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Mercuric Chloride Induced Oxidative Stress and Antioxidant Enzymes in *Labeo Rohita* as Biomarker

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ABSTRACT

In recent years, heavy metal pollution has become a global environmental threat both ocean and inland fresh waste eco systems. Several aquatic systems were polluted by heavy metals from the industrial discharges. The discharges not only pollute the water bodies but also the aquatic plants, animals which related to human health issues. In the present study the fish *Labeo rohita* was exposed to three sub-lethal concentrations of Mercuric chloride which is non-essential metal and hazardous too, representing 5, 25 and 50 % of 96 hr.LC50 respectively. The activity of antioxidant enzymes and oxidative stress due to the exposure of the heavy metal mercuric chloride induces [superoxide dismutase(SOD), catalase CAT), glutathione S.transeferase (GST)] and malondialdehyde (MDA) in the liver were investigated From the results it can be concluded that the activities and expression levels of antioxidant enzymes and oxidative stress can be used as biomarkers to evaluate the influence of heavy metal mercuric chloride as the biochemical pathway and enzymatic function in *Labeo rohita* that can be used for biological monitoring unacceptable levels of environmental contamination.

Keywords: *Mercuric chloride, Labeo rohita ,SOD, CAT, GST, LPO*

INTRODUCTION

Aquatic pollution is a major contributor to oxidative stress in fish, resulting from the redox cycling of pollution. Fish is an important aquatic organism. Fish products are an important source of protein for human consumption (Duran and Talas, 2009). Aquatic organisms can provide model systems for investigation of how reactive oxygen species (ROS) damage cellular compounds, how cells respond, how repair mechanisms reverse this damage, and how oxidative stress can lead to disease. Oxidative stress has become an important item for aquatic toxicology. Fish have been proposed as indicators for monitoring land-based pollution because they may concentrate indicative pollutants in their tissues directly from water through respiration and also through their diet. Fish are frequently subjected to pro-oxidant effects of different pollutants often present in the aquatic environment (Zikic, 2001,Matzinger *et al*., 2007; Vinodhini *et al*., 2009)

Materials and Methods:

In the present experiment, fish were divided into four groups in three replicates each group included 24 fish. Mercuric chloride of various concentration were added to the experimental glass aquaria one hour before the transfer of fish.

Group 1: Exposed to 5% (96 hrLC50)
Group2: Exposed to 25% (96 hr. LC50)
Group 3: Exposed to 50% (96 hr.LC50)
Group 4: Served as a control.
Feeding was allowed in the experimental as well as control groups once per day.

Experimental setup:

At the end of exposure period (5, I5 and 30 days) 8 fish from each group were taken After dissection liver

and gills tissues were carefully removed and washed with ice cold saline (0.7 Nacl). The gill filaments were separated from the gill arches, weighed to the nearest mg. Tissues (liver) were homogenized in 0.25 M sucrose buffer at pH 7.4 using a glass homogenizer and then centrifuged at 8, 000 rpm for 20 min. The supernatant was used for enzymes assays.

Enzymes assays

Super oxide dismutase (SOD)

The activity of super oxide dismutase (SOD) in the liver tissues of the test fishes were determined spectrophotometrically at wave length 480 nm by epinephrine method Misra (1972) and expressed in units of enzymes activities per gram of tissues wet wt.

Catalase activity (CAT)

The activity of catalase CAT) in the liver were determined spectrophotometric at wave length 570 nm followed by the method of Sinha (1972) and was expressed in ml mol of decomposed hydrogen peroxide per sec per gram of tissues wet wt.

Glutathione S transferase (GST)

The effect of glutathione S transferase (GST) was determined spectrophotometric at wave length 340 nm according to the method of Habig *et al.,* (1974) using 1-chloro-2-4 dinitrobenzene (CDNB) as substrate. It was expressed in µmol /min/mg protein wet wt.

