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Short Communication

Effect of Iron Chloride on Peroxidase Activity in Kidney and Liver of *Labeo rohita*

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

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***Labeo rohita*, Iron chloride, Sub-lethal dose, Enzyme activity, Peroxidase.**

ABSTRACT

Present research work was conducted to evaluate the effects of iron chloride on peroxidase enzyme activity in the fish, *Labeo rohita*. For this purpose, four groups of *Labeo rohita* (one-year old) were exposed to different treatments as 96-h LC_{50}, 2/3rd, 1/4th and 1/5th of LC_{50} concentrations of iron chloride for 30 days in the glass aquaria with three replications for each treatment. After 30-day exposure of iron chloride, the fish organs *viz*. kidney and liver were isolated and the peroxidase enzyme activity was analyzed. Activity of peroxidase was assessed by measuring the conversion of guaiacol to tetraguaiacol, spectrophotometrically, at a wavelength of 470 nm. The peroxidase activity was measured in the selected organs exposed to various sub-lethal concentrations of iron chloride. The results revealed that peroxidase activity was increased significantly in both kidney and liver after exposure to iron chloride in all the treatments as compared to the control group. It was found that enzyme peroxidase had activity of 0.364±0.004 and 0.588±0.004 UmL^{-1} in the metal stressed fish kidney and liver, respectively while in control fish, the same for both the organs were observed as 0.085±0.002 and 0.112±0.002 UmL^{-1}, respectively.

Iron (Fe) is an essential micronutrient for normal physiological functioning of the fish because of its vital role in oxygen transport and cellular respiration (Aisen *et al*., 2001). In biological systems, iron occurs in three oxidation states as II, III and IV. These oxidation states bound to haemoglobin, transferrin, ferritin and iron-containing enzymes. Solubility of iron in water depends on pH, redox potential, temperature, oxygen and presence of OH^-, SO_4^{2-} and Cl^- (Valko *et al*., 2005). Toxicity of iron to the fish depends upon the concentration and duration of exposure, as well as the health of fish and its feeding habits (Farkas *et al*., 2003). Elevated concentrations of iron can cause cellular injury and showing negative impact on the fish and other aquatic biota (Orino *et al*., 2001). Iron has been shown to induce oxidative stress in the fish (Sevcikova *et al*., 2011). Toxicity of iron is largely based on its ability to catalyze the formation of radicals through the Fenton reaction. Catalytic amount of iron is sufficient to yield hydroxyl radicals (OH^-) from superoxide (O_2^-) and hydrogen peroxide (H_2O_2), known as reactive oxygen intermediates. These free radicals are highly reactive oxygen species, that can affect the antioxidant enzymatic activities, peroxidation of lipids, oxidation and modification of DNA and eventually cause tissue injury (Papanikolaou and Pantopoulos, 2005; Muller *et al*., 2007).

Oxidative stress is defined as a situation when concentration of reactive oxygen species (ROS) that includes O_2^-, H_2O_2 and OH^- enhanced, disturbing regulation of cellular metabolism and causes cellular injuries (Lushchak, 2011). ROS are generally produced by substances such as chemicals, transition metal ions and pesticides. Antioxidant system acts as front line of defense against oxidative stress, protect the living organisms from oxy-radical damage (Winzer *et al*., 2000). ROS are detoxified by antioxidant defense system including antioxidants like glutathione (GSH) and a set of antioxidant enzymes such as glutathione peroxidase (GPx), glutathione-S-transferse (GST), superoxide dismutase (SOD) and catalase (CAT) that protect the macromolecules against oxidative damage (Ozmen *et al*., 2004). Peroxidases are the enzymes responsible to oxidize molecules at the expense of H_2O_2 and detoxification of organic pollutants (Duran and Esposito, 2000). Peroxidases are widely distributed in the living organisms (Boeuf *et al*., 2000). Fish kidney and liver are pivotal organs involved in osmoregulation, de-toxification, biotransformation and excretion of xenobiotics (Vesey, 2010).

