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RESEARCH ARTICLE

EVALUATION OF LC_{50} AND BEHAVIOURAL RESPONSES OF BISPHENOL A IN THE CICHLID FISH, *ETROPLUS MACULATUS*

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ABSTRACT

Cichlid fish, *Etroplus maculatus* was used in the present study in order to evaluate the median lethal concentration of bisphenol A. Seven different concentrations of bisphenol A at 4 mg, 6 mg, 7 mg, 8 mg, 10 mg, 12 mg and 15 mg/ L for 10 animals per group were exposed for 96 h maintaining a control group. Physico-chemical parameters of water as pH, hardness, dissolved oxygen, and temperature was maintained during the treatment period. LC_{50} was then determined by probit analysis and it was found to be 6.48 mg/ L concentration. Body weight of all treated groups remained unchanged throughout the experiments as compared with the control animal. However, mucous deposition was significantly increased in bisphenol A-treated groups at all concentrations. Bisphenol A treatment altered normal architecture of liver and gill as revealed by irregular or enucleated hepatic cells and degenerated gill epithelium above 6 mg/ L concentrations. Bisphenol A showed altered behavioural changes as erratic activity followed by restricted movements, haemorrhagic on entire body surface, reddening of fins and finally loss of equilibrium. The study disclose that 96 h LC_{50} value of bisphenol A is 6.48 mg/ L and the changes in normal behavioural pattern and histopathology of gill and liver demonstrates the acute toxicity of the compound on *Etroplus maculatus*.

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INTRODUCTION

Bisphenol A is a multipurpose compound that has been widely used in almost all modern industries. It was first synthesized in 1891 and now the current production is estimated at about 4 billion kilograms per year globally (Environment Canada Report, 2009). The chemical structure of bisphenol A is similar to the estrogenic compound diethylstilbestrol and its potential therapeutic estrogenic property was first investigated in 1930s. Bisphenol A, an environmental contaminant have been widely used as a monomer in the manufacture of polycarbonate plastics and epoxy resins where it has also been incorporated into a variety of everyday domestic materials. There has been ongoing debate in both scientific and common community about the potential harmful effect of bisphenol A and its bioaccumulation in human. One of the literatures clearly demonstrates that bisphenol A likely bioaccumulates to some degree in humans where it retains in adipose tissue and excrete a little through sweat and urine (Genuis *et al*., 2012). The main mechanism by which humans are exposed to bisphenol A is through leaching from plastic products.

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The common sources of ingestion include foods stored in food cans, which are lined with bisphenol A epoxy resin films to prevent decaying and also from dental composites and sealants (Braunrath *et al*., 2005). Medical equipments as polycarbonate hemodialysis equipment used for dialysis patients and those plastics used in medical devices when exposed to newborns that are regularly spent time in a neonatal intensive care unit had significantly higher serum bisphenol A levels than the general population (Murakami *et al*., 2007; Calafat *et al*., 2009).

In one of the multi-generation breeding studies on medaka fish, *Oryzias latipes* showed that incorporation of bisphenol A into adult fish found rapid excretion of the compound but on the other hand there was no such mechanism to excrete the incorporated bisphenol A from the eggs (Takao *et al*., 2008). One of the studies in our laboratory have proved that bisphenol A exposure increased production of oxygen free radicals and destruct the normal architecture of gill in *Oreochromis mossambicus* (Chitra and Sajitha, 2014). Bisphenol A also showed an increased lipid peroxidation in hepatic cells and also impaired inter and intracellular membrane transport along with hepatic lesions in tilapia fish (Chitra and Maiby, 2014).

However, biomonitoring of the effects of the compound on behavioural modification remains scanty in piscine model. The present investigation was therefore executed to assess the median lethal concentration of bisphenol A at an exposure period of 96 h and the behavioural alteration and histopathological modification was also incorporated to evaluate its toxicity effect in indigenous cichlid fish, *Etroplus maculatus*.

