International Journal of Fisheries and Aquatic Research
ISSN: 2456-7248; Impact Factor: RJIF 5.44
Received: 25-06-2020; Accepted: 10-07-2020; Published: 26-07-2020
www.fishjournals.com
Volume 5; Issue 3; 2020; Page No. 17-20

Study of defensive enzymatic and non-enzymatic antioxidants in *Labeo rohita*

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Abstract
The aim of the present study deals with the study of defensive enzymatic and non-enzymatic antioxidants in *Labeo rohita* fish. Antioxidants and oxidative stress biomarkers assay illustrated significant variation ($p<0.05$), ($p<0.01$) and ($p<0.001$) while measure up by means of control. Status of antioxidant were evaluated as measuring the levels of GST, GR, SOD, CAT, GSH and GPx activities intended for evaluating occurrence of oxidative stress in *Labeo rohita* fish via revelation to 0.08 and 0.20ppm of mercuric chloride in favor of 15 and 30 days. The oxidative stress biomarkers levels were enhanced subsequent to doses of exposure through an uptrend. The impacts of two 0.08 and 0.20ppm sub lethal doses of mercuric chloride were studies on gills, kidney and liver of *Labeo rohita* fish in substituted exposure duration significantly.

Keywords: enzymatic and non-enzymatic, *Labeo rohita*

Introduction
Oxidative stress studies by antioxidant enzymatic status under dissimilar range of mercuric chloride exposure in aquatic conditions. Extensive occurrence of metals in environment is contributory of a series of deleterious inequities in exposed organisms, show the toxicity. At elevated status, heavy metals can be absorbed through cell membranes, organ and organism. Additionally, at elevated level of biological organization (tissue, organ and whole organism) heavy metals induce changes in metabolism, biochemistry, physiology, histology as well as inhibit the synthesis of proteins and nucleic acid (Gauglhofer & Bianchi 1991 and Wilson & Taylor, 1993) [1, 2]. High status of metals was able to cause oxidative stress which positively harms liver, kidney tissues of fishes. The enzymes activities of fish increases therefore inactivation of reactive molecules appearance were recorded during oxidative stress, which possibly will provide an extra safety against oxidative injure induced through mercuric chloride (Farkas *et al.* 2001) [3].
The antioxidant enzymes in liver and kidney, for instance catalase (CAT), superoxide dismutase (SOD), glutathione S-transferase (GST), glutathione peroxidase (GPX) and glutathione reductase (GR) acknowledged to be receptive to metals (Elia *et al.* 2003) [4]. The sub-acute and chronic mercury concentrations may cause several alterations in biochemical and hormone constraints of monitoring fish thus application of changes represented as ideal biomarkers in regards of mercury detection (Atli & Canli, 2010 and Safahieh & Hedayati, 2011) [5, 6].
The enzymatic antioxidant was settled on to create a possible environmental impact on toxic metal in fish. Amendments in antioxidant cellular systems have often been exhibit as biomarkers of pollutant-mediated toxicity. This prompted us to explore the activities of Catalase, superoxide dismutase, Reduce Glutathione in liver, kidney, gills of mercuric chloride treated fish. Oxidative stress plays a vital role in heavy -metal toxicity. Much experimental data provides evidence in concern to metal interaction by means of nuclear proteins and DNA which cause oxidative deterioration of biological macromolecules. The anti-oxidative defense system is obligatory regarding upholding of biological redox homeostasis. Modifications in antioxidant cellular systems have frequently been advised as biomarker of pollutant arbitrated toxicity.
The reactive oxygen species cause serious pathology in humans and animals at an early stage of illness (Chen *et al.* 1995) [7]. As oxygen was discharged into atmosphere via photosynthetic processes, its toxicity has pretense an immense hazard to existence (Hassan and Schiavone 1991) [8]. Many mammalian species, including aquatic animals like fishes posses suspicious mechanisms to counteract the impact on reactive free from of oxygen species. These systems consist of 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 elements ((Tjalkens *et al* 1998) [9]. The organ liver was chosen for observation as it plays vital function in regulating the overall body metabolism and thus intense involvement in detoxification of xenobiotics takes place. Consequently, evaluation of antioxidant responses to liver is extremely relevant, since toxicants that cause provisional or permanent trouble of homeostasis, can disrupt their functions (Miller, 2002) [10]. Therefore, present study focused to explore the activities of catalase, superoxide dismutase, Reduce Glutathione on liver of mercuric chloride tested *Labeo rohita*.

