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Acute exposure to bisphenol-A altered muscular antioxidant system in cichlid fish, *Etroplus maculatus* (Bloch, 1795)

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ABSTRACT

The toxic effect of bisphenol A on muscular antioxidant system of fish has not been reported till date. Thus the present study was aimed to understand the effect of bisphenol A on the induction of oxidative stress and its action on alkaline phosphatase activity in muscle of cichlid fish, Etroplus maculatus. Animals were grouped and exposed to sub lethal concentration i.e., 648 µg/ L of bisphenol A for 24 h, 72 h and 96 h. Activities of antioxidant enzymes as superoxide dismutase, catalase and glutathione reductase showed a significant reduction only at 96 h whereas no significant changes were observed at 24 h and 72 h. The level of hydrogen peroxide and lipid peroxidation increased significantly at 72 h and 96 h of exposure when compared to control groups. One of biomarker enzymes, alkaline phosphatase decreased in all treatment groups than that of control groups. In conclusion, bisphenol A induced oxidative stress in muscle thereby altered muscular antioxidant system in cichlid fish, Etroplus maculatus.

KEYWORDS : Bisphenol A, Antioxidant enzymes, ROS, Alkaline phosphatase, Muscle, Etroplus maculatus

INTRODUCTION

Bisphenol A (BPA) is a man made potent environmental contaminant used in the manufacture of epoxy resins and also in polycarbonate plastic products. It is widely used in several products including baby bottles, drinking glasses, food storage containers, microwave containers, water storage tanks, supply pipes, toys, sunglasses, lenses, the lining of food beverage containers, medical equipments, tubing, consumer electronics and as dental sealants (Brotons *et al.,* 1995). It has been reported that the US Food and Drug Administration banned the use of bisphenol A in baby bottles in mid of the year 2012. Several countries have taken steps to ban bisphenol A, unfortunately in India there are no regulations even to check the human exposure of bisphenol A and no limits set for the allowable intake. According to USFDA Report the current exposure level of bisphenol A in infants are at 0.2-0.4µg/ Kg body weight/ day (US FDA, 2012).

Bisphenol A is exposed to humans primarily through canned foods and beverage containers that are lined with epoxy resin coatings, and from polycarbonate plastic products. Consequently, the estimated human consumption of bisphenol A from epoxy-lined food cans alone was 6.6 µg/ person/day (Howe and Borodinsky, 1998). Leaching of bisphenol A also occurs when plastic products are warmed at high temperature than the age of the container to which the food is stored. Therefore, it is an important contaminant due to its ubiquitous presence and humans are highly exposed to bisphenol A through environment and food chain. The concern on the ecological impact of bisphenol A has been increasingly raised in the past few years as more studies reveal its toxic effects on aquatic organisms at environmental relevant concentrations (Kang *et al.,* 2007).

Fishes are primarily considered as a relevant experimental model for toxicological studies because of its fecundity, size, economical maintenance and use as protein rich food to humans (Powers, 1989). *Etroplus maculatus* is an indigenous cichlid fish that are used as laboratory model in the present study because of its great demand for cost-effective and ethically acceptable approach to evaluate the toxicity of many industrial chemicals including bisphenol A. It is widely accepted that aquatic ecosystems serve as the final sink for many chemicals and water provide an ultimate vehicle for exposure to many toxic agents. There are relatively few methods exist to assess health risks of aquatic organisms from exposure to pollutants in the aquatic environment.

Many toxic environmental contaminants entering into the aquatic ecosystem may exert their effects through redox cycling. Oxidative stress, incorporating both antioxidant defense system as well as oxidative damage, is a common phenomenon in organisms exposed to toxicants in their environment. Therefore, in the present study the toxic effect of one of the estrogenic environmental contaminants bisphenol A was studied precisely and practically on the muscular antioxidant system of fish to prove its role in the induction of free radicals in muscle tissues.

MATERIALS AND METHODS

Laboratory model:

Adult cichlid fish, *Etroplus maculatus* weighing 7 ± 1 g and length 6.5 ± 1 cm were collected from a fish farm, Kaloos Aquarium, Kotakkal, Malappuram District, Kerala, India. Fishes were acclimatized for a month in laboratory conditions prior to the experiments with constant supply of water and good lighting system. They were maintained in well-aerated tubs (40 L capacity), which was dechlorinated and bath was changed regularly at every 24 h.

Preliminary screening:

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. Preliminary tests were conducted to select the sub lethal concentration of bisphenol A where the median lethal concentration or LC_{50} value of bisphenol A evaluated by probit analysis from our laboratory was 6.48 mg/ L (Asifa and Chitra, 2015).

