

Toxicological Effects of Waterborne Boric Acid and the Protective Role of Dietary Black Cumin (*Nigella sativa* L.) Oil in Nile Tilapia (*Oreochromis niloticus*): Hematological, Biochemical, Oxidative Stress and Histopathological Responses

Mustafa ÖZ

ozmustafa@aksaray.edu.tr

Aksaray University: Aksaray Universitesi <https://orcid.org/0000-0001-5264-7103>

Enes ÜSTÜNER

Aksaray University: Aksaray Universitesi

Suat DİKEL

Cukurova University: Cukurova Universitesi

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Abstract

This study aimed to evaluate the protective effects of dietary black cumin (*Nigella sativa* L.) oil on hematological, biochemical, oxidative stress, and histopathological responses in Nile tilapia (*Oreochromis niloticus*) exposed to waterborne boric acid. After determining the 96-hour LC₅₀ value of boric acid, the experimental design included exposure to 1/20th of this concentration. Fish were fed for 21 days with diets either containing or lacking 1% black cumin oil. At the end of the feeding trial, blood parameters, oxidative stress biomarkers, and tissue histopathology were analyzed. The group receiving black cumin oil without boric acid showed the most favorable physiological and biochemical profiles. In contrast, the group exposed to boric acid alone exhibited significant negative alterations. Importantly, fish fed black cumin oil while exposed to boric acid showed improvements across all measured parameters compared to the toxicant-only group.

The findings indicate that dietary black cumin oil effectively alleviates the toxic effects of waterborne boric acid on Nile tilapia, supporting its potential use as a functional dietary additive in aquaculture.

1. INTRODUCTION

Aquatic ecosystems worldwide face increasing risks from a variety of chemical contaminants emanating from industrial, agricultural, and domestic sources. Among these contaminants, boric acid (H_3BO_3) has emerged as a pollutant of concern due to its widespread use in glass production, ceramics, agriculture, and even in certain antiseptic formulations (Kan & Kucukkurt, 2023). Natural sources of boric acid typically include seawater and geological formations, where it is found in water layers at concentrations exceeding 4 mg/L (Baransi-Karkaby, Bass, & Freger, 2019). In freshwater environments, boric acid can bioaccumulate and may lead to toxicity in aquatic organisms, particularly when concentrations exceed threshold values of 0.5 to 1 ppm (Gaikwad, Gaikwad, & Kamble, 2021). The application of boric acid as a pesticide in urban areas increases the likelihood of surface runoff during rainfall, enhancing its presence in nearby water bodies (See et al., 2010). Boric acid reaching aquatic ecosystems has been shown to cause histopathological damage in fish tissues and negatively affect blood parameters (Öz, Yavuz, & Bolukbas, 2020; Topal, Oruç, Altun, Ceyhun, & Atamanalp, 2016). In recent years, environmental toxicology has witnessed increasing interest in identifying natural protective substances that may reduce the toxic effects of environmental pollutants. Black seed (*Nigella sativa* L.) oil has attracted considerable attention due to its rich composition of bioactive compounds such as thymoquinone, which exhibits strong antioxidant, anti-inflammatory, and immunomodulatory properties (Bordoni et al., 2019). Thymoquinone enhances antioxidant enzyme activities which supports its protective effects against oxidative damage according to multiple animal model studies. (Hosseinizadeh, Monaghash, Ahmadi, Ghasvand, & Shokoohinia, 2017). The toxic effects can be counteracted by using herbal supplements that contain natural antioxidants in aquaculture operations. The bioactive compounds thymoquinone and others in *Nigella sativa* (black cumin) oil provide antioxidant and anti-inflammatory and hepatoprotective effects. Research shows that adding black cumin seeds and oil to fish feed enhances growth rates while decreasing oxidative stress. (Latif, Faheem, Asmatullah,

Hoseinifar, & Van Doan, 2020; Öz, Dikel, & Durmus, 2018; Öz, Üstüner, & Bölükbaş, 2024). The Nile tilapia (*Oreochromis niloticus*) serves as an appropriate model species for toxicological research in aquatic ecosystems because of several key factors. The species stands as a valuable organism for studying environmental stressors because it adapts to different conditions and shows strong physiological responses to pollutants and plays important ecological roles in freshwater habitats. The Nile tilapia stands out because of its ability to adapt to changing water conditions which makes it essential for toxicology research. The species demonstrates wide tolerance to environmental factors including salinity and temperature fluctuations and pollution while thriving in both high and low-quality aquatic environments. (Abdel-Mohsen, 2014). The researchers can study pollution effects under different water conditions because of this natural ability to adapt. This ability enables researchers to study pollution effects under realistic ecological conditions. Research indicates Nile tilapia maintains survival capabilities in polluted environments containing heavy metals which makes it an ideal species for detecting heavy metal exposure in aquatic ecosystems. (Shaaan, 2024). The research aims to assess the hematological, biochemical, oxidative stress and histopathological effects of boric acid exposure in aquatic environment on Nile tilapia (*Oreochromis niloticus*) and to investigate the protective role of dietary black cumin oil against these toxic effects.

2. MATERIALS AND METHODS

2.1. Research Design

Ethical approval was obtained from Çukurova University ethics committee (Ref No: 7/2020). Feeding activities were carried out at Çukurova University, Faculty of Fisheries, Dr. Nazmi Tekelioğlu Freshwater Fisheries Production and Research Station. In order to determine the appropriate boron dosage for our study, we initially estimated the LC50 (96 h) value by probit analysis (Finney, 1971). A total of 120 fish were used to calculate the LC50 value and boron was applied at six different concentrations (0.00, 10.00, 50.00, 100.00, 150.00 and 200.00 mg/L) (Çelik, Dikel, & Öz, 2024). During the evaluation of the LC50 value, the fish were monitored three times a day for 96 hours and the dead fish were immediately removed from the environment. During the LC50 experiment, oxygen was continuously supplied to 50 liter capacity aquariums and water was changed every 24 hours using prepared stocks. In addition, the fish were not fed during the experiment. To determine the LC50 value, 120 fish were used and the experiment was designed in two replicates. In the feeding trial, 108 fish were used. A total of 228 male Nile tilapia with a starting weight of 36.38 ± 0.83 g were used in the study. The research was carried out in 80 liter aquariums with 9 fish in 3 replications and 27 fish were used in each group. Boric acid was added to the water of the research groups at the rate of 1/20 of the determined Lc50 value. Eheim brand 100 Watt thermostat heater was used to keep the water temperature constant and the water temperature was kept constant at 25 0C in all groups during the research. The research groups and fish numbers are shown in detail in Table 1.

