Research Paper

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Oxidative Stress of Bisphenol- A and its Adverse Effect on the Liver of Fresh Water Fish, *Oreochromis Mossambicus*

Fisheries

KEYWORDS : Bisphenol A, Lipid peroxidation, Alkaline phosphatase, Liver

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ABSTRACT

Bisphenol A at sub lethal concentration (1 mg/ L) was given to ten adult freshwater fish, Oreochromis mossambicus for 10 and 20 days maintaining the controls. Exposure to bisphenol A significantly decreased the activities of superoxide dismutase, catalase and glutathione reductase with increased level of lipid peroxidation in time-dependent manner when compared to control groups thereby indicating that bisphenol A induces oxidative stress in liver of Oreochromis. The activity of alkaline phosphatase was significantly decreased in liver of treated fish due to the decreased state of inter and intracellular membrane transport owing to the toxicity of bisphenol A. It also resulted in hepatic lesions including degeneration, necrosis, irregular position of nuclei and vacuolization of cell cytoplasm. The present findings suggest that bisphenol A exerts its toxicity effect in liver of fish by the generation of oxygen free radicals thus inducing lipid peroxidation and modifying the antioxidant status in time-dependent manner.

Introduction

Environmental pollution represents a major problem in both developed and developing countries. Human have long been releasing large amount of various chemical compounds into the environment, and various animals have been exposed to these chemical compounds. Among these chemicals some have reported as not lethal when exposed at low doses, but it has now been suggested by many researchers that these compounds at sub lethal concentrations also produce several toxicity to animals. Such environmental toxicants are called endocrine disrupting chemicals (EDC) or xenoestrogens. One among the EDCs is bisphenol A, which exhibit toxic activity relatively at low concentration.

There has been an increasing awareness that the aquatic pollution and other anthropogenic impacts on water resources may have the potential to damage natural fish stocks. Aquatic contamination by industrial and domestic wastes is nowadays, unfortunately, a great concern in public health, since the direct or indirect consumption of contaminated water can cause serious damages to the organisms.

Fishes are more sensitive to many toxicants and are a convenient test subject for indication of ecosystem health. O. mossambicus is a very important commonly available fish species commercialized in India. It is also a species generally found in fresh water and acknowledged to respond rapidly to environmental alterations. Hence in the present study a commonly available fish Oreochromis mossambicus was selected and subjected to biochemical and histological assessment of liver tissue. The selection of liver cells as appropriate targets is due to their cytological sensitivity as biomarkers of organic contaminants and/ or environmental pollution (Dutta et al. 1993). Ultra structural alterations of fish hepatocytes have repeatedly been used as monitor systems to study sub-lethal effects of organic contaminants with great success. It is the first organ to be exposed by the portal circulation to toxicants ingested by the body. Brusle & Anadon (1996) stated that fish liver histology could serve as a model for studying the interactions between environmental factors and hepatic structures and functions. Alterations in the liver may be useful as markers that indicate prior exposure to environmental stressors or xenobiotics.

Bisphenol A (BPA) is a large volume chemical that is mainly used for synthesis of epoxy resins and other polymers, and also as a stabilizing additive in various plastic products. The environmental occurrence of BPA is of possible concern because the substance has estrogenic properties, and may affect biota as well as humans. There are several evidences reporting the toxicity effect of bisphenol A on freshwater fishes, but limited information is available on the effect of this xenoestrogen on the antioxidant system of Oreochromis mossambicus. Based on the above context the present work has been carried out to study the effect of bisphenol A on the reactive oxygen species generation in the liver of the fresh water fish.

Materials and methods

Collection and maintenance of animal

Fresh water fish, Oreochromis mossambicus weighing 12 ± 2 g and length 7.5 ± 1 cm were collected from a fish farm, Kaloos Aquarium, Kotakkal, Malappuram District, Kerala, India. Fishes were acclimatized to the laboratory conditions for four weeks with constant supply of water and good lighting system. They were maintained in well-aerated tubs (40 L capacity), which was dechlorinated and sustained with fresh water flow and waste water discharge. Bath was changed every 24 h, which was dechlorinated, respectively.

Preliminary tests and LC_{50}

The physico-chemical features of the tap water were estimated as per APHA, 1998. Water temperature in the test ranged from 28 ± 2°C during the experiment, oxygen saturation of water ranged between 70 and 100 %, pH is 6.5 to 7.5 which were monitored using a standardized procedures. The LC_{50} values in the respective time intervals were determined by probit analysis, with a confident limit of 5 % level (Finney et al. 1971). Preliminary tests were conducted to provide guidance on range of concentration of bisphenol A to use in the bioassay. The lethal concentration for 50 % killing (LC_{50}) values was computed on the basis of probit analysis (Finney et al. 1971) for 96 h, which was 10 mg/ L. One-tenth of the dosage (1 mg/ L) of bisphenol A was chosen in the present study.