MATERIALS AND METHODS

The Cichlid fish, *Etroplus maculatus* weighing 10 ± 0.5 g and length 8 ± 1.5 cm were collected from local fish farm, KKF Nursery, Manjeri, Kerala, India. Fishes were acclimatized under laboratory conditions prior to experiments for 10 days and were kept in well aerated 40 L capacity aquarium maintaining constant temperature and good lighting system. The water was dechlorinated and fishes were sustained with standard fish pellets. 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 concentration of the pollutant at which 50 percentage of the test animals dies during a specific period or the concentration lethal to one half of the test population is referred to as median lethal concentration (LC_{50}) or median tolerance limit. The LC_{50} values in the respective time intervals were determined by probit analysis, with a confident limit of 5 % level (Finney, 1971). Ten fishes were maintained in each test and control groups, where monofilament netting was used to cover the tanks to prevent the specimens from jumping out of test solutions. Alterations in the behaviour of specimens were observed in all experimental groups throughout the study.

Bisphenol A (4, 4-Isopropylidenediphenol) of 97% purity was obtained from SISCO Research Laboratories Pvt. Ltd., Mumbai, India. For the present investigation seven different concentrations of bisphenol A (4 mg, 6 mg, 7 mg, 8 mg, 10 mg, 12 mg and 15 mg/ L) were exposed into each aquarium tank maintaining 10 animals per group for 96 h and a control tank was also maintained without toxicant. The lethal concentration for 50 % killing (LC_{50}) values was computed on the basis of probit analysis for 96 h (Finney, 1971). The body weight of each treatment groups were recorded at the end of 96 h and the percentage of mucous secretion was also observed. Differences in body weights and mucous secretion against control group were considered to be significant at $p<0.05$ using students t-test and the data are presented as mean ± SD for ten animals per group. Histopathology of gill and liver above 6 mg/ L were done as per standard procedure and changes at 6 and 7 mg/ L alone were photomicrographed using Cannon shot camera fitted to Carl Zeiss Axioscope 2 Plus Trinocular Research Microscope.

Median lethal concentration or LC_{50} were analyzed with SPSS statistical analysis software (Version 19.0) using Probit Analysis Statistical Method. The LC_{50} values (with 95% confidence limits) were calculated. Differences among the results were considered to be statistically significant when *P* value was < 0.05. Also, the MS Excel 2007 was used to find regression equation (Y = mortality; X = concentrations), the LC_{50} was derived from the best-fit line obtained.

RESULTS AND DISCUSSION

In the present study fishes were randomly selected irrespective of gender difference and were exposed to different concentrations of bisphenol A for 96 h maintaining control group. All specimens selected based on good health in terms of good condition of fins, scales, mouth, eyes and behaviour, as well as their general external appearance. Mortality of the animal were continuously monitored throughout the experiment and it was observed that in 4 mg/ L concentration of bisphenol A did not cause mortality as that of control group. Bisphenol A at 6 mg/ L showed death of 4 animals and at 7 mg/ L showed mortality of 6 fishes at the end of 96 h. At the end of 24 h bisphenol A exposure at 8 mg/ L showed mortality of 7 animals, however, all animals were killed at 10 and 12 mg/ L concentrations after 24 h of bisphenol A treatment. In the last group of 15 mg/ L concentration of bisphenol A all animals were dead immediately after 4 h of treatment (Table 1).

Table 1.Percentage of fish mortality exposed to different concentrations of bisphenol A in cichlid fish, *Etroplus maculatus*

Concentrations (mg/ L)	Mortality (No. of animals)	Hour of mortality
Control	0	96 h
4	0	96 h
6	4	96 h
7	6	96 h
8	7	24 h
10	10	24 h
12	10	24 h
15	10	4 h

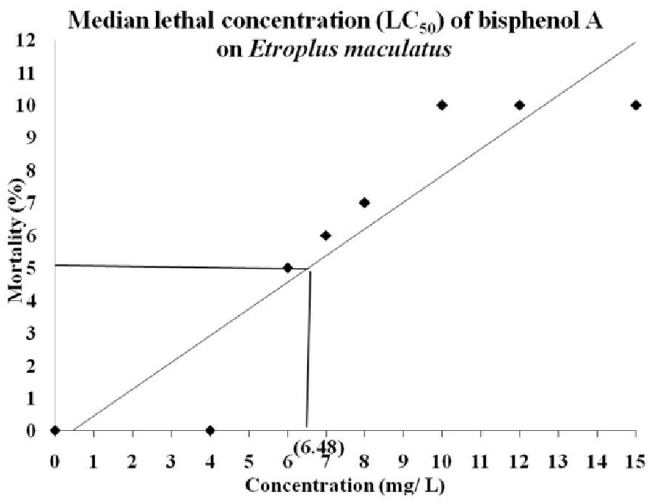

Fig. 1.

