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Short-term boric acid treatment attenuates glyphosate-induced testicular toxicity by restoring redox and hormonal homeostasis

Merve Bolat [1,*], Aslıhan Atasever [1], Ömercan Alat [2], Betül Orhan [3], Furkan Aykurt [1], İsmail Bolat [3], Samet Tekin [1], Burak Çınar [4], Burak Batuhan Laçin [1], Fikret Çelebi [1]

[1] *Atatürk University, Faculty of Veterinary Medicine, Department of Physiology, Erzurum, 25000, Türkiye*
[2] *Atatürk University, Faculty of Veterinary Medicine, Department of Biochemistry, Erzurum, 25000, Türkiye*
[3] *Atatürk University, Faculty of Veterinary Medicine, Department of Pathology, Erzurum, 25000, Türkiye*
[4] *Atatürk University, Faculty of Medicine, Department of Medical Pharmacology, Erzurum, 25000, Türkiye*

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ABSTRACT

Glyphosate (GLY) is a widely used herbicide known to exert toxic effects on the male reproductive system. Boric acid (BA), possessing antioxidant and anti-inflammatory properties, has been investigated for its potential protective effects against oxidative damage in various biological systems. In this study, the shielding effect of boric acid in counteracting testicular impairment caused by glyphosate was assessed. Random allocation of male rats was performed to establish four experimental groups. Control, BA, GLY, and BA + GLY. After the treatment phase, measurements of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone were conducted to explore potential alterations in endocrine function. Testicular malondialdehyde (MDA) and glutathione (GSH) levels were examined by ELISA, while the gene expression of antioxidant enzymes-superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx)-was quantified using RT-PCR. Inflammatory cytokines including, tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and interleukin-10 (IL-10), were evaluated by Western blot, and Caspase-3 immunoreactivity was examined histopathologically. GLY exposure significantly increased MDA, TNF-α, and IL-1β levels while reducing IL-10, GSH, antioxidant enzyme expression, and FSH, LH, and testosterone concentrations. Co-administration of BA restored antioxidant balance, normalized hormonal levels, decreased TNF-α and IL-1β expression, elevated IL-10, and mitigated apoptosis. These results indicate that BA protects the testes from GLY-induced impairment by modulating oxidative stress, inflammation, endocrine disruption, and apoptotic cascades.

1. Introduction

In recent years, reproductive health problems-particularly male infertility-have become an increasing global concern rather than merely an individual health issue. One of the major factors contributing to male infertility is the decline in sperm quality, which has been closely linked to growing exposure to environmental chemicals associated with modern lifestyles [1]. Among these chemicals, pesticides are of particular concern due to their ability to disrupt hormonal regulation, alter gonadotropin secretion, and induce structural damage in testicular tissue [2]. Since hormones such as follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone play critical roles in spermatogenesis and the maintenance of normal testicular function; therefore, disturbances in their levels are considered key indicators of reproductive toxicity [3].

Glyphosate (GLY) is one of the most widely used herbicides worldwide and is extensively applied in agricultural practices. Consequently, humans and animals may be exposed to GLY through the food chain, environmental contamination, and occupational contact [4]. Although GLY is primarily designed to target plant metabolism, increasing evidence suggests that it can also exert toxic effects in mammalian tissues. These effects are largely attributed to oxidative stress, which arises from excessive reactive oxygen species (ROS) production and impaired antioxidant defense mechanisms [5]. In testicular tissue, GLY-induced oxidative stress has been associated with inflammation, mitochondrial dysfunction, and activation of apoptotic pathways. Such alterations may impair Leydig cell steroidogenesis, disrupt FSH- and LH-mediated signalling, promote germ cell apoptosis, and lead to degeneration of seminiferous tubules, ultimately compromising spermatogenesis and sperm quality [6]. Moreover, chronic exposure has increasingly been reported to pose long-term risks to male fertility [7].

*Corresponding author: mervebolat@atauni.edu.tr

Boric acid (BA) is a naturally occurring boron-containing compound that has recently gained attention for its protective effects in biological systems. BA is known for its antioxidant capacity, particularly its ability to reduce oxidative stress by scavenging free radicals and supporting endogenous antioxidant defences. In addition to its antioxidative properties, BA has been shown to exert anti-inflammatory effects by modulating cytokine production and limiting inflammatory responses in damaged tissues [8]. Notably, emerging studies suggest that BA may also play a role in maintaining endocrine balance by supporting steroidogenic processes and preserving physiological levels of testosterone, FSH, and LH. Experimental models of testicular injury-including varicocele, testicular torsion, and ischemia/reperfusion-have demonstrated that BA can attenuate oxidative damage, reduce apoptosis, and help preserve normal testicular architecture [9].

Oxidative stress, inflammation, hormonal imbalance, and apoptosis are critical mechanisms underlying the main pathological alterations in testicular tissue. The toxic effects of GLY through these mechanisms emphasize the need for interventions with antioxidant and anti-inflammatory agents. Investigating the potential protective effects of BA is therefore essential, not only for understanding the harmful impacts of environmental toxins but also for developing strategies to support male reproductive function [10].

