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Study of Superoxide Dismutases (SOD) Activities in *Labeo Rohita* in Response to Dissolved Oxygen

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Abstract: Superoxide Dismutases (SOD) are metal-containing, antioxidant scavenging enzymes that detoxify the reactive oxygens by catalyzing the dismutation of superoxide radicals to oxygen and hydrogen peroxide. SOD exists in virtually all oxygen respiring organisms and has been proposed to be important for stress tolerance during environmental adversity. SOD metabolize Reactive Oxygen Species (ROS) into a safe product, and hence prevents cellular damage. Fishes are exposed to daily and seasonal fluctuations in oxygen availability. Study of the histological activities of SOD in *Labeo rohita* shows significant differences among different tissues types and in response to change in the content of dissolved oxygen from different experimental sites. Results also suggest that the total SOD activities positively correlated with the content of dissolved oxygen in the water samples.

Keywords: Superoxide dismutases, *Labeo rohita* , dissolved oxygen, reactive oxygen.

I. INTRODUCTION

Reactive Oxygen Species (ROS), such as superoxide anion and Hydrogen Peroxide (H_2O_2), are byproducts of metabolism, such as of xenobiotics. These ROS are harmful and affects the cellular integrity and functions. Organisms have developed antioxidant defense systems that metabolize ROS to make it non-toxic, and hence prevent the macromolecular damages. Superoxide Dismutases (SOD) are the important antioxidant scavenging enzymes of the antioxidant defense systems that catalyze the dismutation between two moles of superoxide anion to yield one mole of oxidized product (oxygen) and one mole of reduced product (hydrogen peroxide) (Klug et al., 1972; Halliwell and Gutteridge, 1989; Babich, 1993). SOD form the primary defense against oxygen-derived free radicals (Crapo and Tierney, 1974; Fridovich 1978, 1986). SOD activities rapidly increased in the cells or organisms when exposed to oxidative stress (Crapo and Tierney, 1975). The degree of toxicity also depends on the concentration of pure oxygen. A prolonged elevated concentration of pure oxygen may result in to damage of central nervous system, hepatocellular carcinoma (Elchuri et al., 2005), age-related muscle loss (Muller et al., 2006), earlier incidence of cataracts, and reduced lifespan due to massive oxidative stress (Li et al., 1995). The SOD activities may also vary from tissues to tissues (Fried and Mandel, 1975). The present work describes the study of SOD activities and its comparative analyses in *Labeo rohita* in response to total dissolved oxygen in water of river Ganga. An attempt has also been made to a detail comparative study of SOD activities among different active tissues.

II. MATERIAL AND METHODS

The total content of Dissolved Oxygen (DO) in the collected water at four different points was estimated by Winkler's modified azide method. The measurement was done by precipitating as manganese basic oxide and dissolved by concentrated sulphuric acid forming manganese sulphate. Then it reacts with potassium iodide to liberate iodine which is estimated by titration with sodium thiosulphate (0.025 N).

The SOD activities were studied in *Labeo rohita* which were collected near from four different point of river Ganga: Buxar, Patna, Mokama and Barh. Fishes were sacrificed by decapitation for the extraction of different tissues, viz, liver, adrenal gland and gill. Tissues were thoroughly washed in chilled saline water to clean the blood and adhering tissues. Homogenate of tissues were prepared in the concentration of 10% (w/v) using potassium phosphate buffer (0.05M; pH 7.0). Protein estimation in post nuclear fractions and cytosolic supernatant were done following method of Lowry et. al.,(1951). Standard protein solution was prepared by using 10 mg of crystalline bovine serum dissolved in 100 ml of deionized water. After 30 minutes, 0.5 ml of Folin's reagent was added. Optical density of blue colour developed was read at 625nm exactly after 30 minutes. Standard solution (BSA, 20-100 µg) and blank were run simultaneously.