Table 1. Research groups, black cumin seed oil ratios, boric acid amount and fish numbers

Groups	Black Cumin oil in the feed (%)	The amount of Boric Acid in the water (mg/L)	Numbers of fish
BA1	0.00	0.00	27 (3*9)
BA2	1.00	0.00	27 (3*9)
BA3	0.00	LC ₅₀ /20	27 (3*9)
BA4	1.00	LC ₅₀ /20	27 (3*9)
Total			108

2.2. Preparation of Experimental Diets and Feeding Protocol

The dietary regime used in the trial was based on commercially available tilapia feed (Hem Yem, Gaziantep, Turkey) containing 39% crude protein, 6.7% crude fat, 4.30% crude fiber and 6.79% crude ash. The cold-pressed black seed oil utilised in the study was procured from a commercial supplier. According to the protocol adapted from Öz et al. (2018), 1% black cumin oil was added to the diets of experimental groups BA3 and BA4. The feeds were prepared in 100 g portions to ensure accuracy and uniformity in the supplementation process. To enhance coating quality, black cumin oil was systematically blended with 2 ml of sunflower oil and subsequently uniformly sprayed onto the feed. In the control oil groups, 3 ml of sunflower oil was incorporated to standardise the total lipid volume across all treatments and to maintain dietary consistency among the groups. The feeding trial started one day after the first biometric assessments and continued for 21 days. Throughout the study, fish were fed twice daily - once at 08:30 am and once at 16:30 pm - at a rate equivalent to 2% of their total body weight.

2.3. Blood Sampling and Hematological Procedures

At the end of the feeding trial, fish were anesthetized using 2-phenoxyethanol at a concentration of 300 ppm to ensure minimal stress during sampling. Following anesthesia, each sample was carefully rinsed with 70% ethanol to minimize external contamination. Blood samples were then collected from the caudal vein using heparinized syringes. For hematological analyses, a portion of each blood sample was transferred into lavender-colored tubes containing ethylenediaminetetraacetic acid (EDTA) as anticoagulant. Another portion was placed in serum separator tubes (SST™ II Advance, red cap) for biochemical analysis. These samples were centrifuged at 13,000 × g for 10 min at 4 °C to obtain clear serum. Hematologic parameters were assessed immediately after collection, while serum samples were stored at -80 °C until biochemical assessments were performed. Hematological parameters such as red blood cell count (RBC), hematocrit (Hct), hemoglobin concentration (Hb), mean cell volume (MCV), mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC) were analyzed using an MS4-S hematology autoanalyzer (Melet Schloesing Laboratories, Osny, France). The precision of automated

results was verified through manual counts of K₃EDTA tube samples that followed the protocol established by (Blaxhall & Daisley, 1973).

2.4. Determination of Serum Biochemical Parameters

The biochemical parameters assessed in blood serum comprised alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), total protein (TP), triglycerides (TRG), cholesterol (CHO), and glucose (GLU). These indicators were chosen for their appropriateness in evaluating the metabolic and physiological condition of fish. All biochemical assays were conducted via an automated clinical chemistry analyser (MINDRAY BS-400) following the manufacturer's guidelines.

2.5. Oxidative Stress Parameters

Sera extracted from fish blood samples were analysed for Total Antioxidant Status (TAS), Total Oxidant Status (SOD), Superoxide Dismutase (CAT), Catase, GPx, Glutathione Peroxidase, and MDA (malondialdehyde). Relassay (Cat no: RL0017) commercial assays (Erel, 2004) were employed to quantify TAS levels. Relassay (Cat no: RL0024) commercial assays were employed to assess Total Oxidant Status (TOS) levels. (Erel, 2004). Total Oxidant Status (TOS) levels were evaluated using Relassay (Cat no: RL0024) commercial kits (Erel, 2005). The ratio of TOS to TAS is considered as oxidative stress index (OSI). The TAS unit obtained for calculation was converted to $\mu\text{mol/L}$ and the OSI value was calculated according to the following formula.

$$\text{OSI} = \text{TOS } (\mu\text{mol H}_2\text{O}_2 \text{ equivalent/L}) / \text{TAS } (\mu\text{mol Trolox equivalent/L})$$
 (Harma & Erel, 2003; Kosecik, Erel, Sevinc, & Selek, 2005; Yumru et al., 2009).

Malondialdehyde (MDA) level, a product of lipid peroxidation, was determined according to Alak et al. Superoxide dismutase (SOD) enzyme activity was determined by spectrophotometer (560 nm) according to NBT (nitro blue tetrazolium chloride) reduction method with O-2 under light (Sun, Oberley, & Li, 1988). Measurement of catalase (CAT) activity; samples were mixed with 1 mL H₂O₂ (50 mM) and reacted at 37 °C for 1 min. Then 1 mL of ammonium molybdate was added to terminate the reaction, resulting in the formation of a yellowish complex containing residual H₂O₂. Finally, the UV-vis absorption of this complex was measured at 405 nm by a microplate reader (Aebi, 1984). Measurement of glutathione peroxidase (GPx) activity; Glutathione Peroxidase (GPx) catalyzes the oxidation of glutathione by cumene hydroperoxide. In the presence of glutathione (GSSG), it is immediately converted to the reduced form by simultaneous oxidation of NADPH to NADP. GPx activity was measured by a change in absorbance (decrease in readings over 3 min) at 340 nm (Kraus & Ganther, 1980; Paglia & Valentine, 1967; Prohaska, Oh, Hoekstra, & Ganther, 1977).

2.6. Histopathologic Investigation

Gill, liver, and kidney tissues of Nile tilapia were fixed in 0.1 M phosphate-buffered formaldehyde (pH 7.4). After trimming, tissues were washed under slow running water for 24 hours, dehydrated through an ethanol series, and treated with xylene and xylene-paraffin. Samples were incubated in soft paraffin (46–48°C) for 15 minutes and hard paraffin (56–58°C) for 30 minutes before embedding with a Leica EG 1150 H. Sections of 4 µm thickness were cut using a Leica RM2125 Rotary Microtome and stained with Haematoxylin-Eosin. Sections were mounted on glass slides using Entellan Merck, examined by light microscopy (Leica DM-750), and photographed in lesion areas (Culling, Allison, & Barr, 2014).

Hematoxylin-Eosin (H-E) staining of the gill, liver and kidney tissues was used to score the pathological findings on a scale of “(-) to 3” (-: none, 1: mildly severe, 2: moderately severe and 3: severe).

