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The Influence of Bromelain Administration on Pro-Inflammatory Cytokines and Lipid Peroxidation in a Rat Model of Intestinal Ischemia/Reperfusion Injury

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ABSTRACT

Background: Ischemia-reperfusion injury is a critical problem that can occur following multiple clinical procedures.

Objectives: The study aimed to investigate the effects of bromelain on intestinal damage caused by ischemia-reperfusion injury in rats.

Methods: Wistar albino rats were randomly divided into three groups control (CTR, $n = 6$), intestinal ischemia-reperfusion (I-IR, $n = 6$) and intestinal ischemia reperfusion+bromelain (I-IR+BRO, $n = 6$). Bromelain (40 mg/kg) was given orally 30 min before the ischemia period to the animals in I-IR+BRO group. In the I-IR and I-IR+BRO groups, ischemia-reperfusion was performed with 1-h ligation of the superior mesenteric artery and 1-h reperfusion by opening the ligatures. At the end of the reperfusion period, blood and intestinal tissue samples were collected post euthanasia. Blood samples were analysed for ALT, AST, ALP, LDH, BUN and creatinine to assess liver and kidney function. TNF- α and IL-1 β concentrations were evaluated in both sera and tissue samples to determine the inflammatory response. Finally, Lipid peroxidation of the intestines was assessed by measuring MDA levels.

Results: ALT, AST, ALP, LDH, BUN and creatinine levels were significantly elevated after I/R, but markedly reduced by BRO treatment. Serum and tissue TNF- α , IL-1 β concentrations increased in both IR groups in comparison to the CTR group. However, these values decreased in the I-IR+BRO group when compared to the I-IR group. Histopathological scores were lower in the I-IR+BRO group compared with the I-IR group; however, these differences did not reach statistical significance.

Conclusion: Bromelain administration alleviated the pro-inflammatory cytokines, and the harmful effects experienced by the intestinal tissues.

1 | Introduction

Ischemic intestinal damage is a severe condition in both humans and animals, with survival rates often below 50% due to tissue hypoxia, inflammation and cell infiltration. Intestinal ischemia-reperfusion (I-I/R) injury is generally observed by neonatal necrotizing enterocolitis, mesenteric arterial embolism or vein thrombosis, acute mesenteric ischemia, volvulus,

trauma, shock, aortic aneurysm repair and rejection of the intestinal transplantation in human (Nadatani et al. 2018). In veterinary medicine, although ischemia-reperfusion injury is mostly seen in gastric dilatation volvulus, it also develops in cases such as intestinal volvulus, intestinal incarcerations, colic and abdominal compartment syndrome (Blikslager 2003; McMichael and Moore 2004; Hamilton et al. 2010; Gardner and Schroeder 2022).

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Single-layered intestinal epithelial cells are more susceptible to the effects of ischemia-reperfusion. The ischemic phase leads to tissue hypoxia and organ damage, while the reperfusion phase initiates a cascade of events that leads to increased vascular permeability, cytokine production, disrupts the intestinal mucosal barrier, bacterial translocation and toxin release (Li et al. 2022). Although it has been suggested that female sex hormones may influence ischemia-reperfusion injury, a study by Szabó et al. (2006) showed that reperfusion injury exhibits the same characteristics and consequences in females and males (Szabó et al. 2006). This process leads to a systemic inflammatory response and multi-organ dysfunction (Li et al. 2022).

Ischemia-reperfusion (I/R) studies conducted in recent years focus on agents that are both effective in I/R mechanisms and prevent damage caused by I/R. Bromelain (BRO) is extracted from pineapple and is a mixture of different endopeptidases, phosphatases, glucosidase, peroxidases, cellulases, glycoproteins, carbohydrates and various protease inhibitors. Some studies show that the bromelain reduces the secretion of TNF- α and IL-1 β when excessive cytokine production is stimulated in the inflammation (Chakraborty et al. 2021; Şehirli et al. 2021). In some studies, examining the effects of bromelain on ischemia-reperfusion injury, it has been stated that bromelain protects ischemic tissue and reduces necrosis, as well as reducing factors related to oxidative stress (Weinzierl et al. 2022, Yakut et al. 2025).

