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Evaluation of antioxidant defence system during xenobiotic induced oxidative stress in freshwater fish *Oreochromis mossambicus*

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Abstract
The point of the present study was to assess the impact of xenobiotics on the cell reinforcement status in the liver tissue of freshwater fish *Oreochromis mossambicus*. Amphibian biological system including freshwater are being contaminated with an expansive number of poisons from diverse sources including metropolitan squanders, mechanical effluents, sewage, garbage's, farming squanders, local squanders, and so forth and these toxin rundown might contain tireless chemicals, including bug sprays and pesticides, overwhelming metals, and so forth are being brought through natural way of life cycle, in this manner the focus is expanding at every connection indicate representing a risk people, when expended the hydrobioants from dirtied environs. With the coming of a wide assortment of toxins into the amphibian environ, worldwide fisheries is confronting a steady decrease in their individual stocks. In the present investigation oxidative enzymes, antioxidant enzymes, enzymes involved in scavenging activity and end products of glycolysis including pyruvate and lactate were assayed in the liver tissue of freshwater fish *Oreochromis mossambicus* from the river bed of Pennar from Nellore district of Andhra Pradesh in regions receiving different levels and loads of pollutants. The xenobiotics present in the Aquatic environ showing the hepatotoxic nature and the status of antioxidant nature in the freshwater fish was monitored. The overall hepatotoxic effect of xenobiotics is probably related to a generation of free radicals, which alters the antioxidant status and membrane stability. Therefore, pollution of the aquatic environment by certain heavy metals, pesticides would adversely affect the biology of economically important organisms, including edible species like fishes, prawns, crabs etc. Since *O. mossambicus* significantly exhibits several biochemical stress responses to pollutants this fish species can be taken as a biological indicator of pollution index of Aquatic environment. These results obtained in the present investigation suggests that xenobiotics found in the River bed has a profound effect on the oxidative metabolism of fish, which results in the triggering of compensatory metabolic pathways for sustainability.

Keywords: *O. mossambicus*; Antioxidants; Xenobiotics; Oxidative enzymes

1. Introduction
Present day intensive farming for food and heavy industrialization for production of goods to meet the demands of the need of growing population has led to problem of pollution. Aquatic organisms inhabiting polluted water ways tend to accumulate toxic chemicals on high concentrations even when the ambient environmental concentrations are low. Environmental pollutants are becoming toxicants due to their adverse effects on living beings. The point of the present study was to assess the impact of xenobiotics on the cell reinforcement status in the liver tissue of freshwater fish *Oreochromis mossambicus*. Amphibian biological system including freshwater are being contaminated with an expansive number of poisons from diverse sources including metropolitan squanders, mechanical effluents, sewage, garbage's, farming squanders, local squanders, and so forth and these toxin rundown might contain tireless chemicals, including bug sprays and pesticides, overwhelming metals, and so forth are being brought through natural way of life cycle, in this manner the focus is expanding at every connection indicate representing a risk people, when expended the hydrobioants from dirtied environs. With the coming of a wide assortment of toxins into the amphibian environ, worldwide fisheries is confronting a steady decrease in their individual stocks. Biological mechanisms that can be studied in organisms include those pertaining to the activity of enzymes involved in oxidative metabolism and hydrolysis. Since enzymes are biological

