Journal of Fisheries
Volume 14 Issue 2 August 2026
Peer Reviewed | Open Access

eISSN 2311-3111
pISSN 2311-729X

DOI: https://doi.org/10.17017/j.fish.1292

Original Article

Modulatory effects of dietary vitamin E on bisphenol A toxicity in male *Oreochromis niloticus*: insights into oxidative stress, hepatic dysfunction, reproductive hormone and histological alterations

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Manuscript history
Received 1 May 2026 | Accepted 26 May 2026 | Published online 30 May 2026

Citation
Biswal A, Srivastava PP, Mohanta KN, Gupta S, Kumar P, Varghese T, Jayant M, Kumar AP (2026) Modulatory effects of dietary vitamin E on bisphenol A toxicity in male *Oreochromis niloticus*: insights into oxidative stress, hepatic dysfunction, reproductive hormone and histological alterations. Journal of Fisheries 14(2): 142216. DOI: 10.17017/j.fish.1292

Abstract
The effectiveness of graded dietary vitamin E levels in alleviating bisphenol A (BPA) induced oxidative stress, hepatic dysfunction, reproductive hormone disruption and histological alterations in male *Oreochromis niloticus* was tested through a 45-day study using seven experimental groups: a negative control (C1; no BPA exposure; basal dose of 100 mg vitamin E kg^{-1} diet), a positive control (C2; exposed to 0.04 ppm BPA; basal dose of 100 mg vitamin E kg^{-1} diet), and five treatment groups: T1 (0.04 ppm BPA; 200 mg vitamin E kg^{-1} diet), T2 (0.04 ppm BPA; 400 mg vitamin E kg^{-1} diet), T3 (0.04 ppm BPA; 600 mg vitamin E kg^{-1} diet), T4 (0.04 ppm BPA; 800 mg dietary vitamin E kg^{-1} diet), and T5 (0.04 ppm BPA; 1000 mg vitamin E kg^{-1} diet). Exposure to BPA resulted in haematological and antioxidant enzymes alterations with increased superoxide dismutase and catalase activities. Further, a decrease in 11-ketotestosterone and increase in estradiol levels was observed, which corresponded with pronounced histopathological damage in testicular tissue, including degeneration of seminiferous tubules and disrupted spermatogenesis. The dietary incorporation of 600 mg vitamin E kg^{-1} diet produced most consistent improvement across physiological and endocrine parameters, along with near-normal testicular architecture in T3 group. Collectively, the findings demonstrate that dietary vitamin E effectively mitigates BPA-induced toxicity within an optimal range, emphasizing the importance of dose optimization for maintaining reproductive health in *O. niloticus*.

Keywords: bisphenol A; histology; hormone; *Oreochromis niloticus*; oxidative stress; vitamin E

1 | INTRODUCTION

Driven by rapid industrialization, plastic has become one of the most widely used synthetic materials worldwide. With global production increasing approximately 180-fold between 1950 and 2018 it has reached an estimated 400.3 million tonnes in 2022 (Geyer *et al*. 2017; Pilapitiya and Ratnayake 2024). Owing to its exceptional durability, thermal stability, and resistance to shattering, bisphenol A (BPA; 4,4′-isopropylidenediphenol), a carbon-based synthetic compound is very widely used in various com-

mercial plastic products (Rezg *et al*. 2014).Therefore, in order to cater the increased demand, the global production of bisphenol A (BPA) has escalated rapidly and reached approximately 6.2 million tons in 2020, reflecting its extensive industrial use (Tsai 2023).

BPA undergoes rapid aerobic degradation, resulting in an estimated half-life of about 4.5 days in aquatic environments (Cousins *et al*. 2002; Mishra *et al*. 2023). However, continuous leaching of this chemical from end-use-plastic products, direct discharge of industrial effluents and landfill leachates, contributes to its persistence as a ubiquitous pollutant in aquatic environments (Ighalo *et al*. 2024). Owing to its non-biodegradable property plastic persists in aquatic environment for longer time period, thus freshwater aquatic ecosystem exists as the major sink of bisphenol A (Anderson *et al*. 2016). Pollutants which disrupt the hormonal homeostasis by affecting hormones' production, release, transport, receptor binding, action and elimination are considered to be endocrine disrupting chemical (EDCs) (Lehmler *et al*. 2018). BPA is a xenoestrogenic endocrine disrupting compound, which mimics the structure of natural estrogen and interferes with the hormonal signalling pathways in aquatic organisms affecting the steroidogenesis and reproductive physiology in fish (Liang *et al*. 2024; Nour *et al*. 2024; Peskova and Blahova 2025). Apart from endocrine disruption BPA is also associated with oxidative stress through generation of reactive oxygen species (ROS), which may lead to the disruption of the antioxidant defence systems and causes physiological disturbances in aquatic organisms (Senarath pathirajage *et al*. 2024; Gao *et al*. 2025). Although BPA-induced oxidative stress and endocrine disruptive effects have been demonstrated in several reports, the mechanistic relationship between oxidative stress and endocrine disruption under the environmentally relevant exposure conditions still remains poorly understood.

Owing to its large geographical distribution, fast growth and sensitivity to environmental pollutants the Nile tilapia (*Oreochromis niloticus*) fish is extensively used as a sentinel organism in ecotoxicological studies (Adbel-Tawwab and Hamed 2018). According to Peskova and Blahova (2025) BPA exposure adversely affects both spermatogenic and androgenic processes in fish, highlighting the suitability of male fish as a model for assessing BPA-induced reproductive toxicity. As spermatogenesis is impaired, the reproductive success of the whole population is adversely affected.

Previously reported investigations have mostly focused on acute or high-dose BPA exposure in fishes, while limited information is available regarding its biological effects under environmentally relevant concentrations. In recent years, increasing attention has been given on ameliorative strategies aimed at mitigating pollutant-induced toxicity in aquatic organisms. In addition, studies focusing on nutritional mitigation strategy to ameliorate BPA induced toxicity are still not explored well. Among dietary antioxidants, Vitamin E plays a significant role in maintaining antioxidant defence systems in fish (Hamre 2011). Considering the role of vitamin E as a membrane antioxidant, effect of vitamin E against BPA induced toxicity should be evaluated. Therefore, this present study was conducted with the objective to evaluate the effect of graded dietary vitamin E supplementation against physiological and reproductive alterations in male *O. niloticus* during exposure of environmentally relevant concentration of BPA.

