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Research Paper

STUDY OF BIOMARKERS OF PHYSIOLOGICAL DEFENSE AGAINST REACTIVE OXYGEN SPECIES DURING ENVIRONMENTAL STRESS

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The present study was deal with determination of oxidative stress in fishes of Bisalpur reservoir.. Four areas related to Bisalpur reservoir were selected i.e. Bisalpur, Nasirda, Thadoli and Nagdiya. From each area 20 fishes were collected which are *Clarias batrachus* (10) and *Labeo rohita* (10). Markers of oxidative stress were determined in the tissues like gills, liver, kidney and heart. In present study we found that higher catalase activity in thadoli area was probably due to some stressful condition in aquatic medium leading to excessive production of free radicals, overall higher oxidative stress was found in Thadoli area fishes. The present investigation was planned to find out the physiological defense against reactive oxygen species during environmental stress or due to presence of pollutants in water. This investigation was 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. The findings of the present investigation will provide a rational use of oxidative stress biomarkers in aquatic ecosystem pollution biomonitoring.

Keywords: Oxidative stress, Clarias batrachus, Labeo rohita, Pollution, Biomarkers, Reactive oxygen species (ROS)

INTRODUCTION

Management of India's limited fresh water resources is essential. Environmental pollution, caused by the development of Industry, technology and informal settlements, threaten the health status of many fresh water ecosystems. The health of all living organisms in that system is also subsequently affected by the decline in water quality. The biological integrity of an ecosystem is therefore often reflected by the health of its fauna (Robinson, 1986). It is therefore necessary to identify, monitor and manage the effects of pollution on the health of an aquatic system within a management programmed.

Fishes are relatively sensitive to changes in their surrounding environment including an

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increase in pollution. Fish health may thus reflect, and give a good indication of the health status of a specific aquatic ecosystem. Early toxic effects of pollution may however, be evident on cellular or tissue level before significant changes can be identified in fish behavior or external appearance.

Fish can serve as bio indicators of environmental pollution and therefore can be used for the assessment of the quality of aquatic environment since they are directly exposed to chemicals resulting from agricultural production via surface runoff of water or indirectly through the food chain of ecosystem (Ateeq *et al*., 2002). Fish are endowed with defensive mechanisms to counteract the impact of reactive oxygen species (ROS) resulting from the metabolism of various chemicals. These systems include various antioxidant defense enzymes such as superoxide dismutases which catalyze the dismutation of superoxide radical to hydrogen peroxide, catalase acting on hydrogen peroxide, glutathione S-transferase family possessing detoxifying activities towards lipid hydroperoxides generated by organic pollutants such as heavy metals (Tjalkens *et al*., 1998).

Fish constitute an excellent model to understand the oxidative stress in aquatic ecosystems. Indian catfish and Indian major carps are of great commercial importance and the most common fresh water fishes widely consumed. They can serve as good model to study responses to various environmental contaminants. Fish are relatively sensitive to changes in their surrounding environment, including an increase in pollution. Fish health may as a result reflect, and give a good indication of the health status of the aquatic ecosystem in which the fish occurs. The initial toxic effects of the pollution may, however, only be evident on cellular or tissue level before significant changes can be identified in fish behavior or external appearance. The health of an ecosystem is thus often reflected by the health of its fauna.

The Bisalpur Dam is the important centre of Rajasthan state which is made on river Banas near by Deoli, District Tonk. This dam lies between 26° 28' to 26° 29' north latitudes and 74° 37' 30" to 74° 38' east longitudes. It covers about 500 km perimeter area and its maximum depth is about 30.0 m when full of water. In this dam the water is run off from the surrounding of Banas River during the monsoon season. Bisalpur Dam supplies the water in seven cities i.e. Kekri, Sarvar, Nasirabad, Kishangarh, Ajmer and Beawar. The areas related to Bisalpur reservoir i.e., Bisalpur, Nasirda, Thadoli and negadiya are used for collection of fishes. To carry out such studies, it becomes important to note that areas selected should have proper distance. Bisalpur reservoir fulfills this condition having a variation of distance of approximate 20-25 km from each other. This produces spatial variation with different kind of exposure and level of water quality, stress etc. Various ambiences like moderate, extreme cold, extreme hot and moist warm produce considerable effects on the aquatic ecosystem. Study of Oxidative stress in fish species make useful in assessing the whole aquatic environment of the area. Improvement in the status of water reservoir having fishes especially the major carp and cat fish by proper investigation oxidative stress studies related to fish diseases is an urgent requirement. There is a need of continuous monitoring of fish biological data, physico-chemical conditions and plankton status of the water body like Bisalpur reservoir as it is also related with human health.

