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TOXIC EFFECT OF LEAD CHLORIDE ON ANTIOXIDANT ENZYME IN THE LIVER AND KIDNEY OF FISH

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ABSTRACT

The contamination of aquatic habitats has become a serious problem for sustainability of the fisheries sector in Pakistan. Among different pollutants, heavy metals are very toxic. Their toxicity can be assessed by using antioxidant enzymes as biomarkers. These enzymes protect the living organisms from the oxidative stress caused by the heavy metals. Therefore, the effect of lead chloride ($PbCl_2$) on the peroxidase enzyme activity in the tissues (liver and kidney) of *Cirrhinamrigala* was investigated. Four groups of *Cirrhinamrigala* were exposed, separately, to 96-hr LC_{50} concentration of $PbCl_2$ along with its sub-lethal concentrations, viz.2/3rd, 1/4th and 1/5th for a period of 30 days. Another group of fish was kept in metal free media as a control. After 30 days, fish were dissected and their liver and kidney isolated and preserved for enzyme assay. Peroxidase activity in the tissues of $PbCl_2$ exposed *Cirrhinamrigala* was compared with the control group. Results revealed that the activity of peroxidase enzyme in fish organs increased significantly ($p<0.05$) after exposure to lead chloride, as compared to control.

Keywords: Lead toxicity, Peroxidase activity, Liver, Kidney, Fish, oxidative stress.

INTRODUCTION

Heavy metals pollution in the aquatic environment has become a worldwide problem during the last few decades because of their non-degradable nature (MacFarlane and Burchett, 2000). Generally, heavy metals enter in the aquatic environment through natural sources like atmospheric deposition, erosion of the geological matrix, or anthropogenic activities like industrial effluents, domestic sewage, mining and agriculture wastes (Vutukuru, 2005; Ambreen and Javed, 2015).

Fish are considered to be the most important bio-indicators in aquatic ecosystems for the estimation of metal pollution. Fish are affected by even thesmallest changes in water pollution because it has to live in direct contact with the polluted water. Fish livers and kidneys are pivotal organs involved in osmo-regulation, detoxification, bio-transformation and excretion of xenobiotics (Vesey, 2010). Impacts of heavy metals on aquatic ecosystems can be evaluated by measuring the biochemical parameters in the liver and kidney of the fish that respond specifically to the degree and type of contamination (Barhoumi*et al*., 2012).

Among various heavy metals, lead (Pb) is one of the most harmful contaminants of aquatic environments. The major sources of lead pollution are mining and smelting of lead ores, industrial effluents, fertilizers, pesticides and municipal sewage wastes (Needleman, 2006). Lead may enter into the fish body through different routes, i.e., skin, gills and the respiratory tract (Olaifa*et al*., 2004). Once absorbed, lead becomes distributed in the liver, kidney, gills, heart, and gonads, as well as in the blood of fish (ATSDR, 2005). At higher concentrations, lead induces oxidative damage that may directly affect the cell membrane.

During normal metabolic processes, reactive oxygen species (ROS) are continuously produced at lower

concentrations while over-production of ROS is one of the initial responses against oxidative stress in biological systems (Arora *et al*., 2002). Like all aerobic organisms, fish are also susceptible to the toxic effects of ROS that lead to the oxidation of DNA, lipids and proteins (Rigoulet, 2001). To decrease the negative impacts of ROS, there existed an antioxidant enzyme system which includes peroxidase (POD), catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione reductase (GR) that are responsible for the conversion of harmful ROS to less or non-harmful products (Cossu*et al*., 2000). Among these antioxidant enzymes, peroxidase is the one that protects the tissues from damage caused by hydrogen peroxide. The peroxidase enzyme is comprised of many iso-forms which perform a variety of metabolic processes in organisms including immune cell function, phagocytosis (Rodriguez *et al*., 2003) antioxidants functions (Galloway *et al*., 2001) and cell adhesion (Holmblad and Soderhall, 2000). Antioxidant enzymes may be considered sensitive biomarkers for estimating the environmental stress prior to the onset of harmful effects on the fish (Geoffroy*et al*., 2004). Therefore, in the current study, we aimed to assess the changes in activity of the most important antioxidant enzyme (peroxidase) in the liver and kidney of *Cirrhinamrigala*.

