Effects of Bisphenol A (BPA) in commercially important fish Common Carp (*Cyprinus carpio)* in Kerala

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Abstract

The study is on the toxic effects of Bisphenol A in *Cyprinus carpio*, an Indian freshwater fish, presumably highly nutritious in proteins, and one that finds extensive usage in aquaculture establishments. BPA ranks very high among dispersing contaminants in freshwater systems, chiefly wetlands; hence, pressing attention is warranted. Thereafter, a certain set of hematological, histological, and biochemical parameters had been observed on the seventh day following exposure to BPA at sub-lethal concentrations of 0.1, 0.2, and 0.5 mg/L. The amount of RBC showed a decreasing trend from 2.95×10^6 to 2.3×10^6 Cells/mm^3 in 0.5 mg/L dose. However, the hemoglobin concentration, an opposite trend was observed, from 11.2 g/dL in control to 8.5 g/dL concentration. An opposite trend was noticed in WBC counts that increased from 9.1×10^3 to 13.4×10^3 Cells/mm3 at 0.5 mg/L, thus indicating hematological stress and activation of immune defense. Degeneration in testes and ovaries of fish, implicated through observed histological changes, further corroborates the estrogenic effect of BPA. This was accompanied by a reduction in antioxidant enzyme activities and an elevation in lipid peroxidation, a classical indicator of oxidative stress. Given such findings, the present study emphatically argues against BPA to reduce its effect on aquatic life, thereby affecting the environment and food safety.

Keywords: Bisphenol A (BPA), *Cyprinus Carpio*, Hematological, Histological, Oxidative Stress

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Introduction

Importance of Cyprinus carpio in Kerala's aquaculture

Cyprinus carpio, commonly referred to as common carp, is regarded as one of the most commercially significant freshwater fish species in Kerala. It is extensively cultivated due to its resilient nature, rapid growth rate, and ability to thrive in diverse environmental conditions. The species plays a pivotal role in the livelihoods of countless small-scale farmers and contributes significantly to the aquaculture production of the state. According to the Department of Fisheries, Government of Kerala, *Cyprinus carpio* represents a considerable proportion of freshwater fish production in the region, thereby establishing it as a crucial element of the rural economy. The commercial significance of *Cyprinus carpio* arises from its considerable nutritional value, adaptability to local climatic conditions, and consumer demand. This species is extensively cultivated in ponds, tanks, and reservoirs throughout Kerala, particularly in regions such as Palakkad, Alappuzha, and Thrissur. However, the increasing contamination of these water bodies with industrial pollutants, including BPA, presents considerable challenges to sustainable aquaculture practices (Fazio *et al*., 2015). Numerous studies have demonstrated that BPA exposure adversely impacts the growth performance, reproductive health, and immune functions of *Cyprinus carpio*. The species' susceptibility to endocrine-disrupting chemicals makes it an excellent bioindicator for assessing levels of aquatic pollution (Afzal *et al*., 2022). BPA contamination not only jeopardizes the fish population but also poses significant risks to human health through the consumption of contaminated fish. Despite the growing body of evidence concerning the toxicity of Bisphenol A (BPA) in aquatic organisms, there exists a notable deficiency in research specifically targeting the impacts of BPA on *Cyprinus carpio* under sub-lethal and chronic exposure conditions. Therefore, the present study aims to fill this research gap by evaluating the effects of BPA exposure on the biochemical, histological, and reproductive parameters of *Cyprinus carpio* within the framework of the freshwater ecosystems in Kerala.

BPA as an endocrine-disrupting chemical

Bisphenol A (BPA) is widely recognized as a xenoestrogen, which is a chemical compound that mimics estrogen and disrupts the endocrine systems of aquatic organisms. It exerts its toxic effects by binding to estrogen receptors (erα and erβ), thereby leading to aberrant hormonal signaling pathways. This estrogenic activity results in the feminization of male fish, impaired reproductive performance, and abnormal gonadal development in exposed fish populations (Yamaguchi *et al*., 2015). One of the most significant biomarkers of endocrine disruption in fish is the induction of vitellogenin (VTG), a precursor protein of egg yolk typically produced exclusively in female fish.