MATERIAL AND METHODS

Markers of oxidative stress were determined in total 80 fishes collected from different areas. Fishes were collected during extreme cold condition when water bodies were having peak environmental contamination in reservoir due to fall in water level and feeble current. Four areas related to Bisalpur reservoir were selected i.e. Bisalpur, Nasirda, Thadoli and Negdiya. From each area 20 fishes were collected which constituted *Clarias batrachus* (10) and *Labeo rohita*(10). Markers of oxidative stress were determined in the tissues like gills, liver, kidney and heart.

Markers of Oxidative Stress

Tissue Enzymes

1. Catalase (CAT)
2. Superoxide dismutase (SOD)
3. Glutathione reductase (GR)
4. Peroxidase
5. Xanthine oxidase (XO)

Antioxidants

1. Vitamin E
2. Vitamin A
3. Glutathione (GSH)

RESULTS AND DISCUSSION

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. The markers of oxidative stress included tissue enzymes i.e. catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR), peroxidase and xanthine oxidase (XO) and antioxidants i.e. vitamin E, vitamin A and glutathione.

The mean values of Catalase (Table 1) 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 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 activity of Catalase was highest in gills for both the fishes. Activity was lowest in the heart of both the fishes collected from all four areas. From the results, it can be hypothesized 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.

The mean values of SOD (Table 2) in all the tissues were significantly higher in Thadoli area, followed by Negdiya and Nasirda. It was lowest in Bisalpur area. In Thdoli area concentration of dissolved oxygen was highest. Higher concentration of SOD in fishes of Thadoli area indicated the presence of oxidative stress. In each area, in each tissue, the SOD activity was significantly higher in *Clarias batrachus* than in *Labeo rohita*.

The mean values of Glutathione reductase (Table 3) 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 GR activity

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

Note: n= Number of fishes; All the means values of a parameter superscribed by same letter denotes significant (pd"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)

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	180.00b± 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	200.71b± 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	220.0b± 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	240.0b± 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

Note: n= Number of fishes; SOD= Super oxide dismutase; All the means values of a parameter superscribed by same letter denotes significant (pd"0.05) differences among different areas; C *b* = *Clarias batrachus*; L *r* =*Labeo rohita.*

Table 3: Effect of Varying Ambiences on Glutathione Reductase 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	1.55 e± 0.001	1.50 e± 0.003	1.7 e± 0.002	1.6 e± 0.001	2.9 e± 0.004	2.8 e± 0.003	1.9 e± 0.002	1.8 e± 0.001
Kidney	1.75 e± 0.001	1.60 e± 0.003	1.8 e± 0.002	1.7 e± 0.001	3.9 e± 0.004	3.8 e± 0.003	2.9 e± 0.002	2.8 e±0.001
Liver	1.85 e± 0.001	1.70 e± 0.003	1.9 e± 0.002	1.8 e± 0.001	4.1 e± 0.004	4.0 e± 0.003	3.0 e± 0.002	2.9 e± 0.001
Gills	1.95 e± 0.001	1.80 e± 0.003	2.0 e± 0.002	1.9 e± 0.001	5.3 e± 0.004	4.8 e± 0.003	4.0 e± 0.002	3.5 e± 0.001

Note: n= Number of fishes; SOD= Super oxide dismutase; All the means values of a parameter superscribed by same letter denotes significant (pd"0.05) differences among different areas; C *b* = *Clarias batrachus*; L *r* =*Labeo rohita.*

significantly differed among all the tissues collected i.e. heart, kidney, liver and gills. In each area, the activity of GR was highest in gills and lowest in the heart of both the fishes collected from all four areas. In each area, in each tissue, the GR activity was significantly higher in *Clarias batrachus* than in *Labeo rohita*.

The mean values of Peroxidase (Table 4) 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 peroxidase activity significantly differed among all the tissues collected i.e. heart, kidney, liver and gills. In each area, the acticity of peroxidase was highest in gills for both the fishes. Activity was lowest in the heart of both the fishes collected from all four areas. In each area, in each tissue, the peroxidase activity was significantly higher in *Clarias batrachus* than in *Labeo rohita*.

The mean values of Xanthine oxidase (Table 5) 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 XO in fishes of Thadoli area indicated the presence of oxidative stress. In each area, the XO activity significantly differed among all the tissues collected i.e. heart, kidney, liver and gills. In each area, the activity of XO was highest in gills and lowest in the heart of both the fishes collected from all four areas. In each area, in each tissue, the XO activity was significantly higher in *Clarias batrachus* than in *Labeo rohita.*

The mean values of Vitamin E (Table 6) in all the tissues were significantly lower in Thadoli area, followed by Negdiya and Nasirda. The highest values were obtained in Bisalpur area. In Thdoli area concentration of dissolved oxygen was highest. Lower concentration of vitamin E in fishes of Thadoli area indicated the presence of oxidative stress. In each area, the vitamin E activity significantly differed among all the tissues collected i.e. heart, kidney, liver and gills. In each area, the activity of vitamin E was highest in liver for both the fishes. Activity was lowest in the heart of both the fishes collected from all four areas. In each area, in each tissue, the vitamin E activity was significantly higher in *Clarias batrachus* than in *Labeo rohita*. It can be concluded that in fishes of Thadoli area oxidative stress was there.