This evaluates redox status, inflammatory response, apoptotic activity, and hormonal homeostasis. Levels of FSH, LH, and testosterone were assessed to examine endocrine alterations, while malondialdehyde (MDA) and glutathione (GSH) levels were measured to evaluate oxidative stress. The mRNA expression of antioxidant enzymes (superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx)) was analyzed using RT-PCR, inflammatory cytokines (tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and interleukin-10 (IL-10)) were assessed by Western blotting, and caspase-3 immunoreactivity was examined histologically to determine apoptotic changes. Through this integrated approach, the study aimed to clarify the molecular and hormonal mechanisms underlying GLY-induced testicular damage and to elucidate the potential protective role of BA.

2. Materials and Methods

2.1. Chemicals

A Roundup®-based herbicide (Bayer, USA) comprising 41% glyphosate isopropylamine salt served as the source of GLY for this study. BA was purchased from Tekkim Chemical Company (Türkiye) with a purity of ≥99%. BA was prepared by dissolving in distilled water before administration.

2.2. Animals

Forty adult male Sprague-Dawley rats (3 months old), each weighing between 220 and 250 g, were procured from the Atatürk University Experimental Research and Application Centre (ATADEM). The animals were housed under standard laboratory conditions with a controlled ambient temperature of 25°C, relative humidity of 60±10%, and a 12 h light/dark cycle. Throughout the experimental period, rats had ad libitum access to standard laboratory chow and drinking water. The study procedures were executed following the ethical approval obtained from the Atatürk University Local Ethics Committee (Approval ID: 2025/11; Decision No: 236).

2.3. Experimental Procedure

Prior to initiating the experimental procedures, each rat's weight was measured, and the animals were randomly divided into four distinct groups to ensure unbiased distribution. Each group consisted of n=10 animals. Dose selection was based on previous experimental studies employing similar oral dosing regimens in rodents, where the selected doses were shown to produce toxicological effects without acute lethality.

- **Group I (Control)**: Received physiological saline orally for 7 days.
- **Group II (BA)**: Received 100 mg/kg BA orally every other day for 7 days [11, 27, 28].
- **Group III (GLY)**: Received 150 mg/kg GLY orally every other day for 7 days [11].
- **Group IV (BA + GLY)**: Received 100 mg/kg BA orally every other day, followed 30 min later by 150 mg/kg GLY orally, for 7 days.

At 24 hours following the last treatment, animals were sacrificed in accordance with ethical guidelines. The collected testes were frozen (-80°C) and subsequently used in biochemical and molecular evaluations.

2.4. Evaluation of Biochemical Parameters

2.4.1. Testis tissue homogenization procedure

Testicular tissues were weighed and homogenized in 1.5 mL of ice-cold phosphate-buffered saline (PBS, pH 7.4) using a Magna Lyser homogenizer (Roche Diagnostics, Switzerland) at 5000 rpm for 5 min. Homogenization was performed under cold conditions to minimize protein degradation. The resulting homogenates were centrifuged at 16,000 rpm for 20 min at 4°C. Following centrifugation, the supernatant was carefully collected, transferred into Eppendorf tubes, and stored at -80°C until further biochemical analyses were performed [12].

2.4.2. Measurement of oxidative and hormonal parameters

Assessment of oxidative markers and hormonal parameters was conducted using a plate analyzer (450 nm) (BioTek System, USA). MDA (201-11-0157), GSH (201-11-5134), FSH (YLA0014RA), LH (YLA0015RA) and testosterone (YLA0013RA) levels were quantified in the previously collected supernatants using commercially available ELISA kits, following the protocols [12].

2.5. Molecular Investigation

2.5.1. RT- PCR analysis of antioxidant enzymes

Primers specific for SOD, CAT, and GPx genes were designed using the Primer3 software and further checked with Primer Express® (Table 1).

Table 1. List of primers employed in RT-PCR.

Gene	Sequences (5'-3')	Accession No
SOD	F: AATGTGGCTGCTGGAAAGGA R: GCTTCCAGCATTTCCAGTCT	NM_017050.1
CAT	F: CTGAGAGAGTGGTACATGCA R: AATCGGACGGCAATAGGAGT	NM_012520.2
GPx	F: CAAGGTGCTGCTCATTGAGA R: ATGTCCGAACTGATTGCACG	NM_030826.4
GAPDH	F: ACGGGAAACCCATCACCATC R: CTTCAGGTGAGCCCCAGC	NM_017008.4

To avoid genomic DNA amplification, each primer pair was positioned to span exon-exon junctions, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was employed as the internal control gene. Real-time PCR assays were carried out in duplicate on a LightCycler 480 instrument (Roche, Switzerland) following the thermal profile: an initial denaturation at 95°C for 10 min, succeeded by 45 cycles at 95°C for 15 s and 60°C for 1 min. Each reaction mixture (25 µL total volume) consisted of QuantiNova SYBR Green RT-PCR reagents (QIAGEN, Germany), template cDNA, and primers at final concentrations of 80-160 nM. The specificity of amplification was verified by melting curve analysis, while PCR amplicons were confirmed through 2.5% agarose gel electrophoresis and DNA sequencing. Relative gene expression was evaluated using the RT PCR Miner tool and quantified according to the $2^{-\Delta\Delta CT}$ method [13].

