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Original Research Article

Effects of various doses of copper sulphate on peroxidase activity in the liver, gills, kidney and brain of *Cirrhina mrigala*

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Abstract

Metal ion pollution of water generates addition of metals in the fish organs that leads towards redox reactions, making free radicals, particularly reactive oxygen species (ROS). All organisms have a strong antioxidant defense system to defend their tissues from injurious effects of ROS produced by metallic ions toxicity. During this study, effects of various doses of copper sulphate ($CuSO_4$) on peroxidase activity in the liver, gills, kidney and brain of *Cirrhina mrigala* were measured. One year old *C. mrigala* were subjected to 96-hr LC_{50}, 2/3rd, 1/4th and 1/5th of LC_{50} of $CuSO_4$, discretely, for period of the 30 days in glass aquariums with three replications for each treatment. The control group of the fish did not receive any metal stress. After 30-day exposure of $CuSO_4$, the fish from all treated groups were sacrificed and their liver, gills, kidney and brain is separated for peroxidase enzyme assay. Peroxidase enzyme activity in the $CuSO_4$ treated fish were compared amongst several treatments and with the control fish. Activity of peroxidase enzyme in all organs of the fish increased significantly ($p<0.05$) after exposure of $CuSO_4$ as compared to the control fish. Peroxidase activities in the liver, gills, kidney and brain tissues of metal stressed fish were measured as 0.891±0.002, 0.824±0.004, 0.767±0.004 and 0.334±0.004UmL^{-1}, respectively.

Keywords: Peroxidase activity, Copper sulphate, *C. mrigala*

Introduction

Pollution of freshwater with a diversity of pollutants has become a severe concern in Pakistan (Rauf et al., 2009). Heavy metals are discharged into aquatic habitat that can alter the biodiversity and pollute the water ecosystem due to their toxic and accumulative nature (Olaifa et al., 2004). Many heavy metals are significant for the fish to regulate several biochemical and physiological routes when present in small quantity. However, at larger concentration, more than a tolerant level, they may cause histological and biochemical changes in the fish body (Bu-Olayan and Thomas, 2004). Due to their persistent occurrence in the environment, heavy metals can disturb the growth, reproduction, physiology and survival of aquatic organisms, as well as major carps (Hayat et al., 2007). Heavy metals accumulate in diverse organs like liver, gills, kidney and muscles of the fish body, some of these organs accumulate higher concentration of metals than others (Jabeen and Chaudhry, 2010).

Copper is an important micronutrient for the growth, metabolism and enzyme activities in many organisms whereas it becomes toxic to aquatic organisms when its amount increases above certain levels (Handy, 2003). Copper induces variations in enzymatic activities, which may serve as an initial threat indication, reflecting fish health status and copper pollution level in water bodies (Atli et al., 2006). Some current studies revealed that heavy metals produce

reactive oxygen species (ROS) such as superoxide radicals, hydrogen peroxide (H_2O_2) and hydroxyl radicals "OH-" (Vutukuru et al., 2006). Copper generates direct oxidative damage of the cells by producing the reactive oxygen species through Fenton reactions (Amiard et al., 2006). Reactive oxygen species (ROS) oxidize proteins, lipids and nucleic acids and at the end, result in the apoptotic cell death (Messaoudi et al., 2009).

Antioxidant system of living organisms is affected by ecological chemicals and concentration of oxidants in the living cells act as bio-indicator of pollution (Lin et al., 2001). Antioxidant enzymes contain catalase (CAT), glutathione reductase (GR), superoxide dismutase (SOD), reduced glutathione (GSH), glutathione peroxidase (GPx) and glutathione-S-transferase (GST) that defend the cells and tissues from the detrimental effects of ROS (Pena-Liopis et al., 2001). Peroxidase is an enzyme that neutralize peroxides or hydro-peroxides through its catalytic activity. Peroxides are changed into water whereas hydro-peroxides are reduced into hydroxyl compounds during redox reactions (Pinto et al., 2003; De-Zoysa et al., 2009). Peroxidase defends the red blood cells destruction by hydrogen peroxide (H2O2) and tissues affected by oxidative stress due to lipid peroxidase (Li et al., 2003).

C. mrigala is one of the most important profitable fish species in Pakistan that can be cultured in earthen fishponds under composite and polyculture systems (Yaqub and Javed, 2012). Therefore, this experimental work was demonstrated to find out the effects of various doses of $CuSO_4$ on peroxidase activity in the liver, gills, kidney and brain of *C. mrigala* that would support in its sustainable development in natural habitats.

