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HISTOPATHOLOGICAL EFFECTS OF BISPHENOL-A ON LIVER, KIDNEYS AND GILLS OF INDIAN MAJOR CARP, *CATLA CATLA* (HAMILTON, 1822)

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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 world-wide 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 Hughies, 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'- isopropylidine 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 (Merck, 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 ^{0}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 3and 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 hematopoetic, 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 mucous 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 mucous 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 mucous cells (Figure, 8). Degeneration of chondrocytes and piller cells, necrosis and fiber formation in primary lamellae was observed in gills of fish exposed to BPA.

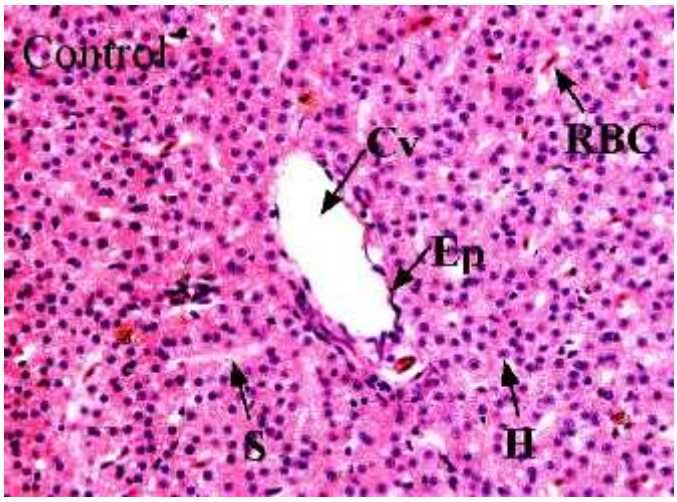

Figure 1.*Catla catla* fish liver tissue from control group: Central vein(CV); Epithelial layer(EP); Hepatocytes with central nucleus(H); Red blood cells(RBC); Sinusoids(S). H & E stain, 400X.

