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Biochemical Activity of Superoxide Dismutase Enzyme in Liver of *Labeo rohita* in River Gomti

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Abstract

Most of aquatic animals are dependent on molecular oxygen. Yet all living forms are prone to oxygen toxicity. This oxygen toxicity has been attributed to reactive oxygen metabolites including the Superoxide radicals(O_2), Hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH-). These highly reactive substances can directly or indirectly cause substantial damage to living cells. The enzyme superoxide dismutase is essential for the survival of all respiring and metabolically active cells. Only respiring and metabolically active cells can generate reactive oxygen metabolites and they are controled at physiological concentration by a repertoire of cellular antioxidant defenses such as Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPx), Glucose-6-Phosphate dehydrogenase (G-6-PDH) and Glutathione-S-transferase (GST).

Keywords: CAT; GPx; G-6-PDH; GST; OH; ROMs.

Introduction

The concept of oxygen as a requirement for aerobic life is mentioned inancient Indian civilization. For example, the Sanskrit word "PRAN VAYU" meaning the "the gas of life" appears frequently in the "VEDAS". For centuries, considered critical to life itself, oxygen remains and will always remain, one of the essential elements. The Biochemical Scientists have envisaged much interest on the role of oxygen derived free radicals in various diseases. "Reactive Oxygen Species (ROS) or Free Radicals" have been implicated in over hundred diseases from arthritis and haemorrhagic shock to AIDS (Southern 1988, Halliwell and Gutteridge 1989). The ROS is also related with plant defence system (Deepmala, 2019). The wide range of diseases implied increased formation of free radicals leading to cell and tissue injury in most, if not all human disease(Halliwell and Gutteridge; 1984 and 1989). The metabolism of oxygen generates reactive intermediates i.e. free radicals (Otto and Moon, 1995). A radical might donate its unpaired electrons to another molecule. It might take an electron from another molecule in order to pair or it might simply join to that molecule.

Superoxide is the best known free radical of all oxygen derived species (Fridovich, 1978) because it is the best intermediate in the sequential univalent reduction of oxygen that leads to formation of H_2O. (Florence, 1990; Harris, 1992; Winkle et al., 2007). The hazardous effects of reactive oxygen species are quite well-known, however their detoxification, which is one of the prerequisite of aerobic life, cannot over sighted for this reason. The nature has equipped the biosystem with multiple defenses. (Otto and Moon, 1996; Winkle et al., 2008; Mishra et al., 2008). The antioxidant systems responsible for cellular protection against oxidative stress are

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diversified as the free radicals themselves (Heffner and Repine, 1989). The Superoxide Dismutase (SOD) catalyzes the dismutation between two moles of superoxide anion to yield one mole of oxidize product (oxygen) and one mole of reduced product (Klug et al., 1972; Babich et al., 1993). This is ananalogoue to the dismutation of hydrogenperoxide to oxygen and water catalyzed by catalase (Mishra et al., 2008; Singh et al., 2009; Mishra et al., 2018; Dar et al., 2019; Abhijith et al., 2012). In rat, mice and fish, the Mn-SOD is localized in mitochondria whereas the Cu-Zn-SOD in cytoplasm. Various studies have been carried out to study the limnological condition and heavy metal pollution in the fresh water bodies and its impact on fish physiology (Prakash et al., 2015a, 2015b, 2015c; Srivastava and Prakash, 2018; Prakash and Verma, 2019a, 2019b, 2019c, 2020a, 2020b, 2020c; Prakash, 2020a, 2020b; Verma and Prakash, 2018, 2019a, 2019b, 2020a,) but no study on activity of superoxide dismutase was done.

The experimental fish *Labeo rohita* is most common fresh water non air breathing edible fish. It has antioxidant defense system which utilizes the enzymatic and nonenzymatic mechanisms. It can be expected that fish antioxidant defense mechanisms depend on oxygen consumptions. This antioxidant defense mechanism of fish will be detected by the Cu-Zn-SOD and Mn-SOD activities in liver (metabolic tissue) of *Labeo rohita.*

Materials and Methods

The fish *Labeo rohita* was collected from different sites of Gomti river at district Jaunpur, U.P. India and stocked in earthen container and acclimatized in laboratory conditions. The physico–chemical parameters of water such as temperature, pH, alkalinity and DO were analyzed by following standard methods.

After acclimatization fishes were sacrificed by decapitation and liver tissues were taken out then homogenized and centrifuged. The clear supernatant was taken for biochemical studies. The protein and superoxide dismutase were estimated bythe method of Lowry et. al. (1954) and Mc Cord and Frodovich, (1969), respectively. The superoxide anion were generated in a system comprised of NADH and PMS. The superoxide anion reduce the nitro blue tetazolium (NBT) forming a blue formazan measured at 560nm optical density. For the assay the tissue homogenate were diluted 1:4 for liver tissues. To find out the amounts of Cu-Zn -SOD and Mn-SOD in tissues, 2mm KCN solution was added to the mixture to inhibit CU, Zn SOD, Manganese SOD remain unaffected. (Fridovich, 1974; Nandi and Chatteree 1988; Crapo et al., 1978; Mishra et al. 2008). The unit of enzyme activity was defined as the amount of enzyme required to inhibit the optical density at 560nm of NBT reduction by 50% in one minute under assay condition. The data in this paper have been presented with mean ± mean standard error and the statistical significance of difference between control and experimental group was calculated by ANOVA.

