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STUDY OF POLLUTION INDUCED OXIDATIVE STRESS IN A CAT FISH (*MYSTUS TENGARA)*

Dr. Reddy P.B.*

PG Department of Zoology, Govt. PG College, Ratlam, M.P. India.

***Corresponding Author: Dr. Reddy P.B.**
PG Department of Zoology, Govt.PG College, Ratlam, M.P. India.

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ABSTRACT
Antioxidant enzyme systems help to correct the balance of free radicals and reactive oxygen species (ROS) and protect the cell against free radical-induced damage. The intensity of oxidative stress can be measured either by measuring the reactive oxygen species (ROS) directly or measuring the presence of antioxidants or by measuring the resulting damage to proteins, lipids, DNA or RNA. The freshwater cat fish Mystus tengara was collected from upstream (non polluted) and downstream (polluted) zones of Chambal River during winter months at Nagda, M.P. India. Homogenate of liver of fish from both the two sited were comparatively assayed for glutathione-*S*-transferase (GST), catalase (CAT), glutathione peroxidase (GPx), glucose-6-phosphate dehydrogenase (G6PDH) and superoxide dismutase (SOD). Lipid peroxidation (LPO) was also estimated by means of malondialdehyde measurements (MDA). Simultaneously with the level of reduced glutathione (GSH) and GSH/GSSG ratio were estimated. All systems were significantly (p<0.1) affected at the polluted site. A comparison was made between the water quality parameters and antioxidant biomarker responses. Results clearly shown that a significant increase ((P<0.001) in the activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione S-transferase, and glutathione reductase (GR) activities in the liver of fish from polluted site was observed. Similarly, the pollution load induced significant increase in the reduced (GSH) and oxidized (GSSG) glutathione content, along with with a significant decrease in [GSH]/ [GSSG] ratio. On the whole, the results demonstrate that alteration in the antioxidant enzymes, glutathione system and induction of lipid peroxidation reflects the presence of heavy metals which may cause oxidative stress in the *Mystus tengara* and other aquatic fauna from Chambal River at Nagda. The increased activity of antioxidant enzymes suggest that fish from polluted site were well protected from oxidative stress.

KEYWORDS: Antioxidant enzymes; Biomarkers. Oxidative stress; biomarkers, pollution.

1. INTRODUCTION

Water bodies serve as sinks as they receive a variety of agricultural and industrial chemicals. The intake of these pollutants by aquatic organisms can happen from water, sediments, suspended particulate matter and food sources. Therefore aquatic organisms like fish may serve as an experimental model in investigation of cellular damage and protection by free radicals.

Earlier research confirms that exposure to pollutants induces the production of powerful oxidants and free radicals which are capable of damaging important cell components like cell membranes, proteins and DNA. In counter, the cell also discharges antioxidant enzyme systems which scavenge free radicals to prevent cellular damage and maintain cell homeostasis.[1,2,3]

Liver is an important metabolic organ that executes many functions essential for survival. It detoxifies dangerous substances which come from both external and internal sources.

Most of the pollutants pass through the body and will have to come across the liver for detoxification process.

The Chambal River at Nagda, Ujjain and (M.P. India) is severely affected due to both municipal and many industrial activities. Both media and public from different residential areas around the industrial zone raised their voice against various health hazards due to aquatic pollution. Therefore, we aimed to study the pollution induced oxidative stress (OS) in locally available cat fish, *Mystus tengara*. The activity of various antioxidant enzymes and the contents of thiobarbituric acid reactive substances TBARS were studied in the livers of *Mystus tengara* from the upstream and polluted downstream of Chambal River at Nagda, Ujjain (M.P. India).

2. MATERIALS AND METHODS

2.1 Study area: Study Area

The Chambal River at Nagda (Ujjain district of Madhya Pradesh, India) is close to the tropic of cancer at 23‟27 N and 75‟25 and 517 meters above MSL. The river gets municipal sewage and effluent water from various industrial units.

2.2 Sampling

The surface water samples from upstream and downstream were collected in winter (December 2015) in glass bottles at about 10 cm below the surface. All collected water samples were refrigerated at 4 ºC, transported to the laboratory and various parameters like pH, COD, BOD and DO, EC, TDS, TSS and hardness were analyzed according to APHA.[4] Surface water sampling was carried out in two different segments of the River during winter months. Samples were collected at 30cm below the water level using a water sampler and acid washed container to avoid unpredicted changes. The samples were immediately transported to the laboratory under low temperature conditions in ice-boxes and stored in the laboratory at 4°C until analysis. Data was analyzed by using computer soft ware http://www.xlstat.com.[5] and have been presented as mean values ± standard error (S.E.).