2.5.2. Western blotting of inflammatory markers

The quantification of total protein extracts was achieved via the BCA assay method (Thermo Fisher, USA). Proteins, loaded in equal concentrations, were resolved on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then electroblotted onto polyvinylidene fluoride (PVDF) membranes. After blocking, membranes were treated with primary antibodies against TNF-α, IL-10, and IL-1β and kept at 4°C overnight. After washing and rinsing, membranes underwent secondary antibody incubation (goat anti-mouse IgG) for 90 minutes at room temperature. Chemiluminescent signals were generated using the Trident Femto Western HRP substrate (Genetex, USA) and imaged via the Bio-Rad Gel Doc XR+ system (Bio-Rad Laboratories, USA). Band intensities were analyzed densitometrically using ImageLab, with at least three independent technical replicates per sample [14].

2.6. Histopathological Assesment

Following the experimental phase, testes were immersed in 10% neutral-buffered formalin and kept for 48 hours for fixation. After routine histological processing, the samples were dehydrated, enclosed in paraffin, and sectioned into 4-µm slices. The sections were then stained with hematoxylin and eosin (H&E) and observed under a light microscope (Leica Flexacam i5, Germany). Microscopic evaluation focused on detecting spermatocyte degeneration, necrosis, and interstitial edema. The degree of tissue damage was classified as absent (-), mild (+), moderate (++), or severe (+++). In addition, spermatogenic activity was quantified using the Johnson scoring method (Table 2) [13].

Table 2. Modified Johnsen scoring system for evaluation of spermatogenesis in testicular tissue.

Score	Histological Description
1	Seminiferous tubules devoid of germinal cells
2	Only Sertoli cells observed, no germ cell layer present
3	Presence of spermatogonia without further maturation
4	Few primary spermatocytes visible within tubules
5	Abundant spermatocytes but no spermatids detected
6	Early spermatids seen in small numbers
7	Numerous early spermatids with limited maturation
8	A small population of late spermatids identified
9	Many late spermatids present; incomplete spermatogenesis
10	Normal spermatogenic process and mature spermatozoa observed

2.7. Immunohistochemical Analysis

During immunohistochemical preparation, paraffinized sections were attached to poly-L-lysine-coated slides and subjected to deparaffinization and rehydration steps following routine protocols. Sections were incubated in 3% H_2O_2 for 10 minutes to suppress endogenous peroxidase activity. Antigen retrieval was carried out by heating the sections in 1% Tris-EDTA buffer (pH 9.0, 1:100) and subsequently allowing them to reach room temperature. To minimize nonspecific binding, a protein-blocking reagent was applied for 5 minutes before antibody incubation. The slides were then exposed to the primary antibody against Caspase-3 (Cat. No. sc-56053, dilution 1:100) according to the manufacturer's protocol. Following primary incubation, a secondary antibody was applied, and the chromogen 3-amino-9-ethylcarbazole (AEC) was used to visualize

the positive immunoreactivity. Stained slides were examined using a microscope (Leica Flexacam i5), and the intensity of immunoreactivity was quantitatively assessed with ImageJ software [15].

2.8. Statistical Analysis

Data derived from biochemical assays, RT-PCR, and Western blot analyses were statistically evaluated using GraphPad Prism. Differences between groups were analyzed by one-way analysis of variance (ANOVA, USA), followed by Tukey's multiple comparison test when applicable. Statistical significance was set at $p<0.05$. For histopathological and immunohistochemical assessments, data distribution was verified through the Shapiro-Wilk normality test. Non-parametric data from H&E-stained samples were examined using the Kruskal-Wallis test, and pairwise differences were determined by the Mann-Whitney U test. Parametric data obtained from immunohistochemical analyses were analyzed via one-way ANOVA coupled with Tukey's post-hoc test. A p-value below 0.05 was considered indicative of statistical significance.

3. Results

3.1. Assessment of ELISA Findings

The GLY group exhibited a significant rise in MDA levels within testicular homogenates as assessed by ELISA, relative to both Control and BA groups ($p<0.05$). BA supplementation effectively mitigated the elevation in MDA levels induced by GLY ($p<0.05$). MDA concentrations in the BA+GLY group were significantly lower than those in the GLY-exposed rats ($p<0.05$), returning to levels similar to the Control group ($p>0.05$). Conversely, GSH concentrations were significantly decreased in the GLY-treated group compared with both the Control and BA groups ($p<0.05$). However, co-administration of BA with GLY resulted in a notable elevation in GSH levels ($p<0.05$), reaching values comparable to those in the Control group ($p>0.05$). Statistical comparisons confirmed significant group variations overall ($p<0.05$). Detailed mean values and comparative results are illustrated in Figure 1.

