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Research Article

Catalase Activity as a Bio-Indicator of Lead+Nickel Toxicity in Carnivorous Fish, *Channa striata*

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Abstract: The freshwater ecosystems are extensively polluted with heavy metals discharged from industrial, domestic, and other human activities. These metals are significant stimulators of oxidative stress in aquatic organisms, especially fish, leading to the creation of reactive oxygen species. Therefore, current research was conducted to elucidate the toxic effect of heavy metals mixture (Pb+Ni) on catalase activity in various organs (gills, liver, kidney, brain, muscle and heart) of carnivorous fish, *Channa striata* exposed to sub-lethal concentrations (1/3rd, 1/4th and 1/5th of LC_{50}) for a period of 14-day. Fish were sampled for enzyme study after 7 and 14-days. Results showed that all concentrations of metals mixture significantly decreased the CAT activity in selected organs of the fish however, maximum depletion was observed in 1/3rd of LC_{50} concentration followed by the order of 1/4th>1/5th. The CAT activity was decreased with increasing the concentration and duration of exposure.

Keywords: Carnivorous fish, Chronic exposure, Metals mixture, Antioxidant enzyme, *Channa striata*

1. INTRODUCTION

Contamination of freshwater bodies with a variety of pollutants is a major worldwide issue [1-2]. These pollutants have harmful effects on the aquatic organisms [3]. Among these pollutants, heavy metals are more toxic due to the tendency of bio-accumulation in aquatic ecosystems [4]. Metals present naturally in water bodies or due to the consequences of human activities, have been proven to be a significant factor of exposure to the organism's lives in water, especially fish [5]. These pollutants have harmful effects on fish and are considered to be the most suitable creature for evaluating the aquatic pollution [6].

Normally, nickel (Ni) occurs in traces in individuals, but at higher amount it would be risky to individuals residing in water [7]. Widespread use of nickel in electroplating, ceramic and steel industries which are producing Ni containing products, are releasing untreated waste into water bodies of the Punjab province [8]. Nickel voluntarily makes compound with many ligands upon release into the environment and becomes more mobile as compared to other metals. Nickel may lead to severe problems such as induction of toxicity in organs, contact dermatitis and nickel allergy [9] and also causes morphological transformations in numerous cellular systems and chromosomal aberrations [10].

According to Sfakianakis et al [11] lead is a highly stable heavy metal and is known as a toxic element. Aquatic animals such as fish accumulate lead from water [12]and deposit in different tissues (liver, spleen, gills, kidney and digestive tract)[13]. Lead inhibits the antioxidant enzymes activities, especially thiol-containing antioxidants, and can also stimulate the production of reactive oxygen species (ROS), inducing ''oxidative stress'' [14]. Organisms have antioxidant enzyme system to counteract the oxidative stress. This system includes superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), and glutathione-S-transferase (GST). Theses enzymes also protect the organism against oxy-radical damage such as lipid peroxidation, oxidation of protein and nucleic acids [15].

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Catalase plays important role to minimize the oxidative stress [16-17] by converting hydrogen peroxide into oxygen and water, and facilitate the redox regulation in various tissues [18]. Antioxidants enzymes can be used as a potential biomarker for detection of metal toxicity in freshwater bodies [19] because of their high sensitivity to metals as their activities are significantly altered and prove to be useful in environmental monitoring program [20]. Many studies were conducted to assess the impact of individual metal on fish whereas aquatic animals are commonly exposed to metals mixtures [21]. Therefore, the aim of present research work was to assess the activity of catalase in various organs of carnivorous fish, *Channa striata* under sub-lethal exposure to Pb + Ni mixture.

2. MATERIALS AND METHODS

2.1. Experimental Lay-out

Freshwater fish, *C. striata* was selected for this experiment. Fingerlings of *C. striata* (90 days old; Average weight, 8.15±0.21) were collected from natural breeding grounds and shifted to the Fisheries Research Farm, University of Agriculture, Faisalabad, Pakistan. Prior to experimental trail, *C. striata* were placed in cemented tanks to acclimatize with laboratory conditions for 14-day. After that, fish were moved to 100-L glass aquarium. A group of fish (n=10) were kept in each aquarium. Control fish were kept in metals mixture free water.

