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IMPACT OF BISPHENOL A ON COMPLETE BLOOD COUNT (CBC) AND HISTOLOGY OF DIFFERENT TISSUES OF CIRRHINUS MRIGALA FINGERLINGS

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Abstract

BPA is highly demanded industrial chemical, which is produced in bulk amount worldwide. It has highly intoxicant nature toward exposed organisms in BPA contaminated environment. To check its adverse effects on lower concentrations, three sublethal doses of 0.5ppm, 1.0ppm and 1.5ppm concentrations were given to C. mrigala for 30 days. Blood of fish were taken and analyzed from each group using standard protocols and then fish were dissected and organs (gills, liver and muscle) were preserved in 10% formalin solution for histology analysis. Histopathological and complete blood count were analyzed to check the severity of damage caused by BPA exposure. Histopathological damage of liver, gills and muscle were analyzed from mild toward severity as concentration of BPA increased from low to high dose. BPA also have inverse correlation with complete blood count of fish, as RBCs, Hb, Hct and other hematological indices of erythrocytes decreases as the concentration of BPA increased. However, WBC increased on lower dose and decreased as the dose increased. Thus, this study concluded that BPA had adverse effect on fish C. mrigala, which is among most consumed fish of Asia.

Keywords
C. mrigala, bisphenol A (BPA), histopathology, complete blood count (CBC), liver, gills, muscle

1. Introduction

Bisphenol A is anthropogenic plastic monomer and is xenobiotic, found in epoxy resin, thermal paper and polycarbonate plastic, adhesive, protective coating and other products (Staples et al, 1998). BPA is high manufacture bulk chemical with a variety of applications globally, its production increases as the demand of plastic and plastic product increases (Corrales et al., 2015) and their production on large scale results in its release in the environment particularly the water bodies which ultimately influence aquatic flora, fauna and the entire surroundings. BPA is among the highest produced chemicals whose global demand was more than 6 billion per annum (Bailin et al., 2008). Toxic Release Inventory, USA release a report which state the annual release of BPA in environment was 2.5 million kg in 2007 and to this total release 13772 kg was directly discharge in water bodies (USEPA. Bisphenol A Action Plan, 2010). Extensive applications of BPA make its existence ubiquitous which is an alarming concern for human health as it is extensively detected in human samples of blood, urine and breast milk at lower Nano molar level (Zimmers *et al*., 2014; Flint et al., 2012). Also, low BPA contamination was detected in aquatic environment which raised the concern of aquatic species health and development (Flint *et al*., 2012; Huang et al., 2012; Kang et al., 2007). BPA enter in the water bodies through effluent discharge, degradation of PVC pipes and plastic, through manufacturing plants, during processing and transport, leaches from landfill site and litter etc. (Flint et al., 2012; Crain et al., 2007; Huang *et al*., 2012). BPA has affinity to interact and alter the titer thyroid and androgen receptor hormones of the body (Faheem et al., 2017b). BPA degrade in 0.5 to 6 days when it got exposed in aquatic atmosphere (Mihaich et al., 2012) and its unremitting release in the water effect the aquatic species in particular fish to be unprotected.BPA as an endocrine disruptor and reported to cause oxidative trauma and reproductive injury in various species of fish (Mandich *et al*., 2007; Madonna *et al*., 2014; Faheem and Lone, 2017; Faheem et al., 2017a, 2017b, 2018b). In North America, Japan and Europe, BPA was detected in surface water with median concentration ranged from 0.001 to 0.081±0.4µg per liter (Klecka *et al.*, 2009) and occurrence of BPA on surface water indicate its characterization as xenoestrogenic (Bonefeld-Jorgensen *et al.*, 2007), also Villeneuve *et al*.,(2012) added aromatase inhibition and antiandrogenic activity as additional mode of endocrine action of BPA.BPA as a xenoestrogen, mess up with the immune defense of an organism either by mimicking to natural estrogens of a body or it may compete with the endogenous estrogen binding to ERs. BPA exposure was reported to modulate the paradoxical immune response of mammalian model by either suppressing or stimulating it (Straub, 2007; Rogers *et al.*, 2013; Schug and Birnbaum, 2014). BPA at its lower concentration is more likely to be susceptible toward pro-inflammatory action in fish species. BPA exposure at low µg/L or mg/L may cause functional change to macrophages and lymphocytes of fish immune system which led to elevate leukocytes count, cell proliferation and enhance cellular

construction of superoxide anions (Rogers *et al.*, 2013; Gushiken *et al*., 2002; Yin et al, 2007). BPA exposure in vivo study at lower concentration on zebra fish highlight several inflammatory mediators that were similar to induced lipopolysaccharide stimulation that was chemokine, nitric oxide and cytokines which reveal that BPA single-handedly may perhaps persuade immune response considerably even at lower concentration (Xu *et al.*, 2013).BPA has been widely spotted in biological matrices and in living being i.e., detected level of BPA in water sample from Europe, Asia and North America was ranged from 4.4 to 8000 ng per liter (Jonkers *et al*., 2010; Staples *et al*., 2000, 2018; Xu et al., 2018). When BPA entered the water body it effects the aquatic species and accumulate in their body. Marine fish of Latium coast of Italy and Gulf of Naples were reported to have burden of 0.5 to 6 mg per kg of BPA in their body (Mita *et al*., 2011), however fish from sea around Taiwan have level of BPA ranged from 0.19 to 25.2 µg per kg in their body (Lee *et al*., 2015). Thus, association concerning fish health and toxicant contact is of chief concern to study the physiological and histological biomarkers to spot the reaction of a fish to that noxious toxicant and to get more realistic understanding on ecotoxicology of BPA on environment. As C. mrigala is an important edible aquatic species of Pak-Indo subcontinent that is consumed commercially so, it is significant to monitor its feasting effects on human physiology. So, the present investigation was designed to explore the risk assessment of sublethal concentrations of BPA to check its effect on characteristics profile of commercially important fish, also to evaluate functional analysis of its toxicity associated to different organs and potential damage in fish body.

2. Materials and Method

This research was conducted in Zoology lab of Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore, Pakistan.

