New Insights Into Hematological, Serum Biochemical and Histopathological Toxicity of Bisphenol a on Bighead Carp (*Aristichthys Nobilis*) Under Long-Term Exposure

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Research Article

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Abstract

Bisphenol A (BPA) is one of the highest volume chemicals produced worldwide and is frequently used in dental sealants, water bottles, food and beverage packaging. Due to persistent application, BPA has become a potential threat to a variety of organisms including public health. In this study, for the first time 80 bighead carps were randomly placed in different four groups (A-D). Fish in groups (B-D) were treated with BPA for 60 days while fish in group A served as control group. Body weight, absolute and relative weight of different visceral organs of fish exposed to higher concentrations (1500 µg/L) of BPA decreased significantly (p < .05). Results on proximate analysis showed significantly decreased in crude proteins, lipid contents and moisture contents in muscles while increased ash contents. Red blood cells count, hemoglobin concentration, lymphocytes and monocytes were significantly decreased while leukocytes counts and neutrophil counts were significantly increased in treated fish. Results on different serum biochemistry parameters like serum albumin and total proteins decreased significantly (p < .05) while alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), urea creatinine, glucose, cholesterol and lactate dehydrogenase (LDH) increased significantly (p < .05) in treated fish. Histopathological ailments like pyknosis, degeneration glomeruli, increased Bowman's space, ceroid formation in kidneys while ceroid formation, hemorrhages, pyknosis, karyorrhexis, karyolysis, binucleated hepatocytes, nuclear hypertrophy and eccentric nuclei in liver were observed in treated fish. Histological observation of different sections of brain of treated fish exhibited degenerated neurons in cerebellum, lipofuscin deposition, microgliosis, necrotic neurons, inflammatory cell and severe hemorrhage. Results on light microscopic observation of different sections of heart of bighead carp revealed necrosis, inflammatory reaction, neutrophilic myocarditis and hemorrhages. In conclusion, it is suggested that BPA induces adverse effects on physical, blood-biochemical parameters and histopathological changes in multiple visceral tissues of exposed fish.

Introduction

Over the past few decades in addition to the pathogenic risk of microbes, the indiscriminate application of synthetic chemicals in agriculture, aquatic life, industries, veterinary practice, protection of environment and to improve public health has become serious threat (Arslan et al., 2017; Richardson et al., 2017; Leem et al., 2017; Hussain et al., 2019). Accidental exposure to industrial chemicals and various environmental pollutants in aquatic and terrestrial ecosystems not only causes deaths but also reduces the life expectancy of several target and non-target organisms (Glassmeyer et al., 2016; Richardson et al., 2017; Ghaffart et al., 2019; Ghaffar et al., 2020). Several studies have indicated that the organisms in aquatic ecosystem are at huge risk than the terrestrial organisms as variety of synthetic compounds from multiple sources including industries and agriculture is shifted directly and easily to water bodies (Sivashanmugam et al., 2017; Verma et al., 2017; Amaroli et al., 2018; Baralic et al., 2019). Many of the environmental contaminants such as by-products of disinfection, fluorinated substances, bisphenol A, phthalates, pesticides and synthetic estrogens are endocrine disruptive (Smarr et al., 2016; Adoamnei et al., 2018; Barakat et al., 2017; Rattan et al., 2017; Karwacka et al., 2017). Exposure to these pollutants
causes disturbances in biodiversity, food web, loss of habitat and multiple organ dysfunctions leading to poor reproductive performance of different organisms (Hussain et al., 2018; Hussain et al., 2019; Rubin et al., 2019; Scarano et al., 2019; Xu et al., 2018; Zhou et al., 2019).

Bio-monitoring and epidemiological reports have shown that bisphenol A is frequently used in paints, protective coatings, mechanical parts and as a liner in plastic food and beverage containers (Prins et al., 2017; Murata et al., 2018; Willhite et al., 2019). It has been reported that the demand of BPA at international market has expanded beyond 10 million tons (Vandenberg et al., 2013, 2019). BPA has been detected in surface water, soil, sediments and different aquatic organisms (Karthikraj et al., 2017; Sun et al., 2017; Wanda et al., 2017; Pal and Reddy, 2018; Staples et al., 2018). Studies have reported that BPA quickly and easily leaches into water (Flint et al., 2012; Bavinck, 2018) and its peak levels have been detected in landfill leachates (17.2 mg/L) and 21 μg/L in surface waters (Flint et al., 2012; Huang et al., 2012). The concentrations of BPA (4.4 to 8000 ng/L) have been detected in fish (0.19 to 25.2 mg/kg) obtained from Taiwan (Ching-Chang et al., 2015), 0.5 to 6 mg/kg in fish from Italy (Mita et al., 2011) and in marine fish 0.83–19.25 from Hongkong (Wong et al., 2017). Among aquatic organisms, fish are considered the most susceptible species to different endocrine disruptive chemicals and are useful biomarkers to track the quality of aquatic environment (Eggen et al., 2003; Qin et al., 2013; Faheem and Lone, 2017). The exact mechanism of toxicity of BPA in exposed organisms is not clear and is still under debate. However, previous studies have shown that PBA induces its toxic effects via rapid generation of free radicals and oxidative stress leading to multiple abnormalities in different tissues of organisms (Bavinck et al 2018; Faheem and Lone, 2017). Moreover, reports are available about the physiological and Bisphenol A may enter into aquatic animals via ingestion and dermal contact with polluted water induces behavior and physical alterations such as loss of locomotion and swimming patterns. Furthermore, few reports are available about the molecular changes due to bisphenol A in liver, brain, kidneys, gills reproductive tissues of fish (Kumari and Khare, 2018; Wei et al., 2018). Hematological and biochemical parameters can be used as useful bio-indicators in fish and are well known targeted organs of toxicity after exposure to xenobiotics (Singh and Srivastava, 2010; Gul et al. 2017; Reddy, 2017; Ratn et al, 2018; Sisodiya et al., 2018). Moreover, estimation of variations in biochemical parameters such as protein, enzymes and glucose are commonly used to evaluate the physiological changes in aquatic environments to explain stress conditions (Abdel-Latif and Khashaba 2017; Abdel-Tawwab and Hamed, 2018). The toxicity of BPA on growth (Abdel-Tawwab et al., 2018), reproduction (Kim et al., 2019), gene expression (Cervantes-Camacho et al., 2020), behavior (Faheem and Lone, 2018) and oxidative stress (Zhang et al., 2020) in fish has been well documented. But to the best of our knowledge, the toxicity of BPA on fresh water fish particularly in Bighead carp is scanty. Therefore, in present study, we attempted to evaluate the toxicity of BPA at sublethal concentrations in bighead carp (Aristichthys nobilis).

**Materials And Methods**

**Toxicant and chemicals**
Analytical grade bisphenol A (BPA) with purity (99.0%) was obtained from the chemical market Lahore, Pakistan. All the other chemicals (analytical grades) were purchased from Sigma Aldrich (St. Louis Missouri, USA) and Merck (Germany). Different commercial kits used for serum analyses were purchased from Randox Company (Pvt.) Pakistan. Initially different stock solutions of bisphenol A were prepared by dissolving in absolute alcohol.

