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Short Communication

Oxidative stress and antioxidant enzymes in tissues of freshwater fishes as biomarkers of aquatic pollution

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Abstract

Our present study deals with the evaluation of oxidative stress due to pollutants which results in disastrous effect in aquatic ecosystems. Lipid hydroperoxide, lipid per oxidation (LPO), and the antioxidant enzyme levels were estimated. The lipid peroxide and lipid hydro peroxide levels were found to be increased in the organs - liver and kidney of Rohu (Labeo rohita) and Tilapia (Oreochromis niloticus) from the polluted site. CAT and SOD activity seems to be reduced in organ samples of both the fishes. Changes in the antioxidant enzymes, lipid per oxide and lipid hydro peroxide levels that was observed indicates the presence of pollutants in the Varahanadhi River.

Keywords: Rohu, Tilapia, SOD, CAT, Lipid peroxidation.

Introduction

Pollution of water is a major environmental problem in the present scenario. Pollutants are introduced into the environment by anthropogenic sources[1] mostly. Aquatic fishes are vulnerable to contaminants of the water and pollutants enter the organs and disrupt the normal physiological and biological processes. Increase in industrialization, and lack of regulations to protect the environment might be the reasons for pollutant accumulation in aquatic system. Aquatic ecosystem receives a number of toxic substances among which heavy metals are of much importance because of their toxicity and bioaccumulation potential. Fishes are delicate to pollution and may be used as bio monitors for ecological assessment of marine environment. Fish population is an integral part of an ecosystem component, but used as food sources and are best bio indicators of pollution. Toxicants interrupts the integrity of biochemical mechanism with the alterations in antioxidants. Oxidative damage occurs due to alterations in pro-oxidants and antioxidants levels. For cellular homeostasis[2] the antioxidant levels and their scavenging actions are of particular importance. Fish are the best marine organisms known for the accumulation of toxicants and are sensitive even to lesser amounts of toxic pollutants.

Materials and methods

Varahanadhi River is located in parts of Villupuram District in Tamil Nadu, India. It lays 12.04^0N latitude and 79.34^0E longitude. It covers a total area of 798 ha and covers within the survey of India, with a total catchment area of about 21Km. Water drains into the Varahanadhi River from areas of Villupuram, Thiruvannamalai, Kancheepuram and Cuddalore districts of TamilNadu and Pondicherry and finally confluences with the ocean Bay of Bengal. This study was carried out over a one year period with the water samples obtained from different sites. Site A (upstream) which is situated in Gingee town and has its source at the hills of Melmalayanur in the South Arcot District of Tamil Nadu, site B (midstream) is the branch of the river that flows through Villupuram District and site C (downstream) part of the river called Sankaraparani that drains into Bay of Bengal. All estimations were done using standard chemicals. (Merck, India).

Freshwater fishes such as Rohu (*Labeo rohita),* and Tilapia (*Oreochromis niloticus*) were collected from the Varahanadhi River located in Villupuram district. Five fishes of each species were collected from both the polluted (downstream site) and the non-polluted site (upstream site and midstream site), without least disturbance were transported in polythene bags filled with water. Fishes that were maintained in the laboratory were termed as control. Different fishes were put in each bag filled with water and were well aerated using pressurized air from a cylinder. This mode of transit has proved successful, since there was no mortality in all consignments throughout the course of this study. Collected fish samples were taken to the laboratory immediately, weighed and length measured. Fishes were forfeited by medullar transection[3] and dissection was done. The organs removed were liver and kidney which was processed for the research. Fishes are profound to anthropogenic pollution and used as finest biomonitors for the evaluation of pollution in marine environment. Toxicants enter the food chains of freshwater ecosystems and becomes incorporated in human tissues and organs, resulting in damage and mortality.

Vital organs such as liver and kidney are involved in the detoxification of xenobiotics[4]. Aquatic ecosystems are drastically affected by pollutants which can be confirmed by biochemical studies using enzymes and oxidative stress condition in the tissues of the fish that responds to the contamination[5].

Tissue homogenate preparation: Organs such as liver and kidney used for the present study were blotted and weighed. The tissues were homogenized using Tris-HCl buffer, in a tissue homogenizer and the homogenate was centrifuged at -4^{o}C and supernatant was decanted and stored at -20^{o}C for future use.