catalysts that enable the most essential metabolic functions to be performed in living cells, disturbances of enzyme functions are most harmful. The enzyme activity levels in aquatic organisms may serve as early indicators of toxicity of pesticides, heavy metals and other pollutants [3, 4, 17, 24, 37, 40]. Fishes being one of the most ancient forms of aquatic life as a food item have been reported to have a nutritional advantage of being able to provide high proportions of their dry weight as proteins of relatively good quality due to the presence of essential amino acids and also being easily digestible unlike those of other livestock. Pollution of aquatic environment from industrial, domestic and agricultural waste has exposed these important aquatic organisms to contaminants which not only endanger their lives but also eventually enter the food chain leading to serious public health hazards. Fishes feed extensively on different varieties of food and so it is important to control the concentrations of likely pollutants in the aquatic habit, so as to reduce their minimal ingestion by fishes there by rendering them unfit for human consumption. Over the last two decades, exploration of modern technology and associated development of chemical industries have resulted in the production and release of vast quantities of manmade chemicals into the environment in liquid, solid and gaseous forms. The Pennar river which flows through Kadapa and Nellore districts of Andhra Pradesh, India receiving effluents from different sources affect the aquatic system by depleting and enhancing different physico- chemical parameters there by affecting the inhabitants various organic and inorganic wastes in industrial and domestic effluents are responsible for polluting the river base. Non– degradable heavy metals are regarded as hazardous to aquatic ecosystem of their environmental persistence and their tendency for bioaccumulation [4, 11, 41]. In the case of vertebrates, the liver tissue is considered to main organ for metabolism and therefore studies on the production of antioxidants against oxidative damage can be conducted by pre-treating the animals with antioxidants then subjecting them to oxidative stress induced by oxidants or toxic substances. The impact of pollutants on aquatic organisms is highly dependent on the biochemical nature of the organisms. The interaction between the pollutants and the organisms can be understood properly. Fresh water fishes are sensitive to contamination of water and pollutants may significantly damage certain physiological and biochemical process when they enter into the organs of these animals. Alteration in the biochemical parameters in fishes due to environmental pollution provides an indication to understand the mode of action and type of pollutants. Lactate dehydrogenase, Succinic dehydrogenase and Glutamate dehydrogenase enzymes are several metabolic functions with great physiological significance. Glutamate dehydrogenase, a mitochondrial enzyme, catalysis the oxidative deamination of glutamate providing α-Ketoglutarate to the Krebs cycle. This enzyme is having several metabolic functions with great physiological significance. GDH in extra-hepatic tissue could be utilized for channelling of ammonia released during proteolysis for its detoxification into urea in the liver. Hence, the activities of GDH are considered as sensitive indicators of stress. Oxy-radical are capable of inducing oxidative tissue damage, lipid peroxidation, nucleic acid damage, Enzyme inactivation and protein degradation. In order to prevent damage to cellular components, numerous enzymatic antioxidant defences act to scavenge oxy-radicals including Superoxide dismutase, Catalase and glutathione s-transferase. So the investigation of enzymatic properties is an essential mission to study the physiological adaptation expressed by organisms in response to different environmental conditions. The pollution of aquatic environment, such as shift in acidity, alkalinity, ionic composition, organic solvents affects the enzyme activity, growth and survival of several aquatic biota. With this back drop, the present investigation is aimed to probe into the aspects of antioxidant mechanisms of freshwater fish *Oreochromis mossambicus* collected from different regions of freshwater river Pennar in the Nellore district with differential levels of pollution.

2. Materials and methods

Three Experimental stations were selected for the procurement of fishes *Oreochromis mossambicus.* Experimental Station-I is located near the dam site where the pollution rate is almost nil. Experimental Station–II is relatively less polluted region at the bank of the Pennar River. The Experimental Station–III is more polluted region and it is receiving the untreated drainage of municipal and domestic sewage from the Nellore city (Latitude 14^0 26' N and Longitude 79^0 58' E). Agrarian squanders additionally go into this zone through waste trenches from the almost farming terrains furthermore aquaculture effluents bringing about generally exceedingly dirtied test station. Physico-chemical parameters including pH, Dissolved Oxygen, Salinity, Alkalinity, Biological oxygen demand, Chemical oxygen demand, Total dissolved Salts, Calcium, Nitrate, Phosphorus, Ammonia, Temperature, were found to be relatively higher in Experimental Station-I i.e zone of no pollution. Fishes of uniform size 125±5 g were separated and are used for experimentation. Oxygen consumption rate (BMR) and tissue respiration were measured. The selected fishes were ice packed and brought to the laboratory and liver tissue was excised and subsequently used for biochemical analysis. The liver tissue was excised immediately and washed with distilled isotonic saline. The liver tissue was homogenized in desired solvents according to the procedures selected for enzyme assay. The liver tissue homogenates were prepared in ice cold 0.1 M Tris – HCl Buffer, pH 7.2 were used for the determination of Lipid Peroxides (LPO), reduced Glutathione (GST), Glutathione-dependent antioxidant enzymes (GPx and GST) and anti Peroxidative enzymes (CAT and SOD) and other oxidant enzymes.

