ABSTRACT

Bisphenol-A (BPA) is a xenoestrogen that mimics the action of natural estrogen, estradiol-17β, and can disrupt the endocrine system. Present study was designed to investigate histopathological effects of BPA on some vital organs of major carp, *Catla catla*. Fish were exposed to various sub-lethal concentrations (1 to 4 ppm) of BPA in a semi-static system for 15 days. After the stipulated time, liver, kidneys and gills were dissected out and fixed immediately in 10 % buffered formalin. After embedding in paraffin wax, 5 micron thick sections were cut and stained with H & E and PAS stains. In liver, BPA caused central vein congestion, inflammation, edema, degeneration and necrosis of hepatocytes. Kidney anomalies included obliteration of bowmen’s space, shrinkage and degeneration of tubules and glomerulus. Gills responded to BPA stress by hyperplasia of mucous cells, clubbing and degeneration of secondary lamellae. The present study revealed that BPA causes degenerative changes in various vital organs of *C. catla* and severity of histological changes were dose-related.

Key words: Histopathology, Endocrine disrupting chemicals, Bisphenol-A, *Catla catla*.

INTRODUCTION

Environmental pollution has become a major concern in the developed as well as under-developed countries (Kazi et al., 2009; Ozden 2010); and aquatic pollution has become a serious concern. Due to worldwide rapid pace of industrialization point and non-point source chemical discharges in water bodies is increasing and causing injurious and harmful effects on aquatic organisms (McGlashan and Hughes, 2001). Approximately 70,000 anthropogenic chemicals are released into the aquatic ecosystems (Routledge et al., 1998, Metzler and Erica, 2001). Among these, there is wide range that shows affinity towards estrogen receptor and are structurally similar to natural or pharmaceutical estrogens; such chemicals are now called endocrine disrupting chemicals (EDCs) (Sumpter, 2002).

Bisphenol A (BPA, 4,4’-isopropylidene diphenol) is an estrogenic endocrine disrupting chemical. It is a commercially used chemical, an additive in the production of polycarbonate plastics as a developing agent in manufacturing of thermal paper and epoxy resins. Bisphenol A is also present in dental sealants, water bottles and baby bottles, paper coatings, adhesives, flame retardants, food and beverage packaging (Staples et al., 1998). Bisphenol A is one of the highest volume chemicals produced worldwide and its demand is increasing due to the ever-increasing demand and production of plastic products.

Since aquatic environments are the ultimate sink of all anthropogenic chemicals, aquatic animals including fish are often exposed to these chemical compounds. In recent years, BPA is extensively used for aquatic toxicity testing because of its adverse effects on wild life, especially fish. Fish are extensively used to determine the health of aquatic systems because their biological responses serve as biological markers of environmental pollution. Histological analysis provides an excellent biomarker to assess toxicity levels because of its broad evaluation (Moeller, 1985) and relationship between molecular and organismal levels (Srivastava et al., 1990).

*Catla catla* is a common food item and commercially an important cyprinid species. It is abundant in Pakistan and is cultured along with other indigenous major carps and exotic Chinese carps (Lone et al., 2009). The aim of the present study was to investigate the effects of BPA on histology of some vital body organs, such as liver, kidneys and gills of major carp, *C. catla*.

MATERIALS AND METHODS

One year old *C. catla* (mean length: 18.8±1.10 cm; mean weight: 94.4± 5.97g) were purchased from a commercial fish farm (latitude 31° 58’N, longitude 74°13’E) located at 40 km in the suburbs of Lahore, Pakistan. Fish were collected by cast nets from ponds and brought to the laboratory alive in plastic bags that contained pond water and compressed air. Fish were acclimatized for 15 days in concrete tanks. Physico-chemical parameters, such as water temperature, dissolved oxygen, electrical conductivity and hardness,
were recorded regularly during the 15 days of acclimation.

After acclimatization, fish were divided into five groups of 10 fish per group. One group served as control, while the other four groups: I, II, III, and IV were exposed to sub-lethal doses (1, 2, 3, and 4 ppm) of BPA, respectively. Bisphenol A was purchased from Sigma (USA) and its different dilutions were prepared in ethanol. Control group was exposed to the maximum level of ethanol used for BPA dilution. Fish were exposed to the toxicant for 15 days; the toxicant solutions were replaced every other day. The experiment was conducted in semi-static condition, following OECD guideline number 203 (OECD, 1992).

