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AMELIORATIVE EFFECTS OF VITAMIN-C AGAINST BISPHENOL-A TOXICITY IN LIVER OF *CIRRHINUS MRIGALA (HAM.)*

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ABSTRACT
180 fishes *Cirrhinus mrigala* 60 ± 5 g were divided into three groups of 50 each. Group 1st received fish diet only and served as control, while group 2nd received Bisphenol-A (2mg/L) and group 3rd received vitamin-C (50 mg/L) along with Bisphenol-A (2mg/L) for 30 and 60 days and the enzymological (GOT, GPT, ACP and ALP), total protein, creatinine levels, histochemical and histopathological studies were estimated in their liver of *Cirrhinus mrigala*. Bisphenol-A significantly elevated the hepatic GOT, GPT, ACP and ALP enzyme activities levels after 30 and 60 days as compared with control group. Apart from this, Bisphenol-A lowered hepatic protein levels insignificantly, whereas its insignificantly elevated hepatic creatinine levels in *Cirrhinus mrigala* after 30 and 60 days in comparison to control group. In connection to this, BPA induced histopathological changes in liver after 30 and 60 days showed hypertrophied, as well as atrophied changes in hepatic cells, characterized by pycknotic nuclei with less amount of cytoplasmic materials. In histochemical studies *i.e.* bromophenol and silver nitrate reactions also denotes that protein and ascorbic contents respectively were lowered in later part of the experiment. However, animals supplementation with vitamin-C along with Bisphenol-A showed recovery in hepatic cells structures, hepatic enzyme activities, protein and creatinine contents in comparison to Bisphenol-A group. Above finding may suggests that vitamin-C ameliorated toxic effects of Bisphenol-A in *Cirrhinus mrigala*

INTRODUCTION

Bisphenol-A (BPA) is mainly used for the production of polycarbonate resins, epoxy resins unsaturated polyester, polysulfone polyetherimide and polyarylate resins (Groshart *et al.,* 2001). Bisphenol-A leaches from plastics consumer's products are widely evidences and contaminated due to Bisphenol-A production is considerable. Initial assessment shows that at low levels, bisphenol-A can harm fish and organisms over time (WHO fact sheet, 2008). Bisphenol-A is targeted at multiple organ systems kidneys, liver, spleen, pancreas and lungs (Petteri, 2002). Eastwood *et al.* (2003) have reported that BPA, acting as an estrogen mimic, inhibits and disrupts estrogen-induced signaling in rats that regulates cell growth and death in the cerebellum. Bisphenol-A tend to be higher in fish liver and other organs (Belfroid *et al.,* 2002). BPA is thought to bind to plasma proteins in rodents, monkeys and humans (Teeguarden *et al.*, 2005).

Vitamin-C (Ascorbic acid) water-soluble antioxidant is extremely powerful and has a multifunction. It helps to strengthen the immune system and it prevents some diseases, plays important roles in the brain, nervous system, and immune system; mobilizes iron in the body; prevents anemia and has many benefits such as prevents several debilitating conditions and increases the body's immunity (Len, 2009). Lipid-soluble antioxidants protect cell membranes from lipid per-oxidation in the cell cytoplasm and the blood plasma (Nordberg and Arner, 2001). In general, they either prevent the formation of free-radicals or neutralize those that are formed or repair the damage done by free-radicals.

Vitamin-C is essential for normal fish growth and has some properties that allow food products to resist oxidation (Ibiyo *et al.*, 2007). The major beneficial actions of vitamin-C and E are due to their antioxidant properties that scavenge reactive oxygen species in biological fluid (Frei *et al.*, 1990) and membranes (Ibiyo *et al.,* 2007).

As we know that there has been less evidence about such type of work available regarding the hazardous effects of bisphenol-A on aquatic organisms and neutralization of these effects, so the objective of this work was to find out the possible toxic effect of bisphenol-A (BPA) plasticizer on freshwater fish *Cirrhinus mrigala* (Ham.) and at same time the ameliorative and protective role of ascorbic acid (vitamin- C) against the BPA.