Materials and Methods
General protocols are described to measure the antioxidant enzyme activity of superoxide dismutase (SOD), catalase, and glutathione peroxidase. The SODs convert superoxide radical into hydrogen peroxide and molecular oxygen, while the catalase and peroxidases convert hydrogen peroxide into water. In this way, two toxic species, superoxide radical and hydrogen peroxide are converted to the harmless product water.
Five fishes were exposed to aquaria containing 70µg/ml and 250µg/ml mercuric chloride. Similarly five fishes (20mg/ml,

30mg/ml, 50mg/ml and 60 mg/ml mercuric chloride) were introduced into the respective tank at 2nd stage, and 5mg/ml, 2.5mg/ml, 2mg/ml, 1mg/ml and 0.5mg/ml at 3rd stage for the period of 96 hrs. Five fishes were introduced into the tanks containing 5%, 10% and 20% of LC50 mercuric chloride as 0.0662mg, 01324mg, 0. 2648mg.

Finally fish were observed for 96h In term of mortality and physical changes. At 96h, equal numbers of fishes were collected from test and from the control, and their liver was dissected. Biochemical assays such as superoxide dismutase, catalase and reduced glutathione were carried out in liver sample. Superoxide dismutase was estimated at the tissue lysate of liver samples (Marklund and Marklund, 1974) [11]. 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 420nm. Reduced glutathione were estimated (Mercy and Nair, 1996) [12] and Catalase was assayed (Ellman and Fiches 1959) [13] from the tissues samples.

Results and Discussion

The fish was taken as sample organism because behavior can be observed easily and tested. Fishes of were randomly introduced into the respective aquarium as control and test. Five fishes for each and 0.5mg/ml, 1mg/ml, 2mg/ml, 2.5mg/ml, 5mg/ml, 20mg/ml, 30mg/ml, 50mg/ml and 60 mg/ml mercuric chloride were introduced into the marked aquarium for period of 96h. Mortalities were recorded at 24th, 48th, 72nd of 96h and the experiment was repeated twofold.

Fishes were observed for 96h including observation of mortality and physical changes. At end of duration (96h.), fishes were collected from test, control and their concern fish liver was allowed for dissection. Enzymatic antioxidant Superoxide dismutase, Catalase and reduced glutathione were determined in liver. Method was used in estimation of Superoxide dismutase at tissue lysate of liver samples (Marklund and Marklund, 1974) [11]. An increase in absorbance was verified at 420nm for 3min. in spectrophotometer. Determination of Catalase was employed through method of Beers and Sizer (Rijijohn and Jayabalan, 1993) [14], Ellman's method were employed for reduced glutathione. Reduced glutathione is a substrate regarding peroxidases of glutathione as detoxification mechanism. Reduction mechanism of glutathione was occurs by means of NADPH under glutathione reductase enzyme. Glutathione Reductase assay enables the spectrophotometric measurement of glutathione reductase activity. Therefore, activity was measured at 412nm by amplify in absorbance grounds through DTNB (5; 5dithio bis (2-nitrobenzoic acid) reduction.