2.1 Fish collection and maintance

50 fingerlings of C. mrigala in weight ranged 39.5±7g and length ranged from 3-4 inch were collected from Fisheries Complex, Manawan, Lahore, Pakistan and were placed in fish aquarium house of Institute of Molecular Biology and Biotechnology (IMBB), UOL. The collected fish fingerlings were washed with 0.01% KMnO4 solution for 15 mins and were checked thoroughly for any injury or diseased condition as only healthy were used for the experiment and then they were acclimatized at laboratory conditions for two weeks. Meanwhile fish were nourished with commercial fish food, once a day. One third of the water was renewed in alternate days with tap water to avoid any type of contamination while maintaining the adapted environment. Also, water physiochemical parameters were checked which include temperature (18-21℃), pH (8.0) etc. Then fish were randomly divided into four groups, 12 in each group and were placed in four aerated aquariums containing 60-liter water and were labelled as A, B, C and D. The first group A was kept as control and was maintained with normal tap water without any treatment. The second group B was treated with 0.5ppm concentration, third group C with 1.0ppm

concentration and fourth group D was exposed to 1.5ppm of BPA for 30 days.

2.2 Dose preparation

Three sublethal doses of BPA of sigma (USA) were prepared of concentration of 1.5ppm, 1.0ppm and 1.5ppm (LC 50 is 7.3 ml/L, Krishnapriya *et al*., 2017) (or LC50 is 4.13ppm, Elvin et al., 2020) and were applied for 30 days. For dose preparation, required amount of BPA was dissolved in 0.75 ml of solvent (ethanol). The solutions were renewed once in 48h exposure period by removing the 30 L volume of water and substituted with the fresh water in order to sustain the drug dose at constant concentration.

2.3 Blood collection and Hematological analysis

After the completion of 30 days trail, blood was sampled by cardiopuncture of fish heart by using 1 CC plastic disposable syringes. Blood was drag up in the syringe, and was transferred immediately into EDTA vials in order to prevent blood from clotting. Hematological variables such as Hb, platelets, RBCs, WBCs, etc. were then estimated using these blood samples. Complete blood count of hematological profile (RBCs, WBCs, platelets, hemoglobin count, Hct etc.,) was done using "sysmex XP-100", while MCH, MCV and MCHC was calculated using standard formulas.

$$\text{MCH (pg)} = \text{Hb (g/dl)} \times 10 \,/\text{RBC count in millions/mm3}$$

$$\text{MCV (fl)} = \text{Hct (\%)} \times 10 \,/\text{RBC count in millions/mm3}$$

$$\text{MCHC (g/dl)} = \text{Hb (g/dl)} \,/\text{Hct (\%)} \times 100$$

2.4 Dissection, organ collection and histology

After taking the required amount of blood, fishes were chloroformed and were dissected. Required organs (gills, muscle and liver) were isolated and were transferred into 25ml falcon tubes containing 10% formalin solution which helped in arresting the cell stage of a tissue by penetrating in tissue and change its physical and chemical state. Also, it hardens and protect tissues for any further subsequent processing steps. After fixing tissues in fixative, they were dehydrated by ethanol in its series of graded concentrations. Then this ethanol was replaced by xylene and tissues were then embedded in paraffin wax. Wax solidified at 20°C to attained the consistency for the sections to be cut in microtomy. Rotatory microtome was used for sectioning purpose. Wedge shaped sharp blade of microtomy trimmed the wax block until the orientation of tissue and 4-5µm thick section was cut and placed in water bath of 45°C and was loaded on glass slide. Slide was then placed on hot plate to adhere section firmly on glass slide.

2.5 Staining and microscopy

Hematoxylin and Eosin staining was used for staining which dye nucleic acid blue and cytoplasm orange red. Slides were first dipped in hematoxylin stain for 15 min and then were placed in eosin solution for 15 min. Slides were then air dried and then a drop of mounting media was applied to fix cover slip that could preserved slides for long. Slides were let to dry overnight at room temperature. Then these prepared slides were observed using "Micros trinocular camera fitted microscope" to examined the histopathological changes of gills, muscle and

liver of all groups (control and treated) and was photographed.

