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Oxidative Stress Biomarker in Assessing the Lead Induced Toxicity in Commercially Important Fish, *Labeo rohita*

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ABSTRACT

Heavy metals considered as the most toxic pollutants in aquatic system. These are important inducers of oxidative stress in aquatic animals, promoting the formation of reactive oxygen species (ROS) which ultimately leads to tissue damage and oxidation of biomolecules. Aquatic organisms possess antioxidant system to cope with the oxidative stress. This research was conducted to see the effect of lead on peroxidase activity (antioxidant enzyme) in the gills and liver of *Labeo rohita*. Experiment was conducted in the laboratory by exposing four groups of *Labeo rohita* to different doses of $PbCl_2$ *viz.* 96-h, 2/3rd, 1/4th and 1/5th of LC_{50}, separately, for 30 days and compared with control group. After 30 days, fish was sacrificed and their gills and liver isolated to determine the effect of $PbCl_2$ on peroxidase activity. Activity of peroxidase enzyme in metal stressed fish was compared with control. The physico-chemical parameters of the test media *viz.* pH, dissolved oxygen, carbon dioxide, total hardness, calcium, magnesium and total ammonia were monitored on daily basis. Significantly increased peroxidase activity was observed in gills and liver of *Labeo rohita* after exposure to $PbCl_2$ due to all doses as compared to the control. All results were statistically significant at $p<0.05$. Fish liver exhibited significantly ($p<0.05$) higher activity of enzyme than that of gills. The physico-chemical variables *viz.* pH, dissolved oxygen, carbon dioxide, total hardness, calcium, magnesium and total ammonia of the test media varied significantly at $p<0.05$, that exerted significant effects on peroxidase activities in gills and liver of fish. This study clearly indicates the defensive nature and adaptive mechanisms of tissues (gills and liver) against free radical induced toxicity.

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SN performed the experiments. FA statistically analyzed the data. MJ and FL wrote the article.

Key words
***Labeo rohita*, Lead, Oxidative stress, Peroxidase, Toxicity.**

INTRODUCTION

The pollution of aquatic environment with heavy metals has been occurring a long time ago, although their impact becoming worst due to industrial resolution and new technologies (Shahid *et al*., 2013; Abbas and Javed, 2016). Metals considered as major environmental pollutants causing cytotoxic, mutagenic and carcinogenic effects in animals (More *et al*., 2003). Majority of heavy metals have long residence time in water sediments or in the body of aquatic organisms because, unlike the organic chemicals, they cannot be metabolized into less toxic compound (Ambreen *et al*., 2015). Heavy metals cause toxicity in fish through various ways out of which one is through the generation of reactive oxygen species (ROS) causing oxidative stress (Valko *et al*., 2005). Metals are able to disrupt the integrity of the physiological and biochemical mechanisms in fish.

Exposure of fish to metals may result in increase in ROS such as hydrogen peroxide (H_2O_2), superoxide radicals and hydroxyl radicals (OH) leading to impairment of normal oxidative metabolism and finally to oxidative stress (Lushchak, 2011). Many xenobiotics use general toxicity mechanism by causing oxidative stress in fish which is a pathological process related to increase production of ROS. Different parameters can be used to evaluate the oxidative stress generated by metal toxicity. These include lipid peroxidation (LPO), formation of protein carbonyls, as well as antioxidant enzymes in fish tissues (Campana *et al*., 2003; Farombi *et al*., 2007). This antioxidant system includes various enzymes such as superoxide dismutase (SOD) which catalyze the conversion of superoxide radicals to H_2O_2, as well as catalase (CAT) and glutathione peroxidase (GPx) which acts to convert H_2O_2 into water and oxygen (Meyer *et al*., 2003; Dauremepuits *et al*., 2004; Farombi *et al*., 2007).

