Review Article

Tissue specific antioxidant response of *Cirrhinus mrigala* exposed to lead chloride

ABSTRACT

Heavy metal toxicity in the aquatic environment has become a universal problem in recent years due to their non degradable nature. Their toxicity can be tested by using antioxidant enzymes as biomarkers. During present study, the effect of Lead chloride ($PbCl_2$) on the peroxidase and catalase activity in the tissues (liver, kidney, gills and muscles) of *Cirrhinus mrigala* was studied. *Cirrhinus mrigala* was exposed to sub-lethal concentrations viz. $1/3^{rd}$ LC_{50} and $1/7^{th}$ LC_{50} of lead chloride for 28 days and sampling of fish was done weekly. After chronic exposure fish was dissected and their organs was isolated and homogenized to determine peroxidase and catalase activity. The activity of peroxidase enzyme was increased significantly ($p<0.01$) in experimental fish organs as compared to control group. Maximum peroxidase activity was measured as 0.997±0.011 UmL^{-1}, 0.676±0.016 UmL^{-1}, 0.489±0.005 UmL^{-1} and 0.339±0.006 UmL^{-1}in the metal stressed liver, kidney, gills and muscles respectively, of fish exposed to $1/3^{rd}$ LC_{50}. The activity of catalase enzyme in fish organs decreased significantly in experimental fish organs as compared to control group. Minimum catalase enzyme activity was measured as 592.55±3.76 UmL^{-1}, 577.32±8.64 UmL^{-1}, 547.10±12.65 UmL^{-1} and 488.21± 28.57 UmL^{-1}in the metal stressed liver, gills, kidney and muscles respectively, of fish exposed to $1/3^{rd}$ LC_{50}. Catalase activity was found significantly higher in the control fish.

Keywords: fish research, aquatic toxicology, *Cirrhinus* spp., lead-fish interaction

INTRODUCTION

Aquaculture is a newly developed industry with significant potential for improvement. Water quality management faces greater problems than at any time in its history due to natural pollutants, varied contaminants exist in surface waters including multiple chemical compounds and different products of industrial and agricultural revolution (Shahid *et al*., 2013). In recent decades, extensive urbanization, industrialization, the use of chemical fertilizers and pesticides has increased the

concentration of heavy metals in the aquatic ecosystem (Ambreen and Javed, 2015). Heavy metals are major toxicants in the aquatic environment. Because of their toxic influence, heavy metals can severely alter the density and diversity of aquatic biota (Nishida, 2011). Functions of vital enzymes are also affected due to oxidative stress caused by heavy metals (Thangam *et al*., 2014).

Lead (Pb) is a common, ubiquitous and persistent environmental pollutant with its increased worldwide production of about 2.5 million tons per year (Osfor *et al*., 2010). Lead act as most hazardous poison in aquatic climate. When the fish exposed to Pb it accumulates in fish organs and cause renal and hepatic dysfunction with retardation in growth. Pb exposure demage the organs of fish and induce pathological changes in hematological and serum biochemical parameters (Patrick, 2006).

Aquatic species have a protective mechanism to minimize ROS before the harmful effects occur. This system consists of antioxidant enzymes, viz. superoxide dismutase, catalase, glutathione peroxidase, glutathione S-transferase, and glutathione reductase are responsible for converting injurious ROS in to such products that are less harmful (Goswami and Das, 2016). Superoxide dismutase (SOD) serves as a first line of protection among these antioxidant enzymes, transforming the superoxide radical into hydrogen peroxide, which is then converted by catalase/peroxidase to oxygen and water (Ahmad *et al*., 2005). Peroxidase is the member of antioxidant family, and considered a major enzyme that is responsible for reducing hydrogen peroxide. Peroxidase enzyme protects the red blood cells from the harm of spoilage and destruction created and enhanced by H_2O_2. Results suggested that this enzyme might guard tissues and defend the body greatly from oxidation troubles produced and propagated by lipid per oxidation. The activity of enzyme may get higher due to environmental toxins (Valavanidis *et al*., 2006).