MATERIALS AND METHODS

This experiment was performed in the laboratories of Fisheries Research Farms, University of Agriculture, Faisalabad, Pakistan. For this purpose, one year old *Cirrhinamrigala* were brought to the laboratory and acclimatized to laboratory conditions. After acclimatization, fish having similar weights and lengths, were transferred to the glass aquaria of 50L water capacity. A chemically pure compound of lead chloride ($PbCl_2$: MERCK) was dissolved in 1000 ml of deionized water and the metal stock solution was prepared. Four groups of fish (n=10) were exposed to 96-hr LC_{50} and its sub-lethal concentrations, viz. $2/3^{rd}$, $1/4^{th}$ and $1/5^{th}$ of LC_{50} values of lead chloride as determined by Abdullah *et al*. (2011). The experiments were conducted with three replications for each test concentration at constant laboratory conditions. Another group of fish regarded as “control” werekept in the metal free media. After 30 days of lead chloride exposure, the fish were dissected and their livers and kidneyswere isolated and stored at -4 ºC for enzyme assay.

Enzyme Analyses

The liver and kidney of the fish were isolated and rinsed with a phosphate buffer of pH 6.5 (0.2 M) and homogenized in cold buffer (1:4 W/V) by using a blender. This was done to remove the red blood cells from the tissues (liver and kidney). After homogenization (Ogawa Seiki Co. Ltd), the organ homogenate was centrifuged (Spectrafuge 24D Lab Net International) at 10,000 rpm for 15 minutes at 4 ºC. After centrifugation, the clear supernatants were preserved at -4 ºC for the enzyme assay. However, the residues were discarded. The samples were subjected to enzyme assay by following the method as described by Civello*et al*. (1995) for the determination of peroxidase activity. The peroxidase enzyme activity was determined spectrophotometrically at a wavelength of 470 nm by measuring the conversion of guaiacol to tetraguaiacol. Guaiacol (750 µL; Sigma-Aldrich) was added to the phosphate buffer (47 ml) and mixed well on the vortex agitator. After agitation, H_2O_2 (300 µL; Sigma-Aldrich) was added to the buffer solution. The reaction mixture contains buffered substrate solution (300 µL), enzymes extract (60 µL) and blank (phosphate buffer).

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A cuvette containing the 3 ml of blank solution was inserted into the Spectrophotometer (OptimaSP-300, Japan) and set to zero at a wavelength of 470 nm. Then a cuvette containing buffered substrate was placed in the Spectrophotometer and an initiation of reaction occurred by adding 0.06 ml enzyme extract. The reaction time was 3 minutes and after that, absorbance was recorded and activity of peroxidase was measured by using the following formula (Civello et al., 1995):

$$Activity\ (U/mL) = \frac{\Delta A/3}{26.60 \times 60/3000}$$

After the calculation of enzyme activity, data were subjected to statistical analyses. Data means were separated by employing Duncan Multiple Range (DMR) test. An alpha level of $p<0.05$ was considered statistically significant.

