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Protective effects of boric acid against glyphosate-induced gastrointestinal toxicity in rats: insights from oxidative stress, inflammatory and histopathological markers

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ARTICLE INFO

Article History:
Received May 28, 2025
Accepted September 8, 2025
Available online September 31, 2025

Research Article

DOI: 10.30728/boron.1708055

Keywords:
Antioxidant
Boric acid
Gastrointestinal toxicity
Glyphosate
Inflammation

ABSTRACT

Glyphosate (GLY), a widely used herbicide, has been associated with gastrointestinal toxicity primarily via oxidative stress and inflammation. This study aimed to evaluate the protective role of boric acid (BA) against GLY-induced stomach and intestinal injury in rats. Adult male Sprague-Dawley rats were divided into control, GLY-treated, BA-treated, and GLY+BA co-treated groups. Both GLY (150 mg/kg/day) and BA (100 mg/kg/day) were administered orally for 7 consecutive days. Gastrointestinal tissues were assessed for oxidative stress markers including superoxide dismutase (SOD) and catalase (CAT), as well as gene expression via real-time polymerase chain reaction (PCR). Inflammatory markers tumor necrosis factor-alpha (TNF-α) and nuclear factor kappa B (NF-κB) were analyzed by Western blot. Histopathological evaluations were performed using hematoxylin-eosin (H&E) staining, and oxidative DNA damage was assessed by immunohistochemical detection of 8-hydroxy-2'-deoxyguanosine (8-OHdG). GLY exposure significantly increased TNF-α and NF-κB expression while downregulating SOD and CAT levels, leading to mucosal injury and marked histopathological alterations. In the GLY+BA group, histopathological evaluation revealed reduced mucosal damage and substantial preservation of glandular architecture. These results suggest that BA confers protection against GLY-induced gastrointestinal toxicity, likely through its antioxidant and anti-inflammatory properties.

1. Introduction

Glyphosate [N-(phosphonomethyl) glycine] is a widely used broad-spectrum herbicide extensively applied in agricultural and non-agricultural settings [1]. Despite its effectiveness in weed control, increasing evidence has raised concerns about its potential toxic effects on non-target organisms, including mammals [2]. Among various organs, the gastrointestinal (GI) tract appears particularly susceptible to glyphosate-induced toxicity due to its direct exposure following ingestion. Glyphosate has been shown to disrupt intestinal epithelial integrity, induce oxidative stress, and promote inflammatory responses, which may contribute to the pathogenesis of gastrointestinal disorders [3,4].

Glyphosate, one of the most widely used herbicides globally, has been the subject of intense scientific and regulatory debate due to its potential adverse effects on human and environmental health. While some regulatory agencies, such as the U.S. Environmental Protection Agency (EPA), have maintained that glyphosate is unlikely to be carcinogenic to humans under normal exposure conditions, others—including the International Agency for Research on Cancer (IARC) have classified it as "probably carcinogenic to humans" (Group 2A) [5]. In addition to concerns about its possible carcinogenicity, glyphosate has also been linked to oxidative stress, inflammation, and organ toxicity in various experimental models. These concerns have led to increasing restrictions or outright bans in several countries, particularly in the European Union [6]. The growing regulatory scrutiny and public concern underscore the need for further studies investigating potential protective agents that could mitigate glyphosate-induced toxicity.

Oxidative stress plays a critical role in glyphosate-induced cytotoxicity, characterized by excessive production of reactive oxygen species (ROS) and impaired antioxidant defense systems [7]. This imbalance can lead to lipid peroxidation, protein oxidation, and damage to cellular structures, including the mucosal barrier of the gastrointestinal tract. In addition to oxidative damage, glyphosate exposure is associated with dysregulation of inflammatory signaling pathways, notably through the activation of NF-κB and related cytokines, further aggravating mucosal injury and compromising gut homeostasis [8,9].

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Boric acid, a naturally occurring compound containing boron, has gained attention for its pharmacological properties, including antioxidant, anti-inflammatory, and cytoprotective effects [10]. Several studies have demonstrated the ability of boric acid to scavenge free radicals, modulate redox-sensitive signaling pathways, and preserve tissue integrity under various models of oxidative injury [11]. Given these properties, boric acid may offer a promising protective strategy against glyphosate-induced gastrointestinal toxicity.

The present study aims to investigate the potential protective effects of boric acid in a rat model of glyphosate-induced GI toxicity by evaluating oxidative stress biomarkers, histopathological alterations, and inflammatory mediators. This research may provide valuable insights into the therapeutic role of boric acid in mitigating environmentally induced gastrointestinal damage.

2. Materials and Methods

2.1. Chemicals

Glyphosate (N-(phosphonomethyl)glycine) was administered in the form of the commercial herbicide Roundup® (Bayer AG, Germany), which was obtained from a local agricultural supplier. The formulation contained 41% glyphosate isopropylamine salt as the active ingredient. It was freshly diluted with sterile distilled water prior to administration and delivered orally via gavage at a dose of 150 mg/kg body weight for 7 consecutive days. Boric acid (H_3BO_3), with a purity of ≥99.5%, was purchased and used without further purification (Sigma-Aldrich, USA). The compound was dissolved in sterile distilled water and administered orally by gavage at a dose of 100 mg/kg body weight, following the same 7-day treatment protocol.