MATERIALS AND METHODS

150 fishes *Cirrhinus mrigala* 60 ± 5g was acclimated in the laboratory condition prior to initiations of the experiment. The fishes were divided into three groups of 50 each. Group 1st received fish diet only and served as control, while group 2nd received Bisphenol-A (2mg/L) and group 3rd received vitamin-C (50 mg/L) along with Bisphenol-A (2mg/L) for 30 and 60 days. Fishes were sacrificed and the liver were dissected out quickly; weighed and homogenized in 0.25 M sucrose solution for enzymological studies *i.e.* glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase

(GPT) by adopting Reitman and Frankel (1957) methodology; and acid phosphatase (ACP) and alkaline phosphatase (ALP) levels were done by Bergmeyer, (1963) method . Apart from this, total protein and creatinine levels were measured in liver by Lowry, 1951 and Picrate, 1945 methods respectively. For histopathological studies, tissues were kept in Bouin's fixative and stained with haemotoxylin and Eosin by using Ehrlich's (1986) methodology. In connection to this, histochemical localization of proteins and ascorbic acid were stained with mercuric bromophenol blue (Ploton *et al*., 1986) and ascorbic acid using silver nitrate technique (Bacchos, 1950) respectively. The Students't' tests (Fisher and Yates, 1953) were applied for statistically analyzed the data.

RESULTS

Fish (*Cirrhinus mrigala*) treated with Bisphenol-A changed the color of the fish skin it becomes light brackish skin, whereas, fishes received vitamin-C (50 mg/L) along with Bisphenol-A (2 mg/L) exposures showed normalcy in their skin color *i.e.* dark blackish color. It has been observed that fish exposed with a Bisphenol-A (2 mg/L) for 30 and 60 days showed alteration in enzyme activities *i.e.* GOT, GPT, ACP and ALP levels. Bisphenol-A significantly elevated the hepatic GOT, GPT, ACP and ALP enzyme activities levels after 30 and 60 days in comparison to control group (Fig. 1, 2, 3, 4). However, the fish supplemented with vitamin-C along with Bisphenol-A showed recovery in hepatic GOT, GPT, ACP and ALP levels after 30 and 60 days as compared to Bisphenol-A groups (Fig. 1, 2, 3, 4).

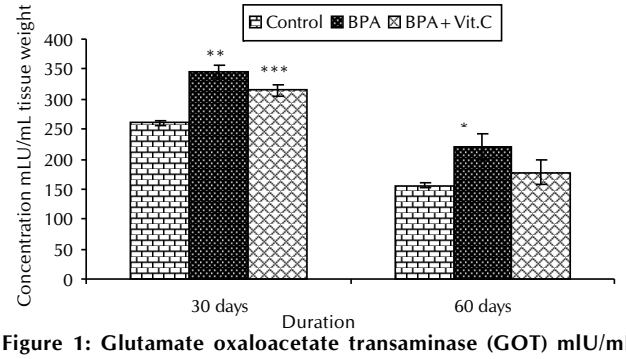

Figure 1: Glutamate oxaloacetate transaminase (GOT) mlU/mL concentration in liver of *Cirrhinus mrigala* after Bisphenol -A and vitamin - C supplemented along with Bisphenol -A

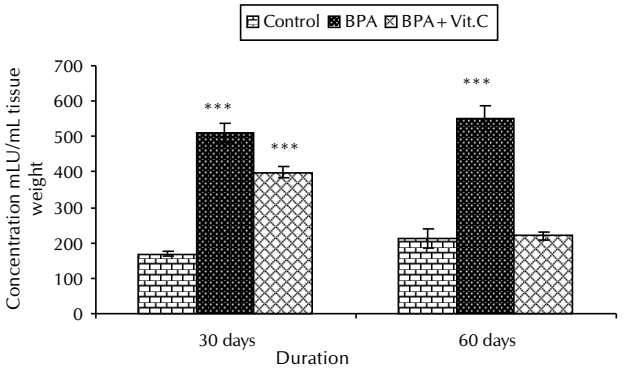

Figure 2: Glutamate Pyruvate transaminase (GPT) mlU/mL concentration in liver of *Cirrhinus mrigala* after Bisphenol -A and vitamin - C supplemented along with Bisphenol -A

