

Analysis of Oxidative Stress Responses in Copper Exposed *Catla catla*

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Abstract: We studied the effect of copper effect on oxidative stress in the liver, kidney of the fish *Catla catla*. Environmental stress is one of the major challenges faced by Indian pisciculture industry, which affects the growth, reproduction and physiology of fishes. The understanding of the biochemical aspects of stress related responses in fishes to reduce the detrimental effects caused by those environmental challenges comes into need. The activities of the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione S-transferase (GST) were studied after 10 days of exposure to three concentrations of copper.

Keywords: copper, *Catla catla*, antioxidative enzymes, oxidative stress

1. Introduction

Currently, environment polluted by a number of chemical, biological pollutants and mainly aquatic environment is a major repository for them. Moreover, fisheries are at risk of toxic contamination because aquatic environment polluted by a wide range chemical mixture and causing disturbance to homeostatic natural systems. Additionally, analysis and monitoring of the local aquatic fauna and behavioral, histological, biochemical responses and patterns of protein levels help the assessing threat posed by toxicant to aquatic life. Moreover, a few studies have reported on bio markers employing western blot due to the lack of available for specific fish antibodies. Heavy metals such as Cu, Cd, Zn, Cr and Hg reach the aquatic systems as a consequence of industrial, agricultural and anthropogenic sources, such as an urban runoff, sewage treatment plants, domestic garbage dumps, and finally they can affect different ecosystems [1, 2]. Metals are able to disturb the integrity of the physiological and biochemical mechanisms in fish that are not only an important ecosystem component, but also used as a food source [3][4].

Oxidative damage after Cu exposure was shown as changes in biomarkers in the liver, kidney, heart and gill of numerous fish species. For instance, zebra fish exposed to Cu led to increase of protein carbonyl content in the gill and liver, along with increases in activity of the ROS defense enzyme catalase [5]. Cu exposure elevated lipid peroxidation levels in the liver and intestine of Indian flying barb (*Esomus danricus*) but resulted a decline in antioxidant enzyme activity [6].

The current study was to analyse effects of copper exposure on oxidative stress response biomarkers in brain, heart and gill of common carp, *Catla catla* exposed to copper.

2. Material & Methods

Experimental animal model: *Catla catla*

Systemic position of *Catla catla* employed as an experimental animal model in this study

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2.2. Collection of test animals and maintenance

Catla catla is a common freshwater fish and widely cultivable species in India. Healthy fishes of average length 12cm and average weight 25.0g was procured from fish ponds; and they were safely transported to the laboratory in well packed polythene bags containing oxygenated water. The physicochemical characteristics of tap water such as temperature, pH, dissolved oxygen, total alkalinity and total hardness were measured and maintained. The water temperature was recorded twice daily, and the other parameters were measured every day interval. All the above mentioned water quality parameters (except water temperature) during the entire experiment period were found to be in the optimum range for fish rearing.

2.3 Experimental design and exposure

The common carp *Catla catla* is a commercially important species. Elevated concentration of copper is a common in aquatic systems which activate stress responses in aquatic organisms. However, copper is received a little attention in *Catla catla*. 28 healthy fish were divided randomly into 4 groups and each group with seven fish. The control group was kept in copper-free water during the exposure period of 10 days. The three treatment groups were exposed to nominal concentrations of 55 ug/L (Test 1), 110 ug/L (Test 2) and 275 ug/L (Test 3) of copper (in the form of copper sulphate pentahydrate) for 10 days.

2.3. Superoxide Dismutase (SOD-EC: 1.15.1.6)

Superoxide dismutase activity was determined according to method of Misra and Fridovich (1972) [7].

2.4. Catalase (CAT-EC 1.11.1.6)

Catalase activity was assessed according to a slightly modified version of Aebi (1984) [8]. **Glutathione-S-Transferase (GST- EC: 2.5.1.18).**

Glutathione-S-transferase levels were analyzed by the method of Habig et al. (1974) using 1-chloro 2, 4-Dinitro Benzene (CDNB) as a substrate. [9]

3. Results

3.1. The effects of copper on super oxide dismutase activity in the brain, heart and gill:

3.1.1. Brain

In response to copper exposure in brain of fish, SOD activity was found to be significantly increased in Test2 treated fish was noticed compared to control fish and it was also found to be significantly increased in Test3 concentration against to Test1 concentration ($p < 0.05$) (Tab.1).

3.1.2. Heart

In copper treatment, SOD activity in heart of fish was exposed to Test2 concentration was shown to be statistically increased when compared to the control and it was also found to be significantly increased in Test3 concentration against to Test1 concentration ($p < 0.05$) (Tab.1).

3.1.3. Gills

In copper exposure, SOD activity in gills of fish was exposed to Test2 concentration was shown to be statistically increased when compared to the control and it was also found to be significantly increased in Test3 concentration against to Test1 concentration ($p < 0.05$) (Tab.1).

3.2. The effects of copper on catalase activity in the liver, kidney, brain, heart and gill:

3.2.1. Brain

In response to Test2 and Test3 copper exposure on brain of fish CAT activity level was found to be significantly decreased in treated fish was noticed compared with control fish but it was observed not significant change in Test1 treated group ($p < 0.05$) (Tab.2).

3.2.2. Heart

The CAT activity was observed to be significantly decreased in the heart of fish was exposed to Test2 and Test3 copper concentration than that of control fish (Tab.2).