2 | METHODOLOGY

2.1 Procurement of experimental fish and acclimatization

A total 200 numbers of Male *O. niloticus* (110±6.5 g) (mean weight ± standard deviation) were commercially procured from Pune, Maharashtra, India. The fishes were then subjected to 30 seconds of dip treatment in 1% salt water followed by dipping in 2 ppm $KMnO_4$ before transferring them to acclimatization tanks. Acclimatization was carried out in big circular tanks of 5000 L capacity for 2 weeks. During the acclimatization the optimum water quality was maintained with dissolved oxygen level of 5.6±0.7 mg L^{-1}, pH 7.8–8.1, temperature 26–30°C, total Ammonia nitrogen, 0.02–0.1 mg L^{-1} with provision of 12:12 h of light: dark photoperiod. The fishes were maintained in continuous aeration with 2% diet fed twice a day.

2.2 Diet preparation

To counteract the adverse effect of BPA, vitamin E (DL-α-tocopherol acetate; CAS No. 7695-91-2) was incorporated in varying dietary inclusion level. Seven isonitrogenous and isocaloric diets were formulated with various levels of vitamin E supplementation. The control groups (C1 and C2) were subjected to a baseline dose (100 mg kg^{-1} diet) of dietary vitamin E, whereas the treatment groups were supplemented with gradually enhanced dose of dietary vitamin E: T1 (200 mg kg^{-1} diet), T2 (400 mg kg^{-1} diet), T3 (600 mg kg^{-1} diet), T4 (800 mg kg^{-1} diet), and T5 (1000 mg kg^{-1} diet).The basal dietary vitamin E concentration (100 mg kg^{-1} diet) was chosen on basis of prior research, indicating that ideal growth and physiological performance in *O. niloticus* take place between 50–100 mg kg^{-1} diet (Rohani *et al*. 2023), whereas male reproductive performance has been demonstrated to be improved within 80–120 mg kg^{-1} (Zhang *et al*. 2021). Given that the experimental fish (~110 g males) were at a sub-adult to adult stage, a functional basal level of 100 mg kg^{-1} diet was used for the control groups. All feed components were precisely measured and thoroughly combined to achieve a uniform mixture. Water was added to the mixture to transform it into a dough, which was then cooked to im-

prove digestibility. In order to protect the heat labile micronutrients, vitamin E (Tocopherol acetate), commercial vitamin mineral mixture & BHT were mixed in oil and blended with the dough evenly, only after the cooling of dough. The prepared dough was formed into pellets with a pelletizer. The pellets were uniformly dried to achieve a moisture content of 10%. Until they were needed again, dried pellets were kept in sealed containers at 4°C. The dietary formulation and proximate composition (AOAC 1995) of the test diets is shown in Table S1 and S2 respectively.

2.3 Experimental protocol and sampling

After the acclimatization, following a completely randomized design (CRD), male fishes were randomly distributed to different experimental groups in which fishes were subjected to co-administration of water added BPA with dietary vitamin E supplementation for evaluating the protective response. Two control groups were present in the experiment, where fishes of C1 (negative control) group were not exposed to BPA in the culture tanks, but C2 (positive control group) was exposed to 0.04 ppm of BPA in culture tanks. The fishes of all the treatment groups (T1 to T5) were exposed to 0.04 ppm BPA. 7 experimental groups *viz*, C1 or negative control (no BPA; baseline vitamin E 100 mg kg^{-1} diet); C2 or positive control (0.04 ppm of BPA; vitamin E 100 mg kg^{-1} diet); T1 (0.04 ppm of BPA; vitamin E 200 mg kg^{-1} diet); T2 (0.04 ppm of BPA; vitamin E 400 mg kg^{-1} diet); T3 (0.04 ppm of BPA; vitamin E 600 mg kg^{-1} diet); T4 (0.04 ppm of BPA; vitamin E 800 mg kg^{-1} diet); T5 (0.04 ppm of BPA; vitamin E 1000 mg kg^{-1} diet) were designed. Analytical grade BPA (CAS 80-05-7) and Tocopherol Acetate/Vitamin E (CAS 7695-91-2) was procured for this experiment. By dissolving BPA in analytical grade ethanol (CAS 64-17-5) stock solution of 1000 ppm was made and with the dilution method experimental dose of 0.04 ppm was derived. Except C1 group, all the fish tanks (Including each treatment groups and C2 group) were subjected to 0.04 ppm BPA treatment. The experiment was conducted for 45 days period, where the fishes were fed with formulated diets containing different levels of vitamin E at 2% biomass twice a day.

A total of 126 healthy male *O. niloticus* were chosen and randomly distributed among seven treatments with three replicate experimental tanks per treatment (six fish per tank). In total 21 tanks and 126 fish were used for the experiment. Considering the relatively large size of the fish (~110 g), this stocking density was maintained to minimize crowding-related stress and aggressive interactions and other potential background influences that could interfere with the physiological responses to BPA exposure and dietary vitamin E supplementation. The experiment was conducted in a completely randomized design. 3 fish were sampled from each replicate tank for biochemical, hormonal, and histological analyses (nine fish were sampled per treatment). Measurements from fish within the same tank were averaged prior to analysis. Replicate means were used for statistical analysis, with the tank considered as the experimental unit (n = 3). The fish were kept starved for one day before sampling and sampling was done on 45^{th} day.

2.4 Haematological parameters

Fish were anaesthetized with MS-222, and blood was drawn from the caudal vein using EDTA-treated syringes. Samples were promptly transferred to anticoagulant vials and gently mixed to avoid clot formation. Haematological parameters, including haemoglobin (Hb), RBC, WBC, packed cell volume (PCV), and red cell distribution width (RDW), were measured using an automated haematology analyser.