RESULTS

Peroxidase Activity

Activity of the peroxidase enzyme was estimated in the liver and kidney of *Cirrhinamrigala*after an exposure of 30 days to various sub-lethal concentrations ($2/3^{rd}$, $1/4^{th}$, $1/5^{th}$ of LC_{50}) of lead chloride. Peroxidase activity of liver and kidney in stressed fish was compared with the control group. Peroxidase activities in the liver and kidney of metal stressed fish were 0.691±0.004 and 0.372±0.003 UmL^{-1}, respectively. Results revealed that activity of the peroxidase enzyme was observed significantly ($p<0.05$) higher in the liver and kidney of *Cirrhinamrigala* due to 96-hr LC_{50} exposure as compared to other treatments ($2/3^{rd}$, $1/4^{th}$, $1/5^{th}$ of LC_{50} and control), indicating dose dependent peroxidase activity in the fish. Table 1 shows the analysis of variance on peroxidase activity (UmL^{-1}) in the liver and kidney of *Cirrhinamrigala* after chronic (30 days) exposure of lead chloride. Comparison of means reveals that the peroxidase activity was significantly higher at all test concentrations as compared to the control group. In the liver of *Cirrhinamrigala*, the peroxidase activity was higher at 0.691±0.004 UmL^{-1}(at 96-hr LC_{50} exposure), while it was significantly lower (0.111±0.004 UmL^{-1}) in the control group. In the kidney of fish, the highest activity of peroxidase (0.372±0.003 UmL^{-1}) was observed at 96-hr LC_{50}, while it was significantly lower (0.107±0.003 UmL^{-1}) in the control fish. Among selected organs, peroxidase activity (UmL^{-1}) was significantly ($p<0.05$) higher in the liver than the kidney of the metal stressed *Cirrhinamrigala* (Figure 1).

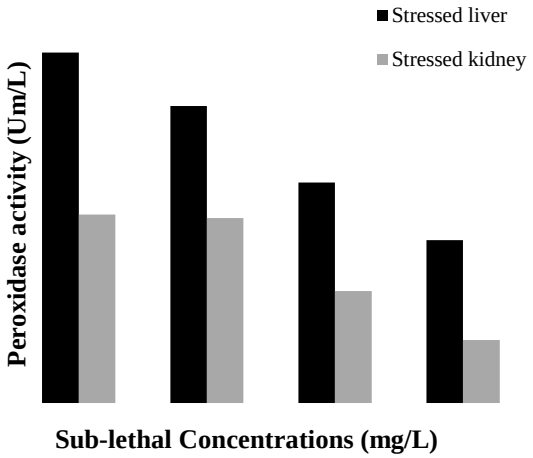

Figure 1: Concentration based peroxidase activity in liver and kidney of fish.

DISCUSSION

In aquatic organisms, heavy metals are known to induce oxidative stress by reducing the activities of antioxidant enzymes which results in the enhanced production of reactive oxygen species (ROS) at the cellular level that leads to oxidation of bio-molecules. Among heavy metals, lead is a redox inactive metal which induces oxidative damage by altering the activities of thiol containing enzymes (Pinto *et al*., 2003).To overcome the toxic effects of ROS, all the aquatic organisms possess antioxidant defense systems (Livingstone, 2001). Toxicants cause disturbances in the physiological

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Table 1: Dose dependent peroxidase activity (UmL^{-1}) in the liver and kidney of *Cirrhinamrigala* after 30-day exposure of Lead Chloride.

Organs	Treatments					
	96-hr LC_{50}	2/3rd LC_{50}	1/4th LC_{50}	1/5th LC_{50}	Control	Means ± SD
Liver	0.691 ± 0.004^{a}	0.586 ± 0.003^{b}	0.435 ± 0.004^{c}	0.321 ± 0.005^{d}	0.111 ± 0.004^{e}	0.429 ± 0.004^{a}
Kidney	0.372 ± 0.003^{a}	0.365 ± 0.005^{b}	0.221 ± 0.002^{c}	0.124 ± 0.003^{d}	0.107 ± 0.003^{e}	0.238 ± 0.003^{b}
Means ± SD	0.531 ± 0.003^{a}	0.475 ± 0.004^{b}	0.328 ± 0.003^{c}	0.222 ± 0.004^{d}	0.109 ± 0.003^{e}	

The means with similar letter in single row are statistically non-significant at $p<0.05$.

state of the fish, affect enzyme activities that causes distortions in the cell organelles and lead to the elevation of various harmful products (Vinodhini and Narayanan, 2009).

