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Assessing the Impact of Xenobiotic (Bisphenol A) on Blood Physiology and Biochemical Alterations Using *Labeo rohita* Fish as a Model Organism

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ABSTRACT

Bisphenol A (BPA) is an emerging pollutant that is extensively used in the manufacturing of various industrial products and is associated with adverse effects on both human and wildlife health. **Objective:** Present study aimed to evaluate the effects of bisphenol A on hematobiochemical biomarkers in freshwater *Labeo rohita*. **Methods:** For the purpose of this investigation, healthy fish were divided into four groups (A-D). Group A was treated as a BPA-free control group, while Group B, Group C, and Group D were exposed to various doses of BPA such as 400, 800, and 1600 µg/L, respectively for 21 days. **Results:** BPA-exposed fish showed different physical and behavioral abnormalities in dose-dependent ways. Results indicated significant increase in the concentrations of various hematobiochemical parameters, such as WBCs, MCHC, RDW, RDW-SD, platelets, neutrophils, triglycerides, cholesterol, ALT, AST, blood glucose, urea, T_3, TSH and creatinine, while HGB, RBCs, HCT, MCV, MCH, PDW, lymphocytes, HDL, LDL, VLDL, total protein, globulin, albumin and T_4 concentrations were decreased. **Conclusions:** The current study concluded that bisphenol A causes deleterious effects by disrupting physiological and hematobiochemical parameters alteration in exposed fish.

INTRODUCTION

The global focus on monitoring the impacts of pollutants, including agricultural and industrial wastes, has grown substantially in recent years [1]. Several contaminants from various industries, including textile mills, pharmaceuticals, paper industry, chemical manufacturing, and plastic industry, add many pollutants, such as pesticides, flame retardants, plasticizers, and heavy metals, to water bodies. A number of these chemicals are endocrine disrupters, such as bisphenols [2]. The release of these chemicals into aquatic ecosystems poses a risk to aquatic organisms, especially fish. Thus, fish are particularly susceptible to these contaminants because they are often exposed to numerous waterborne pollutants throughout their lives, especially during important stages of development [3, 4]. Human activities are increasing the level of these toxic substances in our rivers and lakes, posing a threat to aquatic life [5]. Bisphenol A (BPA) is an organic, colorless, synthetic chemical that has a global application. BPA, an organic compound with two phenol functional groups, is a key precursor in many plastics, including epoxy resins and polycarbonate polymers [6]. BPA is widely utilized in many sectors, including car lenses, compact discs, construction materials, water pipes, electrical components, dyes, protective coatings, paints, plastic bottles and food containers [7]. With a global production of 3 billion kg of BPA per year, around 100 tons of the chemical are released into the environment annually [8]. Government agencies in the United States and Europe have classified bisphenol A as an endocrine disruptor and a "moderately toxic" chemical [9]. Due to its detrimental effects on fish and other aquatic animals, it has recently seen extensive use in aquatic toxicity research. Humans

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and ecosystems, especially aquatic environments, are continually exposed to BPA via discharges from the petrochemical sector, municipal sewage and landfill effluent. The paper recycling and packaging industries release wastewater containing high quantities of BPA, which can also contaminate aquatic ecosystems [10]. Fish are among aquatic animals that are vulnerable to high levels of BPA exposure because surface waters absorb it. BPA at elevated concentrations can harm aquatic life over time, hence the highest quantity recorded at a place is the optimum exposure limit [11]. BPA concentrations in water ranged from 1 to 1000 µg/L [12]. The presence of BPA in aquatic systems leads to significant health issues for aquatic animals, and its negative impact on aquatic ecology has raised considerable alarm [13]. Previous studies reported that BPA causes alteration in different hematobiochemical parameters such as hemoglobin, white blood cells, MPV, hematocrit, RBCs and serum proteins in yellow perch, Korean rockfish and albino rats [14-16]. Fish, as members of the food chain, are sensitive to even small quantities of xenobiotics like Bisphenol A (BPA) and bioaccumulative harmful chemicals. So, they are used as indicators to detect pollutants in water bodies [17]. The carp *Labeo rohita* was used in the present experimental trial. Rohu *(Labeo rohita),* a member of the *Cyprinidae* family, is an economically significant and cultivable fish found in freshwater lakes and rivers in Asia, especially in Pakistan, India, and Bangladesh [18]. Serum biochemical indices and hematological alterations are important biomarkers for detecting physiological changes and assessing toxicity. With these alterations, the fish health status and the toxicological indicators for organisms may be better understood [19].