2.2. Animals and Experimental Design

A total of 24 male Sprague-Dawley rats, aged 9-10 weeks and weighing between 220-250 g, will be used in the experiment. The animals will be housed in standard cages under controlled environmental conditions (temperature: 24-25°C; 12-hour light/dark cycle). They will have ad libitum access to standard rat chow and drinking water. Prior to the initiation of the experimental procedures, the rats will be acclimatized to the laboratory environment for a period of 7 days. All experimental procedures involving animals have been approved by the Atatürk University Local Ethics Committee for Animal Experiments (Approval Number: 2025/06/118). The study was conducted at the Atatürk University Medical Experimental Research Center. Glyphosate and boric acid solutions were prepared by dissolving the compounds in distilled water immediately before administration.

The rats will be randomly divided into four groups, with six animals in each group (n=6):

Control (C): Rats received physiological saline orally once daily for 7 consecutive days.

Boric Acid (BA): Rats received boric acid orally at a dose of 100 mg/kg body weight once daily for 7 consecutive days [12].

Glyphosate (GLY): Rats received glyphosate orally at a dose of 150 mg/kg body weight once daily for 7 consecutive days [13].

Glyphosate + Boric Acid (GLY+BA): Rats first received glyphosate (150 mg/kg) orally, followed by boric acid (100 mg/kg) orally once daily for 7 consecutive days.

2.3. Real-Time PCR Analysis

Primers for SOD and CAT genes were designed using the Primer-BLAST tool from NCBI (Table 1). Tissue samples (50 mg) were homogenized in 1 mL QIAzol reagent (QIAGEN, Germany). Chloroform (200 µL) was added, and samples were centrifuged at 12,000×g for 20 min at 4°C. The aqueous phase was mixed with 500 µL isopropanol and centrifuged at 12,000×g for 10 min. The pellet was washed with 75% ethanol and centrifuged at 7,500×g for 5 min. RNA was dissolved in RNase-free-treated water and quantified by measuring absorbance at 260/280 nm. RNA samples were treated with DNase I (Thermo Scientific, USA) to eliminate genomic DNA contamination. cDNA synthesis was performed using the Omniscript Reverse Transcription Kit (Cat No: 205111, QIAGEN, Germany) following the manufacturer's protocol. cDNA quality and concentration were measured at 260/280 nm and standardized before −20°C storage. GAPDH was used as the reference gene. The reaction mix included SYBR Green PCR Dye Master Mix (Cat No: 208052, QIAGEN, Germany), gene-specific primers, cDNA templates, and nuclease-free water. Reactions were run in triplicate. Gene expression was quantified using the $2^{\Delta\Delta Ct}$ method [14].

Table 1. Primer sequences.

Gene	Sequences (5'-3')	Accession No
SOD	F: AATGTGGCTGCTGGAAAGGA	NM_017050.1
	R: GCTTCCAGCATTTCCAGTCT	
CAT	F: CTGAGAGAGTGGTACATGCA	NM_012520.2
	R: AATCGGACGGCAATAGGAGT	
GAPDH	F: GAGTATGTCGTGGAGTCTAC	NM_017008.4
	R: CAGGATGGATTGCTGACAAT	

2.4. Western Blotting

Western blotting was conducted as previously described with minor modifications (Dogan et al., 2024). Total proteins were extracted from gastric and intestinal tissues using radioimmunoprecipitation (RIPA) buffer (Cat. No: sc-24948, Santa Cruz, USA) supplemented with phenylmethylsulfonyl fluoride (PMSF) and a protease inhibitor cocktail.

Protein concentrations were determined using the Pierce™ BCA Protein Assay Kit (Cat. No: 23225, Thermo Scientific, USA). Equal amounts of protein (30 µg) were mixed with Laemmli sample buffer and separated on 10% SDS-PAGE gels. Proteins were transferred onto polyvinylidene difluoride (PVDF) membranes and blocked in tris-buffered saline with 0.1% Tween® 20 detergent (TBST) containing 5% bovine serum albumin (BSA) (Sigma-Aldrich, USA) for 1.5 hours at room temperature. Membranes were incubated overnight at 4°C with mouse monoclonal primary antibodies against β-tubulin, NF-κB (Cat. No: sc-8008, Santa Cruz, USA) and TNF-α (Cat. No: sc-130349, Santa Cruz, USA). After washing, membranes were incubated with HRP-conjugated goat anti-mouse secondary antibody (1:2000, Cat. No: sc-2005, Santa Cruz, USA) for 1.5 hours. Bands were visualized using Trident femto Western HRP Substrate (Cat. No: GTX14698, GeneTex, USA), and images were captured with the Bio-Rad GelDoc XR system. Band intensities were quantified using Image Lab 6.1 software and normalized to β-tubulin (Cat. No: sc-5274, Santa Cruz, USA). Relative protein expression was calculated as fold change compared to controls [15].