Malondialdehyde (MDA) was determined according to the method of Nair and Turner (1984). MDA derived from lipid peroxidation was determined with thiobarbituric acid (TBA). 0.5 ml homogenate without filtration was taken and 4.5 ml of TBA reagent was added. The mixture was heated using boiling water bath for 20 min, centrifuged at 2500 rpm for 10 min. The absorbance of supernatant was recorded at wave length 525nm MDA results were expressed as µmol of MDA per g. wet wt.in the tissues.

Statistical analyses:

All values were expressed as mean + standard error. The significance of difference between control and experimental data was statistically analyzed using student $((t))$ test (Sendecor and Cochran, 1980).

Results and Discussion:

The oxidative stress due to mercuric chloride exposure of the fish shows changes in SOD, CAT, GST enzymes and lipid peroxidation (MDA) in the liver of *Labeo rohita* exposed to three sub-lethal concentrations (5%, 25% and 50%) of Mercuric chloride were presented in (figures 1&4). Changes was observed in SOD and CAT level of enzymes after 5 days of exposure to different concentrations of $HgCl_2$. However after 15 and 30 days of exposure the activity of SOD was increased significantly to (30.1-38.3%) and (26.7-48.1%), $P<0.05$. Similarly, CAT activity was increased significantly by (37.9–137%) and (65.6 -188%) at low and high concentrations of Mercuric chloride. For GST the activity was significantly increase with the exposure concentration and duration time to (51-77%), (90-184%) and (103-207%) for 5, 15 and 30 days respectively at 5% to 25% of Mercuric chloride ($P<0.05$ and 0.01). No significant changes were observed in low dose of Mercuric chloride in MAD level of *Labeo rohita*. Moreover, there was a significant increase in MAD (57.7 –69%) ($P< 0.01$) at 25% and 50% concentration for 30 days of exposure.

Under normal physiological condition, the antioxidant defense enzymes including SOD, CAT and GST induced by a slight oxidative stress as a compensatory response, and thus the reactive oxygen species (ROS) can be removed to protect organisms from oxidative damage (living stone, 2001). The antioxidant activity may be provoked or inhibited under chemical stress depending on the intensity and duration of stress applied as well as susceptibility of exposure species. Fish liver is an organ that performs various functions associated with the metabolism of xenobiotics (Jiminez and Stegeman, 1990). Hepatocytes cells are dependent on antioxidant enzymes for the protection against reactive oxygen species produced during the bio transformation of xenobiotics (Londis and Yu, 1995). The control values of superoxide dismutase (SOD) and catalase (CAT) enzymes activities in the liver of *Labeo rohita* ranged between (342±2.5-361±1.4 unit/g wet wt.) and (73±1.4 -73±3.6 m mol /g wet wt) respectively and were found to be within the same range compared with other water fishes (Oruce nd sta, 2007;Talas *et al*., 2008; Metwaly 2009;Wenju et al.,2009 and Gad and Yacoub 2009).The present study revealed that SOD and CAT activities in the liver of *Labeo rohita* exposed to Mercuric chloride were increased significantly ($P<0.05$ and 0.01) .

Glutathione S transferase enzyme (GST) facilitates the conjugation of electrophilic substances or groups to tripeptide glutathione in order to make the xenobiotic chemicals more hydrophilic for transportation or excretion (Egaas et al., 1993).The control values of GST in the liver of *Labeo rohita*

ranged between 0.96±0.02-1.0±0.8 µ/mg wet. wt of tissues were found to be within the normal range of freshwater fishes (Oruce and Usta 2007; Talas et al., 2008;Wenju et al., 2009 and Gad 2009). In the present study, GST showed time dependent elevation in the liver tissue of *Labeo rohita* exposed to Mercuric chloride with a significant provoke in the initial exposure and were doubled after 30 days of exposure.

