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Assessment of Biological Effects of Bisphenol A (BPA) On *Labeo Rohita*

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ABSTRACT
Bisphenol A (BPA) is a synthetic chemical extensively used in plastics, resins and food packaging, and is recognized globally as a potent endocrine-disrupting compound. Its continuous discharge into aquatic ecosystems has raised significant ecological and human health concerns. Labeo rohita (rohu), one of the most important freshwater fish in Tamil Nadu's aquaculture, serves as an ideal model organism to assess toxicological stress due to its commercial value and ecological relevance. The present study aimed to investigate the sub-lethal impacts of BPA on the haematological, histological and oxidative stress responses of L.rohita under controlled laboratory conditions. Haematological assessment revealed a significant reduction in red blood cell count, haemoglobin concentration and haematocrit, accompanied by an increase in white blood cell count, indicating anaemia and immune activation. Histopathological investigations demonstrated degenerative changes in the reproductive tissues: male fish exhibited disrupted spermatogenesis, vacuolated germ cells and disorganized seminiferous tubules, while female fish showed increased oocyte atresia, Cytoplasmic vacuolization and severe follicular degeneration. These alterations confirmed BPA's estrogenic and cytotoxic effects on fish gonads. Oxidative stress evaluation further demonstrated a concentration-dependent decline in catalase (CAT) and superoxide dismutase (SOD) activities, along with decreased glutathione (GSH) levels. Conversely, lipid peroxidation (MDA) and reactive oxygen species (ROS) levels increased markedly in BPA-exposed groups compared to the control, signifying elevated oxidative damage and impaired antioxidant defense mechanisms.
Overall, the findings indicate that BPA exposure compromises the haematological balance, disrupts reproductive physiology, and induces oxidative stress in L. rohita. Such toxicological effects not only impair fish health and productivity but also pose potential risks to human consumers through the food chain.
Keywords: *Bisphenol A, Labeo rohita, endocrine disruption, haematology, oxidative stress, histopathology*

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I. INTRODUCTION:

In today's urbanized world, endocrine-disrupting chemicals (EDCs) are regarded as a topic of paramount importance because of their pleiotropic adverse effects on physiological homeostasis across higher to lower trophic levels. EDCs are either natural or synthetic compounds that can profoundly alter metabolism, cellular signalling and excretion of endogenous hormones by mimicking their mode of action due to structural similarities [1]. Among these, Bisphenol A (BPA) is one of the most widely studied EDCs, known for its weak estrogenic activity (xenoestrogen) and extensive industrial use. It is produced in large quantities as a plasticizer in the manufacture of reusable food and beverage containers, baby bottles, epoxy resins, electrical equipment and dental sealants [2]. Primarily due to its lipophilic and estrogenic nature, uncontrolled exposure to BPA in aquatic ecosystems may lead to multidimensional hazardous effects. These include oxidative stress, disruption of endocrine homeostasis, impaired reproductive fitness, reduced developmental potential, genotoxicity, immune alteration and metabolic dysfunction.

BPA exerts its biological actions by functioning as an agonist or antagonist to hormone receptors where it acts as a xenoestrogen, binding to estrogen receptors (ERα and ERβ), which leads to altered hormonal regulation, feminization of male fish, impaired reproductive performance and abnormal gonadal development, with evidence showing both genomic and non-genomic modulation of signalling pathways. Its detrimental impacts have been demonstrated not only on estrogen-responsive reproductive organs but also on non-reproductive targets such as the liver, brain and immune system [2]. In fish, three estrogen receptor (ER) subtypes - ERα (ESR1), ERβ1 (ESR2b) and ERβ2 (ESR2a) - are expressed in the liver [3]. However, studies investigating the influence of BPA on hepatic ER expression, lipid metabolism, insulin signalling and innate immunity remain limited. Disruption of haematological indices, serum biochemical parameters and oxidative stress responses are also significant markers of BPA toxicity in fish. As the liver plays a pivotal role in glucose and lipid homeostasis, xenobiotic metabolism

and detoxification [4], histopathological and biochemical alterations in hepatic tissues represent key biomarkers of BPA-induced toxicity.