2.5 Tissue enzyme assays

2.5.1 Sample preparation

At the end of the experiment, the fishes were anesthetized using clove oil thereafter liver, gill and liver tissues were collected. Collected tissue samples were homogenized (5% w/v) in chilled 0.25 M sucrose solution using Teflon-coated homogenizer under ice cold conditions. The homogenate was centrifuged at 5000 rpm for 10 min and the supernatant was collected and stored at –20°C for subsequent enzyme assay.

2.5.2 Superoxide dismutase (SOD)

Following the procedure of Mishra and Fridovich (1972), based on inhibition of epinephrine auto-oxidation superoxide dismutase (SOD), activity was measured for the gill and liver of fish. The reaction mixture comprised of 50 µL of sample, and 1.5 ml of 0.1 M carbonate-bicarbonate buffer containing 57 mg dl^{-1} EDTA (pH 10.2) AND 0.5 ml epinephrine (3 mM). The reaction mixture was monitored in spectrophotometers at 480 nm for 3 minutes in kinetics method. SOD activity was expressed as unit activity (amount of enzyme required to produce 50% inhibition of epinephrine auto oxidation).

2.5.3 Catalase (CAT)

Catalase activity of gill and liver was determined following Takahara *et al*. (1960) by monitoring the decomposition of H_2O_2 at 240 nm. The tissue sample (50 µL) was mixed with 2.5 ml of 50 mM phosphate buffer (pH 7.0) AND 1.0 ml of H_2O_2 solution was added to it. The decrease in absorbance was recorded at 15 seconds intervals for 3 min at 240 nm. Enzyme activity was expressed as nmol H_2O_2 decomposed min^{-1} mg^{-1} protein.

2.6 Serum biochemical parameters

2.6.1 Serum glutamate pyruvate transaminase (SGPT)

Serum SGPT levels were assessed using a diagnostic kit (Erba Mannheim, Transasia Bio-medicals, Daman, India)

based on a kinetic enzymatic approach.

2.6.2 Serum glutamate oxaloacetate transaminase (SGOT)

Serum SGOT activity was quantified using a commercial kit (Erba Mannheim, Transasia Bio-medicals, Daman, India) following the NAC-activated kinetic method.

2.6.3 11– keto testosterone

Serum 11–KT was estimated through commercial ELISA kit (Krishgen Biosystems) as per the manufacturer's protocol. Absorbance was recorded at 450 nm and values were expressed as pg ml^{-1}.

2.6.4 17 β estradiol

Competitive ELISA Kit (Krishgen biosystems) was used for the assay of serum 17 β estradiol. The absorbance was recorded at 450 nm and values were expressed as pg ml^{-1}.

2.7 Histological analysis

Testicular tissues of fish were carefully excised, trimmed, and fixed in 10% neutral buffered formalin for 24 h, followed by replacement with fresh fixative to ensure complete preservation. The samples were dehydrated through graded alcohol (90% for 1 h and absolute alcohol three times for 45 min each), cleared in xylene (2 × 30 min), and embedded in paraffin wax (3 × 45 min). Sections (~5 µm) were prepared using a rotary microtome, mounted on slides, dewaxed, and rehydrated prior to staining with haematoxylin (12 min) and eosin (4 min). The stained sections were subsequently dehydrated, cleared, mounted with DPX, and examined under a binocular microscope for histo-morphological assessment.

2.8 Data analysis

The statistical analysis of the results from the treatment groups was performed using IBM SPSS software, version 25.0. A one-way ANOVA was conducted, followed by Duncan's test as a post-hoc analysis to identify significant differences between the means of the various treatments and the control group at a 5% significance level ($p < 0.05$). Prior to statistical analysis, homogeneity of variance was assessed using Levene's test. Descriptive statistics were also examined to evaluate the overall distribution of the data and identify potential outliers. As the study employed a balanced completely randomized design with equal replication across all treatments, one-way ANOVA followed by Duncan's multiple range test was considered appropriate for comparing treatment means. Data are presented as mean ± standard error (SE), and differences were considered significant at $p < 0.05$.

3 | RESULTS AND DISCUSSION

3.1 Haematological parameters

Exposure to BPA in the C2 group led to considerable haematological changes compared to the control (C1), demonstrated by a significant ($p < 0.05$) decline in haemoglobin, red blood cell count, and packed cell volume, suggesting severe anaemia. In comparison, white blood cell (WBC) count and red cell distribution width were significantly ($p < 0.05$) increased, indicating systemic stress and anisocytosis (Table 1). Supplementation with Vitamin E led to a dose-dependent changes in blood parameters. The T1 and T2 groups demonstrated partial recovery, exhibiting moderate rises in Hb, RBC, and PCV. Significantly, the T3 group showed almost total recovery of haematological parameters similar to the control group C1 ($p < 0.05$). Nonetheless, additional rises in Vitamin E dosages (T4 and T5) led to diminished recovery efficiency, evidenced by reduced Hb levels and associated declines in RBC and PCV, though these values were still significantly higher ($p < 0.05$) than those observed in the BPA-exposed group. The results suggest a dose-dependent response, with T3 achieving the greatest haematological recovery.

TABLE 1 Effects of graded dietary vitamin E supplementation on haematological parameters (RBC, PCV, WBC, RDW and Hb) in male *Oreochromis niloticus* exposed to BPA. Values are expressed as mean ± SE ($n = 3$).