2.1. Biochemical assays

Oxidative enzymes like Isocitrate dehrogenase (NAD-ICDH, EC No: 1.1.1.41) [26], Lactate dehydrogenase (LDH, EC No: 1.1.1.27) [31], Succinate dehydrogenase (SDH EC No: 1.3.5.1) [31], Malate dehydrogenase (MDH EC No: 1.1.1.40) [31], Glutamate dehydrogenase (GDH, EC No: 1.4.1.2) [27, 44], Cytochrome-c-oxidase (EC No: 1.9.3.1) [3], were assayed in liver tissue. Tissue Lipid peroxidation level was determined as TBA- reactive substances by the method described by Ohkawa *et al* [33]. GSH was determined by the method of Ellman [15]. Glutathione peroxidase (GPx, EC No: 1.11.1.9) activity was measured by the method of Paglia and Valentine [34]. Glutathione-s-transferase (GST, EC No: 2.5.1.18) activity was determined by the method of Habig *et al* [19]. Catalase (CAT, EC No: 1.11.1.6) activity was assayed according to the method of Takahara *et al* [45]. Superoxide dismutase (SOD, EC No: 1.15.1.1) activity was determined according to the method of Misra and Fridovich [29]. Acid Phosphatase (ACP, EC No: 3.1.3.2) and Alkaline Phosphatase (ALP, EC No: 3.1.3.1) were determined the following the modified methods of Bodansky and Butterworth [6, 7]. Glutamate oxaloacetate transferase (GOT, EC No: 2.6.1.1) and Glutamate pyruvate transferase

(GPT, EC No: 2.6.1.2) were assayed by following the method of Reitman and Frankel [36]. The protein content was determined by Lowry *et al* [28]. using Bovine serum Albumen as standard.

2.2. Statistical analysis
Statistical analysis were performed using one-way analysis of variance (ANOVA) followed by Duncan's multiple Range test using statistical package SPSS 17. Differences were considered to be significant at $P \leq 0.05$ against control group.

3. Results and Discussion
Selected enzyme assays of liver tissue of fish *Oreochromis mossambicus* were estimated and presented in Table.1. The fish samples were collected from three different Experimental stations – I, II & III (ES-I, II & III) adjacent to Pennar river near Nellore city of Andhra Pradesh, India. The oxygen utilization at creature level and tissue level in fish were observed to be altogether diminished in fish gathered from ES-II and ES-III contrasted with fish gathered from ES-I. The basal metabolic rate (BMR) and tissue oxygen utilization was lessened in fish furthermore reliant on degree of contamination level. Fish collected from ES-II are ES-III are showing relatively low levels of oxygen consumption compared to ES-I.

Table 1: Selected Oxidative and Antioxidant Enzyme assay values in liver tissue of Fish *Oreochromis mossambicus* collected from three different Experimental Stations – I, II & III.