Labeo rohita (rohu) is one of the most commercially significant freshwater fish species in India, including Tamil Nadu, where it is extensively cultured in districts such as Dharmapuri, Villupuram, Cuddalore and Thanjavur. Valued for its rapid growth, high consumer demand, adaptability to diverse environmental conditions, and nutritional richness, rohu serves as a cornerstone species in freshwater aquaculture and a major contributor to rural livelihoods. Beyond its economic importance, *L. rohita* is recognized as a sensitive bio indicator of aquatic pollutants, making it a suitable model species in ecotoxicological studies.

BPA exposure in fish has been shown to disrupt the hypothalamic–pituitary–gonadal (HPG) axis by altering the expression of key genes such as aromatase (CYP19), estrogen receptor α and androgen receptor genes. This dysregulation leads to impaired reproductive performance, gonadal abnormalities and feminization of male fish, as indicated by the induction of vitellogenin (VTG), a yolk precursor protein normally restricted to females [5]. Chronic BPA exposure has also been linked to growth retardation, immune suppression, oxidative stress and tissue-level histopathological alterations in several freshwater species [6].

Given the increasing industrial contamination of freshwater ecosystems and the socio-economic reliance on aquaculture in Tamil Nadu, understanding the biological impacts of BPA on Labeo rohita is of critical importance. The present study seeks to assess the biological effects of BPA exposure in L. rohita, with a focus on haematological indices, biochemical parameters, oxidative stress biomarkers, hepatic alterations and endocrine disruption. Findings from this investigation are expected to provide significant insights into the ecotoxicological risks of BPA and contribute to sustainable aquaculture practices in polluted freshwater environments.

II. MATERIALS AND METHODS

2.1 Chemical:

Analytical grade Bisphenol A (BPA, 99% purity) was obtained from a certified supplier in Coimbatore. A primary stock solution (10 g/L) was prepared by dissolving BPA in Dimethyl Sulfoxide (DMSO). The stock was stored in amber-coloured bottles under cool, dark conditions to prevent photo degradation.

2.2 Experimental Fish and Acclimatization:

Healthy specimens of *Labeo rohita* were acclimatized in the laboratory for 15 days under controlled environmental conditions prior to experimentation. The fish were maintained in glass aquaria containing dechlorinated water with continuous aeration. Water quality parameters were monitored and maintained at 26 ± 2 °C, dissolved oxygen (DO) 6.5 ± 0.3 mg/L, and pH 7.0 ± 0.2. During the acclimatization period, fish were fed with commercial pellets twice daily, and 90% of the water was renewed every 48 hours to maintain cleanliness and minimize stress.

2.3 Experimental Design:

Following acclimatization, the fish were randomly distributed into four treatment groups (T1, T2, T3, and T4), with three replicate aquaria per group and seven fish per aquarium. Group T1 served as the control, while groups T2, T3, and T4 were exposed to Bisphenol A (BPA) at concentrations of 600, 1200 and 1800 µg/L, respectively. These concentrations were selected based on the 96-h LC_{50} value of BPA for *L. rohita* (5500 µg/L or 5.50 mg/L).

2.4 Biochemical Analysis:

Haematological indices (RBCs, haemoglobin (HGB), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), haematocrit (HCT), mean corpuscular haemoglobin concentration (MCHC) and platelets (PLT)) were examined by haematological analyser to evaluate the physiological responses of the experimental fish, while for serology, samples were left to coagulate over 15 minutes. The serum was separated from blood samples by centrifuging them for 10 min at 5000 rpm, then stored at -20°C for further biochemical indices (blood glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), cholesterol, LDL, HDL, VLDL, total proteins, albumin and globulin) analysed to assess metabolic and hepatic functions [10].

2.5 Histological Analysis

Histopathological examinations was carried out to investigate tissue-level alterations in the reproductive organs of *Labeo rohita* exposed to BPA. The testes and ovaries were carefully excised and immediately fixed in 10% neutral-buffered formalin for 24 hours to preserve structural integrity. Following fixation, the tissues were processed according to the standard histological protocol of Culling et al. (1985), which included dehydration, clearing and embedding in paraffin wax. Thin sections were then prepared, stained with haematoxylin and Eosin (H&E) and examined under a light microscope to identify histological changes and degenerative features associated with BPA exposure.