Results, Histology, Gills

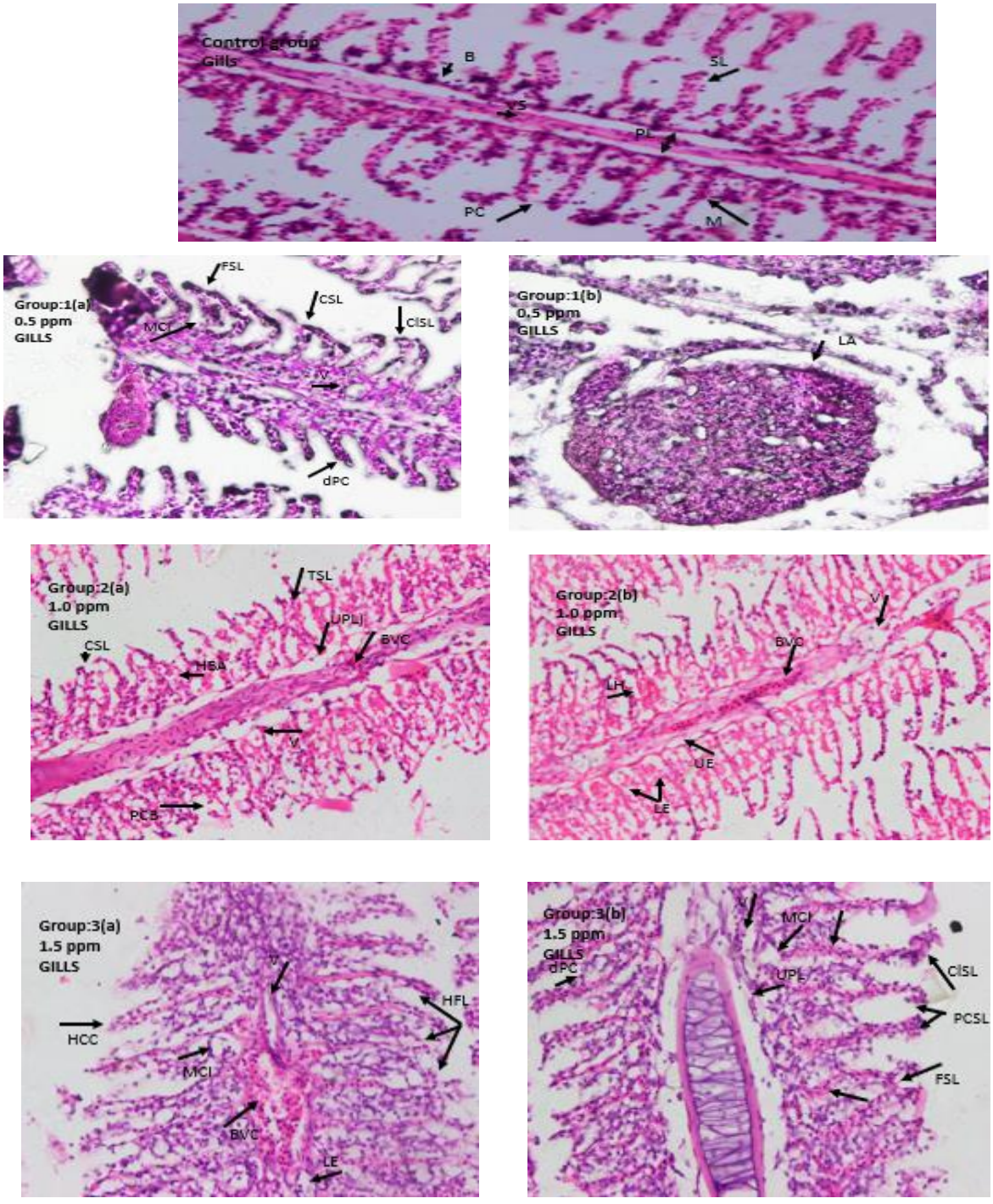
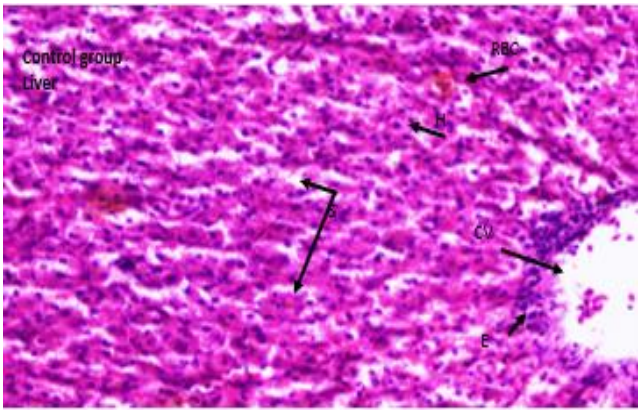
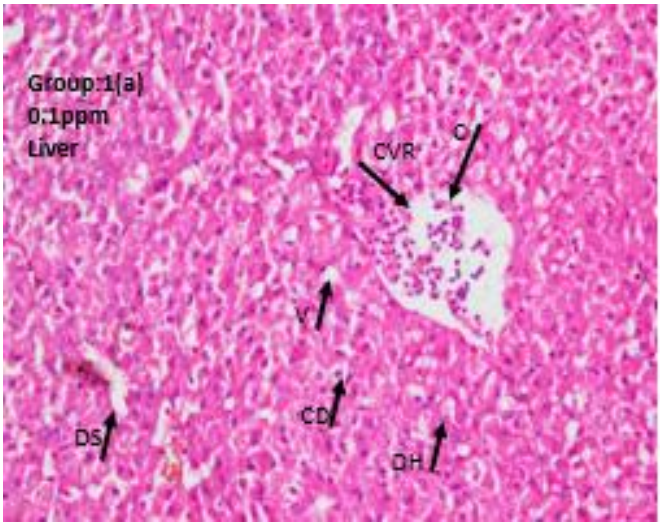


Figure 1. Cirhinnus mriala gills tissue of one control and three groups exposed to 0.5ppm, 1.0ppm and 1.5ppm of bisphenol

A (group 1-3) Abbreviations: basal cell (B), secondary lamella (SL), sinus vein (VS), primary lamella (PL) , mucus cell (M), pillar cell (PC), fusion of s. lamella (FSL), curling of secondary lamella (CSL), Clubbing of secondary lamella (ClSL), increase in mucus cell (MCI), degeneration of pillar cells (dPC), Lamellar aneurysm (LA), Telangiectasis of s. lamella (TSL), hyperplasia of branchial arch and vacuolization (HBA), uplifting of primary lamellae (UPL), Vacuolization (V), breakdown of pillar cells (PCB), lamellar hypertrophy (LH), Lamellar edema (LE), Congestion of blood vessel (BVC), uplifting of the epithelium (UE), hyperplasia chloride cell (HCC), hyperplasia fused in full length (HFL), Pronounced curling of secondary lamella (PCSL),Normal gills histology (fig 1, control) composed of primary and secondary lamella in an arrangement that primary lamella occupy the central position composed of chondrocyte skeleton and was surrounded by a row of thread like secondary lamella which have their base attached with the primary lamella and have free distal ends. Secondary lamella were homogeneously spaced columnar structures that surround at the cellular layer of the primary lamella. The primary lamella was comparatively thick (have chloride cells, blood vessels and was lined with squamous epithelial and have chloride cells) than that of secondary lamella which simply consist of single or double layered epithelial cell, a row of pillar cell, protective mucus cells and blood vessels. Interlamellar region was a space between two adjacent secondary lamellae. Gills when exposed to BPA stress show histopathology alteration. Lowest dose i.e., 0.5 ppm results in partial fusion of secondary lamella, curling, clubbing of s. lamella with enlarged mucus cells (fig 1, group 1a, b). As dose increased to 1.0 ppm concentration more abnormalities were observed that includes hyperplasia, vacuolization, uplifting of primary lamella epithelium and congestion of blood vessels (fig 1, group 2a, b). At concentration 1.5 ppm severe abnormalities with hyperplasia of chloride cells, full length fusion of secondary lamella, pronounced curling of secondary lamella and lamellar hypertrophy were observed (fig 1, group 3a, b).

