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EVIDENCE FOR THE PRESENCE OF BISPHENOL A IN PEARL SPOT FISH (*Etroplus suratensis*) OF VEMBANAD LAKE

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ABSTRACT

The discharge of industrial, agricultural and domestic waste into the environment results in the pollution of aquatic systems. Among several pollutants, Bisphenol - A (BPA) attains much concern because of its persistence in aquatic environment and estrogen mimicking properties. BPA is a breakdown product of epoxy resins and polycarbonate plastics. The discharge of BPA into the aquatic environment from the domestic waste may interfere with the functions of aquatic organisms. The present study designed to assess the biochemical parameters in the liver of pearl spot fish (*Etroplus suratensis*) from Vembanad lake water containing BPA. The observed level of BPA in Vembanad lake is 0.0066 µg/ml. Increased oxidative stress and histopathological changes were observed in pearl spot from the Vembanad lake. The study concluded that further analysis may need to confirm the BPA related hepatotoxicity of pearl spot fishes in Vembanad lake.

KEYWORDS: Vembanad Lake, Bisphenol-A, *Etroplus suratensis*, Hepatotoxicity

The contamination of surface waters including river and wetland ecosystems can take place from the industrial and household products such as plasticizers, pesticides and non-ionic surfactances. Bisphenol – A (BPA) may be released into the environment as a breakdown product of epoxy resins and polycarbonate plastics (Vandenberg et al., 2007). BPA ($C_{15}H_{16}O_2$) is the key monomer used in polymer products (Krishnan et al., 1993). BPA appears to be more resistant to environmental degradation. Temperature, UV radiation, alkaline treatment or intensive washing influence the release of BPA from consumer products in to the water resources (Howdeshell et al., 2003).

Wang and his colleagues (2017), revealed that thermal hydrolysis depolymerizes micro plastics containing ester groups and release building block units like BPA in to environment. Many industrial and agricultural processes have contributed to the micro plastic contamination of wetland eco systems. High levels of micro plastics deposition have been noticed in certain hotspots of the Vembanad lake, largest wetland ecosystem in Kerala (Sruthy and Ramaswamy et al., 2017). The Vembanad lake is most prominent with more than 70 edible species which include mullets, pearl spots, crabs, oysters, clam, cat fishes etc. Out of the above species, pearl spot (Etroplus suratensis) has an outstanding position among the fish eaters (Kurup and Samuel., 1985).

Fishes are highly sensitive to contamination in their environment and are served as bio-indicators of environmental pollution. Fitzgerald and his collegues (2004) have suggested that fish consumption is one of the sources of human exposure to BPA and reported higher level of BPA in human tissues, serum, and milk. Pearl spot is a common edible fish of Kerala and have high economic value than other fresh water and brackish water fishes. The consumption of pearl spot fish is high in Kerala. So there is a chance for BPA contamination, if the water is polluted by BPA containing products. Hence the present study was conducted to identify BPA in Vembanad lake and in pearl spot fishes.

MATERIALS AND METHODS

Sample Collection and Analysis

The water and pearl spot fishes were collected from Vembanad lake, a famous tourist place in Kottayam, Kerala, India. The lake is situated between Lat. 9º30'46''-10º11'11'' N and Long. 76º09'48''- 76º25'45''. An area of 398.12 km² of Vembanad lake is located below the mean sea level (Verma et al., 2002).

Water samples (6L) collected from Vembanad lake filtered and stored in -20^{0}c until analysis. The collected fishes (n=6) were ice packed and brought to the laboratory and hepatic tissue was excised and subsequently used for biochemical analysis. Sample preparation and analysis for water and tissue samples were accordance with the Rezaee et al., (2009) and Aristiawan et al., (2015) methods respectively. Fishes for comparison were obtained from fish hatchery, Neendoor, Kottayam, Kerala. Water samples were also taken for analysis.

Biochemical Analysis

The levels of lipid peroxidation (Beuge and Aust., 1978) and the activities of antioxidant enzymes such as superoxide dismutase (Kakkar et al., 1995), catalase (Aebi 1984), glutathione (Ellman., 1959) were estimated from liver homogenates. The serum ALT and

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AST were analyzed accordance with manufactures instructions from Agappe diagnostic kit, India.

