Effect of Manganese Chloride (MnCl$_2$) on Peroxidase Activity in *Labeo rohita*

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EFFECT OF MANGANESE CHLORIDE (MnCl$_2$) ON PEROXIDASE ACTIVITY IN *LABEO ROHITA*

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ABSTRACT

Four groups (n=10) of one year old *Labeo rohita* were exposed to 96-hr LC$_{50}$ and sub-lethal (2/3rd, 1/4th and 1/5th) concentrations of MnCl$_2$ for a duration of 30 days in the glass aquaria of 50L water capacity. After an exposure period of 30 days, the activity of peroxidase enzyme in the liver and brain of MnCl$_2$ exposed fish was measured and compared with the control group. Physico-chemical parameters of the test media, viz. pH, dissolved oxygen, carbon dioxide, total ammonia, total hardness, calcium and magnesium were also monitored on a 12 hour basis during the whole experimental duration by following the standard protocol. The results showed that peroxidase activity in the fish increased significantly more than the control fish in both organs after exposure of manganese chloride. The enzyme peroxidase had activity of 0.543±0.004 UmL^{-1} and 0.274±0.004 UmL^{-1} in the liver and brain, respectively, in MnCl$_2$ stressed fish. However, in control fish, the enzyme activity was observed as 0.117±0.008 UmL^{-1} and 0.024±0.005 UmL^{-1} in the liver and brain, respectively. The regression analyses revealed a significance variable dependence of increase in liver and brain peroxidase activity on the physico-chemical variables of the metal exposed media.

Key words: Fish, Organs, Enzyme Activity, MnCl$_2$, peroxidase.

INTRODUCTION

Contamination of freshwater ecosystems with a wide range of pollutants due to industrialization and agriculture activities, has become a severe issue of distress all over the World (Vutukuru, 2005). Metallic ions toxicity may also cause destructive effects on the diversity of aquatic fauna (Hayat and Javed, 2008). Heavy metals pollution may alter the function of fish organs, rate of reproduction and the density of aquatic organisms (Hussain et al., 2013). Due to the non-biodegradable nature and tendency of bio-magnification in the food chain, heavy metals are specifically severe in their mode of action (Batool et al., 2014). The native fish fauna of Pakistan in the province of Punjab are affected badly by the presence of heavy metals such as Mn, Zn, Cu, Ni, Hg, Fe, Pb, and Cd which are consistent pollutants of aquatic bodies causing serious health hazards (Javed, 2012). Heavy metals may influence the biochemical and physiological activities in the vital organs of fish (Basha and Rani, 2003). They are generally known to induce toxicity and carcinogenicity in organisms due to their ability to generate reactive oxygen species (ROS) that result in oxidative stress. Due to the severe toxicity of heavy metals, they play an important role in ecotoxicological studies to evaluate the effect of oxidative stress in aquatic organisms (Sobha et al., 2007). Various fish species have been employed to assess the health status of aquatic ecosystems in order to monitor pollution levels (Farkas et al., 2002). Heavy metals gain access to the fish body via the mouth, skin and gills (Yilmaz et al., 2010). Fish could accumulate large amounts of heavy metals from contaminated water, food or sediments (Olaifa et al., 2004). The uptake and retention of metals varied in different body organs of fish (Wong et al., 2001).

Manganese is an essential micro-nutrient that plays an important role as a

constituent and co-activator of several enzymes responsible for biological processes in fish (Maage et al., 2000). However, higher levels of manganese can disturb the sodium balance that might trigger autoimmune responses and neurotoxic effects (Gunter et al., 2006). Increased anthropogenic activities have caused a continuous release of manganese in the natural water bodies of the Punjab (Morillo and Usero, 2008). Different factors affect the toxicity of manganese such as physico-chemical parameters and type of fish species (Fish, 2009). Manganese (Mn) toxicity decreases with increasing water hardness and it can be significantly bio-concentrated by aquatic biota at higher trophic levels (Howe et al., 2004). The excessive concentrations of Mn in the fish can cause neurogenetic disorders through the formation of free radicals that can induce oxidative stress to cause disturbances in the antioxidant defense system of the fish (Aschner et al., 2007). The liver is a prime metabolic organ in the living organisms that detoxifies the exogenous and endogenous substances and the brain is more vulnerable to oxidative stress caused by xenobiotics (Meganathan et al., 2011).