Therefore, we conducted the current study to examine the effects of graded concentrations of BPA on *Labeo rohita* using hematological and biochemical biomarkers.

METHODS

Bisphenol A was obtained from MACLIN, China. We dissolved an adequate amount of BPA in ethanol to prepare the stock solution for use in the experiments following the protocol described by Kwak *et al* [20]. Rohu (*Labeo rohita*) with an average weight of 32.40 ± 4.53 g and length of 18.40 ± 0.28 cm were purchased from Balloki fish farm, Pakistan and transported to fish lab of the University of Okara. No mortality was found during transportation. Fish were acclimatized in a glass aquarium having dimensions of 40" W × 30" H × 35" L with 100 liters of water for one week and fed once a day. 90% of the water was changed after one day during the whole experiment. After acclimatization, four groups were made (A-D). Group A was treated as a BPA-free control group, while groups B, C, and D were exposed to various concentrations of BPA such as 400, 800, and 1600 µg/L, respectively for 21 days. Temperature, pH, hardness of water and dissolved oxygen were maintained. Bisphenol A concentrations were decided based on earlier research [21, 22]. At day 21, fish (n = 12 for each group) were taken from the control and treated groups. Blood was collected through a BD syringe from the abdominal vein and placed in EDTA and gel vials for hematology and serology. This examination was carried out in a laboratory using specific experimental techniques. Fish were anesthetized by using clove oil. All hematological parameters were analyzed by a hematological analyzer. The number of RBCs and WBCs were counted using a hemocytometer. The amount of hemoglobin was measured using a UV spectrophotometer set to 540 nm and a cyanmethemoglobin diagnostic reagent kit. The measurement of HCT was performed using the microhematocrit technique. Erythrocyte indices, viz., MCHC, MCH, and MCV, were calculated from RBC, HCT, and HGB values following the method described by Ramesh *et al* [23]. The lipid profile (cholesterol, LDL, HDL, VLDL, triglyceride), liver enzymes (ALT and AST), and some other biochemical parameters (total proteins, globulin, albumin, urea creatinine, T3, T4, TSH and blood glucose) were analyzed using a chemistry analyzer using the method of Ghaffar *et al* [24]. For the assessment of protein and glucose levels, blood samples were centrifuged at 9392 × g, for 20 minutes at 4 °C to separate the blood plasma. Using a diagnostic reagent kit, blood glucose level was determined as described by Abraham and Gerarge [25]. Blood proteins were assessed by using the method of Kumar *et al* using bovine serum albumin as standard [26]. ALT and AST levels were determined by using a special kit (Spectrum AST – kit, Egypt) [27]. Creatinine and urea were estimated using kits supplied by Biomerieux (France). Using standard kits, serum cholesterol, HDL, and triglyceride levels were measured following the method of Hassan *et al* [28]. VLDL and LDL levels were calculated according to the standard formula described by Zaahkouk, *et al* [29]. T_3, T_4 and TSH values were assessed by following the method of Hadie *et al.*, using standard kits [30]. Statistical analysis was done by applying one-way ANOVA on GraphPad Prism (V 9.5.1) software at $p<0.05$ level of significance. GraphPad Prism (Version 9.5.1) was also used for graphical representations.

RESULTS

In chemical-free group A, no physical and behavioral abnormalities and mortality were observed. Bisphenol-treated low to high-dose groups showed different physical and behavioral responses, from mild to severe. Physical and behavioral responses include loss of equilibrium, faintness, black spots on the whole body surface (changed skin color), operculum movement, fins tremor, gulping of air, mucosa secretion from mouth and gills, eyes bulging, jerking and laying on one side during uneven swimming.