2.6 Oxidative Stress Analysis

Biochemical assays was conducted to assess oxidative stress responses induced by BPA in the liver and gonadal tissues of *Labeo rohita*. Fresh tissues were homogenized in phosphate-buffered saline (PBS, pH 7.4) and centrifuged at 12,000 rpm for 10 minutes at 4 °C, after which the clear supernatants were collected for further analysis. Catalase (CAT) activity was determined using Aebi's method [8]. Superoxide dismutase (SOD) activity was measured following the method of Marklund and Marklund [9], based on its ability to inhibit the autoxidation of pyrogallol. Reduced glutathione (GSH) levels were quantified using Ellman's method, where the sulfhydryl groups react with DTNB to form a yellow-coloured product. The results of these biochemical parameters are summarized in Table.

III. RESULT:

3.1 Biochemical analysis:

The biochemical evaluation of *Labeo rohita* exposed to Bisphenol A (BPA) demonstrated significant alterations in multiple serum indices compared with the control group (Table 1). These findings corroborate the haematological changes and further highlight the systemic toxicity of BPA in freshwater fishes. A marked reduction was observed in RBCs, haemoglobin (HGB), Mean corpuscular haemoglobin (MCH), Mean corpuscular volume (MCV), haematocrit (HCT), Mean corpuscular haemoglobin concentration (MCHC) and platelet (PLT) counts across the BPA-treated groups, confirming dose-dependent haematological suppression. Conversely, WBCs and neutrophils exhibited significant elevation, suggesting an immune response or inflammatory reaction triggered by BPA exposure.

Biochemical indices revealed distinct metabolic and hepatic disturbances. Blood glucose, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels increased significantly with rising BPA concentrations, indicating hepatocellular stress and impaired carbohydrate–lipid metabolism. In contrast, the lipid profile parameters - cholesterol, LDL, HDL and VLDL - were progressively reduced in BPA-treated groups. Similarly, serum proteins (total proteins, albumin and globulin) showed significant reductions, implying impaired protein synthesis and possible liver dysfunction.

Collectively, these biochemical changes emphasize that BPA exposure not only compromises haematological homeostasis but also exerts profound effects on lipid metabolism, glucose regulation, and hepatic function. Such alterations represent critical biomarkers of BPA-induced metabolic dysregulation and hepatotoxicity in *Labeo rohita*.

PARAMETER	T1(control)	T2 (600 µg/L)	T3(1200 µg/L)	T4 (1800 µg/L)
RBC ($\times 10^6$/ µL)	3.01 ± 0.04	2.85 ± 0.06*	2.64 ± 0.08*	2.49 ± 0.07*
HGB (g/dL)	5.02 ± 0.24	4.85 ± 0.16	4.22 ± 0.15*	3.87 ± 0.19*
MCH (pg)	47.32 ± 1.65	44.24 ± 2.03	40.02 ± 1.56*	39.15 ± 1.89
MCV (FL)	143.6 ± 1.39	132.4 ± 4.01*	123.3 ± 3.18*	120.9 ± 3.58*
HCT (%)	16.04 ± 0.83	12.08 ± 0.99*	11.09 ± 0.64*	9.09 ± 0.73*
MCHC (g/dL)	107.9 ± 2.32	101.8 ± 2.46*	98.9 ± 2.98*	91.2 ± 1.99*
PLT ($\times 10^3$/ µL)	192.3 ± 1.26	189.7 ± 2.51*	180.2 ± 3.01*	176.7 ± 4.03*
WBC ($\times 10^3$/ µL)	17.89 ± 2.69	26.34 ± 2.38*	33.48 ± 1.01*	37.89 ± 1.20*
Cholesterol (mg/dL)	196.7 ± 1.48	190.8 ± 1.05*	186.9 ± 1.60*	180.7 ± 1.44*
HDL (mg/dL)	83.25 ± 1.32	77.45 ± 2.06	73.78 ± 1.78*	65.89 ± 1.67*
LDL (mg/dL)	143.4 ± 2.31	137.9 ± 0.98	130.8 ± 3.11*	126.7 ± 3.89*
VLDL (mg/dL)	115.6 ± 1.49	106.9 ± 2.31	105.3 ± 2.86*	100.8 ± 3.01*
ALT (U/L)	22.09 ± 2.01	28.39 ± 2.07*	34.56 ± 1.98*	39.01 ± 3.40*
AST (U/L)	99.02 ± 2.53	104.5 ± 3.81*	121.67 ± 4.08*	130.46 ± 4.52*
Total Protein (mg/dL)	15.59 ± 1.29	11.31 ± 0.99*	9.32 ± 0.86*	7.54 ± 0.88*
Albumin (mg/dL)	4.91 ± 0.32	3.67 ± 0.21	3.22 ± 0.17*	2.87 ± 0.13*

