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# Gastroprotective effects of bromelain on indomethacin-induced gastric ulcer in rats

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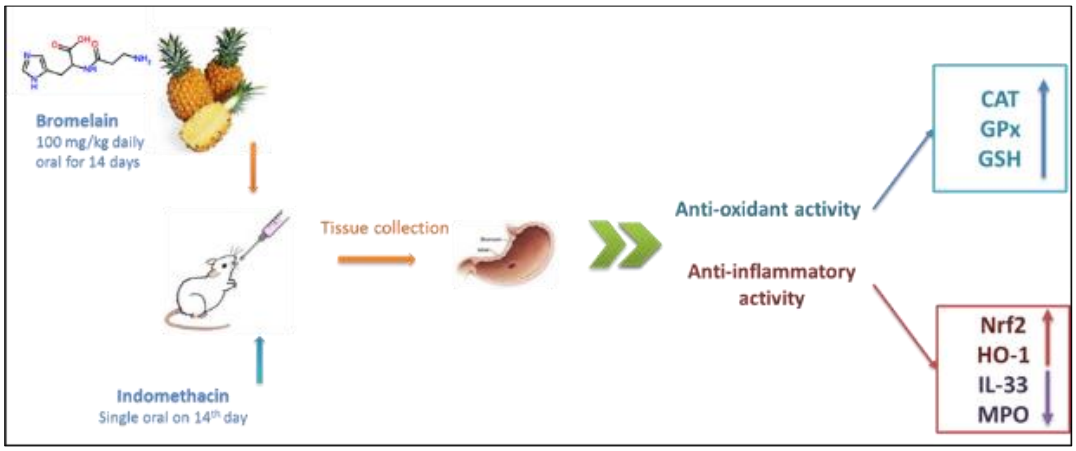
## Abstract

Gastric ulcer is one of the most common diseases of the gastrointestinal system and some of its complications are the main causes of morbidity and mortality. Bromelain (BRM), a sulfhydryl proteolytic enzyme, has multiple activities in many areas of medicine as an antioxidant and anti-inflammatory agent. In this study, it was aimed to investigate the anti-inflammatory and oxidative stress-mediated therapeutic effect of BRM in a gastric ulcer model induced by indomethacin (IND) which is a non-steroidal anti-inflammatory drug (NSAID) with gastric ulcer-forming effects. Gastric mucosal paraoxonase (PON), arylesterase (ARE), catalase (CAT), myeloperoxidase (MPO) and glutathione peroxidase (GPx) activity and total glutathione (GSH) levels were analyzed. Interleukin-33 (IL-33), the nuclear factor erythroid 2-related factor 2 (Nrf2) and heme oxygenase 1 (HO-1) levels were determined by immunohistochemically. Although IND-induced histopathological changes occurred, BRM pretreatment prevented these effects. As a result of the study, it was determined that BRM had an anti-ulcerative effect at a dose of 50 mg/kg. BRM given at this dose significantly decreased gastric tissue MPO activity, and increased CAT, PON, ARE, GPx activity and GSH level. The increased IL-33 and decreased Nrf-2 and HO-1 levels were obtained in IND group and these changes were reversed by BRM. As a result, it was concluded that BRM may have protective effects in IND-induced gastric model by reducing inflammation and regulating oxidant/antioxidant balance.

**Keywords:** Indomethacin; Gastric ulcer; Bromelain; Inflammation; Oxidative stress

### Graphical Abstract

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## 1. Introduction

Gastric ulcer can be briefly defined as sores in the stomach lining (stomach mucosa) (1). Gastric damage occurs as a result of the disruption of the balance between mucosal aggressive factors and protective factors. In addition to acid peptic disorders, disruption of mucosal barrier, genetic susceptibility, stress, trauma, sepsis, hemorrhagic shock, burns (Curling ulcer), pulmonary and liver diseases, smoking, alcohol, NSAIDs, epinephrine and Helicobacter pylori infection are among the factors triggering ulcer (2, 3). NSAIDs can cause gastric injury through inhibition of prostaglandin (PG) synthesis, reduction in local blood flow, regional irritation, and inhibition of tissue regeneration (4). Although many synthetic antiulcer drugs are available, different side effects such as causing thrombocytopenia or enzyme induction have been reported (Piper, 1995). Therefore, it is necessary to encourage the search for non-toxic, easily accessible antiulcer drugs.