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Numerous studies have documented that BPA exposure induces VTG synthesis in male *Cyprinus carpio*, indicating the presence of estrogenic activity (Virk *et al*., 2014). This disruption has been correlated with reduced reproductive success and population decline in natural fish populations. Furthermore, BPA has been demonstrated to alter the expression of key genes involved in the hypothalamic-pituitary-gonadal (HPG) axis, including aromatase (CYP19), estrogen receptor α, and androgen receptor genes (Letcher *et al*., 2005). These molecular alterations have profound implications for the reproductive health of fish populations, particularly in regions such as Kerala, where BPA contamination is prevalent.

Significance of the study

The increasing contamination of freshwater ecosystems in Kerala by Bisphenol A (BPA) represents a substantial threat to aquatic biodiversity, the health of fish populations, and the livelihoods of human communities. This study is of considerable significance as it seeks to produce comprehensive data concerning the toxicological effects of BPA on *Cyprinus carpio*, a species of both ecological and commercial relevance. The results of this investigation will provide crucial insights into the biochemical, histopathological, and reproductive toxicity associated with BPA, thus enhancing the broader understanding of the challenges posed by aquatic pollution in Kerala.

Furthermore, this study endeavors to establish a scientific foundation for the formulation of biomonitoring programs and regulatory measures to address the ecological risks associated with BPA contamination. By identifying critical biomarkers such as oxidative stress enzymes, vitellogenin, and histological alterations, the research will furnish indispensable tools for environmental monitoring and pollution assessment (Holeyappa *et al.,* 2021). Moreover, the study will contribute to the global discourse surrounding endocrine disruptors and their pervasive effects on aquatic ecosystems. The findings will assist in the development of stringent environmental regulations aimed at reducing BPA pollution in Kerala's water bodies, thereby safeguarding the health of aquatic organisms and human consumers.

Materials and methods

Chemical

99% Pure BPA (Bisphenol A) was procured from a local chemist in Coimbatore. The BPA was dissolved in Dimethyl Sulfoxide (DMSO) to make a stock solution at 10 g/L & is stored in a dark and cool place, away from sunlight & in a dark bottle. It was ensured that the final stock solution only contained 0.005% DMSO (Dimethyl Sulfoxide).

Study area and fish collection

The study was conducted using freshwater fish *Cyprinus carpio* collected from selected aquaculture farms in Kerala. The fish were procured from a government-certified hatchery in

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Malampuzha, Palakkad, Kerala. The fish were acclimatised in the laboratory for 15 days under controlled environmental conditions. During the acclimatisation period, the fish were maintained in glass aquariums with proper aeration and dechlorinated water at a temperature of 25±2°C, Dissolved Oxygen (DO) of 6.5±0.3 mg/L, and pH of 7.0±0.2. The fish were fed commercial fish pellets twice a day, and water was changed every 48 hours.

Experimental design

Fish were randomly divided into four experimental groups, each containing 10 fishes. The experimental groups were: 0ml (Control), 1ml, 2ml and 5ml. BPA stock solutions were prepared using analytical-grade BPA (≥99% purity) dissolved in DMSO (Dimethyl Sulfoxide) and was diluted with dechlorinated water. The exposure medium was renewed every 48 hours to maintain the BPA concentrations.