2.3 Fish

The cat fish *Mystus tengara* of more or less of same size and body weight (irrespective of the sex) were randomly collected from both the study stations in December 2015. After morphometry, the livers of the fish were carefully removed and weighed. The liver was carefully taken out and processed for the study of oxidative stress (OS) markers. It was homogenized in 4 volumes of homogenizing buffer (50 mM Tris - HCl mixed with 1.15% KCl and pH adjusted to 7.4), using Teflon Homogenizer (Remi,India). The resulting homogenate was centrifuged at 10,000g for 20 min in a Remi (India) centrifuge at 0–4°C. The supernatant was transferred and stored −20°C until analysis.

2.4 Non Enzymatic assays

Glutathione (GSH) is a frequently studied scavenger of hydrogen peroxide and mainly present in liver. It prevents cell damage caused by reactive oxygen species (ROS).The hepatic ratio of oxidized to reduced glutathione (GSSG/GSH), a value used as an indicator of the “redox status” of the cell, may be appropriate biomarker for oxidative stress.

Total glutathione content (tGSx) and oxidised glutathione (GSSG) were determined with a sensitive assay based on the recycling reaction of GSH with 5, 5’-dithiobis (2-nitrobenzoic acid) (DTNB) in the presence of an excess of glutathione reductase (GR) according to Baker et al.[6] (1990). Measurements were made in a microplate reader as previously described.[7] Reduced and oxidised glutathione ratios were calculated as follows:

GSH/GSSG = (tGSx – GSSG)/(GSSG/2).

Glutathione-protein mixed disulfides (GSSP) in liver was measured according to the method of Rossi et al.[8] (1995) and were expressed as nmol of GSx per mg of protein.

2.5 Malondialdehyde (MDA) assay

MDA were measured by Thiobarbituric Acid Reactive Substances (TBARS) method.[9] The unknown MDA containing samples and standards are first reacted with TBA at 95°C. After a brief incubation, the samples and standards can be read spectrophotometrically at 540nm. The MDA content in unknown samples can be determined by comparison with the predetermined MDA standard curve.

2.6 Enzymes assays

Various antioxidant enzymes were examined spectrophotometrically by the kit provided by OxiSelect™ (BIOGENUIX MEDSYSTEMS PVT. LTD**.** New Delhi). The activity of superoxide dismutase (SOD) at 550 nm, (one unit of SOD activity is the amount of protein required to give 50% inhibition of epinephrine auto oxidation).[10] Catalase (CAT)[11] at 240 nm and GST was measured at 340 nm. Total glutathione assay and MDA were measured by Thiobarbituric Acid Reactive Substances (TBARS) method.[9] All values were expressed as mean ± standard error. The significance of difference between experimental and control data was statistically analyzed using student (T) test.

3. RESULTS

3.1 Water quality parameters

Results of surface water quality parameters of Chambal River at Nagda are summarized in Table.1. Results indicate that almost all water parameters studied were higher than the approved limit by the Central Pollution Control Board (CPCB, 1995).[12]

Table: 1 Physicochemical parameters of water samples collected from Chambal River at Nagda (M.P).

Parameter	Station I	Station II
Colour	Turbid	Grayish
Odor	No specific odour	No specific odour
BOD, mg/L	10.0±0.01	51.2±4.8
COD mg/L	12.0± 1.4	32.0±1,8
DO mg/L	7.5±0.1	5.9±**0.07
EC,umho/cm	85±2.3	419±13.2**
pH	7.1±0.09	8.8±0.9

TDS mg/L	110.10±5.3	392.1±3.2
Temperature ‘C	22.4±0.33	22.8±0.67
Total hardness	200±9.67	1380±34.3
TSS mg/L	26.1±2.2	140.2±8.76
Ca (mg/L)	28±5.3	52±5.3
Mg (mg/L)	84±5.3	218±5.3
Cl (mg/L)	70±5.3	138±5.3
Sulphate (mg/L)	68±5.3	120±5.3

On the whole, the water quality of downstream of Chambal River at Nagda was comparatively poor and not suitable for domestic uses. The level of contamination at downstream of the river is mainly due to indiscriminate discharges of municipal and industrial wastes.