Parameter	ES - I	ES - II	ES - III
Basal Metabolic Rate (BMR)[a]	3.18±0.19 PDC	2.39±0.13 (-24.84)	2.12±0.12 (-33.33)
Tissue Respiration [b]	945.13±29.15 PDC	703.12±23.26 (-25.61)	589.49±15.17 (-37.63)
Isocitrate dehydrogenase (NAD-ICDH)[c]	0.654±0.045 PDC	0.486±0.031 (-25.69)	0.401±0.025 (-38.69)
Malate dehydrogenase (MDH)[c]	0.855±0.064 PDC	0.672±0.055 (-21.40)	0.591±0.032 (-30.88)
Succinate dehydrogenase (SDH)[c]	1.889±0.086 PDC	1.174±0.074 (-37.85)	0.818±0.036 (-56.69)
Glutamate dehydrogenase (GDH)[d]	2.195±0.133 PDC	1.913±0.121 (-12.85)	1.217±0.085 (-44.56)
Lactate dehydrogenase (LDH)[c]	0.875±0.078 PDC	1.348±0.133 (+37.85)	0.494±0.054 (-43.54)
Cytochrome –c-oxidase[e]	82.43±3.46 PDC	63.38±2.43 (-23.11)	49.39±2.04 (-40.02)
Pyruvate[f]	10.04±0.73 PDC	6.18±0.38 (-38.45)	5.25±0.32 (-47.71)
Lactate[g]	0.48±0.05 PDC	0.59±0.07 (+22.92)	0.68±0.07 (+41.67)
Superoxide dismutase (SOD)[h]	60.78±0.97 PDC	69.95±0.95 (+15.09)	28.73±0.42 (-52.73)
Catalase (CAT)[i]	84.32±2.45 PDC	73.43±2.13 (-12.92)	65.43±2.08 (-22.40)
Glutathione S-Transferase (GST)[j]	3.144±0.143 PDC	5.454±0.385 (+73.47)	7.385±0.424 (+134.89)
Acid Phosphatase (ACP)[k]	5.145±0.345 PDC	6.844±0.301 (+33.02)	8.958±0.388 (+74.11)
Alkaline Phosphatase (ALP)[k]	7.743±0.379 PDC	10.945±0.376 (+41.35)	12.313±0.379 (+59.02)
Glutamate Oxaloacetate Transferase (GOT)[l]	0.454±0.024 PDC	0.648±0.132 (+42.73)	0.703±0.129 (+54.85)
Glutamate Pyruvate Transferase (GPT)[l]	0.705±0.035 PDC	0.975±0.101 (+38.30)	1.138 ±0.109 (+61.42)
Glutathione Peroxidase (GPx)[m]	6.83±0.32 PDC	4.94±0.28 (-27. 67)	4.05±0.22 (-40.70)
Lipid peroxidation (LPO)[n]	1.09±0.03 PDC	1.32±0.09 (+21.10)	1.48±0.12 (+35.77)
Reduced Glutathione (GSH)[o]	4.88±0.23 PDC	4.12±0.21 (-15.57)	3.25±0.22 (-33.40)

ES-I: Experimental Station – I
ES-II: Experimental Station – II
ES-III: Experimental Station – III
All values are Mean ± SD of six individual observations
All values are Statistically Significant at P< 0.05
PDC: Percent Deviation over Control

a : ml of oxygen consumed / gram weight of animal / hr
b : µ moles of oxygen consumed / gram wet weight of tissue / hr
c : µ moles of formazan formed / mg protein / minute
d : µ moles of NAD^+ reduced / mg protein / minute
e : µ moles of diformazan formed / mg protein / minute
f : µ moles / gram wet weight of tissue
g : mgs / gram weight of tissue
h : one unit of SOD activity is the amount of protein required to give 50% inhibition of epinephrine autooxidation
i : nano moles of H_2O_2 decomposed / mg protein / minute
j : µ moles 1-chloro 2,4-dinitrobenzene conjugate formed / mg protein / minute
k : µ moles of Pi liberated / mg protein / minute
l : µ moles of pyruvate formed / mg protein / minute
m : nano moles GSH oxidised / mg protein / minute
n : nano moles malondialdehyde released / mg protein / minute
o : nano moles / gram wet weight of tissue

The Lactic dehydrogenase (LDH) activity levels in the liver tissue of fish collected showed differential pattern i.e LDH values obtained for fish collected from ES-II found to be relatively higher as compared to fish collected from ES-I and the values were found to relatively lower as compared to fish collected from ES-I. The results obtained for LDH clearly demonstrate that the disruption of oxidative metabolism at cellular level which in turn compare various biochemical process. Due to the decreased oxygen consumption and its availability at very low levels manifests the production of lactate from pyruvate. Similarly oxidative enzymes assayed including Succinate dehydrogenase (SDH), Malate dehydrogenase (MDH), Isocitrate dehydrogenase (NAD-ICDH), Cytochrome-c-oxidase activity levels in liver tissue of fish collected from ES-I, II & III showed different levels. All the Oxidative enzyme activity values recorded were significantly lower in ES- II & III collected fish compared to fish collected from ES-I. The SDH, MDH, NAD-ICDH, the key enzymes of Krebs cycle representative of oxidative metabolism showed a dose dependent pollutant inhibited activity. The ES-II, representing relatively less polluted environ recorded medium level of oxidative enzyme activity, and fish samples collected from ES-III, a relatively highly polluted environ, recorded very low level of oxidative activity levels, clearly demonstrate the reduction of oxidative metabolic status during pollutant induced stress. Krebs cycle enzymes NAD-ICDH, SDH and MDH exhibited a considerable determinant, indicative of possible impairment in mitochondrial oxidation during xenobiotic induced toxicity. Since the oxygen utilization lessens, the mitochondrial oxidation ought to constantly decrease and this circumstance ought to prompt general decrement in the mitochondrial