**Experimental species and management**

Bighead carp (*Aristichthys nobilis*) approximately of 150-175 g body weight, same age and length (7-8 cm) were purchased from fish breeding center district Bahawalnagne, Punjab province, Pakistan. All the fish were transported in plastic bags supplemented with enough amount of oxygen and stocked at the laboratory of the department of life sciences (Zoology), Islamia University of Bahawalpur. All the fish were placed in tap water in glass aquaria (14” L × 10” W × 12” H) for two weeks for acclimatization purposes. During this period, the fish were offered commercial fish feed containing crude proteins (22% proteins) and groundnut oil cake in the form of pellets. The feed (2-3% of body weight) was provided daily twice a time. The remaining feed and fecal materials from all the aquaria were removed on daily basis. The water chemistry measurements were determined (Table 1).

**Experimental treatments**

After 14 days of acclimatization, all the fish were randomly divided and kept in four different groups (A-D). Each group contained 20 fish. The trial was operated in four glass aquaria containing 100 L water. The fish of aquarium A was maintained as control while fish present in the other three aquaria was exposed to different concentrations of analytical grade bisphenol A (BPA) with purity (99.0%) dissolved in absolute alcohol. The fish in groups B, C and D were exposed to 500 µg/L, 1000 µg/L and 1500 µg/L Bisphenol A for a period of 60 days on the basis of earlier trial (Fukuhori et al. 2005; Huang et al. 2018). During all the experimental duration and toxicity testing, fish kept in different aquaria were fed ad libitum. The rejected feed was strained and removed on daily basis. The fecal material from each aquarium was also removed to prevent water contamination.

**Body mass, organ weight and histopathology**

For estimation of body mass, organ weight and histopathological changes, five fish from each group were randomly weighed, killed and dissected at days 15, 30, 45 and 60 of the trial. Different tissues such as liver, kidneys, gills and brain were removed, weighed and preserved in 10% formaldehyde solution. The absolute and relative weight (% of body weight) of different organs including brain, gills, liver, and kidneys was determined at days 15, 30, 45 and 60 of the trial. For histopathological changes, visceral processed using Hematoxylin and Eosin staining procedures (Hussain et al., 2019).

**Hematological studies**

About 2.5 ml blood was collected from the caudal vein of each fish with sterile 26 gauge hypodermic needle. The collected blood was immediately placed in anticoagulant coated glass test tubes. Different
hematological parameters including red blood cells counts and total and differential white blood cell counts (Islam et al., 2019) while hematocrit %, hemoglobin quantity and total proteins were measured according to earlier protocols (Hussain et al., 2019; Ghaffar et al. 2020) at days 15, 30, 45 and 60 of the experiment.

**Serum biochemical studies**

For different serological parameters, serum was separated from the blood of each fish placing on ice at different experimental intervals at days 15, 30, 45 and 60 of the experiment. Various serum biochemical parameters including ALT, AST, ALP, LDH, urea, creatinine, gulucose, cholesterol and triglycerides were measured using commercially available kits (Randox company Pvt.) using a chemistry analyzer (Randox company Pvt.).

**Statistical analysis**

Data collected during the trial were presented as mean ± S.E. All the collected data in each group was normally distributed and statistical analysis was carried out by one-way analysis of variance (ANOVA) using IBM SPSS statistics (version 20). The difference in mean values (mean ± S.E) of body weight, organ weight, hematological parameters and serum biochemistry of control and treated groups was conducted by using post hoc Tukey’s test at p < 0.05.

**Results**

**Physical parameters**

The results revealed that the body weight of fish exposed to higher concentrations (1500 µg/L) of BPA decreased significantly (p < .05) compared to control group at day 60 of the experiment (Table 1). The absolute weight of visceral organs of fish such as liver, kidneys and gills increased significantly at higher concentrations (1500 µg/L) of BPA (Table 1). The relative weight of liver, kidneys and gills increased significantly (p < .05) at higher concentrations (1500 µg/L) of BPA while non-significant difference was recorded in relative weight of brain as compared to untreated control fish (Table 2).

**Proximate Analysis**

Proximate analysis of A.nobilis revealed that crude protein and lipid contents significantly decreased in muscles of fish exposed to higher concentrations (1000 µg/L and 1500 µg/L) at days 45 and 60 of the study. Results showed that the moisture contents also decreased significantly in muscles of fish exposed to higher concentrations (1000 µg/L and 1500 µg/L) at day 60 of experiment. Ash contents increased significantly in fish meat exposed to higher concentrations (1000 µg/L & 1500 µg/L) at day 45 and 60 of the experiment (Table 6).

**Hematological and serum analysis**
Results of different hematological parameters of blood cells of fish exposed to various levels of bisphenol A are presented in Table 2. The fish exposed to 1000 µg/L and 1500 µg/L bisphenol A showed significantly decreased in red blood cells count at days 45 and 60 of trial. The hemoglobin concentration was significantly decreased in fish exposed to higher concentrations (1000 µg/L and 1500 µg/L) of bisphenol A at days 45 and 60 of the study as compared to control fish. The fish exposed to 1000 µg/L and 1500 µg/L of bisphenol A showed that differential leukocytes counts were significantly higher (neutrophilic leukocytosis) at days 30, 45, and 60 of experiment (Table 3). Results showed that the lymphocytes and monocytes were significantly decreased in fish exposed to 1000 µg/L and 1500 µg/L bisphenol A at days 45 and 60 of the experiment in comparison to untreated control fish. The pack cell volume of blood was also significantly decreased in fish exposed to higher concentrations (1000 µg/L and 1500 µg/L) of bisphenol A at day 45 and 60 of the experiment.

The results on different serum biochemical parameters in fish exposed to various levels of bisphenol A are presented in Table 4. Serum albumin quantity and serum total protein significantly decreased in fish exposed to 1000 µg/L and 1500 µg/L of bisphenol A at days 45 and 60 of the trial in comparison to unexposed fish. The quantity of serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST) and serum alkaline phosphatase (ALP) increased significantly in liver tissues of fish exposed to higher concentrations (1000 µg/L and 1500 µg/L) of bisphenol A at days 45 and 60 of trial (Table 3). The serum triglycerides were significantly increased in fish exposed to 1000 µg/L and 1500 µg/L at days 45 and 60 of trial. The quantity of urea and creatinine significantly increased in kidneys of fish exposed to 1000 µg/L and 1500 µg/L bisphenol A at days 45 and 60 of trial as compared to control fish. The quantity of glucose significantly increased in fish exposed to 1000 µg/L and 1500 µg/L at days 45 and 60 of the trial. The cholesterol and lactate dehydrogenase significantly increased at days 45 and 60 of the trial in fish exposed to 1000 µg/L and 1500 µg/L bisphenol A compared to control fish.