Chemicals:

Bisphenol A (4, 4-Isopropylidenediphenol) of 97% purity was obtained from SISCO Research Laboratories Pvt. Ltd., Mumbai, India. Malondialdehyde, NADPH, glutathione oxidized, thiobarbituric acid, pyrogallol, p-nitrophenol were obtained from Himedia Laboratories, Mumbai, India. All other chemicals were of analytical grade and obtained from local commercial sources.

Experimental design:

After acclimatization, fishes in each treatment groups were sustained separately in aquarium tanks covered with monofilament netting to prevent the escape of specimens. In the present study $1/10^{th}$ of median lethal concentration i.e., 648 µg/ L was selected as sub lethal con-

centration of bisphenol A. It was dissolved in 1 % dimethyl sulfoxide (1% DMSO) and was designated as vehicle control. In each experimental group 10 animals were maintained as follows:

Group I – Negative control: Fishes was maintained in toxicant-free water.

Group II – Vehicle control: Fishes was maintained in 1% DMSO.

Group III: Fishes are treated with 648 µg/ L for 24 h.

Group IV: Fishes are treated with 648 µg/ L for 72 h.

Group V: Fishes are treated with 648 µg/ L for 96 h.

The fish was caught very gently using a small dip net, one at a time with least disturbance. At the end of each exposure time, fishes were weighed and muscle was dissected from both control and treated groups and stored at 4ºC until the biochemical analyses were performed.

Preparation of tissue homogenate and biochemical analyses:

A 1% (w/ v) homogenate of muscle tissue 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 bovine serum albumin as the standard. Activities of superoxide dismutase (Marklund and Marklund, 1974), catalase (Claiborne, 1985), glutathione reductase (Carlberg and Mannervik, 1985), level of lipid peroxidation (Ohkawa *et al.,* 1979), hydrogen peroxide generation (Pick and Keisari, 1981), alkaline phosphatase (Bessey et al., 1946) was estimated in crude homogenate of muscle in control and treatment groups.

Statistical analysis:

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

RESULTS

In the present study when fishes exposed to 1% DMSO did not showed any significant differences in all biochemical parameters (Figures 1-6) and therefore, the organic solvent DMSO is used as a vehicle to dissolve bisphenol A. Acute exposure to sub lethal concentration of bisphenol A (648 µg/ L) significantly decreased the activities of antioxidant enzymes as superoxide dismutase (Figure 1), catalase (Figure 2), glutathione reductase (Figure 3) at 96 h treatment group when compared to control groups. No significant changes were observed at 24 h and 72 h of bisphenol A treatment (Figures 1-3).

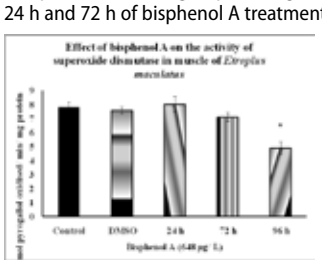

Figure 1

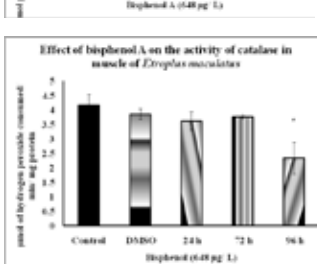

Figure 2

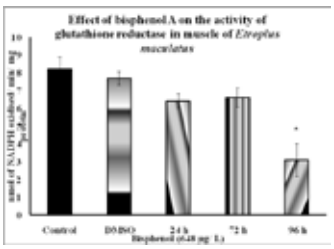

Figure 3

The level of hydrogen peroxide and lipid peroxidation showed a significant increase at 72 h and 96 h bisphenol A-treated groups than that of control animals (Figure 4 and 5).

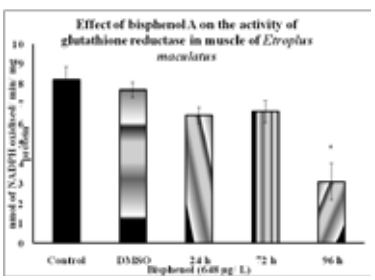

Figure 4

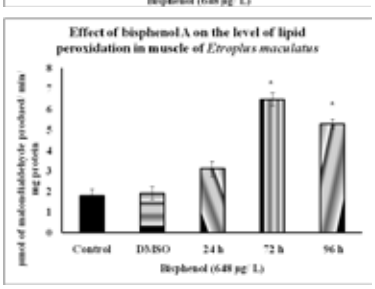

Figure 5

One of the marker enzymes, alkaline phosphatase decreased significantly in time-dependent manner when compared to corresponding control groups (Figure 6).

