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Biochemical vicissitudes of Superoxide dismutase (SOD) and Catalase (CAT) in *Clarias batrachus* & *Labeo rohita*

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Abstract
This paper studies the response to oxidative stress in the fish *Clarias batrachus* and *Labeo rohita* collected from various areas of Bisalpur during extreme cold conditions. Specifically, it investigates the hepatic levels of SOD and CAT as candidate biomarkers of antioxidant defense in this species. SOD and CAT activity are the most widely used measures of oxidative stress. The mean values of catalase & SOD in all the tissues were significantly higher in *Thadoli* area, followed by *Negdiya* and *Nasirda*. The lowest values were obtained in *Bisalpur* area. In Thdoli area concentration of dissolved oxygen was highest. Higher concentration of SOD & catalase in fishes of Thadoli area indicated the presence of oxidative stress. From the results, it can be hypothesised that higher catalase & SOD activity in Thadoli area was probably due to some stressful condition in aquatic medium leading to excessive production of free radicals, which resulted in oxidative stress and an imbalance between oxidant and antioxidant system.

Keywords: Superoxide dismutase (SOD), Catalase (CAT), *Clarias batrachus, Labeo rohita*

Introduction
Water quality, as measured by the physical and chemical parameters, does not give a long-term integrated view of the effect of pollution. One method of bio-monitoring is to use biomarkers. Biochemical biomarkers are molecules that are present in the body fluids, cells and tissues of organisms and whose activity is altered by the presence of toxic agents in the environment (McCarthy and Shugart, 1990) [6]. One important set of biomarkers is the antioxidant defense enzymes that decompose the Reactive Oxygen Species (ROS). These enzymes have an important role in the control, production and elimination of ROS, which in excess can alter the normal functions of the cell and lead to oxidation of the cell membranes as well as lesions in mitochondria, proteins, DNA and other components of the cell. Superoxide dismutase (SOD) and catalase (CAT) are members of the antioxidant defense enzymes. SOD converts superoxide radicals into hydrogen peroxide. It has been shown that SOD activity is altered in mussels living in areas contaminated by heavy metals and organic pollutants (Binelli and Cogni 2010) [2]. The enzyme CAT is widely Tissue levels of the antioxidant enzymes distributed in biological tissues and is involved in the decomposition of hydrogen peroxide into oxygen and water. It is one of the most prominent enzymes involved in defense against oxidative stress in both vertebrates and invertebrates (Goyal and Basak, 2010) [3].
This investigation attempted at providing biomarkers of physiological defense against ROS at one platform. This will provide normal range for future ecotoxicological studies and will be helpful in making the strategies to protect the fishes from oxidative stress. Therefore, the aim of this study was to evaluate for Biochemical vicissitudes of Superoxide dismutase (SOD) and catalase (CAT) in *Clarias batrachus* & *Labeo rohita.* SOD and CAT activity are the most widely used measures of oxidative stress.

Material & method
The present investigation was carried out on eighty fishes of two species i.e. *Clarias batrachus* and *Labeo rohita* collected from various areas of Bisalpur during extreme cold conditions. Specifically, it investigates the hepatic levels of Superoxide dismutase (SOD) and catalase (CAT) as candidate biomarkers of antioxidant defense in this species.

Catalase

It was determined by basic colorimetric method as described by as described by Maan (2010) [5] and Kataria and Kataria (2009) [4]. The supernatant is allowed to react with H2O2 for a specific period of time. The reaction is then stopped by using H2SO4.Then excess of KMnO4solution is added to allow to react with the peroxide not decomposed by the catalase. Within one minute, the excess in KMnO4 is determined photometrically.

In a conical flask, 50 ml cold (4°C) solution of H_2O_2 (0.01 N) was taken and to this 1 ml supernatant was added. Immediately after mixing, 5 ml of this solution was pipetted into a test tube containing 2 ml of the 5N H_2SO_4. The contents of the tube were mixed and 10 ml of the $KMnO_4$ solution was added. After mixing the contents, the optical density was read in a spectrophotometer against the blank at 515 mμ within one minute. The optical density was again recorded after 3 minutes. The change in optical density was determined. The blank was prepared by taking 2 ml of H_2SO_4, 5 ml distilled water and 10 ml of $KMnO_4$ solution. The catalase activity was calculated as follows:

$$UL^{-1} = \frac{\text{Change in OD in 3 minutes X 0.0309 X 1000 X 2260 X1000}}{27.1}$$

Where, 27.1 = mg of enzyme per ml taken in the standard
1000 = Conversion factors for the units, Dilution factor
0.309 lope of the standard curve
2260 = Units per ml of standard

Then units were calculated per ml of supernatant. From protein estimation, total quantity of protein was calculated in one ml of supernatant. Then units per ml were converted to units per mg of protein.