Treatment groups	Hb (g/dL)	RBC (10^6cells/mm^3)	PCV (%)	WBC (10^3 cells/mm^3)	RDW (%)
C_1	7.6^{a}±0.5	2.24^{a}±0.18	32.6^{a}±2.4	28.4^{e}±2.6	13.8^{e}±1.2
C_2	4.2^{e}±0.4	1.28^{e}±0.12	18.4^{e}±1.8	52.6^{a}±4.8	21.6^{a}±1.9
T_1	5.3^{d}±0.4	1.65^{d}±0.14	23.7^{d}±2.1	44.2^{b}±3.9	18.4^{b}±1.6
T_2	6.6^{b}±0.5	2.1^{b}±0.10	28.9^{b}±2.3	35.7^{d}±3.1	15.9^{d}±1.4
T_3	7.8^{a}±0.6	2.48^{a}±0.19	33.4^{a}±2.5	29.6^{e}±2.7	14.2^{e}±1.3
T_4	6.1^{c} ±0.5	1.92^{c}±0.15	26.2^{c}±2.0	38.8^{c}±3.4	16.8^{c}±1.5
T_5	5.7^{cd}±0.4	1.75^{cd}±0.13	24.8^{cd}±1.9	41.3^{bc}±3.7	17.9^{bc}±1.6

C1: no BPA exposure; basal dose of 100 mg vitamin E kg^{-1} diet; C2: exposed to 0.04 ppm BPA; basal dose of 100 mg vitamin E kg^{-1} diet; T1: 0.04 ppm BPA; 200 mg vitamin E kg^{-1} diet; T2: 0.04 ppm BPA; 400 mg vitamin E kg^{-1} diet; T3: 0.04 ppm BPA; 600 mg vitamin E kg^{-1} diet; T4: 0.04 ppm BPA; 800 mg dietary vitamin E kg^{-1} diet; T5: 0.04 ppm BPA; 1000 mg vitamin E kg^{-1} diet.

One-way ANOVA revealed significant differences among treatment groups for the evaluated haematological parameters ($p < 0.05$).The current research shows that in C2 group exposure to bisphenol A (BPA) causes notable haematological imbalances, such as decreased haemoglobin (Hb), red blood cell count (RBC), and packed cell volume (PCV), coupled with increased white blood cell (WBC) count and red cell distribution width (RDW), which signify anaemia, erythrocyte injury, and a systemic inflammatory response. These changes are mainly due to haemolysis caused by oxidative stress and the breakdown of the erythrocyte membrane structure. Comparable haematological reactions have been documented in fish exposed to environmental toxins. Exposure to cadmium (heavy metal) in *Clarias gariepinus* led to notable decreases in erythrocyte count (RBC), haemoglobin (Hb), and haematocrit alteration while causing an elevation in leucocyte (WBC) count, indicating anaemia and immune response triggered by oxidative stress (Samuel *et al*. 2021). Moreover, exposure to aniline in *Channa punctatus* has demonstrated similar reductions in erythrocytic indices and leucocytosis, further reinforcing the presence of toxicant-induced haematological stress (Sharma and Chadha 2023).

In the C2 group, the observed decrease in erythrocyte indices in this study can be linked to the overproduction of reactive oxygen species (ROS), resulting in lipid peroxidation of erythrocyte membranes, heightened cellular fragility, and subsequent haemolysis. Increased RDW further signifies anisocytosis related to impaired erythropoiesis, whereas elevated WBC numbers suggest the activation of immune defence processes under toxic stress. Supplementation with Vitamin E significantly improved ($p < 0.05$) improved these blood-related issues in a dose-dependent fashion, with the T3 group demonstrating the best recovery. This protective effect is ascribed to the antioxidant characteristic of Vitamin E, which stabilizes red blood cell membranes and hinders lipid peroxidation. Comparable beneficial effects have been noted in cases of cadmium-induced toxicity, where Vitamin E supplementation notably returned haematological parameters to standard levels in *Clarias gariepinus* by decreasing oxidative stress and enhancing erythrocyte integrity (Samuel *et al*. 2021).

Nevertheless, the relatively lower recovery noted at elevated supplementation levels (T4 and T5) indicates that high doses of Vitamin E might show reduced effectiveness or possible redox imbalance effects in specific circumstances, consequently hindering full haematological recovery. Conversely, exposure to BPA has been noted to raise erythrocyte levels in certain species, including *Anabas testudineus*, where sub-chronic exposure led to increased RBC and WBC counts as a compensatory reaction to stress (Vadivel *et al*. 2026). These variations can be linked to differences in species, duration of exposure, and concentration of toxicants, as initial stress responses might boost erythropoiesis, whereas extended exposure results in haemolysis. The current results collectively demonstrate that BPA causes haematological alterations mainly through mechanisms involving oxidative stress, whereas Vitamin E reduces these effects by scavenging reactive oxygen species (ROS) and stabilizing membranes, with the T3 group showing significantly better recovery than the BPA-exposed group ($p < 0.05$).

3.2 Antioxidant enzymes

SOD activity in both gill (Figure 1A) and liver (Figure 1B) tissues showed a steady pattern throughout the experimental groups. The fish of positive control (C2) group exposed to the BPA and lowest vitamin E supplementation exhibited the significantly ($p < 0.05$) highest SOD activity in comparison to the negative control C1 group, signifying increased oxidative stress due to the effect of BPA. T3 group exhibited SOD levels significantly similar to C1 control group ($p < 0.05$), indicating successful reduction of oxidative stress. A minor rise was observed in the highest dietary vitamin E fed groups (T4, T5 group). The noted trend shows that antioxidant supplementation aided in lowering oxidative stress and bringing enzyme activity back to normal physiological levels.

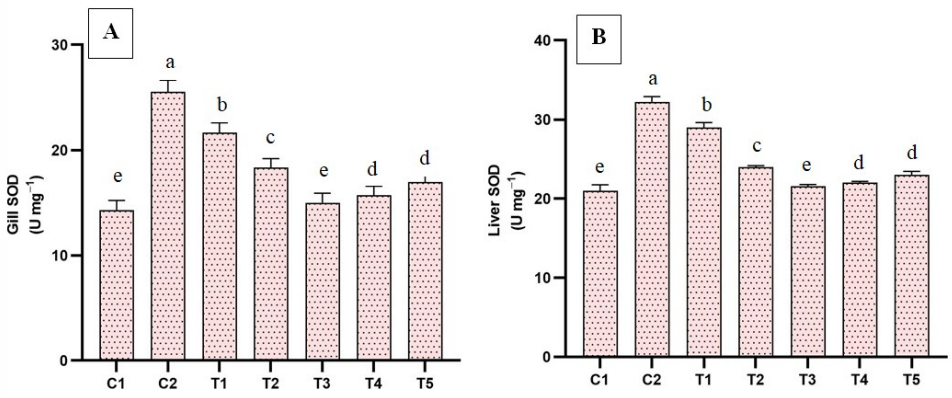

FIGURE 1 Effects of graded dietary vitamin E supplementation on (A) Gill SOD and (B) Liver SOD levels in male *Oreochromis niloticus* exposed to BPA. Different superscripts indicate significant differences among treatments ($p < 0.05$). Data are presented as mean ± SE ($n = 3$). Details of the experimental groups are given in Table 1.