The increase was also demonstrated after exposure of *Labeo rohita* fish to water soluble fraction of Mercuric chloride (Zang *et al.,* 2004) Literatures are also found that the activity of detoxification enzymes such as GST increased in the presence of polycyclic aromatic hydrocarbon (Vander Oost *et al.,*2003).The increase in GST reported were indicated the biotransformation of pathway valid for Mercuric chloride used, as a protective response in fish toward exposure to an oxidative stress inducing xenobiotics. GST activity be a good biomarker for contamination environment. The increase in GST reported in the present study agrees with the results obtained in rainbow trout exposed to phenol (Uguz et al., 2003); in Atlantic cod exposed to sea oil and alkayl phenols for 15 days (Sturve et al.,2006).

Lipid peroxides are formed from the oxidative deterioration of poly unsaturated lipids in the membranes of cells and organelles. It is a bi-products, such as a malondialdehyde (MAD), are used as indicators for increased concentration of cellular reactive oxygen species and a sign of cellular injuries (Christi and Costa, 1984). Diverse contamination can initiate lipid peroxidation, including organic compounds and heavy metals. The control values of MDA in the liver of *Labeo rohita* ranged between (51±3.2-53±2.6) µmol/g wet wt. and was found to be within the same range of other fresh water fishes (Durmaz *et al*., 2006, Sturve *et al*.,2006 and Oruc and Usta 2007).

In the present study, there was no changes in lipid peroxidation level till 15 days of exposure to Mercuric chloride. We evolved MAD as a bi-product of lipid peroxidation after 30 days of exposure. The elevation in lipid peroxidation in the tissue of *Labeo rohita* indicated by increased MAD production which suggested the participation of free radical induced oxidative cell injury mediating the toxicity of Mercuric chloride. The result were correlated with the literature obtained in Atlantic cod exposed to sea oil and alkyl phenol for 15 days (Sturve *et al.,* 2006); (Durmaz *et al.,* (2006)

Conclusion

In conclusion this study demonstrated that crude oil at 5 to 25% concentration levels after 15 -30 days can cause adverse effects on *Labeo rohita* including the induction of SOD, CAT, GST and lipid peroxidation in the liver, The present results suggest that the activities and expression levels of antioxidant enzymes and oxidative stress can be used as biomarker to evaluate the influence of crude oil on the biochemical pathways and enzymatic function in the fish *Labeo rohita* so it can be used as a biological indicator to monitor unacceptable levels of environmental contamination.

