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Effect of various concentrations of Zinc on Peroxidase activity of *Catla catla*

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ABSTRACT

Antioxidant enzymes are the first line of defense against reactive oxygen species (ROS) and other free radicals. When the rate of ROS exceeds the capacity of antioxidant enzymes, non-detoxified radicals begin to attack the bio-molecules. Therefore, present research work was planned to study the effect of zinc on the peroxidase activity in the liver and kidney of *Catla catla.* To check the concentration based effect, *C. catla* were exposed, separately, to 96-hr LC_{50} of zinc and its sub-lethal concentrations viz. $2/3^{rd}$, $1/4^{th}$ and $1/5^{th}$ of LC_{50} for 30 days at constant laboratory conditions. After 30-day, the fish were sacrificed and their liver and kidney were analyzed for peroxidase enzyme activity. Change in activity of peroxidase enzyme in the fish exposed to sub-lethal concentrations was compared with the control. Dose dependent increase in the activities of peroxidase enzyme was observed in the tissues of fish as compared to the control group. Peroxidase activity in the liver of fish from all the treatments was significantly ($p<0.05$) higher than that of kidney. The results of these studies in fish tissues may prove that peroxidase activity can be used as a sensitive bio-indicator of the antioxidant defense system.

Key Words: *Catla catla*, Sub-lethal, Zinc, Antioxidant Enzyme

INTRODUCTION

The fluctuating nature of the environment and human activities are continuously adding pollutants to the water bodies causing deleterious effects on the aquatic organisms (Javed, 2005). Metals are distinctive among various pollutants due to their non-biodegradable nature which helps them to get accumulated in the organs of aquatic organisms and become lethal (Sobha *et al*., 2007). Metal contamination in aquatic environment is considered to be unsafe not only to the inhabitant of aquatic organisms like fish but also to human which are the ultimate consumers in the food chain (Ambreen *et al*., 2015; Perera *et al*., 2015). Among heavy metals, zinc is an essential metal which is used as cofactor in my enzymatic reactions. Although, it is essential for the normal fish growth and bio-mineralization (Clegg *et al*., 2005) but accumulation of zinc can stimulate the production of reactive oxygen species (ROS) in the fish that can oxidize the proteins, DNA and lipids (Oteiza *et al*., 2000). Zinc may also act as an antioxidant being an essential component of Cu/Zn-superoxide dismutase (Dondero *et al*., 2005).

Production of reactive oxygen species (ROS) is an inevitable phenomenon in all aerobically respiring organisms (Nishida, 2011). Reactive oxygen species viz. superoxide anion, hydrogen peroxide and hydroxyl radicals are produced naturally by mitochondrial respiration and other cellular processes that lead to the oxidation of proteins, lipids and nucleic acids (Singh *et al.*, 2006). To minimize the hazardous impacts of ROS on biomolecules, there existed an antioxidant defense system in all aerobically respiring organisms (Geoffroy *et al*., 2004). This system comprises of the enzymes like peroxidase, superoxide dismutase, catalase, glutathione-S-transferase and glutathione peroxidase and low molecular weight antioxidants, like metallothionein, ascorbic acid and vitamin E (Tripathi *et al.*, 2006). Antioxidant enzymes may have variable activity in the liver and kidney of the fish. Liver is the house of redox reactions and bio-transformations that produce maximum amount of free metal ions (Basha & Rani, 2003). Research on oxidative stress in the fish focuses on different toxicological aspects, like effects of different heavy metals on antioxidant enzymes activities, the intensity of lipid per-oxidation as well as the induction of bio-transformational processes and other biomarkers of oxidative damage (Morales *et al*., 2004). Antioxidants are extremely important biomarkers and act as strong indicators in the field of aquatic toxicology (Livingstone, 2003). Peroxidase is