BRM, primary active extract of Ananas comosus, can be an alternative treatment for many diseases because of its antioxidant and anti-inflammatory properties (5). It has been speculated that BRM was effective in colonic inflammation in animal (6). It inhibits inflammatory productions in ulcerative colitis such as interleukin (IL) and TNF-α (7). Also, it enhances elimination of reactive oxygen specials (ROS) by supporting antioxidatant defense systems. It has been reported that excess production of ROS, lipid peroxidation, the reduction of antioxidants and inflammatory cytokines play an important role in gastric ulcer (8). Antioxidant enzymes like GPx, glutathione reductase (GR) and HO-1 may be the main treatment mechanism against oxidative stress-induced gastric ulcers. Nrf-2 is well known to be the main redox sensing transcription factor that plays an important role in the regulation of oxidative stress. Expression of genes coding for many antioxidant defense proteins is regulated by the Nrf2 transcriptional activating factor. Translocation of this protein from the cytosol to the nucleus is stimulated by oxidative stress.

In this study, we aimed to investigate the protective effect of BRM and its relationship with oxidative stress and inflammatory parameters on IND-induced gastric ulcer model in rats. For this purpose, gastric tissue ARE, PON, CAT, MPO, GPx activities as well as GSH levels were investigated. In addition, the anti-ulcerative effect was determined by evaluating IL-33, Nrf-2 and HO levels in immunohistochemically.

## 2. Material and methods

### 2.1. Animals

In this study, twenty-eight adult female albino Sprague-Dawley rats, weighing between 250-300 g, were used. Animals were provided from Atatürk University Laboratory Animals Application and Research Centre. During the experiment, animals were fed rat chow and tap water ad libitum. The animals will be housed in polypropylene cages, in 12-h light / 12-h dark rooms, at 22 ± $0.5^0$C and appropriate humidity.

### 2.2. Chemicals

Bromelain (Sigma-Aldrich International, Germany). Indomethacin (Endol 25 mg; 25 cap., DEVA Holding A.S., Istanbul, Turkey); Esomeprazole (Nexium 40 mg; 28 tablet, AstraZeneca Pharmaceutical Company, Istanbul, Turkey).

### 2.3. Experimental design

The dose determination of the tested agents was determined according to the previous studies considering the protective effects of the products (6, 9, 10). Rats were divided into four experimental groups each consisting of 7 animals: control (no treatment was applied), IND (Gastric ulcer model, 100 mg/kg IND), IND+ ESP (20 mg/kg ESP), IND + BRM (100 mg/kg BRM).

### 2.4. Application of Gastric Ulcer Model

BRM, IND and ESP were dissolved in saline 5% NaOH. BRM (100 mg/kg BRM) and ESP (20 mg/kg of ESP) administered by oral gavage daily for 14 days. The control and IND (100 mg/kg IND) group were given saline in the same way and volume. All experimental groups were fasted 24 hours before drug administration. Except for the control group, ulcer was induced by administering IND to three of the experimental study groups, namely IND, IND+ ESP and IND+ BRM (10, 11). The same volume of physiological saline was administered to the experimental animals in the control group. 50 mg/kg ketamine and 5 mg/kg xylazine were administered to rats 6 hours after IND administration. Anesthetized rats were euthanized by cervical dislocation after tissue samples were taken. Then, the stomach was opened along the greater curvature and washed with physiological saline at 40C. Stomach tissues washed in physiological saline were

stored in tubes containing 10% formalin for histological procedures and at -800C for biochemical analyzes until the working day. Hematoxylin-eosin staining of the taken tissues were evaluated histopathologically and immunohistochemically.

### 2.5. Histopathological Examination

Tissue samples were fixed in 10% formaldehyde solution for 48 hours and embedded in paraffin blocks at the end of routine tissue follow-up procedures. Sections of 4 μm thickness were taken from each block, and the preparations prepared for histopathological examination were stained with hematoxylin-eosin (HE) and examined with a light microscope (Olympus BX 51, JAPAN). A semiquantitative scoring system was used for histopathological evaluation (Schafer et al. 2018) (12) as follows: -, no staining; +, mild staining; ++, moderate staining; +++, strong staining.

### 2.6. Immunohistochemical Examination

Tissue sections taken on adhesive (poly-L-Lysin) slides for immunoperoxidase analysis were deparaffinized and dehydrated. Then, endogenous peroxidase was inactivated by keeping it in 3% H2O2 for 10 minutes. Then the tissues were boiled in 1% antigen retrieval (citrate buffer (pH+6.1) 100X) solution and allowed to cool at room temperature. Sections were incubated with protein block for 5 minutes to prevent nonspecific background staining in tissues. Then, primary antibody (IL33, Cat No: orb6205, Dilution Ratio: 1/100, UK) was dripped onto the tissues and incubated in accordance with the instructions for use. 3-3' Diaminobenzidine (DAB) chromogen was used as chromogen in tissues. The stained sections were examined with a light microscope (Zeiss AXIO GERMANY).

