Short Communication

INVESTIGATION ON THE EFFECTS OF ZINC ON GILLS PEROXIDASE ACTIVITY IN THE FISH, *LABEO ROHITA*

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ABSTRACT

Four groups of fish equal in lengths and weights were exposed, separately, to 96-hr LC_{50}, $2/3^{rd}$, $1/4^{th}$ and $1/5^{th}$ of LC_{50} concentrations of zinc for 30 days in the glass aquaria. Another group was kept as control which was not exposed to any metal concentration. After 30-day zinc exposure, the fish from all treatments were sacrificed and their gills isolated for peroxidase enzyme assay. Physico-chemical parameters viz. pH, dissolved oxygen, carbon dioxide, total hardness, calcium, magnesium and total ammonia of the test media were monitored, twice a day. The data were subjected to statistical analyses by following the factorial design. The correlation and regression analyses were also employed to see the statistical relationships among different parameters under study. The results revealed that peroxidase activity was increased significantly in the gills of *Labeo rohita* after exposure of zinc in all treatments as compared to the control fish gills. The peroxidase activity was measured as 0.353 ± 0.003 UmL^{-1} in the 96-hr LC_{50} metal exposed fish gills while the same was 0.079 ± 0.003 UmL^{-1} in the control fish. The regression of peroxidase activity on physico-chemical variables of the test media showed significantly positive coefficients on all the variables except dissolved oxygen.

Keywords: *Labeo rohita*, gills, enzyme activity, zinc, sub-lethal.

INTRODUCTION

Now-a-days, the most considerable environmental issue is the deterioration of natural resources because of the release of urban and industrial wastes, smelting and mining of natural ores, processed or accidental spillage and discharge of sewage wastewater into the freshwater bodies (Ghosh and Singh, 2005). Among these contaminants, heavy metals are widely present in the aquatic reservoirs, having ability to cause adverse effects on the ecological balance of the recipient environment (Farombi *et al*., 2007). Heavy metals can severely alter the density and diversity of the aquatic habitats, due to their potential toxic effects. Because of their non-biodegradable nature, the metals are concentrated in the aquatic organisms and pass to other living organisms at higher trophic levels that consume these animals as a food source (Ashraf, 2005). Fish are considered as excellent bio-indicator of heavy metal's pollution in the natural aquatic bodies (Dirilgen, 2001). Heavy metals can enter fish body through different routes i.e. (i) directly through the skin (ii) indirectly through food and (iii) through respiratory organs (Rashed, 2001). In trace amounts, most of the heavy metals are important for certain physiological functions in the body of fish (Bu-Olayan and Thomas, 2004). At higher concentrations, all the metals even essential, may become toxic and cause oxidative stress to the fish that lead towards alteration in the functioning of vital enzymes. Therefore, heavy metals are considered as mutagenic, carcinogenic also affect adversely the immune system of fish (Chezhian *et al*., 2010).

Among heavy metals, zinc (Zn) is one of the most important element that is used as cofactor of enzymes involved in carbohydrate, nucleic acid, protein and lipid metabolism, in the transcription of genes and various other biological processes that sustain life (Chung *et al*., 2005). At higher concentrations of zinc, lipid peroxidation increases due to significantly decreased antioxidant enzyme activities in the fish tissues (Dautremepuits *et al*., 2002). Fish gills are the contact sites between water and blood for gaseous exchange and are considered as the major route for the uptake and transfer of toxicants to the internal tissues (Dabas *et al*., 2014). When the fish are exposed to environmental pollutants, their vital functions are altered eventually leading to death (Wilson *et al*., 1999).

Heavy metals are responsible for the induction of oxidative stress by increasing the production of reactive oxygen species (ROS) and decreasing the antioxidant enzyme's potential. Highly reactive hydroxyl radical, superoxide anion radical and hydrogen peroxide are the reactive oxygen species that react with susceptible biological macromolecules causing DNA damage, lipid peroxidation and protein oxidation (Shi *et al*., 2005). To cope with the harmful effects of ROS, fish possess an important defense system consisting of antioxidant enzymes viz. superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) that protect the cells from lethal effects of reactive oxygen species (Aras *et al*., 2009). Enzyme activity is considered as sensitive biochemical indicator to assess the hazardous effects of

toxicants on the fish and serves as vital parameter to evaluate the water quality (Gul *et al.*, 2004). Among antioxidant enzymes, peroxidase reduces the free hydrogen peroxide to water and lipid hydro-peroxides to their relevant alcohols i.e. from highly toxic to non-toxic forms (Ramsey and Sharpless, 2006).