oxidoreductase movement; subsequently all the mitochondrial limited compound exercises likewise reduce. A similar kind of inhibition mitochondrial enzymes by pesticides has been reported [42, 43]. Several investigations linked the decreased activities of Krebs cycle enzymes to the changes in the integrity of mitochondrial membranes as a consequence of pesticide toxicity [8]. Decrease in oxidative enzyme activity as a consequence of xenobiotic induced stress indicates the impaired mitochondrial oxidation of pyruvate. In addition to the above, the NAD-LDH activity was decreased, suggesting lesser formation of pyruvate from lactate, consequently lesser mobilization into citric acid cycle. So the recorded decrement in the activity levels of NAD-ICDH, SDH and MDH in xenobiotic exposed fish tissue clearly indicates the depression of oxidative metabolism at the mitochondrial level leading to an overall depression of the TCA cycle during xenobiotic-induced stress conditions. It is quite possible that the decrease in activity levels of NAD-ICDH, SDH and MDH might be due to the conditions similar to "asphyxia" because the rate of tissue respiration also decreased during xenobiotic exposure. Since SDH plays a vital role in the oxidative metabolism of the cell, any change in its activity is likely to disturb the harmony and coordination of other cellular metabolic processes of the organism. Cytochrome-c-oxidase, which represent the electron transport system, an oxygen – dependent process, was inhibited all the xenobiotic exposed fish tissue. The cytochrome-c-oxidase activity which forms an index of mitochondrial oxidative phosphorylation and ATP production was considerably inhibited. The inhibition of cytochrome-c-oxidase results in respiratory distress which in turn causes reduction in oxidative metabolism. A similar kind of inhibitor pattern of cytochrome-c- oxidase was observed in various animals during pesticide stress [42, 43]. The Glutamate dehydrogenase (GDH) activity levels in liver tissue of fish collected from three different sites ES-I, II & III and are found to be relatively low in fish collected from ES-I and ES-III, when compared to fish collected from ES-I. Glutamate dehydrogenase is also known to play crucial role in ammonia metabolism and is also known to be affected by a variety of effectors [37, 38]. A few metabolic capacities with extraordinary physiological noteworthiness and known not nearly connected with the detoxification components in tissues when presented to distinctive sorts of xenobiotics including pesticides and substantial metals. GDH in extra-hepatic tissues could be utilized for channelling of ammonia released during proteolysis for its detoxification into urea in the liver. Enhancement on GDH activity in the tissue provided ketoglutarate and reduced nucleotides, which may fulfil the energy requirements during toxicity manifestations [5, 12, 13, 22].

The antioxidant enzyme Superoxide dismutase (SOD) activity levels were found to be slightly increased in fish collected from ES-II and significantly reduced in fish collected from ES-III compared to fish collected from ES-I. SOD activity levels in tissues of fishes play an important role in the immune mechanisms. The antioxidant SOD converts this microbiocidal metabolite superoxide anion into oxygen and hydrogen peroxide that passes freely through membranes. SOD activity is further correlated closely with immune stimulation, disease and healthy status of the aquatic organisms including fishes and crustaceans [16, 21, 23, 25]. Thus antioxidant enzymes could also be used as a biomarker for the detection of relative oxygen species in fishes including *Oreochromis mossambicus*. The Catalase activity (CAT) levels in the liver tissue of fishes collected from different sites i.e ES-I, II & III showed differential levels. The CAT activity levels recorded to be maximum in fishes collected from ES-I, compared to fish collected from ES-II and ES-III. The enhanced activity of CAT levels suggest that CAT is efficiently involved in the successful removal of H_2O_2 that was generated by SOD. Hence the elevated trend of CAT was similar to SOD pattern and these observations gains support from earlier reports with fishes and crustaceans exposed to varieties exposed to varieties of xenobiotics. The Glutathione s-transferase (GST) activity levels in liver tissue of fish collected from ES-II and III are more compared to fish collected from ES-I. The activity levels of GST found to be significantly elevated in the fish in liver tissue appears to be maximum with relatively more polluted environ compared to less polluted environs. GST is a family of intracellular multifunctional dimeric protein, plays a major role in the intracellular transport of endogenous compounds, metabolizes various electrophilic xenobiotics, ligand transport and thus protects cell against toxic effects of xenobiotics [18, 39, 46, 47]. In aquatic organisms it is an important component of the detoxification system. GST activity was detected in different tissues of fishes including gills and liver tissue after exposure to a wide variety of xenobiotics [35, 38]. It has a wide substrate specificity. Glutathione (GSH, L-Ɣ-glutamyl-cysteinal-glycin) is a substrate in the GSH-s-transferase framework and the accessibility of GSH can be central point in the digestion system of xenobiotic by this enzymatic framework. It is capable of chelating and detoxifying metals as soon as they enter the cell. It also forms a substrate for GSH peroxidase, an enzyme capable of both remaining hydrogen peroxide (H_2O_2) from the cells and repairing per-oxidatively damaged membranes [5, 9, 14, 38].