Catalase as a primary antioxidant defense component, protects fish from oxidative stress by converting hydrogen peroxide to oxygen and water. This enzyme has important functions such as ion transport, maintenance of electrochemical gradient and regulation of cell volume (Gul *et al*., 2004). Heavy metals accumulate in fish through various routs like skin and gills which are considered as the main entrance of pollutants, oral intake of water and the use of other polluted organisms as food.Metallic ions of high toxicity are known to cause injurious effects on the organs and blood composition of fish. .Fish tissues have antioxidant protection mechanisms which consists of superoxide dismutase, catalase and peroxidase. Superoxide dismutase and peroxidase

which guard the tissues against the demaging effect of heavy metals (Kousar and Javed, 2015).

Gills are the main organ of fish, assuming a multifunctional part in the complex functions performance, for example, acid base equilibrium, osmoregulation, respiration process, and excretion of nitrogenous wastes. By attaching themselves to mucous layer of the gills, metals may enter the gills and cause modifications in the ultrastructure and general morphology of fish gills. Gills are the first target of water borne pollutants due to the direct contact with water (Barhoumi *et al*., 2012).

The organs most associated with the detoxification and biotransformation processes are liver and kidney.Mostly peak absorption of heavy metals is reported in organs liver and kidney of diverse fish forms. Fish tissues, specifically the liver and kidney are endowed with an antioxidant defense systems to protect them from an oxidative stress caused by metals (Azmat and Javed, 2012). Liver is a major site for detoxification of reactive oxygen species (ROS). One of the most important functions of the liver is to detoxify the body from pollutants. Therefore, it may be considered as an indicator of aquatic pollution (Vesey, 2010). Kidney plays an important role because it maintains the homeostasis and responsible for the excretion of chemical wastes from the body of animals. Impact of heavy metals on aquatic ecosystem can be evaluated by measuring the biochemical factors/parameters in the kidney of the fish that respond specifically to the degree and type of contaminents (Bashir *et al*., 2018).

Cirrhinus mrigala commonly known as mrigal carp is native to Pakistan riverine system. Because of nutritive quality of fish the Indian major carp *C.mrigala* is economically important to culture in Pakistan. The population explosion of the world is geometrical where as the increase in food resources is arithmetical, a threat to mankind because of food security (Chavan and Mulley, 2014). *C. mrigala* is not listed in IUCN red list as threatened species.

MATERIALS AND METHODS

The present research entitled “Tissue specific antioxidant response of *Cirrhinus mrigala* exposed to Lead chloride” was conducted in toxicology and limnology laboratories at Fisheries Research Farm, Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad.

Cirrhinus mrigala was chosen as an experimental organism. The samples of fish were collected and brought to the laboratory for acclimatization. Fish was

acclimatized for 15 days in dechlorinated tap water in glass aquaria having 60-liter capacity.

Fig.1 Dissection of *Cirrhinus mrigala*

Experimental Design:

To carry out the chronic toxicity test,Randomly selected *Cirrhinus mrigala*were shifted to those aquaria, 8 fish in each aquarium. Oxygen was maintained by an

automated air pump.

Chronic exposure:

Sub-lethal concentrations ($1/7^{th}$ LC_{50} and $1/3^{rd}$ LC_{50}) were given to different groupsof *Cirrhinus mrigala*for 28 days.

Fish dissection:

After chronic exposure fish were dissected and the vital organs (liver, kidney, gills and muscles) was isolated and stored.

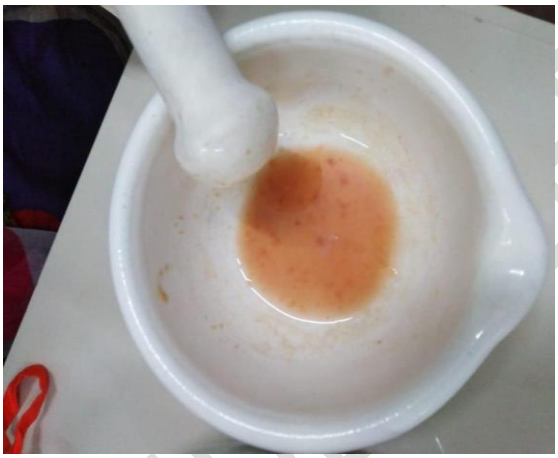
Dissection of *Cirrhinus mrigala*

Antioxidant enzyme study:

To examine the antioxidant enzyme activity organs was isolated and homogenized. Homogenization was done through following steps:

- To study the antioxidant enzyme activity viz. catalase and peroxidase, organs was extracted and stored at -4 °C.
- Weight the stored organs and mixed with phosphate buffer having a pH of 6.5 (0.2M) to remove the RBCs.
- Pestle and mortar was used to homogenize the tissues in the cold buffer.
- To remove the rubbish, homogenized matter was passed through the muslin cloth.
- Whatman filter paper No:1 was used to filter the fluid obtained from muslin cloth.
- Filtrate was centrifuged in a centrifigual machine for 15 minutes at 10,000 rpm.
- After completing the process of centrifiguation, supernatants was preserved at -80 ^{o}C for further examination.

