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ANTIOXIDANT ENZYMES IN *OREOCHROMIS MOSSAMBICUS* AS BIOCHEMICAL INDICATORS OF AQUATIC POLLUTION FROM CHROMPET LAKE AT CHENNAI, INDIA

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ABSTRACT

Antioxidant enzyme activities of fish, *Oreochromis mossambicus* was determined to establish possible environmental impact of toxic effects on anthropogenic pollution of Chrompet Lake. Activities of superoxide dismutase (SOD) and catalase (CAT) in the blood of fish was studies as bioindicator. After sacrificing the fish, fresh blood samples were collected on test tube and stored in ice box. Superoxide dismutase (SOD) activity was measured by the ferricytochrome C method using xantine/ xantine oxidase as a source of superoxide radicals. Catalase (CAT) activity was determinated by measuring the decrease of hydrogen peroxide concentration at 240 nm. Fish have been proposed as indicators for monitoring land-based pollution because they may concentrate indicative pollutants in their tissue, directly from water through respiration and also through their diet. Fish are frequently subjected to prooxidant effects of different pollutants often present in the aquatic environment.

Key words: *Oreochromis mossambicus*, Antioxidant enzymes, SOD, CAT, pollution, Chrompet Lake.

1. INTRODUCTION

Antioxidant enzyme systems are a well developed regulatory mechanism protecting against oxidative stress. Under normal physiological states, ROS are rapidly eliminated by antioxidant enzymes, including superoxidate dismutase (SOD) and Catalase (Yu, 1994; Abele and Puntarulo, 2004; Mohankumar and Ramasamy, 2006). The SOD catalyses the dismutation of two superoxide radicals to hydrogen peroxide (H_2O_2), whereas CAT degrade H_2O_2 (Holmblad and Soderhall, 1999; Mohankumar and Ramasamy, 2006). The analysis of Chrompet Lake nutritient balance clearly point to an eutrophication process, which has led to more than a threefold increase in average P concentration over past century. Although Chrompet Lake is a slow-reacting and oligotrophic system, the ongoing eutrophication was traced and quantified with monitoring, and combination of information on river inputs, lake nutrient concentration, sediment cores, and population development in the catchments (Matzinger *et al.*, 2007). The effects of pollution exposure on the protein expression of SOD and Catalase (CAT) were investigated in tilapia Liver tissue. The results showed that pollution stress induced down regulation of SOD and CAT.

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Environmental condition of the Chrompet Lake is not good due to the continuous dumping of waste materials especially industries, domestic and municipal wastes from neighboring households. From the observed values of DO, BOD, and COD it may safely be concluded that the Bacteriological load in the lake is very high (Ganesan S and Mazher Sultana., 2010).

2. MATERIAL AND METHODS

2.1. Antioxidant enzyme activity

Oreochromis mossambicus fish were collected from Chrompet Lake at Chennai, India. After sacrificing the fishes, fresh blood samples were collected in monovetes with EDTA and transported on ice. Liver were dissected, stored in ice-cold 1X PBS (Phosphate Buffer Saline) and transported on ice. Blood samples were centrifuged for 15minutes at 4000rpm and collected plasma fractions were divided for each analysis separately. Then, blood cells were hemolized with distilledH2O and DNA-se (1 mg/ml), and cells were frozen over night at -80^{o}C. Next day the hemolisate fractions were centrifuged again and hemolisate fractions were divided for each analyses. The specimens of hemolisate were frozen and stored at -80^{o}C until use. Superoxide dismutase (SOD; EC 1.15.1.1) activities were measured by the ferricytochrome C method using xantine/xantine oxidase as a source of superoxide radicals. Enzyme activity of the present analysis can be reported in units of SOD per milligram of

Hb or protein. One unit of activity is defined as the amount of enzyme necessary to produce a 50% inhibition of the ferricytochrome C reduction rate (McCord and Fridovich, 1969). Catalase (CAT; EC 1.11.1.6) activities was determinate by measuring the decrease of hydrogen peroxide concentration at 240 nm according to Aebi (1984). The reaction mixture consisted of 50 mM potassium phosphate buffer (pH=7) and 10.6 mM H_2O_2 freshly added.