2.3. Preparation of Metal Solution

Chemically pure chloride compounds of metals, lead and nickel were dissolved, separately, in deionized water and stock solutions were prepared for required metals and their mixture concentrations (1:1 ratio) on metallic ion equivalence basis.

2.4. Metals Mixture Concentration

The 96-h LC_{50} of lead+nickel mixture for *C. striata* was calculated as 52.147 mg L^{-1} by Anum et al [22]. The sub-lethal values for *C. striata* were about 17.382 ($1/3^{rd}$), 13.036 ($1/4^{th}$) and 10.429 ($1/5^{th}$) mgL^{-1}. Fish, were exposed to sub lethal concentrations *viz.* 1/3, 1/4, 1/5 of LC_{50} for 14-day. Fish sampling was done after 7 and 14 days.

2.5. Water Quality Characteristics

During the experimental period, temperature, total hardness and pH of water was kept constant as 28°C, 230 mg L^{-1} and 7.00, respectively. However, other water variables like magnesium, total ammonia, calcium, sodium, potassium, electrical conductivity and carbon dioxide were also calculated and maintained [23].

2.6. Preparation of Organ Homogenate

To isolate catalase, fish tissues viz. liver, gills, kidney, brain, heart and muscle of *C. striata* were separated. All the organs were weighed and homogenized in phosphate buffer (pH 7.0) in ratio of 1:4 (w/v) for 15 minutes by using homogenizer with short intermissions. The muslin cloth was used to eliminate the debris from homogenized tissues. The obtained filtrate was centrifuged in refrigerated centrifugal machine for 15 minutes at 10,000 rpm and 4°C. Supernatant was separated for enzyme analysis.

2.7. CAT Activity

Catalase activity was measured by its ability to reduce the H_2O_2 concentration at 240 nm [24]. In a cuvette 2 mL of blank solution (60 mM Phosphate buffer used as blank) was taken and put into the spectrophotometer and it was set to zero at wavelength of 240 nm. Buffer substrate solution of 10 mM of H_2O_2 was prepared in 60 mM phosphate buffer. In a cuvette containing buffered substrate solution (1.95 mL), enzyme extract (0.05 mL) was added and placed into the spectrophotometer. The reaction time was 3 minutes and the absorbance was checked after interval of 3 minute.

2.8. Statistical Analyses

Data obtained from this study were statistically analyzed by using Statistix 8.1 and significant difference between treatment groups was tested by one-way analysis of variance (ANOVA).

3. RESULTS

The catalase activity was significantly depleted in all selected organs *viz.* liver, gills, kidney, brain, muscle and heart of exposed fish in comparison of control. This indicates a reduced activity to protect the cells against H_2O_2. Results showed that all concentrations of metals mixture were significantly decreased the CAT activity however; maximum depletion was observed in $1/3^{rd}$ of LC_{50}

concentration followed by that of 1/4th and 1/5th. The CAT activity decreased with increasing the concentration and duration of exposure. Graphical representation of data is given in Fig. 1-6.

4. DISCUSSION

Metals contribute in oxidative stress by producing reactive oxygen species in two ways. Redox active metals (vanadium, iron, copper and chromium) produce ROS via redox cycling. Redox inactive metals (nickel, mercury, lead, and cadmium) have potential to disrupt antioxidants and enzymes which are thiol-containing [25-26]. Fenton reaction is a third important phenomenon of ROS production in which hydrogen peroxide oxidizes the ferrous iron (II) to ferric iron (III), a hydroxyl anion, and a hydroxyl radical [27]. The superoxide radical can reduce iron to its ferrous form. Heavy metals which are involved in Fenton reaction include chromium, titanium, copper, cobalt, vanadium, and their complexes [28]. Antioxidant enzymes are vital to neutralize the oxidative stress induced by heavy metals once the supply of other antioxidant compounds is reduced [29].