To overcome the oxidative stress, organisms have developed protective defense mechanisms to neutralize reactive oxygen species (ROS) before detrimental effects occur in the cell which leads to many disturbances (Tripathi et al., 2006). Antioxidant enzymes, viz. catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD), glutathione reductase (GR) and glutathione-S-transferase (GST) are considered sensitive biomarkers in the fish and are important parameters to monitor the extent of toxicants in the fish (Geoffroy et al., 2004). Peroxidase is an important antioxidant enzyme which protects the biological system from oxidative damage and lipid peroxidation (Winzer et al., 2000). In several studies, including field and laboratory conditions,

the peroxidase activities were analyzed in the aquatic animals that had resulted in either triggering or reducing activity depending upon the dose of metals, mechanism of exposure and type of fish species (Atli et al., 2006).

Labeo rohita (rohu) is the most important major carp due to its high quality meat (Rahman, 2005). It is polycultured in ponds and may serve as an indicator of water quality and environmental pollution (Vutukuru et al., 2007). Therefore, the present study was planned to assess the effect of manganese chloride ($MnCl_2$) on peroxidase activity in the liver and brain of *Labeo rohita*.

MATERIALS AND METHODS

The proposed experimental work was conducted under controlled laboratory conditions at the Fisheries Research Farms, Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad. One year old *Labeo rohita* were obtained from the Fish Seed Hatchery in Faisalabad and brought to the wet laboratory for acclimation. Fish were acclimatized for two weeks in cemented tanks prior to the start of experiment. Fish were fed with pelleted feed having 35% DP and 3.50 Kcalg^{-1} DE twice a day. After the acclimation period, healthy fish of similar weights and lengths were selected for enzymatic studies. A pure chloride compound of manganese ($MnCl_2$) was dissolved in 1000 mL of deionized water for the preparation of the metal stock solution. All glassware and aquaria used in these experiments were washed thoroughly with water prior to use. Before the experiment, all aquaria of 50 liter capacity were filled with dechlorinated tap water. *Labeo rohita* were exposed to the sub-lethal concentrations of manganese chloride as determined by Abdullah et al. (2007) for 30 days by using a static water system with continuous aeration under laboratory conditions (Table 1).

Table 1: Sub-lethal exposure concentrations of MnCl2 to *Labeo rohita*

Metal/Treatment	Sub-lethal levels	Metal exposed concentrations (mgL^{-1})
Manganese	96-hr LC_{50}	73.70±3.64
	$2/3^{rd}$ of LC_{50}	49.13±1.52
	$1/4^{th}$ of LC_{50}	18.42±1.09
	$1/5^{th}$ of LC_{50}	14.74±0.89

After 30 days of manganese chloride exposure, the fish organs, viz. liver and brain, were isolated and peroxidase activity was measured. Each test was conducted with three replications for each concentration/treatment and activity of peroxidase in the selected organs was compared with the control group. The physico-chemical parameters of test media, viz. pH, dissolved oxygen, carbon dioxide, total hardness, total ammonia, calcium and magnesium, were measured on a 12-hour basis to observe the effects of these parameters on enzyme activity. The pH and dissolved oxygen were monitored by using digital meters, viz. HANNA HI-8424 and HI-9146, while other physico-chemical characteristics of water were determined by following the methods of APHA (1998). An optimum range of dissolved oxygen (3-5 ppm) was maintained by using air pumps fitted with a capillary system. After the 30-day exposure of manganese chloride, fish were sacrificed and their liver and brain isolated and preserved at -4°C for the estimation of enzyme assay.

Enzyme assay

Red blood cells were removed from the liver and brain by rinsing these organs with a phosphate buffer of pH 6.5 (0.2 M) and homogenized in cold buffer (1: 4W/V) using a blender. After homogenization, the organ homogenate was centrifuged for 15 minutes at 10,000 rpm at 4°C. After the centrifugation process, the clear supernatant was preserved at -4°C 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.

Required reagents for enzyme assay

1. 0.2 M phosphate buffer, pH 6.5.

2. Guaiacol

3. Hydrogen peroxide

Preparation of 0.2M phosphate buffer of pH 6.5

4g NaH_2PO_4 and 1g Na_2HPO_4 were taken in a flask and dissolved by adding distilled water. The volume was increased to 200 mL and adjusted to a pH 6.5.