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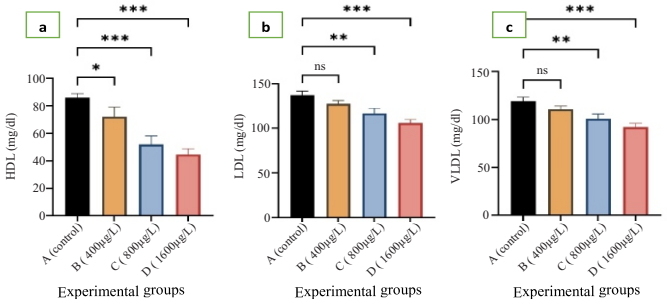
Group C (800 g/L) and D (1600 g/L) BPA-exposed fish showed more severe physical and behavioral signs. In the chemical-free control group, all hematological parameters, including HGB, WBCs, HCT, RBCs, RDW-SD, PCT, neutrophils, lymphocytes, monocytes, MCHC, MCV, RDW, platelets, MCH, MPV, PDW, and eosinocytes were observed as normal. As compared with the BPA-free group, the values of MCHC, WBCs, RDW, RDW-SD, platelets and neutrophils were significantly increased with increasing dose concentration, while HGB, RBCs, HCT, MCV, MCH, PDW and lymphocytes were significantly decreased as compared with chemical-free group A. Other parameters (MPV, PCT, monocytes, and eosinocytes) were not significantly affected by BPA exposure, as shown in table 1.

Table 1: Showing the Hematological Profile Of *Labeo Rohita* Exposed To Various Doses Of Bpa.

Variables	A (Control)	B (400µg/L)	C (800µg/L)	D (1600µg/L)
HGB (g/dl)	5.63 ± 0.35	4.96 ± 0.40	3.16 ± 0.50*	2.36 ± 0.35*
WBC (x10^3/µL)	15.27 ± 2.21	20.83 ± 2.82*	25.27 ± 4.20*	31.80 ± 2.75*
RBC (x10^6/ µL)	2.03 ± 0.14	1.66 ± 0.11	1.29 ± 0.16*	0.71 ± 0.13*
HCT (%)	14.87 ± 0.35	12.13 ± 0.75*	9.85 ± 1.03*	8.61 ± 0.52*
MCV (FL)	142.02 ± 3.48	129.6 ± 4.38	102.9 ± 7.46*	95.57 ± 8.27*
MCH (pg)	47.07 ± 2.12	42.03 ± 2.63	35.30 ± 2.60*	23.53 ± 3.05*
MCHC (g/dl)	30.87 ± 2.13	34.70 ± 1.51	38.10 ± 2.68*	43.67 ± 2.15*
RDW (%)	23.13 ± 3.01	28.03 ± 1.94	36.10 ± 2.45*	42.87 ± 2.85*
RDW-SD (%)	31.90 ± 1.99	36.80 ± 3.03*	45.33 ± 3.11*	49.93 ± 2.30*
PLT (x10^3/µL)	24.35 ± 3.03	30.63 ± 2.84*	39.60 ± 2.09*	45.23 ± 3.27*
MPV (FL)	5.46 ± 0.40	5.03 ± 0.30	4.56 ± 0.25	4.20 ± 0.45
PDW (%)	15.30 ± 0.36	14.40 ± 0.45	12.93 ± 0.41*	12.17 ± 0.30*
PCT (%)	0.33 ± 0.03	0.19 ± 0.05	0.08 ± 0.02	0.06 ± 0.02
Neutrophils (%)	70.70 ± 3.05	87.07 ± 3.64	111.10 ± 4.54*	164.10 ± 4.59*
Lymphocytes (%)	27.00 ± 3.60	23.97 ± 1.15	21.57 ± 1.80*	16.15 ± 1.94*
Monocytes (%)	1.97 ± 0.65	1.70 ± 0.08	1.26 ± 0.08	0.77 ± 0.14
Eosinocytes (%)	1.90 ± 0.07	1.83 ± 0.06	1.39 ± 0.11	1.20 ± 0.08

The values are shown as mean ± SD. Asterisk (*) bearing values show significant differences ($p< 0.05$) as compared to the BPA-free group A (control). The statistical values of biochemical parameters including cholesterol, triglycerides, VLDL, HDL and LDL were presented in figure 1. A significant increase was observed in triglycerides, and cholesterol while a decrease in HDL, LDL and VLDL levels (Figure 1).



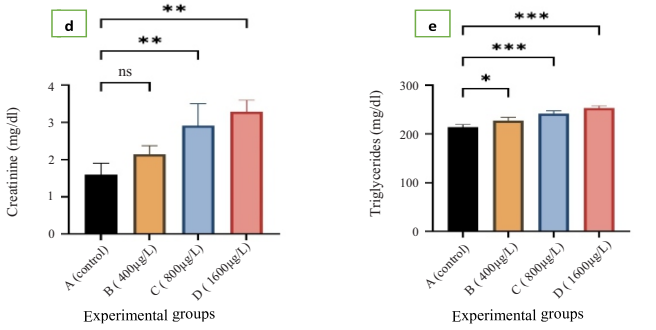


Figure 1: Change in (a) HDL, (b) LDL, (c) VLDL, (d) creatinine and (e) triglycerides in BPA-exposed groups as compared with control group. The data are shown as mean ± SD. Asterisk was shown different significant levels ($p\leq0.05$).

