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Short Communication

Peroxidase Activity in Liver and Kidney of *Labeo rohita* exposed to Zinc Chloride

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ABSTRACT

Four groups of one year old *Labeo rohita* were exposed to 31.37 mgL^{-1}, 20.91 mgL^{-1}, 7.84 mgL^{-1} and 6.27 mgL^{-1} of $ZnCl_2$ for 30 days. The peroxidase activity was found to be significantly increased both in liver and kidney after $ZnCl_2$ treatments. Statistically significant increase in the liver and kidney peroxidase activity of 96-h LC_{50} exposed fish were found as 0.621±0.004UmL^{-1} and 0.219±0.002UmL^{-1}, respectively as compared to the control fish liver (0.123±0.002UmL^{-1}) and kidney (0.056±0.004UmL^{-1}).

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TM performed the experimental work. SA statistically analyzed the data. MJ helped in write-up of this article.

Key words
Peroxidase, Zinc chloride, Sub-lethal concentrations, *Labeo rohita*, ROS.

The presence of metallic ion pollutants in aquatic bodies cause tissue damage in fish leading to abnormal synthesis and degradation of enzymes (Padi and Chopra, 2002; Sevcikova *et al*., 2011). Zinc is a transitional metal and it serves as an essential micro-nutrient that plays an important role in the cellular activities and regulation of proteins (Oteiza and Mackenzie, 2005). It enters the aquatic environments through various anthropogenic activities such as purification of zinc, extraction of minerals, waste water treatment plants, burning of waste, coal as well as many other combustible substances. The small concentrations of zinc are necessary for the normal fish growth, metabolism and bio-mineralization (Clegg *et al*., 2005). The higher concentrations of zinc may cause impairment in several biological processes such as affecting adversely the respiration as well as inhibition of development (Goida *et al*., 2007). Elevated zinc concentrations are also responsible for the destruction of enzymes ultimately causing oxidative stress to the fish, a condition known as "oxygen paradox" (Oteiza, 2012). Oxidative stress in the fish leads toward cellular damage as well as oxidation of DNA, proteins, lipids and other biomolecules (Cao *et al*., 2010).

In general, heavy metals have the ability to produce free radicals in various ways that mainly depend upon the type of metal (Sevcikova *et al*., 2011). The active metals (copper, zinc and iron) produce reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-) and superoxide radical (O_2^-). H_2O_2 is the transitional ROS generated through different oxidative pathways (Davies, 1995). Antioxidant enzymes are natural compounds that are protein in nature and they are important in sustaining animal life (Bairoch, 2000). For protection of cells, antioxidant enzymes such as catalase (CAT), glutathione reductase (GR), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione (GSH) and glutathione-S-transferase (GST) play a vital role in reducing the oxidative stress (Tripathi *et al*., 2006). Peroxidases are broadly present in the microorganisms and animal tissues (Boeuf *et al*., 2000). The amino acid (cysteine) and heme cofactor provides the active site for the enzyme peroxidase. When oxidative stress increases, peroxidase functions as first line of defense towards reactive oxygen species (Kurutas *et al*., 2009). However, alterations in the level of peroxidase may occur due to the toxicity of heavy metals. Therefore, these molecular biomarkers could be widely utilized as diagnostic tool to assess heavy metals toxicity in the fish (Olaifa *et al*., 2004).

The major carp *i.e. Labeo rohita* is an important freshwater species that is extensively consumed in Pakistan due to its better meat quality (Ahmad *et al*., 2000). Consumption of contaminated fish may cause metal accumulation in human body and ultimately affecting their health status (Bahnasawy *et al*., 2008). Elevated levels of heavy metals cause oxidative stress to the fish that may serve as a biomonitoring tool of pollution in natural aquatic bodies. The fish liver and kidney are vital organs involved in various dynamic functions such as osmoregulation, biotransformation, detoxification and excretion of xenobiotics (Vesey, 2010).

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Table I.- Effect of different concentrations of zinc chloride on peroxidase activity (UmL^{-1}) of liver and kidney of *Labeo rohita*.