Globulin (mg/dL)	11.02 ± 0.39	9.34 ± 0.27*	7.44 ± 0.31*	6.11 ± 0.27*
Blood glucose (mg/dL)	62.33 ± 3.48	80.91 ± 3.98*	96.08 ± 4.40*	110.99 ± 4.00*

Table 1. ***Haematological and Biochemical Parameters (Mean Values) of Fish Exposed to Different Concentrations of the Test Compound***

The mean values and standard deviations were obtained using descriptive statistical analysis. Data are presented as mean ± SD. Asterisks (*) indicate significant differences between the control group (T1) and the BPF-treated groups (T2, T3, and T4), as determined by one-way ANOVA followed by Dennett's post hoc test ($*p < 0.05$).

3.2 Histological Observations

Testicular Histology

In the control group, a normal spermatogenesis process was evident. In contrast, BPA-exposed fish exhibited degenerative alterations in the seminiferous tubules (15 - 50%), accompanied by a reduction in spermatocyte numbers (10 - 40%), pronounced Vacuolization of germ cells and structural disarray, particularly at higher exposure levels (1800 µg/L). These pathological changes clearly suggest disruption of spermatozoa formation, which may impair male fertility.

Ovarian Histology

BPA exposure also resulted in profound alterations in ovarian architecture. Notable effects included increased oocyte atresia (20 - 60%), cytoplasmic Vacuolization and massive follicular degeneration (up to 60% at the highest concentration). These observations support the endocrine-disrupting potential of BPA, indicating its ability to interfere with female reproductive physiology and impair fecundity.

3.3 Oxidative Stress Biomarkers

Catalase (CAT) Activity

CAT activity showed a clear dose-dependent decline, decreasing from 6.8 µmol/g protein in the control to 5.1, 4.6 and 3.2 µmol/g protein in the BPA-treated groups. The reduction in CAT activity reflects a diminished capacity to scavenge H_2O_2, thereby enhancing oxidative stress.

Superoxide Dismutase (SOD) Activity

A progressive reduction in SOD activity was also observed, with values declining from 10.6 µmol/g protein (control) to 9.8, 8.6, and 7.1 µmol/g protein in BPA-exposed groups. Reduced SOD activity suggests compromised neutralization of superoxide radicals, further reinforcing the oxidative imbalance triggered by BPA.

Glutathione (GSH) Levels

Similarly, GSH concentrations decreased progressively, from 8.2 µmol/g tissue in control fish to 3.3 µmol/g tissue at the highest BPA dose. Lowered GSH levels indicate impaired antioxidant defense, reflecting elevated oxidative stress and cellular vulnerability in exposed fish.

Reactive Oxygen Species (ROS)

ROS levels increased markedly in a dose-dependent manner, rising from 0.4 (optical density unit) in the control group to 0.6, 0.8, and 0.9 in the BPA-treated groups, respectively. The progressive accumulation of ROS levels are strongly associated with lipid peroxidation, protein oxidation and DNA damage, thereby confirming the role of BPA in inducing oxidative stress-mediated cellular injury in fish.

IV. Discussion & conclusion:

The present study demonstrates that Bisphenol A (BPA) exposure induces profound biochemical, histological and oxidative stress-related alterations in *Labeo rohita*, thereby confirming its systemic toxicity and endocrine-disrupting potential in freshwater fish.

Biochemically, the significant decline in erythrocytic indices (RBC, HGB, HCT, MCH, MCV, MCHC, PLT) reflects haematological suppression, suggestive of anaemia and impaired oxygen-carrying capacity. The concomitant increase in WBCs and neutrophils indicates a stress-mediated inflammatory or immune response. Furthermore, the elevation in glucose, ALT and AST levels strongly suggests hepatocellular stress, metabolic disturbance and disruption of carbohydrate - lipid homeostasis. Conversely, the reductions in serum proteins (albumin, globulin, total proteins) and lipid profile components (cholesterol, HDL, LDL and VLDL) point toward impaired hepatic function, protein synthesis and lipid metabolism. These alterations collectively identify BPA as a potent inducer of metabolic dysregulation and hepatotoxicity in *L. rohita*.