### 2.7. Double-Immunofluorescence Review

Tissue sections taken on adhesive (poly-L-Lysin) slides for immunoperoxidase analysis were deparaffinized and dehydrated. Then, endogenous peroxidase was inactivated by keeping it in 3% $H_2O_2$ for 10 minutes. Then the tissues were boiled in 1% antigen retrieval (citrate buffer (pH+6.1) 100X) solution and allowed to cool at room temperature. Sections were incubated with protein block for 5 minutes to prevent nonspecific background staining in tissues. Then, primary antibody (Nrf-2 Cat No: ab89443, Dilution Ratio: 1/100, UK) was dripped onto the tissues and incubated in accordance with the instructions for use. Immunfluorescence secondary antibody was used as a secondary marker (FITC Cat No: ab6785 Diluent Ratio: 1/1000) and kept in the dark for 45 minutes. Then, the second primary antibody (HO-1 Cat No: ab189491, Dilution Ratio: 1/100, UK) was dripped onto the tissues and incubated in accordance with the instructions for use. Immunofluorescence secondary antibody was used as a secondary marker (Texas Red Cat No: ab6719 Diluent Ratio: 1/1000 UK) and kept in the dark for 45 minutes. Then, DAPI with mounting medium (Cat no: D1306 Dilution Rate: 1/200 UK) was dripped onto the sections and kept in the dark for 5 minutes, and the sections were closed with a coverslip. The stained sections were examined under a fluorescent microscope (Zeiss AXIO GERMANY)

### 2.8. Determination of ARE, PON, MPO, GPx activity and GSH levels

After tissue homogenization, oxidative stress parameters from tissue samples were performed with the following methods. CAT activity in the homogenate supernatant was measured spectrophotometrically according to a procedure previously described (13). GPx activity was evaluated according to Paglia and Valentine. (14) and was measured by reading the absorbance difference during the oxidation of NADPH to NADP+ at a wavelength of 340 nm. MPO is reside in the kinetic altitude of the absorbance of the yellowish-orange complex originate from the oxidation of o-dianiside with MPO in the existence of hydrogen peroxide at a wavelength of 460 nm (15). Paraoxonase is used as a matter for PON activity. The basis of the altitude is the assurance of the absorbance alter of 4-nitrophenol formed by paraoxon hydrolysis at 37° C and 412 nm spectrophotometrically per unit time (Gülcü and Gürsü 2003). Phenylacetate (Sigma Co, UK) is used as a matter to specify ARE activity (Gülcü and Gürsü 2003). Sedlak and Lindsay (1965) method was used for total GSH measurement. After 30 minutes of incubation at 37 °C, absorbance was measured at 412 nm. 2 mM, 1 mM, 0.5 mM, 0.250 mM, 0.125 mM, 0.0625 mM and 0.035 mM reduced glutathione were used as standard. The total amount of GSH was determined by taking the results obtained from standard measurements and their corresponding values in the graph from absorbance. (Sadlak and Lindsay 1968).

### 2.9. Statistical analysis

SPSS 22.00 program was used for statistical analysis SPSS (SPSS for Windows, Inc., Chicago, IL). Shapiro–Wilk test for the normality and Levene test for homogeneity were performed. One-way analysis of variance (ANOVA) was used for the comparison of the groups in parametric condition, Tukey test was used for the post-hoc comparison. The nonparametric Kruskal-Wallis test was used for the analysis of the differences between the groups in the semiquantitative data obtained in the histopathological examination, and the Mann Whitney U test was used for the comparison of the paired groups. In order to determine the intensity of positive staining from the pictures obtained as

a result of immunohistochemical and double immunofluoresence staining; 5 random areas were selected from each image and evaluated in the ZEISS Zen Imaging Software program. Data were statistically defined as mean and standard deviation (mean±SD) for % area. Compared to positive immunoreactive cells and immunopositive stained areas with healthy controls used One-Way ANOVA and after Tukey test. As a result of the test, <0.05 was considered significant and the data were presented as mean ± SD.

## 3. Results

### 3.1. Histopathological Findings

When gastric tissues are examined histopathologically, it was observed that control group have a normal histological structure. IND group: Severe erosion and ulceration in the mucosa, severe necrosis and severe hemorrhage in the epithelium, severe desquamated epithelium in the lumen, severe hyperemia in the vessels in the submucosa, and severe serosal edema were determined. IND+ESP group: Mild erosions, hemorrhage foci, mild mononuclear cell infiltration, mild desquamated epithelium in the lumen, mild edema in the submucosa, moderate hyperemia in the vessels were observed. A statistically significant difference ($p<0.05$) was detected when compared with the IND group. IND+BRM group: Mild erosion of the mucosal layer, necrosis of the epithelium, hemorrhage and mononuclear cell infiltration, mild edema of the submocosa and mild hyperemia of the vessels were detected. A statistically significant difference ($p<0.05$) was detected when compared with the IND group. The results of each dye are demonstrated in Figure 1. Histopathological findings are summarized in Table 1.