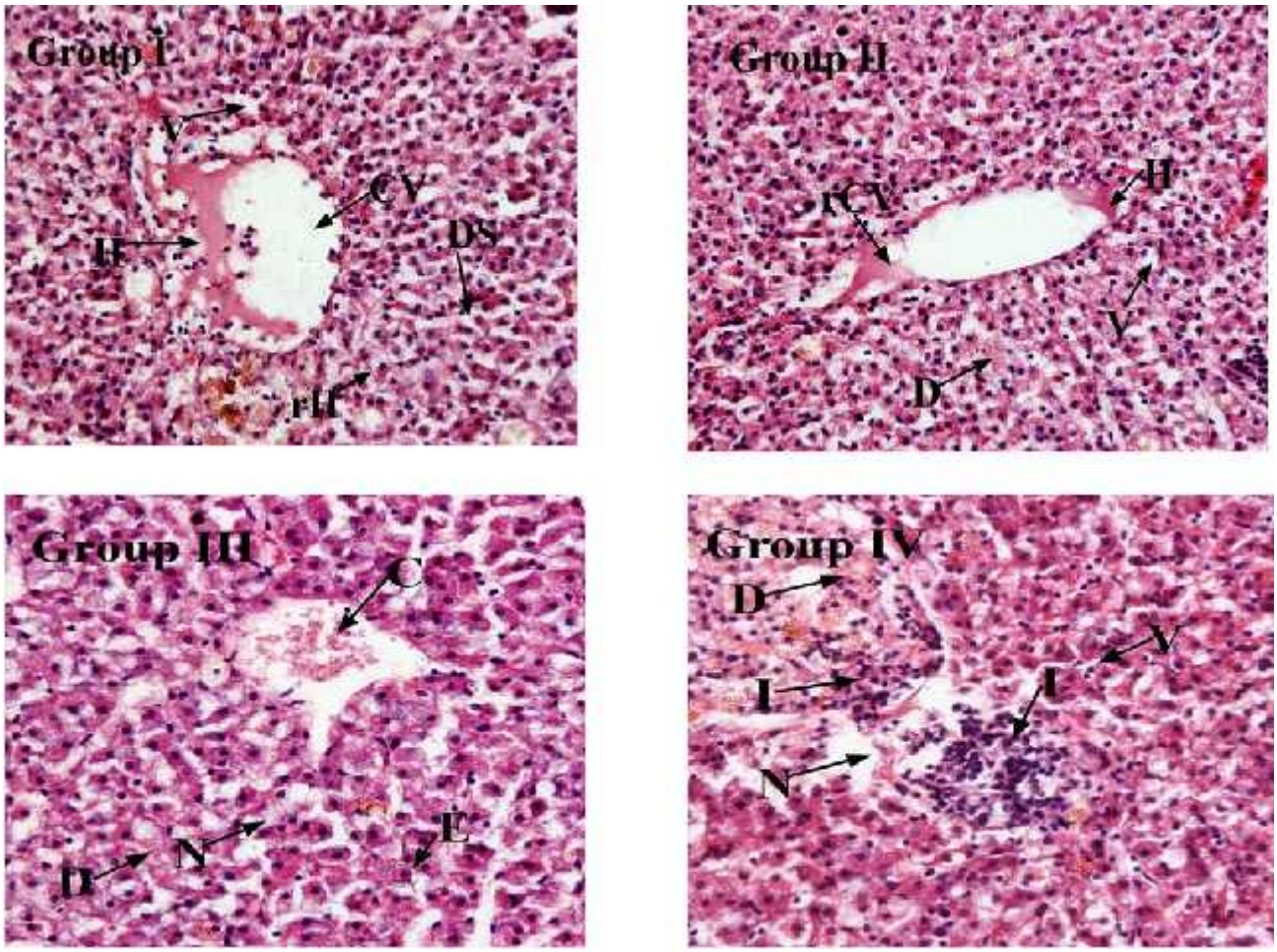

Figure 2.*Catla catla* fish liver tissue of four groups exposed to 1 ppm to 4 ppm of Bisphenol A (group I, II, III and IV, respectively): Hemolysis (H); Dilated sinusoids (DS); Ruptured hepatocytes (rH); Vacuolization (V); Ruptured central vein (rCV); Congestion (C); Necrosis (N); Degeneration (D); Edema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

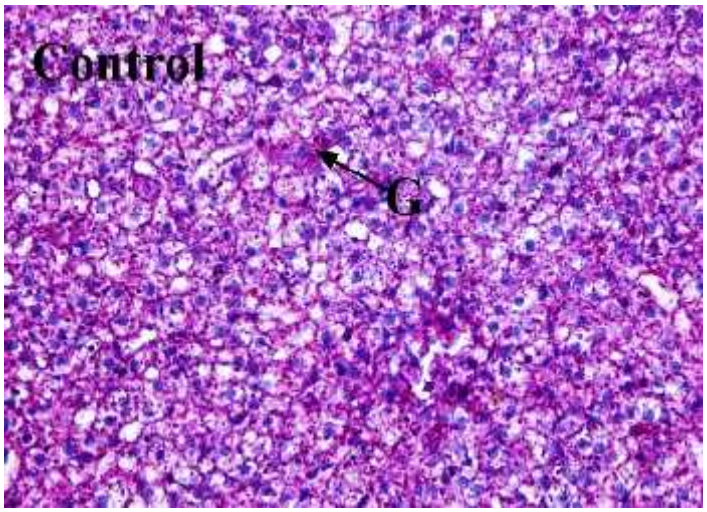

Figure 3. Glycogen granules (G) of liver from control group of *Catla catla* fish in magenta color. PAS staining 400 X.