Impact of heavy metals on aquatic ecosystem can be evaluated by measuring the biochemical parameters in the kidney and liver of the fish that respond specifically

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to the degree and type of contamination (Barhoumi *et al*., 2012; Soufy *et al*., 2007). *Labeo rohita*, an Indian major carp, is economically important species in Pakistan due to its high consumption rate. In the aquatic ecosystems, *Labeo rohita* can help as a bio-indicator of environmental pollution (Vutukuru *et al*., 2007). Therefore, the present study was planned to determine the effect of iron chloride on peroxidase activity in kidney and liver of *Labeo rohita*.

Materials and Methods

The proposed research work was conducted in the laboratories of University of Agriculture, Faisalabad. *Labeo rohita* were procured from the fish ponds and brought to the laboratory for acclimatization for two weeks in cemented tanks. Fish were fed with pelleted feed (30% DP and 3.00 Kcalg^{-1} DE) twice daily. After acclimation period, healthy fish (one-year old), of similar weights and lengths, were selected for these experiments. Pure chloride compound of iron ($FeCl_2$) was dissolved in 1000 mL deionized water for the preparation of metal stock solution. Fish groups were transferred to the glass aquaria of 50 L water capacity to check the effect of iron chloride on peroxidase activity in the selected organs (kidney and liver) of *Labeo rohita*. Fish were exposed to 96-h LC_{50} (56.20±2.34 mgL^{-1}) as determined by Javed and Abdullah (2006) and 2/3rd, 1/4th and 1/5th of LC_{50} $FeCl_2$ concentrations for 30 days in the glass aquaria at constant water temperature (27ºC), pH (8) and total hardness (250 mgL^{-1}). Each test was conducted with three replications for each test dose along with a control treatment (un-stressed). After 30-day exposure of iron chloride, fish were sacrificed and their kidney and liver isolated for the estimation of enzyme assay.

Red blood cells were removed from the kidney and liver by rinsing these organs with phosphate buffer of pH 6.5 (0.2 M) and then by homogenizing the organs in cold buffer (1:4 W/V) using a blender. After homogenization, the organ homogenate was centrifuged for 15 min at 10,000 rpm at 4ºC. After centrifugation process, clear supernatant were preserved at -4ºC for enzyme assay while residue were discarded. For the determination of peroxidase activity, the sample was subjected to enzyme assay by following the methods of Civello *et al*. (1995). Activity of peroxidase was assessed by measuring the conversion of guaiacol to tetraguaiacol, spectrophotometrically, at a wavelength of 470 nm.

Factorial experiment, with three replications for each test concentration, was performed to find out statistical differences among various treatments of $FeCl_2$ under study. The treatment means were compared by using Tukey's / Student Newman-keul test. The relationships among different parameters were determined by Regression and Correlation analyses.

Results

After exposure of sub-lethal concentrations of iron chloride, the peroxidase activity was analyzed in the kidney and liver of the fish, *Labeo rohita*.

The exposure of $FeCl_2$ caused significant increase in the peroxidase activity in kidney and liver of fish as compared to the control group. Peroxidase activity was found to be significantly higher in fish kidney and liver at LC_{50} exposure as compared to other treatments indicating high level of dose dependent peroxidase activity that was enhanced at higher level. Table I shows analysis of variance on peroxidase activity in the kidney and liver of *Labeo rohita* exposed to different concentrations of $FeCl_2$. Statistically significant differences at $p<0.05$ were existed between all the treatments and the organs. In the kidney of *Labeo rohita*, the highest peroxidase activity was measured as 0.364±0.004 UmL^{-1} at LC_{50} concentration while it was significantly lower (0.085±0.002) in the control fish group. Comparison of means revealed that the peroxidase activity was elevated in all the treatments as compared to the control group. In the liver of *Labeo rohita*, the significantly highest peroxidase activity was analyzed at LC_{50} concentration as 0.588±0.004 UmL^{-1} followed by 2/3rd (0.426±0.001 UmL^{-1}), 1/4th (0.398±0.002), 1/5th (0.244±0.001) of LC_{50} and control (0.112±0.002). In *Labeo rohita*, the peroxidase activity was significantly higher in the liver as compared to kidney, having metal's exposure based peroxidase activity that increased significantly to show quick response in preventing the cells against oxidative stress caused by heavy metal.

Table I. Comparison of means on peroxidase activity (UmL^{-1}) in organs of *Labeo rohita* after chronic exposure of $FeCl_2$.