After 15 days, fish were removed from the tanks by a scoop net and anesthetized immediately. Clove oil was used as an anesthesia (Berka, 1986; Kaiser et al., 2006). Anesthesia was prepared fresh by dissolving clove oil into absolute alcohol (Merek, Germany) in a ratio of 1:2. This solution was used as a stock for mixing with water. The fish were removed from anesthetic chamber when completely sedated. Total body weight and total body length, was measured before dissection. Afterwards, fish were dissected to remove liver, kidneys and gills and were fixed in 10% buffered formalin following Troyer (1980).

Preserved tissues were dehydrated in various grades of ethanol, cleared in xylene and impregnated with wax (mp; 58 °C). Five microns thick sections were cut using rotary microtome (Leica RM 2165). Tissue sections were stained with haematoxyline and eosine and PAS stains. Stained slides were studied and photographed by high resolution microscope (Leica, Japan) fitted with a digital camera.

**RESULTS**

**Liver:** Teleost fish liver and hepatocytes are structurally similar to mammals but unlike mammalian liver, fish hepatocytes are not arranged in lobes and liver lacks true portal tracts (Genten et al., 2009). Fish liver from control group showed normal architecture (Figure, 1). The hepatocytes were normal in appearance, with central nuclei, arranged in cords around central vein. Cords of hepatocytes were separated by sinusoids. Liver sections after exposure to 1-4ppm of BPA for 15 days showed various histopathological changes in a dose-dependent manner. Fish exposed to 1mg/l of BPA showed ruptured central vein and few ruptured hepatocytes. Hepatocytes arranged in cords showing comparatively normal architecture. Liver of fish exposed to higher concentrations of BPA showed dilated and congested central vein, ruptured hepatocytes with eccentric nuclei, degeneration of hepatocytes, vacuolization and necrosis (Figure, 2). Massive number of macrophage infiltration was noted in fish liver exposed to 4ppm of BPA (Figure, 2, group IV). Many cells degenerated and transformed to eosinophilic patches with no nuclei or deeply stained nuclei.

Figures 3 and 4 show liver sections stained with periodic acid-schiff stain for detection of glycogen content. Liver of fish from control group (Figure, 3) showed large number of glycogen granules (magenta color), whereas those of treated groups (Figure, 4) showed decreased glycogen content in a dose-dependent manner. As the dose increased, decrease in glycogen granules was observed with complete absence in the fish group (IV) exposed to 4ppm of BPA.

**Kidneys:** Teleost kidney is a mixed organ. It functions as hematopoietic, phagocytic, endocrine and excretory organ and has distinct head and trunk region. Functional unit of kidney is nephron which is composed of glomerulus, proximal and distal renal tubules. Photomicrograph of C. catla kidney from control group showed normal slightly spherical glomeruli with proper bowman space. Brush border of proximal tubules and lumen of distal tubules showed normal structure (Figure, 5). Kidney sections of treated groups with various concentrations of BPA showed necrosis and degeneration of glomerulus with complete obliteration of bowmen’s space, shrinkage of tubules and tubule lumen, loss of brush border and decrease in hematopoietic tissue. Severe necrosis of tubules was noted in fish kidneys exposed to higher concentration of BPA (Figure, 6).

**Gills:** Histologically gills were composed of primary and secondary lamellae and mucus cells. Chloride cells are less in number or completely absent in fresh water teleosts. Figure 7 shows gills from control group, with primary lamellae having chondrocyte skeleton and parallel thread-like secondary lamellae. Secondary lamellae comprise of pillar cells with a protective covering of mucus cells. Several undifferentiated basal cells were noted in gills of fish from control group.

Gills showed histopathological alterations when exposed to BPA stress. The anomalies included hypertrophy and hyperplasia of epithelial cells that resulted in shortening, curling and clubbing of secondary lamellae with increased number of mucus cells (Figure, 8). Degeneration of chondrocytes and pillar cells, necrosis and fiber formation in primary lamellae was observed in gills of fish exposed to BPA.
Figure 1. *Catla catla* fish liver tissue from control group: Central vein (CV); Epithelial layer (EP); Hepatocytes with central nucleus (H); Red blood cells (RBC); Sinusoids (S). H & E stain, 400X.