Results of ALT, AST, total protein, albumin, globulin and blood glucose are presented in figure 2. Results showed significant elevation in ALT, AST and blood glucose levels while a decline in total protein, globulin and albumin levels. In figure 2, the change in blood glucose level, ALT, AST, albumin, globulin, and serum total proteins in BPA-exposed groups compared to the control group is presented as mean ± SD, with asterisks indicating significant differences ($p\leq0.05$).

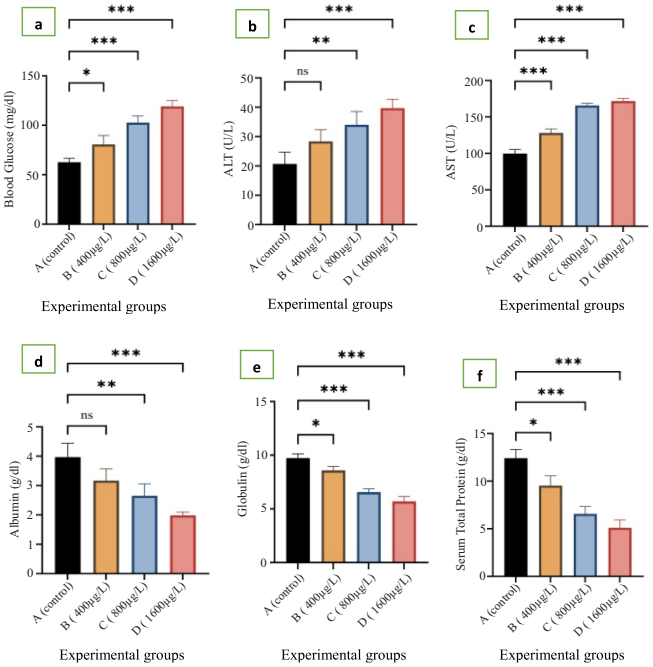


Figure 2: Change in (a) blood glucose level, (b) ALT, (c) AST, (d) albumin, (e) globulin and (f) serum total proteins in BPA-exposed groups as compared with control group. The data are shown as mean ± SD. Asterisk was shown different significant levels ($p\leq0.05$)

T_3, T_4, TSH, urea, creatinine and blood urea nitrogen were presented in figure 3. The result of the one-way ANOVA statistic showed a significant ($p\leq0.05$) increase in, urea, T_3, TSH, blood urea nitrogen, and creatinine levels in treated groups as compared with the chemical-free group A

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(control). A dose-dependent decrease in T4 level was observed

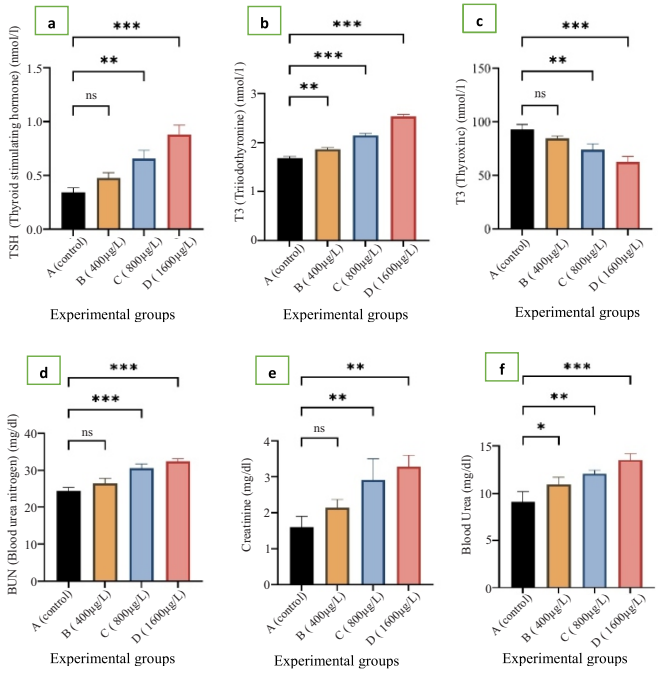

Figure 3: Change in (a) TSH level, (b) T_3, (c) T_4, (d) blood urea nitrogen, (e) creatinine and (f) urea in BPA-exposed groups as compared with control group.The data are shown as mean ± SD. Asterisk was shown different significant levels ($p \leq 0.05$).