Lead is non-essential metal which enter in aquatic organisms from point sources related to lead mining and industrial process. Lead is redox inactive metal and produce oxidative stress in the aquatic organisms by inducing alterations in the activities of antioxidant enzymes leading towards the increase in production of ROS like O_2^-, H_2O_2

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and OH by the Haber-Weiss and Fenton reactions (Ercal *et al*., 2001). Increased production of ROS can cause the oxidation of biomolecules and also produces changes in the redox status of cell and gene expression (Livingstone, 2003). Oxidative stress occurs due to imbalance between production and degradation of ROS by the antioxidant defense system (Nishida, 2011).

Among antioxidant enzymes, one of the enzymes is peroxidase found in the mitochondrial matrix and cell, it has selenium molecules on the active sites and requires glutathione as a cofactor for its functioning and act as a donor and consumer of H_2O_2 and also plays a significant role in the metabolism and phagocytosis (Rodriguez *et al*., 2003; Aruljothi and Samipllai, 2014).

Gills are the most important organ of the fish, play multifunctional role in performing dynamic functions such as osmoregulation, acid-base balance, respiration and excretion of nitrogenous wastes (Evans *et al*., 2005). Metals can enter into the gills by attaching with mucus layer of the gills and cause alterations in the ultrastructure and general morphology of fish gills (Athikesavan *et al*., 2006). Liver is the major place for the detoxification of toxic chemicals and also plays a significant role in the metabolism and excretion of toxic substances (Ferreira *et al*., 2005). Fish liver is the main source of an antioxidant enzyme GPx and shows higher activity of this enzyme as compared to the other organs to overcome the oxidative stress caused by heavy metals (Murugan *et al*., 2008). *Labeo rohita* (rohu) is the most important fish among three Indian major carps belongs to the Cyprinidae family, found in freshwaters of South and South-East Asia. *Labeo rohita* is the most important and widely used in poly-culture systems of carps (Vutukuru *et al*., 2007). Due to its high meat quality, it is widely consumed fish in Asia and it is also suitable to examine the level of water pollution (Ramani *et al*., 2002). Therefore, present research work was planned for assessing the $PbCl_2$ induces toxicity on peroxidase activity in *L. rohita.*

MATERIALS AND METHODS

The present research work was conducted under controlled laboratory conditions at Fisheries Research Farms, Department of Zoology, Wildlife and Fisheries, University of Agriculture Faisalabad. The fingerlings of *L. rohita* were brought to the laboratory for acclimation. After the acclimation period, healthy fish fingerlings of similar weights and lengths were selected for enzymatic studies. Fingerlings of *L. rohita* were exposed to $PbCl_2$ (LC_{50} 34.20±1.80 mgL-1 96 h) at different sub-lethal concentrations as determined by Abdullah *et al*. (2007) for 30 days.

After 30 days of lead exposure, fish organs *viz.* gills and liver were isolated to check the peroxidase activity. Each test was conducted with three replications for each concentration/treatment and activity of peroxidase in the selected organs (gills and liver) was compared with the control group. The gills and liver of fish were rinsed with phosphate buffer of pH 6.5 (0.2M) and homogenized in cold buffer (1:4W/V) by using a blender. The homogenate were centrifuged at 10,000 rpm, at 4ºC, for 15 min. The clear supernatants were preserved at -4ºC for the enzyme assay.

The fish samples were subjected to enzyme assay by following the method as described by Civello *et al*. (1995) for the determination of peroxidase activity. The enzyme peroxidase activity was determined Spectrophotometrically at a wavelength of 470 nm by measuring the conversion of guaiacol to tetraguaiacol. Reagents *viz.* phosphate buffer of pH 6.5 (0.2M), guaiacol and hydrogen peroxide were used in peroxidase enzyme assay. Guaiacol (750 µL) was added to phosphate buffer (47 ml) and mixed well on vortex agitator. After agitation, H_2O_2 (300 µL) was added to buffer solution. Reaction mixture contain buffered substrate solution (300µL), enzymes extract (60µL). A cuvette containing the 3ml of blank solution was placed into the spectrophotometer and set it to zero at wave length of 470 nm. Then a cuvette containing buffered substrate was placed into the spectrophotometer and reaction was started after adding 0.06 ml enzyme extract. The reaction time was 3 min and after that absorbance was recorded and activity of enzyme was calculated by using the following formula:

$$\text{Activity (unit/mL)} = \frac{\Delta A/3}{26.20 \times 60/3000}$$

Water pH and dissolved oxygen were measured and recorded by electronic meter HANNA HI-9146 while pH was recorded by digital meter HANNA HI-8424. However, CO_2, total ammonia, total hardness, calcium and magnesium were measured by following the methods of APHA (1998).