The median lethal concentration or LC_{50} was then analysed using Probit analysis and it was found as 6.48 mg/ L that kills 50% of animals and the data of probit with 95% confidence limits was given in Table 2. The regression line was plotted against mortality (vertical axis) and concentrations (horizontal axis) and the best fit line showed 6.48 mg/ L as the median lethal concentration of bisphenol A for 96 h in cichlid fish, *Etroplus maculatus* (Figure 1).

Table 2. Probit analysis of 95% confidence limits for effective concentrations of bisphenol A in *Etroplus maculatus*

		95% Confidence Limits	
Prob	Concentrations	Lower	Upper
.01	3.76930	1.55304	5.05519
.02	4.01679	1.77632	5.28701
.03	4.18218	1.93372	5.44134
.04	4.31107	2.06084	5.56151
.05	4.41883	2.17007	5.66203
.06	4.51267	2.26734	5.74965
.07	4.59659	2.35598	5.82815
.08	4.67306	2.43810	5.89981
.09	4.74370	2.51508	5.96617
.10	4.80968	2.58792	6.02830
.15	5.09273	2.91048	6.29720
.20	5.32952	3.19196	6.52622
.25	5.54142	3.45185	6.73556
.30	5.73889	3.69990	6.93549
.35	5.92815	3.94204	7.13255
.40	6.11350	4.18239	7.33181
.45	6.29835	4.42419	7.53787
.50	6.48572	4.67025	7.75552
.55	6.67867	4.92326	7.99036
.60	6.88061	5.18604	8.24954
.65	7.09574	5.46182	8.54278
.70	7.32975	5.75463	8.88419
.75	7.59094	6.07009	9.29568
.80	7.89276	6.41699	9.81411
.85	8.25974	6.81126	10.50901
.90	8.74583	7.28815	11.53820
.91	8.86746	7.40009	11.81456
.92	9.00151	7.52036	12.12759
.93	9.15125	7.65109	12.48751
.94	9.32144	7.79537	12.90944
.95	9.51940	7.95793	13.41702
.96	9.75736	8.14661	14.05038
.97	10.05806	8.37581	14.88592
.98	10.47219	8.67721	16.09869
.99	11.15980	9.14873	18.26503

After the respective mortality of fishes at specific time intervals, the body weights were recorded and it was noticed that the body weight remained unchanged (Figure 2).

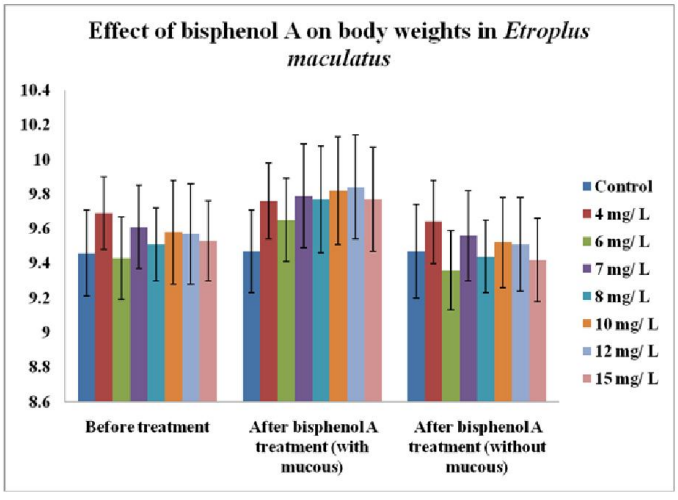

Fig.2.

However, there was tremendous increase in the mucous deposition all over the body as a first line of defense against the toxicant with a significant ($p<0.05$) increase to 29.22 ± 11.29 in all treatment groups when compared to the control group and the results were expressed in percentage (Figure 3). Mucous cells are considered as efficient in removing the toxic agents and it help to prevent the entrance of these agents into the gills (Perry and Laurent, 1993). Hypersecretion of mucous was therefore measured as a defensive mechanism of the fish against the exposure to bisphenol A. Similar results has been observed in our laboratory when octylphenol was exposed to the teleost fish, *Oreochromis mossambicus* (Sreedevi and Chitra, 2014).