The present study aimed to establish an experimental rat model of intestinal ischemia/reperfusion (I/R) injury and to investigate the effects of bromelain on pro-inflammatory cytokines, oxidative stress and histopathological changes. Rats anatomical and physiological properties are similar to humans and are generally used for the experimentally induced disease models. The novelty of this study lies in evaluating bromelain in the setting of intestinal I/R injury, where evidence is scarce. By demonstrating its potential to attenuate biochemical, inflammatory and morphological damage, this work provides new insights and contributes to the growing body of literature supporting bromelain as a promising therapeutic candidate for reducing intestinal and systemic injury associated with I/R.

2 | Materials and Methods

2.1 | Ethical Statement

The local animal ethics committee approved the study protocol using the least number of animals that could produce statistically significant results (Decision No: 2021-5-134). The number of animals used in each group was determined by a priori power analysis and in accordance with ethical committee recommendations to ensure the minimum number of animals necessary for statistically reliable results.

2.2 | Animals

Eighteen outbred Wistar albino rats of both sexes, weighing between 200 and 250 g were used, and housed in a temperature-controlled room ($22 \pm 2^\circ\text{C}$) with a 12-h light/dark cycle (Szabó et al. 2006). The rats had unlimited access to food and water

in conventional cages that were solid plastic, rectangular-shaped and 20 cm high with a wire cap.

2.3 | Experimental Model

Rats were randomly allocated into three groups: control group (rats operated without I/R, $n = 6$), intestinal ischemia-reperfusion group (I-I/R, $n = 6$) and intestinal ischemia/reperfusion + bromelain group (I-I/R+BRO, $n = 6$). Randomization was performed using a simple random number generator. Investigators performing biochemical and histopathological analyses were blinded to group assignments to reduce bias. Exclusion criteria were determined as rats dying during ischemia and/or reperfusion period. No rats were excluded from this study. Bromelain was given 40 mg/kg orally in I-I/R+BRO groups, 30 min before the anesthesia (Yildirim et al. 2018). All groups were anesthetized by intraperitoneal injection with a mixture of Ketamine HCl (100 mg/kg) and Xylazine HCl (10 mg/kg) and fixed in a supine position. Access to the abdominal cavity was performed via a ventral midline incision. In the I-I/R and I-I/R+BRO groups to induce ischemia, ischemia was achieved by ligation of the superior mesenteric artery for 1 h, and the colour changes in the intestines were observed. The intestines were reperfused for 1 h by opening the ligature (Gültekin et al. 2024). The rats were euthanized by overdose of Xylazine+Ketamine anesthesia at the end of the reperfusion periods. The small intestines were excised, and blood samples were collected from all groups. All procedures were performed by the same veterinary surgeon in the experimental working unit under conditions of the housing room.

2.4 | Biochemical Analysis

Blood samples were collected into serum tubes and sera was separated after centrifuge at $2000 \text{ g} \times 10 \text{ min}$. Fresh intestinal tissue samples were initially homogenized using a Dounce tissue grinder set (D8938, Sigma-Aldrich, Missouri, US) and RIPA buffer on ice (<https://www.mdpi.com/1422-0067/22/19/10278>). Sera and tissue homogenizates were kept at a temperature of -80°C until analysis.

Alkaline phosphatase (ALP), lactate dehydrogenase (LDH), alanine transaminase (ALT) and aspartate transaminase (AS) enzyme activities, and blood urea nitrogen (BUN) and creatinine (Crea) levels were measured to assess the hepatocellular injury and renal function. Assays were done using an automated analyser (BS240-Vet, Mindray, Shenzhen, China).