Organs	$ZnCl_2$				Control	*Overall Means±SD
	31.37 mgL^{-1}	20.91 mgL^{-1}	7.84 mgL^{-1}	6.27 mgL^{-1}		
Liver	0.621±0.004 a	0.573±0.006 b	0.497±0.003 c	0.383±0.003 d	0.123±0.002 e	0.439±0.003 a
Kidney	0.219±0.002 a	0.173±0.002 b	0.124±0.003 c	0.097±0.002 d	0.056±0.004 e	0.134±0.002 b
Overall Means±SD	0.420±0.002 a	0.373±0.002 b	0.311±0.002 c	0.240±0.001 d	0.090±0.002 e	

The means with similar letters in a single row and *column are statistically non-significant at $p<0.05$.

Therefore, the present research work was conducted to investigate the effects of zinc chloride on peroxidase activity in the liver and kidney of *Labeo rohita*.

Materials and methods

Fingerlings of one year old fish, *Labeo rohita* (6.88±0.21 g and 77.42±1.15 mm) maintained in the fish ponds of University, Fisheries Research Farms, were acclimatized to the laboratory conditions for two weeks in cemented tanks prior to the start of experiment. Fish were fed with pelleted feed twice a day. Four groups of fish, each containing ten fingerlings were exposed to 31.37±1.70 mgL^{-1} (96-h LC_{50}), 20.91±1.62 mgL^{-1} ($2/3^{rd}$ of LC_{50}), 7.84 ±1.12 mgL^{-1} ($1/4^{th}$ of LC_{50}) and 6.27±0.95 mgL^{-1} ($1/5^{th}$ of LC_{50}) zinc, for 30 days by using static water system with continuous aeration under laboratory conditions. Each test was conducted with three replications for each concentration/treatment and activity of peroxidase in the selected organs was compared with the control fish organs.

Fish were dissected, liver and kidney were taken out for estimation of peroxidase activity. The fish liver and kidney, after rinsing with phosphate buffer of pH 6.5 (0.2 M) to remove RBCs were homogenized in cold buffer (1:4 W/V). The homogenate was centrifuged at 10,000 rpm for 15 min at 4°C, and the supernatant was used for the determination of peroxidase activity, according to Civello *et al*. (1995).

The data were subjected to statistical analyses by using the Factorial experiment, with three replications for each test dose. The means for various parameters were compared by using Least Square Design test.

Results and discussion

Table I shows that statistically highly significant differences ($p<0.01$) existed among all the treatments as well as organs for the peroxidase activity. In both the organs viz. liver and kidney of zinc chloride stressed *Labeo rohita*, the peroxidase activity was found significantly ($p<0.05$) increased as compared to the control fish. The present findings are in conformity with the result of Saliu and Bawa-Allah (2012) who reported that the activity of peroxidase was significantly increased in the liver of zinc chloride stressed (1.120±0.62UmL^{-1}) *Clarias gariepinus* as compared to the control (0.950±0.43UmL^{-1}) fish. Similar type of results have been reported by Palaniappan and Karthikeyan (2009) for liver of *Labeo rohita*, by Farombi *et al*. (2007) for liver and kidney of *Wallagu attu*, and by Ikram (2014) for liver of *Labeo rohita*. During present research work, the peroxidase activity was found increased with an increase in metal exposure concentration that followed the order: 96-h LC_{50} > $2/3^{rd}$ > $1/4^{th}$ > $1/5^{th}$ > control. Aruljothi and Samipillai (2014) also reported that peroxidase activity in the gills and brain of *Labeo rohita* was increased with increasing concentration of arsenic. It was also found that peroxidase activity was significantly higher in the liver of *Labeo rohita* as compared to kidney under metal stress conditions. The present results are in contradiction to those of Talas *et al*. (2008), who reported decreased activity of glutathione peroxidase in the liver tissues of *Oncorhynchus mykiss* (rainbow trout) after exposure of Cd^{+2} and Cr^{+3}.

Conclusion

The peroxidase activity was significantly increased in the liver and kidney after exposure to different concentrations of zinc chloride. However, liver showed significantly higher enzyme activity compared to the kidney.

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Statement of conflict of interest

Authors have declared no conflict of interest.

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