2.7. Statistical Analyses

Statistical analyses were performed using SPSS 18.0 (SPSS Inc., Chicago, IL. USA) to determine the differences between the groups in growth parameters and changes in blood parameters of fish fed with feed supplemented with ALE at different rates.

3. RESULTS

3.1. Determination of LC₅₀ Value

Based on the 96-hour acute toxicity test, the LC₅₀ value of boric acid for Nile tilapia (*Oreochromis niloticus*) was calculated to be 176.446 mg/L. Regression analysis using the Probit method gave the equation $y = 1.24x + 2.2103$ with a coefficient of determination of $R^2 = 0.8846$, indicating a strong correlation between boric acid concentration and mortality (Fig. 1).

3.2. Blood Parameters

The results of hematological analysis of Nile tilapia subjected to different treatments are presented in Table 2. Significant differences in red blood cell indices were observed between the experimental groups, indicating that both boric acid exposure and black cumin oil supplementation affected hematological profiles.

Similarly, serum biochemical parameters including liver enzymes, glucose, lipid profile and total protein levels are summarized in Table 3. The data reflect the physiological responses of fish to boric acid toxicity and the potential ameliorative effects of dietary black cumin oil.

Table 2
Protective effect of black cumin oil on blood parameters parameters against boric acid toxicity in Nile tilapia

	BA 1	BA 2	BA 3	BA 4
RBC (m/mm3)	1.692 ± 0.029 ^c	2.230 ± 0.026 ^a	1.357 ± 0.019 ^d	1.913 ± 0.014 ^b
Hb (g/dl)	13.517 ± 0.306 ^c	16.100 ± 0.167 ^a	11.550 ± 0.176 ^d	14.050 ± 0.259 ^b
Hct (%)	37.550 ± 0.243 ^c	46.733 ± 1.097 ^a	31.733 ± 1.442 ^d	40.750 ± 0.666 ^b
MCV (fL)	222.019 ± 3.635 ^b	209.565 ± 4.178 ^c	233.923 ± 10.689 ^a	212.971 ± 2.459 ^c
MCH (pg)	79.918 ± 2.116 ^b	72.199 ± 0.414 ^c	85.146 ± 1.572 ^a	73.441 ± 1.759 ^c
MCHC (g/dL)	35.998 ± 0.849 ^{ab}	34.463 ± 0.708 ^b	36.469 ± 1.960 ^a	34.491 ± 1.047 ^b

Table 3
Protective effect of black cumin oil on blood biochemistry parameters against boric acid toxicity in Nile tilapia

	BA 1	BA 2	BA 3	BA 4
Cho (mg/dl)	151.370 ± 1.834 ^b	132.530 ± 2.777 ^d	182.027 ± 2.558 ^a	140.270 ± 0.846 ^c
Trg (mg/dl)	154.780 ± 0.551 ^b	128.337 ± 1.382 ^d	166.930 ± 1.048 ^a	142.023 ± 3.835 ^c
Glu (mg/dl)	46.312 ± 0.896 ^b	37.828 ± 0.485 ^d	69.010 ± 0.624 ^a	42.760 ± 0.685 ^c
ALP (U/L)	40.586 ± 0.576 ^c	32.663 ± 0.568 ^d	56.693 ± 2.458 ^a	44.545 ± 0.625 ^b
AST (U/L)	252.640 ± 3.515 ^b	190.717 ± 3.033 ^d	339.439 ± 4.566 ^a	215.582 ± 4.296 ^c
ALT (U/L)	44.090 ± 0.246 ^b	34.327 ± 1.032 ^d	66.393 ± 0.637 ^a	38.448 ± 1.103 ^c
TP (g/dl)	6.070 ± 0.056 ^b	7.707 ± 0.176 ^a	4.167 ± 0.040 ^c	5.997 ± 0.208 ^b

3.3. Oxidative Stress Parameters

In the BA3 group (boric acid exposure), a significant increase was observed in MDA levels, indicating heightened lipid peroxidation and oxidative damage, whereas the activities of antioxidant defense parameters (TAS, CAT, SOD, and GPx) were significantly reduced compared to the control group (Table 4). However, contrary to expectations, TOS and OSI levels were not increased but were actually lower than those in the control group. These findings suggest that, while boric acid exposure predominantly triggers oxidative stress through increased lipid peroxidation (as indicated by elevated MDA levels) and suppression of antioxidant defenses, it does not lead to an increase in total oxidant status or oxidative stress index when compared directly to the control.

Table 4

Protective effect of black cumin oil on oxidative stress parameters against boric acid toxicity in Nile tilapia

Oxidative Stress Parameters				
	BA 1-Control	BA 2	BA 3	BA 4
TAS (mmol/L)	2.142 ± 0.008 ^c	3.259 ± 0.091 ^a	1.999 ± 0.018 ^d	2.475 ± 0.015 ^b
TOS (μmol/L)	26.793 ± 0.628 ^c	34.629 ± 0.198 ^a	21.9991 ± 0.557 ^d	30.228 ± 1.013 ^b
OSI	1.251 ± 0.029 ^a	1.063 ± 0.034 ^b	1.100 ± 0.031 ^b	1.221 ± 0.039 ^a
CAT (U/ml)	216.990 ± 2.062 ^c	291.940 ± 1.901 ^a	180.920 ± 3.304 ^d	263.150 ± 3.847 ^b
SOD (U/ml)	380.877 ± 2.641 ^c	415.010 ± 2.464 ^a	338.500 ± 3.153 ^d	398.430 ± 1.271 ^b
MDA (mmol/L)	79.220 ± 0.886 ^b	63.400 ± 0.704 ^d	94.423 ± 0.666 ^a	74.650 ± 1.759 ^c
GPx (U/ml)	194.063 ± 3.095 ^c	281.317 ± 1.880 ^a	131.903 ± 3.161 ^d	217.283 ± 2.466 ^b