2.2. Protein extraction and western blot

Liver tissue were washed with cold PBS thrice and lysed with 100µl RIPA buffer (25mM Tris HCl pH 7.6, 150mM NaCl, 1mM EGTA, 1% triton-X 100, 1% Sodium deoxy cholate, 1% SDS and 1µg/ml leupeptin), sonicated and centrifuged at 12,000g for 15mins. Then the soup was collected and the protein was quantified using Nano drop. Aliquots of 50µg protein were added to gel loading buffer (5X), proteins were separated in 10% SDS-PAGE run and then western blot was performed. Blots were then probed with specific antibodies of the protein of interest (such as SOD and CAT) followed by appropriate secondary antibody tagged with alkaline phosphatase and the blots were developed by NBT/BCIP method.

2.3. Statistical analysis

Comparison between groups were performed using one-way ANOVA with $p<0.05$ as the criterion for significance. All analysis was done using windows based SPSS statistical package (version 12.0, Chicago, IL).

3. RESULTS AND DISCUSSION

3.1. Antioxidant enzyme activity

Significantly decreased the levels ($p<0.05$) of superoxide dismutase enzyme (SOD) have been detected in experimental group (polluted sites – 1, 2, 3, 4, 5, 6 and 7) as compared to control animals, and similarly in Catalase activity too.

Table 1 Enzyme activity (SOD and CAT) in erythrocytes of *Oreochromis mossambicus* from Chrompet Lake at Chennai, Tamil Nadu.

Sampling sites	Species	SOD, U/mg Hb	CAT, U/mg Hb
Control	*Oreochromis mossambicus*	34.71	361.89
1	*Oreochromis mossambicus*	1.140	172.09
2	*Oreochromis mossambicus*	0.003	23.134
3	*Oreochromis mossambicus*	1.073	96.001
4	*Oreochromis mossambicus*	8.431	49.713
5	*Oreochromis mossambicus*	9.319	53.142
6	*Oreochromis mossambicus*	3.401	17.001
7	*Oreochromis mossambicus*	0.004	13.982

Except for site 3, where in the Catalase activity was observed to be slightly increased as compared to other polluted sites (1, 2, 3, 4, 5, 6 and 7).

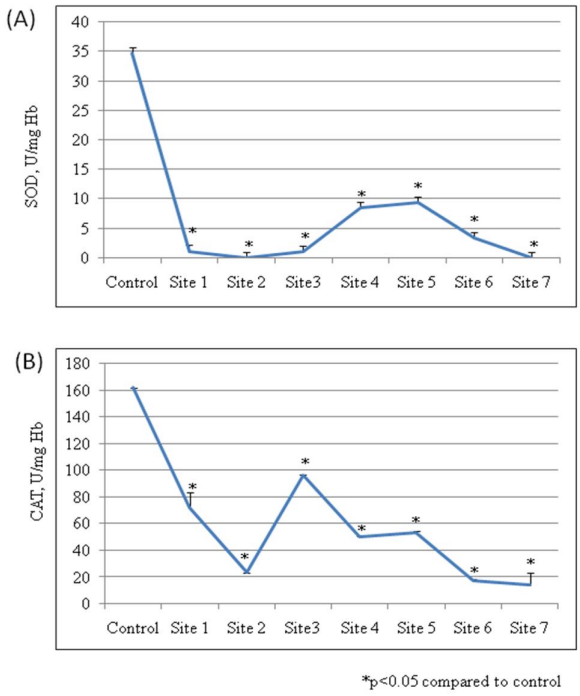

Figure 1 Line graph showing the Enzyme activity (A) SOD and (B) CATALASE in erythrocytes of *Oreochromis mossambicus* from Chrompet Lake at Chennai, Tamil Nadu.

