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Sublethal effect of cyanide on catalase activity in freshwater fishes, *Catla catla* and *Cirrhinus mrigala* (Hamilton)

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ABSTRACT

This study was undertaken to evaluate the catalase activity in two Indian major carps Catla catla and Cirrhinus mrigala. Fishes were exposed to sublethal concentration (0.07 mg/l and 0.115 mg/l) of cyanide and the effect of cyanide on the catalase (CAT) activity was assessed in liver, brain and gill tissues of the fish on day 7 and day 14 of exposure. Among the tissues, brain exhibited maximum decrease in the catalase activity followed by gill and liver. The change was more pronounced on day14 of exposure. Inhibition in the activity of CAT was more pronounced in C. catla. This indicates that sublethal concentration of cyanide can cause changes in activity of key enzyme, the catalase. Results indicate that cyanide is a potential toxicant induces oxidative stress and may bring serious potential health risk. The investigated parameter can be used as biomarker of cyanide toxicity in the freshwater fish.

Keywords: *Catla*, *Mrigala*, CAT, Cyanide, Sublethal studies

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INTRODUCTION

The destructive influence of humans on the aquatic environment may be in the form of sublethal pollution, which results in chronic stress conditions that have a negative impact on aquatic life [Adedeji *et al.*, 2008]. Fishes have been proposed as indicators for monitoring of land-based pollution because they may concentrate in their tissue, directly from water through skin and also through their diet. Fish is the last link in the food cycle [Castano *et al.*, 1996] and frequently subjected to prooxidant effects when exposed to pollutants [Velkova-Jordanoska *et al.*, 2008]. Indian major carps, *Catla catla and Cirrhinus mrigala* are the prime cultured, important staple freshwater fishes generally found in rivers, ponds, and reservoirs.

The sensitivity of aquatic organisms to cyanide is highly species specific, and also influenced by water pH, temperature and oxygen content, as well as the life stage and condition of the organism. Fish and aquatic invertebrates are particularly sensitive to cyanide exposure [Dube *et al.*, 2013]. Cyanide is a potent and rapid-acting asphyxiant; it induces tissue anoxia through inactivation of cytochrome oxidase, causing cytotoxic hypoxia in the presence of normal hemoglobin oxygenation. The effect of the hypoxia causes depression of the central nervous system that can result in respiratory arrest and leading to death [Shwetha and Hosetti, 2009].

Oxidative stress is a common symptom of cyanide toxicity [Dube *et al.*, 2013]. Reactive oxygen species [ROS] have been reported to affect the physiology of aquatic organisms [Pandey *et al.*, 2003]. Fish possess well developed antioxidant defense system neutralizing the toxic effects of ROS [Pedrajas *et al.*, 1995]. The activity of antioxidant enzymes may be increased or inhibited under xenobiotic exposure depending on the intensity and the duration of the

stress induced as well as the susceptibility of the exposed species [Oruc and Usta, 2007]. Due to high reactivity of ROS, most components of the cellular structure and function may become potential targets of oxidative damage. The present study aimed to evaluate the sublethal effect of cyanide on the activity of catalase enzyme, with reference to sensitivity of freshwater fishes, *Catla catla* and *Cirrhinus mrigala*.

MATERIALS AND METHODS

A stock of juvenile fish (*C. catla* and *C. mrigala*), were procured from State Fisheries Department, Bhadra Reservoir Project, Shimoga District, Karnataka State, India. They were acclimatized to the experimental conditions for a week before use and fed with commercial fish food pellets. Only active specimens (5±0.5 g, 7±0.5 cm) with no signs of stress and injury were used in the experiments.

Prior to start of the bioassay, the fishes were treated with 0.1% $KMnO_4$ to remove any dermal adherents and bacterial contamination. Dechlorinated tap water (temperature 27±1°C, pH 7.2±0.2, dissolved oxygen 6.3±0.4 mg/l, hardness 23.2±3.4 mg/l as $CaCO_3$, phosphate 0.39±0.002 µg/l, salinity 0.01ppt, specific gravity 0.001 and conductivity less than 10 µS/cm and a light period of 12 h/day) was used throughout the experimental period and water was renewed for every 24 h. Sodium cyanide (97% purity, Loba chemicals, Mumbai) was used as toxicant, prepared by dissolving in double distilled water. The toxicant solution in the test chambers was replaced with fresh solution of same concentration for every 24 h. The 96h LC_{50} of cyanide to the *C. catla* was found out to be 0.280 mg/l and for *C. mrigala* 0.458 mg/l. For the present study 40% of 96h LC_{50} was selected (0.07 mg/l for *C. catla* and 0.115 mg/l for *C. mrigala*). Fishes were subjected to sublethal levels of cyanide for 7 and 14 days. A group of fishes were maintained without the cyanide served as control. The experiment was run in triplicates.

At the end of day 7 and day 14 of exposure, fishes were sacrificed from control and cyanide treated groups and tissues such as liver, brain and gills were collected immediately, washed with distilled water, blotted and weighed before homogenization. The CAT activity in the tissue was determined by measuring the decomposition of hydrogen peroxide at 240 nm (pH 7.0 and 25 ºC), following the method of Aebi (1984). Enzyme activity was expressed as µmol H_2O_2 decomposed/ min / mg of protein.