3.4. Histopathologic Findings

Histopathological evaluations of the kidney, liver, and gill tissues were performed using light microscopy (Table 5). In Group 1 (control group), no lesions were observed in the gills, and both primary and secondary lamellae exhibited normal histological features. In Group 3, separation of the epithelial cells in the secondary lamellae, fusion of secondary lamellae, and edema in the secondary lamellae were observed (Fig. 4). In Group 2, only minimal lesions were detected in the primary and secondary lamellae, and the overall histological appearance of the gills was close to normal. Group 4 gills exhibited minimal lesions compared to Group 3 and appeared largely similar to the normal histological structure. Examination of the liver tissue revealed that, in Group 1, the liver tissue, exocrine pancreas acinar cells, and sinusoidal spaces displayed normal histological architecture. In Group 3, severe hydropic and vacuolar degeneration, pronounced hemorrhages in the liver sinusoidal spaces, hyperactivated melanomacrophages in the liver (black arrow), vacuolization in pancreatic cells, and edema and erythrocytes in the acinar cells of the exocrine pancreas were observed (Fig. 3). In Group 2, the histological appearance was generally normal, but mild hemorrhage in the liver sinusoids was noted. In Group 4, minimal lesions were found, and the tissue structure resembled normal histology. Renal tissue showed a normal histological appearance in Group 1. In Group 3, hyperactivated melanomacrophages, vacuolization of the epithelial lining of proximal and distal tubules (T), congestion in the glomerulus (red arrow), and interstitial hemorrhage were recorded (Fig. 1). In Group 2, the kidney exhibited a nearly normal histology with only interstitial hemorrhage observed. In Group 4, minimal lesions were detected compared to Group 3, and the general renal tissue architecture was maintained.

Table 5

Semiquantitative histopathological scoring of fish tissues in experimental groups exposed to boric acid and dietary black cumin seed oil

Tissue	Histopathological Alteration	BA1	BA2	BA3	BA4
Gill	Separation of epithelial cells of the secondary lamellae	0	0–1	2–3	2
	Fusion of secondary lamellae	0	0–1	3	1–2
	Edema in secondary lamellae	0–1	1–2	2–3	1
Liver	Hyperactivated melanomacrophages	0	1–2	2	1
	Vacuolization in pancreatic cells	0	0–1	2–3	1–2
	Edema and erythrocytes in the acinar cells of the exocrine pancreas	0–1	1–2	2	1–2
	Congestion in the liver	0–1	1–2	3	2
	Vacuolar degeneration and hydropic degeneration of hepatocytes	0	1–2	3	1–2
Kidney	Hyperactivated melanomacrophages	0	1–2	2–3	1
	Vacuolization of epithelial lining of proximal and distal tubules	0–1	1–2	2–3	1–2
	Congestion in the glomerulus	0	0–1	3	1–2
	Interstitial hemorrhage	0	0–1	2	0–1

0: absent; 1: mild; 2: moderate; 3: severe.

4. DISCUSSION

In this study, the 96-h LC₅₀ value of boric acid for Nile tilapia (*Oreochromis niloticus*) was 176.446 mg/L, indicating moderate acute toxicity. This value provides an important insight into the species-specific sensitivity of Nile tilapia to boron compounds and is consistent with findings from similar studies on Nile tilapia. For example, Acar, İnanan, Zemheri, Kesbiç, and Yılmaz (2018) reported a 96-h LC₅₀ value of 141.42 mg/L for Nile tilapia, while Abdel Aliem, Soliman, Khaled, Mourad, and Dighiesh (2022) found a value of 290 mg/L under different experimental conditions. The observed differences between the experiments demonstrate how water quality and fish physiological state and size together with experimental design affect toxicity results. The evaluation of boron-based compound ecological risks requires site-specific and species-specific assessments for both aquaculture systems and natural water bodies. The observed mortality pattern indicates boric acid toxicity increases with dose and may disrupt cellular and physiological processes including membrane permeability and ion regulation and enzyme activity. Research indicates boron toxicity in aquatic organisms results in oxidative stress and impaired osmoregulation and vital organ histopathological changes in liver gills and kidneys. (Topal et al., 2016).

LC₅₀ values help determine environmental exposure limits for safety purposes and enable the creation of non-lethal toxicity experiments. The researchers chose 1/20th of the LC₅₀ value for chronic exposure experiments to establish an ecologically relevant concentration which would evaluate long-term physiological and histopathological effects. The study enabled researchers to test dietary black cumin oil as a potential mitigation strategy. The health status of fish depends on hematological and biochemical parameters which serve as indicators of environmental pollutant-induced physiological stress. The exposure to boric acid (BA3 group) resulted in substantial decreases of red blood cell count (RBC), hemoglobin (Hb) and hematocrit (Hct) levels which indicated anemia and reduced oxygen delivery capacity. The current study supports earlier research which demonstrated that boron compounds produce adverse effects on fish hematological parameters. (Acar et al., 2018; Çelik et al., 2024). The liver enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were elevated which indicates liver damage. Fish exposed to boric acid have shown similar liver problems. (Topal et al., 2016). By contrast, fish in the BA4 group getting nutritional supplements with 1% black cumin oil (*Nigella sativa*) showed better haematological and biochemical profiles than the BA3 group. Studies on *N. sativa*'s antioxidant and hepatoprotective effects clearly indicate how effective it is in raising haematological markers and liver function in fish. (Mohammed & Arias, 2016; Öz et al., 2024). Environmental toxins create their harmful effects on aquatic organisms through oxidative stress as a widespread mechanism. The study revealed that boric acid exposure led to elevated malondialdehyde (MDA) levels which indicate lipid peroxidation and reduced activities of catalase (CAT) and superoxide dismutase (SOD) and glutathione peroxidase (GPx) antioxidant enzymes. The observed results match previous research which demonstrated that boric acid induces oxidative stress in fish. (Ertürk Gürkan, Gürkan, Sarıtuğ, İbiş, & Güneş, 2025; Öz, 2024).

The *N. sativa* oil supplement in the BA4 group reduced oxidative stress through lower MDA levels and higher CAT, SOD and GPx activities. The antioxidant effects of *N. sativa* stem from its bioactive compounds including thymoquinone which neutralizes free radicals while enhancing antioxidant defense pathways. The Nile tilapia kidney tissue (*Oreochromis niloticus*) functions as a vital organ for both immune system response and detoxification operations. The head kidney of this organ serves as the main hematopoietic center while providing substantial immune defense through its resident lymphocytes and macrophages. The head kidney serves as a vital location for both innate and adaptive immune system development which underscores its essential role in maintaining tilapia health and immune system function. (Gan et al., 2016; Mo et al., 2024). For example, significant increases in chemokines are associated with immune responses against such infections and facilitate the recruitment of immune cells to effectively combat pathogens (Nakharuthai & Srisapoome, 2020). Heavy metal exposure in Nile tilapia kidneys leads to histopathological changes which include glomerular degeneration together with tubular necrosis and vacuolization. The kidneys of fish exposed to di-n-butyl phthalate (DBP) show major histopathological damage through glomerular structural disruption and tubular functional impairment. The observed changes indicate reduced kidney perfusion and filtration efficiency which results in elevated blood levels of urea and creatinine. (Zeid & Khalil, 2014). This deterioration shows a clear relationship between environmental pollutants and impaired kidney function;