Significant alterations in the enzyme contents in the liver tissue was observed in fish exposed to various pollutants as compared to fishes inhabiting unpolluted areas or environs. It indicates environmental stress on biological system [5, 22, 38]. Xenobiotics led to induce severe physiological and biochemical disturbances in experimental animals. ACP is a lysosomal enzyme. Several workers have revealed that toxicant induced alterations release results in more production and release of ACP. The results obtained in the present investigation also gains support from the earlier reports. One of the reasons of increased enzyme activity suggests proliferation of smooth endoplasmic reticulum. Being hydrolytic enzyme taking part in dissolution of dead cells it is stress indicator on biological system. Rise in ALP is also response to hepatotoxicity in the present investigation. Increase in ALP level has been reported to be indicator of damage of cells including liver, kidney, intestine and bone resulting in the liberation of this enzyme in the blood system in many organisms. Elevated levels of ACP and ALP in liver of fish under pollutant stress clearly demonstrate the hepatotoxic nature of the xenobiotic distributed in the aquatic environment. Transaminase are the group of enzymes that elevate activity of enzyme phosphorylase which plays an important role in glycogenolysis and also are major link between protein and carbohydrate metabolism.

Lipid peroxidation *in vivo* has been identified as one of the basic deteriorative reaction in cellular mechanism of xenobiotic induced oxidative stress in freshwater fishes. n the present examination on presentation to contaminated water instigated an expansion in the level of lipid peroxidation in the tissue of fishes gathered from distinctive dirtied environs. This indicates that high vulnerability to peroxidative damage during xenobiotic induced toxicity, is probably due to a decline in the

level of free radicals for scavengers. Antioxidants are necessary for preventing the formation of free radicals and they inhibit some of the deleterious actions of reactive oxygen species that damage lipids, DNA and proteins [20]. Our results also confirmed the same pattern and showed that xenobiotic exposed fishes might be less resistant and more susceptible to Lipid Peroxidation. Three different mechanisms are able to induce Lipid Peroxidation: Autoxidation (by free radicle reaction), Photo-oxidation and enzyme action. Autoxidation is a radical-chain process involving 3 sequences, initiation, propagation and termination. The general process of lipid peroxidation includes initiation, propagation and termination. Initiation occurs when oxygen is partly reduced by Fe^{+2} to species able abstract a hydrogen atom from a methylene carbon.

Resulting alkyl radical reacts rapidly with oxygen to form a peroxy redical (LOO^-), which itself can liberate LOOH via hydrogen abstraction from a neighbouring allyl bond. In this reaction new alkyl radicals produced which propagate lipid peroxidation.

$nFe^{+2}+O_2 \rightarrow n\ Fe^{+3}+\text{Reduced } O_2$ (1)
$I+LH \rightarrow IH+L$ Initiation
$L+O \rightarrow LOO^-$
$LOO^- +LH \rightarrow LOOH+ L$ Propagation

Fe^{+2} can substantially enhance lipid peroxidation by decomposing LOOH to highly reactive lipid alkoxy radicals (LO) that behave as organic initiators and branch lipid peroxidation.

