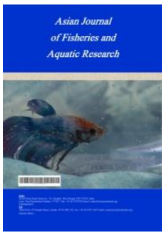

Asian Journal of Fisheries and Aquatic Research

17(3): 18-23, 2022; Article no.AJFAR.83899
ISSN: 2582-3760

Tissue Specific Antioxidant Response of *Cirrhinus mrigala* (Hamilton, 1822) Exposed to Lead Chloride

Amna Abbas [a], Andleeb Zahra [a], Ammar Anwar [b], Hafeez Roy [a*], Umer Liaquat [a], Fatima khan [a], Kalleem Ullah [a], Sahar Saleem [a] and Filza Ghafoor [a]

[a] *Department of Zoology, Wildlife and Fisheries, University of Agriculture Faisalabad, Pakistan.*
[b] *Department of Veterinary Sciences, University of Agriculture Faisalabad, Pakistan.*

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJFAR/2022/v17i330404

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/83899

Original Research Article

Received 03 January 2022
Accepted 06 March 2022
Published 03 May 2022

ABSTRACT

In the 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* were observed at sub-lethal concentrations viz. 1/3rd LC_{50} and 1/7th LC_{50} for 28 days. After chronic exposure fishes were dissected and sampling weekly. The activity of peroxidase enzyme was increased significantly ($p<0.01$) in experimental fish's organs as compared to control group. Through metal stressed maximum peroxidase activity was found 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 liver, kidney, gills and muscles of fishes exposed to 1/3rd LC_{50} respectively. The catalase enzyme activity was 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, in fishes exposed to 1/3rd LC_{50}. Catalase activity was found significantly higher in the control fish.

Keywords: Fisheries research; water quality; aquatic toxicology; Cirrhinus spp.; lead-fish Interaction; antioxidants.

**Corresponding author;*
Email: filzaghafoorjutt111@gmail.com;

1. 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 [1]. In recent decades, extensive urbanization, industrialization, the use of chemical fertilizers and pesticides has increased the concentration of heavy metals in the aquatic ecosystem [2]. 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 [3]. Functions of vital enzymes are also affected due to oxidative stress caused by heavy metals [4].

Lead (Pb) is a common, ubiquitous and persistent environmental pollutant with its increased worldwide production of about 2.5 million tons per year [5]. 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 [6].

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 into such products that are less harmful [7]. 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 [8]. 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 [9].

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 [10]. 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 damaging effect of heavy metals [11].

Gills are the main organ of fish, assuming a multifunctional part in the complex function's 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 [12].

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 system to protect them from an oxidative stress caused by metals [13]. 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 [14]. 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 contaminants [12].

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 whereas the increase in food resources is arithmetical, a threat to mankind because of food security [15]. *C. mrigala* is not listed in IUCN red list as threatened species.

2. 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.

2.1 Experimental Design

To carry out the chronic toxicity test, randomly selected fishes and stocked 8 nos._of fishes in each aquarium with proper oxygenation.

2.1.1 Chronic exposure

Sub-lethal concentrations ($1/7^{th}$ LC_{50} and $1/3^{rd}$ LC_{50}) were given to different groups of fishes for 28 days.

2.1.2 Fish dissection

After chronic exposure fishes were dissected and the liver, kidney, gills and muscles were isolated and properly stored.

2.1.3 Antioxidant enzyme study

To examine the antioxidant enzyme activity, organs were first 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 were 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 centrifuge machine for 15 minutes at 10,000 rpm.
- After completing the process of centrifugation, supernatants were preserved at -80^{o}C for further examination.

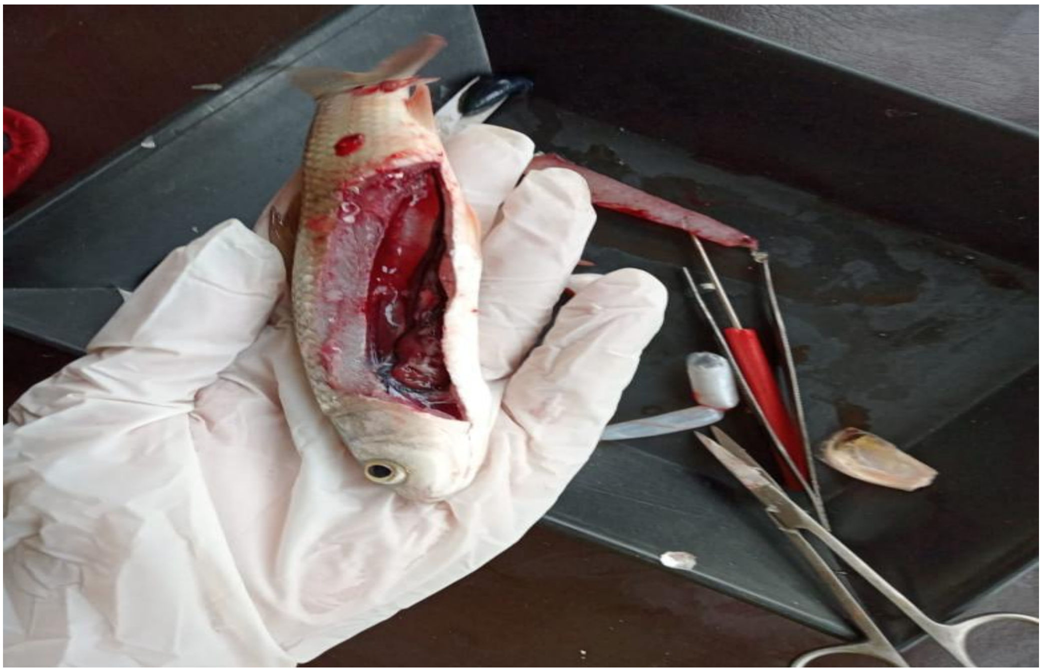

Fig. 1. Dissection of *Cirrhinus mrigala*

2.2 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 into 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 into the spectrophotometer. The reaction time was 3 minutes and the absorbance were noted after interval of 1 minute.