Results and Discussion

Table 1: Physico- chemical parameters of water samples collected from different sites of Gomti river.

Sites	Temp. 0C	PH	Alkalinity mg/lit	DO mg/lit
Kalichabad	24.0±1.35	7.3	194±16.3	11.9±1.83
Katghara	24.2±1.89	7.4	190±17.8	11.3±1.07
Gularghat	24.2±1.18	7.5	199±13.8	7.90±0.99
Hanuman ghat	23.8±1.45	7.1	170±16.8	11.2±0.93
Achalaghat	23.8±0.21	7.0	169±13.6	11.5±0.54
Surajghat	24.1±1.20	7.2	171±13.9	11.9±1.96

Table 2: Total Cu-Zn SOD and Mn-SOD activities (unit mg-1 protein) in liver of *Labeo rohita* collected from different experimental sites. (Activity expressed as mean ±SD of 5 observations).

Experimental sites of River Gomti in Jaunpur	Total SOD	Cu-Zn SOD	Mn-SOD
Kalichabad	9.0±0.381	6.6±0.158	2.3±0.223
Katghara	8.±0.165	6.1±0.007	2.1±0.158
Gularghat	7.2±0.370	5.6±0.212	1.4±0.158
Hanuman ghat	7.1±0.444	5.8±0.244	1.3±0.200
Achalaghat	8.6±0.527	6.2±0.254	2.2±0.273
Surajghat	9.1±0.316	6.8±0.158	2.3±0.158

Table 2a: ANOVA of Cu/Zn SOD of data Table 2.

	S.S.	df	MS	F	P
Total	6.0	28			
Between experimental sites	5.1	5	1.04	27.7	<0.001
Error	0.9	23	0.0373		

Table 2b: ANOVA of Mn-SOD of data Table 2

	S.S.	df	MS	F	P
Total	5.70	28			
Between experimental sites	5.6	5	1.14	21.43	<0.001
Error	1.4	23	0.0539		

The physico- chemical parameters of the water

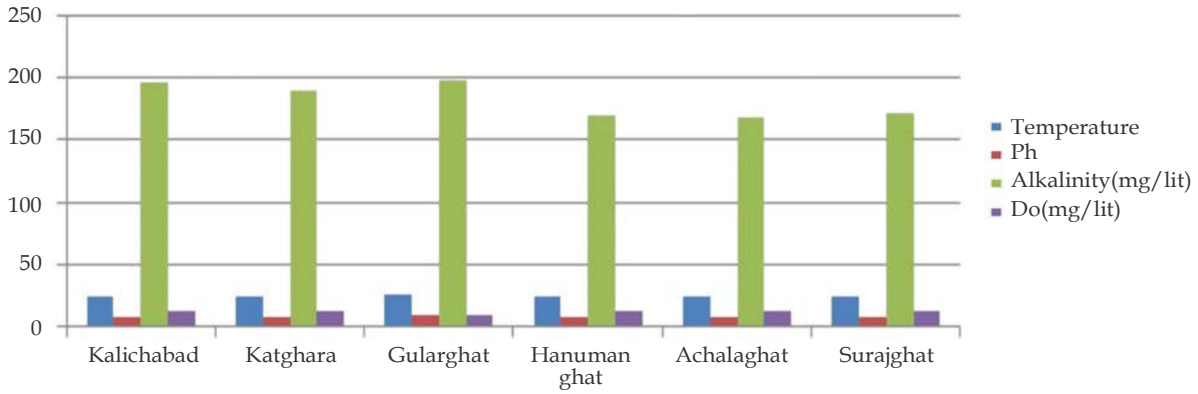

Fig. 1: Physico- chemical parameters of different sites of Gomtiriver at Jaunpur

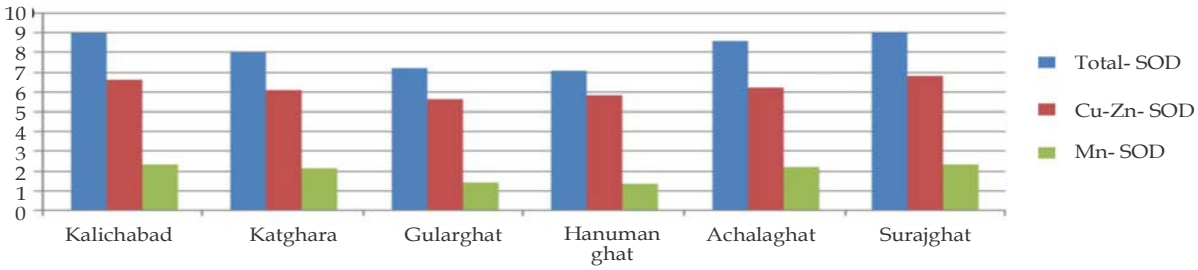

Fig. 2: Cu-Zn SOD &Mn-SOD activities (unit mg-1 protein) in liver of *Labeo rohita* collected from different sites