Material and Methods

Experimental details and enzyme assay

This research work was conducted in the laboratory of Fisheries Research Farms, Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad, Pakistan during September-October of the year 2016. Experiments were accompanied to find out the effects of various doses of $CuSO_4$ on peroxidase activity in the liver, gills, kidney and brain of *C. mrigala*. The fish fingerlings were retained under laboratory conditions in cemented tanks for two weeks, preceding to the start of experiment, for acclimatization and fed to satiation two time a day with pelleted feed. Fish were also fed during the experimental period to avoid stress from starvation. Glass aquaria were washed thoroughly and filled with 50-L dechlorinated tap water and stocked with 10 fish (One-year old) in each aquarium. Fresh air was provided to each aquarium through an air pump fitted with a capillary system. Chemically pure compound of $CuSO_4$ was dissolved in 1000mL deionized water and metal stock solution prepared. Fingerlings were exposed, separately, to 96-hr LC_{50} (48.91±1.11mgL-1) of $CuSO_4$ as determined by Yaqub (2011) and its sub-lethal concentrations viz. 2/3rd, 1/4th, and 1/5th in the aquarium, separately. After 1 month exposure of $CuSO_4$, the fish liver, gills, kidney and brain were isolated for the measurement of peroxidase activity. Every test was conducted with three replications for each dose and peroxidase activity in the tissues of the metal stressed fish was compared with that of the control group. After completion of 30 days of $CuSO_4$ exposure, the fish were sacrificed and liver, gills, kidney and brain of these fish were isolated and preserved at -4ºC for the assessment of enzyme assay.

Enzyme assay for peroxidase

The liver, gills, kidney and brain red blood cells were removed by rinsing these organs with phosphate buffer of pH 6.5 (0.2M) and homogenized in cold buffer (1:4W/V) using a blender. After homogenization, the organs homogenate was centrifuged for 15 minutes at 10,000rpm at 4°C. Clear supernatant was preserved at -4°C for enzyme assay after centrifugation process, while residues were discarded. The sample was exposed to enzyme assay methods of Civello et al. (1995) to determine the peroxidase activity. 3ml of buffer substrate solution (750µl guaiacol, 47ml phosphate buffer and 0.3ml H_2O_2) and 0.06ml of enzyme extract was mixed and peroxidase activity was evaluated by assessing the conversion of guaiacol to tetraguaiacol, at a wavelength of 470nm, spectrophotometrically.

Calculation

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

Statistical analysis

The data were statistically analyzed by using the Factorial design. The means for different parameters were compared by using Least Square Design (LSD).

Results

Peroxidase enzyme activity

After 30 days exposure to sub-lethal concentrations (2/3rd, 1/4th, 1/5th of LC_{50}) of $CuSO_4$, the activity of peroxidase was calculated in the liver, gills, kidney and brain of *C. mrigala*, Peroxidase activities in the selected tissues (liver, gills, kidney and brain) were compared with the *C. mrigala*. Peroxidase activity was observed to be higher in $CuSO_4$ subjected *C. mrigala* when compared to the control. Significantly highest peroxidase activity ($0.891\pm0.002 UmL^{-1}$) was noted in the organs of 96-hr LC_{50} exposed fish followed by 2/3rd, 1/4th, 1/5th and control, showing concentration dependent peroxidase activity. Table 1 shows the analysis of variance on peroxidase activity (UmL^{-1}) in the hepatic, gills, kidney and brain of *C. mrigala* after sub-lethal (30 days) experience of $CuSO_4$. Statistically significant ($P<0.05$) differences existed among peroxidase activity in the particular organs of *C. mrigala* exposed to various treatments (Table 1). The peroxidase activity in the liver of *C. mrigala* was higher as $0.891\pm0.002 UmL^{-1}$ at 96-hr LC_{50} exposure whereas it was significantly reduced ($0.119\pm0.004 UmL^{-1}$) in the control group. The highest activity of peroxidase in the gills of the fish was calculated as $0.824\pm0.004 UmL^{-1}$ at 96-hr LC_{50} concentration of $CuSO_4$ although it was significantly lower ($0.111\pm0.005 UmL^{-1}$) in the control fish. In the kidney tissues of *C. mrigala*, significant maximum activity of peroxidase was observed as $0.767\pm0.004 UmL^{-1}$ at 96-hr LC_{50} concentration however, it was significantly lower ($0.107\pm0.005 UmL^{-1}$) in the control fish group. The highest activity of peroxidase ($0.334\pm0.004 UmL^{-1}$) was observed in the brain of fish at 96-hr LC_{50}, although it was significantly lower ($0.098\pm0.005 UmL^{-1}$) in the control group. Peroxidase activity among organs followed the order: liver > gills > kidney > brain (Figure 1).