Concentrations of TNF- α and IL-1 β in sera and tissue homogenates were determined using commercially available rat-specific enzyme immunoassay kits (Rat TNF- α ELISA Kit Catalogue No: E-EL-R0019; Rat IL-1 β Catalogue No: E-EL-R001, Elabscience Biotechnology Inc., TX, USA). Assays were performed according to the manufacturer's instructions. MDA levels were measured from the intestinal tissue homogenates to evaluate lipid peroxidation levels using commercially available assay kits (TBARS Assay Kit, Item No. 10009055, Batch No. 0510196 and 0502129, Cayman Chemicals, Michigan, USA). The principal measurement was based on the reaction with thiobarbituric acid (TBARS) (Ohkawa et al. 1979). Tissue

TABLE 1 | Plasma ALP, ALT, AST, LDH, BUN and creatinine activities in the control (CTR), intestinal ischemia/reperfusion (I-I/R) and intestinal ischemia/reperfusion +bromelain (I-I/R+BRO) groups.

Parameters	CTR (<i>n</i> = 6)	I-I/R (<i>n</i> = 6)	I-I/R+BRO (<i>n</i> = 6)
ALP (U/L)	92.63 ± 11.26	185.8 ± 18.89 ^{***}	134.3 ± 7.42 ⁺
ALT (U/L)	50.50 ± 12.41	1128 ± 421.9 ^{**}	151.3 ± 21.05 ⁺⁺
AST (U/L)	139.6 ± 41.11	1591 ± 614.3 ^{**}	259.3 ± 31.52 ⁺
LDH (U/L)	1549 ± 160.1	6496 ± 1120 ^{****}	2071 ± 344.8 ⁺⁺⁺
BUN (mg/dL)	35.38 ± 3.32	62.50 ± 4.94 ^{***}	49 ± 2.78 ^{*,+}
Crea (mg/dL)	0.37 ± 0.05	0.96 ± 0.06 ^{****}	0.68 ± 0.04 ^{***,++}

p* < 0.05, *p* < 0.01, ****p* < 0.001, *****p* < 0.0001 compared with the control (CTR) group.

⁺*p* < 0.05, ⁺⁺*p* < 0.01, ⁺⁺⁺*p* < 0.001, ⁺⁺⁺⁺*p* < 0.0001 compared with the I-I/R group.

TNF- α , IL-1 β , MDA concentrations were reported as pg/mg Protein, pg/mg Protein and nmol MDA/mg Protein. Tissue protein concentrations were detected performing Coomassie brilliant blue method (Bradford 1976).

2.5 | Histomorphological Analysis

Excised intestinal tissue were fixed in 10% neutral-buffered formalin solution. The paraffin blocks that contained the intestine samples were serially sectioned (average thickness 5 μ m) and were stained with hematoxylin-eosin (Atalay et al. 2018). Histopathological sections were evaluated using the bright field mode of a light microscope (Zeiss-Axio Scope A1, Carl Zeiss, Gottingen, Germany), and according to each of the criteria were scored using a semi-quantitative system as 0: no, 1: mild, 2: moderate and 3: severe (Gültekin et al. 2024).

2.6 | Statistical Analysis

Statistical analyses were performed using GraphPad Prism 9 (GraphPad Software, San Diego, CA, USA). A one-way analysis of variance (ANOVA) was used for the comparison of the levels of biochemical parameters in the serum and tissue. Tukey's test was used for further analysis for binary comparisons. Histopathologic scores of intestinal tissues were assessed using non-parametric tests: Kruskal–Wallis following Dunn's multiple comparisons test. All results are expressed as the means $\pm$ SEM. *p* values below 0.05 were regarded as significant.

3 | Results

The effects of experimentally created I/R injury serum ALT, AST, ALP and LDH activities, BUN and creatinine values are provided in Table 1. While there was a significant increase in ALT, AST, ALP and LDH activities, BUN and creatinine levels in the I-I/R group in comparison to the CTR group (*p* < 0.01–0.0001). It was found that the increase in the values was less in the I-I/R+BRO group than in the I-I/R group (*p* < 0.01–0.0001).