3.2 Oxidative stress indicators: Mean activities of (MDA) content, glutathione and antioxidant enzymes are shown in Tables 2.

a) ***MDA content***

A significant increase in lipid peroxide (MDA) content (P>0.01) was observed in liver of fish inhabiting in polluted site. The levels of this MDA were significantly increased by 64% and 2.3-fold in liver of fish at downstream. The level of malondialdehyde (MDA) in the liver tissue of the fish from downstream was found to be 1.62 ± 0.02(nano moles / mg protein / minute).

b) Antioxidant enzyme activities

Compared with the control, the activities of Superoxide dismutase (SOD) and GST (2.015 ± 0.003 to 3.285 ± 0.003) in liver of fish from downstream were significantly elevated (P ≤ 0.01) and the increase was 2.2-fold in comparison to the reference site. The superoxide dismutase activities (SOD) in liver were in the range of 4.2713 ± 0.004 in the fish at reference site while it was 6.134 ± 0.004 in the liver of fish at downstream. There was no significant difference in the activity of CAT (4.32± 0.4 and 4.43±0.4) but decreased level of glutathione peroxidase (GPx) (526.83 ± 0.003 and 434.94 ±5.28) was noticed in liver of fish at polluted site. The activity of glutathione-S-transferase (GST) in liver was slightly higher in the fish of polluted site.

c) ***Reduced glutathione (GSH) and GSH to GSSH ratio***

The GSH level was assessed in liver homogenate of M. tengara inhabiting from upstream and downstream in Chambal River at Nagda, M.P. (India). A significant decrease in the level of GSH (p <0.001) was observed in liver homogenate of M. tengara inhabiting at Juna Nagda (downstream) when compared with reference site. The intensity of pollution at downstream of Chambal River at Nagda, provoked significant increase (98%) in the reduced (GSH) and oxidized (GSSG) glutathione content, along with a significant reduction in [GSH]/[GSSG] ratio (29%).

Table.2. Oxidative stress parameters in liver of Mystus tengara at two different sites of Chambal River at Nagda (MP).India.

Parameter	Units	Upstream	Downstream
Superoxide dismutase (SOD)	µmg/ protein/ minute	6.46±0.97	29.19±0.41
Catalase (CAT)	Nano moles/mg protein / minute	4.32±0.41	4.43±.0.4
Glutathione S-Transferase (GST)	(µ mg /mg protein / minute	452.14±0.43	367.11±0.34
Glutathione Peroxidase(GPx)	µmg / mg protein / minute	526.83±3.32	434.94±5.28
MDA content	nano moles / mg protein / minute	1.09±0.11	1.62±0.09
Reduced Glutathione (GSH)	nano moles / gram wet weight of tissue	1.79±0.23	3.32±0.21*
GSH/GSSG	µmol g tissue-1	0.17 ± 0.01	0.26 ± 0.02*
GSSG	µmol g tissue-1	8.33 ± 0.55	6.52 ± 0.58

4. DISCUSSION

Field investigations are helpful for assessing the possible effects of pollutants on aquatic biota. Aquatic sources are used as an ultimate sink of both industrial and municipal effluents which contain a mixture of xenobiotics including heavy metals.[13,14,15,16] The interaction of these pollutants can result in both protective and lethal synergistic effects.[17] For that reason, physico-chemical analysis of aquatic systems has long been engaged to evaluate the quality of water.[14,15] Heavy metals, pesticides and other pollutants in aquatic system have been shown to cause oxidative stress in fish.[13] The free radicals produced due to oxidative stress (OS) are potentially toxic but they are typically inactivated or scavenged by antioxidants before they can impose damage to cellular components and maintain cellular homeostasis[13,18,19,20] Earlier reports reveal that, fish upon exposure to pollutants bring out the production of reactive oxygen species (ROS).[21,22,23] As the ROS levels increase, the biological system develops a first line defense mechanism by adjusting the activities of antioxidants such as catalase (CAT), superoxide dismutase (SOD), and glutathione related enzymes.