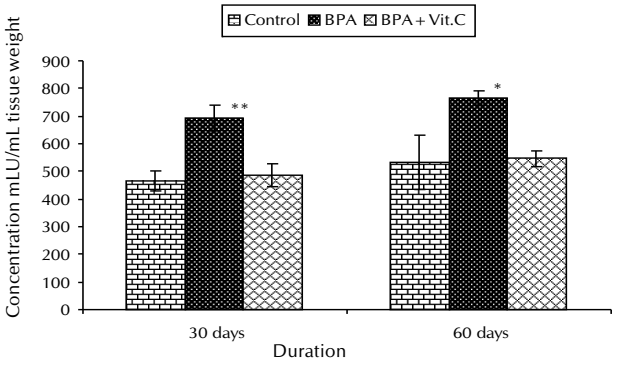

Figure 3: Acid Phosphatase (ACP) mlU/mL concentration in liver of *Cirrhinus mrigala* after Bisphenol -A and vitamin - C supplemented along with Bisphenol -A

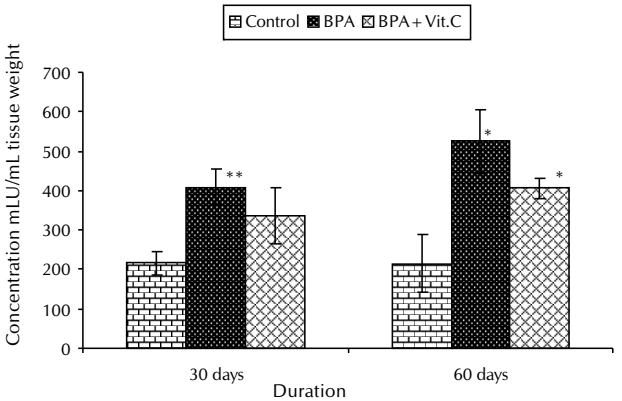

Figure 4: Alkaline Phosphatase (ALP) mlU/mL concentration in liver of *Cirrhinus mrigala* after Bisphenol -A and vitamin - C supplemented along with Bisphenol -A

SEM values of five fishes.*Significant values from student 't' test; **Significant values from student 't' test ; ***Significant values from student 't' test

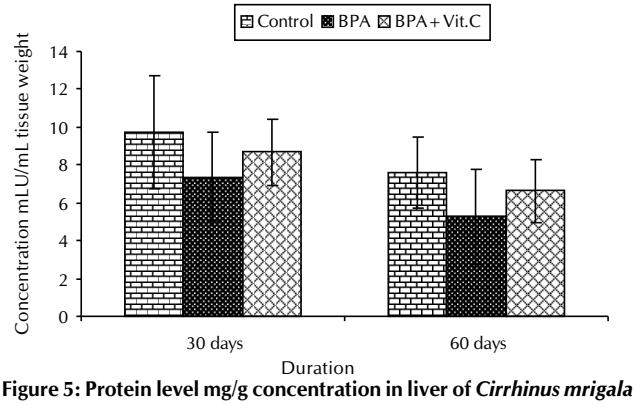

Figure 5: Protein level mg/g concentration in liver of *Cirrhinus mrigala* after Bisphenol -A and vitamin - C supplemented along with Bisphenol -A

In connection to this, Bisphenol-A insignificantly lowered the protein level after 30 and 60 days as compared to control (Fig. 5). Whereas, the changes showed recovery values in protein level after supplemented with vitamin-C along with Bisphenol-A (Fig. 5). Beside this, the creatinine levels were insignificantly elevated in liver after 30 and 60 days as compared to control group (Fig. 6). While, these values were insignificantly lowered when supplemented with vitamin-C along with Bisphenol-A groups (Fig. 6).