Catalase activity in liver (Figure 2A) and gill (Figure 2B) of fish differed significantly among the experimental groups ($p < 0.05$). C2 group fish resulted in a significant elevation ($p < 0.05$) in catalase activity compared to the negative control group (C1 group), indicating an induced oxidative stress response. Among the vitamin E–supplemented groups, Fish of T1 group showed the highest catalase activity, followed by T2, T5 groups, while T4 group fish exhibited a moderate response especially in liver. In contrast, T3 group displayed lowest catalase activity, which was not significantly different from the C1 control group ($p < 0.05$). Overall, vitamin E supplementation modulated BPA-induced alterations in catalase activity, with variable efficacy across treatment levels.

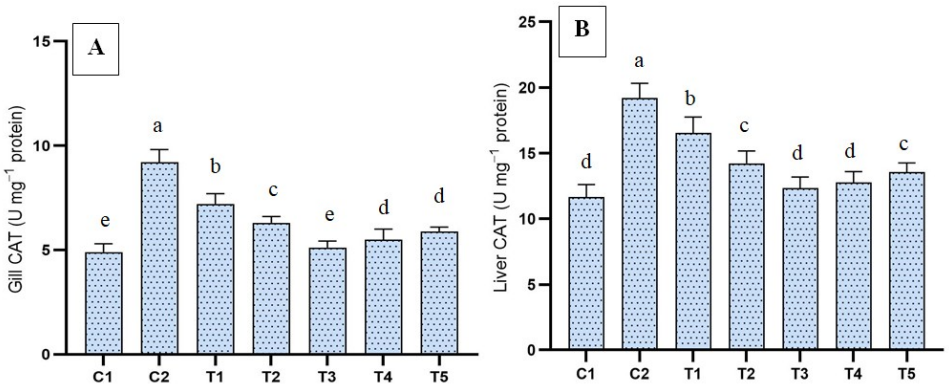

FIGURE 2 Effects of graded dietary vitamin E supplementation on (A) Gill CAT and (B) Liver CAT levels in male *Oreochromis niloticus* exposed to BPA. Different superscripts indicate significant differences among treatments ($p < 0.05$). Data are presented as mean ± SE ($n = 3$). Details of the experimental groups are given in Table 1.

The current study showed that long-term exposure to environmentally relevant conc. of BPA led to significantly increased ($p < 0.05$) increased activities of SOD and CAT, signifying the onset of oxidative stress. The rise in these antioxidant enzymes indicates an adaptive reaction to the overproduction of reactive oxygen species (ROS), with SOD facilitating the conversion of superoxide radicals into hydrogen peroxide, which is then neutralized by CAT. Comparable changes in antioxidant enzyme activities have been observed in fish subjected to BPA. In *O. niloticus*, exposure to BPA notably altered oxidative stress indicators, comprising SOD and CAT, signifying a disturbance in antioxidant defence systems (Abdel-Tawwab and Hamed 2018).

The normalization of SOD and CAT activities noted in the T3 group which were significantly lower than those of the BPA-exposed group (C2) ($p < 0.05$), suggests the beneficial effect of Vitamin E in reducing BPA-induced oxidative stress. Vitamin E as a lipid-soluble antioxidant, safeguards cellular membranes by neutralizing free radicals and preventing lipid peroxidation, thus lowering oxidative stress and re-establishing redox balance. In earlier studies involving *O. niloticus* subjected to zinc oxide, the addition of vitamins E and C significantly lowered the increased activities of SOD and CAT, suggesting a decrease in oxidative stress (Mohamed *et al*. 2021). This decrease signifies a return to normal levels of antioxidant enzymes due to a reduction in reactive oxygen species and a lower need for enzymatic defense. These results reinforce the importance of Vitamin E in reducing ROS-related cellular damage, even as variations in certain antioxidant enzymes may occur based on exposure circumstances.

The relatively higher sod & catalase activity noted at high-level vitamin E supplementation, particularly in the T5 group ($p < 0.05$ compared with T3), in this study indicates that too much Vitamin E might not provide extra protective advantages and could marginally disrupt redox balance. Although the direction of change differs, dose-dependent alterations in antioxidant enzyme activities at higher vitamin E levels have been reported by Hajiani *et al*. (2008) in a rat oxidative stress study, suggesting that supra-optimal supplementation may influence redox homeostasis. Similarly, Hamre (2011) emphasized that the physiological effects of vitamin E in fish depend on maintaining an appropriate dietary balance, as both deficiency and excessive supplementation can affect oxidative status and antioxidant defense mechanisms. A plausible explanation for the relatively higher SOD and CAT activities observed at elevated vitamin E supplementation levels in the present study is that excessive antioxidant supplementation may alter cellular redox regulation and antioxidant defense responses. Although pro-oxidant effects of high vitamin E concentrations have been proposed in other animal models, studies investigating such mechanisms in fish remain limited; therefore, the underlying mechanism warrants further investigation. The current results collectively affirm that BPA triggers oxidative stress in fish, as demonstrated by significantly elevated SOD and CAT activities in the C2 group compared with the C1 control group ($p < 0.05$), while appropriate vitamin E supplementation alleviated toxicant-induced oxidative stress and contributed to the restoration of antioxidant homeostasis.

