuric chloride. *Catla catla* fingerlings had muscle lipids in Group I as 9.73± 0.65 mg/g tissues whereas in mercuric chloride (50 mg/l) exposed Group II it was 8.01± 0.42 mg/g tissues. Because tissues are the end-results of intermediary metabolism, they provide insight into an organism's general state of health (Artacho *et al*., 2007). The stress caused by

heavy metal poisoning reduces the body's lipid content. Since heavy metals accumulate in aquatic ecosystems over time, they are often considered to be very biotoxic (Sharma and Agrawal, 2005). In the development of cell membranes, phospholipids (also known as structural lipids) play a crucial function (Martinez-Pita *et al*., 2011). Lipid changes in response to toxicant exposure have also been investigated by Shaikh (2012).

Mercury chloride also affects oxidative stress indicators. Tissues were analysed for levels of lipid peroxidation, reduced glutathione, superoxide dismutase, glutathione peroxidase, and catalase. Increased MDA in liver were seen in *Catla catla* fingerlings exposed to 50 mg/l mercuric chloride.

Reduced glutathione (GSH) of tissue were detected in Group II *Catla catla* fingerlings exposed to 50 mg/l mercuric chloride (10.01±1.89 U/ml) as compared to Group I (18.76±2.55 U/ml). Group I tissue Superoxide dismutase (SOD) levels were 2.31±0.14 U/ml whereas in *Catla catla* fingerlings exposed to 50 mg/l mercuric chloride (group II) levels were 1.17±0.23 U/ml. *Catla catla* fingerlings exposed to 50 mg/l mercuric chloride showed decreased tissue Catalase (Cat) activity -- groups I 0.62±0.09 U/ml and group II 0.23±0.05 U/ml. Tissue Glutathione peroxidase (GPx) in Group I was 2.23± 0.18 U/ml whereas in Group II it was 1.61± 0.12 U/ml.

Reduction in oxidative stress indicators in *Catla catla* tissue has been noticed after exposure to mercuric chloride (Fig. 2). As a byproduct of mitochondrial molecular oxygen reduction, free radicals are produced in all aerobic species (Siman-Tov *et al*., 2011). The body's natural defences against free radicals are crucial to its health. Reactive oxygen and nitrogen species (RONS) accumulate in the body and cause oxidative stress by damaging essential cellular components. Oxidative stress occurs when there is an imbalance in the body's levels of oxidants and antioxidants, which may be harmful to the organism (Vignini, 2011).

Among the many byproducts of the peroxidation of polyunsaturated fatty acids found in biological membranes, malondialdehyde (MDA) is the most prominent. Tissue damage may be measured by increasing levels of MDA, a byproduct of lipid peroxidation (Ray and Husain, 2002). Lipid peroxidation and its byproduct are now widely believed to contribute to toxicity in organs such as the liver, kidneys, heart, and brain (Lakshmi *et al*., 2005). Two superoxide anions ($O^{\bullet}2^{-}$ and $^{\bullet}OH$) are dismuted by SOD catalysed dismutation to molecular oxygen and hydrogen peroxide (Fridovich, 1998). Both GPx and CAT catalyse the reduction of hydrogen peroxide to water. The degree of oxidative stress may be measured using malondialdehyde (MDA), which is considered a good biomarker (Nesto *et al*., 2007). The removal of $H_2O_2$ by GPx explains why it may mitigate tissue damage (Almeida *et al*., 2002). The effects of heavy metals (e.g. Cd, Cu Zn) on antioxidant enzyme activity in bivalves have been the subject of previous research (Siraj Basha and Usha Rani, 2003). DNA damage, chemical carcinogenesis, lipid peroxidation activation, and enzymatic inactivation, especially CAT, GPx, and SOD, are just some of the toxic processes that can result from oxidative stress caused by reactive oxygen species (ROS) when the rate of ROS production exceeds the rate at which it is decomposed by antioxidant defences and repair systems (Cheung and Chinn, 2001). As a defence, the antioxidant enzymes help to get rid of harmful free radicals

(Solé *et al*., 1998). A variety of bivalve species have been tested, and MDA and these antioxidative enzymes have been found in all of them (Charissou *et al*., 2004). The results of the present study revealed that oxidative stress in fish exposed to Mercuric chloride causes changes in antioxidant enzyme activities, which might be employed as biomarkers in ecotoxicological bioassays of heavy metals.