Histopathology

Results on intensity/severity of different histopathological changes if various tissues of fish exposed to various levels of bisphenol A are presented in the table. Various sections of gills of fish in groups C-D showed severe histopathological abnormalities such as lamellar disorganization, necrosis of lamellar pillar, lamellar atrophy, disruption of primary lamellae, curling of secondary lamellae, fusion of lamellae, severe congestion and degeneration in cartilaginous cores and telangiectasia. Curling and uplifting arrangements in epithelial cells of secondary lamellae were frequently observed in these groups after day 45 and 60 of the experiment (Fig. 1). Mild to moderate histopathological abnormalities like pyknosis, degeneration glomeruli, congestion, increased Bowman's space, atrophic cells, edema, degeneration of tubular epithelium, aggregation of melanomacrophages and atrophy of the lumen of renal tubules were observed in kidneys of treated groups (Fig. 2). Moderate histopathological changes, such as deterioration of glomerulus, increased bowmens space and necrosis of tubular cells were evident in the kidneys of fish in group B at days 45 and 60 of the experiment. Moderate to sever histopathological abnormalities in liver sections including congestion, ceroid formation, hemorrhages, pyknosis, karyorrhexis, karyolysis, binucleated hepatocytes, nuclear hypertrophy and eccentric nuclei, vacuolar degeneration were observed.
in different at days 45 and 60 of the experiment (Fig. 3). Sever histopathological abnormality of liver in fish (A.nobilis) exposed to 1000 µg/L and 1500 µg/L concentrations exhibited degeneration and vacuolar degeneration, karyorrhexis, karyolysis at days 45 and 60 of the experimental study. Microscopic observation of different section of brain of untreated brain tissues showed no pathological changes while different microscopic changes including intracellular edema, congestion, necrosis of neurons and cytoplasmic vacuolization were observed at days 45 and 60 of the experiment. Histopathological analysis of different sections of brain of bighead carp exposed to higher concentrations of BPA exhibited degenerated neurons in cerebellum, lipofuscin deposition, microgliosis, necrotic neurons, inflammatory cell and severe hemorrhage at days 45 and 60 of the trial (Fig. 4). Mild to moderate similar histopathological changes in various sections of brain of fish exposed to 1000 µg/L BPA were observed at days 45 and 60 of the experiment. Histopathological observation of different sections of heart of bighead carp exposed to higher levels of BPA (1000 µg/L and 1500 µg/L) showed neutrophilic infiltration, necrosis, inflammatory reaction, edema, neutrophilic myocarditis, hemorrhages and deposition of fibrin were observed (Fig. 5). Vaious histopathological ailments in intestine such as extensive vacuolation of enterocytes, inflammatory response, congestion, necrosis and sporadic hemorrhages were observed in bighead carp exposed to BPA (1000 µg/L and 1500 µg/L) at days 45 and 60 of the trial.

**Discussion**

Bisphenol A is a commercially used chemical, an additive in the production of polycarbonate plastics as a developing agent in the 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 (Routledge et al., 1998, Metzler and Erica, 2001). In the present study, Absolute organ weight increased and relative organ weight of all of the visceral organs (Gills, Liver, and kidney (Table: 1, 2) except the brain decreased in fish. Many reports on the relative organ weight of different vertebrates exposed to different toxicants are available in previous studies but scanty of information available about bisphenol A effect on absolute and relative organ weight of freshwater fish. In previous research work, similar results showed like decrease in the relative weight of visceral organs (liver, kidneys) was observed in fish (Gaffar et al., 2019; Hussain et al., 2019) rats (Rattus norvegicus) (Rubin et al.,2019; Cervantes-Camacho et al., 2020) exposed to different toxicants. Hematological parameters of blood are considering the greatest indicator of physiological stress in the various aquatic and terrestrial organisms (Ghaффar et al., 2017a). In the present study, decreased hemoglobin concentration, lymphocytes, monocytes, and pack cell volume in fish exposed to bisphenol A has been reported. Parameter values are lowered due to the rapid oxidation of hemoglobin, hemolysis, and destruction of erythrocytes (Ghaффar et al. 2016; Gul et al. 2017). RBCs reduction, increased WBCs and neutrophils also observed in present work at higher concentrations of bisphenol A. An increase in neutrophils count could be due to immunological reactions expressive to injury in tissues.
of exposed big head carp. While in previous reports, similar results like RBCs reduction decreased in Hb, MCHC, and increase in WBCs and neutrophils were studied in common carp (Ghaffar et al., 2018), African catfish (Nashwa, 2014; Sisodiya et al., 2018) Labeo rohita (Krishnapriya et al., 2017) and Clarias gariepinus (Pathania et al., 2019) exposed to sublethal concentrations of bisphenol A. Hematological abnormalities may be due to erythrocyte destruction in blood-forming cells, increase the production of free radicles, and poor supply of oxygen through gills. Moreover, many reports of hematological parameters are also available on other vertebrates like the Albino mouse (Moselhy, 2015), Rats (Kamam et al., 2015) Yellowfin seabream (Yaghoobi., 2017), and adult cockerels (Hussain et al., 2019) exposed to toxicants. In the present research work, tissue damage observed in bighead carp caused by a higher concentration of BPA. Damage may occur due to stress conditions which induced the inflammatory response of fish tissues led to overproduction of white blood cells. Serum biochemistry analysis gives a clear indicator of pollutant exposure which is a mirror image of environmental contamination, which is useful for tissue pathophysiological status identification (Sayed and Hamed 2017; Abdel-Tawwab and Hamed, 2018). Furthermore, it has been reported that bisphenol A induces adverse effects on serum biochemical index in adult fish, leading to a defect in growth performance and fish health (Wang et al. 2016). In the present study, serum biochemical parameters like ALT, AST, and ALP increased significantly in treated fish in association to stress induced by bisphenol A. Serum biochemical parameters like serum albumin quantity and serum total protein decreased in the present investigation. However, increased glucose, cholesterol, and lactate dehydrogenase level were observed due to stress conditions in treated fish. Serum creatinine and uric acid are essential factors for muscle and purine metabolism for renal safety and kidney function (Hamed and Tawwab, 2017). Urea and creatinine levels were also increased in the liver and kidney which indicated that disturbance in filtration mechanisms and damages of kidneys and liver tissues of fish exposed to bisphenol A in the current experiment. Many previous reports are also available in other species exposed to bisphenol A. Previously, abnormal liver, kidney enzymes, an increase in hepatic enzymes as ALT, ALP, AST, abnormal urea ad creatinine, fatty liver disease, edema, vacuolation of hepatocytes, abnormal structure of cells, degeneration of structural protein due to increase in hepatic enzymes were observed in O. niloticus (Abdul-Tawwab and Hamed, 2018), Zebrafish (Renaud et al., 2017; Ngo et al., 2017 ), C. catla (Faheem et al., 2019), C. gariepinus (Makinwa & Uadia, 2017) H. fossilis (Pal & Reddy, 2018) due to exposure of bisphenol A. Moreover, in literature, many reports of serum biochemistry are present on other species like rats (Pal et al., 2017; Geetharathan & Josthna, 2016).