Table 1: Peroxidase activity (UmL^{-1}) in the organs of *C. mrigala* after 30-day exposure of copper sulphate

Organs			Treatments			
	96-hr LC_{50}	**2/3rd LC_{50}**	**1/4th LC_{50}**	**1/5th LC_{50}**	**Control**	**Overall Mean**
Liver	0.891±0.002 a	0.703±0.005 b	0.511±0.003c	0.333±0.003 d	0.119±0.004 e	**0.511±0.003 a**
Gills	0.824±0.004 a	0.642±0.003 b	0.457±0.006 c	0.283±0.002 d	0.111±0.005 e	**0.463±0.004 b**
Kidney	0.767±0.004 a	0.581±0.005 b	0.376±0.002 c	0.207±0.003 d	0.107±0.005 e	**0.407±0.005 c**
Brain	0.334±0.004 a	0.251±0.006 b	0.207±0.002 c	0.167±0.003 d	0.058±0.007 e	**0.203±0.003 d**
Overall Mean	**0.704±0.003 a**	**0.544±0.004 b**	**0.387±0.002 c**	**0.247±0.002 d**	**0.098±0.005 e**	

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

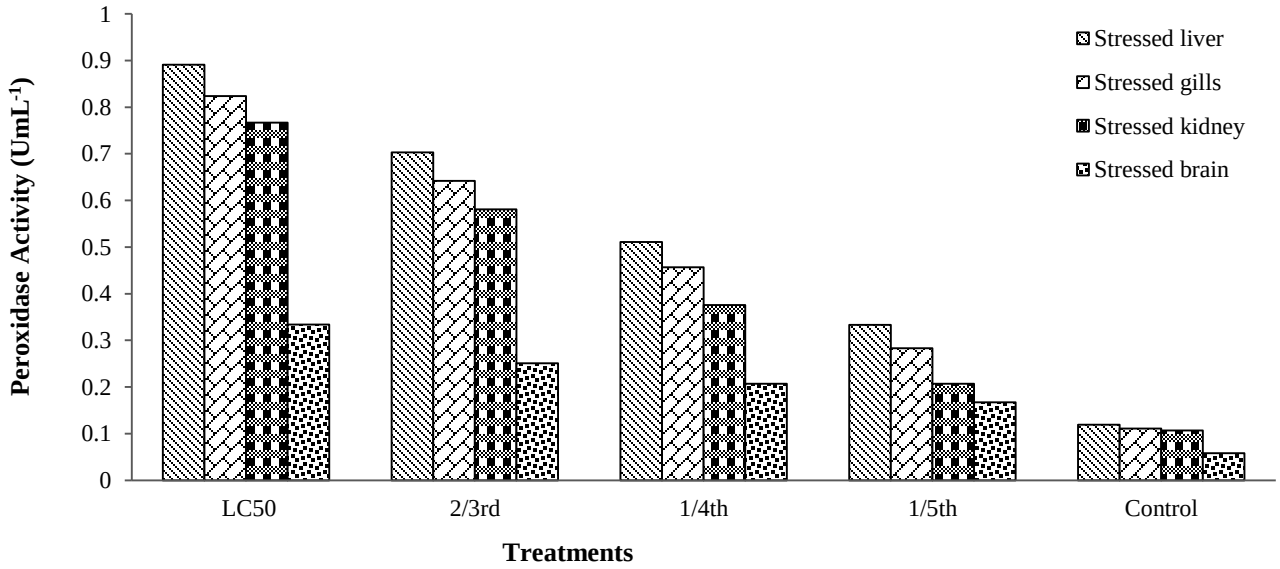

Figure 1. Comparison of peroxidase activity (UmL^{-1}) of the fish at different treatments of the copper sulphate.

Discussion

Every living organisms depend on production of ATP by oxygen based metabolism, but the extra production of this oxygen in the form of reactive oxygen species (ROS) can change in the redox status of the cell (Zhang et al., 2003). This oxidative stress produced by highly reactive oxygen species (ROS) viz. hydroxyl radical, hydrogen peroxide and superoxide radical can oxidize proteins, lipids, nucleic acids, frequently harm cell structure and ultimately cause cell death (Dewez et al., 2005; Cao et al., 2010). Living organism's antioxidant system comprises of peroxidase (POD), catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), and lipid peroxidase (LPO) (Tripathi et al., 2006). Peroxidase is a major antioxidant enzyme that catalyzes the oxidation of glutathione-S-transferase (GST) into glutathione by converting hydrogen peroxide (H_2O_2) into water (H_2O) and oxygen (O_2) (Aruljothi and Sankur, 2014). Glutathione peroxidase enzyme is accountable for the removal of ROS and reduction of H_2O_2 by changing it into oxygen and water in various metabolic pathways (Senchez et al., 2005). These antioxidant enzymes are measured as biomarkers of pollution and delicate tool to study pollutant content in a variety of marine and freshwater organisms (Brokovic et al., 2005).