The mean values of Vitamin A (Table 7) in all the tissues were significantly lower in Thadoli area, followed by Negdiya and Nasirda. The highest values were obtained in Bisalpur area. In Thdoli area concentration of dissolved oxygen was highest. Lower concentration of vitamin A in fishes of Thadoli area indicated the presence of oxidative stress.

In each area, the vitamin A activity significantly differed among all the tissues collected i.e. heart, kidney, liver and gills. In each area, the activity of vitamin A was highest in liver for both the fishes. Activity was lowest in the heart of both the fishes collected from all four areas. In each area, in each tissue, the vitamin A activity was significantly higher in *Clarias batrachus* than in *Labeo rohita.* The decreased levels of vitamin A indicated towards the presence of oxidative stress in fishes.

The mean values of Glutathione (Table 8) in both the types of fish tissues obtained from different areas were compared with the control values of earlier researchers. The mean values were more or less similar in fishes from Bisalpur

Table 4: Effect of Varying Ambiences on Peroxidase 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	11.5 f± 0.10	9.4 f± 0.14	12.0 f± 0.13	10.0 f± 0.16	17.0 f± 0.13	15.0 f± 0.19	13.0 f± 0.11	12.0 f± 0.12
Kidney	12.5 f± 0.21	10.5 f± 0.12	13.0 f± 0.12	11.0 f± 0.11	18.0 f± 0.12	16.0 f± 0.12	14.1 f± 0.12	13.0 f± 0.14
Liver	13.3 f± 0.11	11.6 f± 0.13	14.0 f± 0.11	12.0 f± 0.10	19.0 f± 0.10	17.0 f± 0.14	15.0 f± 0.13	14.0 f± 0.13
Gills	14.6 f± 0.10	12.8 f± 0.10	15.0 f± 0.10	13.0 f± 0.10	20.0 f± 0.12	18.0 f± 0.15	16.2 f± 0.11	15.1 f± 0.11

Note: n= Number of fishes; All the means values of a parameter superscribed by same letter denotes significant (pd"0.05) differences among different areas; C *b* = *Clarias batrachus*; L *r* =*Labeo rohita.*

Table 5: Effect of Varying Ambiences on Xanthine Oxidase 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	0.55 g± 0.001	0.50 g± 0.001	1.30 g± 0.002	1.20 g± 0.001	2.10 g± 0.001	2.00 g± 0.002	1.70 g± 0.002	1.60 g± 0.001
Kidney	0.65 g± 0.002	0.55 g± 0.002	1.40 g± 0.001	1.30 g± 0.002	2.20 g± 0.002	2.10 g± 0.002	1.80 g± 0.002	1.70 g± 0.003
Liver	0.75 g± 0.002	0.76 g± 0.001	1.50 g± 0.001	1.40 g± 0.002	2.30 g± 0.001	2.20 g± 0.001	1.90 g± 0.001	1.80 g± 0.002
Gills	0.85 g± 0.001	0.86 g± 0.002	1.60 g± 0.002	1.50 g± 0.001	2.40 g± 0.002	2.30 g± 0.002	2.00 g± 0.002	1.90 g± 0.001

Note: n= Number of fishes; SOD= Super oxide dismutase; All the means values of a parameter superscribed by same letter denotes significant (pd"0.05) differences among different areas; C *b* = *Clarias batrachus*; L *r* =*Labeo rohita.*

Table 6: Effect of Varying Ambiences on Vitamin E Concentration 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	18.0 b± 0.11	17.0 b± 0.2	16.0 b± 0.20	15 b± 0.41	12.0 b± 0.33	11.2 b± 0.16	14.1 b± 0.14	12.1 b± 0.21
Kidney	19.0b± 0.12	18.0 b± 0.1	17.0 b± 0.20	16.0 b± 0.40	13.0 b± 0.13	12.3 b± 0.11	15.2 b± 0.11	13.3 b± 0.23
Liver	23.0 b± 0.30	22.0 b± 0.3	21.0 b± 0.21	19.0 b± 0.25	16.0 b± 0.10	15.4 b± 0.12	18.1 b± 0.12	17.0 b± 0.22
Gills	21.0 b± 0.02	20.0 b± 0.2	19.0 b±0.22	18.0 b± 0.25	15.0 b± 0.30	14.2 b± 0.13	17.0 b± 0.13	16.2 b± 0.24