Histopathology

Gross appearance and color change in the internal organs were checked in the sacrificed fishes using a magnifying glass. Histology assessment of hepatic tissue was done with hematoxylin and eosin staining. The slides were examined under phase contrast microscope (Olympus, India)

Data Analysis

The data were analyzed by using one way analysis of variance in SPSS 16 software (Chicago, IL, USA). The results were represented as means ± standard deviations (SD). Values were considered significantly different if $P<0.05$.

RESULTS

Quantification of BPA from Vembanad Lake Water

Identification and quantification of BPA from lake water was made by comparing HPLC retention time of sample peak in Fig.1 (a) with those of the authentic standard in Fig.1 (b). The BPA concentration was measured from the regression equation y = 6866 x + 2247. The regression coefficient (R^2) value of the calibration curves was 0.997. The obtained BPA concentration from Vembanad lake was depicted in Fig.1 (c).

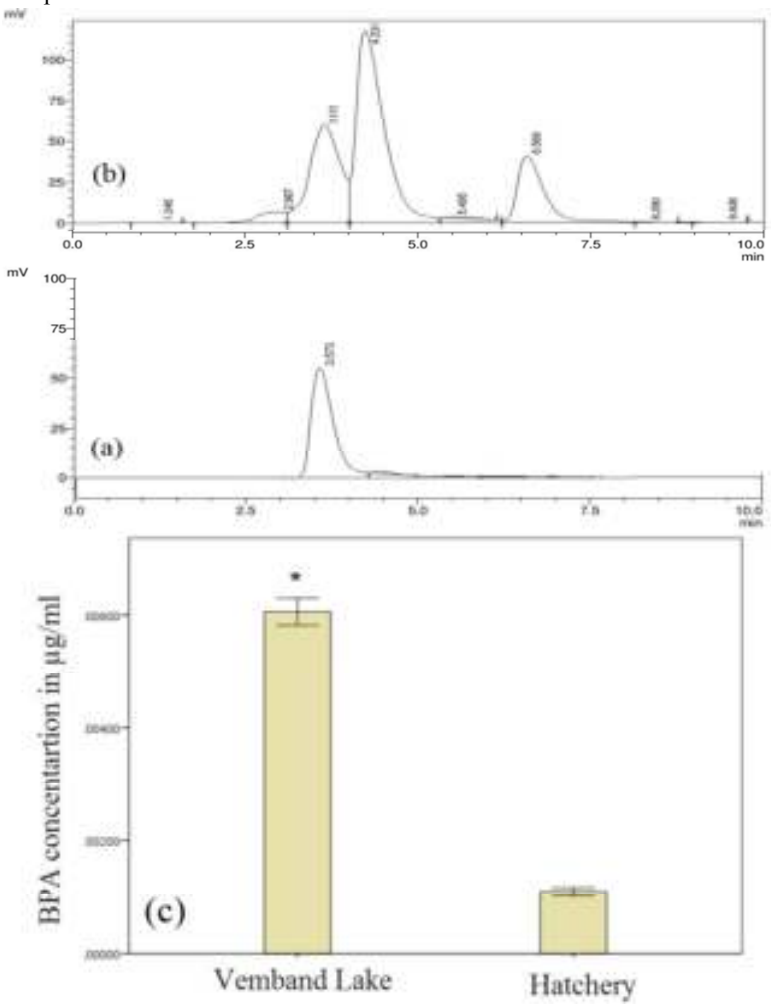

Figure 1: HPLC analysis for bisphenol-A (a) Chromatogram of standard bisphenol-A (b) Chromatogram of bisphenol-A from Vembanad lake water (c) Comparison of bisphenol-A concentration from Vembanad lake and hatchery.

Identification of BPA from Pearl Spot Liver

The identification of Bisphenol A from the hepatic tissue was done under the negative ionization mode of LC QTOF analysis. The mass chromatogram and fragmentation pattern of BPA was shown in the Fig.2.