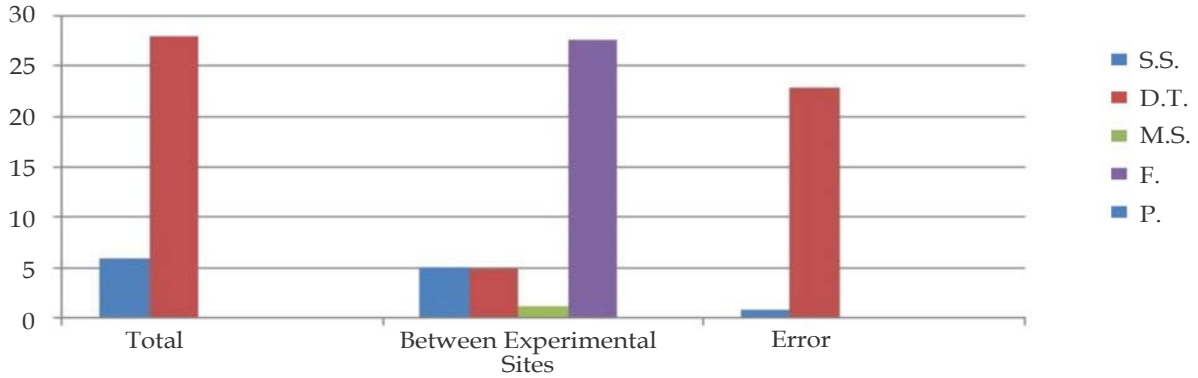

Fig 3: ANOVA of Cu/Zn SOD of data

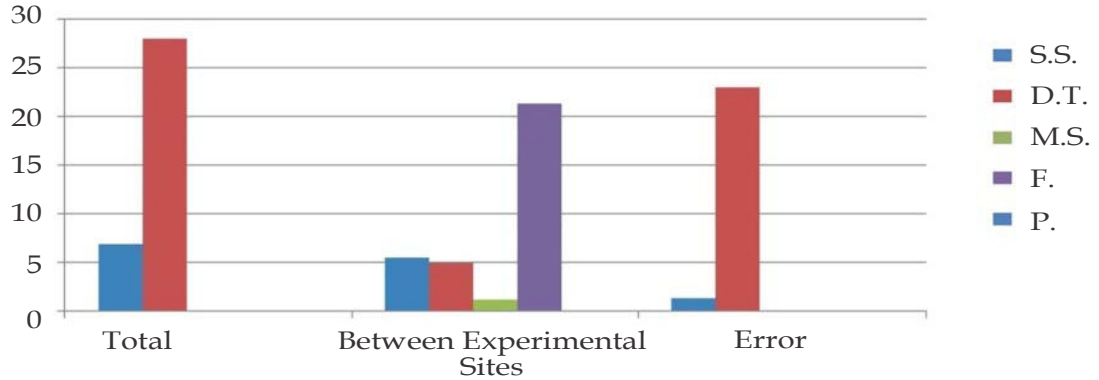

Fig 4: ANOVA of Mn-SOD of data

samples collected from six different sites of Gomti river at Jaunpur. In these experimental sites temperature was almost identical (23.8-24.020C). There was a nominal difference in pH and alkalinity in all the sites but dissolve oxygen show variation in different sites (Table 1).

The liver plays very important role in metabolism. The Superoxide dismutase, Cu-Zn-SOD Mn-SOD activities in liver of *Labeo rohita* are presented in table and Graph 2, Table 2a and Table 2b, respectively. The highest SOD activities was seen in fish collected from experimental site Kalichabad and lowest SOD activities observed in fishes collected from Hanuman ghat (Table and Fig. 2). The highest Cu-Zn-SOD was found in fishes collected from Surajghat site and lowest in Gularghat site (Table and Fig. 2). Similarly the Mn-SOD activity was observed highest and lowest in fishes collected from Kalichabad and Hanuman ghat respectively (Table and Fig. 2). The ANOVA results indicate that SOD activities of liver in *Labeo rohita* collected from different experimental sites were significantly with each other (Table and Fig. 1- 4).

The result shows that the liver of this more active non air breathing fish, *Labeo rohita* possess an enhanced antioxidant defense system while less active fishes have poor antioxidant defense system. More active fish are metabolically active tissue higher O_2 consumption cause oxidative stress which is inhibited by SOD. SOD forms the primary line of defense against oxidative stress.

Conclusion

The present study reveals the role of dissolved oxygen in the activity of superoxide dismutase enzyme in fresh water non air breathing fish *Labeo rohita*. High DO contents produces oxyanion that cause high oxidative activity of SOD. The present study clearly indicated that the physico-chemical parameters affect the SOD, Cu-Zn-SOD and Mn-SOD activities resulting the severe damage of cellular compounds and also conformational changes in nucleic acid, protein, lipids and carbohydrates. The fishes facing such severe problem of reactive oxygen species also produce the ADS (Antioxidant Defense System) to protect themselves.

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