where heavy metals accumulate in kidney tissues, causing cellular damage and functional deficiencies. (Abdel Hakim, Helal, Salem, Zaghloul, & Hanbal, 2016). The exposure of fish to dimethoate pesticides results in major histopathological changes that affect the structural integrity of *Garra mullia* kidneys. The kidneys of control group fish remain healthy with their glomeruli clearly visible and intact. The toxic substance causes kidney function impairment which results in the development of tubular degeneration and necrosis and glomerular alterations after exposure. (Borane, 2016). Heavy metals have been shown to produce harmful histological changes in fish kidneys. The toxic effects of metals such as lead, copper, and zinc have been demonstrated through histopathological analyses that show glomerular atrophy, tubular necrosis, and interstitial inflammation. (Al-Balawi et al., 2013; Al-Kshab, 2023). The importance of pesticides at sub-lethal concentrations, especially malathion and cypermethrin, is of critical importance. These chemicals are known to cause various histological changes in kidney tissues of species like *Channa gachua*, including hyperplasia, vacuolar degeneration, and cellular apoptosis. (Preeti & Garg, 2023; Singh, 2021). The study demonstrates that melanomacrophage hyperactivation in kidney tissue results from the inflammatory response which occurs because of elevated ROS levels produced by boric acid-induced oxidative stress. The vacuolization of proximal and distal tubules develops because boric acid disrupts membrane integrity and reduces Na^+/K^+ -ATPase activity which causes ionic imbalance and increased osmotic pressure. The damage to capillary endothelial cells by boric acid leads to increased vascular permeability which results in blood accumulation and leakage. The toxicological effects of heavy metals and pesticides on fish gill tissues constitute a major concern in aquatic biology because these substances severely affect fish health and ecosystem integrity. The toxic substances cause consistent histopathological changes which appear as hyperplasia together with necrosis and edema. The use of chlorpyrifos pesticides leads to major changes in gill structure and operational capabilities. The exposure to chlorpyrifos results in long-term damage to fish *Cyprinus carpio* through necrosis and filament fusion of their gills. (Edwin, Ihsan, Rahmatika, & Darlis, 2019). Similar observations were documented in research on the Indian Flying Barb, showing increased mucus secretion and filament degeneration following exposure to Endosulfan (Das & Gupta, 2013). Studies have shown that lead exposure causes severe gill damage in *Tilapia* species, particularly leading to edema and necrosis (Batista, Triastuti, & Pursetyo, 2021). The research indicates epithelial cell separation in secondary lamellae of gill tissue occurs because boric acid weakens intercellular junction complexes. The fusion of secondary lamellae occurs through epithelial hyperplasia together with increased mucus secretion and inflammatory cell accumulation. The swelling of secondary lamellae occurs because boric acid makes capillary endothelial cells more permeable and disrupts the osmotic balance. The toxicological effects of heavy metals and pesticides on fish liver tissue result in numerous histopathological changes. The liver remains most vulnerable to chemical changes and structural damage because of its detoxification and metabolism roles after toxic substance exposure. Histopathological studies show that heavy metal exposure causes vacuolization of hepatocytes, degeneration of hepatic structure, and infiltration of immune cells which indicates significant tissue damage and stress responses. (Akter et al., 2020; Bibi, Naz, Saeed, & Chatha, 2021). Exposure to paraquat has been shown to cause liver necrosis and hemorrhage in African catfish (*Clarias gariepinus*) (Ladipo, Doherty, & Oyebadejo, 2011). The combined exposure to pesticides and heavy metals produces synergistic toxic effects which intensifies

histopathological damage. Fish exposed to both types of pollutants show higher levels of hepatocyte degeneration and necrosis. (Ghafariarsani et al., 2024; Ogbeide & Okoduwa, 2024). The research indicates that boric acid leads to liver tissue degeneration through membrane peroxidation in hepatocytes which disrupts mitochondrial function and reduces ATP production. Hemorrhages in sinusoidal spaces are believed to be associated with structural damage to endothelial cells. Oxidative stress, mitochondrial dysfunction, ion imbalance, and protein denaturation caused by boric acid are thought to play roles as common molecular mechanisms in the formation of all these histopathological lesions.

CONCLUSION

The present study revealed that even non-lethal exposure to waterborne boric acid can significantly impair the physiological, biochemical and histopathological integrity of Nile tilapia (*Oreochromis niloticus*). In particular, fish exposed to 1/20 of the 96-hour LC_{50} (176,446 mg/L) exhibited hematological suppression, elevated hepatic enzyme levels, increased oxidative stress biomarkers, and marked tissue damage in gill, liver, kidney and muscle tissues.

Dietary supplementation with 1% black cumin (*Nigella sativa*) oil showed a remarkable protective effect against boric acid-induced toxicity. The black cumin supplemented group showed improved hematological profiles, recovered antioxidant enzyme activity and markedly reduced histopathological lesions compared to the toxicant-only group. These results support the functional role of black cumin oil as a natural antioxidant and tissue protective agent in aquaculture nutrition (Fig. 5).

Taken together, the findings highlight the dual importance of this research: first, contributing to the toxicological understanding of boric acid as an emerging aquatic contaminant; second, highlighting the efficacy of a phytogenic feed additive in reducing such toxicity. Future research should investigate the dose-dependent effects of *Nigella sativa*, examine its long-term physiological consequences and assess the interaction between environmental stressors and dietary antioxidants in aquaculture systems.

Declarations

Author Contributions

Mustafa OZ: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Software; Validation; Visualization; review & editing Writing- Original draft preparation.

Enes ÜSTÜNER: Taking tissue samples, taking histological sections, pathological evaluation, writing histopathology findings, Data curation , review & editing Writing- Original draft preparation

Suat DIKEL: Methodology; Project administration, Data curation

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The availability of data and material

The data supporting the findings of this study can be obtained from the corresponding author upon a reasonable request.

Competing interests

The authors explicitly affirm that they have no identifiable financial conflicts of interest or personal ties that could have potentially influenced the findings presented in this research.