Preparation of buffer substrate solution

Guaiacol (750 µL) was added to the phosphate buffer (47 mL) and mixed well on a vortex agitator. After agitation, H_2O_2 (0.3 mL) was added to the buffer solution.

Procedure

A cuvette containing 3 mL of blank phosphate buffer solution was inserted into the spectrophotometer and set to zero at wavelength of 470 nm. A cuvette containing buffered substrate solution was then put into the spectrophotometer and an initiation of reaction occurred by adding

0.06 mL of enzyme extract. The initiation of reaction occurred and the reaction time was 3 minutes. Hence, the absorbance was noted after 3 minutes. The enzyme activity was calculated by employing the following formula:

Activity (Unit/mL)

$$= \frac{\Delta A \, / \, 3}{26.60 \, \times \, 60 \, / \, 3000}$$

Statistical analyses of data

The data were subjected to statistical analyses by using the Factorial experiments with three replications for each test dose while Correlation and Regression analyses were also performed to find-out possible relationships among various parameters defined for this study.

RESULTS

The experiments were conducted to evaluate the effect of manganese chloride on enzyme peroxidase activity in the liver and brain of *Labeo rohita*.

Peroxidase enzyme activity

The exposure of $MnCl_2$ resulted in a significant increase over the control group in the enzyme peroxidase activity in fish organs. The peroxidase activity was higher in the liver and brain at LC_{50} exposure, compared to the other treatments. Peroxidase activity was significantly higher at elevated concentrations of $MnCl_2$. Table 2 shows a comparison of means on the activity of peroxidase in the organs (liver and brain) of *Labeo rohita* under various exposure concentrations of manganese chloride. Comparison of means revealed that the activity of peroxidase was increased more than the control group at all exposure concentrations. In the liver of *Labeo rohita*, peroxidase activity was highest (0.543 ± 0.004 UmL^{-1}) at LC_{50}

concentration, while it was significantly lower (0.117 ± 0.008 UmL^{-1}) in the control group of fish. In the brain of *Labeo rohita*, the highest peroxidase activity was analyzed at LC_{50} concentration (0.274 ± 0.004 UmL^{-1}), followed by $2/3^{rd}$ (0.251 ± 0.005 UmL^{-1}), $1/4^{th}$ (0.148 ± 0.005 UmL^{-1}) and $1/5^{th}$ (0.072 ± 0.003 UmL^{-1}) of LC_{50} concentration exposures. However, the enzyme activity was least (0.024 ± 0.005 UmL^{-1}) in the brain of the control fish. It was found that in the liver of *Labeo rohita*, the peroxidase activity was more pronounced as compared to the brain, showing the quick response of liver antioxidant enzymes (peroxidase activity) to prevent the cells from oxidative damage caused by manganese chloride.

Physico-chemistry of the test media

Table 3 shows analysis of variance on all the physico-chemical characteristics, viz. water, pH, dissolved oxygen, carbon dioxide, total ammonia, total hardness, calcium and magnesium, of the test media. Analysis of variance showed significant ($p<0.05$) variability in pH observed during the whole experiment. The values of pH varied from 8.27 ± 0.04 to 9.08 ± 0.06 at different sub-lethal concentrations of $MnCl_2$. The water pH increased significantly at LC_{50} (9.08 ± 0.06) treatment compared to that of control (8.08 ± 0.03) treatment. Analysis of variance revealed significant differences at $p<0.05$ for dissolved oxygen contents of the test media during these experiments. The dissolved oxygen contents of the water decreased significantly with the increased concentration of the manganese. A significant decrease (4.78 ± 0.05 mgL^{-1}) in dissolved oxygen was observed at LC_{50} treatment compared to the control (5.76 ± 0.04 mgL^{-1}). Analysis of variance showed that there existed statistically significant ($p<0.05$) variations among the treatments for carbon dioxide concentration of the test media. A gradual

rise in carbon dioxide concentration with the increase in exposure concentrations was found.

Table 2: Comparison of means on peroxidase activity (U mL^{-1}) in the organs of *Labeo rohita* after chronic MnCl$_2$ exposure.