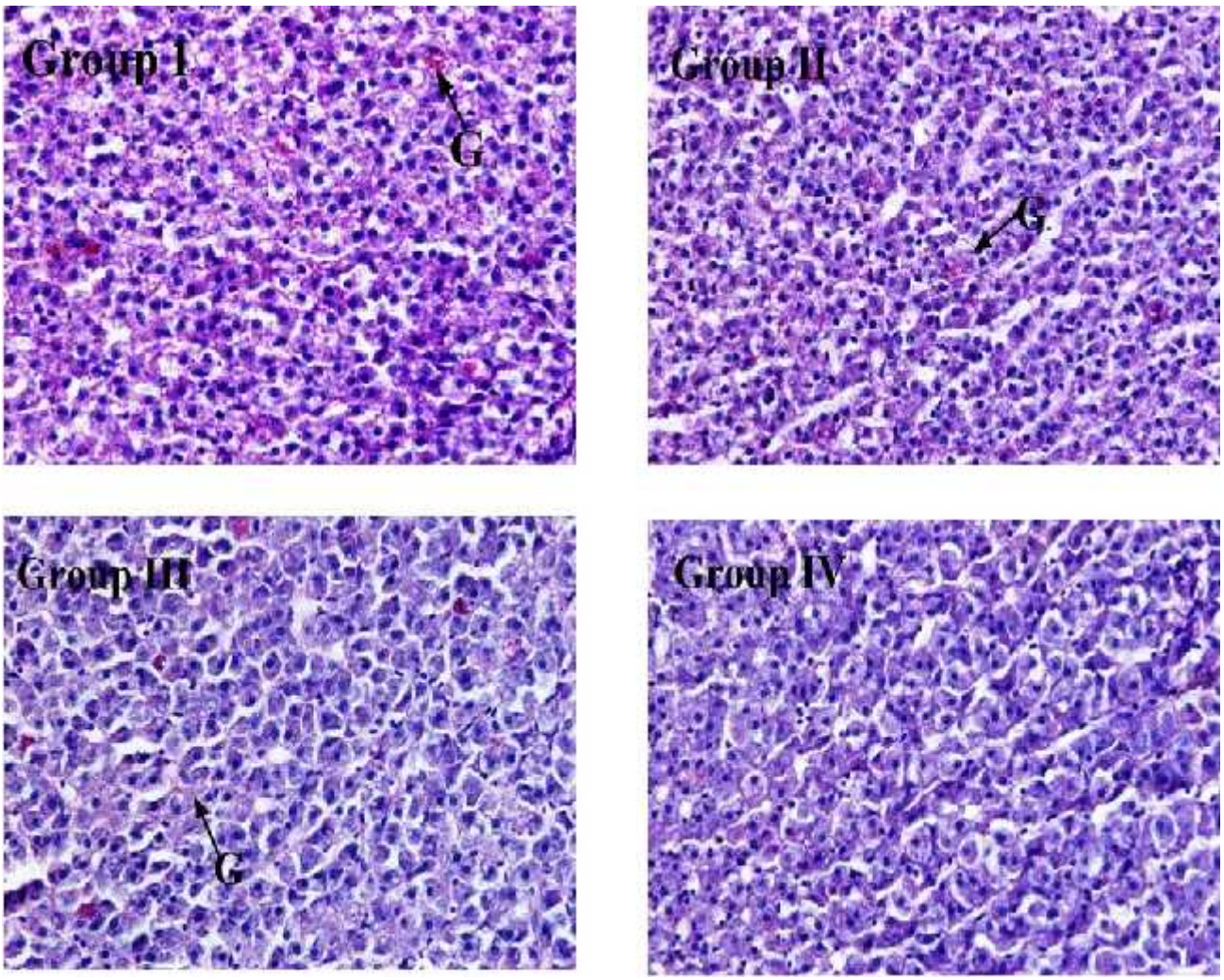

Figure 4. Decrease in glycogen granules (magenta color) in *Catla catla* fish liver exposed to 1 to3 ppm of Bisphenol A (group I, II, and III). Note complete absence of glycogen granule (magenta color) from the fish liver exposed to 4ppm of BPA (group IV). PAS staining 400X.

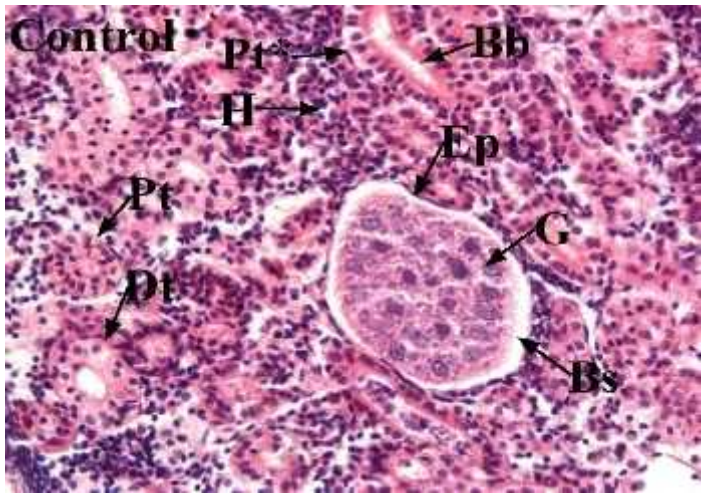

Figure 5. Kidney tissue from control group of *Catla catla* fish: Glomerulus(G); visceral epithelium of the renal capsule (Ep); Bowmen's space (Bs); Hematopoietic tissue (H); Distal tubules (Dt); Transverse section of Proximal tubule (Pt); Longitudinal section of Proximal tubule (Pt*); Brush border (Bb). H&E stain, 400X.