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antioxidant enzyme that converts highly reactive H_2O_2 into non-toxicant forms, water and oxygen (Hansen *et al*., 2006). Peroxidase has been reported in the elimination of ROS produced in pathological and physiological processes and plays a significant role in the stimulation of inflammation, apoptosis and signal transductions (Imai & Nakagawa, 2003). Fish liver and kidney are considered as vital organs, involved in metabolism, bio-transformation and excretion of contaminants (Figuiredo-Fernandes *et al*., 2006). *C. catla*, commonly called Thaila, an Indian Major Carp which is preferred due to its higher growth potential, consumer preference and its compatibility with other major carps in poly-culture system (FAO, 2005). These fish species are being highly affected with increasing pollution level in the freshwater bodies of Pakistan. To conserve them, it is essential to check the effects of metallic ions toxicity on these important edible fish species. Therefore, this research work was conducted to assess the concentration based effect of zinc on peroxidase activity in the liver and kidney of *C. catla*.

MATERIALS AND METHODS

This research work was conducted in the laboratories of Fisheries Research Farms, Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad, Pakistan. One year old *Catla catla* were procured from the ponds and brought to the laboratory for acclimatization in cemented tanks. Fish were fed with pelleted feed (30%DP and 3.00Kcalg^{-1} DE) twice daily. After acclimation period, *C. catla* of similar weights and lengths, were selected for these experiments. Pure chloride compound of zinc ($ZnCl_2$) was dissolved in 1000ml de-ionized water for the preparation of metal stock solution. Four fish groups (n=10) were transferred to the glass aquaria of 50L water capacity to check the effect of Zn on peroxidase activity in the selected tissues viz. liver and kidney of *C. catla*. To check the concentration/dose based enzyme activity, these fish groups were exposed for 96-hr to LC_{50} of zinc (25.88±1.28mgL^{-1}) as determined by Abdullah & Javed (2006) and to 2/3rd, 1/4th and 1/5th LC_{50} values for 96 hrs, separately, for 30 days. Each test was conducted with three replications for each conentration along with a control group. After 30-day exposure period, fish were sacrificed and their tissues viz. liver and kidney were isolated and preserved at 4ºC for the estimation of enzyme assay.

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

Preparation of 0.2M phosphate buffer (pH 6.5): The 4g NaH_2PO_4 and 1g Na_2HPO_4 were taken in a flask and dissolved by adding distilled water and volume was raised up to 200ml and pH was adjusted at 6.5.

Preparation of buffer substrate solution: Guaiacol (750µl) was added to phosphate buffer (47ml) and mixed well on vortex agitator. After agitation, H_2O_2 (0.3ml) was added to buffer solution. Reaction mixture contained buffer substrate solution (3 ml), enzyme extract (0.06ml) and blank (phosphate buffer). A cuvette containing 3ml of blank solution was placed in the spectrophotometer and set it to zero at wavelength of 470nm. Then a cuvette containing buffered substrate solution was placed in the spectrophotometer and the reaction was initiated by adding 0.06ml of enzyme extract. After 3 minutes of reaction time, absorbance was observed and activity of enzyme was calculated by using the following formula:

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

Statistical Analyses: Factorial experiments, with three replications for each test concentration, were performed to find out statistical differences among various treatments of zinc under study. The means were compared by using Least Square Design (LSD). Possible relationships among different parameters were determined by Correlation and Regression analyses.

RESULTS

The peroxidase activity in the liver and kidney of zinc stressed fish was analyzed after 30-day metal exposure and represented in Table I.

Table I: Peroxizdase activity (UmL^{-1}) in liver and kidney of *Catla catla* after sub-lethal exposure to zinc

Organs	Sub-lethal Levels					
	96-hr LC_{50}	2/3rd LC_{50}	1/4th LC_{50}	1/5th LC_{50}	Control	Overall Means±SD
Liver	0.550±0.007 **a**	0.451±0.006 **b**	0.381±0.006 **c**	0.249±0.005 **d**	0.060±0.003 **e**	**0.338±0.005**
Kidney	0.147±0.002 **a**	0.085±0.002 **b**	0.060±0.002 **c**	0.040±0.001 **d**	0.022±0.003 **e**	**0.070±0.002**
Means ± SD	**0.348±0.004**	**0.268±0.004**	**0.220±0.004**	**0.144±0.003**	**0.041±0.003**	