2.5. Histopathological Examination

At the end of the experimental period, stomach and intestinal tissue samples were fixed in 10% neutral buffered formalin. Following routine tissue processing, the samples were embedded in paraffin. Sections of 4 µm thickness were obtained from paraffin blocks and stained with hematoxylin and eosin (H&E) for light microscopic examination (Leica, Flexacam i5, Germany). In the stomach sections, the presence of desquamation in surface epithelial cells, degeneration and necrosis in parietal cells were evaluated. For the intestinal sections, desquamation of surface epithelial cells, degeneration and necrosis of epithelial cells, and findings indicative of enteritis were assessed. The severity of histopathological findings was scored semiquantitatively as absent (-), mild (+), moderate (++), or severe (+++) [16].

2.6. Immunohistochemical Analysis

For immunohistochemical analysis, after routine tissue processing, sections were placed on adhesive slides (poly-L-lysine-coated), then deparaffinized and rehydrated. Endogenous peroxidase activity was blocked by incubating the sections in 3% hydrogen peroxide (H_2O_2) for 10 minutes. Antigen retrieval was performed by boiling the sections in 1% TRIS-EDTA buffer (pH 6.1, 100X), followed by cooling at room temperature. To prevent nonspecific background staining, sections were incubated with a protein block for 5 minutes. Subsequently, primary antibody against 8-hydroxy-2′-deoxyguanosine (8-OHdG, Cat. No: sc-66036, dilution 1:100, Santa Cruz, USA) was applied and incubated according to the manufacturer's instructions. After secondary antibody incubation, 3-amino-9-ethylcarbazole (AEC) was used as the chromogen. The stained sections were examined under a light microscope (Leica, Flexacam i5, Germany). The intensity of immunopositivity was quantified using the ImageJ analysis software [17].

8-OHdG is one of the most widely recognized and sensitive indicators of oxidative DNA damage, particularly relevant in studies investigating toxicant-induced oxidative stress in gastrointestinal tissues. Its accumulation reflects ROS-mediated nuclear and mitochondrial DNA damage, making it a reliable endpoint for evaluating the genotoxic potential of glyphosate exposure.

2.8. Statistical Analysis

Statistical analyses were performed using SPSS (Version 28.0.1.1., IBM, USA) Data were tested for normality using the Shapiro-Wilk test and for homogeneity of variance using Levene's test. For group comparisons, one-way analysis of variance (ANOVA) was applied. If the variance was homogeneous, Tukey's post hoc test was used for pairwise comparisons. Results were expressed as the mean ± standard deviation of the mean (SEM) and $p < 0.05$ was considered statistically significant.

3. Results

3.1. Antioxidant Status of the GI Following GLY and BA Treatment

Real-time PCR analysis revealed that GLY administration significantly downregulated the mRNA expression levels of SOD and CAT in both gastric and intestinal tissues compared to the control group ($p<0.001$). In contrast, co-treatment with boric acid (GLY+BA group) markedly restored the expression of both antioxidant enzymes toward normal levels in both tissues. No significant differences in SOD and CAT expression were observed between the control and BA-only groups. These results indicate that boric acid mitigates GLY-induced oxidative stress by enhancing antioxidant gene expression in gastrointestinal tissues (Figure 1).

3.2. Effects of GLY and BA on NF-κB and TNF-α in the GI Tract

In gastric tissue, Western blot analysis demonstrated that GLY exposure significantly increased the expression levels of NF-κB and TNF-α compared to the control group ($p<0.001$), indicating the activation of inflammatory pathways. However, co-administration of boric acid (GLY+BA group) effectively reduced the elevated protein levels of both markers, restoring them toward baseline values ($p<0.001$). No statistically significant difference was observed between the control and BA-only groups (Figure 2).