Factorial experiment with the three replications for each test concentration was performed to find-out statistical differences among various parameters. The treatment means were compared by using Tukey's/Student Newman-Keul test while the relationships among different parameters were determined by using regression and correlation methods.

RESULTS

The experiment was designed to determine the effect of lead on peroxidase activity in the gills and liver of *L. rohita*. The fish were exposed to $PbCl_2$ for 30 days and physico-chemical parameters *viz.* water temperature, pH, dissolved oxygen, carbon dioxide, total ammonia, total hardness, calcium and magnesium were also monitored throughout the trial.

Peroxidase biomarker

Peroxidase belongs to the family of antioxidant enzymes which provides protection to the aquatic organisms against toxic effects of ROS which inhibits a situation of oxidative stress. To check the variations in the peroxidase activity at different concentrations of $PbCl_2$ in the gills and liver of *L. rohita,* the data were subjected to the statistical analysis by following the factorial designs. A significant increase was observed in the peroxidase activity in the lead treated groups as compared to control group. Peroxidase activity was found to be higher in the gills and liver at LC_{50} exposure as compared to other treatments (96- hr, 2/3rd, 1/4th, 1/5th of LC_{50}) indicating high level of dose dependent peroxidase activity to overcome the toxic effects of ROS produced due to exposure of metal. Table I represents the activity of peroxidase in the gills and liver of *L. rohita* with different treatments of lead. In the gills of *L. rohita*, the significantly highest peroxidase activity was observed at LC_{50} concentrations that was 0.485±0.004UmL^{-1} and second highest was found due to 2/3rd concentration exposure (0.343±0.003 UmL^{-1}) while this was least in control fish with the value of 0.112±0.007 UmL^{-1}. Comparison of means indicated that the peroxidase activity tends to be elevated in all treatments as compared to control. In liver of *L. rohita*, the highest activity of peroxidase was measured as 0.615±0.005UmL^{-1} at LC_{50} concentration while it was significantly lower (0.143±0.005 005 UmL^{-1}) in control fish groups. In *L. rohita* the peroxidase activity was more pronounced in the liver than in gills. Therefore, after exposure to metal the peroxidase activity was significantly increased to show quick response in preventing the cells against oxidative stress caused by heavy metal therefore, the activity of peroxidase was found to be higher in the liver as compared to gills (Table II).

Table I.- Effect of chronic exposure to lead on peroxidase activity (UmL^{-1}) in the gills and liver of *Labeo rohita*.

Organs	Treatments					Overall means ± SD
	96-hr LC_{50}	2/3rd LC_{50}	1/4th LC_{50}	1/5th LC_{50}	Control	
Gills	0.485±0.003a	0.343±0.002b	0.275±0.004c	0.123±0.003d	0.112±0.004e	0.268±0.004
Liver	0.615±0.005a	0.425±0.007b	0.358±0.002c	0.286±0.004d	0.143±0.005e	0.366±0.005
Overall Means ± SD	0.550±0.004	0.384±0.005	0.317±0.005	0.205±0.004	0.126±0.006	

Source of variation	Degree of freedom	Sum of squares	Mean squares	F-value
Replications	2	0.00011	0.00005	NS
Treatments	4	0.64504	0.16126	9179.91$^{p<0.05}$
Organs	1	0.07193	0.07193	4094.80$^{p<0.05}$
Treatments × organs	4	0.01530	0.00382	217.67$^{p<0.05}$
Error	18	0.00032	0.00002	
Total	29	0.73269		

The mean with similar letter in single row and column are statistically non-significant at p<0.05. NS, non-significant; Significant at p<0.05; SE (treatments) = 1.711; SE (organs) = 1.082; SE (Treatments×Organs) = 2.420

Table II.- Effect of different concentrations of lead on physico-chemical characteristics of the test media.