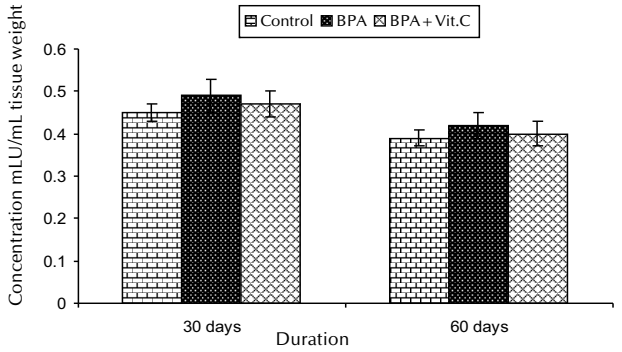

Figure 6: Creatinine level mg/g concentration in liver of *Cirrhinus mrigala* after Bisphenol -A and vitamin - C supplemented along with Bisphenol -A

Apart from this, histopathological studies have been also showed that Bisphenol-A induced degenerative changes, vacuolization and necrosis in hepatic after 30 days as compared to control (Fig. 7, 8). However, these changes were more prominent in later part of the experiment. While, the liver of fish supplemented with vitamin-C along with Bisphenol-A exposures showed recovery in hepatic nuclei (Fig. 9, 10). In histochemical localization, it has been observed that protein stained by Bromophenol blue and ascorbic acid stained by silver nitrate on Bisphenol-A exposures after 30 and 60 days showed less reaction on hepatic cells as compared to control (Fig. 12, 13, 14, 17 18, 20). Beside this, fish supplemented with vitamin-C along with Bisphenol-A showed more reaction in hepatic cells in comparison to Bisphenol-A group (Fig. 12, 13, 16, 17, 19, 21).

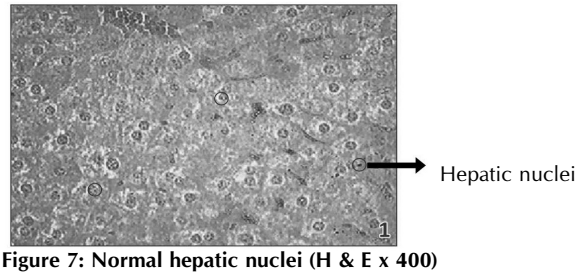

Figure 7: Normal hepatic nuclei (H & E x 400)

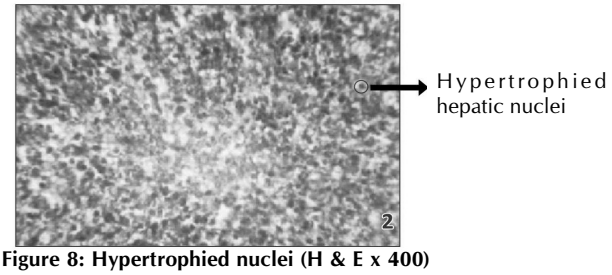

Figure 8: Hypertrophied nuclei (H & E x 400)

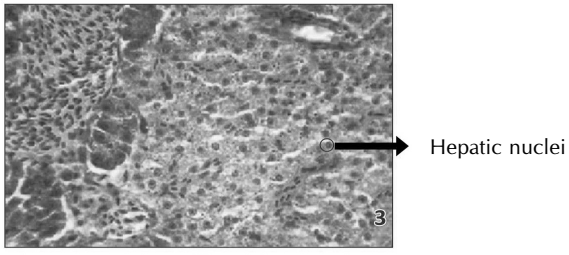

Figure 9: Hepatic cells with prominent nuclei (H & E x 400)

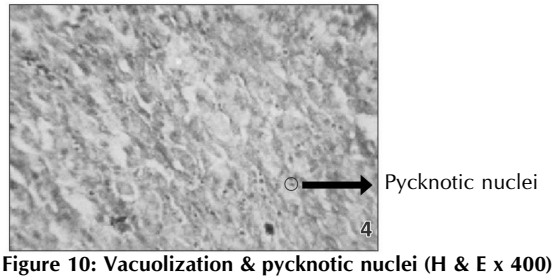

Figure 10: Vacuolization & pycknotic nuclei (H & E x 400)

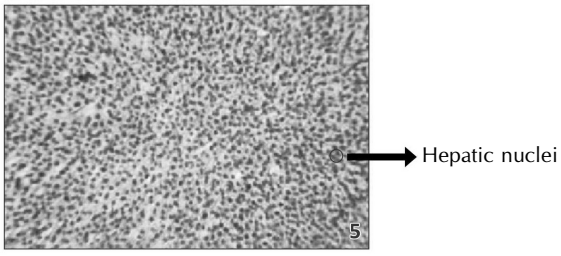

Figure 11: Hepatic Nuclei (H & E x 400)