Liver





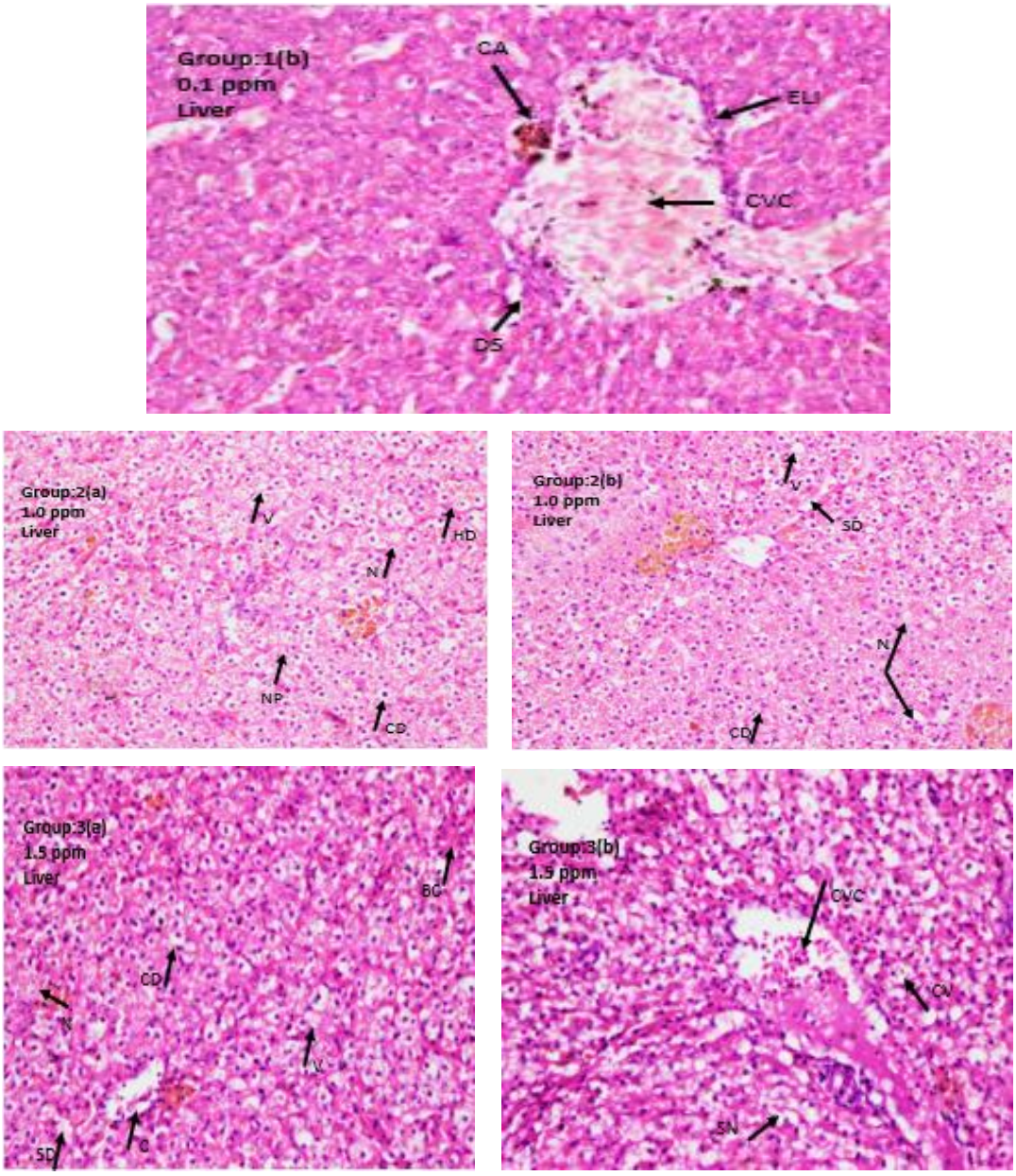


Figure 2. Cirhinnus mriala liver tissue of one control and three groups exposed to 0.5ppm, 1.0ppm and 1.5ppm of bisphenol A (group 1-3)

Abbreviations: Red blood cell (RBCs), Sinusoid (S), Hepatocytes (H), Central vein (CV), Epithelial layer (E), Rupture central vein (CVR), Congestion (C), Dilated sinusoids (DS), Vacuolization (V), Cytoplasmic degeneration (CD), Degeneration of hepatocyte (DH), Central Vein congestion (CVC), Chemical accumulation (CA), Endothelial lining inflammation (ELI), Vacuolization (V), Hepatocyte's degradation (HD), Cellular necrosis (N), Cytoplasmic degradation (CD), Nuclear pyknosis (NP), Necrosis(N), Sinusoid damage (SD), Blood congestion (BC), Cytoplasmic vacuolization (CV), Severe necrosis (SN)Normal histology of liver (fig 2, control) composed of hepatocytes which have

round polygonal appearance with centrally located nuclei, sinusoids and blood capillaries. Hepatocytes were organized in cord around central vein. Hepatic cell cords are cord like structure which were separated by sinusoids from the arrangement of hepatocytes. Between the hepatocytes, blood sinus were irregularly distributed. The changes observed in liver histology after 0.5 ppm BPA exposure was rupture of central vein, damaged hepatocytes, dilated sinusoids and vacuolization (fig 2, group 1a, b). On higher account of concentration i.e., 1.0 ppm, more damage was analyzed including hepatocytes degeneration, cytoplasmic vacuolization, central vein congestion, hypertrophy and clumping of hepatocytes, acute necrosis etc. (fig 2, group 2a, b) and on highest BPA exposure of 1.5 ppm concentration severe damages were observed with severe cell necrosis, extensive vacuolization, cytoplasmic degradation, pyknotic nuclei, cell wall of hepatocytes were also damaged and have indistinct boundaries, and many hepatocytes were degraded and eosinophil patches were observed with no or deep stained nuclei (fig 2, group 3a, b).