Assay of lipid per oxidation: Nichans and Samuelson[6] method was used to determine lipid peroxide level. Isolated tissues were homogenized with Tris–HCl buffer. Added measured volume of TBA-TCA-HCl reagent to tissue homogenate and boiled in a water bath for 15 minutes and cooled. The pink colour developed was measured at 535nm in a spectrophotometer. The results were presented as nmoles/mg protein.

Assay of Hydro peroxide: By using the method of Mair and Hall[7] lipid hydro peroxide was estimated.5ml of chloroform: methanol mixture was added to 1ml of sample homogenate and centrifuged at 1000g. Lower chloroform layer was separated, 3ml of which is taken and dried at 45^{o}C by placing on a water bath. 1ml of acetic acid: chloroform mixture and 0.05ml of potassium iodide was added, stoppered, mixed and placed at room temperature in dark for 5 minutes and 3ml of cadmium acetate was added, mixed and centrifuged at 1000g. The upper phase of the reaction mixture was measured at 353nm. Results were presented as nmoles/mg protein.

Estimation of SOD: It was assayed by the method of Misra and Fridovich[8].

To 0.05ml of tissue homogenate, 1.5ml of carbonate – bicarbonate buffer containing EDTA was added. 0.4ml of 3mM epinephrine was used to initiate the reaction and change in absorbance per minute was recorded at 480nm. One unit of SOD activity is the amount of protein required to give 50% inhibition of epinephrine auto oxidation.

Estimation of CAT: Sinha[9] method was used to determine the activity of catalase. 1.0ml of phosphate buffer was added to 0.1ml of enzyme followed by the addition of 0.4ml of hydrogen peroxide. 2ml of dichromate - acetic acid reagent was added to stop the reaction after the duration of 30 and 60 seconds. Then it was boiled for 10 minutes in a water bath, cooled and read at 620nm. The values were represented as micromoles H_2O_2 consumed /min/mg protein.

Total Protein: It was measured by the standard procedure of Lowry *et al*[10].

Results and discussion

Contamination in the river causes adverse effects on aquatic ecosystem[11] which might be due to heavy industrialization and dense population along the coastal areas[12]. Excessive free radicals are formed during stress which is a threat to cellular homeostasis of organisms. Stress in the environment causes an imbalance in the antioxidant systems[13] that results in oxidative stress. To assess the marine environment fish are the simplest organisms and thus indicates pollution in the environment[14]. Normally reactive oxygen species are produced at reduced concentrations which increases in biological systems during oxidative stress[15]. Fish are more prone to the toxicity induced by ROS that causes the macromolecules like DNA, lipids and proteins to undergo oxidation due to three reasons: i. Increased formation of oxidants, ii. decrease in antioxidant levels, and iii. failure of oxidative damage repair systems[16]. Superoxide (O_2), a highly reactive free radical molecule, and superoxide dismutase (SOD) converts it into oxygen and water and catalase (CAT) converts H_2O_2 into oxygen and water. Therefore, alteration in activities of SOD and CAT is a nominal method of determining oxidative stress and other biomarkers could be the possible tools in marine toxicological research. Therefore, observation of the alteration in activities of SOD and CAT shall be an effective method of assessing oxidative stress and other biomarkers could be the possible tools in marine toxic research. Cell membrane destruction and damage was triggered by lipid peroxides and hydro peroxides. Heavy metal pollution leads to the generation of free radicals through a chain of reactions.

Table-1: Estimation of LPO and Lipid Hydro peroxide content in fish tissue samples**.**

Experiment	Organs	Experimental fish	Control	Upstream	Midstream	Downstream
LPO (nmol MDA/mg protein)	Liver	Rohu	03.13±0.03	03.12±0.03	03.14±0.03	06.25±0.03*
		Tilapia	03.69±0.02	03.70±0.02	03.73±0.03	05.84±0.03*
	Kidney	Rohu	01.67±0.02	01.66±0.04	01.74±0.03	03.85±0.03*
		Tilapia	01.75±0.03	01.81±0.03	01.84±0.04	03.93±0.03*
Lipid Hydro peroxide (nmol/mg protein)	Liver	Rohu	09.84±0.03	10.04±0.04	10.13±0.03	18.45±0.03*
		Tilapia	07.13±0.03	07.24±0.03	07.75±0.03	16.35±0.03*
	Kidney	Rohu	10.32±0.03	11.15±0.03	12.15± 0.04	19.44±0.03*
		Tilapia	9.38±0.04	10.85±0.04	10.65± 0.03	18.17 ±0.03*

Values were expressed as Mean ± SD, *p* value: *p<0.05 control vs different sample sites.