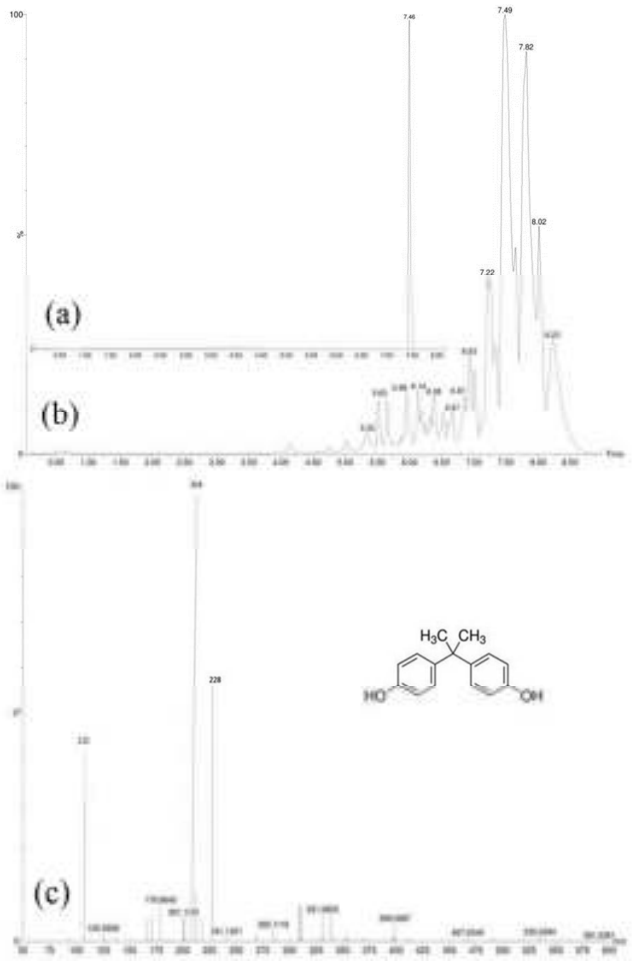

Figure 2: Chromatogram and Mass spectrum of Bisphenol A: (a) Chromatogram of BPA standard (b) Chromatogram of bisphenol A from hepatic tissue (c) The mass spectrum of BPA exhibit the molecular ion peak at m/z 228.

Biochemical Analysis of Hepatic Tissue

The level of MDA (malondialdehyde) and antioxidant enzymes in hepatic tissue was assessed from pearl spot fishes collected from Vembanad lake and hatchery. The obtained differences in the parameters were showed in Fig.3.