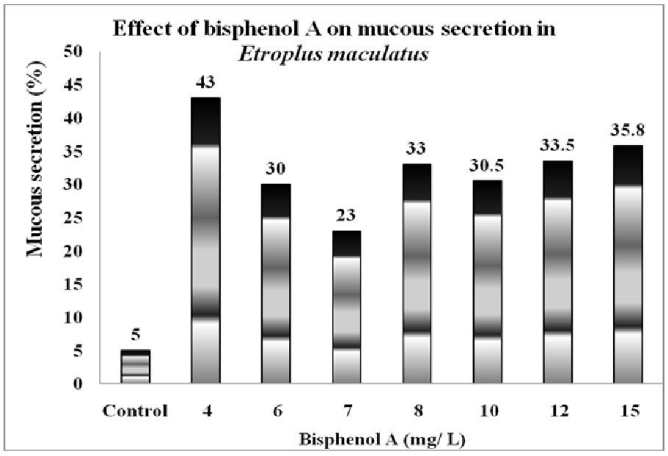

Fig.3.

Environmental pollutants pose serious risks to most of the aquatic organisms, however, its effects on fish behaviour are less frequently studied. Median lethal concentration or 96 h LC_{50} is considered as one of the acute lethality tests to find the concentrations at which the compound is said to be lethal and it depends upon many contributing factors as species, water quality and mode of exposure. Even if animals are not overtly harmed by a contaminant, they may be unable to function in an ecological context if their normal behaviour is altered. Therefore, study of behavior of an organism may be considered as an important ecologically relevant indicator to monitor the effect of pollutants on aquatic ecosystem (Atchison *et al*., 1987).

Although numerous literatures has considered the effects of anthropogenic pollutants on different fish behaviours, interbreeding and hyperactivity following bisphenol A exposure was well studied in zebrafish (Saili *et al*., 2012). Nevertheless, the behavioural modification due to bisphenol A exposure on cichlid fish *Etroplus maculatus* remains inadequate. The present study showed bisphenol A treatment showed altered behavioural changes as erratic activity followed by restricted movements immediately after the exposure, haemorrhage on entire body surface, reddening of fins and finally loss of equilibrium at the end of 96 h of exposure in all treatment groups (Figure 4).

Histopathological examination of bisphenol A treatment showed that in control, the hepatocytes of *Etroplus maculatus* was composed of distinct hepatic walls separating the cells with spherical nucleus (Figure 5). In the treatment group from 6 mg/ L onwards the hepatocyte showed loss of shape of hepatocytes, necrosis, irregular nucleus, vacuolization and damaged blood vessels (Figure 6 and 7).

Fig.4.

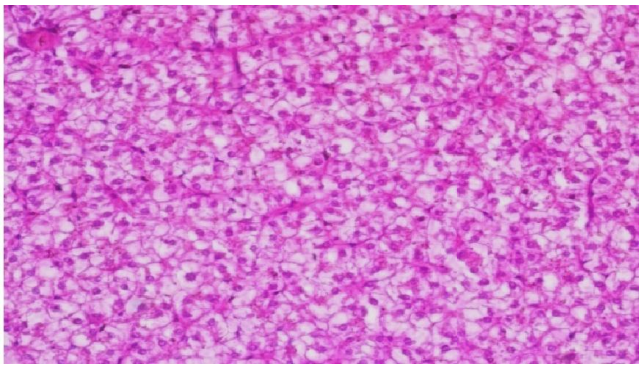

Fig.5. Histopathology of control hepatocytes of *Etroplus maculatus* (40X magnification)

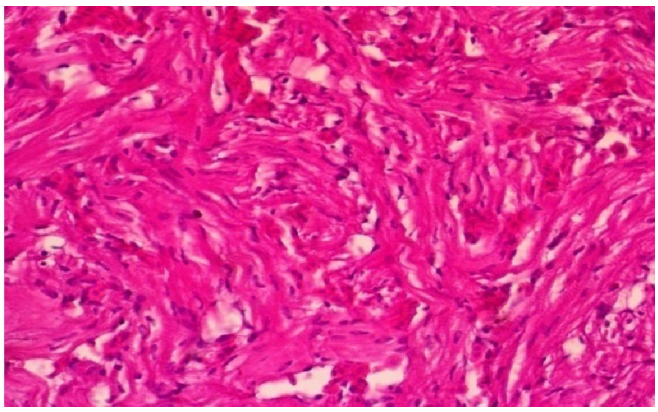

Fig. 6. Bisphenol A exposed hepatocytes showing irregular nucleus at 6 mg/ L for 96 h (40X magnification)