3.3 Serum hepatic biomarkers

Serum SGOT and SGPT levels exhibited a significant rise ($p < 0.05$) in the positive control (C2) group in comparison to the negative control group (C1 group), indicating hepatic

damage due to BPA exposure (Figure 3A and 3B). Supplementing with vitamin E led to a gradual decrease in serum transaminase levels and caused the highest restoration of SGOT & SGPT in the T3 group, where enzyme levels were not significantly different from those of the control group C1 ($p < 0.05$). Nonetheless, a minor increase was observed in the higher vitamin E supplementation groups (T4 and T5), although the figures remained significantly lower than those of the C2 group ($p < 0.05$).

The notable rise in SGOT and SGPT levels in the BPA-exposed C2 group which were significantly higher than those of the control group (C1) ($p < 0.05$), suggests liver cell injury caused by BPA, aligning with previous research indicating oxidative stress and liver damage related to BPA in fish (Srivastava and Reddy 2020). Gradual dietary vitamin E supplementation (T1–T3) led to a dose-related reduction in serum transaminase levels, with the T3 group nearing control values, supporting the hepatoprotective role of vitamin E in tilapia and other species under toxicant stress (Elkaradawy *et al*. 2021). However, a slight increase in SGOT and SGPT in the highest vitamin E supplemented groups (T4 and T5) suggests that beyond an optimal level, vitamin E may stop providing additional benefits and could slightly impair the liver's function. This pattern resembles the findings reported by El-Hak *et al*. (2019), which indicated that excess dose of vitamin E (2000 mg kg^{-1} body weight for 30 days) resulted in significant ($p < 0.05$) increases in ALT and AST alongside histological damage in rat liver, while lower doses (500–1000 mg kg^{-1}) seemed to be comparatively safer. Overall, these results indicate that vitamin E supplementation for tilapia subjected to BPA should be adjusted to a moderate level (similar to T3), while high doses (T4–T5) should be used cautiously to avoid potential redox imbalance or hepatotoxic negative effects.

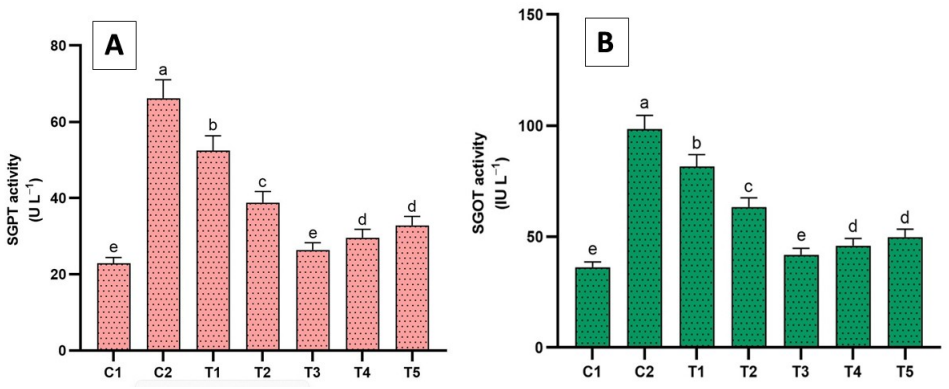

FIGURE 3 Effects of graded dietary vitamin E supplementation on (A) SGPT and (B) SGOT levels in male *Oreochromis niloticus* exposed to BPA. Different superscripts indicate significant differences among treatments ($p < 0.05$). Data are presented as mean ± SE ($n = 3$). Details of the experimental groups are given in Table 1.

3.4 Endocrine function

Serum 11-ketotestosterone levels exhibited a significant ($p < 0.05$) decline in the BPA exposed C2 group, in comparison to the C1 group, highlighting a disturbance in androgenic activity due to BPA exposure (Figure 4). Estradiol level was also significantly ($p < 0.05$) increased in the C2 group, indicating hormonal imbalance (Figure 5). Similar endocrine disruption has been observed in experimental research on fish subjected to various endocrine-disrupting substances. In male zebrafish (*Danio rerio*) subject to environmental oestrogens, it has been recorded that 11-ketotestosterone synthesis is suppressed while estradiol levels rose (Zhang *et al*. 2025). Additionally, bisphenol compounds have been found to enhance aromatase expression and interfere with steroidogenic pathways in zebrafish, resulting in hormonal disruption (Kinch *et al*. 2015; Qiu *et al*. 2019). Oxidative stress caused by BPA and similar toxic substances increases reactive oxygen species (ROS), which disrupt Leydig cell steroidogenesis by inhibiting essential enzymes like 17β-hydroxysteroid dehydrogenase, resulting in diminished androgen (11-KT) production. Simultaneously, redox-sensitive signalling pathways is often triggered by ROS, that increase the expression of aromatase (cyp19), facilitating the transformation of androgens into estradiol. This dual ROS-driven process leads to reduced 11-ketotestosterone and elevated estradiol levels in fish that are exposed with BPA derivatives. (Ji *et al*. 2013; Yang *et al*. 2018).

Vitamin E supplementation led to a gradual recovery of hormone levels among the treatment groups. The T3 group showed the most significant recovery, with levels of 11-ketotestosterone rising and estradiol levels falling to approach control values ($p < 0.05$). Nonetheless, a minor divergence from ideal levels was observed in the higher vitamin E supplementation groups (T4 and T5), yet the values still showed improvement in comparison to C2, T1 and T2 groups. As a strong lipid-soluble antioxidant, vitamin E probably protects steroidogenic tissues by stabilizing cellular membranes, scavenging reactive oxygen species, and maintaining their structural and functional integrity. This defence may inhibit excessive aromatization of androgens to oestrogens and promote the reactivation of steroidogenic pathways. However, excess vita-

min E intake may inhibit physiologically necessary ROS, disrupting redox-sensitive processes involved in sperm growth and function. Study on Gangetic *Mystus* fish show that supra-optimal levels do not improve or even decrease reproductive performance (Shaha *et al*. 2022). It emphasizes the significance of preserving an ideal redox balance and provide additional evidence that excessive exposure to antioxidants can hinder gonadal development. In general, spermatogenesis and sperm functional competence are supported by moderate dietary vitamin E levels, while excessive amounts may cause a reductive redox imbalance and negatively reproductive results.