Treatments

Single dose with double durations were used in present study. Ten fish specimens were used for every test and also in control groups. The first group of fishes was maintained in toxicant-free water and was used as control and the second and third group was treated with bisphenol A at 1 mg/ L for 10 and 20 days, respectively. Biochemical estimation of liver was performed at the end of 10 and 20 days and the histology of liver was done at the end of 20 days of bisphenol A treatment by maintaining the control group.

Biochemical analysis

Fish at the end of every treatment was caught very gently using a small dip net, one at a time with least disturbance, weighed and the liver of both control and treated groups were dissected and stored at 4ºC until the analyses were performed. A 1% (w/ v) homogenate of liver was prepared in ice-cold normal saline with the help of a motor-driven glass Teflon homogenizer on crushed ice for a minute. The homogenate was centrifuged at

8000 g for 15 min at 4°C to obtain the supernatant, which was then used for the analyses.

Protein was estimated by the method of Lowry et al. 1951 with BSA as the standard. Activity of superoxide dismutase (Marklund & Marklund, 1974), catalase (Claiborne, 1985), glutathione reductase (Carlberg & Mannervik, 1985), alkaline phosphatase (Bessey et al. 1946) and level of lipid peroxidation (Ohkawa et al. 1979) was measured.

Histology of liver tissues

Liver tissue was collected by sacrificing the fish and it was fixed in 10 % buffered formalin. After processing tissue was impregnated with wax then the blocks were cut in a rotary microtome to prepare sections of thickness 4 to 6 microns. The sections were stained with haematoxylin and eosin and mounted in DPx. The structural alteration was observed under light microscope and was compared with those of control tissues. Photomicrographs were taken using Cannon shot camera fitted to the Carl Zeiss Axioscope 2 Plus Trinocular Research Microscope.

Statistical analyses

Statistical analyses were performed using one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range test using statistical package SPSS 17.0. Differences were considered to be significant at $p<0.05$ against control group. Data are presented as mean ± SD for ten animals per group. All biochemical estimations were carried out in duplicate.

Results and Discussion

In India, several thousands of man-made chemicals have been released into the environment in vast quantities since the chemical industry began to bloom around 1950s. This has brought many, often unforeseeable, problems to the environment. Only very recently evidence reported about the threat of environmental estrogens in the ecosystem. Food packaging has been reported to be contaminated with bisphenol A and it is also widely used in the production of plastics and epoxy resins that come into contact with a wide variety of foodstuffs. Once it is discharged into the environment they do not always remain close to where they are released, but instead may be transported over long distances in air currents or by water. So it is readily leached into the aquatic ecosystem and aquatic animals are most exposed. In the present investigation the sub-lethal toxicity of bisphenol A were evaluated in the fresh water fish, Oreochromis mossambicus, which is a widely farmed freshwater food fish in India.

Administration of bisphenol A showed a significant reduction in the body weight after 20 days of treatment whereas no changes were observed after 10 days (Table 1). Bisphenol A decreased hepato-somatic index of all treated groups in dose-dependent manner (Table 1). This could be due to the result of atrophy or necrosis of hepatocytes (Busacker et al. 1990) and it was confirmed by the monitorization of histological changes, as necrosis or atrophy of hepatocytes in bisphenol A-treated fish (Fig. 1B), which is considered as a highly sensitive and accurate way to assess the effects of xenobiotic compounds in experimental studies.

Table 1 Effect of bisphenol A on the body weight, hepato-somatic index and biochemical parameters in liver of freshwater fish, Oreochromis mossambicus

Parameters	Control	Bisphenol A (1 mg/ L)	
		10 days	20 days
Body weight (g)	12.54 ± 0.22	12.53 ± 0.86	8.05 ± 0.81*
Hepato-somatic index (HSI) (g)	1.732 ± 0.05	1.631 ± 0.047*	0.808 ± 0.1*
Activity of superoxide dismutase [a]	21.794 ± 2.15	14.164 ± 0.88*	12.155 ± 1.084*
Activity of catalase [b]	223.86 ± 10.7	184.57 ± 10.24*	141.45 ± 7.59*
Activity of glutathione reductase [c]	2.26 ± 0.241	0.757 ± 0.11*	0.605 ± 0.08*
Level of lipid peroxidation [d]	165.77 ± 3.64	191.38 ± 3.03*	221.35 ± 11.21*
Activity of alkaline phosphatase [e]	17.59 ± 0.87	12.38 ± 0.57*	8.45 ± 0.51*