Because of its sensitivity, blood is a biomarker that responds to changes in its surrounding environment. As a result, fish health may be monitored using haematology analysis. For the purpose of diagnosing illness and poisoning in fish, biochemical alterations in blood values are especially crucial. A variety of blood measures, including red blood cell (RBC) count, white blood cell (WBC) count and haemoglobin (Hb)

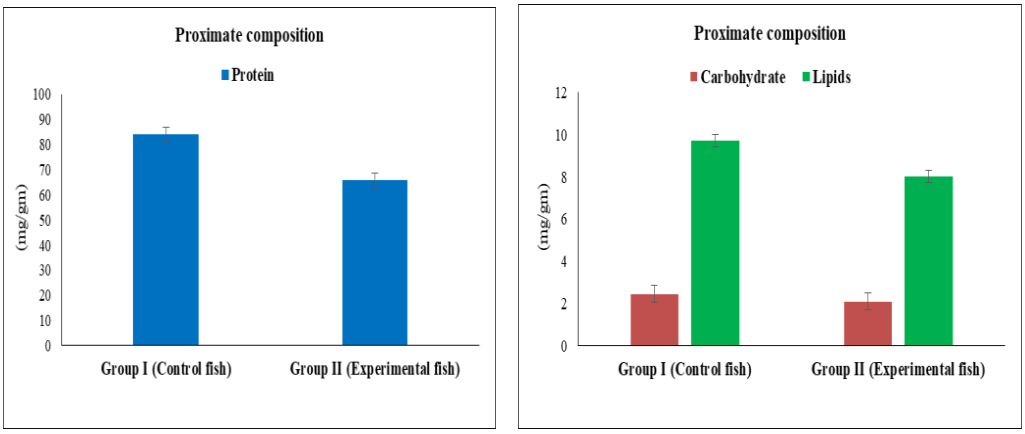


Fig. 1: Effect of Mercuric chloride on proximate composition in *Catla catla* fish muscles

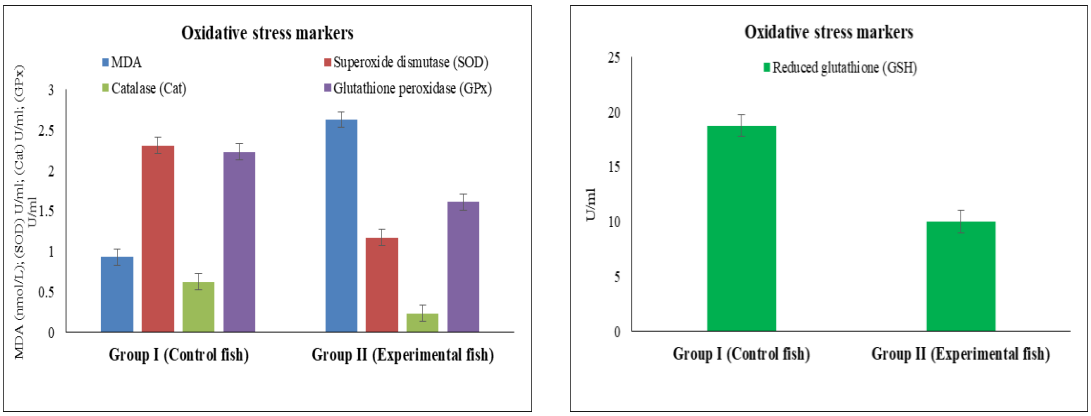


Fig. 2: Effect of Mercuric chloride on oxidative stress markers in *Catla catla* tissue.