Organs	Treatments					*Overall Means±SD
	LC$_{50}$	2/3rd	1/4th	1/5th	Control	
Liver	0.543±0.004 a	0.471±0.005 b	0.332±0.002 c	0.266±0.003 d	0.117±0.008 e	0.345±0.004 b
Brain	0.274±0.004 a	0.251±0.005 b	0.148±0.005 c	0.072±0.003 d	0.024±0.005 e	0.153±0.002 a
Means±SD	0.408±0.003 a	0.361±0.005 b	0.240±0.003 c	0.169±0.003 d	0.070±0.006 e	

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

Table 3: Analysis of variance on various physico-chemical characteristics of the test media.

Treatments	pH	Dissolved oxygen	Carbon dioxide	Total ammonia	Total hardness	Calcium	Magnesium
LC$_{50}$	9.08±0.06 a	4.78±0.05 e	1.45±0.03 a	1.63±0.05 a	296.80±0.05 a	27.55±0.04 a	56.97±0.06 a
2/3rd	8.74±0.05 b	5.13±0.04 d	1.35±0.05 b	1.54±0.06 b	280.76±0.04 b	26.53±0.06 b	53.59±0.05 b
1/4th	8.45±0.06 c	5.34±0.03 c	1.24±0.04 c	1.47±0.07 c	270.71±0.05 c	25.31±0.05 c	51.86±0.03 c
1/5th	8.27±0.04 d	5.64±0.06 b	1.16±0.05 d	1.36±0.03 d	261.95±0.03 d	23.62±0.07 d	51.72±0.04 cd
Control	8.08±0.03 c	5.76±0.04 a	1.07±0.03 e	1.17±0.04 e	250.38±0.04 e	21.19±0.03 e	49.35±0.04 d

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

The carbon dioxide of the test media increased (1.45 ± 0.03 mgL^{-1}) significantly at LC$_{50}$ exposure of manganese chloride compared to the control (1.07 ± 0.03 mgL^{-1}). Analysis of variance revealed significant differences at $p<0.05$ among all the treatments for the total ammonia contents of water. During stress trials, along with an increase in manganese concentrations, the total ammonia contents of the test media also increased significantly. The total ammonia contents of 96-hr LC$_{50}$ treatment was highest (1.63 ± 0.05 mgL^{-1}), followed by 2/3rd (1.54 ± 0.06 mgL^{-1}), 1/4th (1.47 ± 0.07 mgL^{-1}) and 1/5th (1.36 ± 0.003 mgL^{-1}) of LC$_{50}$ treatments. However, the total ammonia contents of all the manganese exposed media were higher as compared to the control media. Statistically significant $p<0.05$ differences existed between all the treatments. With an increase in exposed manganese concentration, a significant increase in total hardness was also observed. A significant rise in the total hardness was found as 296.80 ± 0.05 mgL^{-1} in LC$_{50}$ exposed media as compared to the control media for which the same was observed as 250.38 ± 0.04 mgL^{-1}. Analysis of variance on calcium contents of the test media revealed significant variations at $p<0.05$. It was observed that with an increase in manganese concentration, the calcium contents of the exposed test media also increased significantly. The mean value of calcium was found as 21.19 ± 0.03 mgL^{-1} in the control media, while the mean value of the same was estimated as 27.55 ± 0.04 mgL^{-1} in the LC$_{50}$ treated media. Analysis of variance on magnesium contents of the test media showed significant ($p<0.05$) differences during the 30-day experimental period. It was observed that as the concentration of manganese in the different test media increased, the magnesium contents of that particular test media also increased significantly. The maximum and minimum mean values of magnesium were estimated as 56.97 ± 0.06 and 49.35 ± 0.04 mgL^{-1} for LC$_{50}$ exposed and control media, respectively.