3.2.3. Gills

The CAT activity was remains same and observed significantly not changed in the gills was exposed to Test1 and Test2 copper concentration than that of control fish, but CAT activity was found changes in Test3 group ($p < 0.05$) (Tab.2).

3.3. The effects of copper on glutathione S transferase activity in the liver, kidney, brain, heart and gill:

3.3.1. Brain

In exposure to Test1 and Test2 and Test3 copper concentration, GST activity in brain of fish was found to be a significant increase was noticed compared to control fish, but GST activity was observed not significantly change in Test1 group in comparison to control group ($p < 0.05$) (Tab.3).

3.3.2. Heart

In copper exposure, GST activity in fish heart was exposed to Test2, and Test3 concentrations were shown to be statistically increased when compared to the control, but GST activity was observed not significantly change in Test1 group in comparison against control group ($p < 0.05$) (Tab.3).

3.3.3. Gills

The GST activity was remains same and it was not significantly changed in the fish gills were exposed to Test3 copper than that of control group, but GST activity was observed significantly changed in Test2 in comparison to control group ($p < 0.05$) (Tab.3).

4. Discussion & Conclusion

In living organisms, oxidative stress can induced by metals exposed to them through the elevation of free radicals and the changes in antioxidant defense mechanisms, including detoxification and scavenging enzymes. Protein carbonylation and lipid peroxidation are considered consequences of ROS-induced oxidation of protein side chains and lipids, respectively. Generally, a high content of ROS induction can oxidize cell constituents, such as lipids and proteins, causing lipid peroxidation and protein oxidation which are always evaluated by MDA and PC, respectively [10].

Antioxidant enzymes SOD, CAT, GST, GPx and GR scavenge increased ROS and MDA, and protects organisms from possible oxidative damage by this immediate emergency response mechanism. SOD is the first enzyme to deal with oxyradicals and responsible for catalyzing the dismutation of highly super oxide radical O_2^- to O_2 and H_2O_2 . In our study, the increased SOD activity in fish organs suggested that oxidative stress resulted from the accumulated superoxide radicals induced by the copper and Increased SOD activity may be an adaptive response to Cu exposure (Table. 1). CAT is an enzyme located in peroxisomes and facilitates the removal of the H_2O_2 , which is metabolized to molecular oxygen and water and protection from the oxidation of unsaturated fatty acids in cell membrane. In the present work, CAT activities in different organs of carp inhibited with the concentrations of copper exposure and suggest that copper induced oxidative stress responses by suppressing or inactivating the antioxidant activity of CAT (the H_2O_2 scavenger) in fish (Table. 2) GST, a biotransformation phase II enzyme, has peroxidase activity and is capable of detoxifying ROS by becoming increasingly active. In the present work, GPx activities were in different organs of carp were activated because of an adaptive mechanism to slight oxidative stress induced by copper. (Table. 3).

5. Tables

Table 1: Effect of sub lethal exposure of copper on SOD activity in common carp *C. catla* tissue.

Exposure levels	BRAIN	HEART	GILL
CONTROL	0.098± 0.014	0.035± 0.005	0.018± 0.001
TEST1 (55 µg/L)	0.039± 0.002	0.050± 0.004	0.026± 0.001
TEST2 (110 µg/L)	0.052 ±0.008 ^a	0.038± 0.003 ^a	0.068± 0.006 ^a
TEST3 (275 µg/L)	0.083± 0.0129	0.077± 0.001	0.120± 0.004

Comparison of SOD activity in tissue: Liver, Kidney. Values were expressed as means of \pm SD of observations, N=4: P<0.005 significant. The data was showed with upper script "a" letter indicate significant between exposure and control tissues respectively.

Table 2: Effect of sub lethal exposure of copper on catalase in common carp *C. catla* tissue.

Exposure levels	Brain	Heart	Gill
CONTROL	1.51± 0.20	2.40±0.51	4.30±0.87
TEST1 (55 µg/L)	0.98±0.20 ^a	1.16±0.31 ^a	3.16±0.47
TEST2 (110 µg/L)	0.98±0.27 ^a	0.61±0.05 ^a	2.1a6±0.23 ^a
TEST3 (275 µg/L)	1.13±0.30	0.48±0.20 ^a	1.37±0.25 ^a

Comparison of catalase activity in tissue: Liver, Kidney. Values were expressed as means of \pm SD of observations, N=4: P<0.005 significant. The data was showed with upper script "a" letter indicate significant between exposure and control tissues respectively.

Table 3: Effect of sub lethal exposure of copper on GST activity in common carp *C. catla* tissue.

Exposure levels	BRAIN	HEART	GILL
CONTROL	0.92 ± 0.20	1.64 ±0.39	1.90 ± 0.41
TEST1 (55 µg/L)	0.60 ± 0.22	1.31 ± 0.41	1.03 ± 0.31 ^a
TEST2 (110 µg/L)	2.06 ±0.48 ^a	2.63 ± 0.51 ^a	1.95 ±0.40
TEST3 (275 µg/L)	2.91 ± 0.64 ^a	3.12 ± 0.73 ^a	3.40 ± 0.69 ^a

Comparison of GST activity in tissue: Liver, Kidney. Values were expressed as means of \pm SD of observations, N=4: P<0.005 significant. The data was showed with upper script "a" letter indicate significant between exposure and control tissues respectively.

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