Fig. 2 Grinding and Separation of organs through homogenation



1) Catalase Assay:

Catalase activity was evaluated according to the method of Chance and Maehly, 1955. Catalase activity was determined by measuring its ability to decline the hydrogen peroxide concentration per minute at 240nm. 0.224 g NaH_2PO_4 and 0.1632 g Na_2HPO_4 was taken in a flask and dissolved by adding distilled water. Then volume was raised upto 50 ml and adjusted the pH 7.0. 2ml buffer solution was prepared. A cuvette containing the 2 ml of blank solution (buffer) was placed in to the spectrophotometer and set it to zero at wavelength of 240 nm. In a cuvette containing buffered substrate solution 0.05 ml of enzyme extract was added and put in to the spectrophotometer. The reaction time was 3 minutes and the absorbance was noted after interval of 1 minute.

$$\text{Catalase activity (Units/mL)} = \frac{\Delta A/\text{min} \times \text{dilution} \times 2\text{ml}}{(0.04\ M^{-1}cm^{-1} \times 0.05\text{ml}}$$

2) Peroxidase Assay:

Peroxidase enzyme action was concluded by assessing its capability to reduce the hydrogen peroxide concentration at 470nm. Prepared 0.2 M phosphate buffer solution. 4g NaH_2PO_4 and 1g Na_2HPO_4 was taken in a flask and dissolved by adding distilled water. Then volume was raised upto 200 ml and adjusted the pH at 6.5. Also prepared buffered substrate solution 3 ml.

A cuvette containing the 3 ml of blank solution was placed in to spectrophotometer and set it to zero at wavelength of 470 nm. In a cuvette containing buffered substrate solution, 0.06 ml of enzyme extract was added and put into the spectrophotometer. The reaction time was 3 minutes and so absorbance was noted after 3 minutes.

$$\text{Peroxidase activity (Units/mL)} = \frac{\Delta A/3\text{min}}{26.6 \times 60\mu\text{l}/3000\mu\text{l}}$$

RESULTS AND DISCUSSION

The laboratory experiments was performed to evaluate the effect of lead chloride on peroxidase and catalase enzyme activity in the tissues (liver, kidney, gills and muscles) of *Cirrhinus mrigala*. *Cirrhinus mrigala* was exposed to various sub-lethal concentrations of lead chloride ($PbCI_2$.

Liver:

Peroxidase activity in the liver of the fish during chronic exposure of lead chloride sub-lethal concentrations $1/7^{th}$ LC_{50} was 0.648±0.007 $UmL^{-1}$and at $1/3^{rd}$ LC_{50} was 0.997±0.01 UmL^{-1}. Group treated with $1/3^{rd}$ LC_{50} show higher peroxidase activity in fish liver then other group $1/7^{th}LC_{50}$.

Catalase activity in the liver of the fish during chronic exposure of lead chloride sub-lethal concentrations $1/7^{th}$ LC_{50} 619.80±4.51 $UmL^{-1}$and at $1/3^{rd}$ LC_{50} was 592.55±3.76 UmL^{-1}.$_0$. The activity of catalase is lower in both the treated groups as compared to the control group and the activity of catalase is lower in $1/3^{rd}$ LC_{50} as compared to $1/7^{th}$ LC_{50}.

Kidney:

Peroxidase was high in the kidney of fish which was treated with $1/3^{rd}$ LC_{50} as compared to the group treated with $1/7^{th}$ LC_{50} during exposure of lead chloride. The

activity of peroxidase in control group is 0.266±0.01 UmL^{-1} in $1/7^{th}LC_{50}$ was 0.485±0.01 and $1/3^{rd}$ LC_{50} was 0.676±0.01 UmL^{-1} which is higher then control group.