During present study, metal (Cu) stressed fish exhibited significantly increased activity of peroxidase in the selected organs viz. liver, gills, kidney and brain as compared to the control fish. Liver and kidney displayed significantly higher tendency for the accumulation of metals as compared to the gills and muscles (Javed, 2005; Rauf et al., 2009). Pollutants and toxicity effects of heavy metals in the water ecosystems can be evaluated by measuring the physiological and biochemical parameters in the liver and kidney tissues of the fish (Barhoumi et al., 2012). In comparison to the exposed fish, the unstressed fish has showed minimum values for their peroxidase activity. In control group, these low values may be due to normally less generations of reactive oxygen species (ROS). Naz (2013) also detected increased peroxidase activity in the copper strained fish, *C. mrigala*. Increase in liver peroxidase activity of the goldfish, *Carrassius auratus* subjected to $CuSO_4$ was interpreted to reveal damage due to toxicant (Trivedi et al., 2012). Firat and Kargin (2010) described that copper can initiate oxidative distruction in the common carp (*Cyprinus carpio*). Increased glutathione activity was also detected with the production of ROS in crucian carp, *Carassius auratus*, when exposed to copper (Jiang et al., 2013). Waheed et al. (2014) observed that copper can generate oxidative stress in the *Labeo calbasu*, *Rita rita*, *Cirrhina riba*, *Clupisoma naziri* and *Securicola gora* through increased production of ROS. Peroxidase activity was enlarged in these fish species. The present study are also in accordance with the results of Eroglu et al. (2015) who also detected increased glutathione peroxidase activity in the river fish, *Oreochromis niloticus*. Wang et al. (2014) also proved that after exposure to $CuSO_4$, activity of glutathione significantly reduced in the fish, *Epinephelus coioides*. Min et al. (2014) demonstrated dose dependent increased glutathione peroxidase activity in the black rockfish, Sebastes schlegeli after exposure to higher concentrations of copper.

In the present study, the peroxidase activity was also observed to be increased in the liver of the fish with increasing $CuSO_4$ concentration in water. Among the organs, peroxidase activity followed the order: liver > gills > kidney > brain. The increased activity of peroxidase in the liver (6.4±0.27U/mg) has been perceived by Liu et al. (2010) in the copper stressed fish, *Synechogobius hosta*. Atli and Canli (2010) described an increase in peroxidase activity (86.8±8.14µmol/mg) in the kidney of copper stressed freshwater fish, *Oreochromis niloticus* as compared to the kidney in control fish (42.9±4.43µmol/mg) whereas peroxidase activity reduced significantly in the liver of the copper showing fish. Sivikova et al. (2016) detected the increase in glutathione peroxidase activity in the gills, liver and kidney of the common carp *Cyprinus carpio* after exposure to the $CuSO_4$ as similar results of the gills and liver were gained in the present study. The current study is also comparable to the findings of Trivedi et al. (2012) who also noticed increased peroxidase activity (61.73±0.24nmol/mg) in the liver of copper exposed fish, *Carrassius auratus*. In the course of the present study the liver showed significantly ($p<0.05$) higher peroxidase activity (0.891±0.002U mL^{-1}) than both of the kidney and gills, which is correlated to the findings of Min et al. (2014) who examined increased peroxidase activity in the liver (5.67±1.27nmolg^{-1}tissue), gills (1.00±0.04nmolg^{-1} tissue) and kidney (0.91±1.17nmolg^{-1}tissue) of the black rockfish, *Sebastes schlegeli* subjected to various concentrations of copper. In agreement with these results, increased glutathione peroxidase enzyme activity in the liver resulting copper exposure has been examined in *Gasterosteus aculeatus* by Sanchez et al.

(2005). Increased peroxidase activity (374.7±0.08 nmol/mg) was also detected with the production of ROS in the liver of the *Prochilodus lineatus*, after exposure of copper. Simonato et al. (2016) as similar results of the liver were observed in present study.

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