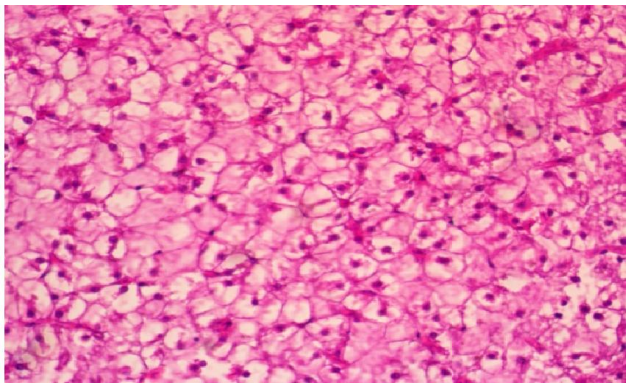

Fig. 7. Bisphenol A exposed hepatocytes showing vacuolization at 7 mg/ L for 96 h (40X magnification)

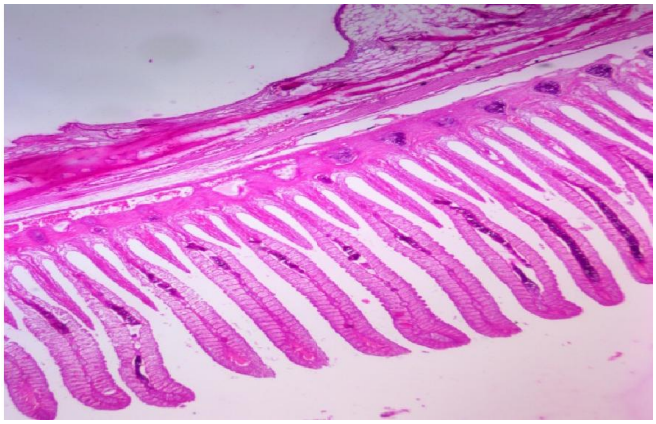

Fig. 8. Histopathology of gill of control fish, *Etroplus maculatus* (10X magnification)

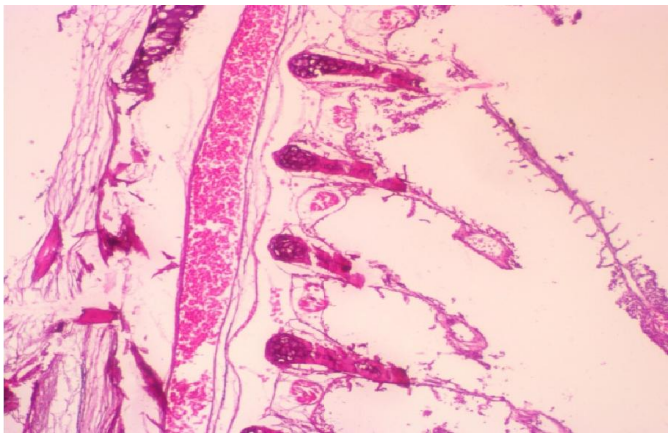

Figure 9. Bisphenol A exposed gill showing upliftment of epithelium, necrosis of primary and secondary lamellae at 6 mg/ L for 96 h (10X magnification)

Gills play a very significant role in the absorption of toxic compounds so ultimately those pollutants have a potential part to cause harm to the organ which are in first contact with the aquatic environment (Timbrell, 1991). Unexposed fish gill histology revealed a normal architecture with the presence of normal primary and secondary gill lamellae and gill arches (Figure 8), whereas in bisphenol A exposed fishes showed atrophy of gill lamellae, upliftment of gill epithelium and blubbing at the tip of gill filament (Figure 9). The severity of damage increases as the dose increases from 6 mg/ L and the upliftment or hyperplasia of lamellar epithelium could be the defensive mechanism of the fish against the aquatic toxicant and may serve as a barrier to the entrance of toxicant (Pandey *et al*., 2008).

The current findings clearly demonstrate the negative consequence of bisphenol A at acute toxicity phase where its median lethal concentration by probit analysis was found to be 6.48 mg/ L. The toxicity of the compound is also revealed by the modifications in normal behaviour of the animal as well as histopathological findings provide illustrative confirmation of the above facts. In conclusion, the present data suggested that median lethal concentration for 96 h in *Etroplus maculatus* caused altered behavioural pattern and changed the normal architecture of vital organs as an outcome of bisphenol A toxicity.

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