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Figures

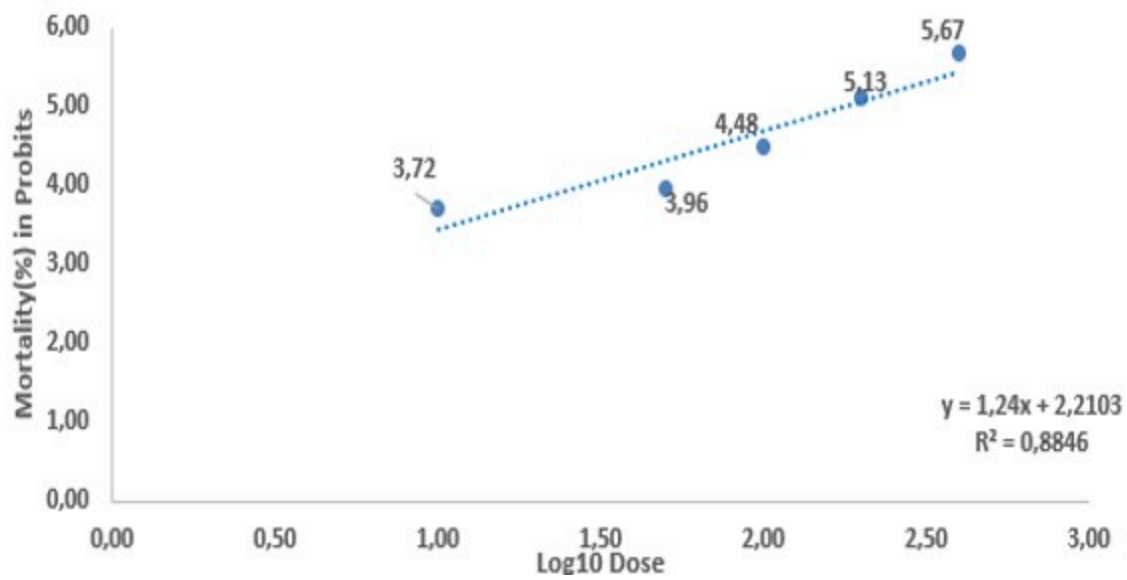


Figure 1

Probit regression curve used to determine the 96-hour LC_{50} of boric acid in Nile tilapia (*Oreochromis niloticus*).

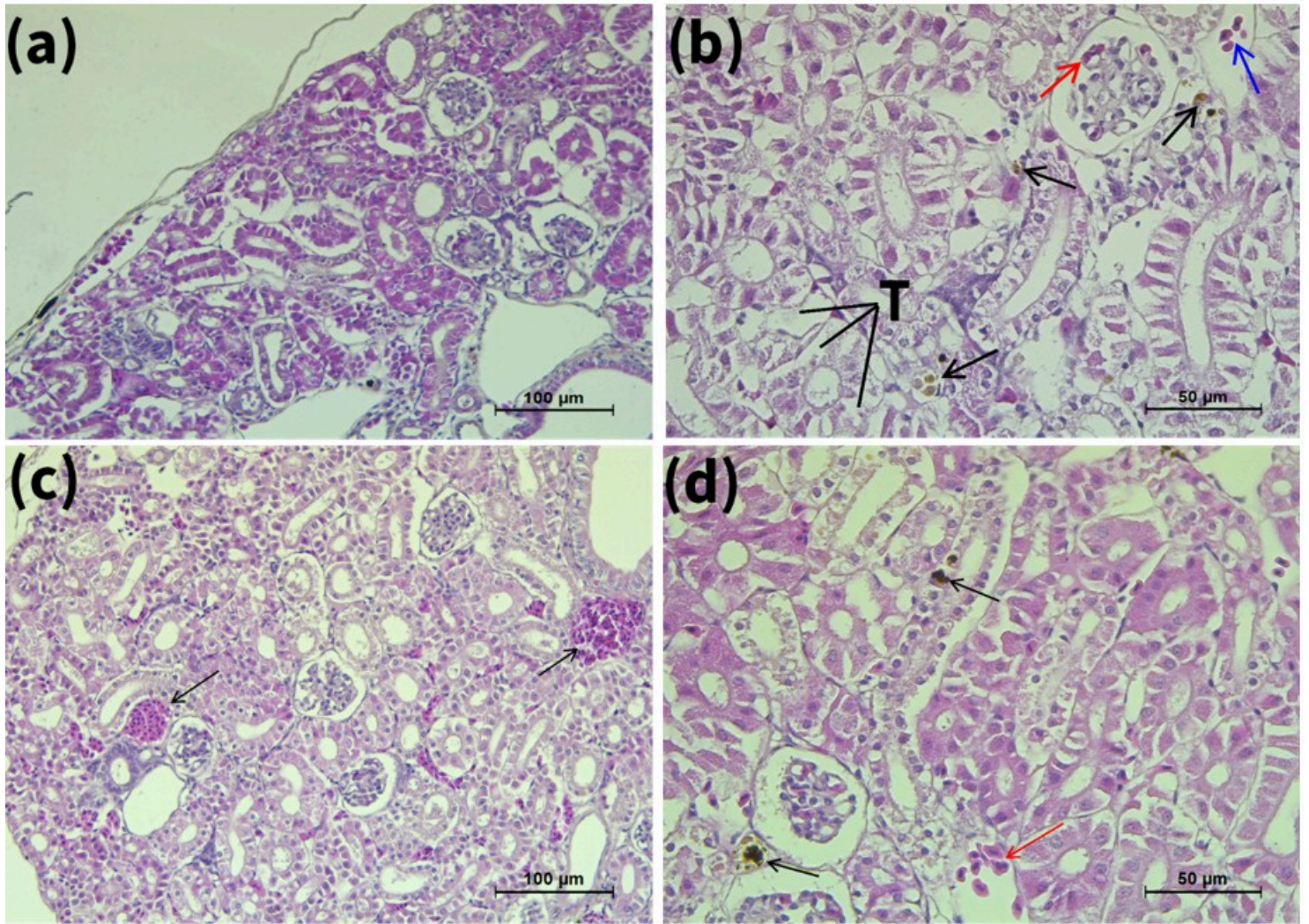


Figure 2

(A) Normal histological appearance of the kidney of Nile tilapia (*O. niloticus*); Group 1, hematoxylin and eosin stain (H&E). **(B)** Hyperactivated melanomacrophages (black arrow), vacuolization of the epithelial lining of proximal and distal tubules (T) (black lines), congestion in the glomerulus (red arrow), and interstitial hemorrhage (blue arrow) in the kidney; Group 3, hematoxylin and eosin stain (H&E). **(C)** Interstitial hemorrhage (black arrow) in the kidney; Group 2, hematoxylin and eosin stain (H&E). **(D)** Hyperactivated melanomacrophages (black arrow) and interstitial hemorrhage (red arrow) in the kidney; Group 4, hematoxylin and eosin stain (H&E).