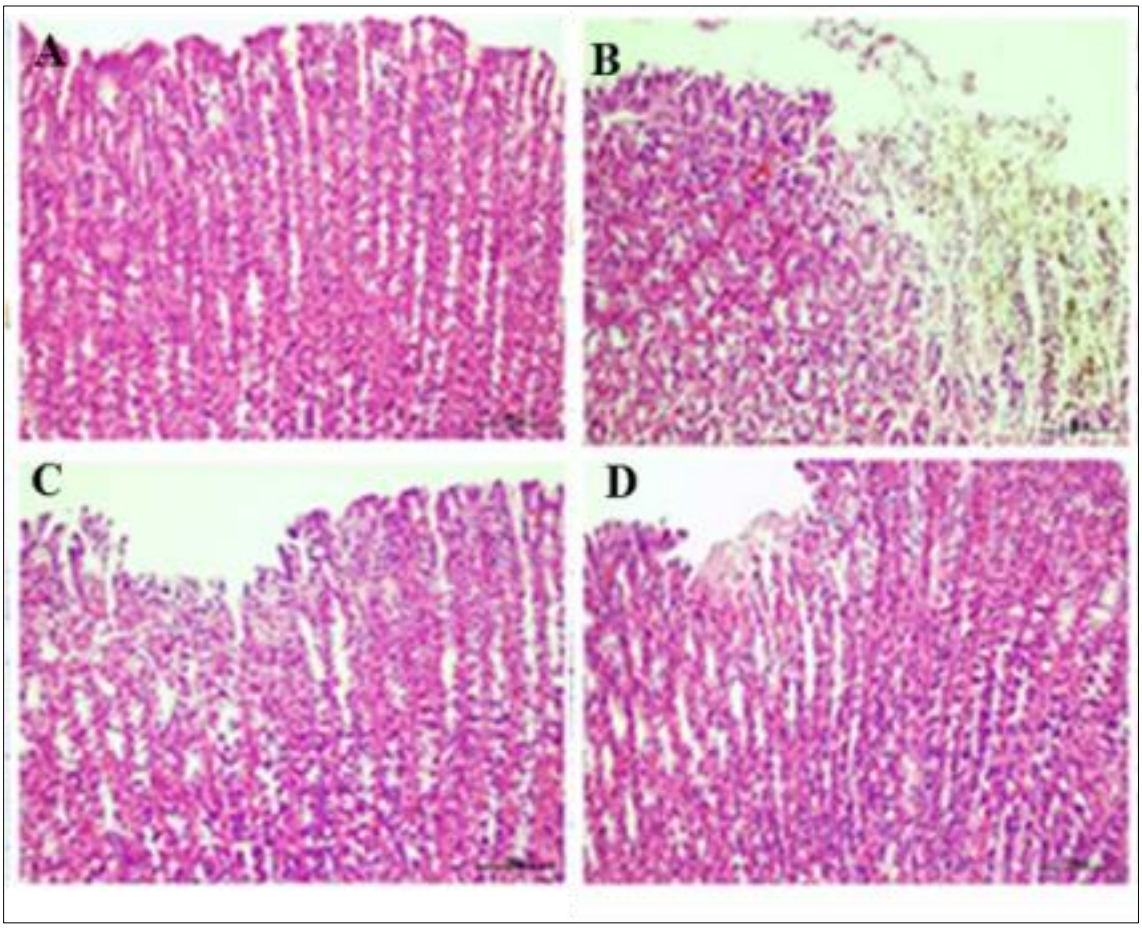


**Figure 1** Gastric tissue, histopathological results, H&E, Control (A), IND (B), IND+ESP (C) and IND+ BRM (D). IND: indomethacin, ESP: esomeprazole, BRM: Bromelain

**Table 1** Scoring of Histopathological findings observed in stomach tissues

| | Control | IND | IND+ESP | IND+BRM |
|---|---|---|---|---|
| Bleeding in mucosa | - | +++ | + | + |
| Erosion in mucosa epithelium | - | +++ | + | + |
| Necrosis in mucosa epithelium | - | +++ | + | + |
| Desquamated epithelium in the lumen | - | +++ | + | - |
| Mononuclear cell infiltration | - | +++ | + | + |
| Hyperemia in vessels | - | +++ | ++ | + |
| Oedema in submucosa | - | +++ | + | + |

### 3.2. Immunohistochemical and immunofluorescent Findings

When gastric tissues are examined by immunohistochemical and immunofluorescent methods, expressions of IL 33, Nrf-2 and HO-1 in the gastric wall were evaluated as negative in control group (Figure 2-3). IND group: Severe IL33 expression was detected in the gastric wall, mucosal layer, interstitial spaces, cytoplasm of inflammatory cells, and around vessels (Figure 2). Severe Nrf-2 and HO-1 expressions were detected in the erosive-ulcerative regions (Figure 3). IND+ESP group: Mild IL33 expression was observed in erosive-ulcerative regions, mucosa interstitium, cytoplasm of inflammatory cells and around vessels, mild Nrf-2 and HO-1 expressions were observed in and around the erosive-ulcerative region (Figure 2-3). A statistically significant difference ($p<0.05$) was detected when compared with the IND group. IND+ BRM group: mild IL33 expression was observed in the mucosa layer around the necrotic area, in the inflammatory cells and around the vessels, and mild Nrf-2 and HO-1 expressions were observed in the erosive-ulcerative region in immunofluorescent staining (Figure 2-3). A statistically significant difference ($p<0.05$) was detected when compared with the IND group. Immunohistochemical and immunofluorescent assessments are summarized in Table 2.

