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Catalase Activity in Response to Metals in Freshwater Fish *Catla catla*

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Abstract

Catalase activity in response to binary metal mixture of Zn + Ni was studied in gills, hepatic, renal and cardiac tissues of *Catla catla*. A total of 30 fish fingerlings were kept in two glass aquaria (15 in controlled condition and 15 in metal mixture containing aquarium) for the period of two weeks. After the completion of the trial period, fish were dissected for different organ collection. The extracted organs were homogenized in phosphate buffer (50 mM; pH 7.0). The activity of catalase was determined by absorption at 240 nm by using the standard methods. The inferences showed higher catalase activity in liver (223.33 ±1 UmL^{-1}), kidney (163.33 ± 0.7 UmL^{-1}), gills (123.33 ±0.9 UmL^{-1}) and cardiac (120 ±3 UmL^{-1}) tissues of Zn + Ni treated fish in comparison to controlled fish liver (116.66 ±2 UmL^{-1}), kidney (101.66 ±1 UmL^{-1}), gills (96.66 ±0.66 UmL^{-1}) and cardiac tissues (70 ±0.33 UmL^{-1}) in this study. Statistically, significant differences at $p \leq 0.05$ was observed for catalase activity between Zn + Ni stressed and control fish groups. While, in different organs of both group of fishes the catalase activity order was observed as hepatic > renal > gills > heart. Findings of this study would be helpful in monitoring aquatic ecosystems using fish antioxidant system which acts as a bio-indicator of metal contamination.

Keywords: Heavy metals, Antioxidants, Major carp, Aquatic pollution, Biomarkers.

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1. INTRODUCTION

Aquatic pollution has been considered a major threat to the aquatic life. The agricultural drainage water containing pesticides and fertilizers, effluents of industries and households are the most important sources of aquatic pollution [1]. Due to their ability, heavy metals are ingested and deposited by aquatic species from various sources, including industrial runoff, wastewater discharges, sediments, aerosols and soil erosion [2]. Tissues of fishes show response against contaminations and hence are helpful in eco-toxicological studies.

Aquatic organisms especially fishes are helpful in monitoring surrounding environmental conditions, temperature changes and water quality [3]. The absorbed and accumulated metal pollutants in the various parts of fish body can be taken up by human beings through food that may cause chronic or acute diseases [4].

Catla carp (*Catla catla*) is an important South-Asian freshwater fish species which is intensively cultured in controlled conditions and captured from natural reservoir of its endemic range due to its market value [5]. Normal fish body functioning is affected due to Zinc (Zn) and Nickel (Ni) toxicity. Gills of fish are the main intention of water born Zn toxicity that cause death of a fish due to hypocalcaemia because the Ca^{2+} uptake is disturbed [6]. In fresh and marine water fish species, various toxic effects of Zn vary with the most common disturbing is in hatching, reproduction, growth and survival rate [7]. Ni occur in biosphere (soil, water and air) as a trace metal and is released by industries and mining. Ni is toxic for fish and may cause change in cell morphology and chromosomal aberrations [8].

Toxic reactive oxygen species (ROS) are produced by the absorption of heavy metals in fish tissues which can trigger oxidative stress to the environment [9]. In all living organisms, there is a homeostatic mechanism called the antioxidant mechanism that helps to neutralize the ROS created by metal pollutants [10]. Catalase enzyme protects the organism from oxidative stress and is one of the primary antioxidant defence components whose activity may be either inhibited or induced due to metal contamination [11].

Examining the catalase activity in selected body tissues of freshwater fish *C. catla* under the exposure of Zn+Ni was the objective of this study. This study will be helpful in monitoring aquatic ecosystem by using fish antioxidant system which acts as a bio-indicator of metal contamination.

2. MATERIALS AND METHODS

2.1. Experimental animal

The Cala carp (*Catla catla*) commonly called Thaila; an important South Asian freshwater fish species was selected as an experimental animal. For the span of two weeks in cemented tank the fish fingerlings were acclimatized to the laboratory environment and fed twice a day with standard fish feed.

2.2. Preparation of metal mixture

Stock solutions of Zn+Ni were prepared by dissolving 4.0503 g nickel and 2.083 g Zinc in 1000 mL of distilled H_2O by following the methods of Hayat et al [12]. Both mixtures were added in the same flask and stir well to homogenize them.

2.3. Conduction of experimental trial

Two glass aquaria having length 24 inch, width 12 inch and depth 24 inch were selected for the binery metal mixture stress group and contol one. The selected glass aquaria were washed and rinsed with dilute HCl solution. The diluted HCl treated aquaria were washed with distilled water to remove the effect of acid. The glass aquaria both for both experimental and control was filled with tap water. The acclimatized fish fingerlings were shifted in both control and metal mixture stress glass aquaria (15 fingerlings in each aquaria).