ELISA-based hormonal evaluation of testicular tissues showed a reduction in FSH levels in rats exposed to GLY when in comparison with the Control and BA ($p<0.05$). Co-administration of BA led to a significant elevation in FSH values relative to the GLY ($p<0.05$), whereas the difference from the Control remained significant, but not from the BA ($p>0.05$). Similarly, LH concentrations exhibited a marked decline in the GLY-treated animals compared with the Control and BA ($p<0.05$). In the BA+GLY, LH levels increased in comparison with the GLY ($p<0.05$), showing no substantial variation relative to the Control and BA ($p>0.05$). Testosterone levels followed a comparable

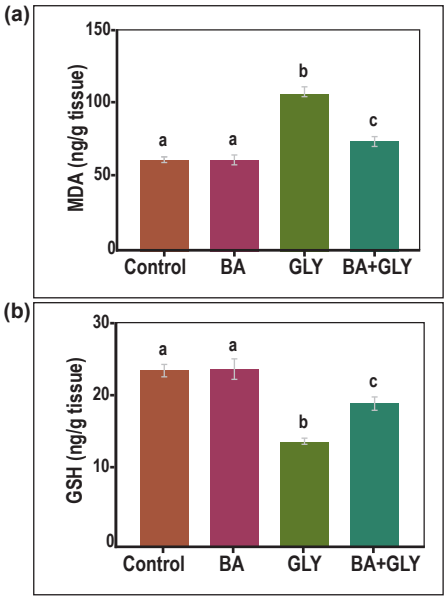

Figure 1. Measurement of MDA (a) and GSH (b) in testicular tissue among the study groups. Different superscript letters identify statistically distinct groups in comparison with the Control ($p<0.05$). Mean ± SEM values are shown, and statistical significance was determined through one-way ANOVA with Tukey's multiple comparison test.

pattern, being significantly lower in the GLY group in comparison with both Control and BA ($p<0.05$). Administration of BA markedly improved testosterone levels ($p<0.05$). In the BA+GLY, testosterone concentrations were higher than in the GLY ($p<0.05$) and differed statistically from the Control ($p<0.05$). Comparative analysis among the experimental groups demonstrated significant differences across treatments ($p<0.05$). The corresponding mean values and statistical data are illustrated in Figure 2.

3.2. Evaluation of RT-PCR Findings

RT- PCR analysis suggests that the mRNA levels of the antioxidant genes SOD, CAT, and GPx were markedly downregulated in the GLY-treated group in comparison with the Control and BA ($p<0.05$). Supplementation with BA enhanced the transcription of these antioxidant enzymes ($p<0.05$). In the BA + GLY, SOD, CAT, and GPx mRNA expressions were considerably higher than those observed in the GLY ($p<0.05$), although they did not fully return to the Control levels. Detailed quantitative results and statistical comparisons are illustrated in Figure 3.

3.3. Evaluation of Western Blot Findings

Western blot findings revealed a marked upregulation of TNF-α and IL-1β proteins in the GLY group relative to both the Control and BA groups ($p<0.05$). Administration of BA effectively reduced the overexpression of these pro-inflammatory cytokines ($p<0.05$). In rats receiving both BA and GLY, the expression levels of TNF-α and IL-1β were significantly lower than in the GLY-only group ($p<0.05$), though

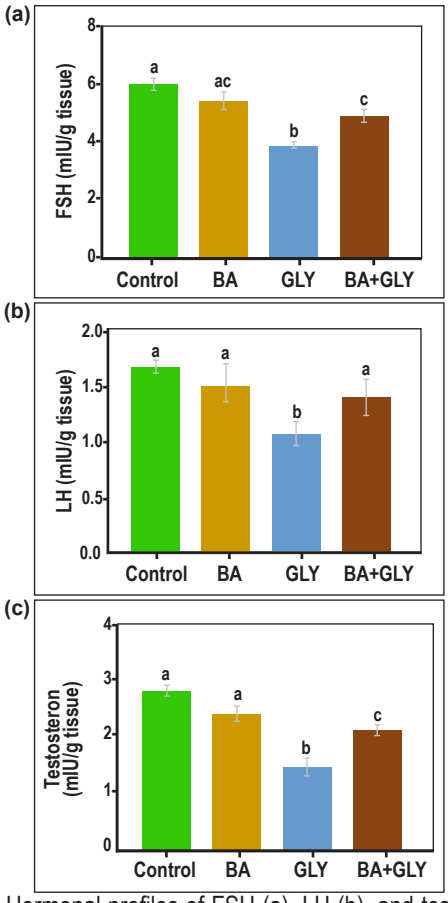

Figure 2. Hormonal profiles of FSH (a), LH (b), and testosterone (c) measured in testicular tissue. Groups denoted by distinct letters differ significantly from the control ($p \leq 0.05$). Results are shown as mean ± SEM, and data were analyzed through one-way ANOVA with Tukey's post hoc test.