TNF- α and IL- β levels as pro-inflammatory cytokines were evaluated in the measurement of the response to the injury caused by I/R (Figure 1a,c). TNF- α (*p* < 0.0001) and IL-1 β (*p* < 0.0001)

levels were significantly higher in the I-I/R group than in the CTR group. However, it was observed that TNF- α and IL-1 β levels decreased in the I-I/R+BRO group when compared to the I-I/R group, respectively *p* < 0.01 and *p* < 0.001. TNF- α and IL-1 β levels obtained from tissue samples were significantly higher in the I-I/R group than in the CTR group, respectively *p* < 0.001 and *p* < 0.001. However, it was observed that TNF- α and IL-1 β levels in the I-I/R+BRO group decreased compared to the I-I/R group, respectively *p* < 0.01 and *p* < 0.01 (Figure 1b,d).

MDA levels were evaluated to determine lipid peroxidation and oxidative damage in tissue samples (Figure 2). MDA levels increased significantly in the I-I/R group when compared to the CTR group (*p* < 0.01). Although there was a decrease in the I-I/R+BRO group compared to the I-I/R group, no statistically significant difference was determined between the groups (*p* > 0.05).

According to histopathological examination and semi-quantitative scoring system, it was determined that the CTR group had a regular epithelial and glandular structure, almost no inflammatory cell infiltration (Figure 3 and Table 2), but a large number of inflammatory cells, and destructive morphological degenerations of both epithelial and glandular structures in the I-I/R group (Figure 3b and Table 2). On the other hand, in the I-I/R+BRO group, it was observed that had epithelial regeneration of both epithelium and glandular structure compared to the I-I/R group (Figure 3c). Although the reductions did not reach statistical significance, the I-I/R+BRO group exhibited visibly improved villus morphology, reduced epithelial desquamation and lowered inflammatory cell infiltration scores compared with the I-I/R group (Table 2).

4 | Discussion

Intestines perform a number of different roles, with digestion, nutrient absorption and the discharge of waste products being part of its major function. Today, many more have been revealed one of which is its ability to eliminate bacterial formations and toxins generated by the gastrointestinal system. The structure called the intestinal mucosal barrier, consists of a single epithelial layer, which plays an important role in fulfilling this function. I-I/R injury disrupts the intestinal mucosal barrier,