Table-2: Estimation of CAT and SOD content in fish tissue samples**.**

Experiment	Organs	Experimental fish	Control	Upstream	Midstream	Downstream
SOD (Units/mg protein)	Liver	Rohu	20.14±0.03	19.18±0.03	19.38±0.04	9.25±0.03*
		Tilapia	16.36±0.04	15.17±0.04	14.88±0.06	3.06±0.04*
	Kidney	Rohu	21.36±0.03	20.26±0.04	18.16±0.04	10.30±0.02*
		Tilapia	15.26±0.04	14.36±0.03	13.88±0.04	2.06±0.03*
CAT (µmoles O_2/min/mg protein)	Liver	Rohu	37.76±0.08	35.81±0.04	35.14±0.44	25.26±0.03*
		Tilapia	27.3±0.03	26.79±0.04	25.91±0.04	17.37±0.04*
	Kidney	Rohu	40.12±0.05	39.85±0.04	42.2±0.03	21.04±0.03*
		Tilapia	28.21±0.06	27.32±0.03	25.58±0.06	15.21±0.04*

Values were expressed as Mean ± SD, *p* value: **p*<0.05 control vs different sample sites.

Estimation of lipid per oxidation is of great importance in environmental risk assessment[17]. Free radicals that contain PUFA causes peroxidation of lipids and oxidative stress condition is characterized by increased lipid peroxide formation[18]. Many studies showed diverse results in the organs of aquatic organisms exposed to contaminants. Increased oxidation of lipids in membranes results in elevated concentration of MDA that indicates oxidative stress and therefore used as a marker of oxidative damage[19]. Elevated MDA content indicates poor quality of water and environment[20]. Reactive oxygen species attacks the lipids present in cell membranes and their oxidation results in oxidative stress and variation in antioxidant enzymes as an adaptation mechanism to stress induced by pollutants in organisms[21]. Various environmental pollutants increases the ROS formation within the cells which in turn resulted in the physiological alterations induced by ROS, in fishes[2]that was given by the earlier studies. Antioxidant enzyme induction represents a defense strategy to thwart the free radicals to determine the magnitude of pollution present in natural water resources[21]. Sayeed et al[22] observed elevated lipid peroxides in experimental animals after cadmium intoxication. Reports have shown increased formation of oxygen free radicals during oxidative damage.

Catalase (CAT) is a ubiquitous enzyme present in cells of aerobic organism. It degrades hydrogen peroxide into molecular oxygen and two molecules of water. Catalase, a scavenger of hydrogen peroxide, converts it into water and oxygen to protect the cells. Catalase activity in liver and kidney tissues under study showed significant decrease when compared with control fishes. In particular, samples from the downstream region expressed significant decrease in catalase activity. Similar results were reported in fishes subjected to deltamethrin[23].

A significant reduction in the activity of SOD was observed in the organs studied. The formation of oxy radicals was suppressed by the action of SOD[21,24]. Since SOD stands first in protection to oxygen toxicity, because of its suppressive function on oxyradical formation. Variations in antioxidant enzyme activities upon exposure to pollutants were reinforced by the results of Jasmin Lena et al[25,26] who analyzed the water sample collected from Varahanadhi River and found the downstream site of the river to be polluted with the increased levels of hardness, alkalinity, turbidity, TDS, EC, BOD and COD. This was further confirmed by the hematological studies done by Aiwan et al[27], Bhatkar[28] and Jasmin Lena et al[29] in freshwater fishes collected from the polluted river site and reported alterations with decreased RBC Count, Hb, MCH, MCHC and increased WBC Count due to pollution by Arsenic, Chromium, Lead and Cadmium. Decreased SOD activity indicates the deficiency of antioxidant enzymes upon metal exposure. Barata et al[30] observed alterations in the measure of SOD and CAT in the freshwater cladoceron *Daphnia magna* upon exposure to pollutants depending upon the concentration of metal and reported alterations in the oxidative stress condition because of their ability to produce ROS.

Conclusion

Our present study concludes that fishes such as Rohu and Tilapia being main inhabitants of Varahanadhi River are affected due to contamination by pollutants. Research provides evidence of enzymatic biomarkers being sensitive indicators of aquatic pollution. If measures are taken against pollution the earth's natural resources can be protected. This study provides fundamental basis in environmental research and marine organisms. Future research should be focused to study the genes that are expressed in response to pollutants in aquatic organisms.

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