Haematological analysis

Haematological parameters were measured to assess the physiological status of the fish. Blood samples were collected from the caudal vein using sterile heparinised syringes following the method of Svobodová *et al*. (2007). The Red Blood Cell (RBC) Count was determined using a Neubauer hemocytometer under a light microscope. White Blood Cell (WBC) Count was measured using the same hemocytometer. Haemoglobin (Hb) Concentration was estimated by Sahli's hemoglobinometer method (Witeska and Wargocka, 2003). Hematocrit (PCV) was determined by the microhematocrit method using a centrifuge at 12,000 rpm for 5 minutes

Histological analysis

Histological examinations were conducted to assess the structural changes in the reproductive tissues of fish exposed to BPA. Testis and ovary tissues were excised and fixed in 10% neutral-buffered formalin for 24 hours. The tissues were processed following the protocol by Culling *et al*. (1985).

Oxidative stress analysis

Biochemical markers were analyzed to evaluate the oxidative stress induced by BPA in fish tissues. Liver and reproductive tissues were homogenized in phosphate-buffered saline (PBS, ph 7.4) and centrifuged at 12,000 rpm for 10 minutes at 4°C. The supernatants were collected for biochemical assays. Catalase (CAT) Activity was measured using Aebi's method (1984) with hydrogen peroxide as the substrate (Livingstone, 2001). Superoxide Dismutase (SOD) Activity was determined by the Marklund and Marklund method (1974) based on the inhibition of pyrogallol autoxidation. Glutathione (GSH) Levels were estimated using the method of Ellman (1959). Lipid Peroxidation (MDA Levels) was measured by the Thiobarbituric Acid Reactive Substances (TBARS) method (Afzal *et al*., 2022) (Table 1) (Figs. 1 to 5).

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Table 1: BPA concentration and exposure period.

Group	BPA concentration	Exposure period
Group I	0 (Control)	1 week
Group II	0.1 ml	1 week
Group III	0.2 ml	1 week
Group IV	0.5 ml	1 week



Figure 1: Bisphenol A (BPA).



Figure 2: Stock solution.

Results

Hematological parameters

All the fish haematological parameters of *Cyprinus carpio* observed above indeed showed significant differences between the groups, following similar changes in a typical dose-dependent manner with respect to BPA exposure.

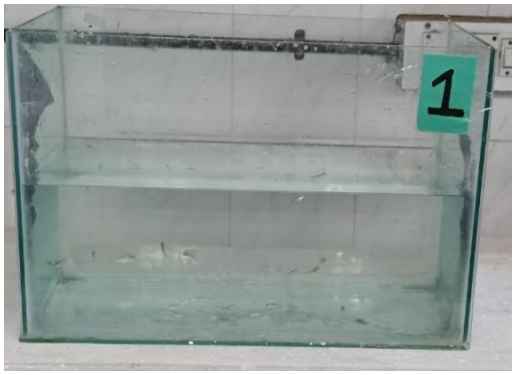


Figure 3: Tank-1.



Figure 4: Tank-2.

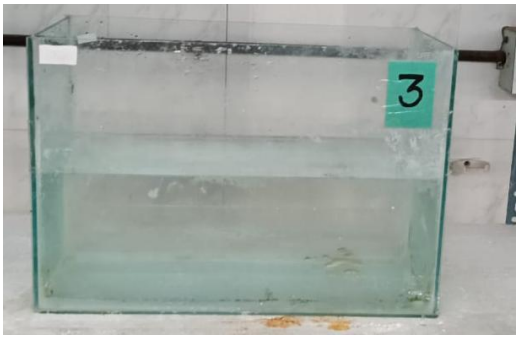


Figure 5: Tank-3.

Red blood cell (RBC) count

The variations shown in the increments of RBC count observed did not support an uptrend toward increases in BPA concentration. The fishes of the CTS groups accounted for an RBC count value of nearly 2.95×10^6 cells/mm³, which decreased to 2.85×10^6, 2.65×10^6,

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and 2.30×10^6 cells/mm³ for those fishes treated with BPA doses of 0.1, 0.2, and 0.5 mg/L, respectively. This fall in RBC shows anemia, either down-regulating RBC formation or up-regulating RBC destruction. In this, it upholds the previous observations of other researchers regarding the detrimental effects of BPA on blood parameters (Afzal *et al*., 2022).