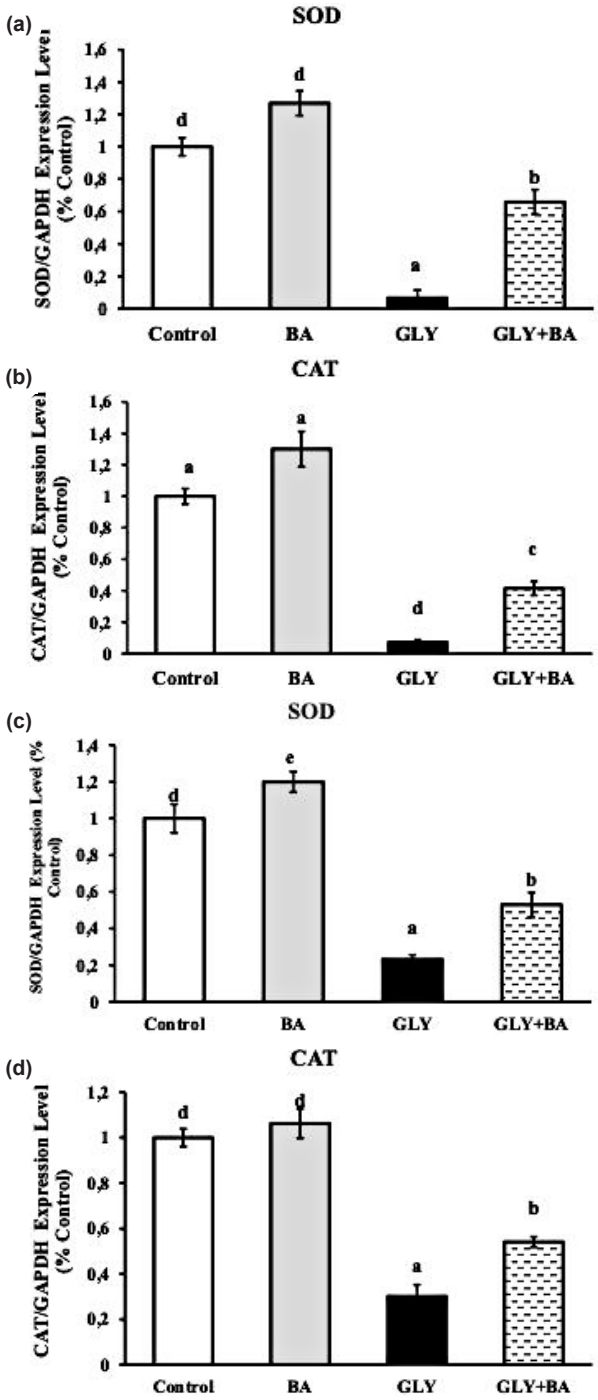

Figure 1. Relative mRNA expression levels of antioxidant enzymes in gastric and intestinal tissues as determined by real-time PCR. (a) Gastric SOD expression, (b) gastric CAT expression, (c) intestinal SOD expression, (d) intestinal CAT expression. a,b,c,d means with different letters in the same row ($p<0.001$; $p<0.01$; $p<0.05$ NS: Non-significant). Data are presented as fold changes relative to the Control group and normalized to GAPDH. Values are expressed as mean ± standard deviation (n = 6).

Similarly, in intestinal tissue, NF-κB and TNF-α protein levels were markedly upregulated in the GLY group compared to the control group ($p<0.001$), confirming the presence of intestinal inflammation. Treatment with boric acid in the GLY+BA group significantly suppressed this ($p<0.001$). Again, the BA-only group

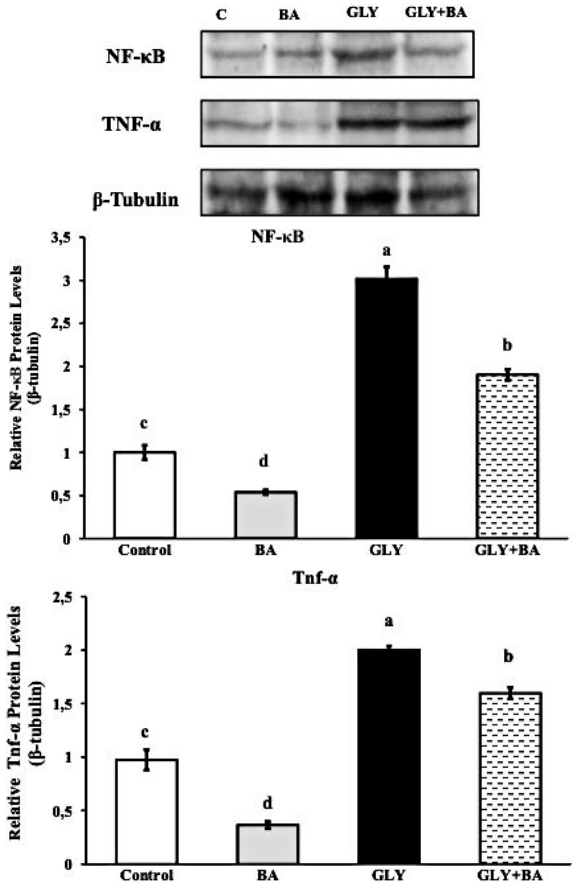

Figure 2. Protein expression levels of NF-κB and TNF-α in gastric tissue as determined by Western blot analysis. Representative immunoblot images and corresponding densitometric quantification are shown for each group: Control, BA, GLY, and GLY+BA. GLY exposure significantly increased NF-κB and TNF-α levels compared to control, while co-treatment with boric acid attenuated these elevations. Data are expressed as mean ± standard deviation. [a,b,c,d] means with different letters in the same row ($p<0.001$; $p<0.01$; $p<0.05$ NS: Non-significant). (n = 6).

showed no significant deviation from the control, indicating that BA alone does not alter inflammatory status under normal conditions (Figure3).

3.3. Tissue Morphological Changes in the Stomach and Intestines Following GLY and BA Treatment

Histopathological examination of gastric tissue revealed no apparent morphological alterations in the control and BA groups. In contrast, the GLY-treated group exhibited severe desquamation of the surface epithelial cells, along with marked degeneration and necrosis in parietal cells. However, co-administration of BA significantly attenuated these histopathological changes compared to the GLY group ($p<0.05$) (Figure 4).