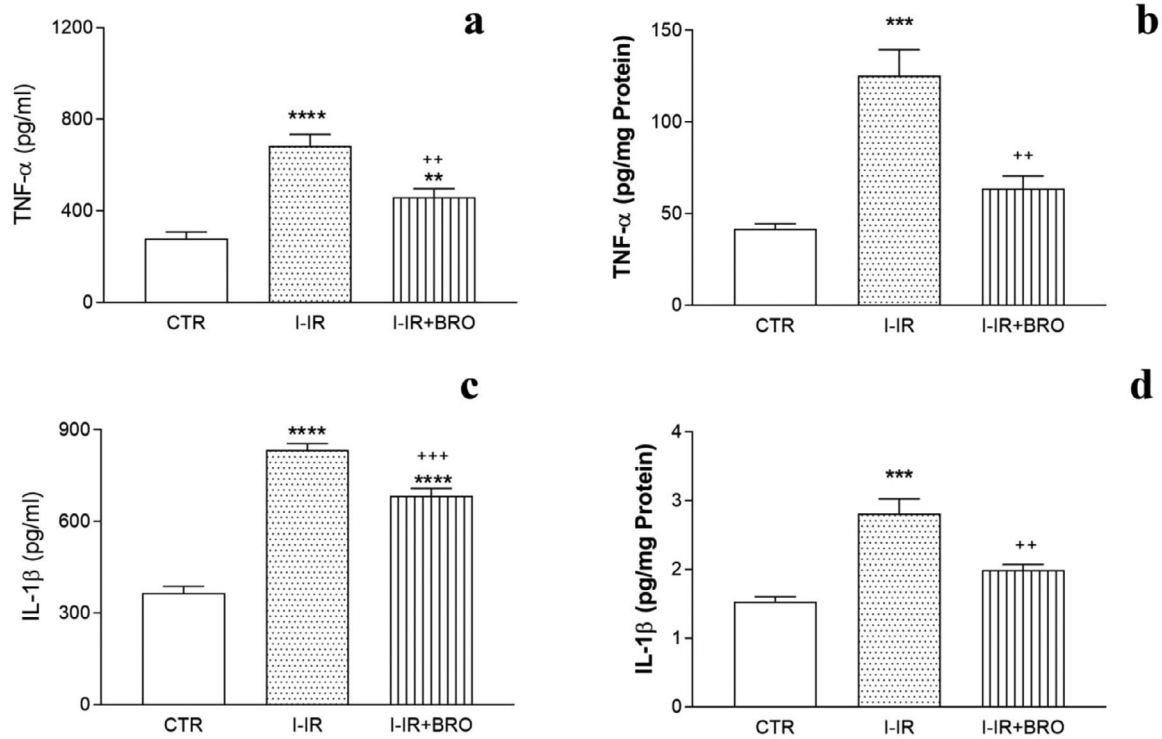


FIGURE 1 | Blood (a and c) and tissue (b and d) concentration of TNF- α and IL-1 β in the control (CTR), intestinal ischemia/reperfusion (I-I/R) and intestinal ischemia/reperfusion +bromelain (I-I/R+BRO) groups. ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$ compared with the control (CTR) group. + $p < 0.05$, ++ $p < 0.01$, +++ $p < 0.001$, ++++ $p < 0.0001$ compared with the I-I/R group.

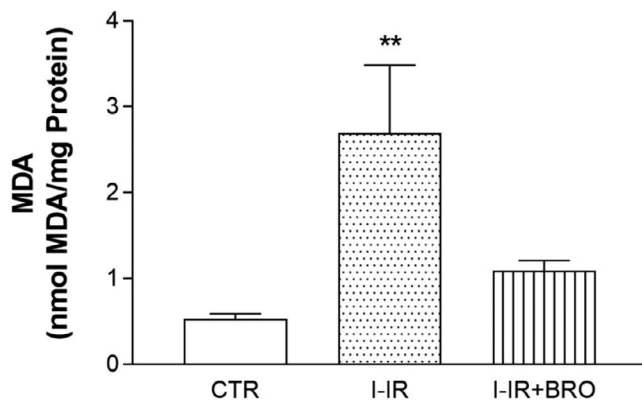


FIGURE 2 | MDA activity in intestinal tissues of control (CTR), intestinal ischemia/reperfusion (I-I/R) and intestinal ischemia/reperfusion +bromelain (I-I/R+BRO) groups. ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ compared with the control (CTR) group.

where bacterial toxins cannot be removed and consequently pass into the systemic circulation. Additionally, I-I/R injury causes the release of pro-inflammatory cytokines formed in response to inflammation in the intestinal mucosal barrier. As a result of all these cascades, which increase over time in the systemic circulation, the damage to the tissues and organs intensifies (Nadatani et al. 2018). Accordingly, recent studies have focused on strategies to prevent and reduce factors that play a role in I/R injury or to protect tissues and organs from the harmful effects of I/R injury (Soares et al. 2019; VanderBroek et al. 2021). In this study, bromelain was trialled as a new strategic agent, and its

effect on the important intestinal cytokines TNF- α and IL-1 β in I/R injury, was investigated.