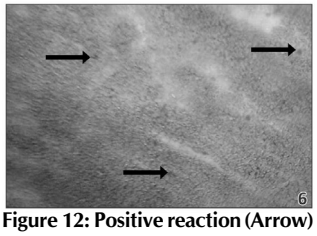

Figure 12: Positive reaction (Arrow) show protein contain in hepatic cells. (Bromophenol blue x 100)

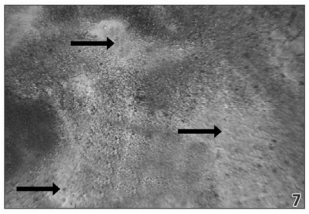

Figure 13: Less reaction (Arrow) show protein contain in hepatic cells (Bromophenol blue x 100)

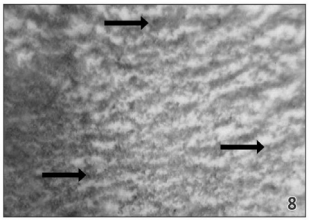

Figure 14: Positive reaction (Arrow) show protein contain in Hepatic cells (Bromophenol blue x 100)

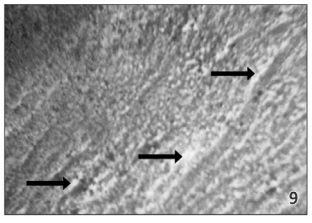

Figure 15: Less reaction (Arrow) show protein contain in hepatic cells (Bromophenol blue x 100)

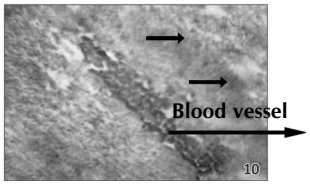

Figure 16: Positive reaction (Arrow) show protein contain in hepatic cells (Bromophenol blue x 100)

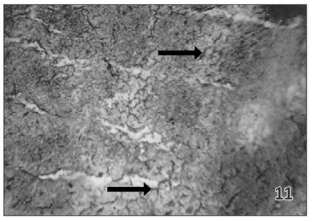

Figure 17: Dark brown clour reaction for ascorbic acid in hepatic cells (Silver nitrate x 100)

DISCUSSION

Fish species are sensitive to enzymic and hormone disruptors. Liver, kidney, brain and gills are the most vulnerable organs of a fish exposed to the medium containing any type of toxicant (Jana and Bandyopadhyaya, 1987). In humans and other non-human primates, bisphenol-A administered orally enters first

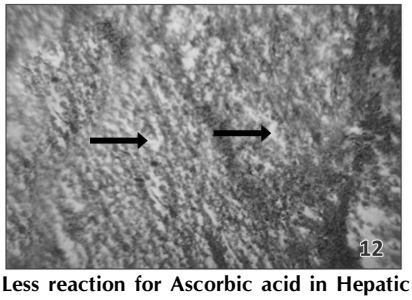

Figure 18: Less reaction for Ascorbic acid in Hepatic cells (Silver nitrate x 100)

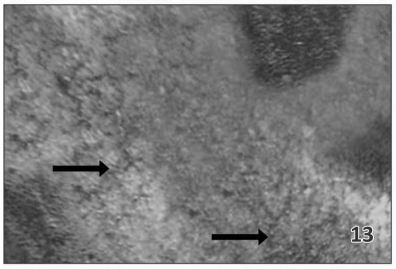

Figure 19: Positive reaction for ascorbic acid in Hepatic cells (Silver nitrate x 100)

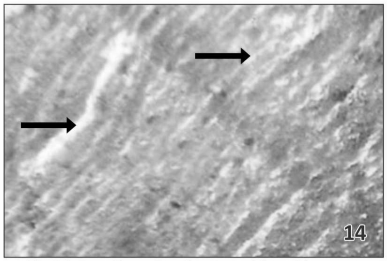

Figure 20: Less reaction for ascorbic acid in Hepatic cells (Silver nitrate x 100)

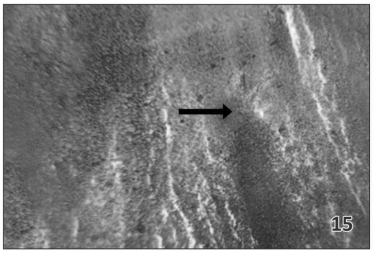

Figure 21: Positive reaction for ascorbic acid in Hepatic cells (Silver nitrate x 100)

pass metabolism in the gut wall and the liver and is quickly metabolized to bisphenol-A monoglucuronide, which has no endocrine activity and is rapidly excreted in urine with a half-life of less than 6 hours (Tominaga *et al*., 2006). In rats, bisphenol-A administered orally also undergoes glucuronidation, but the resulting bisphenol-A glucuronide is excreted from liver into bile.