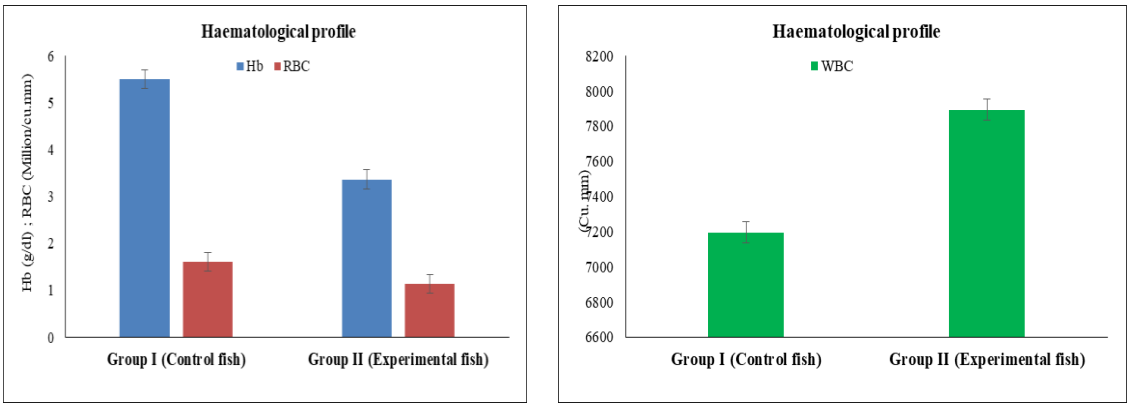


Fig. 3: Effect of Mercuric chloride on haematological profile in *Catla catla.*

concentration, have been employed as markers of metal contamination in water. Catla catla exposed to Mercuric chloride was tested for changes in their RBC, WBC, and haemoglobin content. Group had haemoglobin levels of 5.51±0.29 g/dl and red blood cell counts of 1.61±0.09 Million/cu.mm,whereas group II had haemoglobin levels of 1.14±0.02 g/dl and RBC counts of 1.22±0.07 Million/cu.mm after exposure to 50 mg/l mercuric chloride. Group II exposed fish had a considerable decreased value as compared to the control group (group I). The white blood cell (WBC) counts in the blood of *Catla catla* fingerlings exposed to 50 mg/l Mercuric chloride was 7194.07±192.39 Cu. mm whereas in Group I it was 7894.43±183.65 Cu. mm. Similarly, there was a statistically significant increase in the number of WBC in exposed fish (group II) as compared to the control (group I).Mercuric chloride's impact on *Catla catla* fingerlings haematological profile is depicted in Figure 3. The blood's haematological composition may reveal a lot about the body's internal climate. The growing interest in pisciculture and the growing concern about human contamination of marine water resources have boosted the importance of haematological research on fishes. This kind of research has traditionally served as a reliable and sensitive indicator of physiological and pathological status (Li *et al*., 2010). Because blood characteristics are very susceptible to environmental pollutants and rapidly react to unwanted elements, we were able to discover variations in blood cell counts and other haematological parameters in our investigation. *Catla catla*'s haematological profile was drastically changed by exposure to $HgCl_2$. According to Babatunde *et al*. (1992), the metabolic and health condition of an animal may be inferred from a change in the constituent component (Gabriel *et al*., 2011). It was determined from this research that *Catla catla* exposed to mercury chloride had changes in many haematological parameters.

## Conclusion

The toxicity of mercuric chloride to Catla catla may be regarded as high. The estimated biochemical, haematological, and oxidative stress indicators suggest that fish exposure to mercuric chloride may have a substantial impact. We come to the conclusion that fish can serve as trustworthy markers of metal concentrations in water systems. Additionally, it is possible to conclude that biochemical, haematological, and haematological signs could be considered potential biomarkers of metal pollution.

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