Muscle

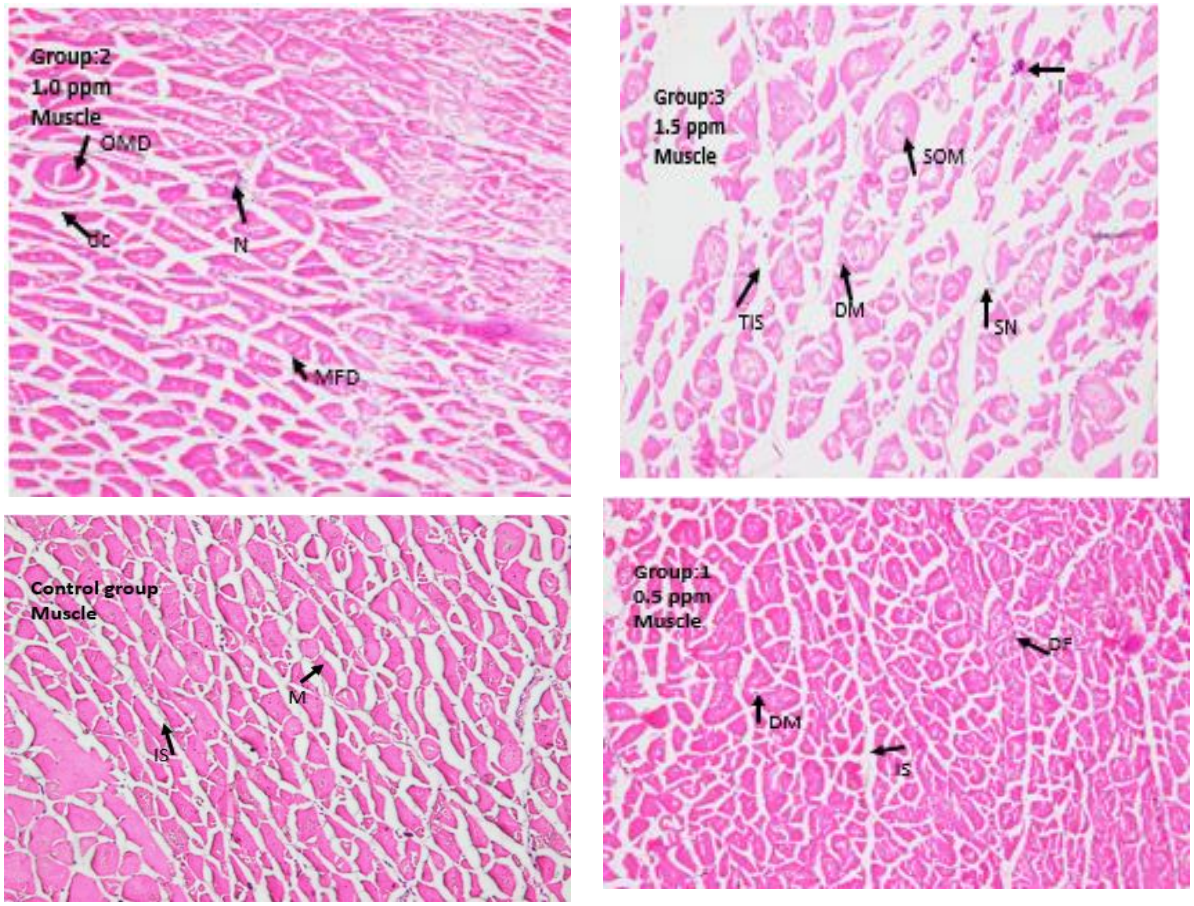
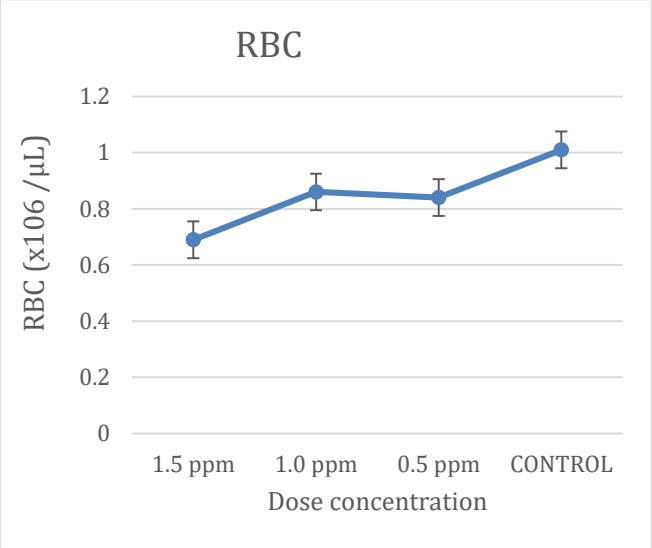
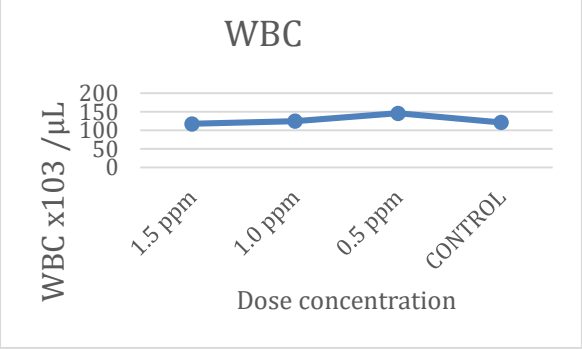


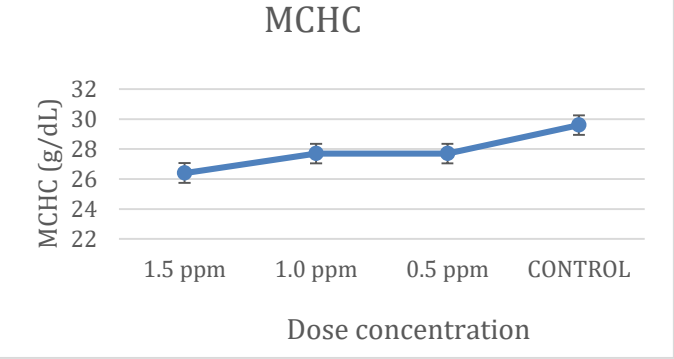
Figure 3. Cirhinnus mriala muscle tissue of one control and three groups exposed to 0.5ppm, 1.0ppm and 1.5ppm of bisphenol A (group 1-3)