Treatments	pH	Dissolved oxygen (mgL^{-1})	Carbon dioxide (mgL^{-1})	Total hardness (mgL^{-1})	Calcium (mgL^{-1})	Magnesium (mgL^{-1})	Total ammonia (mgL^{-1})
96-hr LC_{50}	8.58±0.06 a	4.83±0.04 e	0.96±0.03 a	298.18±0.06 a	28.44±0.03 a	58.27±0.05 a	1.70±0.04 a
2/3rd LC_{50}	8.51±0.04 a	5.15±0.06 d	0.86±0.03 b	284.32±0.04 b	25.84±0.05 b	56.28±0.04 b	1.63±0.03 b
1/4th LC_{50}	8.38±0.07 b	5.28±0.04 c	0.75±0.03 c	278.52±0.07 c	23.54±0.04 c	54.70±0.05 c	1.37±0.02 c
1/5th LC_{50}	8.23±0.05 c	5.46±0.03 b	0.65±0.02 d	267.28±0.07 d	21.70±0.07 d	52.37±0.03 d	1.34±0.04 d
Control	8.18±0.03 d	5.75±0.02 a	0.53±0.032 e	250.40±0.06 e	20.86±0.07 e	51.69±0.04 e	1.06±0.05 e

Physico-chemistry of lead test media

Water pH, dissolved oxygen, carbon dioxide, total ammonia, total hardness, calcium and magnesium of the test media were monitored on 12 hourly basis. The pH is an important parameter of water which determines the acidic or basic nature of water. The significantly acidic or basic water may interrupt the biochemical reaction of the aquatic organisms. The pH value of the water found to be increased with increasing concentrations of lead. Aquatic organisms require dissolved oxygen (DO) for metabolism and respiration. They also have to pay the costs of dependence on DO when reactive oxygen species are produced by various pollutants results in oxidative stress. During lead exposure the consumption of DO by the fish decreased significantly with the increased concentration of the lead. DO of water decreased (4.83±0.04 mgL^{-1}) significantly at LC_{50} as compared to the control (5.75±0.02 mgL^{-1}). The maximum and minimum mean values of carbon dioxide contents of the test media ranged from 0.96±0.03 to 0.53±0.02mgL^{-1} at LC_{50} and control group, respectively. A gradual increase in total ammonia concentration was observed with the increase in concentrations of lead. Total ammonia of water increased (1.75±0.04 mgL^{-1}) significantly at LC_{50} treatment as compared to the control (1.06±0.05 mgL^{-1}). The regression of peroxidase activity in the gills and liver on pH of the test media was significantly positive with R^2 values of 0.958 and 0.901, respectively. The peroxidase activity in both liver and gills was found to be increased with the increasing pH of the lead exposed test media. The dependence of peroxidase activity in both gills and liver on DO contents of the test media was significantly inverse with R^2 values of 0.924 and 0.994, respectively. Due to lead exposure, the DO decreased in the test media and the peroxidase activity of both gills and liver increased significantly. Linear regression between peroxidase activity in the gills and liver on carbon dioxide showed that the enzyme (peroxidase) activity was positively significant at $p<0.05$ with R^2 values of 0.927 and 0.962, respectively. Carbon dioxide concentration increased resulting into enhanced activity of peroxidase in gills and liver of the fish. Statistically significant and direct dependence of peroxidase activity in liver and gills and total ammonia was observed with the computed R^2 values of 0.834 and 0.927, respectively. Fitted line plots of peroxidase activity in both the organs against total ammonia revealed that with an increase in total ammonia of the metal exposed test media, the enzyme activity also increased. A strong and direct dependence of peroxidase activity in both the organs *viz.* gills and liver was found, on total hardness of lead exposed media. The regression analysis between peroxidase activity in gills and liver on calcium contents exhibited a positive and significant relationship with R^2 values of 0.979 and 0.946, respectively. Peroxidase activity was increased with the increasing magnesium contents of the test media (Supplementary Table 1).