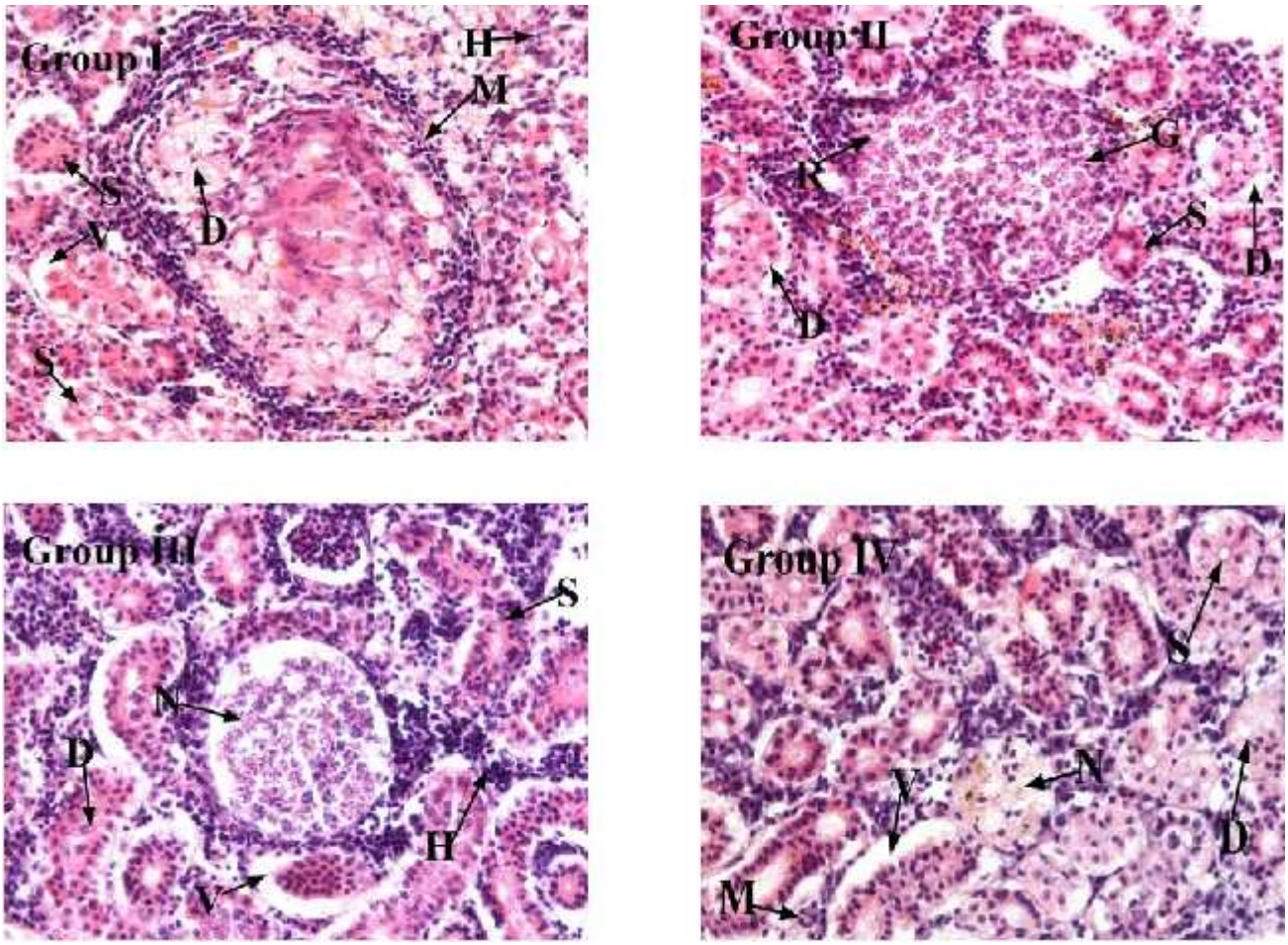

Figure 6. Kidney tissue of *Catla catla* fish exposed to 1ppmto 4ppm of Bisphenol A (groups I, II, III and IV, respectively): Vacuolization (V); Degeneration (D); Hematopoietic tissue (H); Macrophage infiltration (M); Shrinkage of lumen of tubules (S); Degeneration (D); Rupture of glomerulus (R); Necrosis (N). H & E stain, 400 X.