DISCUSSION

In toxicological studies, exposing organisms to specific dosages at different acute or sublethal concentrations helps better understand the hazardous levels of chemicals, including bisphenol A [31]. The extent to which environmental contaminants harm aquatic life is crucial [32]. Pollutants may harm fish fauna via physiological, biochemical and histological alterations [33]. Over the last few decades, there has been a worldwide rise in efforts to monitor and record the impacts of environmental toxins such as herbicides, pesticides, and industrial effluents [24]. Many pollutants from different kinds of sources readily and instantly enter water bodies. Therefore, aquatic organisms are more vulnerable to damage than terrestrial animals [34]. Many of these synthetic substances, such as bisphenol A, are endocrine disruptors that damage fish tissues [35]. Therefore, to minimize the public health concerns associated with bisphenol A, it is important to conduct ongoing monitoring and evaluation of its toxicological impacts at low levels. The current study aimed to evaluate the sublethal toxicity of bisphenol A in *L. rohita* concerning clinical, behavioral, and hematobiochemical alterations in exposed fish. In present study, results showed that fish exposed to low levels of BPA developed a variety of physical and behavioral symptoms such as loss of balance, faintness, black spots on the whole-body surface (changed skin color), operculum movement, trembling of fins, gulping of air, increased production of mucus from mouth and gills, eyes bulging, and jerking of body and laying on one side during irregular swimming. Previous research revealed that the same findings were seen in *Cirrhinus mrigala* [36], *Ctenopharyngodon* [37], bighead carp [21], zebrafish [38], and *Channa punctatus* [39]. The same observations were also reported by Cervantes *et al.*, and Namratha *et al.*, in vertebrates [40, 41]. Blood serves as a pathophysiological indicator of the health status of an organism [42]. Therefore, hematobiochemical profiles are vital indicators of health, that are frequently employed to understand and diagnose the harmful effects of external environmental stressors and harmful chemicals on fish morphology and physiology [43-45]. Some hematological parameters, like hemoglobin level, hematocrit, white blood cell count, mean corpuscular hemoglobin concentration, mean corpuscular volume and red blood cell count, can be used to find out which organs in fish are most affected by metals, pesticides, and endocrine disruptors [46, 47]. Hematological analysis revealed that in the BPA-treated groups, the concentrations of platelets, neutrophils, White Blood Cells (WBCs), MCHC, RDW, RDW-SD, and RDW significantly increased with increasing dose concentration, compared to the BPA-free control group. Similar findings were reported by Afzal *et al.*, and Asenuga *et al* [48, 49]. Andujar et al., and Senthil *et al.*,reported that bisphenol [33, 50]. A causes reductions in hemoglobin, lymphocytes, PCV, RBCs, and monocytes . Higher levels of WBCs, leucocytes, neutrophils, cholesterol, triglycerides, urea, creatinine, blood glucose, ALT, and AST were observed by exposure to BPA [15, 48, 51]. Various kinds of stressors in animals can increase white blood cells, and MCHC due to immune system activation and inflammation [15]. A rise in WBC level is associated with direct activation of immunological responses and tissue injury exposed to BPA. The elevation in the count of WBCs in treated fish is indicative of a state of toxemia that indicates impairment of the defense system [52]. Increases in WBC concentration may lead to higher numbers of neutrophils. HCT, MCH, RBC, HGB, lymphocytes, MCV and PDW levels were reduced significantly in BPA-treated fish as compared to unexposed fish, leading to anemia in *L. rohita*. A decrease in hematopoiesis and an increase in erythrocyte breakdown in hemopoietic organs like the liver and kidneys can lead to anemia. Similar results are reported by Andujar *et al.*, Abid *et al.*, Hassan *et al.*,and Yaghoobi *et al.*, [33, 51, 53]. One possible explanation for the reduced HGB concentration might be the detrimental impact of BPA on HGB formation. BPA may inhibit HGB synthesis by interfering with the actions of enzymes necessary for HGB formation. Erythrocytes are crucial in assessing the health status of fish in the presence of toxic substances. Changes in the red blood cells of fish are exceptionally reliable indicators of the accumulation of hazardous substances in various