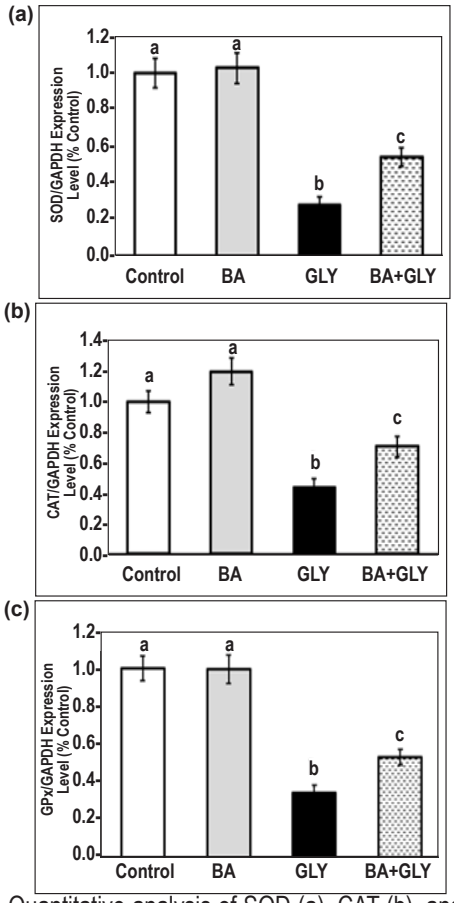

Figure 3. Quantitative analysis of SOD (a), CAT (b), and GPx (c) transcripts in testicular tissue by RT-PCR. Distinct letters above columns indicate significant group differences ($p<0.05$). Data represent mean ± SEM. Statistical significance was determined via one-way ANOVA followed by Tukey's test.

they did not fully return to baseline Control values. In contrast, the anti-inflammatory cytokine IL-10 exhibited a marked decline in the GLY, while BA administration partially restored its expression ($p<0.05$). Comparative quantitative data and detailed statistical findings are illustrated in Figure 4.

3.4. Evaluation of Histopathological and Immunohistochemical Findings

Microscopic evaluation of the testicular tissues showed normal structural integrity in both the Control and BA groups. In contrast, the GLY group displayed pronounced spermatocytic degeneration and necrosis, accompanied by evident intertubular edema. These pathological alterations were considerably alleviated in the BA + GLY group ($p<0.05$) (Figure 5). Immunohistochemical detection of Caspase-3 demonstrated minimal staining in the Control and BA groups, whereas strong positive immunoreactivity was evident in the GLY-treated rats. Co-treatment with BA markedly decreased Caspase-3 expression when in comparison with the GLY ($p<0.05$) (Figure 6). Quantitative assessments of histopathological grading, Caspase-3 immunoreactivity, and Johnsen's spermatogenesis scores, together with their statistical evaluations, are presented in Figure 7.

The present investigation demonstrated that GLY exposure causes significant oxidative stress, inflammation, apoptosis, and hormonal imbalance in rat testicular tissue, while BA co-treatment substantially counteracted these deleterious changes. These findings support existing literature describing GLY-induced alterations in spermatogenic activity, hormone secretion, and testicular structure [7,10,16]. Importantly, the current study extends these observations by showing that BA provides robust protection through the modulation of oxidative, inflammatory, and apoptotic pathways as well as by maintaining endocrine homeostasis.

Oxidative imbalance appears to be a pivotal factor in GLY-induced reproductive toxicity. The observed rise in MDA levels, coupled with a reduction in GSH and decreased expression of antioxidant enzymes, implies that GLY facilitates excessive ROS generation and compromises the intrinsic antioxidant system. These findings are consistent with previous reports demonstrating that chronic GLY exposure induces lipid peroxidation and impairs cellular redox homeostasis [6,16]. Testicular tissue is particularly vulnerable to oxidative damage due to its high content of polyunsaturated fatty acids and elevated oxygen