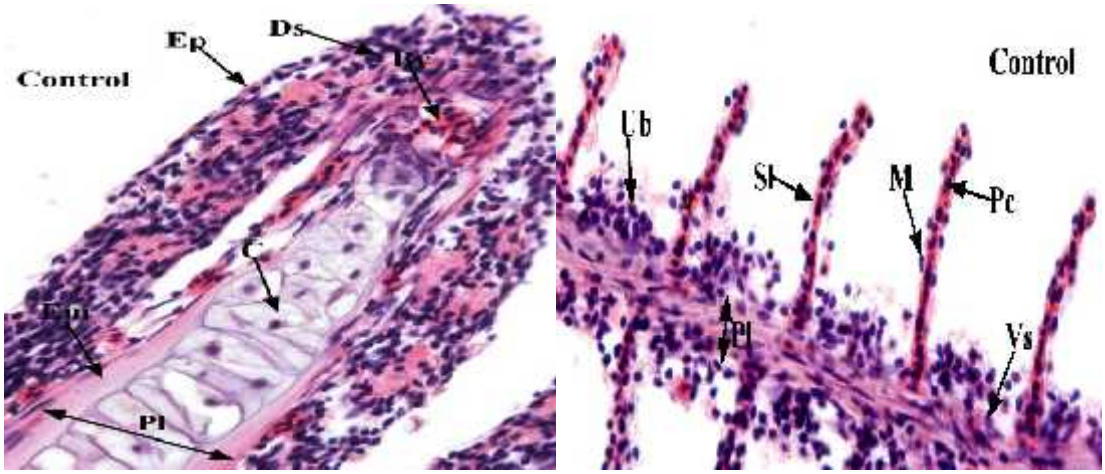

Figure 7. Gill tissue of *Catla catla* fish control group: Epithelial covering (Ep); Distal end of Primary lamella (Ds); Blood vessel (Bv); Chondrocytes (C); Extracellular matrix (Em); Primary lamella (Pl); Secondary lamella (Sl); Pillar cells of secondary lamella (Pc); Mucous cells (M); Central venous sinus (Vs); Undifferentiated basal cells (Ub). H&S stain, 400X.