Statuses of mean antioxidant in liver of *Labeo rohita* exposed to diverse attentions of mercuric chloride in different concentration were studies. The concentration were made from 5, 10, 15, 20, 25, 30, 35 and 40% to estimate the three antioxidant enzymes viz. Catalase, Super oxide dismutase and reduced glutathione in specific range of spectrophotometric measurement. Values were expressed in mean experiments were carried out in triplicate. In which elevated level of Catalase was obtained with increase the percentage of LC50 concentration. The mean Catalase value was found in control fish (0.118) liver it was increase as 0.106, 0.211, 0.279, 0.332, 0.367, 0.394, 0.412 and 0.456 in concentration having the different 5, 10, 15, 20, 25, 30, 35 and 40% respectively (Table 1), whereas decrease level of superoxide dismutase were observed in graded concentration of mercuric chloride exposure as concern to LC50 (Table 1). In control liver sample 0.295 SOD value was recorded which represent 0.266, 0.242, 0.203, 0.184, 0.168, 0.142, 0.131 and 0.103 absorbance in favor of graded LC50 concentration respectively, similarly the another studies antioxidant enzyme like reduced glutathione was also determined for control and other design 5% LC50 -40% LC50 concentration of mercuric chloride treated fish and their organs. The absorbance of 0.723 was found in control whereas the variable ranges from 5% LC50 to 40% LC50 following value were recorded 0.630, 0.579, 0.507, 0.478, 0.419, 0.378, 0.314 and 0.257. There are no significant changes were observed only in 5 % of LC50 of Catalase activity (Table 1). Here finding justify accordance in the midst of findings (Velkova-Jordanoska, 2008) [15]. A direct reply to ecological hazard and their toxic impact in aquatic resource exhibit a suitable possible cause.

Table 1: Status of mean antioxidant in liver of *Labeo rohita* exposed to diverse attentions of mercuric chloride.

Concentration	Reduced glutathione absorbance (412nm)	Catalase absorbance (570nm)	SOD absorbance (420nm)
Control	0.723±0.11	0.118±0.14	0.295±0.15
1% of LC50	0.673±0.26	0.113±0.26	0.279±0.26
5% of LC50	0.630±0.16	0.106±0.16	0.266±0.24
10% of LC50	0.579±0.19	0.211±0.28	0.242±0.24
15% of LC50	0.507±0.24	0.279±0.33	0.203±0.31
20% of LC50	0.478±0.28	0.332±0.10	0.184±0.39
25% of LC50	0.419±0.12	0.367±0.41	0.168±0.14
30% of LC50	0.378±0.15	0.394±0.19	0.142±0.18
35% of LC50	0.314±0.124	0.412±0.25	0.131±0.27
40% of LC50	0.257±0.21	0.456±0.24	0.103±0.20

Values were expressed in Mean ± SE, experiments were carried out in triplicate.

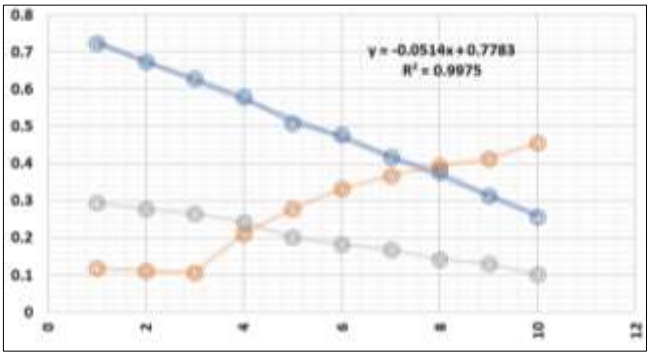

Fig 1: Graphics analysis of antioxidant in liver of *Labeo rohita* exposed to diverse attentions of mercuric chloride

Researcher reported suggested that the status of antioxidant enzymes depend on epoch, nourishment along with eggs of fish. Combined impact of zinc and lead studies on liver superoxide dismutase and Catalase in carp fish, results showed an elevated value in activity of Catalase and superoxide dismutase under 24h exposure as well as a decline activity were recorded subsequent to 5 days of contact. Mercuric chloride was creating to restrain the activity of this enzyme antioxidant for duration of oxidative stress (Dimitrova *et al.* 1994) [16].