In the current study, histopathological responses of the fish indicate the degree of damage caused by BPA to the liver of fish (A. nobilis). In this present research work, histopathological lesions in liver tissues of fish were congestion, decreased cytoplasmic space, vacuolar degeneration, increased sinusoidal space, karyolysis of hepatocytes, and necrosis exposed to the higher concentration of BPA. Similar results are available in previous other species of aquatic organisms like ruptured central vein, lipids like vacuolization, macrophage, and lymphocytes infiltration, ruptured and degenerated hepatocytes in Ctenopharyngodon Idella (Faheem et al., 2017), seabream (Carnevali et al., 2017) exposed to sublethal concentration of BPA. The current study suggests that bisphenol-A is capable of causing damage to vital organs (brain, gills, lungs, and liver) of fish at biologically appropriate concentrations, contributing to
altered rates of enzymes that could potentially affect fish health and reproduction. If these fish with high BPA load are routinely eaten by humans may also cause similar health problems. In the current study, kidneys of bisphenol A treated fish also showed microscopic lesions as edema, ceroid formation, glomerular degeneration, Bowman's space, congestion atrophy of tubules, and atrophy of lumen of renal tubules. However, similar results as necrosis, vacuolation, aggregation of melanomacrophages, degeneration, blood congestion, cellular rupture, nuclear hypertrophy degeneration, pyknotic nucleus, and reduction of lumen were observed in other species of fish like Heteropneustes fossilis (Pal & Reddy, 2018), tilapia (Vasu et al., 2019), Catla Catla (Faheem et al., 2017) exposed to BPA has been reported in previous studies. Like previous studies, bisphenol A is responsible for kidney damage in bighead carp in current research because kidneys are primary organs to be infected by any type of pollutant (chemical, insecticide, pesticide, etc) in water bodies (Hussain et al., 2017). The degree of the damage caused and the degenerative changes that have occurred in the brain of the fish due to BPA toxicity have been progressive over the exposure, indicate that the histopathological responses depend not only on the concentration of chemicals but also on the duration of the fish exposure time to this toxicant. Several authors have recorded various histopathological changes in fish brains after exposure to different chemical substances (Ayoola & Ajani, 2008; Lakshmaiah, 2017; Ding et al., 2018; Murali et al., 2018; Gobi et al., 2018). Scanty of the latest information available on histopathological differences in brain tissues of fish exposed to bisphenol-A. However, few reports are present in our assessed data on histopathological changes of the brain of fish like C. gariepinus (Ayoola et al., 2008), L. rohita (Das et al., 2000), O. punctatus (Pugazhvendan et al., 2009), C. carassius (Mattsson et al., 2017), C. catla (Bose et al., 2013), O. niloticus (Ayoola et al., 2008; Ding et al., 2018), O. mossambicus (Gobi et al., 2018; Murali et al., 2018), C. carpio (Lakshmaiah, 2017) exposed to toxicants.

Literature exhibited that histopathological lesion formation in the gills of fish is a suitable tool to screen the effect of different contaminants in the freshwater ecosystem. It is because the gills are facing direct contact with water pollutants and gills are the 1st organ in which contaminants enter. Gills are those important organs that act as a medium for gaseous exchange, boundary between water and fish, ionic compounds balancer, and are responsible for osmoregulation mechanism (Gaffar et al., 2018). In the present study, histopathological lesions in the gills of fish include lamellar fusion atrophied lamellae, uplifting of lamellae, congestion, and disorganization of primary, secondary lamellae. Likewise, results as Necrosis, lamellar deformation, loss of epithelium, vacuolations, hyperplasia, tubular alteration, neoplasia, hemocyte infiltration, hypertrophy, pyknosis, and histological aberrations were observed in other organisms like Van fish (Oguz et al., 2018) and C. fluminea, (Benjamin et al., 2019) exposed to different concentration of BPA. In current research work edema, neutrophilic myocarditis, hemorrhages and deposition of fibrin were observed in heart of big head carp exposed to different concentrations of bisphenol A. In one of the previous reports, calcific aortic valve disease (CAVD), including extra-cellular matrix (ECM) alteration were confirmed by histopathology for high-level of BPA exposure, and structural defects (abnormal curvature) of the atrio-ventricular valves corresponded with impaired cardiovascular function (reduced ventricular beat rate and blood flow) were observed in zebra fish (Brown et al, 2019) exposed to bisphenol A. Few reports are available on histological changes of heart in fish exposed to
bisphenol A. However, many reports of heart histology are available on rats. Potential Toxic Effect of Bisphenol A on rats (Bahey et al., 2019, Amin, 2019, Eweda et al 2020, Rasdi et al., 2020) as myocardium in the form of disarrangement of myofibers, hypertrophy of myocytes, myocardial fibrosis, and dilatation of intramyocardial arterioles were observed in previous research work. In the present study histopathological changes in intestine are extensive vacuolation of enterocytes, inflammatory response, congestion, necrosis and sporadic hemorrhages in fish exposed to different concentrations of bisphenol A. like heart and brain scanty of information about BPA effect on intestine of fish is available in my assessed reports. Previously, histological Intestinal alterations in fish Dicentrarchus labrax (peda et al., 2016) was reported. Similarly, histopathological alteration in intestine of fish Lates niloticus (Ibrahim et al 2014), Sparus aurata (Rathee and Radha, 2015), H.fossilis (Pradip et al., 2019) were exposed to toxicants have been reported. The fish under toxicant stress started the utilization of immediate sources of energy like protein, lipid, and carbohydrate, resultantly depleting the levels of these nutritive sources in the muscles as these all are interrelated in metabolism during the citric acid cycle (Sulekha and Marcy, 2011; Muralidharan, 2014. In the present study, protein contents depletion in the fish muscle might be because of the diversion of energy due to the toxic stress of bisphenol A (Sweilum, 2006; Sobha et al., 2007; Sulekha and Marcy, 2011; Karmai et al., 2016). The decrease in protein in the meat of fish could be due to a reduction in salt and water-soluble (Chonnawang et al., 2007) or because of autolytic degradation combine with endogenous enzymes and bacteria (Hultmann and Rsted, 2004). The decrease in protein content was probably due to the leaching of soluble components especially water proteins (Osibona and Ezekiel, 2014; Ihanacho et al., 2017). Scanty of work available on body composition on freshwater fish exposed to BPA In the present study lipid contents of fish decreased at a higher concentration of bisphenol A. Previously, similar reports available on C. gariepinus (Mahboob et al., 2018), Tilapia (Sana et al., 2017) exposed to toxicants. Presently moisture contents were also decreased like protein and lipid contents in fish exposed to bisphenol-A. However, increased moisture content was observed in C.catla, L. rohita, and C. mrigala (Ghazala et al., 2018). In the present study, Ash content increased after exposure of fish to high concentrations of bisphenol A. Similar results available previously, (Rao et al., 2010; Hussain et al., 2019). Limited information is available in the literature about an effect of industrial effluents on the proximate composition and amino acid profile of freshwater fishes and their use as a biomarker of toxicant contamination (Hussain et al., 2019). The findings of this research work have indicated that industrial contaminant (bisphenol A) probably had adversely affected the proximate composition of fish meat in A.nobilis. hence, more research work is required to verify these findings.

**Conclusion**

The results showed that BPA at sublethal concentration changes the hematological and biochemical parameters of fish, A.nobilis, and these parameters can be used to detect adverse effects of BPA in aquatic environments and to determine the physiological condition of fish. Histopathological studies are therefore conducted to confirm the degree of damage in vital organs of fish especially the liver. In contrast, the introduction of such compounds into rivers should be restricted, although carps (A.nobilis) are natural inhabitants of freshwater environments and are desired species in countries like Pakistan as
food. The findings of this research work have indicated that environmental contaminants probably had adversely affected the proximate composition of fish meat in A. nobili. However, more research work is required to verify these findings.