Activities of the serum enzymes like GOT, GPT, ACP and ALP represent the functional status of the liver. Liver is the richest sources of both GOT and GPT are used as sensitive indicator of liver damage (Ozaki *et al*., 1995). Any damage to the liver cells will result in the increase of both these enzymes (Cole and Bradley, 1973).The increase in quantity usually reflects the severity of hepatic damage (Ginsberg, 1970). When any of these organs are damage, the serum GOT levels rises in proportion to the severity of damage. Besides this, increase in serum activities of GPT consider liver specific enzymes in rat are use as markers of hepatocellular necrosis or increased cell membrane permeability (Travlos *et al.,* 1996). AST (Aspartate transaminase), ALT (Alanine transaminase) levels were increased in BPA groups. Hepatic necrosis and congestion were observed in livers of rats treated observed by (Korkmaz *et al.,* 2010).

Acid phosphatase belongs to the class of enzymes called hydrolases and they are characterized by their ability to hydrolyse a large variety of organic phosphatase esters with the formation of an alcohol and a phosphate ion. Alteration in the enzyme activity is due to adverse effect of xenobiotics on the cell and its organells (Jana *et al.,* 1985). Alkaline phosphatase, a brush border enzyme mediates membrane transport (Goldfisher *et al.,* 1964). It is known to be involved in a variety of metabolic activities such as permeability (Seth *et al.,* 1969) growth and cell differentiation protein synthesis and gonadal maturation (Shaffi *et al.,* 1974) and steroid ogenesis. Alkaline phosphatase elevated in liver diseases portal cirrhosis is associated with minimal increase in ALP which is not as high as that with post necrotic cirrhosis (Musser *et al*., 1966), striking elevations of plasma ALP are usually confined to patients with cholestatic disorders and is due to increase and is due to both an increased synthesis and reduced bilary excretion of ALP (Kaplan, 1986). Hathway (1986) TEM (Transmission electron microscope) also revealed significant destruction changes in sub-cellular organelles and ultra-structural organization of liver in the carcinogen fed mice, but signs of recovery were noticeable in the mice also fed AA (Ascorbic acid). The reduction of protein is due to decrease of hepatic protein synthesis and the hyperactivity of hydrolytic enzymes (Sivaprasada *et al*., 1983).

In present study, it has been observed that Bisphenol-A increased the level of GOT, GPT, ACP and ALP enzyme activities in liver of *Cirrhinus mrigala* after 30 and 60 days durations which denotes that the Bisphenol-A induced liver functions. However, these GOT, GPT, ACP and ALP levels were ameliorated to some extent when vitamin-C were supplemented with Bisphenol-A. In connection to this, it has been noticed that protein and creatinine levels were also altered in liver after 30 and 60 days treatment of Bisphenol-A. Beside this, histopathological studies have been also showed that Bisphenol-A induced degenerative changes, vacuolization and necrosis in hepatic cells after 30 days as compared to control. These changes were more prominent in later part of the experiment. While some part of the recovery were noticed in these changes when the fishes were supplemented vitamin-C along with Bisphenol-A. These results were also supported by histochemical observations. In which, it has been observed that protein stained by Bromophenol blue and ascorbic acid stained by silver nitrate on Bisphenol-A exposures for 30 and 60 days showed less reaction on hepatic cells. Whereas these reaction were showed more reactions when the animals were supplemented with vitamin-C along with Bisphenol-A. All these results suggest that the Bisphenol-A induced histopathological and histochemical changes in liver of *Cirrhinus mrigala* by modulating the enzyme activities. These changes may be

ameliorated to some extent by using vitamin-C in *Cirrhinus mrigala*.

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