DISCUSSION

Contamination of aquatic environment due to the industrial, domestic and human activities has severe effects on the aquatic fauna (Canli and Atli, 2003). Metals can cause oxidative damage to the cell due to higher production of ROS by Fenton- and Haber-Weiss type mechanisms, that can cause an induction in lipid peroxidation, protein modifications and DNA damage (Baysoy *et al*., 2012). Heavy metals have ability to generate oxidative stress in the fish because of their strong oxidative nature. Peroxidase is one of the important antioxidant enzymes which plays an important role in the cellular defenses by the decomposition of H_2O_2 into water and oxygen and prevents the tissues from oxidative damage (Dursun *et al*., 2001).

During the present study, peroxidase activity at all sub-lethal concentrations was significantly increased in both the gills and liver of *L. rohita* as compared to the control fish. Liver is the main detoxifying organ as it contains antioxidant enzymes to overcome the lethal effects of ROS produced by the toxicity of heavy metals (Fernandes *et al*., 2007). Gills are more susceptible to oxidative damage because of direct contact with water and thin epithelial cells; therefore, metals can easily penetrate in the gills (Evans *et al*., 2004). Bangeppagari *et al*. (2014) also observed that the peroxidase activity significantly increased in the liver and gills of lead exposed *L. rohita*. Activity of lipid peroxidase was increased significantly in the liver of cadmium exposed *L. rohita* (Dabas *et al*., 2014). The results are also in line with the findings of Mohanty *et al*. (2013) who found that the cadmium exposure can cause a significant increase in the activity of lipid peroxidase in the gills and liver of *L. rohita*. Cadmium induced significant alterations in the activities of antioxidant enzymes in the gills and liver of the freshwater fish, *L. rohita,* leading towards oxidative stress (Kumari *et al*., 2014).

Lead caused a significant alteration in the activity of GPx in the liver of freshwater fish, *Oreochromis niloticus* (Eroglu *et al*., 2014). Similar results were obtained by Maiti *et al*. (2010) who found that lead exposure caused an increase production of reactive oxygen species at cellular level and leads towards increased activity of lipid peroxidase in the brain of fish (*Clarias batrachus*). Similarly, Awoyemi *et al*. (2014) observed that lead chloride caused a significant induction in the glutathione peroxidase activity in the liver and gills of *Oreochromis niloticus* and *Clarias gariepinus*. GPx was significantly

increased in the liver of lead exposed *Clarias gariepinus* (Saliu and Bawa-Allah, 2012). Baysoy *et al*. (2012) also reported a significant increase in the activity of lipid peroxidase in the liver of the lead stressed *Oreochromis niloticus*. Similarly Brucka-Jastrzebska (2010) observed enhanced activity of lipid peroxidase activity in the liver and kidney of lead stressed fish as compared to control group. In contrast to our findings, Sujatha *et al*. (2013) observed significantly decreased GPx activity in *L. rohita* after cadmium exposure.

The physico-chemical parameters of the test media also altered by the lead exposure leading towards more toxic effects on the metal stressed fish. During the whole experiment, the concentration of dissolved oxygen was observed to be decreased and the level of carbon dioxide and total ammonia was decreased with the increasing lead concentrations. These results are confirmed by the findings of Abdullah *et al*. (2007) who observed that DO concentration was decreased when the concentrations of total ammonia, carbon dioxide, and total hardness were increased after exposure of lead. Leung and Furness (2001) observed that the pH, carbon dioxide, total ammonia and total hardness significantly induced increase in peroxidase activity in the metal exposed fish. Sampaio *et al*. (2012) observed decrease oxygen consumption rate of fish while level of carbon dioxide and total ammonia increased significantly leading towards oxidative stress in the fish, *Piaractus mesopotamicus*.

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Supplementary material

There is supplementary material associated with this article. Access the material online at: http://dx.doi.org/10.17582/journal.pjz/2018.50.2.735.741

Statement of conflict of interest

There is no conflict of interest.

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