Superoxide dismutase (SOD)

It was determined by colorimetric method as described by Maan (2010) [5]. The method disbased up on the ability of super oxide dismutaseto inhibit the reduction of nitroblue tetrazolium by superoxide. One unit is defined as that amount of enzyme causing half the maximum inhibition of nitroblue tetrazolium reduction.

A series of ten test tubes were set and various quantities of each supernatant sample (0.1, 1,2,3,4,5,6,7,8,9 and 10 µl) were added. In each tube 0.2 ml of EDTA-cyanide reagent, 0.1 ml NBT and 3 ml of phosphate buffer were added. The tubes were incubated by placing them in light box providing uniform light intensity. For this a foil-lined box (4' long X 8" X 6") with an internally mounted 40 W fluorescent bulb was used. The tubes were incubated for 5 minutes to achieve a standard temperature. Then 0.05 ml riboflavin was added. All tubes were again incubated in the light box for 12 minutes. Then at 560 mμ wavelength, the % transmission of each tube was determined at one minute interval in an increasing order. The amount of sample resulting in 50% of transmission was determined by a curve and put in the formula to calculate SOD units as follows:

$$\text{Units/ ml} = \frac{1000}{\text{µl of serum resulting in 50 \% transmission}}$$

Here, 1000 = Conversion factor

After calculation the values were converted into kU L^{-1}. Then units were calculated per mg of proteins as discussed for other enzymes.

Result & Discussion

On the basis of results of enzymological parameters of fishes obtained from other three areas were compared from respective parameters of fishes from Bisalpur area.

Catalase (CAT)

Mean ±SEM values of catalase in tissues of *Clarias batrachus* and *Labeo rohita* are presented in table 1. The mean values of catalase in various tissues obtained in the present study showed more or less similar pattern of distribution in various tissues and control values were comparable to the values obtained from Bisalpur and Nasirda areas. On the basis of available control values it was inferred that the mean values of catalase in all the tissues of both the types of fishes from Thadoli and Negdiya areas showed oxidative stress. The mean values of catalase in all the tissues were significantly higher in Thadoli area, followed by Negdiya and Nasirda. The lowest values were obtained in Bisalpur area. In Thdoli area concentration of dissolved oxygen was highest. High oxygen concentration can be related with development of oxidative stress (Ansaldo *et al*., 2000) [1]. Higher concentration of catalase in fishes of Thadoli area indicated the presence of oxidative stress. In each area, the catalase activity significantly differed among all the tissues collected i.e. heart, kidney, liver and gills. In each area, the acticity of catalase was highest in gills for both the fishes. From the results, it can be hypothesised that higher catalase activity in Thadoli area was probably due to some stressful condition in aquatic medium leading to excessive production of free radicals, which resulted in oxidative stress and an imbalance between oxidant and antioxidant system. Further it can be inferred that higher catalase activities showed activation of defense system of fishes whereas, its lowered activities could have a negative impact on cellular resistance against the oxidant induced damage of the cell.

Superoxide dismutase (SOD)

Mean ±SEM values of superoxide dismutase in tissues of Clarias batrachus and Labeo rohita are presented in table 2.The mean values of SOD obtained from fishes of Bisalpur and Nasirda areas were more or less similar to the available control values (Stephanie *et al*., 2006) [7]. The higher values in Thadoli and Negdiya areas over and above the control values reflected the oxidative stress. The mean values of SOD in all the tissues were significantly higher in Thadoli area, followed by Negdiya and Nasirda. The lowest values were obtained in Bisalpur area. In Thdoli area concentration of dissolved oxygen was highest. In each area, the SOD activity significantly differed among all the tissues collected i.e. heart, kidney, liver and gills. In each area, the acticity of SOD was highest in gills for both the fishes. Activity was lowest in the heart of both the fishes collected from all four areas. The higher value of tissue SOD in the fishes from Thadoli area was probably to scavenge the free radicals produced due to some environmental or ambient factors. To correlate, in Thadoli area, water samples showed the higher levels of pH, alkalinity, turbidity, hardness etc. Higher activity of SOD in tissues of fishes indicated towards oxidative stress as

environment induced oxidative stress was confirmed in some previous studies on the basis of higher activity of SOD during by Kataria and Kataria (2009a) [4] in mammals.