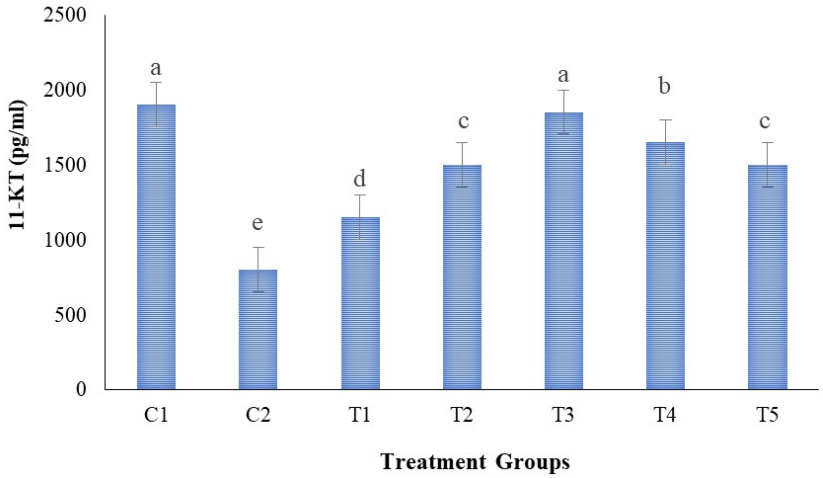

FIGURE 4 Effects of graded dietary vitamin E supplementation on 11KT levels in male Oreochromis niloticus exposed to BPA. Different superscripts indicate significant differences among treatments ($p < 0.05$). Data are presented as mean ± SE ($n = 3$). Details of the experimental groups are given in Table 1.

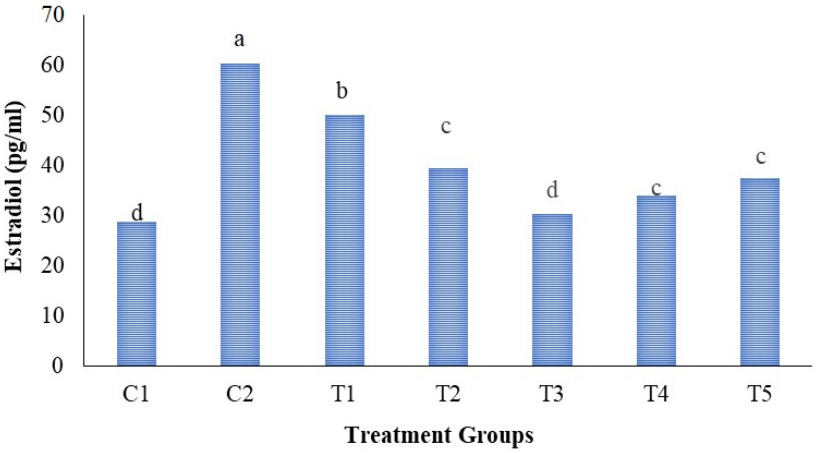

FIGURE 5 Effects of graded dietary vitamin E supplementation on estradiol levels in male *Oreochromis niloticus* exposed to BPA. Different superscripts indicate significant differences among treatments ($p < 0.05$). Data are presented as mean ± SE ($n = 3$). Details of the experimental groups are given in Table 1.

3.5 Testicular histoarchitecture

The C1 control group fish testes showed well-structured seminiferous tubules with intact tuble wall, featuring well-structured germinal epithelium, signifying normal spermatogenesis (Figure 6A). In comparison, the BPA-exposed C2 control group fish testes (Figure 6B) exhibited atrophy, germ cell degradation causing cellular debris deposition in the centre, altered tubule structure and lack of spermatogenic cells. Groups receiving increased level of vitamin E showed changes in a dose dependent manner. Fish testes of T1 group (Figure 6C) featured seminiferous tubules with partially restored germinal epithelium, however tubular walls appear damaged and irregular, with reduced spermatozoa in the lumen. Fish testes of T2 (Figure 6D) revealed gradual restoration of seminiferous tubule structure with a moderate quantity of spermatids, but widened interstitial space between the seminiferous tubule and sloughed germinal epithelium was observed at many places. The fish testes of T3 group (Figure 6E) exhibited nearly normal histology, featuring round seminiferous tubules and lumens filled with a high density of mature spermatozoa. T4 group fish testes (Figure 6F), however, showed minor variations, including lower mature spermatozoa density and a rise in immature spermatocytes, although the tubule structure remained relatively intact. The T5 group fish testes (Figure 6G) featured the reduced luminal space and very few spermatozoa. T4 and T5 group showed spermiogenic arrest that might be caused by excess vitamin E. This study shows that exposure to BPA causes notable histopathological changes in testicular tissue, whereas vitamin E supplementation provides a dose-dependent protective effect, resulting in negative effects at elevated doses.