Figure 2. *Catla catla* fish liver tissue of four groups exposed to 1 ppm to 4 ppm of Bisphenol A (group I, II, III and IV, respectively): Hemolysis (H); Dilated sinusoids (DS); Ruptured hepatocytes (rH); Vacuolization (V); Ruptured central vein (rCV); Congestion (C); Necrosis (N); Degeneration (D); Edema of hepatocytes (E); Inflammation (I). H & E stain, 400 X.
Figure 3. Glycogen granules (G) of liver from control group of *Catla catla* fish in magenta color. PAS staining 400 X.

Figure 4. Decrease in glycogen granules (magenta color) in *Catla catla* fish liver exposed to 1 to 3 ppm of Bisphenol A (group I, II, and III). Note complete absence of glycogen granule (magenta color) from the fish liver exposed to 4 ppm of BPA (group IV). PAS staining 400X.
Figure 5. Kidney tissue from control group of *Catla catla* fish: Glomerulus (G); visceral epithelium of the renal capsule (Ep); Bowmen’s space (Bs); Hematopoietic tissue (H); Distal tubules (Dt); Transverse section of Proximal tubule (Pt); Longitudinal section of Proximal tubule (Pt*); Brush border (Bb). H&E stain, 400X.

Figure 6. Kidney tissue of *Catla catla* fish exposed to 1ppm to 4ppm of Bisphenol A (groups I, II, III and IV, respectively): Vacuolization (V); Degeneration (D); Hematopoietic tissue (H); Macrophage infiltration (M); Shrinkage of lumen of tubules (S); Degeneration (D); Rupture of glomerulus (R); Necrosis (N). H & E stain, 400X.
Figure 7. Gill tissue of *Catla catla* fish control group: Epithelial covering (Ep); Distal end of Primary lamella (Ds); Blood vessel (Bv); Chondrocytes (C); Extracellular matrix (Em); Primary lamella (Pl); Secondary lamella (Sl); Pillar cells of secondary lamella (Pc); Mucous cells (M); Central venous sinus (Vs); Undifferentiated basal cells (Ub). H&S stain, 400X.

Figure 8. Gill tissue of *Catla catla* fish exposed to 1ppm to 4ppm of Bisphenol A (groups I, II, III and IV, respectively): Increase in mucous cells (IM); Uplifting of primary lamella (Upl); Curling of secondary lamella (Cu); Uplifting of epithelium (Ue); Vacuolization in secondary lamella (V); Clubbing of secondary lamella (Cl); Fusion of secondary lamella (Fu); Fiber formation in Primary lamella (Fi); Degeneration of Chondrocytes (Dc); Degeneration of pillar cells (Dp). H&E stain, 400X.
DISCUSSION

Histopathological investigation is a useful tool for determining effects of different anthropogenic pollutants on organisms. Histopathological biomarkers reflect the overall health status of population in an ecosystem (Khoshrood \textit{et al.}, 2010). Various anthropogenic wastes are released in water bodies adversely affecting aquatic life, especially fish. Histopathological changes in more than one tissue are always instructional in assessment of the biological effects of a toxicant and allow for diagnoses of the observed changes (Adeyemo, 2008). Toxic potential of a toxicant is directly related with severity of damage, it causes.

Bisphenol A is an anthropogenic endocrine disrupting chemical. Previously, studies have been conducted to elucidate the effects of various anthropogenic toxicants on fish liver, kidneys and gills (Osman \textit{et al.}, 2007).

Fish liver is the main organ for detoxification of xenobiotics, including BPA. Therefore, the changes in liver of aquatic fauna such as fish are reflective of aquatic pollution of their habitat (Moon \textit{et al.}, 2012). Liver histology is highly sensitive and is an accurate way to assess the effect of any pollutant on fish. In the present study, \textit{C. catla} exposed to different concentrations of BPA showed changes in normal architecture of liver, dilated blood vessels and sinusoids, congestion of central vein, increased vacuolization and necrosis. El-jawaher (2012) observed similar changes in hepatocytes of \textit{Oreochromus spilurus} exposed to nonylphenol (endocrine disrupting chemical). Change in normal liver architecture with dilated sinusoids may be due to loss of structural proteins. Abdelaziz \textit{et al.} (2006) observed abnormal liver architecture of \textit{Siganus rivulatus} exposed to heavy metals. Vacuolization of hepatocyte is a nonspecific response of fish due to toxic conditions (Roberts, 1978). The vacuolization of hepatocytes might indicate an imbalance between the rate of synthesis of substances in the parenchyma cells and the rate of their release into the circulation (Gingerich, 1982). Blood flows from hepatic portal vein and hepatic artery into the central vein, congestion in the central vein makes flow of blood difficult. Cellular degeneration and necrosis may be as a result of the congestion of central vein. Present study revealed that BPA caused cytoplasmic degeneration and rupture of central vein. Radhialah and Rao (1992) reported hepatocyte degeneration, ruptured blood vessels, vacuoles formation, and pyknotic nuclei in liver of \textit{Tilapia mossambica} exposed to the insecticide, chlorpyrifos (Tilak \textit{et al.}, 2005). Results of the present study revealed decrease in glycogen content in liver of fish exposed to BPA. Glycogen decrease was directly dose-related. Murty and Priyamvada-Devi (1982) observed decreased lipid, protein and glycogen levels in liver of \textit{Channa punctatus} exposed to endosulphane. Similar decrease in liver glycogen was recorded by Gluth and Hanke (1985) in \textit{Cyprinus carpio} exposed to different insecticides.