Table 1: Effect of varying ambiences on catalase activity in tissues of fishes collected from different areas /villages of Bisalpur reservoir (n=10)

Catalase,U/mg protein	Areas							
	Bisalpur		Nasirda		Thadoli		Negdiya	
	C *b*	L *r*	C *b*	L *r*	C *b*	L *r*	C *b*	L *r*
Heart	78.00 ^{c} ± 1.9	70.00 ^{c} ± 1.8	101.1 ^{c} ± 1.0	90.00 ^{c} ± 1.0	150.01 ^{c} ± 3.00	140.23 ^{c} ± 2.00	127.00 ^{c} ± 2.0	126.21 ^{c} ± 2.0
Kidney	83.00 ^{c} ± 1.9	75.00 ^{c} ± 1.8	107.1 ^{c} ± 1.0	95.00 ^{c} ± 1.0	156.01 ^{c} ± 3.00	144.5 ^{c} ± 2.00	132.00 ^{c} ± 2.0	129.00 ^{c} ± 2.0
Liver	88.00 ^{c} ± 1.3	95. 00 ^{c} ± 1.4	127. 00 ^{c} ± 1.0	115.00 ^{c} ± 1.0	176.91 ^{c} ± 3.03	164.83 ^{c} ± 2.03	152.80 ^{c} ± 2.5	149.91 ^{c} ± 2.3
Gills	108.00 ^{c} ± 1.3	125. 01 ^{c} ± 1.4	147. 01 ^{c} ± 1.0	135.00 ^{c} ± 1.0	196.11 ^{c} ± 3.03	184.13 ^{c} ± 2.03	172.80 ^{c} ± 2.5	168.01 ^{c} ± 2.3

n= Number of fishes

All the means values of a parameter superscribed by same letter denotes significant (p≤0.05) differences among different areas.

C *b = Clarias batrachus*

L *r =Labeo rohita*

Table 2: Effect of varying ambiences on SOD activity in tissues of fishes collected from different areas /villages of Bisalpur reservoir (n=10)

SOD, U/mg protein	Areas							
	Bisalpur		Nasirda		Thadoli		Negdiya	
	C *b*	L *r*	C *b*	L *r*	C *b*	L *r*	C *b*	L *r*
Heart	180.00 ^{b} ± 5.00	160.00 ^{b} ± 5.11	290.20 ^{b} ± 6.21	260.00 ^{b} ± 5.11	380.00 ^{b} ± 4.14	360.00 ^{b} ± 4.11	330.00 ^{b} ± 4.14	310.50 ^{b} ± 4.11
Kidney	200.71 ^{b} ± 5.00	180.54 ^{b} ± 5.11	310.20 ^{b} ± 6.21	280.00 ^{b} ± 5.11	401.00 ^{b} ± 4.14	382.54 ^{b} ± 4.11	352.00 ^{b} ± 4.14	329.50 ^{b} ± 4.11
Liver	220.0 ^{b} ± 5.00	200. 0 ^{b} ± 5.11	332. 00 ^{b} ± 6.21	301. 00 ^{b} ± 5.11	419.00 ^{b} ± 4.14	400.04 ^{b} ± 4.11	371.10 ^{b} ± 4.14	349.00 ^{b} ± 4.11
Gills	240.0 ^{b} ± 5.00	220. 0 ^{b} ± 5.11	352. 00 ^{b} ± 6.21	321. 00 ^{b} ± 5.11	439.00 ^{b} ± 4.14	420.05 ^{b} ± 4.11	391.80 ^{b} ± 4.14	379.90 ^{b} ± 4.11

n= Number of fishes

SOD= Super oxide dismutase

All the means values of a parameter superscribed by same letter denotes significant (p≤0.05) differences among different areas.

C *b = Clarias batrachus*

L *r =Labeo rohita*

Conclusion

On the basis of above discussion it can be reiterated that higher dissolved oxygen, high pH, higher alkalinity, higher turbidity etc. generated free radicals to produce oxidative stress in fishes of Thadoli area.

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