**Declarations**

**Conflict of interest:** All the authors carefully read the paper and declare that they have no financial/personal conflict of interests.

**Ethical Approval:** This study was approved by the bio ethical committee of Institute of Pure and Applied Biology, Zoology Division, Bhauddin Zakariya University, Multan, Pakistan.

**Consent to Participate:** All the authors equally participated. and gave their consent to publish the study in this journal.

**Consent to Publish:** All the authors gave their consent to publish the study in this journal.

**Authors Contributions:** Riaz Hussain and Rehana Iqbal planned and designed the experiments. Rabia Akram and Riaz Hussain conducted the experiments. Rabia Akram, Riaz Hussain and Rehana Iqbal involved data collection and interpretation. Rehana Iqbal and Muhammad Ali involved in manuscript preparation.

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**Availability of data and materials:** The datasets used and/or analysed during the current study are available from the first author on reasonable request (Rabia Akram).

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Tables
Table 1
Body weight and absolute weight of different visceral tissues of A.nobilis exposed to different concentrations of bisphenol A.

| Parameters/day | Groups/Treatments |
|----------------|-------------------|
|                | A (0.0)           | B (500 µg/L)  | C (1000 µg/L) | D (1500 µg/L) |
| Body weight (g) |                  |                |               |               |
| 15             | 163.35 ± 2.59     | 161.62 ± 2.20  | 161.42 ± 2.00 | 162.95 ± 1.01 |
| 30             | 174.60 ± 2.63     | 173.12 ± 0.86  | 172.67 ± 1.00 | 170.32 ± 1.27 |
| 45             | 184.67 ± 1.97     | 182.25 ± 1.67  | 183.05 ± 0.87 | 177.42 ± 1.85 |
| 60             | 202.62 ± 2.36     | 195.45 ± 1.01  | 192.97 ± 0.70 | 184.65 ± 1.46*|
| Absolute weight of liver (g) |  |                |               |               |
| 15             | 2.30 ± 0.02       | 2.38 ± 0.01    | 2.38 ± 0.01   | 2.42 ± 0.01   |
| 30             | 2.39 ± 0.01       | 2.46 ± 0.00    | 2.48 ± 0.01   | 2.53 ± 0.01   |
| 45             | 2.43 ± 0.00       | 2.49 ± 0.00    | 2.55 ± 0.01   | 2.62 ± 0.01   |
| 60             | 2.48 ± 0.00       | 2.51 ± 0.00    | 2.58 ± 0.01   | 2.95 ± 0.03*  |
| Absolute weight of gills |  |                |               |               |
| 15             | 3.96 ± 0.02       | 3.98 ± 0.04    | 4.03 ± 0.02   | 4.14 ± 0.01   |
| 30             | 4.16 ± 0.02       | 4.20 ± 0.00    | 4.25 ± 0.01   | 4.32 ± 0.00   |
| 45             | 4.26 ± 0.01       | 4.27 ± 0.00    | 4.33 ± 0.02   | 4.40 ± 0.00   |
| 60             | 4.30 ± 0.01       | 4.38 ± 0.01    | 4.46 ± 0.01   | 5.53 ± 0.02*  |
| Absolute weight of Kidneys |  |                |               |               |
| 15             | 1.95 ± 0.01       | 2.01 ± 0.03    | 2.03 ± 0.02   | 2.12 ± 0.01   |
| 30             | 2.09 ± 0.00       | 2.16 ± 0.00    | 2.19 ± 0.01   | 2.26 ± 0.01   |
| 45             | 2.15 ± 0.00       | 2.18 ± 0.00    | 2.24 ± 0.01   | 2.32 ± 0.01   |
| 60             | 2.19 ± 0.01       | 2.24 ± 0.00    | 2.30 ± 0.01   | 2.90 ± 0.02*  |
| Absolute weight of brain |  |                |               |               |
| 15             | 0.75 ± 0.01       | 0.78 ± 0.01    | 0.79 ± 0.00   | 0.81 ± 0.03   |
| 30             | 0.84 ± 0.02       | 0.84 ± 0.01    | 0.83 ± 0.01   | 0.82 ± 0.21   |
| 45             | 0.96 ± 0.04       | 0.95 ± 0.01    | 0.91 ± 0.03   | 0.90 ± 0.01   |
| 60             | 0.98 ± 0.04       | 0.98 ± 0.03    | 1.01 ± 0.00   | 1.09 ± 0.01   |
Table 2
Relative weight of different visceral tissues of A. nobilis exposed to different concentration of bisphenol A.

| Parameters/days | Groups/Treatments | A (0.0) | B (500 µg/L) | C (1000 µg/L) | D (1500 µg/L) |
|-----------------|-------------------|---------|--------------|---------------|---------------|
|                 |                   | 15      | 30           | 45            | 60            |
| Relative weight of liver | | 1.41 ± 0.03 | 1.47 ± 0.02 | 1.47 ± 0.01 | 1.49 ± 0.01 |
|                 |                   | 1.38 ± 0.01 | 1.45 ± 0.01 | 1.44 ± 0.01 | 1.45 ± 0.01 |
|                 |                   | 1.32 ± 0.01 | 1.36 ± 0.01 | 1.39 ± 0.00 | 1.48 ± 0.01 |
|                 |                   | 1.22 ± 0.01 | 1.28 ± 0.00 | 1.33 ± 0.00 | 1.751 ± 0.02*|
| Relative weight of gills | | 2.43 ± 0.02 | 2.46 ± 0.03 | 2.49 ± 0.02 | 2.54 ± 0.02 |
|                 |                   | 2.39 ± 0.02 | 2.47 ± 0.02 | 2.46 ± 0.02 | 2.48 ± 0.01 |
|                 |                   | 2.30 ± 0.01 | 2.34 ± 0.01 | 2.36 ± 0.00 | 2.48 ± 0.02 |
|                 |                   | 2.12 ± 0.02 | 2.24 ± 0.01 | 2.31 ± 0.00 | 2.70 ± 0.02*|
| Relative weight of Kidneys | | 1.19 ± 0.01 | 1.24 ± 0.02 | 1.26 ± 0.02 | 1.30 ± 0.01 |
|                 |                   | 1.20 ± 0.02 | 1.27 ± 0.00 | 1.27 ± 0.01 | 1.29 ± 0.01 |
|                 |                   | 1.16 ± 0.01 | 1.20 ± 0.01 | 1.22 ± 0.00 | 1.30 ± 0.01 |
|                 |                   | 1.08 ± 0.00 | 1.14 ± 0.00 | 1.19 ± 0.00 | 1.37 ± 0.01*|
| Relative weight of brain (%) | | 0.45 ± 0.01 | 0.48 ± 0.00 | 0.49 ± 0.00 | 0.49 ± 0.00 |
|                 |                   | 0.48 ± 0.00 | 0.49 ± 0.00 | 0.50 ± 0.00 | 0.51 ± 0.00 |
|                 |                   | 0.49 ± 0.00 | 0.52 ± 0.00 | 0.52 ± 0.00 | 0.53 ± 0.01 |
|                 |                   | 0.46 ± 0.00 | 0.50 ± 0.00 | 0.52 ± 0.00 | 0.57 ± 0.01 |
Table 3
Various hematological parameters of fish exposed to different concentrations of bisphenol A.