White blood cell count

Conversely, the WBC count was at an undue rise by the presence of BPA. In the Control group, it remained constant at 9.1×10^3 cells/mm³, but in BPA-treated fishes, the count was recorded to have increased in a graded manner of 0.1, 0.2, and 0.5 mg/L-
successively above being 10.5×10^3, 11.8×10^3, and 13.4×10^3 cells/mm³. This WBC increase may have an immunological response, probably triggered as a result of stress or inflammatory action induced by BPA (Qiu *et al*., 2016) (Fig. 6).

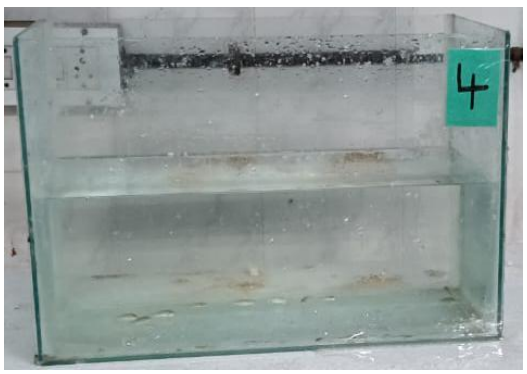


Figure 6: Tank-4.

Hemoglobin concentration

Hemoglobin concentration decreases with increased level of BPA in water. For the fish in control, the Hb level recorded was 11.2 g/dL. Hb levels decreased with increasing concentrations of BPA exposure at 10.5, 9.7, and 8.5 g/dL with 0.1, 0.2, and 0.5 mg/L of BPA, respectively. The fall observed in Hb levels pointed towards the reduced oxygen carrying capacity, which corroborates other findings of blood damage caused by BPA (Holeyappa *et al.,* 2021).

Packed cell colume

The PCV decreased as the BPA dose increased, going from 34.5 in control to 32.8, 30.5, and 27.1 with an increase in dose concentration. Decreasing PCV values suggest anemia and hence correspond to a report that BPA inhibits blood cell formation (Afzal *et al*., 2022).

Histological observations

Testicular histology

A normal spermatogenesis process was observed in control fish. In contrast, BPS-exposed fish endured degenerative changes in the seminiferous tubules (15%–50%), reduction in the numbers of spermatocytes (10%–40%), vacuolation in germ cells, and disarray, mainly at higher concentrations. Such changes indicate that the process of spermatozoa formation has been disturbed and the ability to impregnate females may also have been decreased, which corroborates previous findings of the estrogenic and cytotoxic action of BPA in male fishes (Al-Sakran *et al*., 2016).

Ovarian histology

Ovaries showed increased oocyte atresia (10–40%), cytoplasmic vacuolation, and

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massive follicular degeneration (up to 50% at the highest dose) in BPA-exposed fish. These pathological changes support the proposed mechanism of action of BPA as an endocrine disruptor affecting female reproductive organs (Holeyappa *et al*., 2021).

Oxidative atress in biochemical parameters

CAT analysis

In a concentration-dependent manner, there was a decrease in the activity of CAT, beginning from 5.8 µmol/g protein in control to 5.3, 4.7, and 3.9 µmol/g protein in the treatment groups, respectively. The decreased activity of CAT means decreased scavenging of $H2O_2$ and increased oxidative stress inducted by BPA (Afzal *et al.,* 2022).

SOD activity

SOD activity decreased with increasing concentration of BPA from the control value of 12.5 to 11.3, 9.8, and 7.5 µmol/g protein. Decreased SOD activity would imply weak defense against superoxide radicals, thereby further confirming the oxidative imbalance created by BPA (Gu *et al.,* 2021).