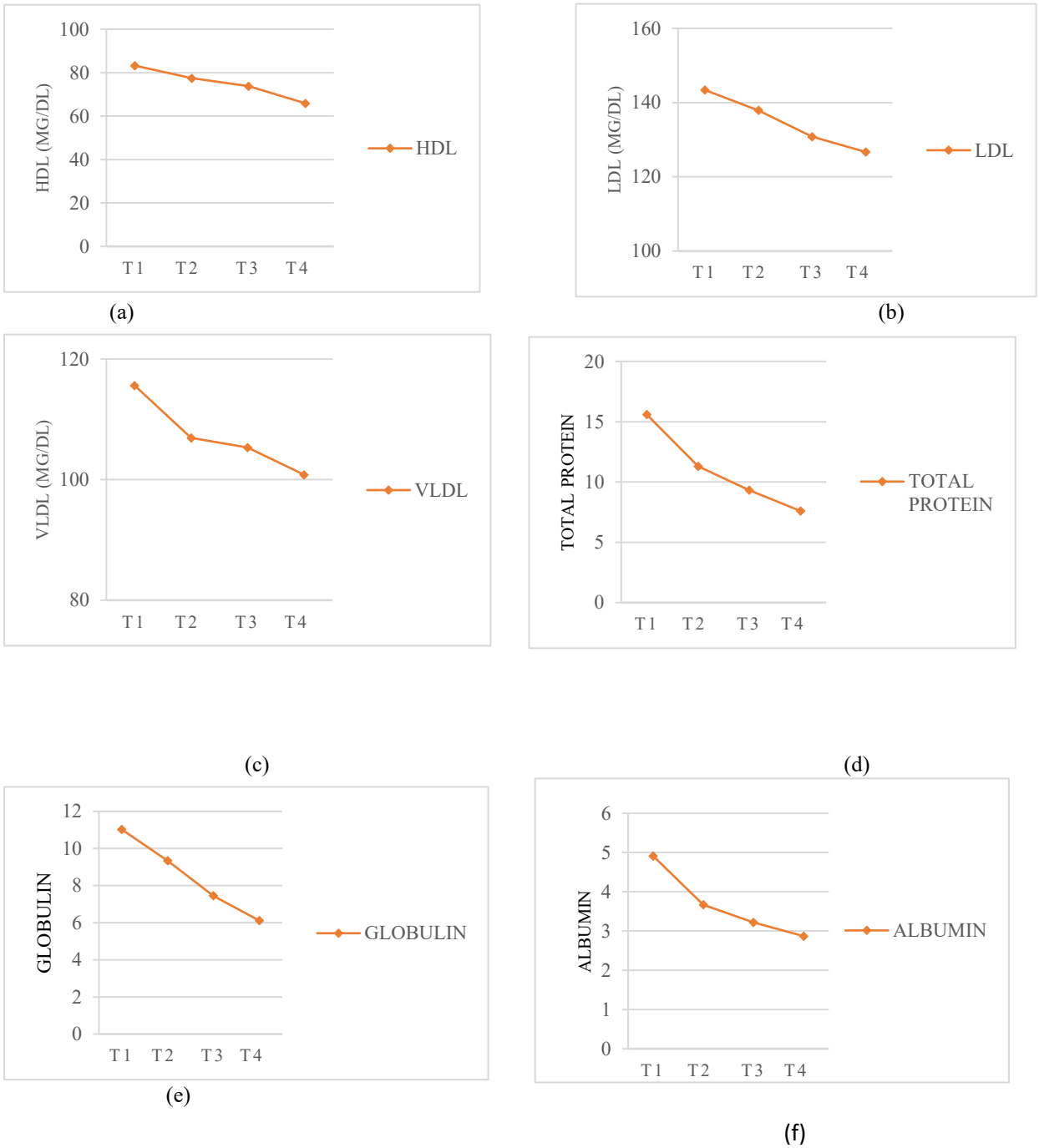
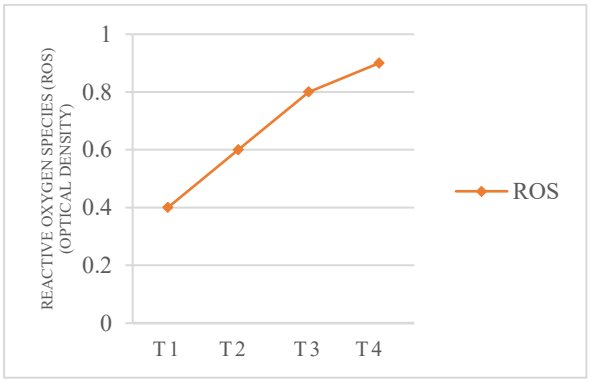
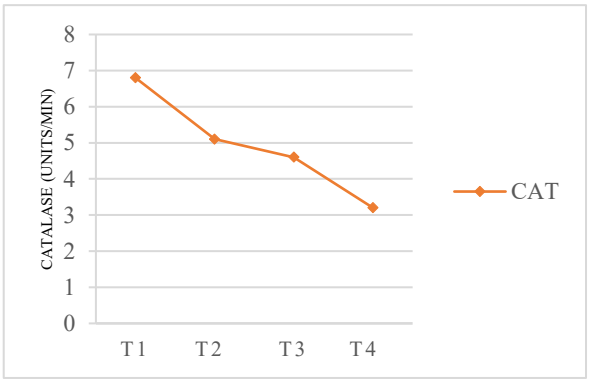