Histopathological examination of intestinal tissues showed normal histoarchitecture in both the Control and BA groups. In the GLY-treated group, severe desquamation, degeneration, and necrosis of intestinal epithelial cells were observed, along with pronounced

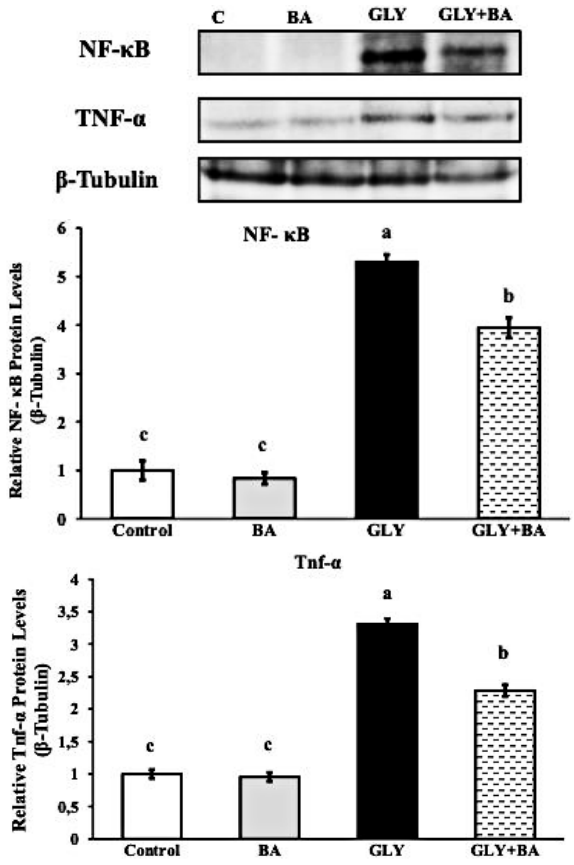

Figure 3. Protein expression levels of NF-κB and TNF-α in intestinal tissue as determined by Western blot analysis. Representative immunoblot images and corresponding densitometric quantification are shown for each group: Control, BA, GLY, and GLY+BA. GLY exposure significantly increased NF-κB and TNF-α levels compared to Control, while co-treatment with boric acid attenuated these elevations. Data are expressed as mean ± standard deviation. ^{a,b,c,d} means with different letters in the same row ($p<0.001$; $p<0.01$; $p<0.05$ NS: Non-significant). (n = 6).

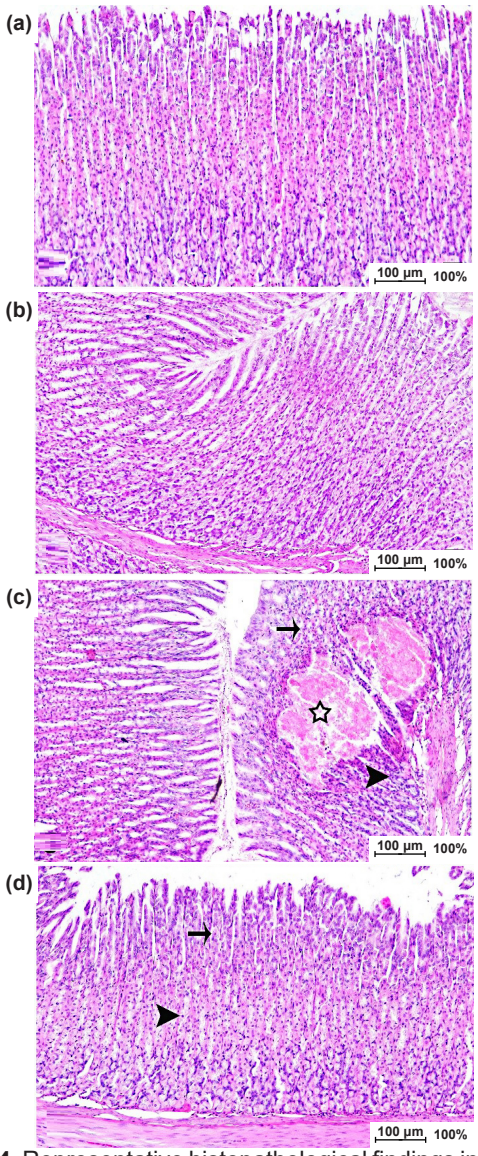

Figure 4. Representative histopathological findings in gastric tissue. (a) control, (b) BA, (c) GLY and (d) GLY+BA groups. Degeneration in parietal cells is indicated by arrows, while necrotic areas are marked with arrowheads. Necrotic regions within the lamina propria are denoted by asterisks. H&E staining, objective: 10X, scale bar: 100 µm, zoom: 100%.

enteritis. However, these pathological alterations were significantly attenuated in the GLY+BA group ($p < 0.05$) (Figure 5). Histopathological scoring of gastric and intestinal tissues, along with corresponding statistical analyses, is presented in Figure 6.

3.4. 8-OHdG Immunoreactivity as a Marker of Oxidative DNA Damage in Gastrointestinal Tissues

Immunohistochemical analysis of gastric (Figure 7) and intestinal (Figure 8) tissues showed negative 8-OHdG expression in the Control and BA groups. In contrast, strong 8-OHdG immunoreactivity was observed in the GLY group. However, co-treatment with BA significantly reduced 8-OHdG expression levels compared to the GLY group. Quantitative 8-OHdG expression data obtained using the ImageJ analysis software, along with corresponding statistical analysis, are presented in Figure 9.

4. Discussion

The current study demonstrated that GLY exposure leads to significant gastrointestinal damage in rats, primarily mediated by oxidative stress and inflammatory responses. Elevated levels of TNF-α and NF-κB, along with downregulation of key antioxidant enzymes such as SOD and CAT point to a disruption in redox balance and the activation of pro-inflammatory pathways. These molecular disturbances were consistent with histopathological alterations observed in both the stomach and intestinal tissues, further confirming GLY's deleterious effects on the gastrointestinal mucosa.