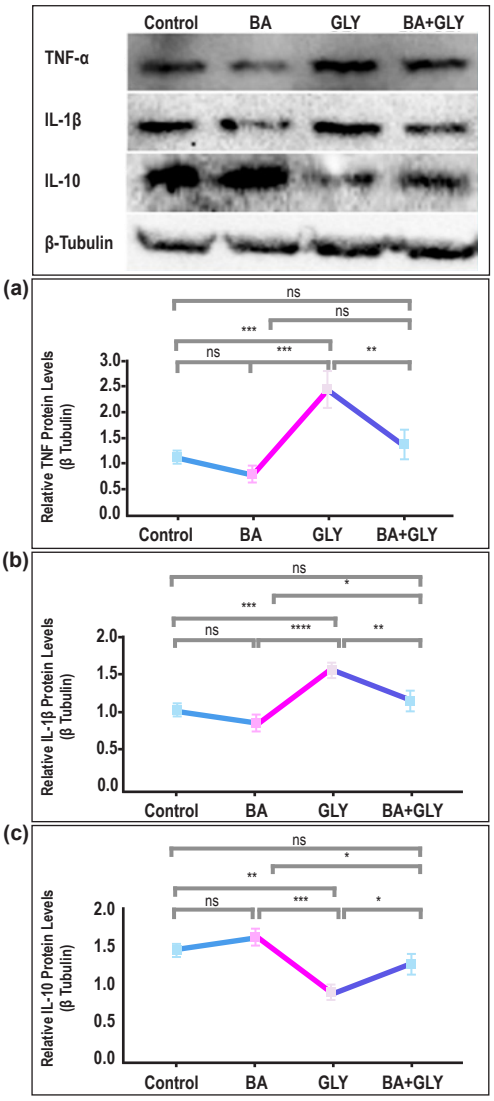

Figure 4. Western blot analysis illustrating the expression patterns of TNF-α (a), IL-1β (b), and IL-10 (c) in testicular tissue. Bars with different letters represent groups differing significantly ($p<0.05$). Values are expressed as mean ± SEM, and group comparisons were assessed by one-way ANOVA with Tukey's post hoc test.

consumption rate [10]. In contrast, BA supplementation markedly ameliorated oxidative stress, as evidenced by decreased MDA levels, restored GSH content, and the upregulation of SOD, CAT, and GPx mRNA expression. Although enzyme activities were not directly measured in the present study, the observed transcriptional changes suggest a potential enhancement of antioxidant defense mechanisms. Previous studies have demonstrated that BA stabilizes cellular membranes, supports glutathione metabolism, and strengthens enzymatic antioxidant defense [17-19]. Collectively, these findings indicate that BA may attenuate GLY-induced oxidative injury by suppressing ROS generation and positively regulating antioxidant-related gene expression in testicular tissue.

Inflammation represents another important mechanism contributing to GLY-induced toxicity. In the GLY group, the marked increase in TNF-α and IL-1β, along with a reduction in IL-10, indicates an intensified inflammatory

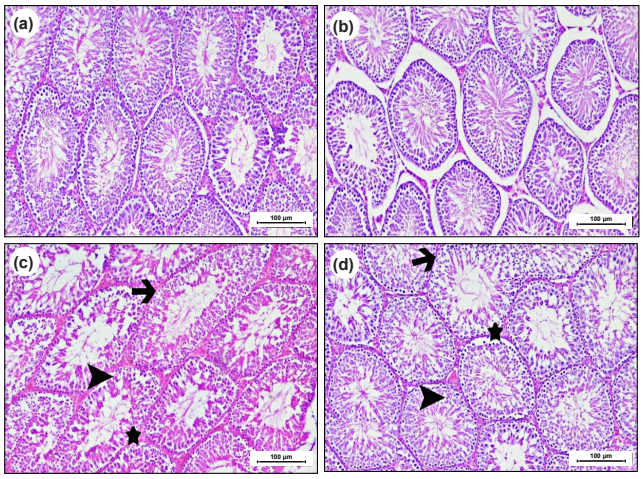

Figure 5. Representative H&E-stained images of testicular tissue. Images are shown for Control (a), BA (b), GLY (c), and BA + GLY (d) groups. Histological images display spermatocyte degeneration (arrows), necrotic changes (arrowheads), and intertubular edema (asterisks). Staining: H&E; Objective: 10X; Scale bar: 10 µm; Magnification: 100%.

response. This imbalance in cytokine levels may cause microvascular disruption, detachment of germ cells, and impairment of spermatogenesis [20,21,23]. BA administration effectively suppressed the elevation of TNF-α and IL-1β while restoring IL-10 expression, demonstrating its well-documented anti-inflammatory potential [11,22]. Previous research has reported that BA limits the release of inflammatory mediators and helps maintain structural integrity within the tissue. Therefore, the anti-inflammatory effects of BA, acting synergistically with its antioxidant capacity, play a crucial role in protecting testicular tissue from GLY-induced inflammation and degeneration.

Apoptosis, often triggered by oxidative and inflammatory stress, also plays a pivotal role in GLY-mediated testicular damage. Increased caspase-3 immunoreactivity observed in the GLY group reflects

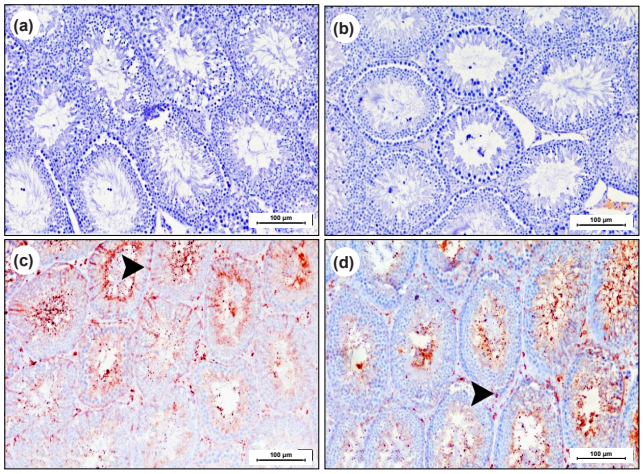

Figure 6. Representative immunohistochemical (IHC) images of testicular tissue. Images are shown for Control (a), BA (b), GLY (c), and BA + GLY (d) groups. Caspase-3 expression is demonstrated by arrowheads. Staining: IHC-P; Objective: 10X; Scale bar: 10 µm; Magnification: 100%.