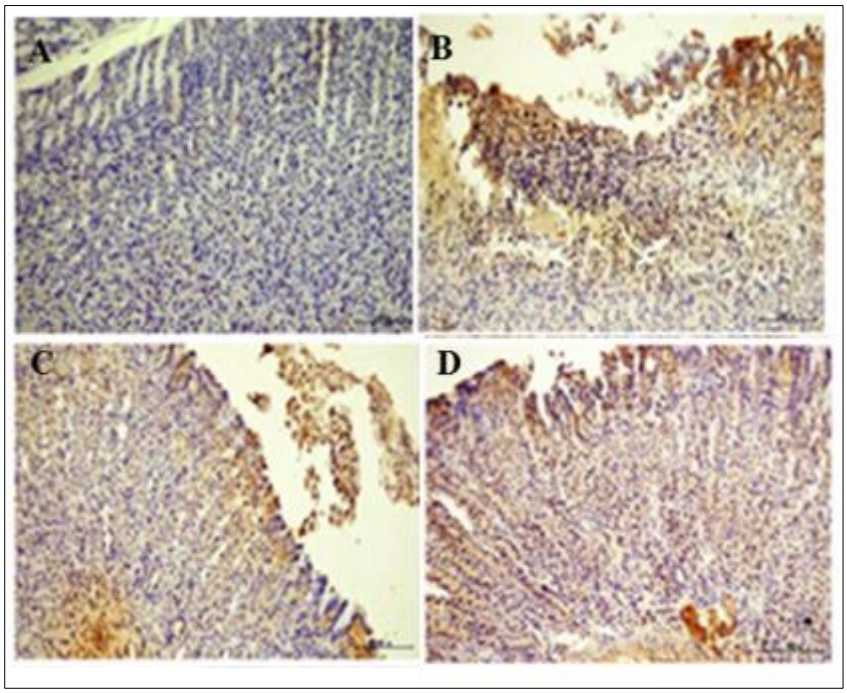

**Figure 2** Gastric tissue, Control (A), IND (B), IND+ESP (C) and IND+ CA (D), IL-33 expression, IHC-P, IND: Indomethacin, ESP: Esomeprazole, BRM: Bromelain. Bar: 100µm