Statistical analysis

Each assay was repeated five times, and differences between experimental and control groups, at each exposure time were converted to percentage of the respective control and analysis of variance (ANOVA) was employed using SPSS 13.0 for windows. In all the cases, differences were considered statistically significant at $P<0.05$ [Daniel, 1987].

RESULTS AND DISCUSSION

In the present study no mortality was observed during acclimation and experimental period. Sublethal exposure of cyanide to *C. catla* and *C. mrigala* exhibited significant ($P<0.05$) inhibition in the activity of catalase enzyme in all the tissues studied at both the exposure period when compared to control. The decrease in the activity of CAT was more pronounced on day 14 of exposure to cyanide in both the fishes. Maximum percent inhibition in the activity of CAT in *C. catla* was observed in brain (52.25%) followed by gill (41.93%) and liver (36.83%) (Table 1). Similarly, *C. mrigala* also exhibited maximum inhibition of CAT activity in brain (30.87 %) followed by gill (26.58 %) and liver (21.97%) (Table 2).

Knowledge of the sublethal effects of toxic compounds appears to be highly important to identify early warning as bio-indicators of exposure before irreversible damage occurs [David *et al.*, 2008]. Aquatic organisms can provide model systems for cellular damage caused by ROS and how oxidative stress can lead to disease. Evaluation of oxidative stress has become an important parameter in the field of aquatic toxicology [Dube *et al.*, 2013]. Among the aquatic animals, fishes have proved to be excellent bioindicators of aquatic contamination, since the biochemical responses under toxic stress are similar to those of mammals and other vertebrates [Sancho *et al.*, 2000].

Table 1. Changes in the CAT activity (µmol H_2O_2 decomposed/ min / mg of protein) in the *Catla catla* fingerlings exposed to sublethal concentration (40% of 96h LC_{50}) of sodium cyanide

Tissue	Exposure Periods in days		
	Control	7	14
Liver	15.3429	10.9503	9.6928
±SD	0.05	0.07	0.03
% change	---	-28.63	-36.83
Brain	12.443	9.6313	5.9419
±SD	0.06	0.05	0.03
% change	---	-22.6	-52.25
Gill	11.212	8.9498	6.5112
±SD	0.06	0.04	0.09
% change	---	-20.18	-41.93

Data are average of n=5 with ±SD, statistically sig nificant (p≤0.05)

Table 2. Changes in the CAT (µmol H_2O_2 decomposed/ min / mg of protein) activity in the *Cirrhnus mrigala* fingerlings exposed to sublethal concentration (40% of 96h LC_{50}) of sodium cyanide

Tissue	Exposure Periods in days		
	Control	7	14
Liver	8.907	7.9763	6.9498
±SD	0.04	0.06	0.05
% change		-10.45	-21.97
Brain	9.187	7.5341	6.3513
±SD	0.07	0.02	0.03
% change		-17.99	-30.87
Gill	12.6518	11.1315	9.2893
±SD	0.10	0.06	0.09
% change		-12.02	-26.58

Data are average of n=5 with ±SD, statistically sig nificant (p≤0.05)

CAT is a prospective biomarker of cyanide toxicity, mainly located in the peroxisomes and responsible for the reduction of hydrogen peroxide produced from the metabolism of long-chain fatty acids [Atli and Canli, 2007; David *et al.*, 2008]. Exposure to sublethal levels of cyanide inhibited catalase activity, indicating a typical response to stress. In our study, reduction in the CAT activity in tissues can be attributed to recorded high superoxide dismutase [SOD] activity due to cyanide induced O_2^- production, which has been reported to inhibit CAT activity [Kono and Fridovich, 1982]. Bainy *et al.* (1996) reported inhibition of CAT activity in Nile tilapia [*Oreochromis niloticus*] and attributed this decline to excess production of O_2^-. Similar findings have been reported by many workers in different fishes exposed to different toxicants [Daya *et al.*, 2002; Atli and Canli, 2007; and David *et al.*, 2008]. Among the tissues brain exhibited maximum inhibition in the levels of catalase activity followed by gill and liver. The brain is susceptible to oxidative damage through free radicals as it contains high amounts of unsaturated lipids and utilizes about 20% of total oxygen demand of the body [Song *et al.*, 2006]. Fish brain has been proposed as highly sensitive organ and is one of the most important target under cyanide toxicity [Bhattacharya *et al.*, 2009]. The tissue specific differences in activity of CAT may assign to difference in uptake of toxicant by the fish [David *et al.*, 2008].

CONCLUSION

The antioxidative enzymes are important in controlling the oxidative stress in animals. The activity of one or more of these enzymes is generally increased or decreased in animals exposed to stressful conditions. In the present study, CAT activity in fish tissues was decreased by cyanide exposure. Inhibition in the activity of CAT was more pronounced in *C. catla* than *C. mrigala*. The investigated parameter can be used as a biomarker of cyanide toxicity in the freshwater fishes studied above.

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