Note: n= Number of fishes; SOD= Super oxide dismutase; All the means values of a parameter superscribed by same letter denotes significant (pd"0.05) differences among different areas; C *b* = *Clarias batrachus*; L *r* =*Labeo rohita.*

Table 7: Effect of Varying Ambiences on Vitamin A Concentration 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	21.30 h± 0.40	20.0 h± 0.22	19.0 h± 0.34	18.0 h± 0.30	15.0 h± 0.19	13.1 h± 0.21	17.2 h± 0.14	16.2 h± 0.19
Kidney	22.00 h± 0.30	21.0 h± 0.23	20.0 h± 0.34	19.0 h± 0.30	16.0 h± 0.19	14.1 h± 0.12	18.1 h± 0.12	17.2 h± 0.16
Liver	25.90 h± 0.40	24.0 h± 0.31	23.0 h± 0.34	22.0 h± 0.30	19.0 h± 0.19	17.1 h± 0.11	21.9 h± 0.13	20.2 h± 0.15
Gills	24.00 h± 0.20	23.0 h± 0.22	22.0 h± 0.34	21.0 h± 0.30	18.0 h ± 0.19	16.1 h± 0.11	20.2 h± 0.11	19.2 h± 0.16

Note: n= Number of fishes; SOD= Super oxide dismutase; All the means values of a parameter superscribed by same letter denotes significant (pd"0.05) differences among different areas; C *b* = *Clarias batrachus*; L *r* =*Labeo rohita.*

Table 8: Effect of Varying Ambiences on Glutathione Concentration on 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	6.2 d± 0. 09	6.1 d ± 0. 08	6.0 d± 0. 09	5.9 d± 0. 08	3.9 d± 0. 07	3.8 d± 0. 08	4.3 d± 0. 09	4.0 d± 0. 08
Kidney	5.8 d± 0. 09	5.7 d ± 0. 08	5.6 d± 0. 09	5.5 d± 0. 08	3.6 d± 0. 07	3.2 d± 0. 08	3.8 d± 0. 09	3.7 d± 0. 08
Liver	8.0 d± 0. 09	7.5 d ± 0. 08	7.3 d± 0. 09	7.2 d± 0. 08	4.4 d± 0. 07	4.0 d± 0. 08	4.7 d± 0. 09	4.3 d± 0. 08
Gills	5.0 d± 0. 09	4.8 d ± 0. 08	4.8 d± 0. 09	4.7 d± 0. 08	2.4 d± 0. 07	2.3 d± 0. 08	2.9 d± 0. 09	2.7 d± 0. 08

Note: n= Number of fishes; SOD= Super oxide dismutase; All the means values of a parameter superscribed by same letter denotes significant (pd"0.05) differences among different areas; C *b* = *Clarias batrachus*; L *r* =*Labeo rohita.*

and Nasirda areas. The mean values were very lower in Thadoli and Negdiya areas suggesting oxidative stress in these two areas. The mean values of glutathione in all the tissues were significantly lower in Thadoli area, followed by Negdiya and Nasirda. The highest values were obtained in Bisalpur area. In Thdoli area concentration of dissolved oxygen was highest. Lower concentration of glutathione in fishes of Thadoli area indicated the presence of oxidative stress. In each area, the glutathione activity significantly differed among all the tissues collected i.e. heart, kidney, liver and gills. In each area, the activity of glutathione was highest in liver for both the fishes. Activity was lowest in the gills of both the fishes collected from all four areas. On the basis of glutathione concentration in the tissues, it was concluded that in the fishes from Thadoli and Negdiya areas, glutathione levels were significantly lower when compared to Bisalpur and Nasirda areas in present study and earlier available literature. The findings clearly reflected the presence of oxidative stress in fishes of Thadoli and Negdiya areas.

CONCLUSION

It was concluded that out of four areas studied under Bisalpur reservoir, two areas were having comparatively higher water pollution. Although concentration of a single pollutant was not alarming but few limnological parameters were high consequently affecting health of the fishes. Oxidative stress is one measure to assess the health of the fishes in the water bodies. Many times symptoms of a particular disease may not arise in the fishes but repeated and chronic exposure of fishes to pollutants may interfere with the physiological mechanisms leading to the development of stress. It may be dangerous in the future to the extent that threat may appear to the existence of the species. Timely scientific attention should be paid on the biomonitoring of these fauna. To support this hypothesis many markers of oxidative stress were determined in the tissues of fishes.

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