In Pakistan, most significant freshwater culturalable fish species are the major carps viz. thaila (*Catla catla*), mori (*Cirrhina mrigala*) and rohu (*Labeo rohita*). In ecotoxicological research, *Labeo rohita* is used as a suitable model for enzyme bioassays (Chavan and Muley, 2014). Considering these points, the present study was conducted to investigate the effects of various concentrations of zinc on the gills peroxidase activity in the freshwater fish, *Labeo rohita.*

MATERIALS AND METHODS

Experiments were conducted to determine the effects of various zinc concentrations on the gills peroxidase activity in *Labeo rohita.* The fish were kept under laboratory conditions in cemented tanks for two weeks, prior to the start of experiment for acclimatization and fed to satiation twice a day with pelleted feed. Glass aquaria were washed thoroughly and filled with 50-L dechlorinated tap water and stocked with 10 fish (90-day old) in each aquarium. Fresh air was supplied to each aquarium through a pump fitted with capillary system. Chemically pure compound of zinc chloride ($ZnCl_2$) was dissolved in 1000 mL deionized water and metal stock solution prepared. One year old fish were exposed, separately, in the aquaria to 96-hr LC_{50} (31.37±1.70 mgL^{-1}), $2/3^{rd}$, $1/4^{th}$ and $1/5^{th}$ of LC_{50} of zinc as determined by Abdullah *et al.* (2007). After 30 days of zinc chloride exposure, the fish were sacrificed and their gills isolated for the estimation of enzyme assay. Each test was conducted with three replications for each concentration and activity of peroxidase in the gills was compared with that of control fish. The pH and dissolved oxygen of the test media were monitored twice a day by using digital meters, viz. HANNA HI-8424 and HI-9146 while the other physico-chemical characteristics were determined by following the methods of APHA (1998).

Preparation of Enzyme Extract: Red blood cells were removed from the gills by rinsing this organ with phosphate buffer of pH 6.5 (0.2 M) and homogenized in cold buffer (1:4 W/V) using a blender. After homogenization, the organ homogenate was centrifuged for 15 minutes at 10,000 rpm. After centrifugation process, clear supernatant was preserved at -4℃ for enzyme assay while residues 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.

Guaiacol (750 μL) was added to 0.2 M phosphate buffer of pH 6.5 (47 mL) and mixed well on vortex agitator. After agitation, H_2O_2 (0.3 mL) was added to buffer solution. The reaction mixture contains buffered substrate solution (3 mL) and enzyme extract (0.06 mL). The phosphate buffer was used as blank.

A cuvette containing 3 mL of blank solution was inserted into the spectrophotometer. Then a cuvette containing buffered substrate solution was put into the spectrophotometer and initiation of reaction was occurred by adding enzyme extract. The reaction time was 3 minutes and the absorbance noted after the specified reaction time.

The peroxidase activity was calculated by employing the following formula:

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

Statistical Analyses: The data were subjected to statistical analyses by using the Factorial design, with three replications for each test concentration. The means for various parameters were compared by using Least Square Design test. The correlation and regression analyses were also performed to find-out possible relationships among different parameters defined for this study.

RESULTS

Peroxidase Activity: After exposure of sub-lethal concentrations of zinc, the peroxidase activity was assessed in the gills of fish, *Labeo rohita*, by measuring its ability to reduce the concentration of H_2O_2 at a wavelength of 470 nm. The data were subjected to statistical analyses by following the Factorial experiment under RCBD with three replications for each treatment. Table 1 shows analysis of variance on peroxidase activity in the gills of *Labeo rohita* under different exposure concentrations of zinc. Statistically significant (P<0.05) variations existed among peroxidase activities in the gills of *Labeo rohita* exposed to various treatments. Comparison of means revealed that the activity of enzyme "Peroxidase" was increased due to all exposure treatments than the control fish. In the gills of *Labeo rohita*, the significantly highest peroxidase activity was found at 96-hr LC_{50} exposure followed by $2/3^{rd}$, $1/4^{th}$ and $1/5^{th}$ of LC_{50} exposures revealing that peroxidase activity increased significantly with an increase in zinc exposure concentrations. However, the enzyme activity was significantly least (0.079±0.003 UmL^{-1}) in the control fish gills.

Regression Analyses: Table 2 presents the regression analyses for peroxidase activity in gills of zinc exposed

Labeo rohita on the physico-chemical characteristics of the test media. The linear regression computed between peroxidase activity in the gills and pH, carbondioxide, total hardness, calcium, magnesium and total ammonia of the test media were found as statistically significant at P<0.05 and positive. However, statistically significant and inverse regression of peroxidase activity in the gills on dissolved oxygen contents of the zinc exposed test media was observed.