4.1 Lipid peroxidation (LPO)

Lipid peroxidation is measured usually as a level of thiobarbituric acid reactive substances (TBARS) and has been used most often to analyse the effect of pollutants.[16,24,25] Malondialdehyde (MDA) is one of the most identified secondary products of lipid peroxidation. Elevated level of MDA due to increase in ROS, frequently used as a marker of oxidative stress and the antioxidant status.[26] It is usually measured as a level of thiobarbituric acid reactive substances (TBARS). In the present study, the soluble thiobarbituric acid reactive substances (TBARS) level was significantly increased in the liver of Mystus tengara at Downstream of Chambal River. Similar results were reported in H.fossils.[27] Our results are also in agreement with the earlier reports of many workers.[28,29,30,31,32,33] The results of the present study point out that, the mixture of various xenobiotics present in downstream of the River stimulated an increase in the lipid peroxidation and antioxidant enzymatic activities.

4.2 Reduced glutathione (GSH) and GSH to GSSH ratio: Glutathione is a tripeptide and exists in reduced (GSH) and oxidized (GSSG) states. With higher levels of oxidative stress, intracellular GSSG will accumulate, and the hepatic GSH/GSSG ratio will decrease and that can be used as an appropriate biomarker of oxidative stress.[34] Elevated level of GSH and reduced hepatic GSH/GSSG ratio in Mystus tengara at downstream confirms that River is highly polluted at this site. Previous reports also confirm that high level of GSH was indicated in fish, *Pleuronectes vetulus*[35], *Wallago attu*[35], *C. gariepinus*[36], and killifish (*F. heteroclitus*)[37] from contaminated site compared to the reference site.The decrease in the ratio of hepatic GSH/GSSG in fish may be due to either direct scavenging of radicals or increased peroxidase activity. The increase of GSH level can hold benefit to fish in its capability to stay alive in contaminated and unclean environment, while reduction in glutathione is usually associated to enhancing of peroxidation processes in the cell membrane and leads to stress and can prominently contribute in hepatotoxicity.[38]

4.3 Antioxidant enzyme activities: Results of various enzyme activities are summarized in Table.2. The liver is the most frequently targeted organ in terms of xenobiotics induced toxicity. It is the most active organ in antioxidant defence, playing also an important role in redox metabolism.[39] The SOD–CAT system provides the first line defense against reactive oxygen species (ROS). Typically a synchronized stimulation response in the activities of SOD and CAT is observed when exposed to pollutants.[40,41]

In the present study hepatic SOD, GST and GPx activities were elevated in Mystus tengara from downstream while there was no significant difference was found in second main antioxidant enzyme catalase (CAT).[39] In fish, *C. carpio*, brown trout (*S. trutta*) and *Acipenser ruthenus* collected in two different sites of polluted area showed higher SOD and GST and reduced catalase and also GPx activities.[42,3] The changes in antioxidant enzyme levels may be due to reaction and toxic impact of the pollutants in the aquatic environment. However, the antioxidant defense status varies from tissue to tissue depending on habitat, age, sex, thermoregulatory capacity, metabolic rate and nutritional status of the organism.[43] The severity of oxidative stress or injury depends on duration and concentration of the active toxic compound(s) present in polluted site and also depends on the ability of the organism to recover.

CONCLUSIONS

The fish, Mystus *tengara* were subject of activities of hepatic superoxide dismutase (SOD) and catalase (CAT) and marker to prooxidant effects of various pollutants present in the Chambal River at Nagda. The present data in our paper show an increase of superoxide dismutase activity and decrease of level of catalase enzyme in the liver of Mystus tengara at polluted site.

The overall hepatotoxic effect of pollutants at Chambal River is probably related to a production of free radicals (ROS), which alters the antioxidant status and membrane stability. Water chemistry analysis at two sites indicated that the downstream at Juna Nagda is highly polluted with higher biochemical oxygen demand (BOD), chemical oxygen demand (COD), pH and low dissolved oxygen (DO) when compared to the upstream (Methwasa). Strong industrial activity coupled with intensive use of chemicals in agricultural practices and discharges of untreated municipal effluent directly into the River are the major sources of the pollution. The study therefore provides a balanced use of biomarkers of oxidative stress in biomonitoring of aquatic pollution.

On the whole, the results of physico-chemical characterization and oxidative stress parameters when compared between the two sites we observed induction of oxidative stress in the fish collected from polluted downstream (Juna Nagda). The sampling site is the source of municipal, industrial and dyeing effluent. This could be a reason for higher pH, BOD and COD levels in this site.[14,15] Our results demonstrate that it is needed much more investigation on fish populations to resolve the issue of pollution impact in Chambal River at Nagda.

This outcome of the present investigation suggests that pollutants found in the River bed has a strong effect on the oxidative metabolism of fish, which was reflected in the activating of antioxidant defence system for survival.

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