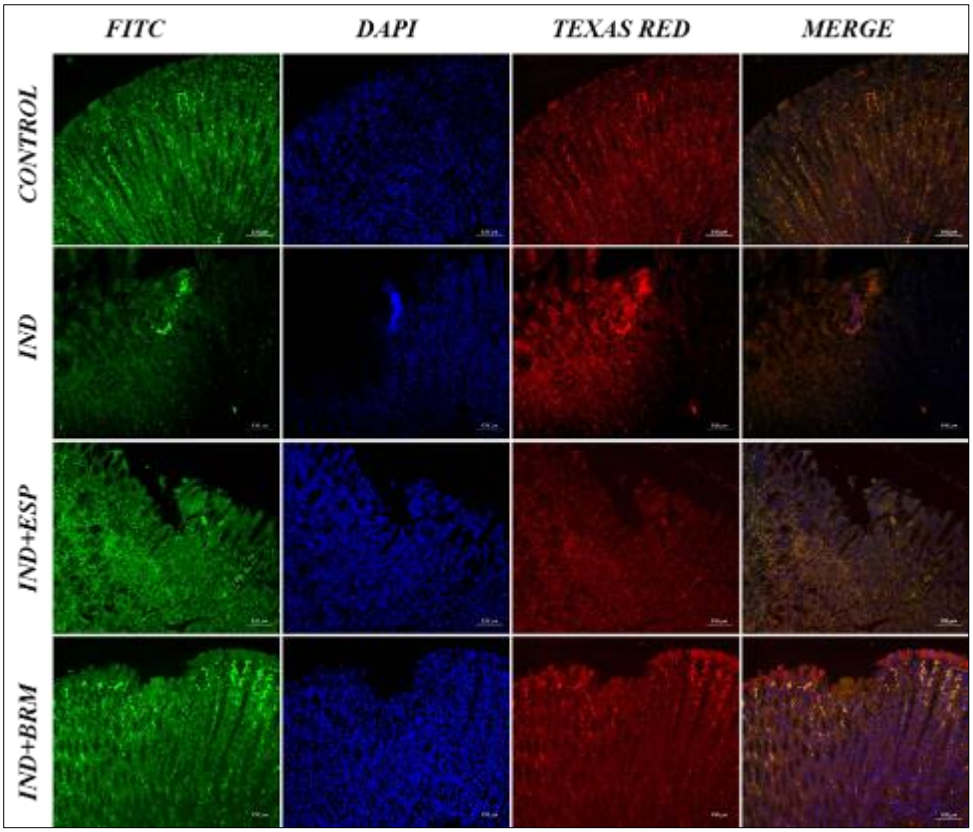


**Figure 3** Double immunofluorescence labeling and merged images in stomach tissue Nrf2 expression (FITC), HO-1 expression (Texas Red), D-IF, Bar:100µm. Control group showed negative expression. IND showed severe Nrf-2 and HO-1 expressions. IND+ESP, IND+BRM showed mild expression of Nrf-2 and HO-1. 4',6-diamidino-2-phenylindole (DAPI), fluorescein isothiocyanate (FITC), IND: indomethacin, ESP: esomeprazole, BRM: Bromelain

**Table 2** Scoring of immunohistochemical and immunofluorescent findings

| | **Control** | **IND** | **IND+ESO** | **IND+BRM** |
|---|---|---|---|---|
| IL-33 | 19,28±0,14$^{a}$ | 98,12±3,64$^{b}$ | 53,74±3,68$^{c}$ | 52,44±2,56$^{c}$ |
| Nrf-2 | 105,14±3,47$^{a}$ | 34,26±1,51$^{b}$ | 68,74±3,12$^{c}$ | 64,18±3,5$^{c}$ |
| HO-1 | 99,12±3,74$^{a}$ | 26,74±1,24$^{b}$ | 59,18±2,12$^{c}$ | 61,26±2,75$^{c}$ |

Data shown for ulcer scores are mean ± SE (n=7 rats/group). a,b,c; Different letters on the same line represent a statistically significant difference. ($p<0.05$)

### 3.3. Biochemical results

*3.3.1. Oxidant, antioxidant and neutrophil infiltration parameters on gastric tissue*

The oxidant and antioxidant parameter levels of rat stomach tissue induced by IND are given in Table 3. CAT activity was found to be statistically significant increased in all other experimental groups compared to the IND ($p<0.001$). Gastric tissue MPO activity was found to be higher in IND group when compared to other experimental groups, and higher in IND+ESP group compared to control ($p<0.01$). Compared with the IND, the GSH level was found to be significantly higher in the control, IND+ESP and IND+BRM groups ($p<0.001$). When the gastric GPx activities in our

study were compared, the GPx activity in control, IND+ESP and IND+BRM was significantly higher than the control ($p< 0.001$).

*3.3.2. ARE and PON-1 levels*

ARE and PON-1 levels of the IND that received ESP and BRM pretreatment was found to be significantly increased when compared to IND ($p< 0.01$). Also, it was determined that ARE activities of IND+ESP and IND+BRM were significantly lower than the control ($p< 0.01$), (Table 4).

**Table 3** Effects of Bromelain on oxidative stress markers in gastric tissue. Data are means ± SEM, n=7

| Groups/Parameters | Control | IND | IND+ESP | IND+BRM |
|---|---|---|---|---|
| ARE (U/mg protein) | 79333.91 ± 2988.70[a] | 14832.06 ± 1441.56[b] | 60368.95 ± 1500.73[c] | 71035.32 ± 2997.11[c] |
| PON (U/mg protein) | 5243.45 ± 710.99[a] | 1016.89 ± 25.72[b] | 3437.94 ± 443.50[a,c] | 4620.52 ± 284.67[a,c] |
| CAT (U/mg protein) | 728.90 ± 48.63[a] | 243.20 ± 33.76[b] | 605.11 ± 32.52[a,c] | 764.44 ± 29.67[a,c] |
| MPO (U/mg protein) | 6.28 ± 0.66[a] | 12.46 ± 1.03[b] | 9.66 ± 0.23[c] | 8.13 ± 0.14[a,c] |
| Total GSH (nmol/ mprotein) | 5.30 ± 0.23[a] | 1.38 ± 0.10[b] | 3.89 ± 0.27[c] | 4.66 ± 0.18[a,c] |
| GPX (U/ mg protein) | 8.79 ± 0.17[a] | 2.88 ± 0.37[b] | 6.44 ± 0.54[c] | 7.12 ± 0.44[a,c] |

a,b,c; Different letters on the same line represent a statistically significant difference ($p<0.001$). PON, paraoxonase; ARE, arylesterase; CAT, catalase; MPO, myeloperoxidase; GSH, glutathione; GSH-GPx, glutathione peroxidase. IND: indomethacin, ESP: esomeprazole, BRM: Bromelain. Data are presented as mean ± SD