| Parameters/days | Groups/Treatments | A (0.0) | B (500 µg/L) | C (1000 µg/L) | D (1500 µg/L) |
|----------------|-------------------|---------|--------------|---------------|---------------|
| Red blood cell count |                   | 3.77 ± 0.11 | 3.71 ± 0.15 | 3.69 ± 0.07 | 3.66 ± 0.10 |
| 15              |                   | 3.74 ± 0.13 | 3.67 ± 0.08 | 3.60 ± 0.09 | 3.34 ± 0.06 |
| 30              |                   | 3.74 ± 0.09 | 3.63 ± 0.12 | 3.26 ± 0.15* | 3.04 ± 0.11* |
| 45              |                   | 3.73 ± 0.15 | 3.51 ± 0.13 | 3.13 ± 0.12* | 2.86 ± 0.07* |
| 60              |                   | 3.74 ± 0.09 | 3.63 ± 0.12 | 3.26 ± 0.15* | 3.04 ± 0.11* |
| Hemoglobin concentration (g/dl) |     | 9.13 ± 0.25 | 9.13 ± 0.15 | 9.08 ± 0.12 | 9.03 ± 0.17 |
| 15              |                   | 9.23 ± 0.3  | 9.14 ± 0.13 | 8.99 ± 0.14 | 8.58 ± 0.12 |
| 30              |                   | 9.25 ± 0.19 | 9.05 ± 0.32 | 8.71 ± 0.23 | 7.90 ± 0.16* |
| 45              |                   | 9.21 ± 0.31 | 8.95 ± 0.11 | 7.54 ± 0.16* | 7.12 ± 0.11* |
| 60              |                   | 9.20 ± 0.24 | 21.8 ± 0.35 | 24.1 ± 0.67* | 25.4 ± 0.79* |
| White blood counts |               | 19.6 ± 0.27 | 20.5 ± 0.46 | 20.7 ± 0.47 | 21.4 ± 0.36 |
| 15              |                   | 19.6 ± 0.41 | 21.2 ± 0.38 | 21.7 ± 0.71 | 23.0 ± 0.42* |
| 30              |                   | 19.7 ± 0.28 | 21.7 ± 0.54 | 23.7 ± 0.53* | 25.0 ± 0.32* |
| 45              |                   | 19.8 ± 0.24 | 21.8 ± 0.35 | 24.1 ± 0.67* | 25.4 ± 0.79* |
| Pack cell volume |                  | 33.5 ± 0.52 | 32.9 ± 0.53 | 32.5 ± 0.88 | 31.5 ± 0.74 |
| 15              |                   | 33.5 ± 0.43 | 32.6 ± 0.61 | 31.0 ± 0.98 | 30.1 ± 0.52 |
| 30              |                   | 33.7 ± 0.34 | 32.1 ± 0.77 | 28.4 ± 0.73* | 28.1 ± 0.74* |
| 45              |                   | 33.6 ± 0.77 | 31.1 ± 0.88 | 27.3 ± 1.15* | 27.0 ± 1.21* |
| Lymphocytes (%) |                 | 21.9 ± 0.17 | 21.4 ± 0.25 | 19.7 ± 0.20 | 19.6 ± 0.11 |
| 15              |                   | 22.1 ± 0.17 | 20.6 ± 0.20 | 19.3 ± 0.30 | 19.0 ± 0.17 |
| 30              |                   | 22.0 ± 0.24 | 20.6 ± 0.13 | 19.1 ± 0.24 | 17.7 ± 0.17* |
| 45              |                   | 22.1 ± 0.18 | 19.8 ± 0.12 | 18.4 ± 0.43* | 17.1 ± 0.19* |
| Parameters/days | Groups/Treatments | A (0.0) | B (500 µg/L) | C (1000 µg/L) | D (1500 µg/L) |
|----------------|-------------------|---------|--------------|---------------|---------------|
| Monocytes (%)  |                   |         |              |               |               |
| 15  | 4.15 ± 0.12 | 4.10 ± 0.10 | 4.06 ± 0.15 | 4.06 ± 0.17    |               |
| 30  | 4.16 ± 0.09 | 4.10 ± 0.09 | 3.97 ± 0.11 | 3.95 ± 0.21    |               |
| 45  | 4.16 ± 0.17 | 4.04 ± 0.12 | 3.91 ± 0.22*| 3.74 ± 0.25*   |               |
| 60  | 4.23 ± 0.07 | 3.83 ± 0.15 | 3.47 ± 0.23*| 3.38 ± 0.14*   |               |
| Neutrophils (%)|                   |         |              |               |               |
| 15  | 21.4 ± 0.78 | 22.7 ± 0.28 | 23.6 ± 0.26 | 23.6 ± 0.17    |               |
| 30  | 21.8 ± 0.40 | 22.9 ± 0.23 | 24.0 ± 0.29 | 26.9 ± 0.22*   |               |
| 45  | 21.8 ± 0.56 | 23.5 ± 0.27 | 24.8 ± 0.25 | 27.1 ± 0.24*   |               |
| 60  | 22.2 ± 0.49 | 23.4 ± 0.60 | 26.4 ± 0.38*| 29.2 ± 0.25*   |               |
Table 4
Various serum biochemical parameters of fish exposed to different concentrations of bisphenol A.