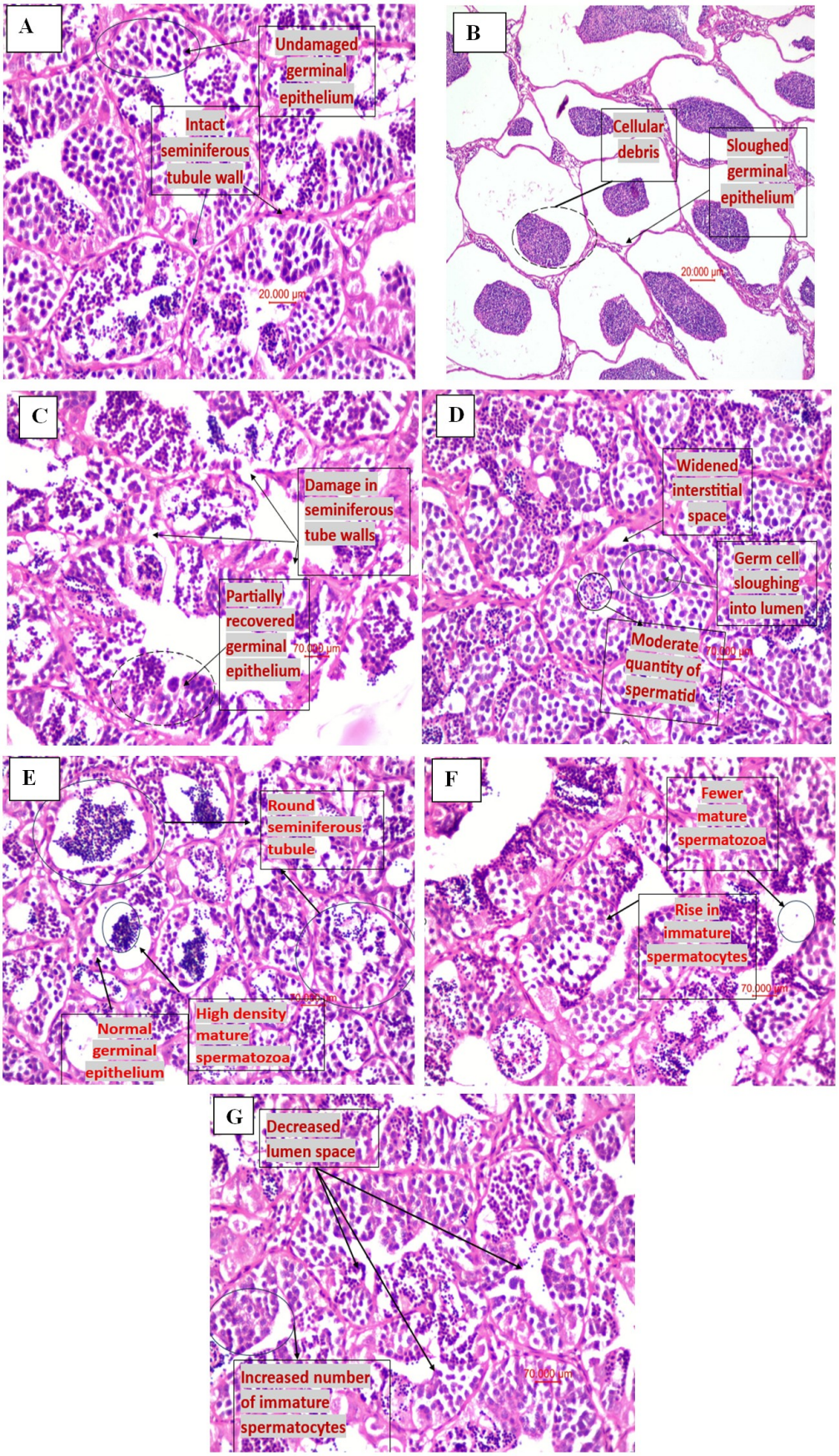

FIGURE 6 Histology of *Oreochromis niloticus* testes in different treatments (A) C1 (B) C2 (C) T1 (D) T2 (E) T3 (F) T4 (G) T5 observed at 40X. Details of the experimental groups are given in Table 1.

The noted degeneration of seminiferous tubules and interruption of spermatogenesis after BPA exposure (in C2 group) aligns with results from rat studies assessed through histological (H&E) and stereological techniques, where BPA-related oxidative stress resulted in the loss of germ cells and caused atrophy of cells in tubules (Mourad and Khadrawy 2012). These effects mainly result from heightened lipid peroxidation and diminished antioxidant defence, which ultimately disrupt testicular function. Supplementing with vitamin E led to a gradual improvement in testicular structure (In T1, T2, T3 groups), confirming its recognized function as an antioxidant. Comparable protective benefits have been observed in Wistar rats exposed to toxins, evaluated via histological and histomorphometry analyses, with vitamin E enhancing the integrity of seminiferous tubules and spermatogenesis (Sukmawati *et al*. 2019). In studies involving rats exposed to BPA, effective doses of vitamin E re-established the structure of the germinal epithelium and improved antioxidant levels (Akbaş and Kum 2022), probably by scavenging reactive oxygen species and stabilizing cellular membranes.

Nonetheless, higher vitamin E Supplementation (T4 and T5) led to compromised spermiogenesis. Seminiferous tubules show relatively intact architecture but reduced luminal space due to accumulation of immature germ cells. The decreased presence of mature spermatozoa indicates spermiogenic arrest, likely associated with excessive vitamin E supplementation. Experimental studies in rodents have demonstrated that reactive oxygen species (ROS) play a dual role in testicular physiology, where controlled levels are essential for normal spermatogenesis, while both excessive ROS and excessive antioxidant activity may impair sperm function (Aitken and Roman 2009). This could be attributed to Reductive stress, in which an overabundance of antioxidants inhibits the physiologically necessary ROS needed for normal spermatogenic functions.

4 | CONCLUSIONS

From the above results it can be concluded that even in environmental concentration chronic exposure of BPA can cause oxidative stress mediated endocrine disruption in fish along with haematological alteration in male *O. niloticus*. The results suggest a biphasic role of vitamin E; appropriate supplementation reduces BPA-induced testicular damage, whereas high levels could interfere with spermatogenesis by changing redox equilibrium. Thus, optimization of dietary vitamin E is essential for maintaining reproductive health and ensuring breeding success of male *O. niloticus*.

ACKNOWLEDGEMENTS

The authors express heartfelt thanks to Director, ICAR-Central Institute of Fisheries Education, Mumbai, and Head of the Department, FNBP Division for providing necessary facilities to conduct the research work.

ETHICAL APPROVAL

This is to state that all methods and all the experimental protocols were carried out in accordance with relevant guidelines and regulations and all the experimental protocols were approved by ICAR-Central Institute of Fisheries Education (Deemed University), Mumbai, India.

CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

AUTHORS' CONTRIBUTION

Abhilipsa Biswal: Writing- original draft, Investigation; Prem Prakash Srivastava: Conceptualization, Methodology & Investigation; Kedarnath Mohanta: Supervision; Subodh Gupta: Investigation; Prem Kumar: Methodology; Tincy Varghese: Software and data curation; Manish Jayant: Writing- review & editing; Annam Pavan Kumar: Supervision.

DATA AVAILABILITY STATEMENT

Data will be made available on request.

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