Organs	Treatments					
	LC_{50}	2/3rd	1/4th	1/5th	Control	*Overall Means±SD
Kidney	0.364±0.004 a	0.289±0.002 b	0.211±0.004 c	0.145±0.001 d	0.085±0.002 e	0.281±0.002 b
Liver	0.588±0.004 a	0.426±0.001 b	0.398±0.002 c	0.244±0.001 d	0.112±0.002 e	0.353±0.002 a
Means±SD	0.476±0.004 a	0.357±0.001 b	0.304±0.003 c	0.194±0.001 d	0.098±0.002 e	

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

Discussion

Contamination of aquatic bodies with heavy metals stimulates the production of ROS (Orun *et al*., 2008; Kurutas *et al*., 2009). ROS disturb the basic mechanisms of the body resulting into oxidative stress (Dautremepuits *et al*., 2002). The living organisms develop both enzymatic and nonenzymatic defensive mechanisms against the oxidative stress (Hu *et al*., 2007; Kakoolaki *et al*., 2013). The enzymatic defense system comprises of various antioxidants *viz.* CAT, SOD and peroxidase that are produced against reactive oxygen species (Pinto *et al*., 2003). These enzymes act as biomarkers to overcome the toxic effects of heavy metals in the aquatic organisms also (Geoffroy *et al*., 2004). Kidney is responsible for the elimination of compounds that form ROS in *Labeo rohita* (Radovanovic *et al*., 2010). Liver is the primary site where detoxification of pollutants takes place (Vinay and Yadav, 2014). Among antioxidant enzymes, peroxidase plays an important role in the defense system from the harmful effects of ROS in order to catalyze the H_2O_2 into water molecules.

During the course of this experiment, it was observed that iron stressed fish exhibited significantly higher peroxidase activity in both kidney and liver as compared to the control fish. The present results are in parallel to the findings of Ercal *et al*. (2001). They concluded that iron caused significantly higher production of ROS that lead to the oxidative stress. They found that the peroxidase activity in iron exposed fish (*Labeo rohita)* was significantly increased as compared to the un-exposed fish. The present results are also parallel to the findings of Rajeshkumar *et al*. (2013) who reported increased peroxidase activity in iron treated fish, *Chanos chanos* (1.70±1.00 UmL^{-1}) than that of control fish (0.70±0.10 UmL^{-1}). It was also concluded that enhanced peroxidase activity in iron chloride $FeCl_2$ exposed fish helped in the removal of oxyradicals. Sevcikova *et al*. (2011) reported that sub-lethal exposure of iron chloride resulted into increased peroxidase activity in the tissues of fish (*Carassius auratus*). In relation to the iron exposed fish, the control fish showed minimum values of peroxidase activity during the whole experimental period. Contrary to the present findings, Talas *et al*. (2014) reported that, as a result of sublethal arsenic exposure (0.01 mgL^{-1}) to *Cyprinus carpio*, the enzymatic activities in the selected organs (liver, gills and muscles) were decreased significantly as compared to the control fish group. During present investigation, it was found that with a rise in concentration of iron, the enzyme (peroxidase) activity also increased significantly. The present results are also in conformity with the findings of Ruas *et al*. (2008). They observed that the enzyme activities in red blood cells of cichlid fish (*Oreochromis niloticus*) were increased (1.02±0.04 UmL^{-1}) with increasing concentration of iron in water. Atli and Canli (2010) studied the effect of iron on peroxidase activity in the kidney and liver of *Oreochromis niloticus*. They reported that the levels of antioxidant enzyme "peroxidase" increased with the increase in metal's concentration. The present research work revealed that liver exhibited significantly higher activity of peroxidase as compared to kidney. As heavy metals can cause oxidative stress in the liver by generating free radicals such as ROS. Hence, liver serves as the main site for multiple oxidative reactions and show the quick response of peroxidase activity during exposure of metals (Avci *et al*., 2005). Vinay and Yadav (2014) observed that liver was the major organ for the production of antioxidant enzymes and to protect the organisms from oxidative stress caused by various pollutants.

Conclusions

The peroxidase activity was found increased significantly in both the organs *viz.* kidney and liver after exposure of $FeCl_2$ in all the treatments as compared to the control group. Fish liver showed significantly higher production of peroxidase than kidney to countere oxidation in the body of fish.

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Statement of conflict of interest

There is no conflict of interest.

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