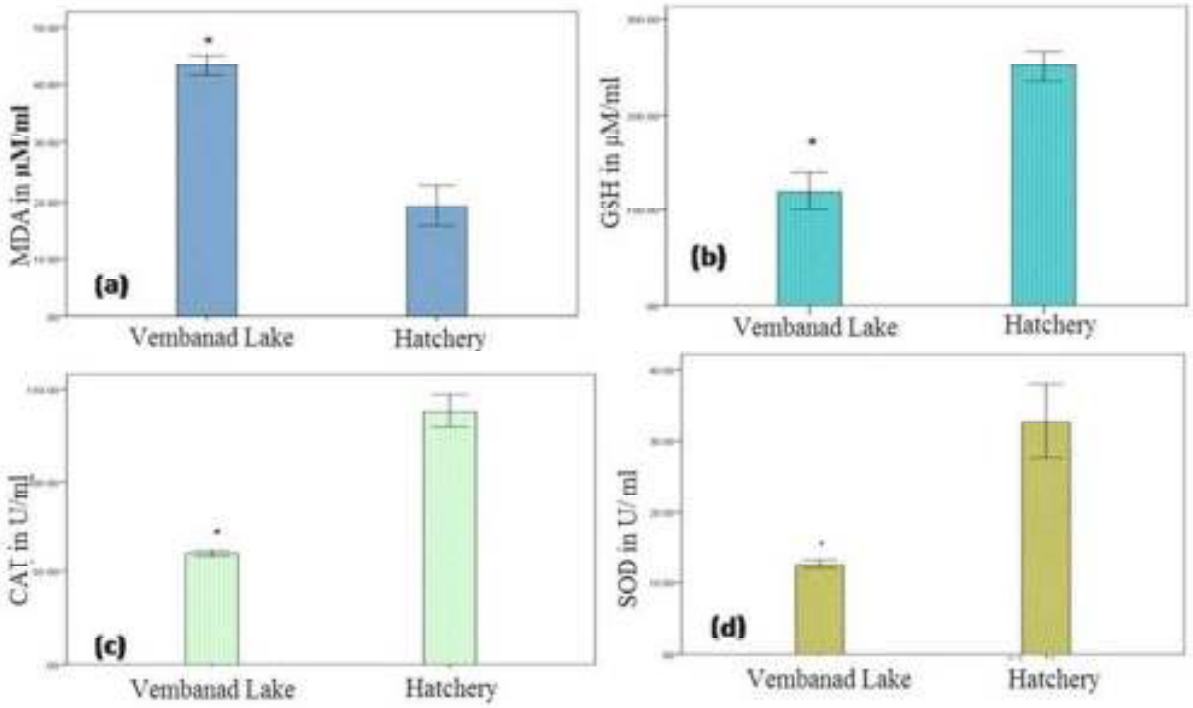

Figure 3: Analysis of lipid peroxidation and antioxidant enzymes. (a) MDA (b) GSH (c) CAT (d) SOD.
('*' P<0.05 Pearl spot fishes from Vembanad lake vs Neendoor. The data represented as mean ± SD, n=6)

Serum Hepatic Markers

The AST and ALT level of the hepatic tissue was assessed from the Vembanad lake and Neendoor pearl spot fishes. The obtained differences in the AST and ALT level were depicted in Fig.4. A significant ($p<0.05$) increase of hepatic markers was observed in the hepatic tissue of pearl spot collected fromVembanad region.

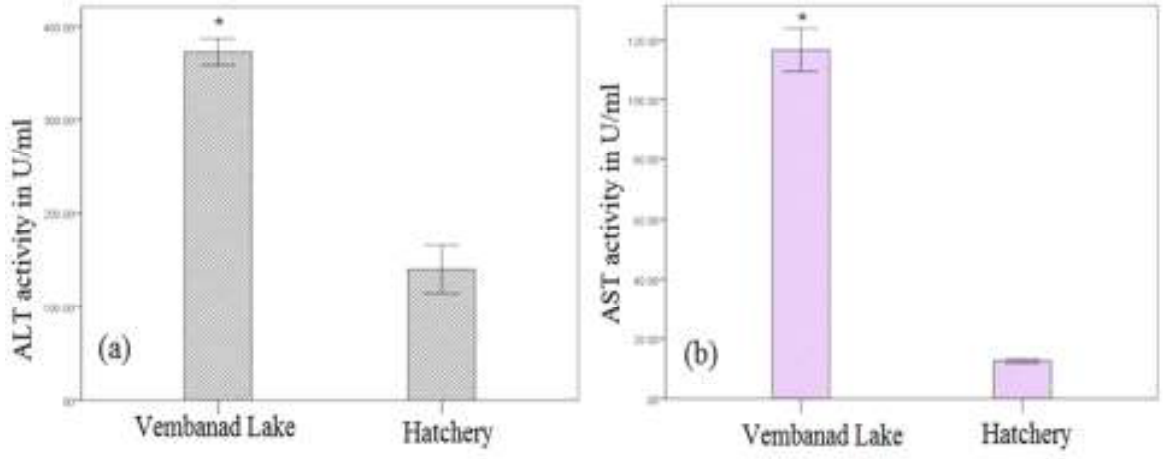

Figure 4: Serum hepatic markers in pearl spot fishes (a) ALT level (b) AST level;
('*'p<0.05, Vembanad lake vs Neendur)

Liver Histology

The H& E stained sections of liver shows normal architecture for pearl spot collected from fish hatchery. While the hepatic tissue of pearl spot collected from BPA contaminated Vembanad water samples shows severe hepatic cell damage. The distortion represented as cytoplasmic vaculation (green arrow) and enlarged interlobular duct (red arrow).