GSH levels

GSH levels progressively decreased with increased concentration of BPA from 9.2 to 6.3 µmol/g tissue. A lowered GSH level as a major antioxidant indicates oxidative stress, leading to impaired cellular defense in BPA-exposed fish (Qiu *et al*., 2016) (Table 2 to 7; Figs. 7 to 12).

Table 2: SOD activity.

BPA Concentration (mg/L)	SOD Activity (µmol/g protein)	Observation	Interpretation
0.0	12.6	Highest antioxidant enzyme activity	Normal cellular oxidative balance
0.1	11.2	Moderate decrease	Mild oxidative stress begins
0.2	9.8	Significant reduction	Antioxidant defense being suppressed
0.5	7.5	Sharp decline	Severe oxidative stress due to BPA toxicity

Table 3: WBC count in blood.

BPA Concentration (mg/L)	WBC Count (×10³ cells/mm³)	Observation	Interpretation
0.0	9.1	Baseline count	Normal immune function
0.1	10.5	Moderate increase	Immune response activation begins
0.2	11.8	High WBC level	Stress-induced leukocytosis
0.5	13.4	Peak WBC count	Possible inflammation or BPA-induced immune stress

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Table.4: RBC count in blood.

BPA Concentration (mg/L)	RBC Count ($\times 10^6$ cells/mm^3)	Observation	Interpretation
0.0	2.95	Normal erythrocyte count	Normal oxygen transport
0.1	2.85	Slight reduction	Initial hepatotoxic effects
0.2	2.65	Noticeable decline	BPA interferes with erythropoiesis or causes lysis
0.5	2.3	Severe reduction	Risk of anemia and hypoxia

Table 5: Hemoglobin content in blood.

BPA Concentration (mg/L)	Hemoglobin (g/dL)	Observation	Interpretation
0.0	11.2	Normal Hb level	Effective oxygen delivery
0.1	10.5	Slight decrease	Reduced RBCs and impaired Hb synthesis
0.2	9.7	Sharp drop	Hematological toxicity progressing
0.5	8.5	Critically low	Severe anemia risk

Table 6: GSH activity.

BPA Concentration (mg/L)	GSH (µmol/g tissue)	Observation	Interpretation
0.0	9.2	Highest GSH levels	Optimal antioxidant protection
0.1	8.5	Moderate decline	GSH used up in combating BPA-induced ROS
0.2	7.6	Significant reduction	Detoxification capacity getting compromised
0.5	6.3	Substantial depletion	High oxidative stress and depleted antioxidant reserve

Table 7: Catalase activity.

BPA Concentration (mg/L)	Catalase Activity (µmol/g protein)	Observation	Interpretation
0.0	5.8	Normal enzymatic function	Efficient H_2O_2 breakdown
0.1	5.3	Mild decrease	BPA begins inhibiting CAT activity
0.2	4.7	Significant suppression	Weakened ROS defense
0.5	3.9	Strong inhibition	Potential for hydrogen peroxide accumulation

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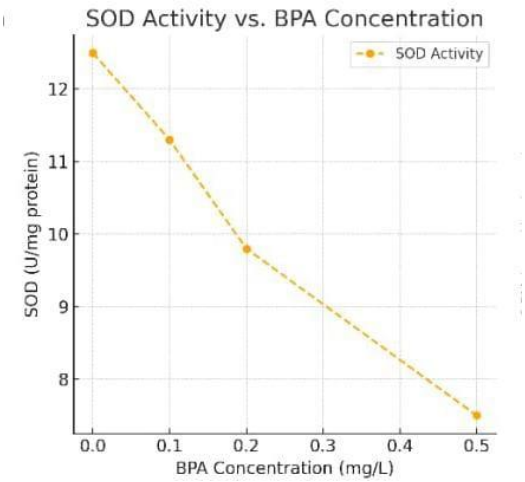


Figure 7: SOD activity.