Abbreviations: Intercellular spaces/ connective tissue spaces (IS), Myofibril (M), Disintegrated myofibril (DM), Disintegrated muscle fiber (DF), Inter myofibril spaces (IS), Dystrophic change (dc), Oedema of muscle fiber (OMF), Necrosis (N), Muscle fiber disintegration (MFD), Severe oedema of myofibril (SOM), Inflammatory cells in connective tissue spaces (I), Pronounced thickening of inter myofibril spacing (TIS), Severe necrosis (SN)Muscle histology photograph represent muscle fiber which were held together by connective tissues, intramuscular spaces. A muscle bundle was formed by the arrangement of various muscle fibers which contains many individual muscles and these muscles were separated by the presence of connective tissues in between them (fig 3, control). In least treated group of 0.5 ppm concentration mild histology changes were observed that include disintegration of muscle fiber, myofibril degradation, mild increased in intramuscular spaces (fig 3, group 1). While as the concentration increased to 1.0 ppm, more histopathological changes were found which were oedema of muscle fiber, dystrophic changes in muscle tissue with increased intramuscular spaces, vacuolar degradation of muscle fiber (fig 3, group 2). Treated group 1.5 ppm concentration have even severe damage including severe oedema of myofibrils, inflammation in connective tissue spaces, severe necrosis and pronounced thickness in inter myofibril spaces, intramuscular oedema and atrophy was also observed (fig 3, group 3).



HEMATOLOGY





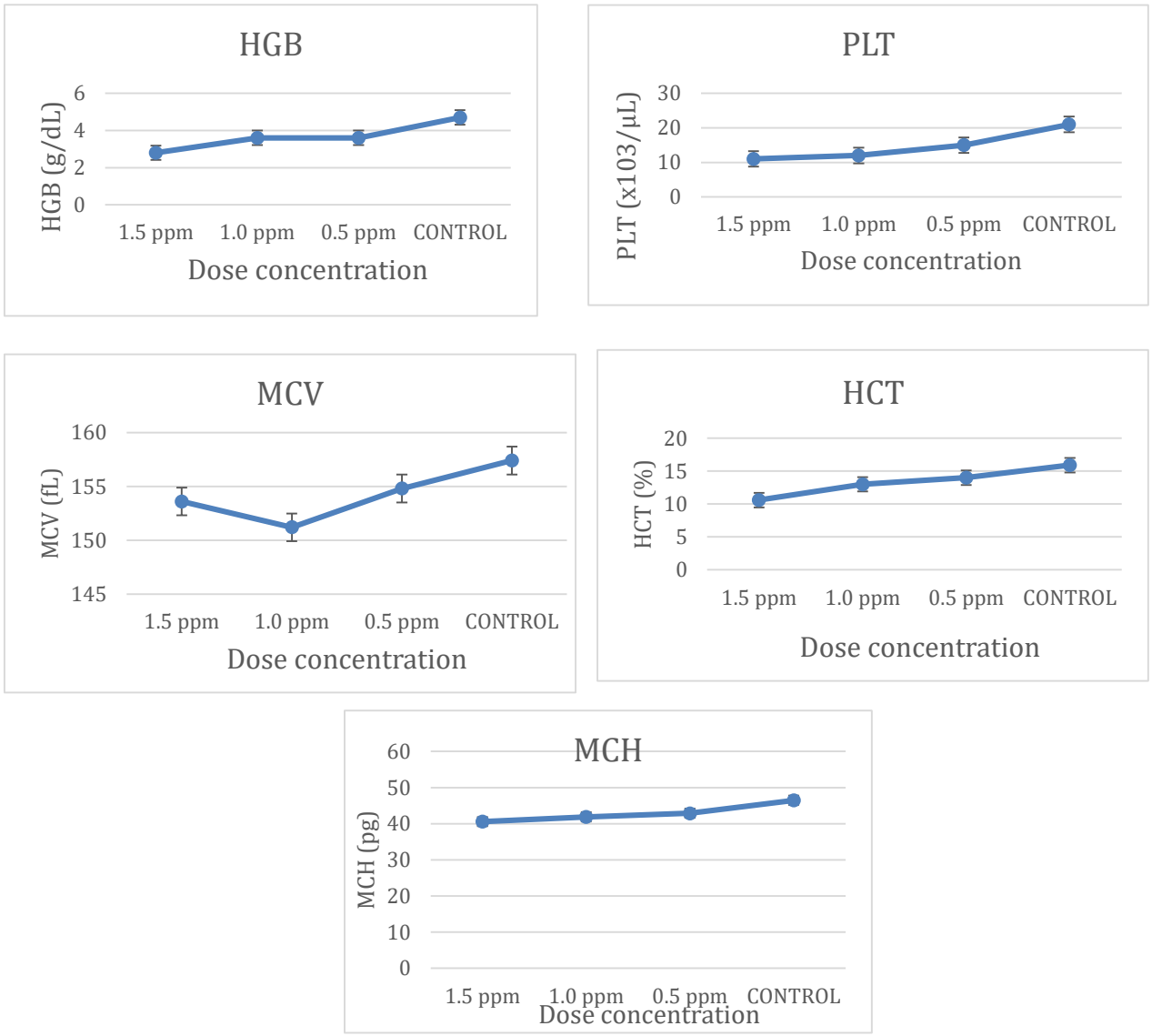


Figure 4. Comparison of average concentration of blood parameters (RBC, WBC, HGB, HCT, MCV, MCH, MCHC and PLT) of Cirhinnus mriala between control and three groups exposed to 0.5ppm, 1.0ppm and 1.5ppm of bisphenol

A From present study, complete blood count of C. mrigala were analyzed after 30 days exposure with 0.5 ppm, 1.0 ppm and 1.5 ppm dose concentration of BPA and results observed were gradually decrease in RBCs as concentration of dose increased on comparison with the control. However, WBCs count increased on 0.5 ppm exposure and then decreased as the dose of BPA increased. A significant difference was recorded WBCs in dose dependent manner to that of the control group. Also, mean corpuscular volume (MCV), hematocrit (HCT), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH) also decreased in dose dependent manner when compared to control group. Hematological indices (MCH, MCV, MCHC) help to determine the anemia

type, erythrocytes shape and size. MCV and MCHC values reflect the normal or abnormal division of cell during erythropoiesis. In this study, MCV, MCHC and MCH values decreased in experimental groups from lower to higher concentration compared to control group (fig 4).No mortality was observed in any sublethal concentration of BPA exposure in C. mrigala throughout the trial period. Some behavioral abnormalities were observed which include more mucus secretion, depressed swimming behavior, feeding behavior was also low.