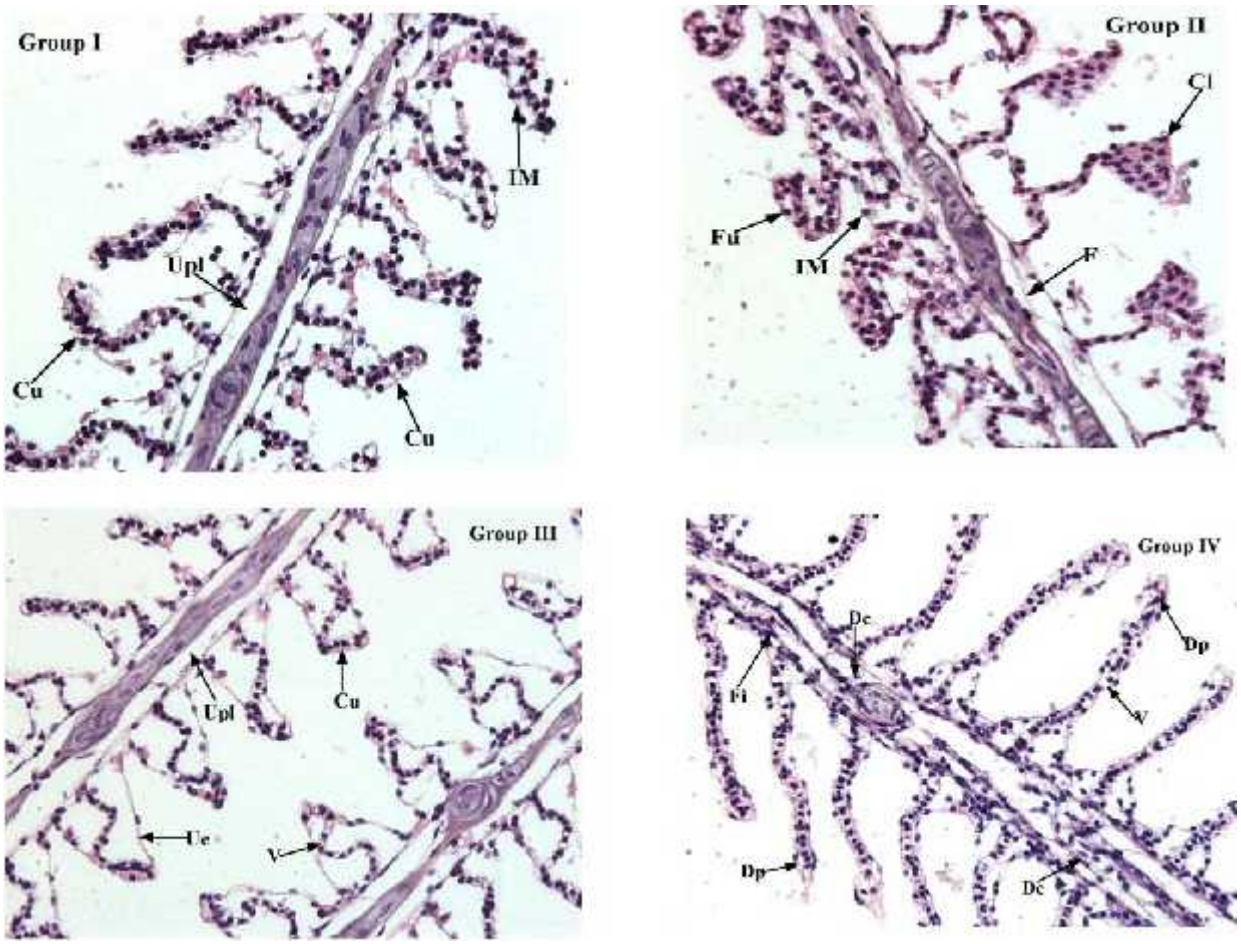

Figure 8. Gill tissue of *Catla catla* fish exposed to 1ppmto 4ppm of Bisphenol A (groups I, II, III and IV, respectively): Increase in mucous cells (IM); Uplifting of primary lamella (Upl); Curling of secondary lamella (Cu); Uplifting of epithelium (Ue); Vacuolization in secondary lamella (V); Clubbing 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. Histopathalogical biomarkers reflect the overall health status of population in an ecosystem (Khoshnood *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 *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 *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, *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 *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 *et al.* (2006) observed abnormal liver architecture of *Siganus rivulates* exposed to heavy metals. Vacuolation 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. Radhaiah and Rao (1992) reported hepatocyte degeneration, ruptured blood vessels, vacuoles formation, and pyknotic nuclei in liver of *Tilapia mossambica* exposed to the insecticide, fenvalerate. Similar changes were observed in the liver of *C. catla* exposed to the organophosphate insecticide, chloropyrifos (Tilak *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 *Channa punctatus* exposed to endosulphane. Similar decrease in liver glycogen was recorded by Gluth and Hanke (1985) in *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 *et al.*, 1997; Oliveira Ribeiro *et al.*, 2005; Vigliano *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 *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 *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 *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 *et al.* (2002) had noted similar changes in gill epithelium of fresh water fish, *Prochilodus scrofa*, when exposed to copper. Gill degeneration and necrosis reflects direct effect of pollutants on fish health (Garcia-Santos *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 haemopoieatic 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

Abdel-aziz, S.H., N. El-ghazaly, and G.A. Bindohaish (2006). Effect of pollutants in coastal water of Jeddah on the histological structure of liver of the fish *Siganus rivulatus*. Saudi Arabia. Egypt. J. Aquat. Res., 32: 316-333.

Adeyemo, O.K (2008). Histological Alterations Observed in the Gills and Ovaries of *Clarias gariepinus* exposed to environmentally relevant lead concentrations. J. Environ. Health., 70 (9): 48-51.

Arellano, J.M., V. Storch, and C. Sarasquete (1999). Histological changes and copper accumulation in liver and gills of the Senegales Sole, *Solea senegalensis*. Ecotoxicol. Environ. Saf., 44: 62–72.

Banerjee, S. and S. Bhattacharya (1994). Histopathology of kidney of *Channa punctatus* exposed to chronic nonlethal level of elsan, mercury and ammonia. Ecotoxicol. Environ. Saf., 29 (3): 65–275.

Berka, R (1986). The transport of live fish. A review. EIFAC Technical Paper 48. Food and Agriculture Organization of the United Nations, Rome, pp.51.

Cengiz, E. I (2006). Gill and kidney histopathology in the freshwater fish *Cyprinus carpio* after acute exposure to deltamethrin. Environ. Toxicol. Pharm., 2: 200–204.

Coutinho, C. and K. S. Gokhale (2000). Selected oxidative enzymes and histopathological changes in the gills of *Cyprinus carpio* and *Oreochromis mossambicus* cultures in secondary sewage effluent. Water Res., 34, 2997–3004.

El-jawaher, A. B. D (2012). The effects of 4-nonylphenol contamination on liver of Tilapia fish (*Oreochromus spilurus*) in Jeddah. Biol. Res, 45: 15-20.