| Parameters/days | Groups/Treatments |            |            |            |            |
|-----------------|-------------------|------------|------------|------------|------------|
|                 | A (0.0)           | B (500 µg/L) | C (1000 µg/L) | D (1500 µg/L) |
| Albumin quantity (mg/dL) |            |            |            |            |            |
| 15              | 2.83 ± 0.07       | 2.78 ± 0.11 | 2.76 ± 0.05 | 2.72 ± 0.08 |
| 30              | 2.87 ± 0.09       | 2.76 ± 0.09 | 2.72 ± 0.07 | 2.70 ± 0.06 |
| 45              | 2.87 ± 0.05       | 2.74 ± 0.07 | 2.68 ± 0.09 | 2.25 ± 0.07* |
| 60              | 2.86 ± 0.08       | 2.70 ± 0.07 | 2.32 ± 0.08* | 2.22 ± 0.04* |
| Total proteins (mg/dL) |            |            |            |            |            |
| 15              | 3.83 ± 0.02       | 3.80 ± 0.02 | 3.75 ± 0.00 | 3.68 ± 0.00 |
| 30              | 3.89 ± 0.01       | 3.75 ± 0.01 | 3.61 ± 0.01 | 3.49 ± 0.02 |
| 45              | 3.89 ± 0.02       | 3.74 ± 0.01 | 3.17 ± 0.02* | 3.01 ± 0.04* |
| 60              | 3.90 ± 0.00       | 3.65 ± 0.03 | 3.12 ± 0.10* | 2.84 ± 0.08* |
| Aspartate aminotransferase (U/L) |            |            |            |            |            |
| 15              | 14.2 ± 0.35       | 15.0 ± 0.23 | 15.6 ± 0.14 | 15.9 ± 0.19 |
| 30              | 14.1 ± 0.17       | 15.6 ± 0.17 | 16.3 ± 0.16 | 16.5 ± 0.18 |
| 45              | 14.0 ± 0.19       | 15.9 ± 0.16 | 16.7 ± 0.21 | 18.2 ± 0.25* |
| 60              | 14.3 ± 0.24       | 16.3 ± 0.19 | 18.9 ± 0.35* | 19.5 ± 0.28* |
| Alkaline phosphatase (U/L) |            |            |            |            |            |
| 15              | 25.8 ± 0.27       | 26.0 ± 0.22 | 26.1 ± 0.21 | 26.7 ± 0.20 |
| 30              | 25.1 ± 0.25       | 26.4 ± 0.23 | 26.6 ± 0.26 | 27.3 ± 0.28 |
| 45              | 25.1 ± 0.25       | 26.9 ± 0.23 | 28.9 ± 0.19 | 29.3 ± 0.23* |
| 60              | 25.2 ± 0.31       | 27.4 ± 0.19 | 29.8 ± 0.52* | 31.7 ± 0.65* |
| Alanine aminotransferase (U/L) |            |            |            |            |            |
| 15              | 22.6 ± 0.23       | 22.8 ± 0.18 | 23.3 ± 0.19 | 23.7 ± 0.14 |
| 30              | 22.8 ± 0.19       | 23.1 ± 0.18 | 23.8 ± 0.18 | 24.3 ± 0.16 |
| 45              | 22.9 ± 0.23       | 23.4 ± 0.21 | 24.0 ± 0.17 | 26.0 ± 0.15* |
| 60              | 22.6 ± 0.36       | 24.2 ± 0.19 | 27.4 ± 0.20* | 28.7 ± 0.22* |
| Parameters/days | Groups/Treatments | A (0.0) | B (500 µg/L) | C (1000 µg/L) | D (1500 µg/L) |
|----------------|-------------------|---------|--------------|---------------|---------------|
| Lactate dehydrogenase (U/L) |                  | 242.9 ± 2.40 | 247.0 ± 1.72 | 251.7 ± 1.64 | 252.6 ± 1.23   |
| 15              |                   | 246.3 ± 1.08 | 249.7 ± 1.31 | 254.0 ± 1.22 | 255.4 ± 0.71   |
| 30              |                   | 248.5 ± 1.80 | 253.5 ± 0.89 | 256.5 ± 1.21 | 268.5 ± 2.23*  |
| 45              |                   | 247.8 ± 0.86 | 256.4 ± 0.42 | 273.9 ± 2.40*| 280.0 ± 0.85*  |
| Urea (mg/dL)    |                   | 8.43 ± 0.10  | 8.68 ± 0.17  | 8.75 ± 0.03  | 9.14 ± 0.01    |
| 15              |                   | 8.55 ± 0.07  | 8.84 ± 0.16  | 9.31 ± 0.17  | 9.43 ± 0.04    |
| 30              |                   | 8.59 ± 0.09  | 8.93 ± 0.17  | 9.51 ± 0.12  | 10.74 ± 0.10*  |
| 45              |                   | 8.64 ± 0.10  | 9.15 ± 0.01  | 10.97 ± 0.01*| 11.2 ± 0.31*   |
| Creatinine (mg/dL) |                 | 1.11 ± 0.00  | 1.14 ± 0.00  | 1.16 ± 0.00  | 1.18 ± 0.00    |
| 15              |                   | 1.13 ± 0.00  | 1.15 ± 0.00  | 1.19 ± 0.00  | 1.22 ± 0.00    |
| 30              |                   | 1.15 ± 0.00  | 1.17 ± 0.00  | 1.22 ± 0.01  | 1.31 ± 0.02*   |
| 45              |                   | 1.16 ± 0.00  | 1.20 ± 0.008 | 1.35 ± 0.04* | 1.53 ± 0.02*   |
| Cholesterol (mg/dL) |                 | 151.4 ± 0.72 | 154.3 ± 0.74 | 155.0 ± 0.83 | 157.8 ± 0.59   |
| 15              |                   | 150.6 ± 0.77 | 155.8 ± 0.19 | 157.6 ± 0.50 | 160.5 ± 1.36   |
| 30              |                   | 152.1 ± 1.14 | 156.2 ± 0.46 | 158.6 ± 0.44 | 164.4 ± 1.64*  |
| 45              |                   | 152.4 ± 1.06 | 157.5 ± 0.34 | 165.9 ± 0.47*| 170.3 ± 1.99*  |
| Glucose (mg/dL)  |                   | 28.0 ± 0.57  | 29.4 ± 0.57  | 30.4 ± 0.57  | 32.1 ± 0.57    |
| 15              |                   | 29.1 ± 0.58  | 30.4 ± 0.38  | 31.6 ± 0.21  | 32.4 ± 0.43    |
| 30              |                   | 29.1 ± 0.51  | 31.1 ± 0.51  | 32.7 ± 0.51  | 33.9 ± 0.51*   |
| 45              |                   | 29.8 ± 0.36  | 31.7 ± 0.47  | 34.7 ± 1.22* | 37.8 ± 0.74*   |
| 60              |                   | 172.9 ± 0.83 | 173.1 ± 1.14 | 175.9 ± 0.62 | 177.2 ± 0.85   |
| Triglycerides (mg/dL) |               | 172.9 ± 0.83 | 173.1 ± 1.14 | 175.9 ± 0.62 | 177.2 ± 0.85   |
| Parameters/days | Groups/Treatments |
|----------------|-------------------|
|                | A (0.0)           | B (500 µg/L) | C (1000 µg/L) | D (1500 µg/L) |
| 30             | 172.8 ± 3.64      | 174.5 ± 2.45 | 177.1 ± 2.73  | 180.1 ± 1.06  |
| 45             | 173.7 ± 2.65      | 175.9 ± 2.25 | 188.3 ± 3.70  | 193.8 ± 1.62* |
| 60             | 174.1 ± 0.32      | 177.1 ± 0.31 | 189.2 ± 1.76* | 198.9 ± 1.90* |
Table 5
Severity of different histopathological changes in various tissues of bighead carp exposed to various concentrations of bisphenol A.