Data are expressed as mean ± SD for ten-animals/ group
Asterisks (*) denotes p values set significant at 0.05 against the control groups

[a] nmol pyrogallol oxidized/ min/ mg protein
[b] µmol H_2O_2 consumed/ min/ mg protein
[c] nmol NADPH oxidized/ min/ mg protein
[d] µmol malondialdehyde produced/ min/ mg protein
[e] µmol p-nitrophenol liberated/ 30 min/ mg protein

Environmental pollutants are generally known to cause an increase in peroxidative processes within cells, causing oxidative stress (Cheung et al. 2001). Aquatic organisms have developed a comprehensive antioxidant defence system, comprising both molecular and enzymatic defences, against the dangers of oxygen radicals, thereby preventing excess oxidation and damage. A disturbance in the balance between the pro-oxidants and antioxidants leading to detrimental biochemical and physiological effects is known as oxidative stress. This is a harmful condition in which increases in free radical production, and/or decreases in antioxidant levels can lead to potential damage. Indicator of oxidative stress include changes in antioxidant enzyme activity, damaged DNA bases, protein oxidation products, and lipid peroxidation products and finally leads to several human diseases (Thannickal & Fanburg, 2000).

In the present study a significant ($p<0.05$) decrease in the activity of superoxide dismutase (Table 1) was observed that has been shown to increase the level of superoxide anion, which is turn inactivate catalase activity (Kono & Fridovich, 1982). The elevated superoxide radicals by themselves or after their transformation to $H_2O_{2\ could\ have}$ caused an oxidation of the cysteine in the enzyme and decreased the activity of superoxide dismutase. Thus in the present study the activity of catalase as well as glutathione reductase was significantly decreased (Table 1). Similarly, catalase or glutathione has been shown to eliminate hydrogen peroxide from the cell leading to the inactivation of superoxide dismutase and therefore, led to the generation of lipid peroxides (Bray et al. 1974). In the present study the level of lipid peroxidation was increased significantly ($p<0.05$) at 10 and 20 days of bisphenol A treatment when compared to control group (Table 1). Increased lipid peroxidation may indicate an increased oxygen free radical generation (Thiele et al. 1995). Malondialdehyde is a major oxidation product of peroxidized polyunsaturated fatty acids and increased malondialdehyde content is an important indicator of lipid peroxidation.

On the other hand, the present study also showed a significant

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decrease in the marker enzyme, alkaline phosphatase in the liver after 10 and 20 days of bisphenol A exposure. Alkaline phosphatase serves as diagnostic tool to assess the toxicity stress of chemicals in the living organisms. Alkaline phosphatase is a hydrolytic lysosomal enzyme and is released by the lysosomes for the hydrolysis of foreign material. Subsequently the enzyme activity may begin to drop either as a result of having partly or fully encountered the toxin or as a result of cell damage. Alkaline phosphatase is also involved in the mediation of membrane transport and transphosphorylation. A decreased alkaline phosphatase activity in liver of bisphenol A-treated fish indicate the decreased state of inter and intracellular membrane transport (Asifa et al. 2014) and possibly this could be due to the toxicity of bisphenol A.

The teleost liver is one of the most sensitive organs with regard to showing alterations in histoarchitecture, biochemistry, and physiology following exposure to various types of environmental pollutants. In the present study, the control liver exhibited a normal architecture and there were no pathological abnormalities, where hepatocytes present homogenous cytoplasm, and a large central or subcentral spherical nucleus (Figure 1A). Tilapias exposed to bisphenol A showed hepatocellular necrosis (Figure 1B). Treatment of bisphenol A decreased the number of nucleus in hepatic tissues (Figure 1C). Additionally, hepatic parenchyma of fish exposed to bisphenol A after 20 days showed an increase in cytoplasmic vacuolization (Figure 1D).