3. Discussion

3.1 Histology

The present study showed the effect of different concentration of BPA (0.5ppm, 1.0ppm and 1.5ppm) in different organs (gills, muscle and liver) of C. mrigal and many histopathological defects were examined. Histological analysis is significant for determining the effect of pollutant on an organism, that was taken as a biomarker which reflect the general health grade of inhabitants of an ecosystem (Khoshnood et al., 2010). Liver is a chief organ for detoxification of toxic pollutants (heavy metals, drugs, pesticides, other anthropogenic pollutants) in living body (Roberts and Rodger, 2001). Various biotransformation also occurs in liver, which is why it enhances the toxicity of various metabolites therefore, it is a common target for tumorigenesis and cytotoxicity (Faheem *et al*., 2016; Hinton *et al*., 2017). Health status of fish can be determined on examining the histopathology of liver. Therefore, histopathology of liver of aquatic fauna reflects the presence of toxicants in their environment (Moon *et al*., 2012). In this study, C. mrigal was exposed to three concentration of BPA for 30 days. Several significant histopathological changes were examined on dose dependent manner from least to higher concentration with slightly to severe changes. Pathological changes include rupture of central vein, congestion, dilated sinusoid, hepatocytes degeneration and cytoplasmic vacuolization. Similar results were reported by Radhaiah and Rao (1992) in liver of Tilapia mossambica who was exposed with fenvalerate (insecticide) and Maharajan *et al* (2016) observed in Lates calcarifer liver when exposed to copper. Cell necrosis, inflammation and nuclei pyknotic were also observed in severe dose exposure. El-jawaher (2012) reported similar results in Oreochromus spiluruus hepatocytes when they were exposed to nonylphenol. Faheem et al. (2016) stated analogous histological changes in Catla catla on 15 days exposure of BPA and Sakr *et al*. (2005) also observed comparable hepatic pathology in Clarias gariepinus fish which include blood vessel congestion, necrosis, cytoplasmic vacuolization of hepatocytes, fatty infiltrations and inflammation. Damage in normal histology of liver upon toxicant exposure may cause toward the loss of structural protein (Faheem and Lone, 2017). Excessive necrosis may occur to get rid off BPA in fish body (Elvin et al., 2020). Or may be due to inability of liver to regenerate after toxification of BPA might cause severe widespread necrosis (Elvin *et al*., 2020). These histopathological degenerative changes of hepatocytes were similar to the study reported by Faheem *et al*., (2019) in Catla catla, and Sisodiya *et al*., (2018) in Heteropneustes fossilis on exposure to BPA. Gills are respiratory room for fish,

also it plays part in ionic regulation of fish body. Gills are considered as a bio monitoring tool that respond against environmental pollutant due to high contact with water (Laurent and Perry, 1991; Au, 2004; Oliveira Ribeiro et al., 2005; Vigliano *et al.*, 2006). In this study, gills of control group have normal histology of primary lamella, secondary lamella, pillar cells etc., while treated groups showed damaged histology and this damaged increased with the severity of BPA concentration. Hypertrophy of gills epithelial cells, fusion, clubling and curling of secondary lamella, vacuolization, oedema, uplifting of primary lamella were some abnormalities observed after 30 days exposure of BPA. These abnormalities were severe with higher concentration e.g., fusion of secondary lamella with its neighborhood's lamella is partial in case of lower concentration and hyperplasia fused in full length with higher concentration, similarly uplifting of primary lamella was more pronounced in higher concentration as compare to the lower concentration exposure. Hyperplasia and uplifting of epithelium are protective mechanism of fish against their polluted environment (Tietge *et al.*, 1988). The lifting of lamellar epithelium was also observed by Muller and Lloyd (1994) and Heath (1995) in gills of fish when fish were exposed with metal, detergents, oil and ammonia etc. Clubbing, fusion and curling of secondary lamella was reported by Faheem et al., (2016) on exposure of C. catla with BPA, Maharajan et al (2016) also reported the similar pathology of gills in L. calcarifer on exposure with different concentration of copper. Patnaik *et al*. (2011) also reported similar gills damage with lamellar fusion, congestion in blood vessels, hyperplasia of bronchial arch, vacuolization etc. in Cyprinus carpio communis when treated with different doses of lead and cadmium. Gill's damage, degradation and necrosis are indicators of toxic health of fish in polluted environment (Garcia-Santos *et al*., 2007). Lamellar telangiectasis of blood vessels of secondary lamella results to damage the pillar cells (Alazemi et al., 1996). Lamellar fusion result in reduced surface area for gaseous exchange. So, histopathological change of gills leads to hypoxia and respiratory problems along with ionic imbalance (Alazemi *et al*., 1996). Like gills, muscle also have a close contact with the toxins of environment and show pathology in its normal histology. In our study histological damage of muscles were seen which include muscle intracellular oedema which increases muscle bundle spaces and this space amplified as the concentration of doses increased. At highest concentration, intracellular spaces thickened, inflammation, degradation and necrosis of muscle fiber occur. Similar observations were reported by Fatma (2009) which include muscle bundle degradation, inflammation, atrophy, necrosis and vascular degradation of muscle bundles in fish when exposed to different pollutants. Maharajan *et al*. (2016) also stated similar histopathology in L. calcarifer when exposed to copper doses. Toxic copper effect to cause hyperactivity and excitability of muscle cell which led to be the reason muscle fatigue (Das and Mukherjee, 2000) along with degradation, atrophy and necrosis of muscle cells. Similar to our investigation, alteration in muscle histology were

observed in different fish species on exposure to different metals by Thophon *et al*. (2003), Nagarajan and Suresh (2005), Kaoud and El-Dahshan (2010), Patnaik BB *et al*., (2011) and Maharajan *et al*. (2016). A stressed organism from a polluted environment shows different histopathological changes of organs which lead toward abnormality in their functions and ultimately cause stressed behavior and abnormal physiology to that organism. Histological biomarker helps to determine the adverse effects of xenobiotics in an environment by measuring the healthy normal tissue with injured diseased tissue or organs of species from different environments. In this present study, adverse effect of BPA was observed in gills, muscle and liver of C. mrigal with different doses. Least pathology were observed on lowest 0.5ppm concentration and adverse effects were examined on high 1.5ppm concentration.