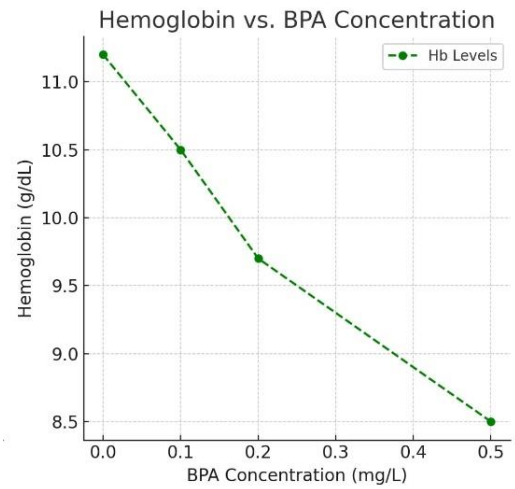


Figure10: Haemoglobin content in blood.

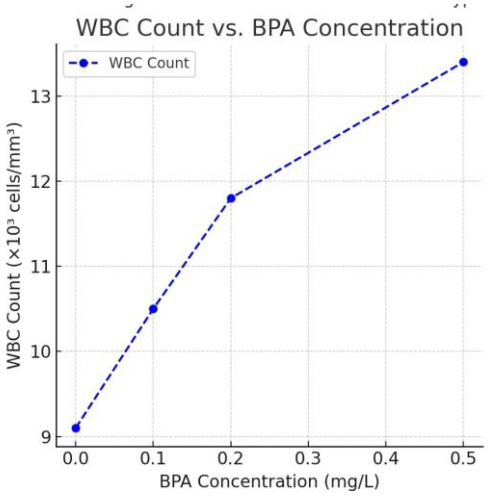


Figure 8: WBC count in blood.

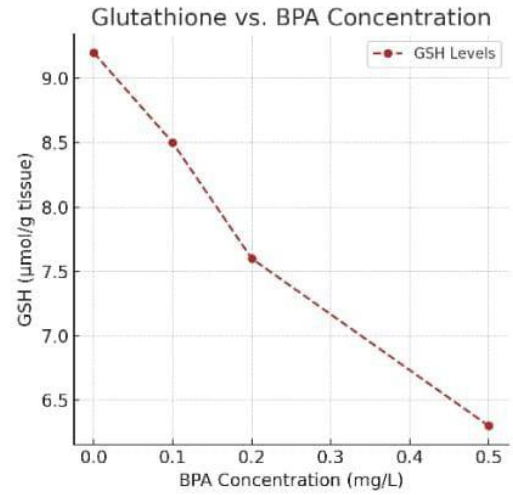


Figure 11: GSH activity.

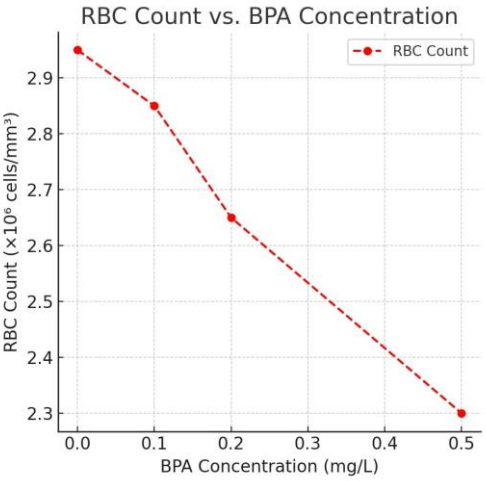


Figure 9: RBC count in blood.

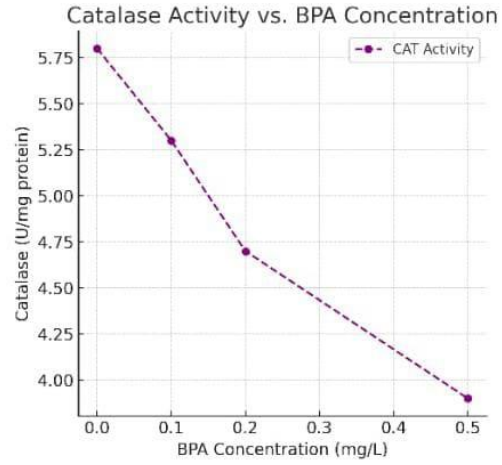


Figure 12: Catalase activity.