Figure 1. The Changes in (a) HDL, (b) LDL, (c) VLDL, (d) Total Protein, (e) Globulin and (f) Albumin levels in BPA-exposed groups compared to the control group. Data are expressed as mean ± SD.

Histopathological findings provide direct evidence of BPA-induced reproductive toxicity. In males, degenerative changes in seminiferous tubules, germ cell vacuolization and disrupted spermatogenesis confirm impaired testicular function and reduced fertility potential. In females, massive follicular degeneration, cytoplasmic vacuolization and increased oocyte atresia underscore the endocrine-disrupting action of BPA on ovarian physiology.



(a)



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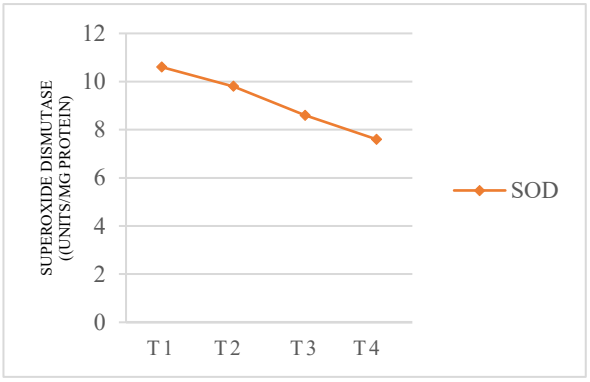

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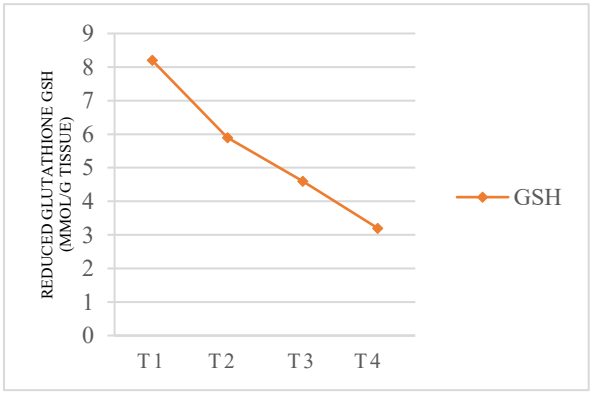

(d)

Figure 2. The Changes in (a) ROS, (b) CAT, (c) SOD and (d) GSH levels in BPA-exposed groups compared to the control group. Data are expressed as mean ± SD.

Oxidative stress biomarkers further strengthen these findings, as CAT, SOD and GSH levels declined progressively, demonstrating a weakened antioxidant defense system. Meanwhile, ROS levels rose sharply in a concentration-dependent manner, confirming enhanced free radical generation. The imbalance between pro-oxidants and antioxidants indicates that BPA induces redox imbalance, lipid peroxidation, protein oxidation and potential DNA damage, mechanisms that may underlie the observed hepatic and reproductive impairments. In conclusion, the findings reveal that BPA exposure in *L. rohita* leads to haematological suppression, metabolic dysfunction, hepatic injury, oxidative imbalance and reproductive toxicity.

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