4. Hematology

Hematological indices are monitoring parameters for observing pathological and physiological health of fish as well as to evaluate the ecotoxicological status of an environment (Narra, 2017; Kumar et al., 2019). Because blood is a transport medium of necessary elements along with the toxicant at intercellular and intracellular medium to various tissues and organs of body. Red blood cell act as an interorgan which communicate to transport oxygen and other nutrients to organs according to their demand (Kuhn et al., 2017). BPA is known to impact negatively on health of living bodies including fish (Cano-Nicolau et al., 2016, Pal and Reddy, 2018, Wang et al., 2019). In this study, BPA caused to decreased the total erythrocytes count, Hb, Ht and other secondary indices of RBCs including MCV, MCH, MCHC were also significantly lower in treated groups of C. mrigala than that of the control group.From our study, it was found that when fish groups were exposed to BPA, the quantity of RBCs reduced significantly according the concentration gradient. This finding was similar to Keum et al., (2005) and Elvin *et al*., (2019). This decrease might be because of oxidative damage that BPA induces to bone marrow as it produces RBCs (Tiwari and Vanage, 2017) or it might also be because of rapid hemolysis. This reduction in RBCs lead to result in the reduction of Hb and Ht level (Javed *et al.,* 2016) which ultimately cause anemic condition to exposed fish. Another study has showed that BPA also causes disturbances in human body i.e., increases phosphatidylserine translocation into RBCs and causes to increase in caspase-3 and calpain activity. These changes cause increased eryptosis i.e., programmed death of RBCs in human body (Macczak *et al*., 2016). BPA also inhibits the signaling and secretion of erythropoietin hormone which impairs the mechanism of erythropoiesis. In this way BPA disrupts the endocrine system (Pal et al., 2018). From these results we assumed that the exposure of BPA might have induced the carbon dioxide toxicity which led to caused anemic hypoxia in the exposed tissues (liver and muscle) of C. mrigala.The hemoglobin found in the fish erythrocytes have the same heme content and tetrameric molecular structure as that of the hemoglobin found in mammalian erythrocytes (Giardina et al., 1973). Our study showed that the

hemoglobin concentration was reduced in the fish cells due to BPA exposure. This decrease in hemoglobin concentration was due to the decreased number of erythrocytes as reported by our study was same as reported by another study by Elvin et al., (2019). Many other studies have also showed similar results of hemoglobin reduction due to BPA exposure (Kaliappan *et al*., 2017; Keum *et al*., 2005 and Elvin et al., 2019). Also decreased hemoglobin content in BPA exposed fish might be an indication of the cellular damage or injury or destruction of haemopoietic tissue of the exposed fish (Kumar and Banerjee, 2016). Reduced hematological indices i.e., MCH, MCV and PCV also suggest the possible RBCs shrinkage caused by BPA toxicity (Elvin et al., 2019). Anemia was observed in liver and muscle tissue of C. mrigala in the present study might be due to the erythropoiesis caused by BPA toxication. Similar observation was made in H. fossilis by Aiswarya and James (2016), in Labeo rohita by Krishnapriya *et al.* (2017) and in Acanthopagrus latus by Yaghoobi *et al.* (2017) due to BPA toxication. WBCs are the immune cells in living body which defend body against various foreign agents and hence help fighting diseases and infections. So, when an infectious agent enters the body, the number of WBCs increase. An increase in WBCs determines the presence of a toxic agent studies (Kaliappan *et al.*, 2017; Rogers and Mirza, 2013). Our study however, showed contrary results with a decrease in WBCs. Although, WBCs increased in lower dose exposure of 0.5ppm but then it decreased in high dose exposure. This decreased in WBCs were consistent with the study conducted Elvin et al., (2019). This result suggests that our fishes might had weak immune system or some environmental stress e.g., low temperature may cause to decreased in WBC values. Sugita-Konishi et al., (2003) have also showed similar results of decreased in number of lymphocytes and macrophages due to BPA exposure. BPA is also known to delay the lymphocytes mitogenesis in in-vitro (Sakazaki *et al*., 2002) and for the production f MCP -1 (Monocyte chemoattractant protein -1) (Inadera *et al*., 2000). Even the low doses of BPA disrupted the immunity and cytokine level (Özaydin et al., 2018). Similarly, reduction in Hct values designates anemic conditions in fish on BPA exposure, which is a reflex reaction of fish body in stressed environment (Krishnapriya *et al.,* 2017). There is a possibility that when Hb level down, it led to decreased the MCHC value (Sarma 1990). Srivastava and Reddy, (2020) also reported to have decrease MCHC values in H. fossilis on BPA exposure. But there is conflict with MCH and MCV values, which in our study decreases but in Srivastava and Reddy, (2020), Aiswarya and James (2016) and Yaghoobi *et al* (2017) studies, they increased. Also, Yaghoobi *et al.* (2017) reported contrary correlation of BPA with RBCs, Hb, Ht and other secondary indices of erythrocytes count in Acanthopagrus latus. Concludingly, BPA exposure impart negatively on fish histology, physiology and hematology on low dose contact, which is very alarming because ultimately it enters to human body directly or indirectly through food web.

5. Conclusion

The current study investigation reveals that BPA impart its severe effect on histopathology and CBC of C. mrigala. BPA is high risk pollutant as has high toxic potential and severe damage were observed at any concentration. Instability of complete blood count of C. mrigala and mild to severe damage in dose dependent manner in histopathological analysis of muscle, liver and gills concluded the severity of toxicant nature of BPA. Eventually these pathological changes may influence the metabolism and physiological damage to the fish. Thus, BPA induced environment can make health and survival of fish and other aquatic species at risk.

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