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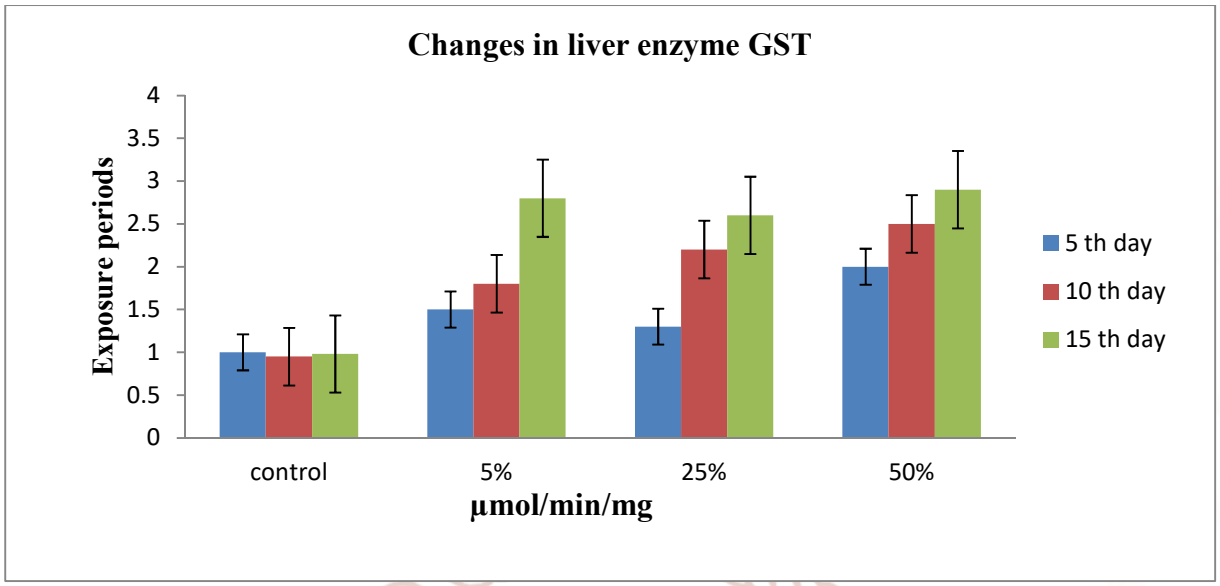
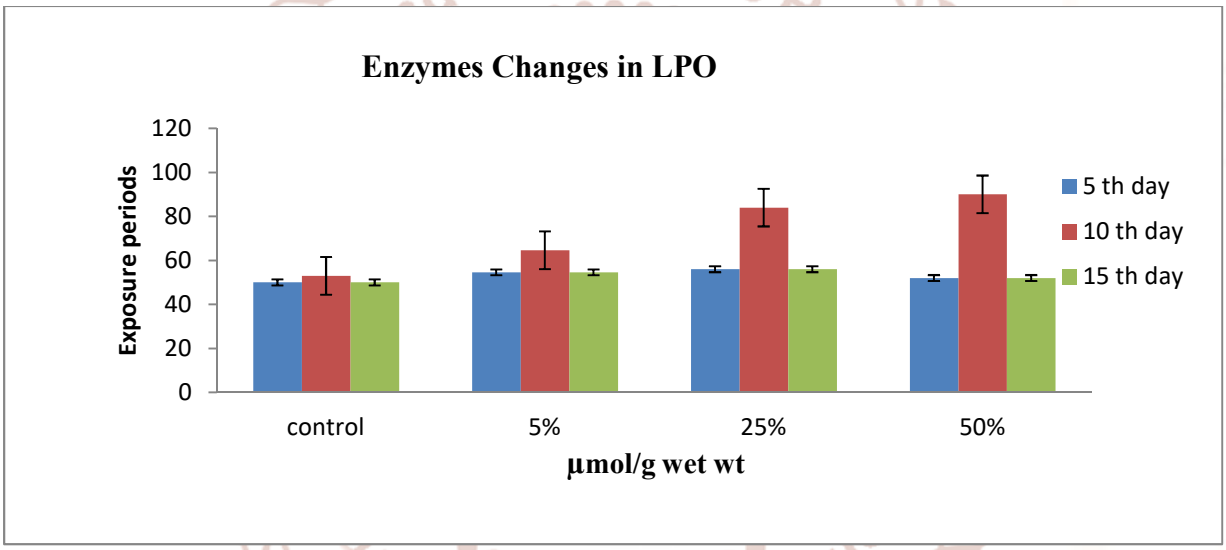
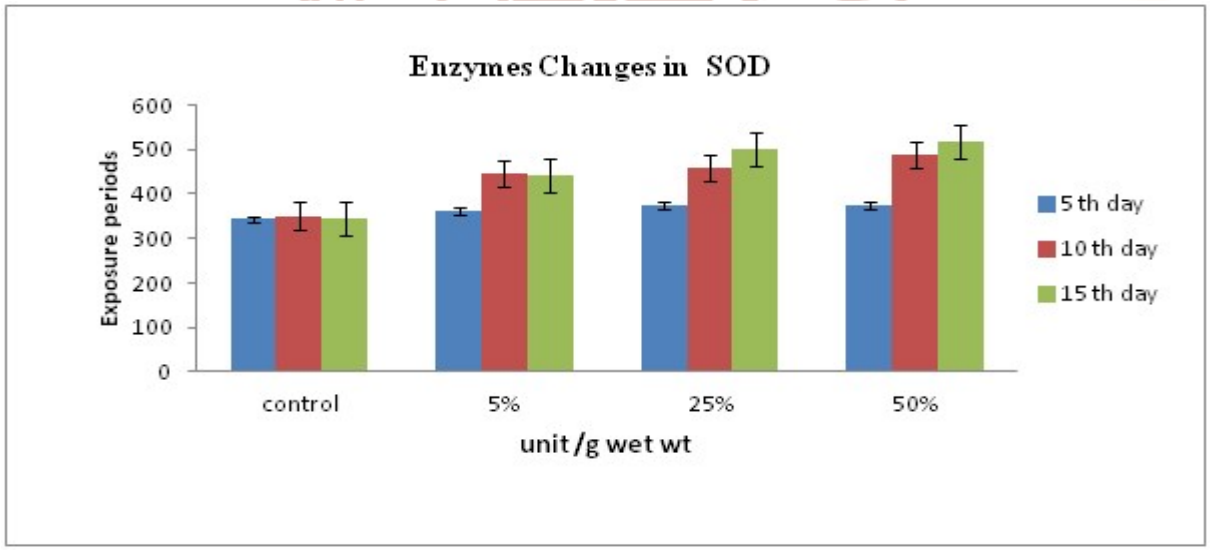