Fig. 1 A Photomicrograph showing histology of liver of the control fish (X 40 magnification)

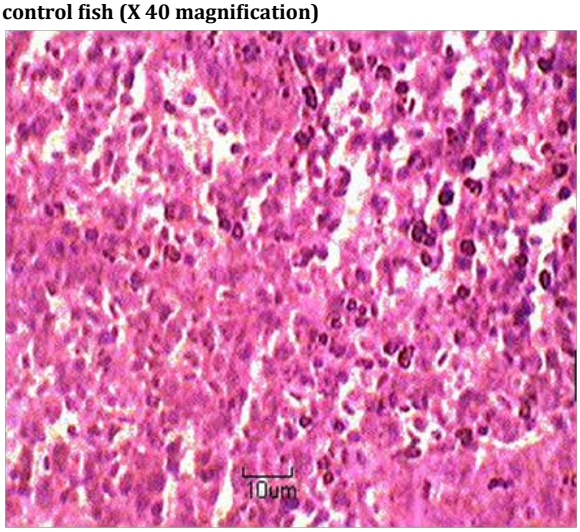
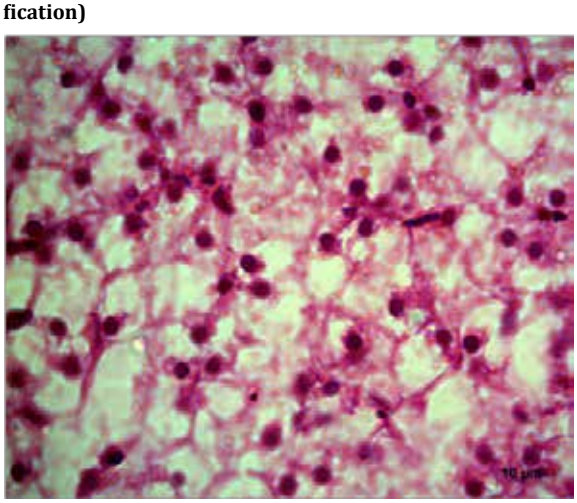


Fig. 1 B Photomicrograph of liver histology in bisphenol A-treated fish showing necrosis in hepatocytes (X 40 magnification)



sFig. 1 C Photomicrograph showing liver histology of bisphenol A-treated fish showing enucleated liver cells (X 40 magnification)

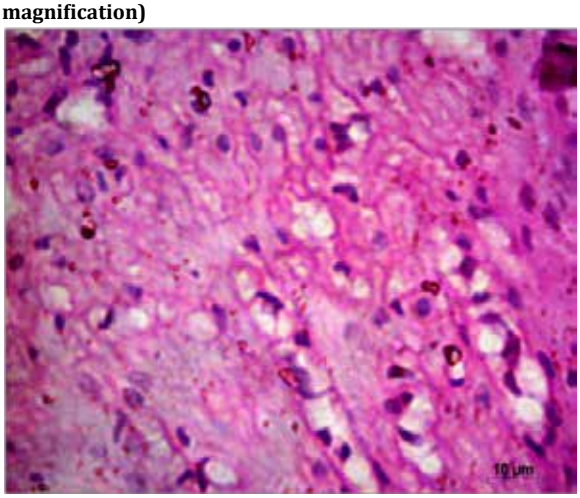
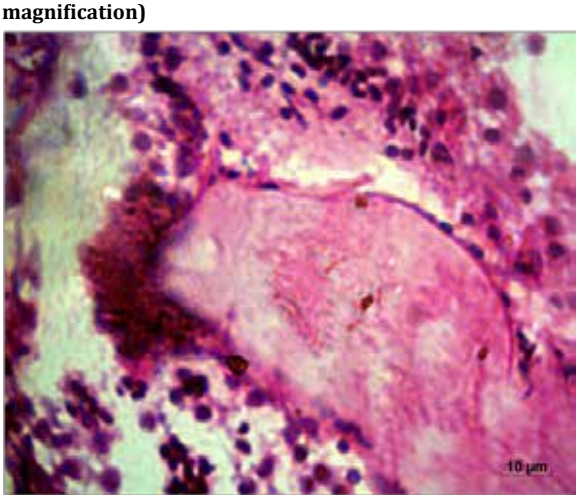


Fig. 1 D Photomicrograph showing liver histology of bisphenol A-treated fish showing cytoplasmic vacuolization (X 40 magnification)



The widespread vacuolization might be likely due to accumulation of lipids and glycogen in hepatocytes as a result of aquatic pollution. Deposition of glycogen in the hepatocytes has been found in stressed animals, because the glycogen acts as a reserve of glucose to supply the higher energetic demand occurring in such situations (Hinton & Laurén, 1990). Anomalies such as irregular shaped hepatocytes, cytoplasmic vacuolization and nucleus in a lateral position, close to the cell membrane, were also described in Oreochromis mossambicus contaminated by malathion (Chitra & Abdu, 2013).

Conclusion

It is evident from the present study that the generation of oxygen free radicals induced lipid peroxidation and this could be the possible mechanism for the sub lethal toxicity of bisphenol A and it was proved by histological alterations in the liver of the fresh water fish, Oreochromis mossambicus.

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