Distribution of antoxidant enzyme SOD in Gomti and Ganga river in liver and skin of *Cirrhinus mrigala* (Ham.)

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SUMMARY

Laboratory experiments were carried out to expose the activity of superoxide dismutase in the liver and skin of fresh water teleost fish, *Cirrhinus mrigala*(Ham.) from sample of water collected from river Gomti at Ramghat, Jaunpur and river Ganga at Ghazipu. The fishes were acclimatized in both types of water sample for 14th days. The superoxide dismutase activities were significantly higher in the sample of water collected from river Gomti than river Ganga and control. The physico-chemical parameters of four samples indicated that sample from Ganga river had high biological oxygen demand in comparison to Gomti river, chemical oxygen demand and pH where as the same samples was low in dissolved oxygen when compared with control sample.

Key words : Antioxidant Enzyme (SOD), Water and fish, *Cirrhinus mrigala*, Superoxide dismutose.

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Oxygen is an essential element required by all aerobic forms of life; Recently, this essential gas has become a focus of controversy which has resulted from observations by several laboratories that oxygen though essential for aerobic form of life can be hazardous and deleterious to biological system (Bert, 1978).

The oxygen toxicity effect lies in chemical reaction between oxygen and other molecules with in both the living body and environment; which generates harmful reactive oxygen species (ROS) or Free radicals that have been implicated in hundreds diseases from arthritis to haemorrhagic shock to AIDS (Southern, 1988; Halliwell and Gutteridge, 1989; Halliwell and Cross, 1991 and Risberg *et al.*, 1991). There few comparative studies of vertebrates antioxidant defense (AD) in the literature specially in the case of fresh water teleost fish.

In 1969 biomedical community was introduced new enzyme conceived in mystery and dedicated to the an boliation of superoxide free radicals (McCord and Fridovich, 1969). The mechanism is the production of Superoxide radical and its dismutation reaction catalysed by enzyme superoxide dismutase (SOD) (Halliwell and Gutteride, 1989 and Otto and Moon, 1995).

Superoxide dismutase SOD is metalloenzyme playing a key role in the defense against the toxic effect of reactive oxygen species by disproportionating superoxide anion (Fridovich, 1988).

The fishes are exposed to daily or seasonal changes in both temperature and oxygen availability. The fishes posses antioxidant defense system (AD) which utilizes enzymatic and non-enzymatic mechanisms. It can be expected that fish antioxidant defense system depends on oxygen consumptions. The antioxidant defense system of fish we have detected Cu-Zn SOD and Mn-SOD activities in fish metabolic tissues.

MATERIALS AND METHODS

Fishes were collected from Gomti river at Jaunpur and Ganga river at Ghazipur. They were stacked in earthen container (Capacity about 20 lit) in the water of site from where they were collected. Fishes were acclimatized to laboratory conditions under normal photoperiod and temperature for three days. They were fed adlibitium.

Preparation of homogenate:

10% (W/V) homogenate of tissues liver and skin were prepared with aid of Yorks homogenizer fitted with Teflon plunger in potassium phosphate buffer (0.05 M pH 7.0). The homogenate was first centrifuged at 2500 xg for ten minutes in a refrigerated centrifuge (Electric Rc 4100). The pellete consisting of a nuclear fraction and cell debris was discarded. The clear supernatant was taken for enzyme studies.

For the assay of Superoxide dismutase (SOD) was estimated by the method of McCord and Fridovich (1969).

Superoxide anions were generated in a system comprised of NADH and PMS. The superoxide anion reduced the nitro blue lerozolium (NBT) forming a bluformazon which was measured at 560 nm.

SOD inhibited the reduction of NBT and thus enzyme activity was measured by monitoring the rate of decrease in optical density at 550 nm.

To determine the amounts of Cu-Zn SOD and Mn-SOD in tissues, 2 mM KCN solution was added to the mixture to inhibit Cu.Zn-SOD, Mn-SOD remain uneffected (Fridovich, 1975; Nandi and Chatterji, 1988 and Crapo *et al.*, 1978). Protein content in enzyme source was also determined. The unit of enzyme activity was defined as the amount of enzyme required to the inhibit the optical density at 560 nm of NBT reduction by 50% in one minute under assay condition. Results were expressed in units/mg protein.

RESULTS AND DISCUSSION

The physico-chemical parameters of water is given in Table 1 and 2. It clearly showed that the value of BOD and COD in the experimental samples were significantly higher and DO was lower when compared with control samples whereas the value of pH did not vary significantly.