Increased activities of peroxidase were observed in the liver and kidney of the fish exposed to various concentrations of zinc as compared to the control. Significantly higher peroxidase activities in the liver and kidney of fish were observed at 96-hr LC_{50} exposure as compared to other treatments. Statistically significant differences at $p<0.05$ existed among all the treatments and organs. The overall means exhibit that the peroxidase activity increased with an increase in metal exposure concentrations that followed the order: 96-hr LC_{50} > 2/3rd > 1/4th > 1/5th > control. Significantly higher enzyme activity was measured in the liver of fish (Fig., 1) exposed to 96-hr LC_{50} (0.550±0.007UmL^{-1}) while it was lower in the liver of control fish group as 0.060±0.003UmL^{-1}. However, in the kidney of *C. catla*, the enzyme peroxidase activity under 96-hr LC_{50}, 2/3rd, 1/4th and 1/5th ofLC_{50} exposures were recorded as 0.147±0.002, 0.085±0.002, 0.060±0.002 and 0.040±0.001UmL^{-1}, respectively while the enzyme activity in control fish was observed as 0.022±0.003UmL^{-1} (Fig., 2). The overall means computed for organs indicated that the peroxidase activity was more pronounced in the liver of *C. catla* as compared to the kidney (Fig., 3).

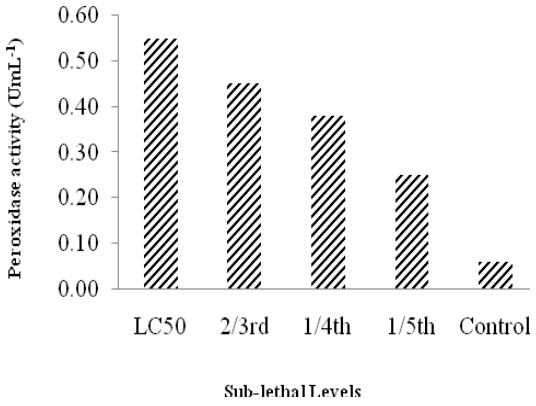

Fig., 1: Liver peroxidase activity in stressed and controlfish.

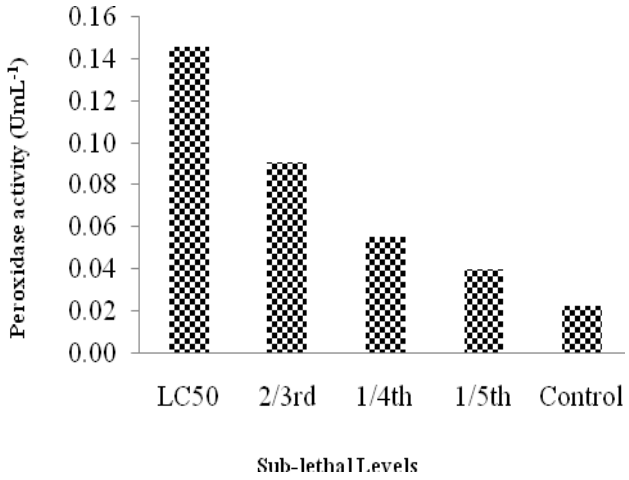

Fig., 2: Kidney peroxidase activity in stressed and control fish.

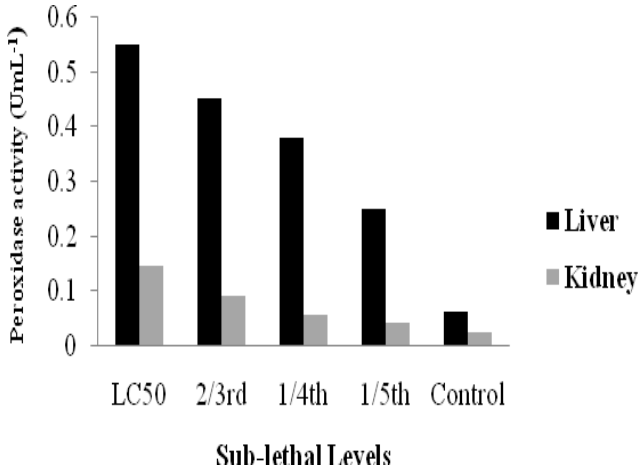

Fig., 3: Concentration base peroxidase activity in liver and kidney of fish.