$Fe^{+2} + LOOH \rightarrow Fe^{+3} + OH^- + LO$
$LO +LH \rightarrow LOH + L$ Chain branching

Excess Fe^{+2}can also complete, as electron donors for LOO^- and LO inhibiting both the proportion and chain branching reactions and causing the Fe^{+2} dependent termination of lipid peroxidation.

$Fe^{+2}+LOO^-/LO \rightarrow Fe^{+3}+$ LOOH / LOH Termination

The results of the present study demonstrated that Xenobiotic present in the aquatic environment might have stimulated lipid peroxidation by influencing a variety of these reactions i.e. xenobiotics might have enhanced the initiation process not only by producing OH^- but also by activating the Fe^{+2} autoxidation. The action of xenobiotics may alter other molecules of biological relevance in cellular and sub-cellular membranes. The xenobiotics present in the aquatic environment might have activated the Fenton- like reaction that causes the formation of the alkoxy radical's initiator of lipid peroxidation. It elevates the amount of Fe^{+2} oxidized probably by acting with a site specific mechanism similar to that described for other OH^- producers stimulating the Fenton reaction. The alteration of redox recycling of iron, affects the Fe^{+2} / Fe^{+3} ratio in the reaction mixture. Both of the above mentioned phenomena may account for the activations exerted by the xenobiotic on the peroxidation of cell membranes. It is already reported that xenobiotic alters the Ca^{+2} binding sites on membrane acidic phospholipids, in particular of the Phosphatidyl serine and Phosphatidylinositol classes. Glutathione is one of the abundant tri peptide non-enzymatic biological antioxidants present in the liver [2]. It acts as a substrate for H_2O_2 removing enzyme glutathione peroxidase and for dehydroascorbate reductase [1]. It also plays a critical role in cellular function, which includes the maintenance of membrane protein. The removal of free oxygen radicals such as peroxyl radical, superoxide radical, alkoxy radical, translocation of amino acids across cell membranes, the detoxification of foreign compounds and biotransformation of drugs [10, 30]. The GSH activity was found to be decline in fishes exposed to xenobiotic stress and the tissue antioxidant might be operating at a diminished level during xenobiotic induced stress condition. Reduction in GSH levels during xenobiotic induced stress was either due to increased degradation or decreased synthesis of glutathione. The GSH dependent antioxidant enzymes, GPx and GST activity were found to decline significantly in the liver tissue of fishes exposed to xenobiotic stress, reflecting an increased oxidative stress due to xenobiotic presence in the body of fishes. GPx alters the protection to the cellular and subcellular membranes from the Peroxidative damage by eliminating H_2O_2 and Lipid Peroxide. GST binds to many different lipophilic drugs; so it would be expected to bind xenobiotic molecules and act as an enzyme for GSH conjugation reactions. Inhibition of these enzymes may lead to the accumulation of these oxidants and makes liver cell membranes more susceptible to oxidative damage. GSH and GSH-dependent enzyme systems may be directly related to the pathogenic mechanisms of xenobiotic induced fishes.

The present investigation may be presumed that presentation of freshwater fish *Oreochromis mossambicus* to xenobiotics contained Aquatic environment seriously influences different physiological and biochemical systems and this is reflected in adjustments of different biochemical constituents and a few protein measure exercises. The raised levels of GST proposes that these was an actuation of an instrument to detoxify xenobiotics. However, the toxic effects of xenobiotics were not fully neutralized and there was an evidence of protein denaturation, disturbances of cellular metabolic activities and impairment on neural transmission. The overall hepatotoxic effect of xenobiotics is probably related to a generation of free radicals, which alters the antioxidant status and membrane stability. Therefore, pollution of the aquatic environment by certain heavy metals, pesticides etc. would adversely affect the biology of economically important organisms, including edible species like fishes, prawns, crabs etc. Since *O. mossambicus* significantly exhibits several biochemical stress responses to pollutants this fish species can be taken as a biological indicator of pollution index of Aquatic environment. These results obtained in the present investigation suggests that xenobiotics found in the River bed has a profound effect on the oxidative metabolism of fish, which results in the triggering of compensatory metabolic pathways for sustainability.

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