Fish body organs are gifted with defensive mechanism [30] which includes antioxidant enzymes (i.e. superoxide dismutase, catalase, glutathione peroxidase, glutathione S transferase and glutathione reductase) to save them from oxidative stress. Catalase is primarily present in peroxisomes and trapped hydrogen peroxide (H_2O_2) [31]. Catalase transforms the hydrogen peroxide

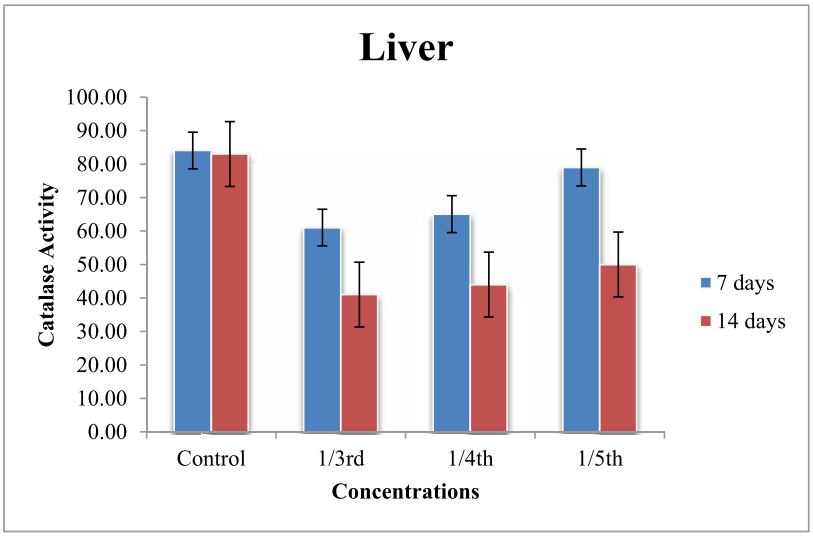

Fig. 1. Activity of CAT (U/mL) in liver of *C. striata* exposed to metals mixture for different time intervals

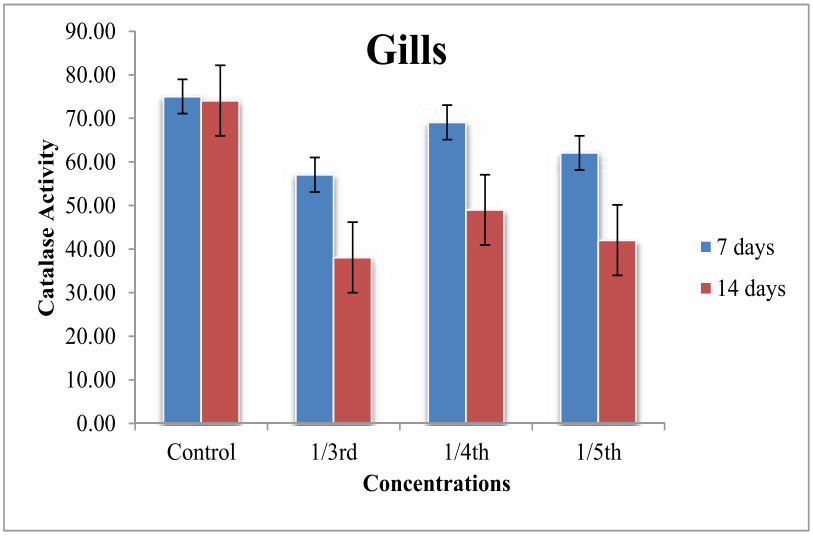

Fig. 2. Activity of CAT (U/mL) in gills of *C. striata* exposed to metals mixture for different time intervals