Catalase activity (Units/mL) = (ΔA/min×dilution×2ml / (0.04 $M^{-1}cm^{-1}$×0.05ml))

2.3 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 up to 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 into 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.

$$\text{Peroxidase activity (Units/mL)} = \frac{\Delta A/3\text{min}}{26.6\times60\mu l/3000\mu l}$$

3. RESULTS AND DISCUSSION

The laboratory experiments were 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 ($PbCl_2$.

3.1 Liver

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

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

3.2 Kidney

In this study it was observed that peroxidase activity was high in the kidney at 1/3rd LC_{50} as compared to 1/7th LC_{50} in the fishes when exposure to lead chloride. The activity of peroxidase in control group was 0.266±0.01 UmL^{-1}, in 1/7thLC_{50} was 0.485±0.01 and 1/3rd LC_{50} was 0.676±0.01 UmL^{-1} which was higher than the control group.

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

3.3 Gills

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

Catalase activity was lower in the gills of fish which was treated with 1/3rd LC_{50} as compared to at 1/7th LC_{50} and control group during exposure of lead chloride. The activity of peroxidase in 1/3rd LC_{50} was 577.32±8.64 UmL^{-1} and at 1/3rd LC_{50} was 608.01±2.40 UmL^{-1} and in control group is 621.04±0.74 UmL^{-1}.

3.4 Muscles

Peroxidase activity in the muscles of the fish during chronic exposure of lead chloride sub-lethal concentrations 1/7th LC_{50} was 0.206±0.006

Fig. 2. Grinding and Separation of organs through homogenation

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

Durations	Tissues	Treatments		
		Control	$1/7^{th}$ LC_{50}	$1/3^{rd}$ 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)*

$UmL^{-1}$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.

4. CONCLUSION

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

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Shahid M, Ferrand E, Schreek E, Duma EC. Behavior and impact of zirconium in the soil-plant system: Plant update and phytotoxicity. Rev. Contam. Toxicol. 2013; 221:107-127.
2. Ambreen F, Javed M. Assessment of acute toxicity of pesticide mixtures for *Cyprinus carpio* and *Ctenopharyngodon idella*. Pak. J. Zool. 2015;47:133-139.
3. Nishida Y. The chemical process of oxidative stress by copper (II) and iron (III) ions in several neurodegenerative disorders. Monat. Fur. Chem. 2011;142: 375-384.
4. Thangam Y, Jayaprakash S, Perumayee M. Effect of copper toxicity on haematological parameters to freshwater fish *Cyprinus carpio*. J. Environ. Sci. Toxicol. Food Technol. 2014;8:2319-2402.
5. Osfor MM, Ibrahim HS, Mohamed YA, Ahmed SM, Abd-El Azeem AS, Hegazy AM. Effect of alpha lipoic acid and vitamin E on heavy metals intoxication in male albino rats. J. Am. Sci. 2010;6:56-63.
6. Patrick L. Lead toxicity part II: the role of free radical damage and the use of antioxidants in the pathology and treatment of lead toxicity. Altern. Med. Rev. 2006; 11:114-127.
7. Goswami S, Das S. Copper phytoremediation potential of *Calandula officinalis* and the role of antioxidant enzymes in metal tolerance. Ecotoxicol. Environ. Saf. 2016; 126:211-218.
8. Ahmad I, Oliveira M, Pacheco M, Santos MA. *Anguilla Anguilla* oxidative stress biomarkers responses to copper exposure with or without β-naphthoflavone pre-exposure. Chemosphere. 2005;61:267-275.
9. Valavanidis A, Vlahogianni T, Dassenakis M, Scoullos M. Molecular biomarkers of oxidative stress in aquatic organisms in relation to toxic environmental pollutants. Ecotoxicol.Environ. Saf. 2006;64:178-189.
10. Gul S, Belge-Kurutas E, Yıldız E, Sahan A, Doran F. Pollution correlated modifications of liver antioxidant systems and histopathology of fish (*Cyprinidae*) living in Seyhan Dam Lake, Turkey. Environ. Int. 2004;30:605-609.
11. Kousar S, Javed M. Diagnosis of metals Induced DNA damage in fish using comet assay. Pak. Vet. J. 2015;35:168-172.
12. Bashir MA, Javed M, Latif F, Ambreen F. Effects of various doses of copper sulphate on peroxidase activity in the liver, gills, kidney and brain of *Cirrhinus mrigala*. Asian J. Agric. Biol. 2018;6:367-372.
13. Azmat H, Javed M. Acute toxicity of aluminium to the fish (*Catla catla, labeo rohita* and *Cirrhina mrigala*). Pak. Vet. J. 2012;32:85-87.
14. Vesey DA. Transport pathways for cadmium in the intestine and gills proximal tubule: Focus on the interaction with essential metals. Toxicol. Lett. 2010;198:13-19.
15. Chavan VR, Muley DV. Effect of heavy metals on liver and gills of fish *Cirrhinus mrigala*. Int. J. Curr. Microbiol. Appl. Sci. 2014;3:277-288.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/83899