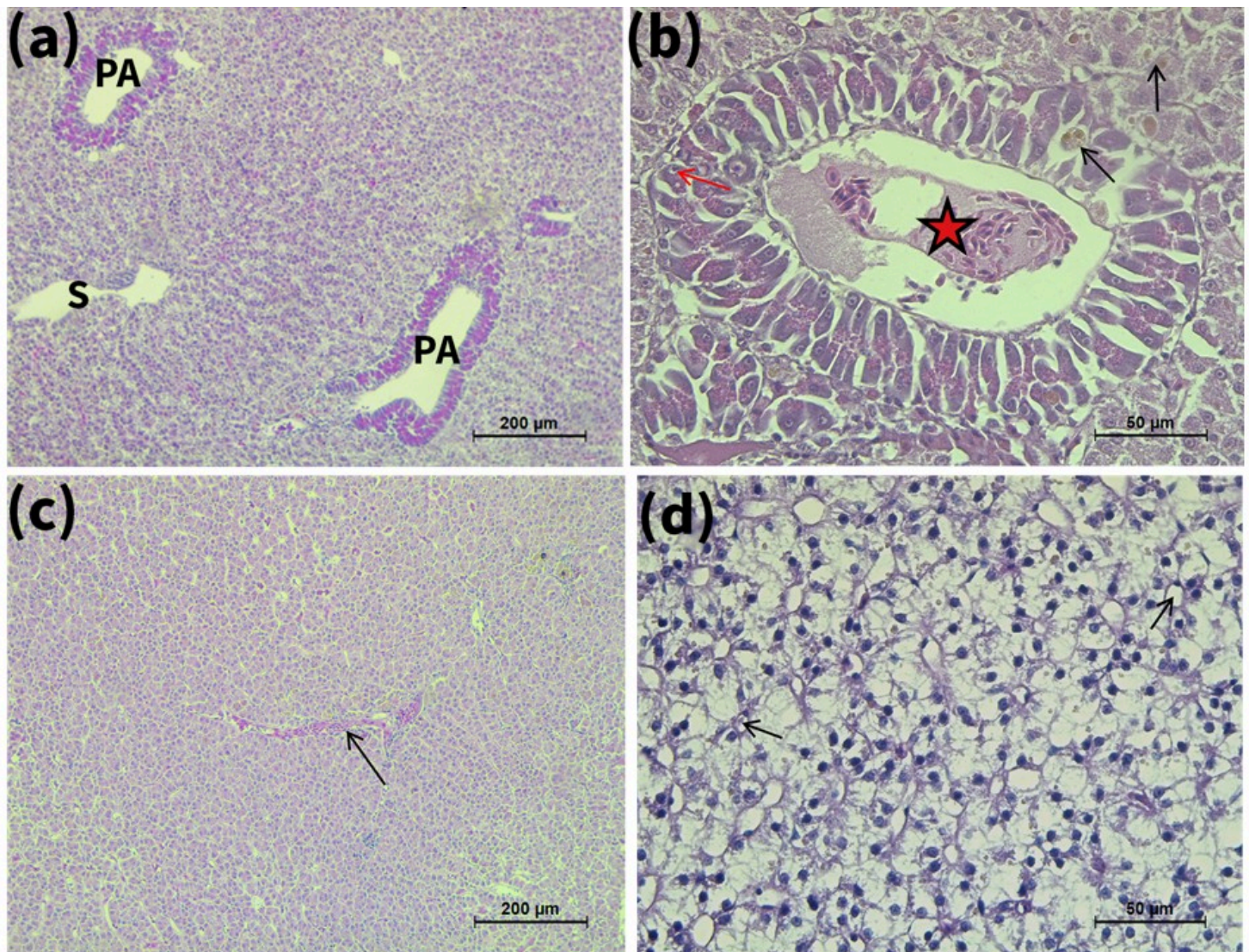


Figure 3

(A) Histological appearance of normal liver in Nile tilapia (*O. niloticus*), showing acini of the exocrine pancreas (PA) and liver sinusoids (S), Group 1, stained with hematoxylin and eosin (H&E). **(B)** Hyperactivated melanomacrophages observed in the liver (black arrow), vacuolization in pancreatic cells (red arrow), and edema and erythrocytes in the acinar cells of the exocrine pancreas (asterisk); Group 3, stained with hematoxylin and eosin (H&E). **(C)** Congestion in the liver (black arrow); Group 2, stained with hematoxylin and eosin (H&E). **(D)** Vacuolar degeneration and hydropic degeneration of hepatocytes in the liver (black arrow); Group 4, stained with hematoxylin and eosin (H&E).