It evident from Table 3 the activity of SOD (Total) was significantly higher in experimental samples than the control. The fish, *Cirrhinus mrigala* (Ham.) is a not airbreathing fish. This was collected from different experimental sites and following metabolically active tissues liver and skin were carefully dissected out, washed in chilled fish saline and used for estimation of total SOD, Cu-Zn-SOD, Mn-SOD activities, Total SOD, Cu-Zn-SOD and Mn-SOD activities in the metabolically active tissues of liver and skin of fish *C. mrigala*. The highest Cu-Zn-SOD activities were obtained in river Ganga than river Gomti in tissues of liver. The tissues dependent Cu-Zn-SOD comparisons show that highest Cu-Zn-SOD activity was found in the liver of fish and than skin. Above Table 3 also shows that the different tissues of *Cirrhinus mrigala* collected from different experimental sites have Cu-Zn-SOD and Mn-SOD activities significantly different from each after.

Unlike other vertebrates, fishes are exposed to daily or seasonal fluctuations in both temperature and oxygen availability. This situation is well exemplifed by fresh water teleost fish living in unstable environments such as tropical waters (Kramer, 1987 and Graham, 1990). Like other aerobic organisms, fishes posses an elaborate antioxidant defence system which utilizes enzymic and non-enzymic mechanisms to fight oxidative stress. Mitochonadria are the main sites for production of reactive oxygen species. In most fishes red muscles are relatively assent/scare and tissues such as liver, kidney, blood, skin, gills, swim bladder, roe, crystalline and chroids are more important

Table 1 : Physico-chemical parameters of water and river Gomti

Samples	pH	DO (mg/l)	COD (mg/l	BOD (mg/l)
Control	6.7±0.08	11.9±1.83	16.6±0.93	12.0±0.16
Experimental	7.6±0.10	8.4±0.06	39.2±0.06	26.8±2.30

Table 2 : Physico-chemical parameters of water and river Ganga

Samples	pH	DO (mg/l)	COD (mg/l	BOD (mg/l)
Control	6.5±0.07	7.6±0.98	14.6±0.83	10.3±0.15
Experimental	7.4±0.10	6.4±0.07	33.6±1.99	24.0±1.80

Table 3 : Total Cu-Zn SOD and Mn SOD activity (units mg^{-1} protein) in liver and skin of *Cirrhinus mrigala* collected from different experimental sites (Activity expressed as mean ± SD of observation)

Experimental Sites	Samples	Liver		
		Total SOD	Cu-Zn SOD	Mn SOD
River Gomti, Jaunpur	Control	7.4±0.258	5.87±0.158	1.6±0.254
	Experimental	9.5±0.454	6.9±0.156	2.6±0.223
River Ganga, Ghazipur	Control	8.8±0.381	6.6±0.233	2.2±0.158
	Experimental	9.9±0.391	7.1±0.213	2.8±0.158
Experimental Sites	Samples	Skin		
		Total SOD	Cu-Zn SOD	Mn SOD
River Gomti, Jaunpur	Control	3.7±0.399	2.7±0.187	1.0±0.291
	Experimental	4.2±0.607	3.2±0.316	1.0±0.221
River Ganga, Ghazipur	Control	4.1±0.345	2.7±0.127	0.9±0.339
	Experimental	5.2±0.511	3.3±0.187	0.8±0.223

reactive oxygen species generating organs (Wilhelm *et al.*, 1993 and Wilhelm and Mcercon, 1996).

Conclusion :

The present study was under taken to gain an insight into the role of dissolved oxygen content on the superoxide dismutese activities in fresh water teleost fishes. From two experimental sites of two districts of Uttar Pradesh in two rivers fresh water teleost fishes were collected and two tissues were dissected out. Total SOD (*i.e.* Cu-Zn-SOD and Mn-SOD), Cu-Zn-SOD and Mn-SOD were studied on effect of physico-chemical parameters especially, level of DO content; water temperature, water pH on the quantitative level of SOD activities. It was observed that high DO content; high temperature and alkalinity or acidic pH may results into high SOD activities. Metabolically active tissue *i.e.* liver higher SOD activities and less metabolically active tissue *i.e.* skin have lower SOD activities. Both the SODs (Cu-Zn-SOD and Mn-SOD) scavenge reactive oxygen species and protect the organisms. SOD activities are higher in higher vertebrates because they lack L-gulanolactone oxidase activity while lower vertebrates have lower SOD activities and act synergistically with L-gulanolactone oxidase scavange, the oxy anions (Izokun - Etiobhio *et al.*, 1990; Scott and Harrington, 1990; Rodriguez - Ariza *et al.*, 1993; Pedrajas *et al.*, 1993; Hai *et al.*, 1995 and Nandi *et al.*, 1997).

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