With the formation of I-I/R damage, oxygen deficiency in intestinal epithelial cells and enterocytes with high oxygen activity destroys cells, therefore degenerative changes are observed in the intestinal mucosal barrier. A complex pathophysiological cascade is therefore initiated, which causes the release of pro-inflammatory cytokines such as TNF and IL-1, with the stimulation and degradation of inflammatory cells (Gharishvandi et al. 2020). TNF- α and IL-1 β , which play an important role in this inflammatory cascade are produced by inflammatory cells in the intestinal mucosa and cause local cell damage, triggering systemic inflammation, are observed as a result of the early inflammatory response after I-I/RI injury (Alexandropoulos et al. 2017). In this study, tissue and blood TNF- α and IL-1 β values were found to be higher in I-I/R and I-I/R+BRO groups, that underwent 1-h ischemia and 1-h reperfusion, compared to the CTR group (Figure 1a–d). Additionally, this is supported by the biochemical analyses in terms of cell infiltration and tissue damage from histomorphological evaluation (Figures 3a–c). These results indicated that 1 h of ischemia-reperfusion injury caused an increase in TNF- α and IL-1 β levels, devastating morphological changes, and inflammatory cell infiltration in the intestine. BRO significantly suppressed TNF- α and IL-1 β levels in serum and tissue. Although histopathological scores did not show statistically significant improvement, BRO-treated rats exhibited visibly better villus organization and reduced epithelial injury.

This damage triggers the peroxidation of membrane lipids which results in tissue damage, inflammatory cell activation and the

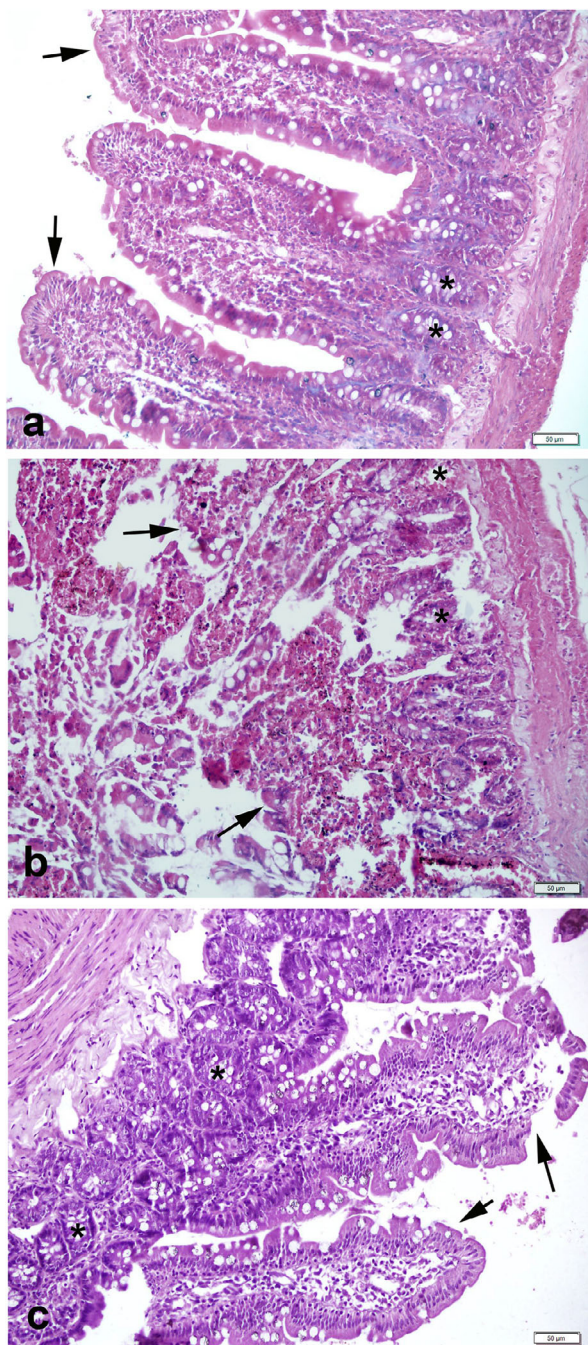


FIGURE 3 | (a) CTR group, regular epithelium of the villi (arrows) with regular structure of the intestinal glands (*). (b) I-I/R group, severe loss of villi structure as a result of heavy desquamation of epithelium (arrows) and glands (*). (c) I-I/R+BRO group, reorganization of villi structure with regeneration of epithelium (arrows) and glands (*).