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McCord and Fridovich (1969) method was used for the estimation of total SOD from different tissues The tissue homogenate were diluted 1:9 for gills and adrenal gland, 1:4 for other tissues. The tubes were centrifuged at 1000 rpm for 15 minutes at 4°C. Solid 313 mg/ml ammonium sulphate was added into supernatant of each tube to make the final concentration of 50%. After thoroughly shaken and kept for 4 hrs at 4°C, the supernatant was dialyzed three times against deionized water using 0.3 ml nitroblue tetrazolium, 0.2 ml phenazine methosulphate, 1.0 mi pyrosulphate buffer, and 2.0 ml enzyme source. After 90 second, 1.0 ml of glacial acetic acid was added for checking the reaction. The absorbance were read at 560 nm on a spectrocolorimeter (Elico CL 171) against blank (NBT + PMS + Buffer + deionized water). Protein content in enzyme sources was also estimated by the method of Lowry et al., (1951). The unit of enzyme activity was defined as the amount of enzyme required to inhibit the optical density at 560 nm in one minute under the assay condition. Results were expressed as units/mg protein.

III. RESULTS ANS DISCUSSION

Total 20 different water samples were collected from each of the four different point of river Ganga. Variation in the content of total dissolved oxygen (DO) among four sampled site were found (Table 1). Highest DO content (12.0± 1.97 mg/lt) was observed in the water samples collected from river Ganga near Barh, while lowest content (7.6.0± 0.98 mg/lt) was in the sample collected near Buxar.

Table 1: Content of dissolved oxygen (DO) in water samples collected from four sites of river Ganga.

Experimental site	DO (mg/lit)*
Near Buxar	7.5 ± 0.78
Near Patna	10.9 ± 1.03
Near Mokama	11.2 ± 0.33
Near Barh	12.1 ± 1.17

* Value indicate mean ± standard deviation of 20 measurement

Labeo rohita is a common edible fish in India widely distributed in the river, lake, and ponds. Metabolically active tissues from liver, adrenal gland, kidney and gills were carefully dissected out from for the determination of total SOD activities. Tissues samples were collected from five fishes. The measured SOD activities of all three tissues are shown in Table 2.

Table 2: Total SOD activities (units. mg^{-1} protein)* in the liver, adrenal gland, kidney and gill tissues of *Labeo rohita* collected from river Ganga near four different sites.

Experimental site	Liver tissue	Adrenal gland tissue	Kiudney tissue	Gill tissue
Near Buxar	7.2 ± 0.370	3.6 ± 0.322	2.1 ± 0.427	1.8 ± 0.441
Near Patna	7.2 ± 0.444	4.1 ± 0.496	2.3 ± 0.399	2.5 ± 0.477
Near Mokama	8.7 ± 0.527	7.4 ± 0.381	4.0 ± 0.498	2.8 ± 0.412
Near Barh	9.3 ± 0.312	7.8 ± 0.489	4.8 ± 0.316	4.3 ± 0.669

* Value indicate mean ± standard deviation of 5 measurement

Measurement shows significant differences in the total SOD activities among the four tissues of *Labeo rohita* across four different experimental sites near river Ganga. The total SOD activities were not much different between samples collected near Buxar and Patna, particularly in liver and kidney tissues. The highest total SOD activity (9.3 (± 0.312) units. mg^{-1} protein) was observed in the liver tissues of sample collected near Barh, while lowest activity (1.8 (± 0.441) units. mg^{-1} protein) was observed in the gill tissue collected near Buxar.

Overall the data and results indicate that the SOD activities in *Labeo rohita* are much difference between different tissues among four sampled sites of river Ganga. However, there is almost no difference in SOD activities in the liver tissues near Buxar and Patna. Further, the observed data suggest that SOD activities positively correlates with the DO content among the four experimental sites. Means increase in dissolved oxygen increase the SOD activities. Effect of physiochemical parameter of water, such as freely available oxygen, temperature and pH changes have a direct influence on the SOD activities. It has been also shown that the lack of Vitamin C and other anti-oxidant systems may enhance SOD activity in the metabolically active tissues. Further, the presence of xenobiotics may also additionally results into increase in SOD activities.

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IV. CONCLUSION

The above study suggests that the SOD activities vary significantly among the different tissues of *Labeo rohita* from different sampled sites of river Ganga. The SOD activities are relatively higher in liver tissues than in the gill and kidney tissues. Further, the total SOD activity positively increases with the increase in the content of DO in the water samples.

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