The observed decrease in SOD and CAT gene expression following glyphosate exposure in this study is in agreement with previous findings that implicate

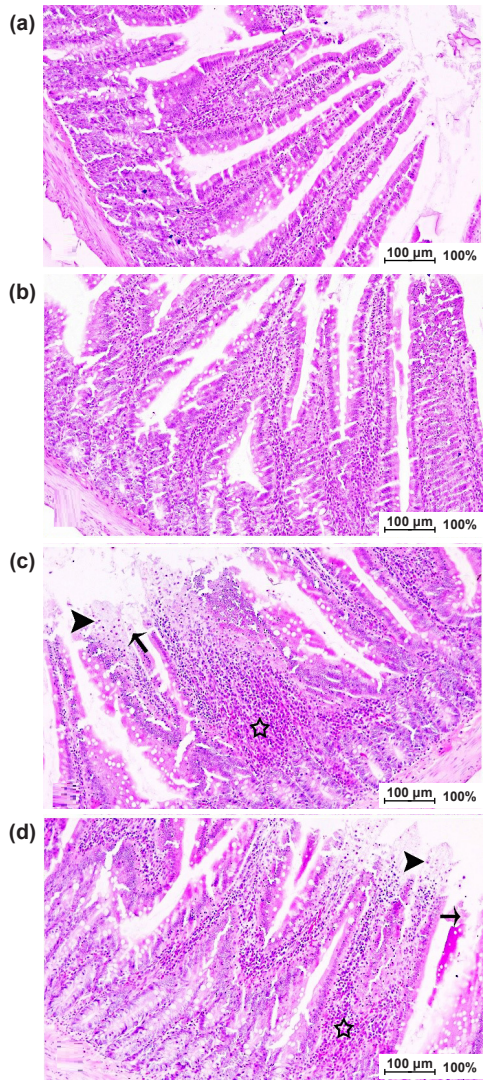

Figure 5. Representative histopathological findings in intestinal tissue. (a) control, (b) BA, (c) GLY and (d) GLY+BA groups. Degeneration in villous epithelial cells is indicated by arrows, necrotic areas by arrowheads, and enteritis is denoted by asterisks. H&E staining, objective: 10X, scale bar: 100 µm, zoom: 100%.

glyphosate in the suppression of endogenous antioxidant systems. Several studies have reported that glyphosate increases the generation of ROS, leading to oxidative stress by overwhelming cellular antioxidant defenses. For instance, Benedetti et al. (2004) demonstrated that glyphosate exposure significantly reduced SOD and CAT activity in liver and kidney tissues of rats, highlighting its systemic oxidative impact [18]. Similarly, Astiz et al. (2009) noted that chronic exposure to environmental toxicants, including herbicides, alters antioxidant enzyme expression, rendering tissues more susceptible to oxidative injury [19]. In our study, the downregulation of these key enzymes in gastrointestinal tissues suggests that the mucosa is particularly vulnerable to glyphosate-induced redox imbalance. Conversely, co-treatment with boric acid reversed these effects, as evidenced by restored SOD and CAT levels. This finding is supported by previous reports on the antioxidant potential of boron compounds.

The upregulation of TNF-α and NF-κB expression observed in the gastrointestinal tissues of glyphosate-treated rats aligns with previous studies that identify these markers as central mediators in herbicide-induced inflammation. Glyphosate has been reported to activate NF-κB signaling, leading to enhanced transcription of pro-inflammatory cytokines such as TNF-α, which plays a pivotal role in initiating and sustaining mucosal inflammation. For example, Mesnage et al. (2017) demonstrated that glyphosate exposure in rodents triggered a significant inflammatory response marked by increased NF-κB activation in gut-associated lymphoid tissue [20]. Similarly, de Winstone et al. (2022) reported elevated TNF-α levels following glyphosate treatment [21].

In the present study, boric acid co-treatment effectively suppressed the overexpression of both TNF-α and NF-κB in gastric and intestinal tissues. This finding supports earlier evidence of boron's anti-inflammatory potential. Research by Scorei and Rotaru (2011) indicated that boric acid downregulates pro-inflammatory cytokine production by modulating intracellular signaling pathways such as MAPK and NF-κB [22]. The current data suggest that boric acid's protective effect may stem from its ability to interfere with glyphosate-induced inflammatory cascades, thereby mitigating mucosal injury.

In addition to its well-established antioxidant and anti-inflammatory effects, boric acid may exert its protective role through other cellular mechanisms [23,24]. Recent studies have suggested that boron compounds can influence mitochondrial function by stabilizing mitochondrial membrane potential and reducing the release of pro-apoptotic factors such as cytochrome c. Moreover, boron has been shown to modulate apoptotic signaling pathways by regulating caspase activity and the expression of Bcl-2 family proteins, which could further contribute to its tissue-protective effects. Although our study did not directly assess apoptosis or mitochondrial integrity, these mechanisms may have played a role in the observed mucosal protection and should be investigated in future studies [10].