Heavy metals accumulations have capacity to synthesize superoxide anions, SOD scavenge the superoxide radicals which work as mediator of toxicity and it also offer protection beside portion of oxygen toxicity (Farombi *et al* 2007) [17]. Oxidative stress acts as bioindicator of aquatic contaminations in fish due to indirect or direct association of heavy metals (Sies, 1993) [18]. GSH reduction suggest as harmful oxidative modification due to heavy metals (Pandey *et al.* 2003) [19]. Heavy metals show positive response as mechanism of induction of lipid per-oxidation along with certain amendment in status of enzymes of antioxidant related to fish organ. Configuration of ROS competent of injure tissues for instance proteins, lipids and DNA also (Kadar *et al.* 2005) [20].

Combined glutathione work as a delivery service of mercury and as antioxidant along with detailed shielding results in body which affected from toxicity of mercury. Specifically, glutathione binding with methyl mercury forms a complex that put offs mercury as of binding to cellular proteins in addition to cause harm to tissue and enzymes (Kromidas *et al.* 1990) [21]. Produce complexes of glutathione and mercury reduces the cellular break through anticipation of mercury ingoing tissue cells furthermore becoming a toxin. Diminish in glutathione stage and SOD capacity be owing to poisonous outcome of impurities that could devastate the defense of antioxidant. Increases in Catalase movement correspond to first line of protection beside oxidative strain (McCord, 1996) [22].

Second last aim of this study was performed by way of determination of oxidative stress biomarkers and parameters of antioxidant as a comeback to mercuric chloride exposure at low and moderate doses in three specific vital organs of *Labeo rohita* following 15 and 30 days of exposures. In this regard fish, is a biomonitoring organism of aquatic ecosystem which plays growingly vital responsibility in supervising of aquatic situation, as a result of its immense compassion in direction of environmental alterations. In study, a part of investigation were conducted with a specific aim which based on determination of oxidative stress biomarkers and parametric cellular defensive enzymatic antioxidant system as a low and moderate dose exposure of mercury chloride and their impacts on vital organs of fish after 15 and 30 days of exposures. Therefore, next aim was taken as objective determination of oxidative stress and antioxidant parameters under mercury chloride exposure at variable doses and their impacts on vital organs of fish.

For the performing of following mention investigative objective 36 *Labeo rohita* fish species were allowed to fed on commercially available pellet diet according to their 2% of body weight/day and kept in rectangular tanks contain tap water with specific pH value of 7.4 under 12:12 light and dark routine life cycle. Experiment was performed after two week of acclimation, then fishes were allowed to classify or distributed into three groups (12 fish in each). First group was untreated or control whereas two others were experimental tested groups tested for 15 and 30 days of mercury chloride exposure with two designs 0.08 and 0.20ppm doses.

Three vital Organs gills, kidney and liver were collected after the end of experimental duration and carefully excised to prepare for observation specimen study. After scarification of tissues surface were allowed to dry with a suitable absorbing filter paper, issues were thoroughly washed by means of freshly prepare 50 mM phosphate buffer having value of pH 7.4. Tissues were further homogenized in presence of same buffer. Process of Homogenous crushing was occur at 4°C using mechanical homogenizer, then it furthermore allowed to centrifuge at 10,000 rpm for 20 min at 4°C. Obtained supernatants were exploited in antioxidants and oxidative stress biomarkers assay.

Conclusion

concluded the enzymatic and non-enzymatic antioxidants under dissimilar range of mercuric chloride exposure in aquatic conditions. Sub-acute and chronic mercuric chloride status may cause several alterations in biochemical and hormone constraints of monitoring fish therefore request of changes correspond to ideal biomarkers in regards of mercury detection.

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