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Discussion

The research work is aimed at exploring the hematological, histopathological, and biochemical effects of Bisphenol A (BPA) exposure when exposed to fishes of the *Cyprinus carpio* species. The evidence proves great physiological and cellular changes that verify the harmful impacts of BPA at environmentally potential levels. The gradual decreases in the number of red blood cells (RBCs), concentration of hemoglobin (Hb) and packed cell volume (PCV) and an increase in the BPA concentrations demonstrate development of the anemia. Such condition can be developed due to the oxidative damage of erythrocytes or due to disrupted erythropoiesis- the processes noticed in fish in conditions of xenobiotic stressors. Such hematological changes indicate the loss of the oxygen-delivery ability and overall health decline in BPA-exposed fish. At the same time, in all a treatment groups, the increase in the level of white blood cells (WBC) testifies to an active immune response, which is a typical physiological response to physical stress and inflammation caused by chemicals in fish. Gonadal tissues showed sign of enormous structural degeneration under histopathological examination. Seminiferous tubules in the males showed elevated vacuolization of tubules, degeneration and disorganization, which positively correlated with the BPA level. The lowered number of spermatozoa is indicative of impaired spermatogenesis that could have been caused by either endocrine disruption or oxidation. With an increased amount of BPA, there was increased atresia and cytoplasmic vacuolization state in the ovarian tissues. At the level of severe follicular degeneration at 0.5 mg/L, it confirms the estrogenic potential of BPA and confirms its propensity in interfering with reproductive capacity. These data are in agreement with the previous findings that determine that BPA is an endocrine disruptor that can affect the reproductive physiology of enclosed organisms. Biochemical evaluations depicted the fact that oxidative stress is behind the BPA-induced toxicity. Abnormalities of antioxidant defense mechanism are characterized by a substantial decrease in catalase (CAT) and superoxide dismutase (SOD) activities, and reduced glutathione (GSH) levels. These enzymes deactivate reactive oxygen species (ROS) and inhibition of such enzymes allow reactive damage to lipids, proteins and nucleic acids. The gradual deterioration of antioxidant status of *Cyprinus carpio* with the increased addition of BPA proves an increased burden on fish of oxidative load. When taken together, these numbers strongly confirm the argument that BPA causes systemic toxicity to C. carpio by interfering with hematological parameters, impairing the reproductive organs, and causing oxidative stress. Such observations are consistent with previous studies that demonstrate an immune interference, genotoxic, and reproductive effects caused by BPA exposure in fish (e.g., Kang *et al*., 2007; Oehlmann *et al.,* 2009; Corrales *et al*., 2015). The

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apparent dose-dependency demonstrates the ecological hazard of BPA pollution in the aquatic environments and highlights the increased sensitivity of fish species to endocrine-disrupting chemicals.

Conclusion

The research identified herein leaves no doubt that bisphenol A (BPA) has a significant biological impact on the *Cyprinus carpio* fish, and it induces considerable changes to the hematological indices and the structure of reproductive tissues and the oxidative-stress reactions in a dose-dependent manner. The development of erythrocyte reduced, hemoglobin concentrations, packed-cell volume and the level of the activity of antioxidant enzyme, along with increased in the white-blood-cell level and gonadal atrophy statements demonstrate the multifactorial nature of BPA toxicity. These results show the presence of systematic- physiological disruption, immunological stress, and loss of reproductive capability thus showing severe ecological hazard on fresh water fish populations. The implications of the findings indicate that there is a high need of intensified regulation analysis of endocrine-disrupting substances within the water spaces.

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