2.4. Metals toxicity to experimental animal

For two weeks the toxicity stress of the chronic binary metal mixture was given to experimental fish at optimum water temperature, pH and total hardness. A total of 233.0 mL Zn+Ni solution was added from the stock solution in the aquarium having *C. catla* fingerlings for metal stress. The total solution quantity

was applied in a period of 6 hours, so fish didn't die [13]. During the experimental trial, various physico-chemical parameters were examined using the methods listed in [14].

2.5. Harvesting of fish and extraction of various organ tissues for Catalase assay

After two weeks trial completion, the fish fingerlings were collected both from control and metal mixture stressed group. Various selected organs were extracted from both groups after dissection. The extracted organs were homogenized in phosphate buffer (50mM; pH 7.0). The activity of catalase was determined by absorption at 240nm by using Bergmeyer [15] modified methods of Chance and Mehaly [16].

2.6. Statistical analyses

The data obtained from this experimental trial were subjected to statistical analysis by following the methods described by Steel et al. [17].

3. RESULTS AND DISCUSSIONS

Measuring the response of antioxidant enzyme catalase in selected body tissues of metal mixture stressed and controlled *C. catla* under laboratory conditions was the objective of this study. The current study inferences showed higher catalase activity in in liver (223.33±1 UmL^{-1}), kidneys (163.33±0.7 UmL^{-1}), gills (123.33±0.9 UmL^{-1}) and cardiac (120±3 UmL^{-1}) tissues of Zn+Ni stressed fish in contrast to control fish liver (116.66±2 UmL^{-1}), kidneys (101.66±1 UmL^{-1}), gills (96.66±0.66 UmL^{-1}) and cardiac tissues (70±0.33 UmL^{-1}) as shown in Fig. 1. Significant differences at $p \leq 0.05$ between Zn+Ni metal mixture stressed, and control fish organ catalase activity was recorded in the present study (Table 1).

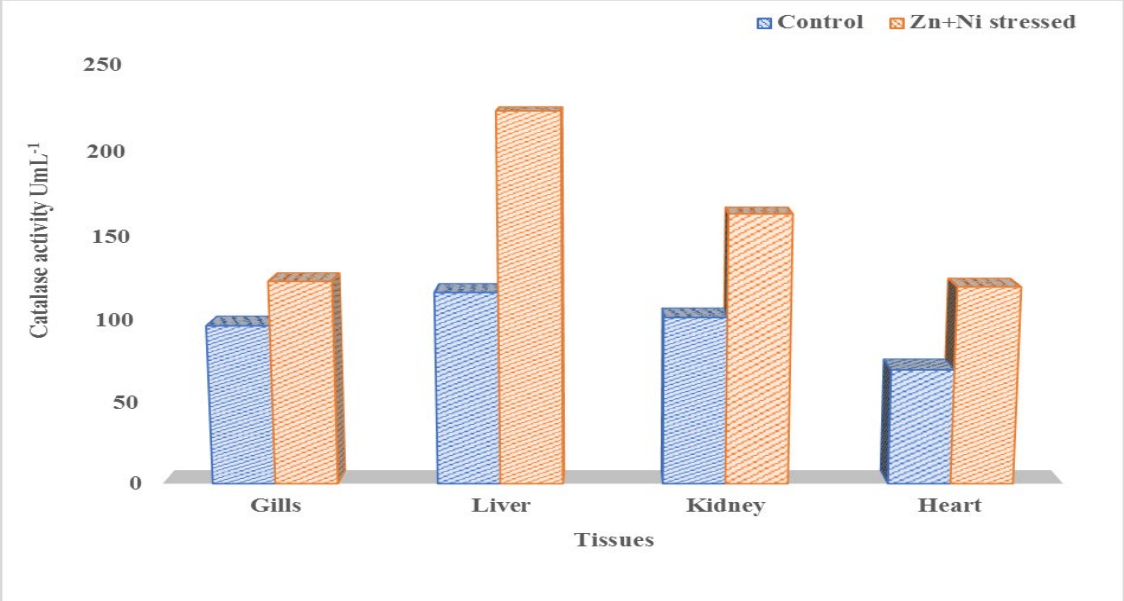

Fig. 1. Comparative gills, hepatic, renal and cardiac tissues catalase response in controlled and Zn+Ni stressed *C. catla*

Table 1: Analysis of variance table for catalase activity

SOV	DOF	SS	MSS	F value	P value
Groups	1	23855.6	23855.6	4211.12	0.0000**
Tissues	3	20583.0	6861.0	1211.14	0.0000**
Groups x Tissues	3	5950.3	1983.4	350.13	0.0000**
Error	16	90.6	5.7		
Total	**23**	**50479.6**			