DISCUSSION

During the normal cell metabolism, endogenous cellular process causes the production of free

radicals. However, the over production of reactive oxygen species (ROS) can cause changes in the cell redox status and alternation of gene expression, oxidation of lipids and proteins (Cao *et al*., 2010). Antioxidant defense system has been evolved in the aerobic organisms to protect them against toxicity of heavy metals and other substances that generate oxidative stress (George *et al*., 2004). Peroxidase is well known antioxidant enzyme present in mitochondrial matrix and cell which catalyzes the oxidation of gluthathione-S-transferase into glutathione by converting H_2O_2 into the water and oxygen (Aruljothi & Samipillai, 2014). Antioxidant enzymes are sensitive biomarkers, and are considered as significant diagnostic tools for testing water for the presence of toxicants in the aquatic environment (Geoffroy *et al*., 2004).

During present study, increased activities of peroxidase were observed in the liver and kidney of the fish exposed to various concentrations of zinc as compared to the control. Significantly higher peroxidase activities in the liver and kidney of fish were observed at 96-hr LC_{50} exposure as compared to other treatments. The overall means exhibit that the peroxidase activity increased with an increase in metal exposure concentrations that followed the order: 96-hr LC_{50} > 2/3rd > 1/4th > 1/5th > control. Liver and kidney plays important role in the excretion and detoxification of heavy metals ingested in the body (Marijic & Raspor, 2006). Effect of contaminants and toxicity of heavy metals in the aquatic ecosystem can be assessed by measuring the physiological and biochemical parameters in the kidney and liver of the fish (Barhoumi *et al*., 2012). Farombi *et al*. (2007) also observed increased activity of peroxidase in zinc stressed African catfish, *Calarias gariepinus*. During present study, liver exhibited significantly ($p<0.05$) higher peroxidase activity than that of kidney. In agreement with this study, increased peroxidase enzyme activity in liver, following zinc exposure has been reported in *Labeo rohita* by Palaniappan *et al*. (2009). Increase in hepatic peroxidase activity of the fish exposed to zinc was interpreted to reflect hepatocytes damage due to toxicant (Devi & Gupta, 2014). Banni *et al*. (2011) reported that zinc can induce oxidative stress in the liver of zebra fish, *Brachydanio rerio*. Alkaladi *et al*. (2014) reported that Zn can induce oxidative stress in the liver and kidney of Nile tilapia, *Oreochromis niloticus* and may cause damage to the cell membrane and mitochondria through over production of ROS. The present results are also in accordance with the findings of Saliu & Bawa-Allah (2012) who also observed higher activity of peroxidase (1.120±0.62UmL^{-1}) in the liver of zinc chloride stressed *Clarias graiepinus* as compared to the control (0.950±0.43UmL^{-1}) fish. However, Saddick *et al*. (2015) concluded from their research as peroxidase activity decreased in the liver of *Oreochromis niloticus* after exposure to higher concentration of zinc. Hao and Chen (2012) reported decreased activity of peroxidase in the liver of carp after exposure to higher concentration of zinc. Liver, a primary organ for various metabolic processes, may act as major target organ for zinc toxicity as zinc oxide induce ROS triggered mitochondrial mediated apoptosis, thus increasing antioxidant activity in response to it (Sharma *et al*., 2012). Change in the activity of peroxidase in kidney may be attributed to the fact that kidney is one of the major organs for detoxification and elimination of metallic toxicants (Gupta & Srivastava, 2006).

In conclusion the evaluation of metal's toxicity in freshwater organisms is one of the imperative areas of research and there is an emergent concern on the development of new techniques for detecting toxic effects of metals in aquatic organisms, especially fish. Oxidative biomarkers are useful in assessing the health of aquatic life. Therefore, this experiment was conducted on fish to see the effect of zinc by using oxidative stress biomarker (peroxidase) in the liver and kidney of fish. The acquired information would further help in making the strategies for treating zinc polluted water bodies and making the water safe for the survival of fish species.

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