Figueiredo-Fernandes, A., J.V. Ferreira-Cardoso, S. Garcia-Santos, S.M. Monteiro, J. Carrola, P. Matos, and A. Fontainhas Fernandes (2007). Histopathological changes in liver and gill epithelium of Nile tilapia, *Oreochromis niloticus*, exposed to waterborne copper. Pesqui. Vet. Bras., 27 (3), 103– 109.

Garcia-Santos, S., S. M. Monteiro, and J. E. Carrola and A. Fontainhas-Fernandes (2007). Histological alterations in gills of Nile tilapia *Oreochromis niloticus* caused by cadmium. Braz. J. Vet. Anim. Sci., 59 (2), 376–381.

Genten F., E. Trewinghe, and A. Danguy (2009). Digestive System. In: Atlas of Fish Histology, Science Publishers. 75-91.

Gingerich, W.H (1982). Hepatic Toxicology of Fishes, In: L. J. Weber, Ed., Aquatic Toxicology, Raven Press, New York. pp. 55-105.

Gluth, G. and W. Hanke (1985). A comparison of physiological changes in carp, *Cyprinus carpio*, induced by several pollutants at sublethal concentrations. The dependency on exposure time. Ecotoxicol. Environ. Saf., 9:179-188.

Heath, A.G (1995). Water Pollution and Fish Physiology. Boca Raton, FL: CRC Press, Lewis Publisher.

Kaiser, H., G. Brill, J. Cahill, K. Collet, K. Czypionka, K. Green, P. Orp, R. Pattrick, R. Scheepers, M. Stonier, A. Whitehead, and R. Yearsley (2006). Testing clove oil as an anesthetic for long-distance transport of live fish: the case of the lake Victoria Cichlid *Haplochromis obliquidens*. J. Appl. Ichthyol., 22: 510-514.

Kazi, T.G., N. Jalbani, J.A. Baig, G.A. Kandhro, H.I. Afridi, B.M. Arain, M.K. Jamali and A.Q. Shah (2009). Assessment of toxic metals in raw and processed milk samples using electrothermal atomic absorption spectrophotometer. Food. Chem. Toxicol., 47 : 2163 -2169.

Khidr, M. B. and I.A.A. Mekkawy (2008). Effect of separate and combined lead and selenium on the liver of the cichlid fish *Oreochromis niloticus*: ultrastructural study. Egypt. J. Zool., 50: 89-119.

Khoshnood, Z., S. Khodabandeh, S. Mosafer, and R. Khoshnood (2010). Effects of Cortisol on Gill Chloride Cells in Persian Sturgeon, *Acipenser persicus*, Fry. Yakhteh., 11(4): 424-431.

Lone, K.P., S. Fatima, and S. Sahar (2009). Gross and histological variations in testes of a major carp, *Catla catla* (Hamilton, 1822), during its first maturation cycle in pond culture system. Pakistan J. Zool., 41(6): 483-494.

Mazon, A. F., C.C. Cerqueira, and M.N. Fernandes (2002). Gill cellular changes induced by copper exposure in the South American tropical freshwater fish *Prochilodus scrofa*. Environ. Res., 88:52–63.

McGlashan, D.J., and J.M. Hughies (2001). Genetic evidence for historical continuity between populations of the Australian freshwater fish *Craterocephalus stercusmuscarum* (Atherinidae) east and west of the Great Diving Range. J. Fish. Biol., 59: 55–67.

Metzler, M. and P. Erika (2001). Chemistry of natural and anthropogenic endocrine active compounds. In: The Handbook of Environmental Chemistry, Vol 3, Part L, Endocrine Disruptors. Ed Springer -Verlag, Berlin Heidelberg.

Moeller, H (1985). "A Critical Review on the Role of Pollution as a Cause of Fish Diseases," In: A. E. Ellis, Ed., Fish and Shellfish Pathology, European Association of Fish Pathology, Academic Press, London, 1985, pp. 169-182

Moon, M.K., M.J. Kim, I.K. Jung, Y.D. Koo, H.Y. Ann, K.J. Lee, S.H. Kim, Y.C. Yoon, B.J.Cho, K.S. Park, H.C. Jang, and Y.J. Park (2012). Bisphenol A impairs mitochondrial function in the liver at doses below the no observed adverse effect level. J. Korean. Med. Sci., 27: 644-652.

Müller, R., and R. Lloyd (eds.). (1994). Sublethal and Chronic Effects of Pollutants on Freshwater Fish. FAO and Fishing News Books, Oxford, UK. 371 p.