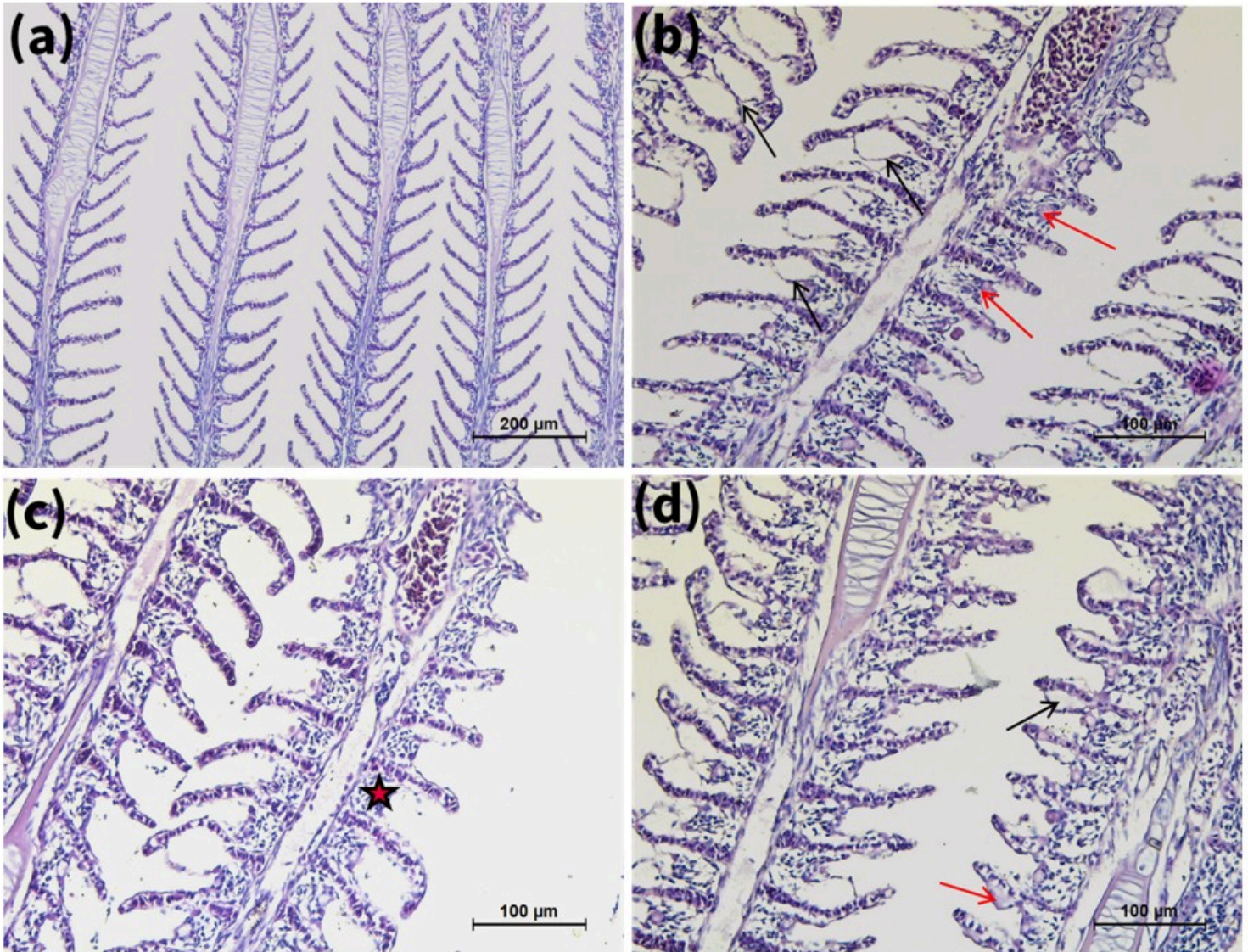


Figure 4

Gill tissue of Nile tilapia (*Oreochromis niloticus*). **(A)** Normal histological image of Nile tilapia (*O. niloticus*) gills; Group 1, hematoxylin-eosin staining (H&E). **(B)** Separation of epithelial cells of the secondary lamellae (black arrow), fusion of secondary lamellae (red arrow); Group 3, hematoxylin-eosin staining (H&E). **(C)** Fusion of secondary lamellae (star); Group 2, hematoxylin-eosin staining (H&E). **(D)** Separation of epithelial cells of the secondary lamellae (black arrow), edema in secondary lamellae (red arrow); Group 4, hematoxylin-eosin staining (H&E).

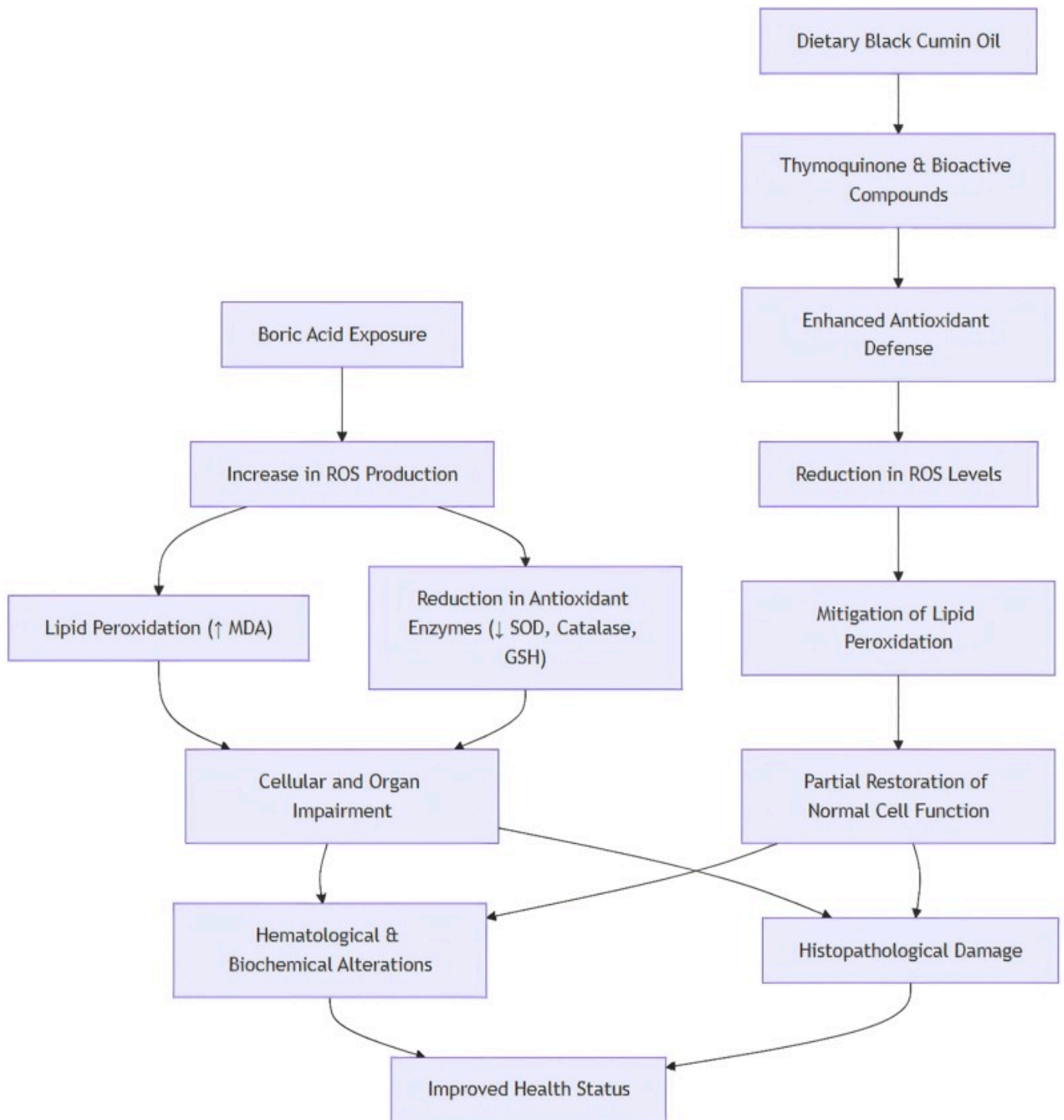


Figure 5

Schematic representation of the toxic mechanisms induced by waterborne boric acid exposure and the protective effects of dietary black cumin (*Nigella sativa L.*) oil in Nile tilapia (*Oreochromis niloticus*).