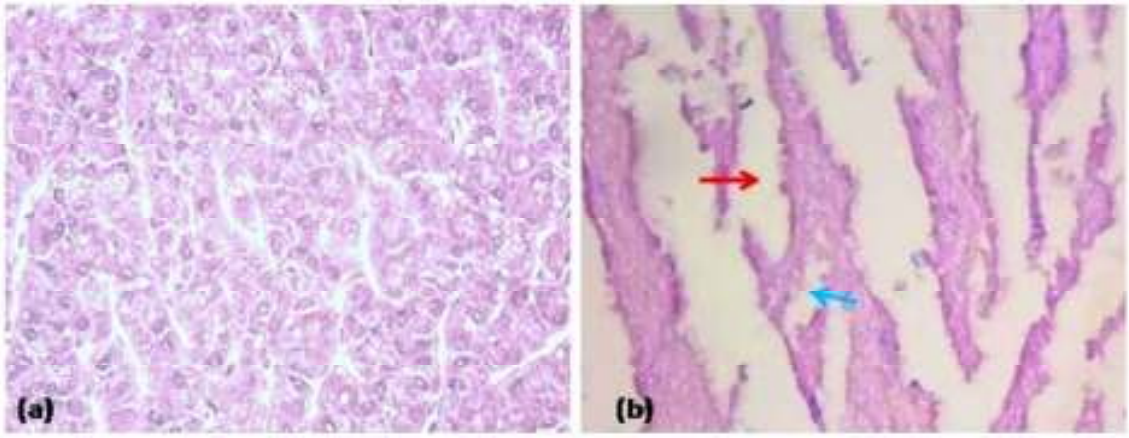

Figure 5: Light photomicrographs of hepatic sections from different experimental groups of pearl spot fishes. Hematoxylin and Eosin stained microscopic sections, magnification as 20×. (a) Pearl spot fish from hatchery (b) Pearl spot from Vembanad lake water.

DISCUSSION

Analysis of Vembanad lake water revealed the presence of BPA. The level of BPA found to be higher in Vembanad lake (0.006µg/ml) compared to hatchery (<LOQ). The results may reflect the contamination of Vembanad water ways with BPA containing domestic waste and it might be higher than the artificial ecosystem like hatchery. When xenoestrogens like BPA is released in to the aquatic bodies there may be an immediate impact on the fishes. The low level discharge may activate the xenobiotic mechanisms in fish liver. Studies have shown that BPA conjugate with glucuronide and are excreted immediately (James 2011). But presence of BPA is detected in pearl spot fish from Vembanad lake. Exposure of endocrine disruptors like BPA to aquatic organisms can enhance reactive oxygen specious production with a subsequent damage to lipid bilayer membrane (Wu et al., 2011). The MDA serves as a convenient index for determining the extent of lipid peroxidation. The present investigation exhibited elevated level of MDA in pearl spot liver.

The peroxidation reactions in the cellular pathogenesis can exert by depleted status of antioxidants like superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH) (Farombi et al., 2007). The analyzed hepatic tissue of pearl spot shows decreased activities of SOD, CAT and GSH. Superoxide is the primary free radical produced from membrane peroxidation, which converts superoxide anion radical in to hydrogen peroxide (Alvarez et al., 1987). Enzymes responsible for the reduction of H_2O_2 also include CAT and GSH. The present study showed decreased activity of SOD, CAT and GSH level. The reduced activity of SOD and CAT may reflect inability of liver cells to eliminate the hydrogen peroxide. The excess H_2O_2 can increase superoxide level via NADPH oxidase pathway (Gauduel et al., 1989). The generation of superoxide radicals may exaggerate the level of MDA. Therefore, we assume that the cellular level peroxidation rate in pearl spot fish liver is augmented by the depletion antioxidant enzymes.

The consequences of elevated MDA level have been considered in relation to oxidation of pyridine nucleotide and uptake of calcium. The intake of excess calcium leads to the release of hydrolytic enzymes from lysosomes and decrease plasma membrane integrity can contribute to further damage to hepatocytes (Poli et al., 1987). The elevated serum level of hepatic markers in our study may relate the hepatotoxicity in pearl spot fishes in Vembanad lake. The obtained histopathology of pearl spot liver reflects severe hepatic damage. The cytoplasmic vaculation and enlarged interlobular duct are the main characteristics of liver injury (Dahi et al.,1971).

The study results conclude that, Pearl spot (*Etroplus suratensis*) fishes in Vembanad lake is highly susceptible to oxidative stress related liver damage. Further studies are needed to clarify the role of BPA in hepatotoxicity.

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