Table 1. Peroxidase activity (UmL^{-1}) in the gills of *Labeo rohita* after sub-lethal zinc exposure.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F-value
Replication	2	0.00001	0.00001	NS
Treatments	4	0.14517	0.03629	1667.33[P<0.05]
Error	8	0.00017	0.00002	
Total	14	0.14535		

NS = Non-significant, P<0.05 = Significant
Comparison of Means.

Treatments	Mean ± SD
LC$_{50}$	0.353±0.003 a
2/3rd	0.288±0.002 b
1/4th	0.199±0.008 c
1/5th	0.135±0.002 d
Control	0.079±0.003 e

The means sharing similar letters in a column are statistically non-significant at P<0.05.

Table 2. Relationships of peroxidase activity in the gills of *Labeo rohita* with physico-chemical characteristics of the test media.

Regression equation (y = a+bx)	r	R^2
Peroxidase activity = -1.694+0.216 (pH) SE = 0.01$^{(P<0.05)}$	0.740	0.861
Peroxidase activity = 1.588-0.258 (Dissolved oxygen) SE = 0.01$^{(P<0.05)}$	0.990	0.991
Peroxidase activity = -0.881+0.988 (Carbon dioxide) SE = 0.05$^{(P<0.05)}$	0.801	0.892
Peroxidase activity = -2.190+0.009 (Total hardness) SE = 0.01$^{(P<0.05)}$	0.990	0.991
Peroxidase activity = -1.073+0.058 (Calcium) SE = 0.03$^{(P<0.05)}$	0.923	0.957
Peroxidase activity = -2.217+0.049 (Magnesium) SE = 0.01$^{(P<0.05)}$	0.992	0.994
Peroxidase activity = -0.993+0.933 (Total ammonia) SE = 0.02$^{(P<0.05)}$	0.962	0.974

r = Regression Co-efficient, R^2 = Co-efficient of Determination, SE = Standard Error, P<0.05 = Significant

DISCUSSION

During present investigation, it was found that the activity of enzyme peroxidase in the gills of zinc treated fish, *Labeo rohita*, increased significantly as compared to the control fish. Rajeshkumar *et al*. (2013) also reported enhanced activity of lipid peroxidase in the gills of zinc exposed milk fish (*Chanos chanos*) as compared to the control fish. The present results are also parallel to the findings of Firat *et al*. (2009). They observed a significant increase in the gills peroxidase activity of zinc exposed fish, *Oreochromis niloticus* as compared to the non-exposed fish. Qu *et al*. (2014) concluded that zinc exposure caused an increase in liver glutathione peroxidase activity in the fish, *Carassius auratus*, as compared to the control fish. Eroglu *et al*. (2015) also demonstrated that after exposure to zinc, glutathione peroxidase activity was significantly elevated in the liver of fish, *Oreochromis niloticus*. Doherty *et al*. (2010) also observed an increase in the activity of enzyme glutathione peroxidase in the gills of zinc exposed Nile tilapia (*Oreochromis niloticus*). An increased peroxidase activity in the gills of fish, *Salmo*

trutta was reported by Hansen *et al*. (2007). The present results are also parallel to the findings of Atli *et al*. (2006). They reported that the glutathione peroxidase activity was lesser in the gills of control Nile tilapia (*Oreochromis niloticus*) as compared to the zinc exposed fish.

Zinc exposure induced significant alterations in the physico-chemical parameters viz. water pH, dissolved oxygen, carbon dioxide, total hardness, calcium, magnesium and total ammonia of the test media that ultimately affect the peroxidase activity in gills of *Labeo rohita*. It was found that carbon dioxide and total ammonia concentrations were increased while the dissolved oxygen contents of the test media decreased significantly with the increasing zinc exposure. These findings are in accordance with English and Storey (2003). They observed that physico-chemical parameters viz. pH, dissolved oxygen, carbon dioxide and total hardness play an important role in the induction of oxidative stress to the fish by the heavy metals exposure. Qu *et al*. (2014) reported that zinc exposure caused significant increase in pH of the test media that resulted into enhanced activity of peroxidase in the liver of metal exposed fish, *Carassius auratus*. Findings of Carvalho *et al*. (2015) showed that the activity of lipid peroxidase was increased significantly in the gills of copper exposed fish, *Prochilodus lineatus*, at higher pH. Sampaio *et al*. (2012) reported that metal exposure caused significant increase in the carbon dioxide contents of the test media that exerted stress on the fish, *Piaractus mesopotamicus*. The oxidative stress caused by the heavy metals exposure enhanced the antioxidant enzyme activities in the fish. Saglam *et al*. (2014) observed that exposure of cadmium and copper caused significant increase in total hardness of the test media that ultimately resulted into increased activity of glutathione in the liver and kidney of freshwater fish, *Oreochromis niloticus*.

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