**Highly significant differences at $p \leq 0.05$

In selected body tissues of binary metal stressed fish higher catalase activity was due to high production of ROS which may include Hydrogen peroxide, superoxide ion and hydroxyl radicals. These ROS are destructive to various components of cells and their rapid and efficient elimination is necessary. To cope with these oxidative radicals the level of catalase was observed higher in all the selected Zn+Ni stressed fish tissues in this study. Barat et al. [18] stated that biochemical responses of living organisms are useful for the determination of pollution status by measuring the catalase activity in *Hydopsyche exocellato*. Higher hepatic catalase enzyme activity was reported by Avci et al. [19] in fish captured from River Krikkale, Turkey. Response of liver and kidney antioxidant enzymes CAT was measured higher in Fe, Cu, Ni, Pb and Cd stressed *Leuciscus cephalus* by Hermenean et al. [20]. Kiran et al. [21] stated that fish species kept under metal stressed condition revealed higher antioxidant enzyme activities in comparison to controlled group by examining the response of gills, liver and kidneys tissues of *C. catla*. Joseph and Bawa-Allah [22] reported higher hepatic catalase activity in $ZnCl_2$ and $Pb(NO_3)_2$ streesed *Clarias gariepinus*. The inferences of the current study are opposed to the findings reported by Tufail et al. [23, 24] in pesticide mixture stressed *Channa striata*.

The order of catalase activity in both control and metal stressed *C. catla* in the current study was recorded as hepatic > renal > gills > heart tissues. Higher CAT activity in the hepatic tissues of *C. catla* are due to accumulation of metals as stated by Lin et al. [25] by studying the effect of metal toxicity in the *Oreochromis niloticus*. Jabeen et al. [26] reported the inferences of selected heavy metals on antioxidant defensive system of *C. catla, Labeo rohita* and *C. mrigala*. They stated that hepatic tissues are affected significantly than other body tissue due to metal pollutants. Elevated level of CAT was observed by Pretto et al. [27] in hepatic tissues of Cd stressed *Labeo rohita.* Fadhlaoui et al. [28] reported higher catalase and glutathione reductase activity in muscles, liver and brain tissues of Yellow perch by examining the toxic effect of nickel and cadmium. Similarly, Amado et al. [29] reported elevated level of antioxidants in gills of *Liza aurata* under metal exposure. Atli and Canli [30] proposed that antioxidant enzymatic system may be used as a bioindicator of metal contamination in freshwater ecosystem by examining the activity of catalase of *Oreochromis niloticus.* Farombi et al. [31] reported the elevated level of antioxidant enzymes including CAT, GSH, SOD and GST against Pb, Cd, Zn, Cu and As in gills, renal, hepatic and cardiac tissues of *Clarias gariepinus*. Elevated level of antioxidant enzymes was observed in Mn stressed freshwater fish species including *C. catla*, *Cirrhina mrigala* and *Labeo rohita* as compared to control group by Hayat et al. [12]. Anushia et al. [32] observed higher catalase activity in muscle tissues of *Tilapia mossambicus* kept under CU and Cd stressed. Similarly, enhanced level of oxidative stress biomarkers including CAT, GPX, and GST was observed in Cd stressed Japanese flounder by Cao et al. [33].

The inferences of the current study are not according to Ahmed et al. [13, 34] who reported lower catalase activity in kidney and hepatic tissues of *Oreochromis niloticus* kept under Pb+Cd metal mixture stressed fish. Similarly, present study inferences are also not according to the findings of Partiban and Muniyan [35] who also reported decrease catalase activity in *Cirrhinus mirgala* kept under Ni stress for the period of 96 hours; Paul et al. [36] who observed lower CAT activity in Pb stressed *Channa punctatus*; Ameur et al. [37] who measured lower hepatic CAT activity in Sea bass (*Dicentrarchus labrax*) and Mullet (*Mugil cephalus*); Dabas et al. [38] who noted lower gills, renal and hepatic CAT activity in $CdCl_2$ stressed freshwater murrel (*Channa punctatus*).

4. CONCLUSION

The current study was performed to examine the response of antioxidant important enzyme catalase against the binary metal mixture of Zn+Ni contamination in various body tissues of *C. catla*. The study inferences showed enhanced catalase activity due to oxidative stress in all the selected body tissues of metal mixture stressed *C. catla* as compared to control group. The present study inferences would be helpful in monitoring aquatic ecosystems by using fish immune system part catalase which act as a bio-indicator in eco-toxicological studies.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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