The immunohistochemical analysis of 8-hydroxy-2'-deoxyguanosine (8-OHdG), a well-established marker of oxidative DNA damage, showed strong nuclear immunopositivity in both gastric and intestinal epithelial cells following glyphosate exposure. This finding underscores the extent of oxidative stress imposed by glyphosate at the genomic level, consistent with previous reports linking herbicide toxicity to increased 8-OHdG accumulation in vulnerable tissues [25]. In contrast, rats co-treated with boric acid demonstrated significantly reduced 8-OHdG expression, suggesting that BA exerts a protective effect by minimizing DNA oxidative lesions. The reduction in immunopositivity may be attributed to enhanced antioxidant defense

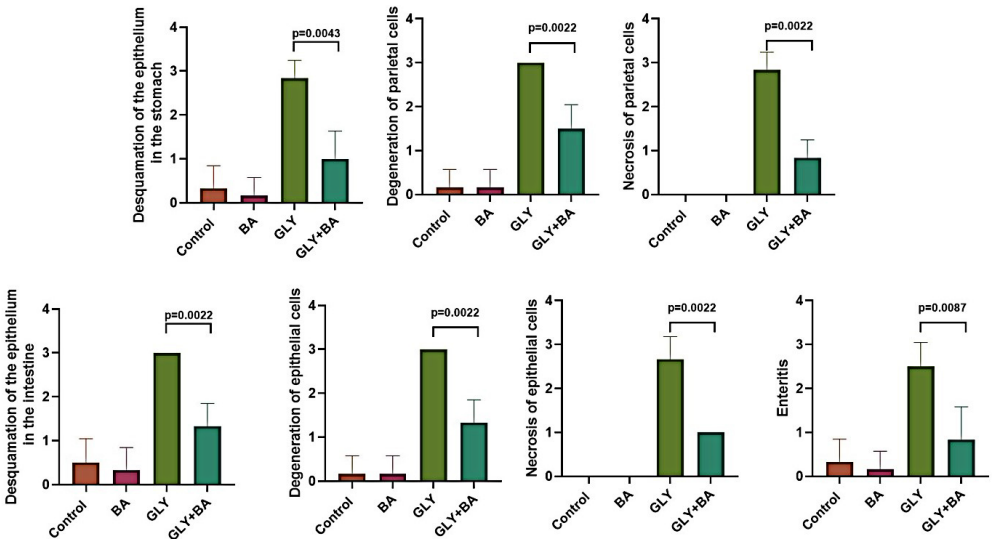

Figure 6. Histopathological scoring of gastric and intestinal tissues and corresponding statistical analysis. Data were analyzed using the non-parametric Mann-Whitney U test.

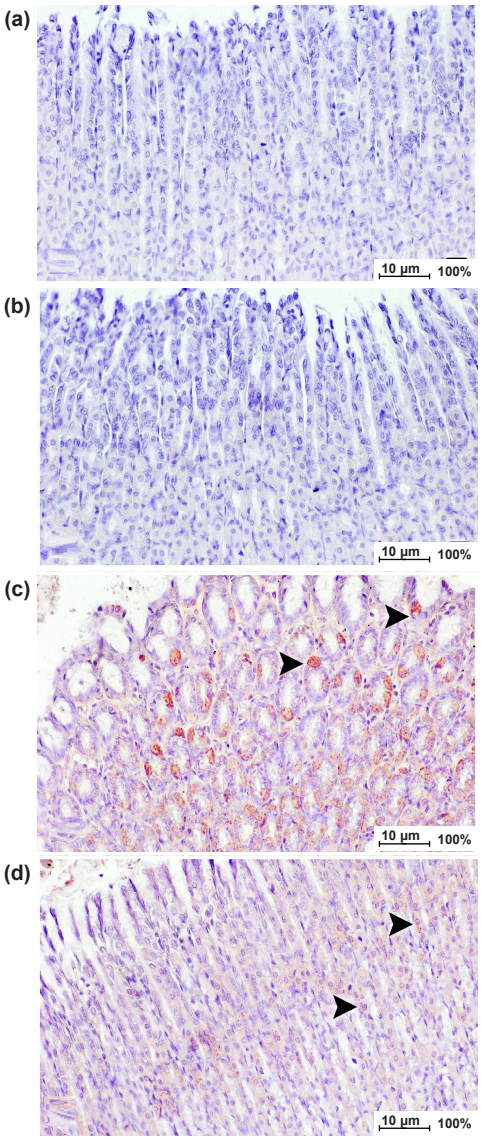

Figure 7. Immunohistochemical detection of 8-OHdG expression in gastric tissue. (a) control, (b) BA, (c) GLY, and (d) GLY+BA groups. 8-OHdG-positive staining in parietal cells is indicated by arrowheads. IHC staining, objective: 20X, scale bar: 10 µm, zoom: 100%.