release of cytokines such as $\text{TNF-}\alpha$ and $\text{IL-1}\beta$, especially during the reperfusion period after ischemia. Lipid peroxidation causes changes in cell membrane permeability, leading to cell lysis (Zu et al. 2018). MDA, which is a marker of lipid peroxidation, is widely used in studies because of its sensitivity, safety and being a reliable indicator of oxidative damage to lipids (Sayiner et al. 2019; Şehirli et al. 2021). In this study, MDA activity was evaluated in the determination of lipid peroxidation, thus oxidative degradation of lipids, in intestinal tissue. The results obtained showed an

increase in the I-IR and I-IR+BRO groups compared to the CTR group. The increase in MDA levels was lower in the I-IR+BRO group than in the I-IR group, even though statistical significance could not be determined between the I-IR and I-IR+BRO groups. When the results of MDA, $\text{TNF-}\alpha$ and $\text{IL-1}\beta$ in the intestinal tissue, serum $\text{TNF-}\alpha$ and $\text{IL-1}\beta$ activity and histomorphological examinations are evaluated, BRO is effective on inflammatory cells, cytokine release and lipid peroxidation in the intestinal tissue.

The sequential, but complex pathophysiological events of ischemia and reperfusion cause devastating effects on the intestine. Changes observed during the ischemic phase are mostly energy metabolism disorders, mitochondrial dysfunction, cell damage and activation of multiple cell death. The pathophysiological events observed during the reperfusion phase can be summarized as oxidative stress, inflammatory response, neutrophil infiltration and complement system activation. This cascade of events leads to increased vascular permeability and impaired intestinal mucosal barrier function, leading to systemic effects that can result in multiple organ failure (Xu et al. 2025). In this study, intestinal villus damage, glandular hyperplasia and cellular infiltration observed in the I-I/R group demonstrate the effects of I/R injury. Similar changes were observed in the I-I/R+BRO group, but the I-I/R+BRO group exhibited more regular villus structure and cellular infiltration. Although bromelain did not produce statistically significant improvements in semi-quantitative histopathological scores, the I-I/R+BRO group showed more organized villus architecture, reduced epithelial desquamation and milder inflammatory cell infiltration compared with the I-I/R group. These morphological improvements, together with the significant reductions observed in serum and tissue cytokines ($\text{TNF-}\alpha$ and $\text{IL-1}\beta$), suggest that BRO attenuates early tissue injury at the microscopic level even when statistical significance is not achieved in scoring systems.

I/R injury not only causes anatomical deformity and local inflammatory response in the ischemic organ but also affects other organs and tissues because of the systemic effects that result from the ischemia-reperfusion cascade. According to studies on I-I/R injury, the liver and kidney are among the organs most affected by the systemic damage caused by ischemia-reperfusion (Alexandropoulos et al. 2017). Alterations in ALP, ALT, AST and LDH enzyme activities, BUN and Crea levels are used to determine the degree of tissue damage (Sookoian and Pirola 2015). While these enzyme activities, which are frequently used clinically in the determination of ischemia-reperfusion injury, show more and specific increases, especially in the advanced stages of the disease, it is stated that early increases in serum values are due to the inflammatory response (Bertoni et al. 2018). In the presented study, ALP, ALT, AST, LDH, BUN and Crea enzyme activities were significantly increased in both I-I/R and I-I/R+BRO groups compared to the CTR group. However, BRO administration caused a remarkable reduction in these values. Accordingly, it suggests that the damage in the intestine is caused by the formation of proinflammatory cytokines and is similar to the findings of the mentioned studies. Besides, it has been demonstrated that BRO administration reduces the effects of I/R injury by decreasing enzyme activities and $\text{TNF-}\alpha$, $\text{IL-1}\beta$ and MDA levels, which are markers of organ damage, in this initial study.

TABLE 2 | Histomorphological scoring results of control (CTR), intestinal ischemia/reperfusion (I-IR) and intestinal ischemia/reperfusion +bromelain (I-IR+BRO) groups. Each of the criteria was scored semi-quantitatively as 0: none, 1: mild, 2: moderate, 3: severe.