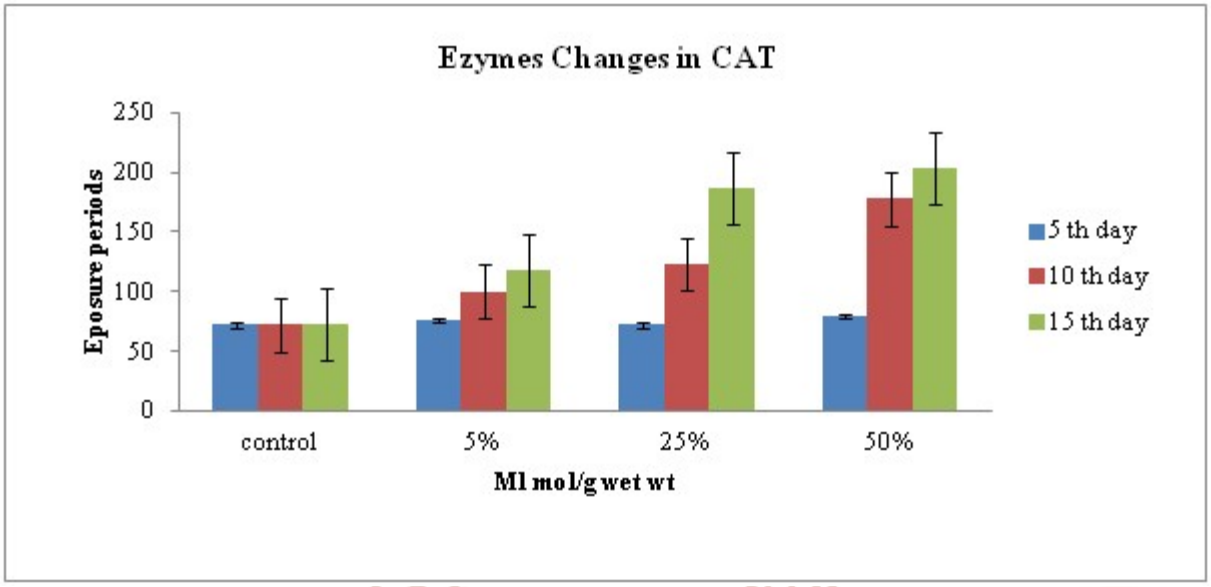
1.1 The oxidative stress due to mercuric chloride exposure of the fish shows changes in GST



1.2. The oxidative stress due to mercuric chloride exposure of the fish shows changes in LPO



1.3. The oxidative stress due to mercuric chloride exposure of the fish shows changes in SOD



1.4. The oxidative stress due to mercuric chloride exposure of the fish shows changes in CAT