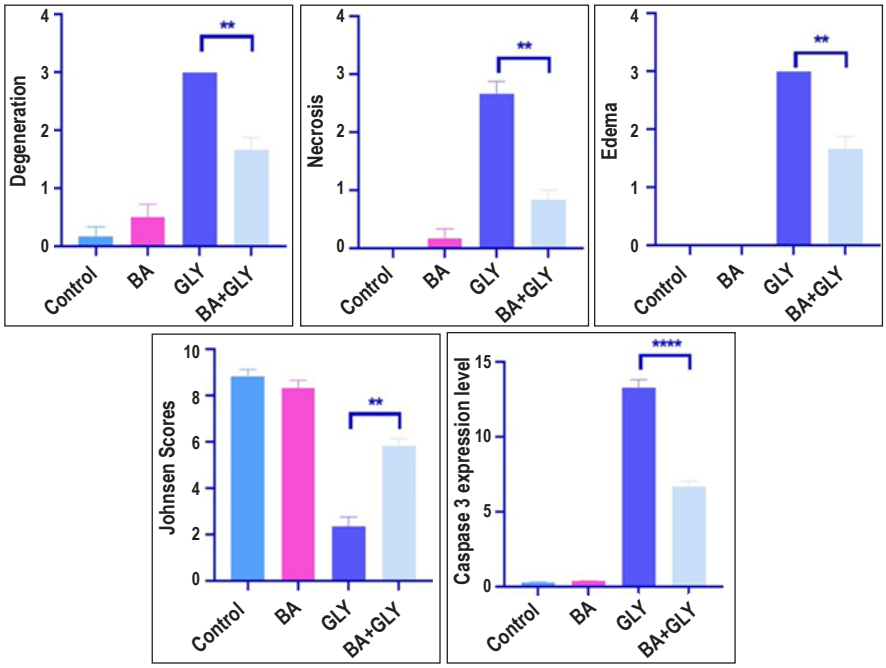

Figure 7. Histopathological scores, IHC expression levels, and Johnsen scores, along with their corresponding statistical analyses (**p<0.01, **p<0.0001), are presented. Kruskal-Wallis and Mann-Whitney U tests were used for the evaluation of histopathological and Johnsen scores. One-Way ANOVA followed by Tukey's post-hoc test was applied for the analysis of Caspase-3 expression. Data are presented as mean ± SEM.

enhanced apoptotic activity in germ cells, supporting prior findings that GLY induces mitochondrial dysfunction and cell death [6,10]. Conversely, BA markedly reduced caspase-3 expression, indicating suppression of apoptosis. This outcome aligns with earlier studies showing that BA preserves mitochondrial integrity, prevents cytochrome c release, and maintains intracellular calcium balance [25]. Through these mechanisms, BA preserves cellular viability and spermatogenic continuity.

Histopathological evaluation further confirmed biochemical and molecular results. GLY exposure resulted in severe spermatogenic disruption, degeneration of germinal epithelium, and interstitial edema, whereas BA co-treatment largely preserved the seminiferous tubular structure and minimized degenerative changes. Similar protective outcomes have been documented in models of testicular ischemia, torsion, and chemical injury, where BA maintained tissue organization and reduced necrotic cell loss [13,26].

In addition to oxidative, inflammatory, and structural alterations, GLY exposure resulted in a pronounced disruption of the hypothalamic-pituitary-gonadal (HPG) axis, as evidenced by significantly reduced serum levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone [29]. The suppression of gonadotropins suggests that GLY may interfere not only with testicular function but also with upstream hypothalamic and pituitary signalling. Oxidative stress-mediated neuroendocrine dysfunction has been proposed as a central mechanism underlying GLY-induced impairment of gonadotropin release, leading to secondary testicular failure [7,9]. Reduced LH levels are particularly critical, as LH directly regulates testosterone synthesis in Leydig cells through activation of key steroidogenic enzymes, including steroidogenic acute regulatory protein (StAR) and cytochrome P450 family members [30]. Consequently, diminished LH stimulation may result in impaired steroidogenesis and reduced intratesticular testosterone availability, which is essential for the maintenance of spermatogenesis [9,10]. Furthermore, decreased FSH levels may adversely affect Sertoli cell function, thereby compromising germ cell nourishment, blood-testis barrier integrity, and paracrine signaling required for normal spermatogenic progression [31]. Sertoli cell dysfunction, in combination with reduced testosterone support, may synergistically exacerbate germ cell apoptosis and spermatogenic arrest. Accumulating evidence indicates that oxidative and inflammatory insults impair both Leydig and Sertoli cell viability by disrupting mitochondrial function, altering calcium homeostasis, and downregulating steroidogenic and reproductive gene expression [7,10,16]. Notably, BA co-treatment effectively restored circulating levels of FSH, LH, and testosterone, indicating a protective role against GLY-induced endocrine disruption [8,25]. The normalization of gonadotropin levels suggests that BA may preserve HPG axis integrity, potentially through attenuation of oxidative stress and inflammatory signaling at both central and peripheral levels. By reducing ROS burden and inflammatory mediator release, BA may indirectly stabilize hypothalamic and pituitary function, thereby ensuring adequate gonadotropin secretion. In