Parameter	Control (n = 6)	I-I/R (n = 6)	I-I/R+Bro (n = 6)
Desquamation of villus tip	0.41 ±0.08	2.78 ± 0.07***	1.63 ± 0.06
Hiperplasia of intestinal glands	0.43 ± 0.07	2.40 ± 0.11***	1.65 ± 0.05
Inflammatory cell infiltration	0.38 ± 0.05	2.65 ± 0.05***	1.61 ± 0.06

***p < 0.001 compared with the control (CTR) group.

Our results demonstrated that bromelain treatment mitigated intestinal tissue injury and systemic biochemical disturbances following I/R. These findings are consistent with studies in poultry and rodent models showing that bromelain reduces intestinal lesions, modulates inflammatory responses and improves barrier integrity. In addition, experimental data on food additives such as Sunset Yellow also emphasize that villus rupture, crypt degeneration and haemorrhage are critical histological indicators of intestinal injury. In the present study, histological evaluation of the small intestine was based on widely accepted criteria, including villus morphology, epithelial integrity, crypt structure and haemorrhage formation (Şensoy 2024, Gharib-Naseri et al. 2024). By linking our observations to these established parameters, the protective effects of bromelain can be more clearly interpreted in the context of intestinal barrier function and systemic injury prevention.

Inflammatory response, which plays an important role in the pathophysiological mechanisms that develop because of I-I/R injury, is one of the parameters used in the early diagnosis of a developing problem, and also affects the success of the medical and/or operative treatment used to eliminate the problem. However, the search for medical treatment methods that can be used against the destructive effects of I/R injury and studies on this is continuing (Gültekin et al. 2024). Although the efficacy of BRO in the inflammatory response has been demonstrated in some studies, its effect on pro-inflammatory cytokines in intestinal ischemia-reperfusion injury has not been investigated before (Şehirli et al. 2021). This study highlights the effect of BRO on proinflammatory cytokines in I-I/R injury and its protective or preventive effect on organ damage after ischemia-perfusion. However, to clarify the full impact of BRO in I-I/R injury, all aspects of biochemical pathways need to be explored, and more work is needed in this direction.

This study has certain limitations. Bacterial translocation, a systemic consequence of prolonged intestinal barrier dysfunction, was not assessed. Since the present model involved only short-term ischemia and reperfusion, our focus remained on early inflammatory and biochemical changes. In addition, the lung—a well-recognized remote organ affected by intestinal I/R injury—was not evaluated, and other antioxidant parameters beyond MDA were not measured. These limitations should be addressed in future studies to comprehensively evaluate the protective potential of bromelain in intestinal I/R injury.

The present study demonstrates that bromelain administration attenuates the production of pro-inflammatory cytokines (TNF-α, IL-1β), reduces biochemical markers of hepatic and renal

injury, and improves histopathological outcomes in a rat model of intestinal ischemia/reperfusion injury. These findings support our initial hypothesis that bromelain can exert protective effects on intestinal tissue exposed to I/R. Although the study was designed as an experimental model, the results suggest that bromelain may represent a promising adjunctive approach in veterinary medicine to reduce I/R-related tissue damage. Future research involving longer reperfusion periods, additional antioxidant parameters and evaluation of remote organs will be necessary to further clarify the therapeutic potential and translational applicability of bromelain.

Author Contributions

Çağrı Gültekin: conceptualization, Investigation, methodology, writing – original draft, writing – review & editing, resources, validation, project administration. **Serkan Sayiner:** conceptualization, writing – review & editing, formal analysis, resources. **Şule Çetinel:** formal analysis. **Ahmet Özer Şehirli:** formal analysis, methodology, supervision.

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The authors have nothing to report.

Ethics Statement

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received.

Conflicts of Interest

The authors declare they have no conflicts of interest.

Data Availability Statement

The data presented in this study are available via the corresponding author's e-mail.

Peer Review

For transparency, the peer review documents associated with this article are available at <https://doi.org/10.1002/vms3.70805>.

[Correction added on 6 April 2026, after first online publication: Peer review history has been added].

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