Catalase activity in the kidney of the fish during exposure of lead chloride sub-lethal concentrations $1/7^{th}$ LC_{50} was 577.06±1.88 $UmL^{-1}$and at $1/3^{rd}LC_{50}$ was 547.10±12.65 UmL^{-1} and in control group is 605.19±0.65 UmL^{-1}. Activity of catalase was low in group with $1/3^{rd}$ LC_{50} as compared to $1/7^{th}$ LC_{50} and control group.

Gills:

Activity of peroxidase was high in the gills of fish which was treated with $1/3^{rd}$ LC_{50} as compared to $1/7^{th}$ LC_{50} and control group during exposure of lead chloride. The activity of peroxidase in $1/3^{rd}$ LC_{50} was 0.489±0.005 UmL^{-1}, in $1/7^{th}$ LC_{50} was 0.296±0.022 UmL^{-1} and in control 0.197±0.004 UmL^{-1}

Catalase activity was low in the gills of fish which was treated with $1/3^{rd}$ LC_{50} as compared to $1/7^{th}$ LC_{50} and control group during exposure of lead chloride. The activity of peroxidase in $1/3^{rd}$ LC_{50} was577.32±8.64 UmL^{-1} and at $1/3^{rd}$ LC_{50} was 608.01±2.40 UmL^{-1} and in control group is 621.04±0.74 UmL^{-1}.

Muscles:

Peroxidase activity in the muscles of the fish during chronic exposure of lead chloride sub-lethal concentrations $1/7^{th}$ LC_{50} was 0.206±0.006 $UmL^{-1}$and at $1/3^{rd}$ LC_{50} was 0.339±0.006 UmL^{-1}. Peroxidase activity was higher in the muscles of fish group treated with $1/3^{rd}$ LC_{50} as compared to $1/7^{th}$ LC_{50} and control group.

Catalase activity in the muscles of the fish during chronic exposure of lead chloride sub-lethal concentrations $1/7^{th}$ LC_{50} was 526.24±7.66 $UmL^{-1}$and at $1/3^{rd}LC_{50}$ was 488.21±28.57 UmL^{-1}. Activity of catalase was low in the muscles of fish I group treated with $1/3^{rd}$ LC_{50} as compared to $1/7^{th}$ LC_{50} and in control group.

Table 1 show Peroxidase activity (UmL^{-1}) in the tissues of *Cirrhinus mrigala* after chronic exposure of lead chloride.

Durations	Tissues	Treatments		
		Control	1/7th LC_{50}	1/3rd LC_{50}
After 7 days	Liver	0.275±0.005	0.394±0.014	0.649±0.021
	Kidney	0.19±0.003	0.279±0.017	0.436±0.010
	Gills	0.189±0.006	0.239±0.007	0.419±0.006
	Muscles	0.085±0.006	0.167±0.006	0.219±0.006
After 14 days	Liver	0.278±0.004	0.562±0.007	0.871±0.007
	Kidney	0.223±0.004	0.491±0.009	0.628±0.032
	Gills	0.19±0.001	0.298±0.002	0.481±0.003
	Muscles	0.110±0.007	0.189±0.006	0.318±0.004
After 21 days	Liver	0.283±0.005	0.732±0.004	1.114±0.007
	Kidney	0.291±0.051	0.549±0.008	0.749±0.013
	Gills	0.198±0.004	0.286±0.071	0.514±0.004
	Muscles	0.141±0.007	0.222±0.006	0.391±0.009
After 28 days	Liver	0.287±0.006	0.903±0.003	1.354±0.009
	Kidney	0.358±0.002	0.622±0.013	0.891±0.008
	Gills	0.209±0.004	0.362±0.008	0.543±0.006
	Muscles	0.172±0.016	0.246±0.008	0.432±0.005
Overall Mean±S.D	**Liver**	**0.281±0.005**	**0.648±0.007**	**0.997±0.011**
	Kidney	**0.266±0.015**	**0.485±0.012**	**0.676±0.016**
	Gills	**0.197±0.004**	**0.296±0.022**	**0.489±0.005**
	Muscles	**0.127±0.009**	**0.206±0.006**	**0.339±0.006**

NS= Non significant (P>0.05); *=Significant (P<0.05); **= Highly Significant (P<0.01)

Conclusion

It was observed that peroxidase enzyme activity was found significant effect ($p<0.01$) increased and catalase enzyme activity was found significantly lower in the lead stressed fish as compared to the control fish. Among the tissues, fish liver exhibited significantly higher activity of peroxidase and catalase show decline trend Liver > gills > kidney > muscles.

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