Regression analyses

Table 4 shows the dependence of increase in peroxidase activity in both the liver and brain of MnCl$_2$-stressed *Labeo rohita* on physico-chemical variables of the media. The regression of peroxidase activity of the liver and brain on the pH of the test media was statistically significant and positive with R^2 values of 0.982 and 0.946, respectively. It was found from the fitted line plots that peroxidase activity in both the liver and brain of the fish increased with a rise in pH of manganese exposed test media. The regression of peroxidase activity in the liver and brain on dissolved oxygen contents of the test media was significantly inverse with R^2 values of 0.962 and 0.939, respectively. It was found that enzyme peroxidase activity in both the liver and brain decreased with the increase in dissolved oxygen contents of the test media. Linear regression computed for peroxidase activity in the fish organs on carbon dioxide showed that the enzyme peroxidase activity was positively significant at $p<0.05$ with R^2 values of 0.994 (liver) and 0.971 (brain). A strong and direct dependence of peroxidase activity in both the liver and brain on carbon dioxide concentrations of the test media was observed. The enzyme peroxidase activity in the fish liver and brain exhibited statistically significant and positive dependence with R^2 values of 0.926 and 0.917, respectively, on the total ammonia contents of the test media. The total ammonia concentrations of all the metal exposed media increased resulting in enhanced peroxidase activity in both the liver and brain of *Labeo rohita*. The regression of peroxidase activity of the liver and brain on total hardness of the test media was significantly positive at

Table 4: Regression of liver and brain peroxidase activities on physico-chemical characteristics of the test media.

Regression equation (y = a + bx)	R	R^2
Liver		
Peroxidase activity = -2.828 + 0.374 (pH)	0.990	0.982
SE = 0.02$^{(p<0.05)}$		
Peroxidase activity = 2.122 - 0.370 (Dissolved oxygen)	0.980	0.962
SE = 0.02$^{(p<0.05)}$		
Peroxidase activity = -0.881 + 0.988 (Carbon dioxide)	0.996	0.994
SE = 0.01$^{(p< 0.05)}$		
Peroxidase activity = -0.993 + 0.933 (Total ammonia)	0.962	0.926
SE = 0.02$^{(p< 0.05)}$		
Peroxidase activity = -2.190 + 0.009 (Total hardness)	0.986	0.973
SE = 0.02$^{(p< 0.05)}$		
Peroxidase activity = -1.073 + 0.058 (Calcium)	0.970	0.941
SE = 0.04$^{(p< 0.05)}$		
Peroxidase activity = -2.217 + 0.049 (Magnesium)	0.068	0.938
SE = 0.04$^{(p< 0.05)}$		
Brain		
Peroxidase activity = -2.122 + 0.267 (pH)	0.972	0.946
SE = 0.02$^{(p<0.05)}$		
Peroxidase activity = 1.574 - 0.226 (Dissolved oxygen)	0.969	0.939
SE = 0.02$^{(p< 0.05)}$		
Peroxidase activity = -0.737 + 0.711 (Carbon dioxide)	0.985	0.971
SE = 0.02$^{(p< 0.05)}$		
Peroxidase activity = -0.685 + 0.585 (Total ammonia)	0.957	0.917
SE = 0.03$^{(p< 0.05)}$		
Peroxidase activity = -1.452 + 0.006 (Total hardness)	0.969	0.940
SE = 0.03$^{(p< 0.05)}$		
Peroxidase activity = -0.888 + 0.041 (Calcium)	0.970	0.942
SE = 0.03$^{(p< 0.05)}$		
Peroxidase activity = -1.659 + 0.034 (Magnesium)	0.936	0.877
SE = 0.04$^{(p< 0.05)}$		

SE = Standard Error; r = Regression Co-efficient; R^2 = Co-efficient of Determination; $p<0.05$ = Significant

$p<0.05$ with R^2 values of 0.973 and 0.940, respectively. The peroxidase activity was enhanced with the increase in total hardness of the test media. Statistically significant and direct dependence of peroxidase activity in the liver and brain of *Labeo rohita* on calcium contents of the test media was observed with the computed R^2 values of 0.941 and 0.942, respectively. The regression line computed for peroxidase activity in both the organs against calcium contents revealed that with an increase in calcium contents of the metal exposed test media, the enzyme activity also increased. The magnesium contents of the test media exerted significantly direct impacts on the peroxidase activity in both liver and brain of the fish with R^2 values of 0.938 and 0.877, respectively. It was found that with the increase in magnesium contents of test

media, the enzyme peroxidase activity of both the organs increased concomitantly.