## 4. Discussion

Gastric ulcer, which occurs as a result of the erosion of the inner surface of the stomach, especially results from the imbalance between damaging factors (hydrochloric acid, H. pylori and free radicals) and defense systems (1). In gastric ulcer, it is important to eliminate the damage to the gastric mucosa at the cellular level and maintain the oxidant/antioxidant balance. NSAIDs, which are frequently preferred for pain and inflammation, cause gastric reactions such as gastric ulceration, perforation and bleeding. IND, which is one of the NSAID, causes injury to the gastric mucosa by suppressing gastro-duodenal bicarbonate secretion, decreasing prostaglandin synthesis or increasing ROS and proinflammatory cytokines (16). The IND-induced ulcer model is widely used to evaluate the potential effects of pharmaceutics. At the cellular level, it is important to focus on ROS production in the design of new antiulcer drugs, because it plays the pivotal role in the pathogenesis of gastric ulceration. Bromelain has been accepted as a phytotherapeutic agent due to its safety and minimal side effects. It is also widely used for anti-inflammatory, antioxidant, anticancer activity, and immunomodulatory effects (17). In the present study, the potential gastro-protective effects of BRM against IND-induced gastric ulcers in rats were investigated (5).

Antioxidant enzymes are used as indicators of oxidative stress and are crucial in maintaining normal cell function and homeostasis. Rats exhibited an imbalanced oxidant/antioxidant status as apparent in depletion in enzymatic (CAT, GPx) and non-enzymatic antioxidants (GSH) in gastric tissue indicating the failure of antioxidant defense system. Plant extracts are considered critical steps in gastric tissue antioxidant levels and antioxidant defense system can be a possible target of BRM. Gastric tissue GSH level decreases due to ulceration, so mucosal injury may occur (18). Also, decreased CAT activity has been associated with impaired gastro-protection because of the function of it to protect cells from apoptosis (19). In different studies, it was stated that the addition of BRM protects tissue from oxidative stress and inflammation (10, 20). Similarly, in our study, increased CAT level in the treatment group indeed suggests possible gastro-protective properties of BRM. MPO enzyme is a lysosomal enzyme secreted from leukocytes in response to oxidative stress, but is also secreted during inflammatory condition (15). Active neutrophils produce MPO, cytokines, ROS and reactive nitrogen species that cause oxidative stress in gastric endothelial cells. Increased MPO level regressed with BRM treatment. At variance from MPO levels suggests a prognostic role and confirming that inflammation is an essential phenomenon in gastric ulcer. In a previous study, it was reported that ESP, a standard drug control, reduce oxidative stress, consistent with our results (6, 9, 10). The findings of our study are consistent with the studies revealing that BRM inhibits oxidative stress. There was no difference between ESP and BRM in terms of reducing oxidative stress.

The well-known common properties of PON1 and ARE are the functions as an intracellular protector against oxidative stress, the antioxidant effect on cell membranes and against lipid peroxidation, and the anti-inflammatory process (21). The increase in PON1 and ARE activity caused by IND in gastric tissue was prevented by BRM. In the ulcer group pre-treated with BRM, significantly reduced ulceration and near-normal histological structure were observed compared to the untreated ulcer group. Bromelain pretreatment showed a protective effect on the histopathological damage induced by indomethacin.

Inflammation is another important issue accompanied by increasing in ROS and plays an essential role in gastric ulcer pathogenesis. There is an increment in the production of activated neutrophils, pro-oxidative, pro-inflammatory enzymes, free radicals that cause oxidative stress, and also an increase in the levels of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin (IL) in gastritis and peptic ulcer (22, 23) . Recent research has been reported that gastric epithelial cells can secrete IL-33 and IL-33 can support mast cells to release pro-inflammatory mediators (24). Also, it has been shown to increase specifically in the mucosa of patients with ulcerative colitis (25). Studies suggest that proinflammatory cytokines has a vital role in ulcer development. Pretreatments with BRM significantly prevented the increase of IL-33 in IND group by inhibiting inflammatory response. Compared with both the normal control and standard drug used; the results of our study revealed the possible gastro-protective effects of BRM.

HO-1 catalyzes the degradation of heme and is one of the key defense mechanisms against oxidative damage. Downstream regulation of Nrf2 signaling involves the antioxidant enzyme HO-1, CAT and reduced GSH. In the present study, immunoblotting showed a decrease in the expression of Nrf2 and HO-1 in IND-exposed group as compared to control. Whereas, pretreatment with BRM increased the expression of Nrf2 and that facilitated the increase in the expression of HO-1 compared to the IND. It has been showed that HO-1 has a protective role against IND-induced gastric ulcer in rats through the Nrf2/HO-1 pathway. It has been reported that inflammatory factors (IL, CRP and TNF-α) are significantly increased in patients with gastric ulcer, and even elevated CRP levels are a positive predictive value (26). Therefore, in this study, IL-33 levels were evaluated, and it was determined that these inflammation factor increased significantly in the IND group compared to the control group.