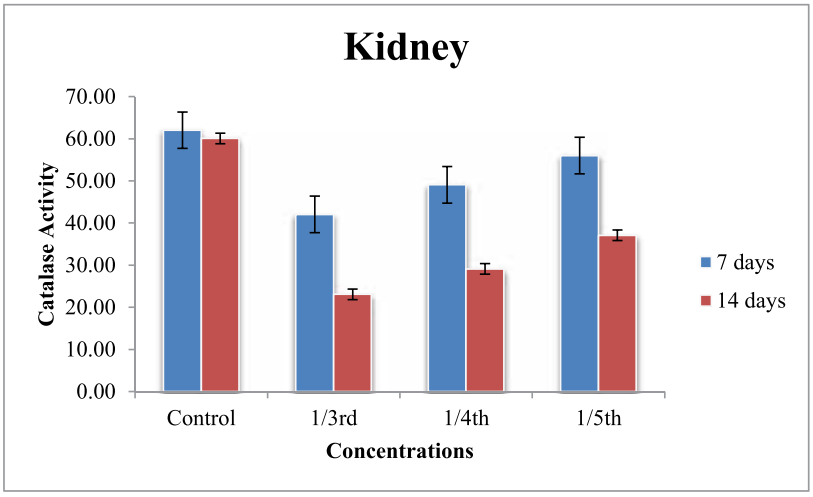

Fig. 3. Activity of CAT (U/mL) in kidney of *C. striata* exposed to metals mixture for different time intervals

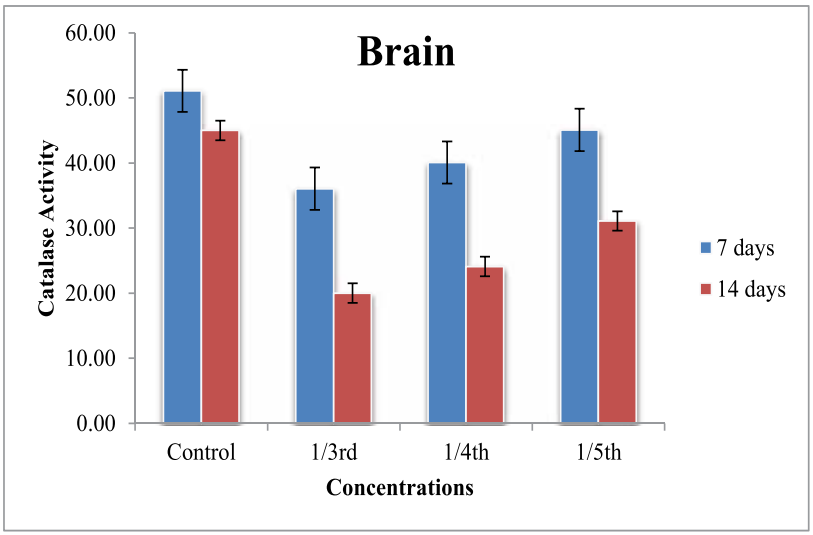

Fig. 4. Activity of CAT (U/mL) in brain of *C. striata* exposed to metals mixture for different time intervals

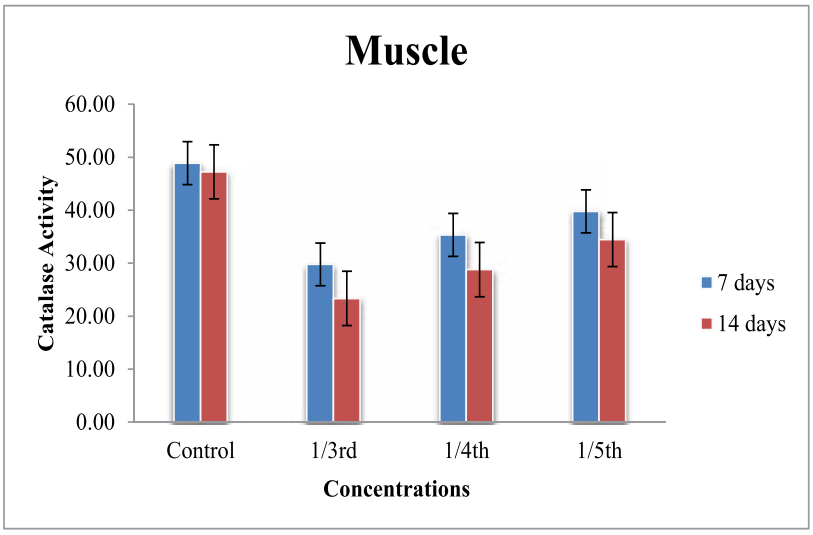

Fig. 5. Activity of CAT (U/mL) in muscle of *C. striata* exposed to metals mixture for different time intervals