addition, BA-mediated protection of Leydig cells likely contributes to the restoration of testosterone synthesis through preservation of mitochondrial integrity and steroidogenic enzyme activity [8,25]. Previous studies have also suggested that BA supports endocrine balance by modulating oxidative pathways and enhancing cellular resilience in reproductive tissues [9]. Collectively, these findings demonstrate that BA's protective effects extend beyond local antioxidant and anti-inflammatory actions to encompass systemic endocrine regulation. It is important to emphasize that although boric acid demonstrated significant protective effects in the present short-term experimental model, its long-term safety in reproductive systems remains controversial. Previous studies have reported that prolonged or high-dose exposure to boron compounds may exert reproductive and testicular toxicity, including impaired spermatogenesis and altered sperm parameters [32,33]. Therefore, the beneficial effects observed in this study should be interpreted within the context of acute or short-term administration. Long-term exposure to boric acid may produce different, or even adverse, reproductive outcomes [34]. Consequently, BA should not be considered a universally safe or long-term therapeutic agent without further comprehensive toxicological evaluation [35]. By preserving HPG axis signalling and maintaining hormonal homeostasis, BA provides a favorable hormonal milieu essential for spermatogenesis, testicular integrity, and male reproductive function.

Although the present study focused on early-stage alterations, longer exposure periods may be required to fully evaluate spermatogenesis and long-term reproductive outcomes.

We acknowledge that the lack of sperm analysis represents a limitation of the present study. However, the primary objective of this work was to investigate early biochemical, molecular, and hormonal alterations in testicular tissue following short-term glyphosate exposure. Future studies incorporating comprehensive sperm parameters will be important to further elucidate the functional reproductive outcomes.

In the present study, oxidative stress was evaluated using a combined methodological approach. While MDA and GSH levels were assessed using established biochemical assays to reflect lipid peroxidation and antioxidant capacity, the expression of antioxidant enzymes (SOD, CAT, and GPx) was evaluated at the transcriptional level. This approach provides complementary information regarding oxidative damage and antioxidant defense regulation. However, assessing all antioxidant-related parameters at the same molecular level may further enhance methodological consistency and should be considered in future studies.

4. Conclusion

In conclusion, glyphosate exposure induces multifactorial testicular toxicity involving oxidative stress, inflammatory activation, apoptotic cell death, and hormonal imbalance, leading to impaired spermatogenesis and structural degeneration. Boric acid exhibits comprehensive protective effects by enhancing antioxidant defense, suppressing proinflammatory signalling, preventing apoptosis, and preserving endocrine equilibrium. This integrated protective profile suggests that BA could serve as a promising therapeutic or preventive agent against pesticide-induced reproductive toxicity.

Nevertheless, the present study has limitations. Only a single dose of GLY and BA was tested, and the study did not examine downstream steroidogenic enzymes or fertility outcomes. Future investigations should include dose-response designs, molecular analyses of steroidogenic pathways, and reproductive performance assessments to better elucidate the mechanisms underlying BA-mediated protection. Overall, this research provides valuable evidence supporting BA's potential as a protective compound against environmental toxicants affecting male reproductive health.

Importantly, the protective effects of boric acid observed in this study are limited to short-term exposure conditions. Given the documented reproductive toxicity associated with prolonged boron intake, caution should be exercised when extrapolating these findings to long-term use. Further long-term and dose-dependent studies are required to clarify the safety profile of boric acid and to determine whether its protective properties persist or reverse under chronic exposure conditions.

5. Author Contribution

Merve Bolat: Methodology, data analysis and editing, visualization, and writing draft.

Aslıhan Atasever: Methodology, laboratory work, original draft writing.

Ömercan Alat: Methodology, laboratory work, data analysis and editing.

Betül Orhan: Methodology, laboratory work, data analysis and editing.

İsmail Bolat: Methodology, laboratory work, data analysis, writing analysis.

Samet Tekin: Methodology, laboratory work, data analysis, writing analysis

Burak Çınar: Methodology, laboratory work, graphic design.

Burak Batuhan Laçin: Methodology, laboratory work, graphic design.

Furkan Aykurt: Methodology, laboratory work, graphic design.

Fikret Çelebi: Methodology, data analysis and editing, visualization, and writing draft.

6. Conflict of Interest

The authors have no conflict of interest.

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