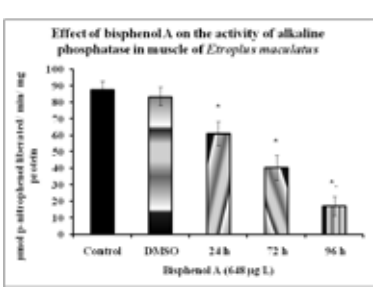

Figure 5

DISCUSSION

The present study focused mainly on the toxic effect of bisphenol A at sub lethal concentration could elevate reactive oxygen species (ROS) generation in muscle tissues. It is well known that ROS are a class of oxygen derived molecules that have detrimental effects on cells or tissues and mediates several pathophysiological processes in muscle tissues such as muscle-specific activity like growth, migration, contraction, apoptosis etc (Clempus and Griendling, 2006). Some of the physiological stress conditions such as exposure to toxicants, nutrient or energy depletion due to anorexia or treatment related intake of food, sheer stress and hypoxia could cause oxidative stress in skeletal muscle (Clanton, 2007). Generation of ROS results in oxidative stress that is commonly associated with changes in antioxidant capacity of the affected tissues and also link to the alteration in the biomarker enzymes.

Free radicals generated as a byproduct due to exposure of environmental contaminant are enormously reactive, but all biological systems have well developed endogenous antioxidant defense mechanisms against the generated free radicals. Antioxidant enzymes such as superoxide dismutase, catalase, glutathione reductase/ peroxidase primarily involved in the elimination of free radicals as superoxide radical, hydroxyl radical and hydrogen peroxide (Jamieson, 1989).

However, due to the toxic effect and the stress induced by environmental contaminant could inactivate the antioxidant enzymes. In the present study exposure to bisphenol A at 96 h significantly decreased the activities of antioxidant enzymes as superoxide dismutase, catalase and glutathione reductase when compared to control groups.

Production of superoxide results from the reduction of one-electron of molecular oxygen (O_2) and, within the cell, is rapidly converted by superoxide dismutases 1 and 2 (SOD1 and 2) into H_2O_2. SOD1 is primarily located in the cytosol and mitochondrial intermembrane space, whereas SOD2 localizes to the mitochondrial matrix (Schieber and Chandel, 2014). In general, superoxide dismutases prevent the accumulation of superoxide radical and it is rapidly eliminated. In the present study the reduction in the activity of superoxide dismutase reveals the failure of the enzyme superoxide dismutase to eliminate superoxide radicals. Accumulation of superoxide radical is therefore associated with oxidative stress in muscle tissues.

H_2O_2 generated from superoxide is produced by mitochondria and NADPH oxidases and it is further reduced to water by the enzymes catalase and glutathione reductase/ peroxidase system (Brand, 2010). Catalase exists as a tetramer composed of four identical monomers, each of which contains a heme group at the active site. The decrease in the activity of catalase and glutathione reductase due to bisphenol A exposure suggest that H_2O_2 generated is not eliminated and it was confirmed in the present study by a significant increase in the level of hydrogen peroxide in the muscle tissue.

H_2O_2 is one of the major ROS in the cell and thus leads to the induction of lipid peroxidation which disrupts the membrane lipid bilayer arrangement that may inactivate membrane-bound receptors and enzymes and increase tissue permeability (Girotti, 1985) as revealed by the increase in malondialdehyde level in the present study. ROS can also cause fragmentation of the peptide chain, alteration of electrical charge of proteins, cross-linking of proteins and oxidation of specific amino acids, and therefore lead to increased susceptibility to proteolysis by degradation by specific proteases (Kelly and Mudway, 2003). Therefore, bisphenol A exposure may also cause disintegration and putrification of protein myofibrils in muscle tissues and further histopathological study is needed to diagnose the effects.

Alkaline phosphatase serves as a stress marker enzyme and a diagnostic tool to assess the toxicity stress of chemicals in the living organisms (Harper, 1991). Alkaline phosphatase is a hydrolytic lysosomal enzyme and is released by the lysosomes for the hydrolysis of foreign material and it is also involved in the mediation of membrane transport and transphosphorylation. In the present study a significant decrease in the activity of alkaline phosphatase at all treatment groups in time-dependent manner indicate the decreased state of inter and intracellular membrane transport. This could be possibly due to the acute toxicity effect of bisphenol A.

CONCLUSION

The present findings conclude that bisphenol A altered muscular antioxidant system in cichlid fish, *Etroplus maculatus*. Acute exposure to bisphenol A also decreased the state of inter and intracellular membrane transport across the muscle tissue as evidenced by the decrease in the activity of alkaline phosphatase.

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