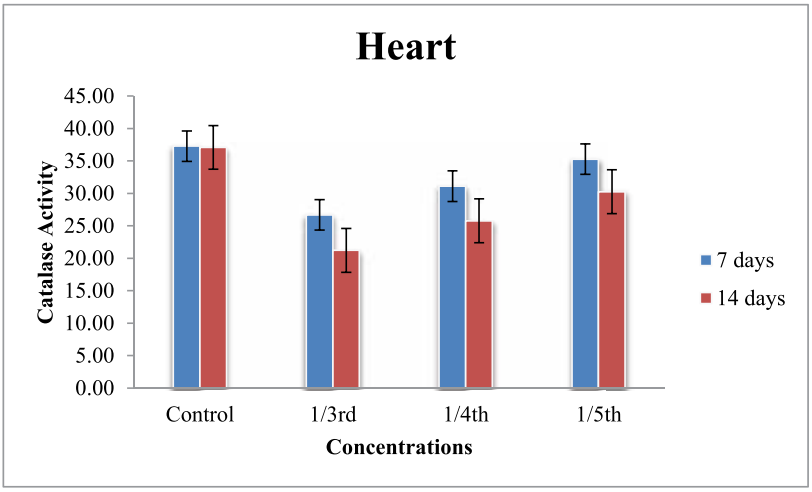

Fig. 6. Activity of CAT (U/mL) in heart of *C. striata* exposed to metals mixture for different time intervals

into water and oxygen, to protect the cell from the accumulation of H_2O_2. This indicates that the decreased activity may be due to protection of cell against H_2O_2.

Many authors have studied the effect of heavy metals on antioxidant systems of herbivorous and omnivorous fish. However, very little information is present in literature in which metals mixture effect was studied on carnivorous fish antioxidant systems. Our results showed that metallic ion concentration had negative impact on activity of catalase. CAT activity was depleted in metals mixture stressed fish in comparison of control. These finding are in accordance to Farombi et al [32]who observed the lower level of CAT in hepatic, gills, cardiac and renal tissues of African catfish exposed to heavy metals(cadmium and copper). The response of CAT activity may vary with the environmental factors, duration of exposure and type of toxicants [20].

Fish increased/decreased the level of antioxidants to overcome the oxidative stress [33]. According to Madhavan and Elumalai [34] the activity of CAT decreased in the gill and kidney of fish, *Clarias batrachus* under chromium exposure. Velma and Tchounwou [35] observed decreased CAT activity with increasing the concentration of chromium. According to Shen et al [36], CAT activity decreased with increasing the exposure concentration and duration. Depleted CAT activity in liver, gills and kidney of goldfish under chromium exposure was recorded by Kubrak et al [37].The activity of CAT decreased in chromium exposed fish [38]. Sub-lethal exposure of cadmium chloride significantly inhibited the CAT activity in gills, muscle, heart and liver of *Oreochromis niloticus* [39]. According to Saliu and Bawa-Allah [40] CAT activity decreased in hepatic tissues of *Clarias gariepinus* after sub-lethal exposure to lead and zinc. Yilmaz et al [41] reported the lower CAT activity in *Cyprinus carpio* in comparison to uncontaminated area. Exposure of cadmium significantly inhibited the CAT activity in renal tissues of the sea bass. This reduction in activity may be due to the direct binding of cadmium to CAT [42]. Similar result was observed by Sunaina and Ansari [43] who reported the reduced CAT activity in liver of zebra fish exposed to cadmium.

5. CONCLUSION

The findings of current study revealed that chronic exposure of heavy metals mixture can cause an imbalance in the antioxidant enzymes activities such as catalase in fish. Furthermore, it was concluded that these enzymes could be successfully used as prospective biomarkers of heavy metal toxicity to the freshwater fish in aquatic environment.

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