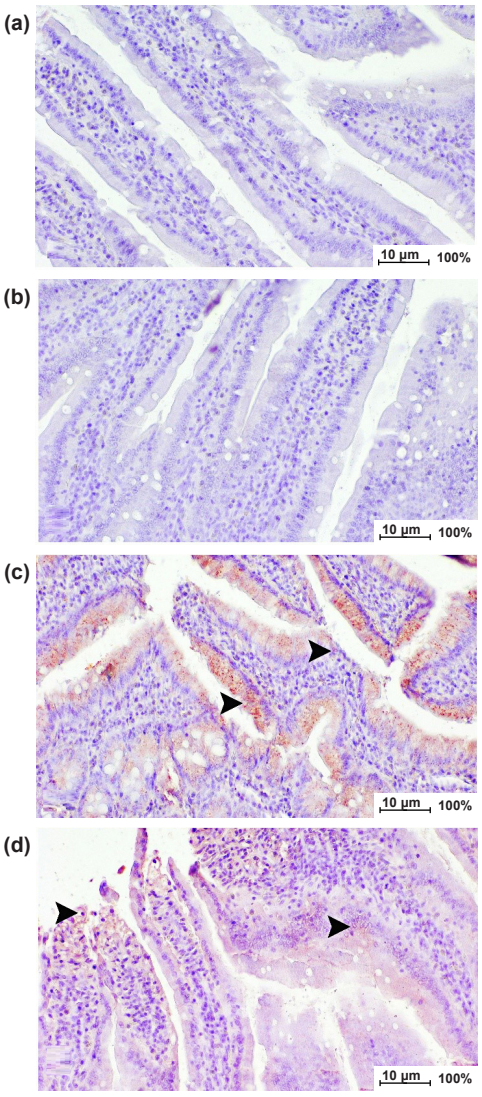

Figure 8. Immunohistochemical detection of 8-OHdG expression in intestinal tissue. . (a) control, (b) BA, (c) GLY, and (d) GLY+BA groups. 8-OHdG-positive staining in the villus epithelial cells of the intestine is indicated by arrowheads. IHC staining, objective: 20X, scale bar: 10 µm, zoom: 100%.

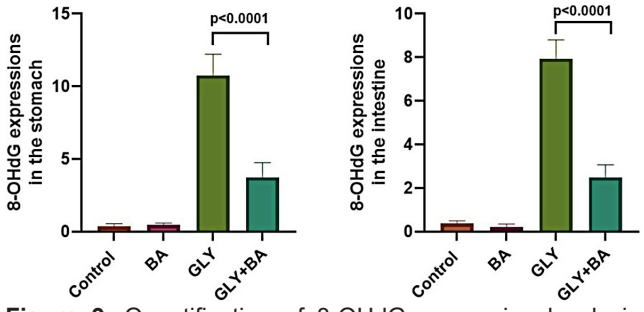

Figure 9. Quantification of 8-OHdG expression levels in gastric and intestinal tissues using the ImageJ analysis software. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test.

mechanisms, as supported by the upregulation of endogenous antioxidant enzymes in the same group. These results reinforce the role of oxidative DNA damage in GLY-induced tissue injury and highlight the potential of boric acid in preventing genotoxic stress within the gastrointestinal system.

One of the limitations of the present study is the use of a single dose of both glyphosate (150 mg/kg/day) and boric acid (100 mg/kg/day). Although these doses were selected based on previously published studies demonstrating toxic and protective effects, respectively, the absence of a dose-response assessment limits our ability to determine whether 100 mg/kg/day of boric acid represents the most effective dose. It also remains unclear whether lower or higher doses would produce comparable or superior outcomes. Similarly, the use of a single glyphosate dose precludes evaluation of how boric acid might modulate toxicity across varying levels of exposure. Future studies incorporating multiple dose groups are warranted to establish dose-effect relationships and optimal therapeutic ranges.

An important limitation of this study is the use of a commercial glyphosate formulation containing 41% active ingredient and unspecified co-formulants, which may have their own toxic effects. Thus, the protective effects of boric acid may reflect mitigation not only of glyphosate toxicity but also of other formulation components. Further studies using pure glyphosate are needed to clarify its specific role.

5. Conclusions

In conclusion, this study demonstrates that glyphosate exposure induces marked oxidative stress, inflammation, histopathological damage, and oxidative DNA injury in the gastrointestinal tissues of rats. The downregulation of key antioxidant enzymes such as SOD and CAT, along with the upregulation of pro-inflammatory markers TNF-α and NF-κB, underscores the dual role of oxidative and inflammatory mechanisms in glyphosate-induced toxicity. These molecular disturbances were corroborated by histopathological findings, which revealed mucosal disruption, epithelial degeneration, and inflammatory infiltration in both the stomach and intestines. Additionally, the elevated expression of 8-OHdG highlights the genotoxic impact of glyphosate through DNA oxidation. Importantly, co-administration of boric acid significantly ameliorated these adverse effects. BA effectively restored antioxidant enzyme levels, suppressed inflammatory responses, reduced oxidative DNA damage, and improved tissue architecture. These findings suggest that boric acid exerts protective effects against glyphosate-induced gastrointestinal toxicity through its antioxidant and anti-inflammatory properties. Further studies are warranted to elucidate the precise molecular mechanisms involved and to explore the potential therapeutic application of boric acid in preventing pesticide-induced gastrointestinal injury.

6. Author Contribution Statement

Tuba Dogan: Methodology, data analysis and editing, visualization, and writing draft.

Omercan Alat: Methodology, project management.

İsmail Bolat: Methodology, project management.

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