Fish gills are important organ for respiration and ionic regulation and because of their high permeability and contact area with water, gills are considered to be an efficient tool for bio-monitoring potential impacts of pollutants (Zeeman and Brindley, 1981; Schwaiger \textit{et al.}, 1997; Oliveira Ribeiro \textit{et al.}, 2005; Vigliano \textit{et al.}, 2006). In the present study, gills of fish in the control group showed normal structure, while BPA-exposed fish groups showed degenerative changes in their gills. Severity of damage increased with increasing concentration of BPA. Gill anomalies of fish exposed to BPA included hyperplasia, fusion, clubbing and uplifting of secondary lamellae and degeneration of primary lamellae. Tietge \textit{et al.}, (1988) reported that hyperplasia (increase in cells of the secondary lamellae) and epithelial lifting (elevation of the external layer of the lamellar epithelium) are protective mechanisms of fish towards pollutants. In the present study, epithelial lifting and hyperplasia was observed in gills exposed to a gradient of BPA concentrations. The first change in the gills under acute exposure to the toxicant included lifting of the lamellar epithelium. Similar epithelium lifting was observed by Muller and Lloyd (1994) and Heath (1995) in fish gills exposed to oils, ammonia, detergents, acids, and metals like mercury, and phenols.

Fusion and clubbing of secondary lamellae were observed in fish gills exposed to 2ppm of BPA. Similar changes in fish gills were recorded as a response to copper by Arellano \textit{et al.}(1999), effluents from a bleaching paper mill (Pacheco and Santos, 2002) and sewage from a secondary treatment plant (Coutinho and Gokhale, 2000). Figueiredo-Fernandes \textit{et al.} (2007) explained that fusion of some secondary lamellae causes reduction of the branchial superficial area that is in contact with the external environment and this fusion is an example of defense mechanism.

When exposed to higher concentrations of BPA, gill cells underwent degeneration and necrosis. Mazon \textit{et al.} (2002) had noted similar changes in gill epithelium of fresh water fish, \textit{Prochilodus scrofa}, when exposed to copper. Gill degeneration and necrosis reflects direct effect of pollutants on fish health (Garcia-Santos \textit{et al.}, 2007).

Fish kidneys are the major hematopoietic and osmoregulatory organs. Altered fish histology is a good indicator of environmental pollution because largest proportion of post-branchial blood goes to fish kidneys (Cengiz, 2006).

Many studies used histological characteristics of kidney as an indicator of pollution especially, the
nonylphenol (Srivastava et al., 1990; Banerjee and Bhattacharya, 1994; Ortiz et al., 2003; Cengiz, 2006). Results from present work revealed histological changes in C. catla kidneys after exposure to BPA were necrosis, hypertrophy of glomerulus, degeneration and dissociation of renal tubules and Bowman’s capsule, proliferation in the renal tubule and haemopoietic tissue, shrinkage of glomerulus, pyknosis, dilated blood vessel, rupture of Bowman’s capsule, and obliterated Bowman’s space. Similar results were reported in fishes after exposure to other pollutants (Cengiz, 2006; Khidr and Mekkawy, 2008). Very limited literature is present regarding the histological effects of BPA on fish tissues. Therefore, the results obtained and reported here are the first for this EDC on C. catla and will be useful for future work in elucidating the detailed effects of BPA.

REFERENCES