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organs of the fish. RBCs may exhibit responses to certain environmental stressors [54]. Furthermore, decreased levels of HCT, RBC, and HGB suggest the initiation of a defensive reaction in response to exposure to bisphenol A [55]. A low level of red blood cells can lead to oxidative damage in the body, resulting in membrane impairment and eventual cell death [56, 57]. The excessive accumulation of bisphenol A, which results in internal destruction, hemorrhage, and decreased erythrocyte formation, maybe the cause of the observed change in hematological parameters [58]. The decrease in MCV was caused by changes in RBC volume associated with exosmosis and a rise in the concentration of electrolytes inside RBCs following exposure to BPA [59]. Since RBC and HGB produce MCHC and MCH, changes in RGB and HGB concentrations also affect the levels of MCHC and MCH [60]. Highly significant reductions in MCV and MCH levels indicate hypochromic microcytic anemia [61]. This study demonstrated that BPA exposure had no significant effects on mean platelet volume, procalcitonin, monocytes, or eosinocytes. Therefore, work carried out by Asenuga *et al.,* supported present study [49]. Assessing biochemical indicators, such as lipid profile, glucose, and protein levels, is commonly employed to monitor the health of fish in aquatic environments and to understand the physiological responses shown by aquatic species under stress [58]. Aquatic pollutants have the potential to alter the function of enzymes in fish serum, which can serve as an indicator of fish health [62]. Biochemical examination revealed that the current study work showed a substantial drop in HDL, LDL, and VLDL levels and a rise in triglycerides and cholesterol. Similar findings were reported by Ozaydin *et al.*,and Pinafo *et al.,* [63, 64]. A rise in blood total lipids and cholesterol may cause catecholamines, which enhance lipolysis and fatty acid production. Due to an increase in total blood cholesterol, the liver bile duct may become blocked, reducing its secretion to the duodenum and causing cholestasis. ALT, AST, and blood glucose levels were increased by increasing the BPA dose. The present results are supported by Abid and Hassan [51]. The higher levels of ALT (alanine aminotransferase) and AST (aspartate aminotransferase) may be due to the oxidative stress caused by BPA exposure [65]. BPA impacts glucose metabolism via oxidative damage, inflammation, insulin resistance, and β cell malfunction [66]. A significant rise in urea, creatinine, BUN, T_3 and TSH levels while a significant decrease in albumin, total protein, globulin, and T_4 levels, were observed in the BPA-treated groups as compared with the BPA-free group. Similar results have been reported on other fish and rats [67-70]. High levels of urea and creatinine may be due to renal tubule damage, as shown in histological alterations, which indicated that bisphenol A affects muscle and purine metabolism [71]. Damage to the glomerulus, a decrease in glucose metabolism, and an increase in muscle tissue catabolism may all contribute to an elevated blood creatinine concentration [72]. BPA can disrupt the production of thyroid hormones by causing changes in blood protein transporters or by increasing the breakdown of thyroid hormones [70]. Administration of BPA may result in hypothyroidism through the processes of thyroid dyshormonogenesis and dysgenesis [73]. Abdel *et al.,* and Qiu *et al.,* reported that BPA causes a decrease in globulin, total proteins, and albumin in *Cyprinus carpio* and *Oreochromis niloticus* [68, 74]. The liver and kidneys have significant functions in protein metabolism. The liver and kidneys have the role of synthesizing, breaking down, and excreting blood proteins [75]. Therefore, the reduction of total protein, globulin, and albumin in the BPA-treated fish may be a result of liver and kidney damage, as shown by histological changes. Albumins are a group of globular proteins that play an important role in antioxidation, immune function and homeostasis [76, 77]. A reduction in albumin production is linked with inflammation [78].

CONCLUSIONS

Results of the current investigation concluded that bisphenol A causes harmful effects on the hematological and biochemical parameters of *Labeo rohita*. Exposure of *Labeo rohita* to BPA at 800 µg/L and 1600 µg/L induces alterations in hemoglobin, RBC, hematocrit, MCV, MCHC, lipid profile, kidney and liver functioning. Moreover, BPA altered thyroid functioning by altering T_3, T_4 and TSH levels in a dose-dependent manner. According to the findings, BPA is undoubtedly toxic to aquatic life. To lessen their harmful impacts, there is an urgent need to find other eco-friendly chemicals with higher degradation abilities and decrease BPA use.

Authors Contribution

Conceptualization: KS
Methodology: SA
Formal analysis: HA
Writing, review and editing: SA, HA, KS

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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