The response to environmental pollution and toxic impact of the pollutant in the aquatic environment represents one of the possible reasons for decreased in antioxidant enzyme. According to Zikic (2001) cadmium induces the appearance of anemia and alters the metabolism of carbohydrates and proteins in goldfish. Their results also show the decreased activity of SOD in erythrocytes of goldfishe during acute exposure to cadmium, which indicates the presence of ROS-induced peroxidation, which leads to the destruction of RBC membrane (Table 1).

Inflammatory processes were evidenced in some of the investigated individuals, but with even distribution in all investigated polluted sites. The link between the environmental pollution and the stress response in fish indicates that infectious diseases arise when the host is exposed to certain conditions of environmental pollution. The hepatic-toxic impact of the pollutants in the aquatic environment upon liver in barbel fish represents one of the possible reasons for the above inflammatory processes (Velkova-Jordanoska, 2003). Although Chrompet Lake generally resists the negative influences of the anthropogenic factor for the time being, certain localities of the littoral region display loading with contaminants from the ground, especially in the course of the summer period. This implies the need of a greater seriousness in

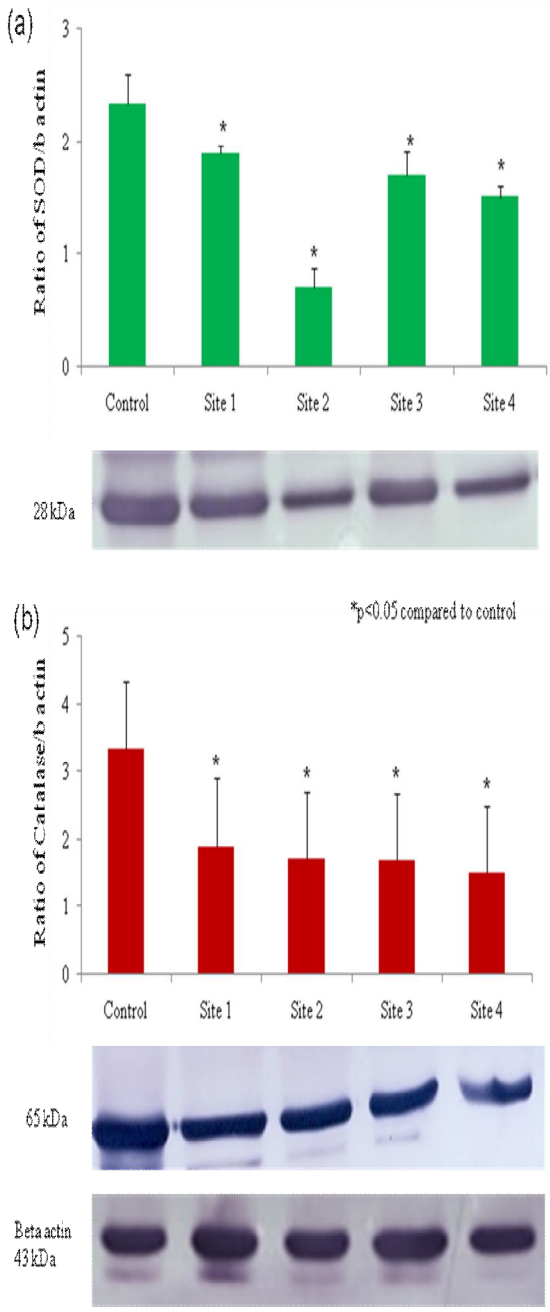

Fig. 2. The Protein expression of SOD (a) and CAT (b) at different pollution sites. Values are expressed as means ± SD.

terms of protection of the lake and more efforts towards eliminating the constant sources of pollution. The present investigation revealed a remarkable change in the antioxidant enzyme activity, which has lead to the stage of eutrophication of the Chrompet Lake.

3.2. Protein expression of SOD and CAT

The effect of pollution stress on the protein expression of SOD and CAT in tilapia Liver cells is shown in Fig. 2 (A and B). A clear different pollution sites decreasing expression pattern of the antioxidant enzyme protein was observed and compared with the control groups. All are statistically significantly compared with the control groups.

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