DISCUSSION

A large number of pollutants, particularly heavy metals, are released from domestic and industrial sources that are severely affecting the quality of water (Andhale and Zambare, 2012). The natural freshwater streams in the Punjab province have been intensely polluted with iron, lead, nickel, manganese, cobalt, cadmium and zinc due to bulk discharges of untreated industrial effluents and sewage water, which is affecting the fish populations by producing toxic effects (Jabeen et al., 2012). The heavy metals enter the food chain through accumulation in the body tissues of fish. The increased metallic ions pollution in the aquatic environment leads to the production of reactive oxygen species (ROS) in the fish that can cause bimolecular destruction and ultimately result in cell death (Pinto et al., 2003). To cope with the toxic effects of reactive oxygen species, all the aquatic organisms possess the defensive mechanism involving antioxidant enzymes such as peroxidase (POD), catalase (CAT) and superoxide dismutase (SOD). Therefore, these antioxidants can be used as biomarkers of aquatic pollution (Varanka et al., 2001). Peroxidase is an antioxidant enzyme that is present in the cell mitochondria to provide protection to the cells against oxidative stress–a condition of imbalance in the production and destruction of ROS due to an alteration in the activities of antioxidant enzymes (Boeuf et al., 2000).

During the present study, the peroxidase activity was found significantly (p<0.05) higher in the fish liver and brain under all metal exposure treatments as compared to the control group. Manganese-exposed fish showed higher peroxidase activity due to the production

of reactive oxygen species as compared to the control fish in which peroxidase activity remained lesser due to the balanced production of ROS and optimum peroxidase activity. Bangeppagari, et al. (2014) also observed that lead exposure induced alterations in the gills and liver antioxidant enzyme activities of the metal stressed fish, *Labeo rohita*. Kumari, et al. (2014) also observed an increase in the activity of peroxidase in the liver and gills of cadmium exposed fish, *Labeo rohita*. The present results are also in conformity with Aruljothi and Samipillai (2014). They observed that arsenic exposure caused an increase in lipid peroxidase activity in the fish, *Labeo rohita*. Vieira, et al. (2012) reported the activity of glutathione peroxidase (GPx) was elevated in the liver and brain of manganese stressed gold fish (*Carassius auratus*) as compared to the control group. Falfushynska, et al. (2011) found that manganese caused inhibition of lipid peroxidase (LPO) activity in the liver of *Carassius auratus* as compared to the unstressed group. The present findings showed that the activity of peroxidase (POD) was found to be significantly higher in the liver as compared to the fish brain due to the exposure of manganese. Velma and Tchounwou (2010) also found that the antioxidant enzyme "lipid peroxidase" activity in the liver was higher than in the brain of manganese stressed gold fish (*Crassius auratus*). The liver is the main organ for various metabolic pathways that can undergo more oxidative damage as compared to the brain. Gabriel, et al. (2013) reported that the antioxidant enzyme activity was significantly higher in the liver while it was lower in the brain of manganese exposed tambaqui (*Colossoma macropomum*).

During the present investigation, manganese exposure induced significant changes in the physico-chemical parameters, viz. water pH, dissolved oxygen, carbon dioxide, total ammonia,

total hardness, calcium and magnesium of the test media, exerting significant effects on the metal stressed fish. The carbon dioxide and total ammonia concentrations were increased, while dissolved oxygen contents decreased significantly with the increase in manganese concentrations of the test media. These findings are in conformity with the result of Abdullah, et al. (2007). They found that manganese exposure caused a significant decrease in the oxygen consumption by the metal stressed fish, *Labeo rohita,* while other parameters such as carbon dioxide, total ammonia and total hardness increased significantly in the metal exposed test media*.* Serafim, et al. (2002) reported that heavy metals can induce oxidative stress due to alterations in the antioxidant enzyme activity as a result of an increase in CO_2 and total ammonia concentrations, while it decreased in test media. English and Storey (2003) found that pH, CO_2, dissolved oxygen and total hardness play an important role in inducing oxidative stress in the metal-stressed fish. The physico-chemical parameters contributed significantly towards inducing oxidative stress in the metal-treated fish, resulting in enhanced activity of enzyme peroxidase (Nussey, et al., 1995).

CONCLUSIONS

The enzyme peroxidase activity of *Labeo rohita* increased in both the liver and brain as a result of exposure to manganese chloride in all the treatments, i.e. 96-hr, $2/3^{rd}$, $1/4^{th}$ and $1/5^{th}$ of $LC_{50,}$ compared to the control fish. In all the treatments, the peroxidase activities were significantly higher in the liver than that of brain. The regression of peroxidase activity in the liver and brain of *Labeo rohita* on the physico-chemical parameters, except dissolved oxygen, was found statistically significant at $p < 0.05$.

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