Murty, A.S., and A. Priyamvada Devi (1982). The effect of endosulfan and its isomers on tissue protein, glycogen and lipids in fish (*Channa punctatus*). Pestic. Biochem. Physiol., 17: 280-286.

Oliveira Ribeiro, C.A., Y. Vollaire, A. Sanchez-Chardi, and H. Roche (2005). Bioaccumulation and the effects of organo- chlorine pesticides, PAH and heavy metals in the eel (*Anguilla anguilla*) at the Camargue Nature Reserve, France. Aquat. Toxicol., 74: 53–69.

Organization for Economic Cooperation and Development (1992). Fish acute toxicity test. Test Guideline 203. OECD Guidelines for the Testing of Chemicals. Paris, France

Ortiz, J.B., M.L.G. De Canales, and C. Sarasquete (2003). Histopathological changes induced by lindane (gamma-HCH) in various organs of fishes. Sci. Mar., 67 (1): 53–61

Osman, A.G.M., I.A. Mekkawy, J. Verreth, and F. Kirschbaum (2007). Effects of lead nitrate on the activity of metabolic enzymes during early developmental stages of the African catfish, *Clarias gariepinus* (Burchell, 1822). Fish. Physiol. Biochem., 33:1-13.

Ozden, O (2010). Micro, macro mineral and proximate composition of Atlantic bonito and horse mackerel: a monthly differentiation. Int. J. Food. Sci. Tech., 45:578-586.

Pacheco, M. and M.A. Santos (2002). Biotransformation, genotoxic, and histopathological effects of environmental contaminants in European eel (*Anguilla anguilla*). Ecotoxicol. Environ. Saf., 53: 331–347.

Radhaiah, V. and J.K. Rao (1992). Fenvalerate toxicity to the liver in a freshwater teleost, *Tilapia mossambica* (Peters). Comp. Physiol. Ecol., 17(2): 48-53.

Roberts, R.J (1978). Fish pathology. Bailliere tindall, London, pp. 489.

Routledge, E. J.,D. Sheahan, C. Desbrow, G.C. Brighty, M. Waldock and J.P. Sumpter (1998). Identification of estrogenic chemicals in STW effluent. 2. In vivo responses in trout and roach. Environ. Sci. Technol., 32: 1559-1565.

Schwaiger, J., R.S. Wanke, S. Adam, M. Pawert, W. Honnen, and R. Triebskorn (1997). The use of histopathological indicators to evaluated contaminant related stress in fish. J. Aquat. Ecosyst. Stress recovery., 6: 75–86.

Srivastava, S. K., P.R. Tiwari, and A.K. Srivastav (1990). Effects of chlorpyrifos on the kidney of freshwater catfish. *Heteropneustes fossilis*. Bull. Environ. Contam. Toxicol., 45: 748–751.

Staples, C.A., P.B. Dome, G.M. Klecka, S.T. Oblock, and L.R. Harris (1998). A review of the environmental fate, effects, and exposures of bisphenol A. Chemosphere., 36 (10): 2149–73.

Sumpter, P. J (2002). Endocrine disruption in the Aquatic Environment. In: The Handbook of Environmental Chemistry, Vol 3, Part M, Endocrine Disruptors. Ed. Springer-Verlag, Berlin Heidelberg.

Tietge, J. E., R. D. Johnson, and H. L. Bergman (1988). Morphometric changes in gill secondary lamellae of brook trout (*Salvelinus fontinalis*) after long term exposure to acid and aluminum. Can. J. Fish. Aquat. Sci., 45: 1643–1648.

Tilak, K.S., R. Koteswara, and K. Veeraiah (2005). Effects of chlorpyrifos on histopathology of the fish *Catla catla*. J. Ecotoxicol. Environ. Monit.,15(2): 127-140.

Troyer, H (1980). Principles and technology of histochemistry. Little Brown , (Boston).431p.

Vigliano, F.A., N. Aleman, M.I. Quiroga and J.M. Nieto (2006). Ultrastructural Characterization of Gills in Juveniles of the *Argentinian Silverside*, *Odontesthes bonariensis* (Valenciennes, 1835) (Teleostei: Atheriniformes). Anat Histol Embryol., 35:76–83.

Zeeman, M.G., and W.A. Brindley (1981). Effects of toxic agents upon fish immune systems: a review. In: Immunologic Considerations in Toxicology. (R. P. Sharma, ed.).Vol. II Boca Raton, FL: CRC Press. Lewis Publisher. pp. 1–60.