| Histopathological lesions                          | Groups                     |
|---------------------------------------------------|----------------------------|
|                                                   | B (500 µg/L) | C(1000 µg/L) | D(1500 g/L) |
| **Liver**                                         |              |              |             |
| Congestion                                        | +            | ++           | +++         |
| Ceroid formation                                  | +            | ++           | +++         |
| Vauolar degeneration                              | ++           | +++          | +++         |
| Hemorhages                                        | +            | ++           | +++         |
| Pyknosis                                          | ++           | ++           | +++         |
| Nuclear hypertrophy                               | ++           | +++          | +++         |
| Karyorrhesis                                      | ++           | +++          | +++         |
| Karyolysis                                        | ++           | ++           | +++         |
| Degeneration of hepatocyte                        | ++           | +++          | +++         |
| Hepatocytes with eccentric nuclei                 | ++           | +++          | +++         |
| **Kidneys**                                       |              |              |             |
| Congestion                                        | +            | +++          | +++         |
| Increased Bowman's space                         | ++           | +++          | +++         |
| Ceroid formation                                  | +            | ++           | +++         |
| Edema                                             | +            | +            | ++          |
| Necrosis of tubular cells                         | ++           | +++          | +++         |
| Melanomacrophage aggregates                       | +            | ++           | +++         |
| Nuclear hypertrophy                               | +            | ++           | +++         |
| Deterioration of glomerulus                       | ++           | +++          | +++         |
| Dneration and obilitation of renal tubule          | +            | ++           | +++         |
| Thyroidization                                    | +            | ++           | +++         |
| **Brain**                                         |              |              |             |
| Necrosis of neurons                               | +            | ++           | +++         |
| Cytoplasmic vacuolization                         | ++           | +++          | +++         |
| Histopathological lesions                  | Groups          |
|------------------------------------------|-----------------|
|                                          | B (500 µg/L)    | C(1000 µg/L) | D(1500 g/L) |
| Intracellular oedema                     | ++              | ++           | +++          |
| Congestion of neural cells               | +               | ++           | +++          |
| **Gills**                                |                 |              |              |
| Congestion                               | +               | +++          | ++++         |
| Congested cartilaginous core             | +               | ++           | ++++         |
| Lamellar fusion                          | ++              | +++          | ++++         |
| Necrosis of lamellar pillar cells        | +               | +++          | ++++         |
| Degeneration of cartilaginous core       | ++              | +++          | ++++         |
| Lamellar atrophy                         | ++              | +++          | ++++         |
| Telangiectasia                           | +               | ++           | +++          |
| Uplifting of lamellae                    | ++              | +++          | ++++         |
| Disruption of primary lamellae           | ++              | +++          | ++++         |
| Curling of secondary lamellae            | ++              | +++          | ++++         |
| Lamellar disorganization                 | ++              | +++          | ++++         |
Table 6
Some change in meat composition of *A. nobilis* exposed to different concentrations of Bisphenol A.

| Parameters/days | Groups/Treatments          |          |          |          |
|-----------------|----------------------------|----------|----------|----------|
|                 | A (0.0)                    | B (500 µg/L) | C (1000 µg/L) | D (1500 µg/L) |
| Crude protein (%) | 15 16.55 ± 0.75            | 16.40 ± 0.49 | 15.60 ± 0.54 | 15.00 ± 0.30 |
|                 | 30 16.67 ± 0.38            | 16.07 ± 0.16 | 15.15 ± 0.45 | 14.35 ± 0.25 |
|                 | 45 16.62 ± 0.26            | 15.37 ± 0.11 | 14.82 ± 0.26 | 13.12 ± 0.17* |
|                 | 60 16.25 ± 0.29            | 15.15 ± 0.15 | 12.52 ± 0.24* | 12.71 ± 0.11* |
| Lipids (%)      | 15 5.28 ± 0.12             | 5.18 ± 0.01 | 5.14 ± 0.01 | 5.13 ± 0.00 |
|                 | 30 5.24 ± 0.01             | 5.14 ± 0.01 | 5.02 ± 0.02 | 4.96 ± 0.00 |
|                 | 45 5.29 ± 0.01             | 5.05 ± 0.01 | 4.81 ± 0.01* | 4.79 ± 0.02* |
|                 | 60 5.32 ± 0.02             | 4.74 ± 0.24 | 4.50 ± 0.07* | 4.36 ± 0.05* |
| Moisture (%)    | 15 78.02 ± 0.45            | 76.85 ± 0.32 | 75.92 ± 0.49 | 75.20 ± 0.55 |
|                 | 30 77.65 ± 0.49            | 74.95 ± 0.60 | 73.07 ± 0.66 | 72.07 ± 0.50 |
|                 | 45 77.22 ± 0.46            | 74.20 ± 0.65 | 71.05 ± 0.96 | 63.60 ± 0.29* |
|                 | 60 77.60 ± 0.34            | 73.27 ± 0.41 | 70.57 ± 1.30 | 62.20 ± 1.02* |
| Ash (%)         | 15 4.17 ± 0.07             | 4.21 ± 0.02 | 4.31 ± 0.00 | 4.38 ± 0.02 |
|                 | 30 4.16 ± 0.01             | 4.33 ± 0.01 | 4.43 ± 0.02 | 4.67 ± 0.04 |
|                 | 45 4.35 ± 0.02             | 4.35 ± 0.02 | 4.57 ± 0.07 | 4.91 ± 0.04* |
|                 | 60 4.22 ± 0.01             | 4.42 ± 0.03 | 4.64 ± 0.07 | 5.23 ± 0.07* |

Figures
Figure 1

Photomicrograph of gills of bighead carp exposed to BPA (@1000 µg/L) and 1500 µg/L showing different microscopic lesions. A) gills showing necrosis of primary lamellar epithelial cells (arrow heads), secondary lamellar disorganization (thick arrows), disorganization of cartilaginous core (dcc) and sloughing of lamellar epithelium (thin arrow). B) sloughing of lamellar epithelium (thin arrow), disorganization of cartilaginous core (dcc), necrosis of primary lamellar epithelial cells (arrow heads) and aneurysm (thick arrow). C) necrosis of primary lamellar epithelial cells (arrow heads), disorganization of cartilaginous core (dcc), secondary lamellar disorganization (thick arrows) and necrosis of epithelium (thin arrow). D) aneurysm (thick arrow), lamellar epithelial cells (arrow heads) and disorganization of cartilaginous core (dcc).
Figure 2

Photomicrograph of liver of bighead carp exposed to BPA (@1000 µg/L) and 1500 µg/L showing various microscopic changes a) congestion (arrow) and necrosis of hepatocyte (arrow heads). b) edema (*), heamorrhages (arrow) and necrotic hepatocytes (arrow heads). c) edema (*) and necrosis of hepatocyte (arrow heads). d) heamorrhages (arrow) and necrosis of hepatocyte (arrow heads) at day 45 and 60 of trial.
Figure 3

Photomicrograph of kidneys of bighead carp exposed to BPA (@1000 µg/L) and 1500 µg/L showing various microscopic changes a) ceroid formation and inflammatory cell accumulation (arrows). b) severe hemorrhage (**), ceroid formation (arrow head), expansion of Bowman's space (thin arrows) and inflammatory cell (thick arrow) at day 45 and 60 of experiment.
Figure 4

Photomicrograph of heart of bighead carp exposed to BPA (@1000 µg/L) and 1500 µg/L showing a) inflammatory exudate (arrows) and disorganization of cardiac muscles (arrow head). b) inflammatory exudate (arrows) and disorganization (arrow head). d) hemorrhage (arrow), edema (*) and disorganization of cardiac muscles (arrow head) at day 45 and 60 of experiment.
Figure 5

Photomicrograph of brain of bighead carp exposed to BPA (@1000 µg/L) and 1500 µg/L showing a) hyperemic vessels (*), necrosis of neurons (arrows) and degeneration of neurons (arrow heads). b) severe necrosis of neurons (arrows). c) necrosis of neurons (arrows) and degeneration of neurons (arrow heads). d) inflammatory cells (arrows). e) severe edema (*), severe hemorrhage (arrow), inflammatory cell acomulation (**) and necrosis of neuron (arrow head). j) ceroid formation (arrow heads), inflammatory cell acomulation (**) and necrosis of neurons (arrow) at day 45 and 60 of experiment.