During the present study, increased activity of the peroxidase enzyme was found in the metal stressed fish as compared to the control. In the control group, lowered values of peroxidase activity may be due to the lower production of ROS. It is known that exposure of metals to the fish would enhance the production of ROS in response to which antioxidant activity increases. In the present study, peroxidase activity was significantly increased in the liver and kidney of the fish, *Cirrhinamrigala*, after chronic exposure to lead chloride, while the fish from the control group showed minimum values for peroxidase activity in these tissues. Increased activity of peroxidase in the liver may be attributed to the fact that many biochemical processes as well as bio-transformational events are confined to the liver. During these processes/events reactive oxygen species are formed. Fish liver is the main source of antioxidant enzymes to detoxify the environmental pollutants.Therefore, it has been used as an indicator of environmental pollution (Siscar*et al*., 2014). Kidneys are responsible for the elimination of harmful compounds from the body (Radovanovic*et al*., 2010). Peroxidase belongs to the antioxidant enzymes family and causes the oxidation of a particular substrate at the expense of H_2O_2.Peroxidase can act as a scavenger to reduce the harmful effects of ROS and converts the H_2O_2 into water and oxygen (Aruljothi and Samipillai, 2014). Therefore, after lead exposure, activity of this enzyme was significantly increased to overcome the severe effects of ROS on bio-molecules. It was higher in the liver than kidney because the liver is the main detoxification site and has a high affinity to accumulate the higher amount of metal as compared to other organs (Murugan*et al*., 2008). Increased activity of peroxidase in the liver and kidney of metal stressed *Cirrhinamrigala*may be explained as a defensive mechanism against oxidative stress, which was similar to the findings of Vinay and Yadav (2014) who reported that the liver is a major organ for the production of antioxidant enzymes and therefore protects organisms from oxidative stress.

During the present study, peroxidase activity was found to be increased significantly ($p<0.05$) in the liver of lead exposed fish as compared to the control fish. Bangeppagari et al. (2014) also observed increased activity of lipid peroxidase in the liver of lead exposed *Labeorohita*. Similarly, increased production of reactive oxygen species was observed in the brain of *Clariasbatrachus* after lead exposure by Maiti*et al*., 2010. The present results are in-conformity with the earlier studies on the fish exposed to toxic metals (Vinodhini and Narayanan,

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2009) which also reported a significant, direct relationship of glutathione activity with lead ions in *Cyprinuscarpio*. Farombi*et al*. (2007) observed a significant increase in the activity of lipid peroxidase in the liver and kidney of lead exposed fish (*Clariasgariepinus*). The present results are inconformity with the findings of Jastrzebska (2010), who observed that lead stressed fish (*Cyprinuscarpio*) exhibited significantly higher peroxidase activity than the unstressed fish. Present results are also parallel with the findings of Baysoy*et al.* (2012), who reported reduced lipid peroxidase and increased peroxidase activity in the liver of lead stressed *Oreochromisniloticus* than that of control fish. Abedi*et al*. (2013) found that lead exposed *Cyprinuscarpio* exhibited the maximum enzyme activity than that of unstressed fish.

Significant reductions in the level of lipid peroxidase was observed in the liver and kidney of *Halobatrachusdidactylus* after exposure to lead (Campana*et al*., 2003). Peroxidase activity was decreased in various tissues of fish due to heavy metal exposure (Fatima *et al*., 2000). Saliu and Bawa-Allah (2012) reported reduced activities of antioxidant enzymes in the liver of the lead stressed fish, *Claris garpiepinus*. Lead may cause oxidative stress to the fish that eventually altersthe glutathione peroxidase activity in the fish, *Channapunctatus* (Paul and Sengupta, 2013). Significant decreases in the activity of glutathione was observed in lead stressed fish as compared to the control. A similar report has been observed in *Oreochromisniloticus* when subjected to heavy metals stress (Atli and Canli, 2007). Diaconescu*et al*. (2008) observed a decline in the glutathione activity and a relationship of enzyme activity with concentrations of lead. They concluded that metal contaminated fish exhibited less enzyme activity than that of control fish.

CONCLUSION

Peroxidase enzyme activity was found to be significantly ($p<0.05$) higher in the lead stressed fish as compared to the control fish. Among two selected tissues, the fish liver exhibited significantly higher activity of peroxidase than that of the kidney. Humans and fish exhibit similar toxicological and adaptive responses to oxidative stress; therefore, this study will be used for ongoing future understanding of mechanisms underlying the oxidative stress response.

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