In this study, increased inflammation parameters coupled with decreased antioxidant activities indicated that the protective ability of the stomach was reduced, thus causing tissue damage in IND-ulcerated rats. This finding points out that the ability of the gastric mucosa to counteract to the harmful effects of IND is decreased. Drugs that protect the mucus layer and prevent the progression or formation of the ulcer would promote the healing of gastric with antioxidant as well as anti-inflammatory effects. Pretreatment with BRM facilitated the ulcer amelioration process associated with reduced inflammation and increased antioxidant level. This highlights the important role of BRM at a dose of 100 mg/kg body weight in the ulcer healing process and performs a better ulcer healing capacity compared favorably with the reference drug used. On the whole, BRM can be evaluated as a pharmaceutical raw material in the prevention of stomach diseases and support new perspectives for nutraceutical applications. Preventing stomach diseases before they occur is of great importance in terms of increasing the quality of life. In this regard, herbal food supplements provide convenience in terms of both ease of use and cost.

## 5. Conclusion

The aim of this study was to investigate the effect of BRM on oxidant and inflammatory parameters in an IND-induced gastric ulcer model. For this reason, PON, ARE, CAT, MPO, GPx, GSH, IL-33, Nrf2 and HO-1 values were examined. We have shown that exogenous BRM treatment increases CAT, PON, ARE, GPx and GSH values and decreases MPO. The increased IL-33 and decreased Nrf-2 and HO-1 levels were obtained in IND group and these changes were reversed by BRM. In summary, BRM is a potential gastroprotective agent with important therapeutic effects, which can be used as an alternative to prevent gastric ulcer.

## Compliance with ethical standards

### *Disclosure of conflict of interest*

The authors have no relevant financial or non-financial interests to disclose.

### *Statement of ethical approval*

This study was approved by Atatürk University Experimental Animal Ethics Committee with the number (E-42190979-945-2200025420).

### *Data Availability*

We declare that all data supporting the findings of this study are available.

### *Author Contributions*

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed and the first draft of the manuscript was written by all authors. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

## References

[1] Cheng Y-T, Wu C-H, Ho C-Y, Yen G-C. Catechin protects against ketoprofen-induced oxidative damage of the gastric mucosa by up-regulating Nrf2 in vitro and in vivo. The Journal of nutritional biochemistry. 2013;24(2):475-83.

[2] Farzaei MH, Abdollahi M, Rahimi R. Role of dietary polyphenols in the management of peptic ulcer. World journal of gastroenterology: WJG. 2015;21(21):6499.

[3] Banerjee S, Hawksby C, Miller S, Dahill S, Beattie A, McColl K. Effect of Helicobacter pylori and its eradication on gastric juice ascorbic acid. Gut. 1994;35(3):317-22.

[4] Hudson N, Hawthorne A, Cole A, Jones P, Hawkey C. Mechanisms of gastric and duodenal damage and protection. Hepato-gastroenterology. 1992;39:31-6.

[5] Bhattacharyya BK. Bromelain: an overview. 2008.

[6] Chakraborty AJ, Mitra S, Tallei TE, Tareq AM, Nainu F, Cicia D, et al. Bromelain a potential bioactive compound: a comprehensive overview from a pharmacological perspective. Life. 2021;11(4):317.

[7] Hikisz P, Bernasinska-Slomczewska J. Beneficial Properties of Bromelain. Nutrients. 2021;13(12):4313.

[8] Park S, Hong H, Han Y, Kangwan N, Kim S, Kim E, et al. Nonsteroidal anti-inflammatory drugs (NSAID) sparing effects of glucosamine hydrochloride through N-glycosylation inhibition; strategy to rescue stomach from NSAID damage. J Physiol Pharmacol. 2013;64(2):157-65.

[9] Koc K, Cerig S, Ucar S, Colak S, Bakir M, Erol HS, et al. Gastroprotective effects of oleuropein and thymol on indomethacin-induced gastric ulcer in Sprague-Dawley rats. Drug and Chemical Toxicology. 2020;43(5):441-53.

[10] Ferah Okkay I, Okkay U, Bayram C, Cicek B, Sezen S, Aydin IC, et al. Bromelain protects against cisplatin-induced ocular toxicity through mitigating oxidative stress and inflammation. Drug and Chemical Toxicology. 2021:1-8.

[11] Wu J-Z, Liu Y-H, Liang J-L, Huang Q-H, Dou Y-X, Nie J, et al. Protective role of β-patchoulene from Pogostemon cablin against indomethacin-induced gastric ulcer in rats: Involvement of anti-inflammation and angiogenesis. Phytomedicine. 2018;39:111-8.

[12] Schafer KA, Eighmy J, Fikes JD, Halpern WG, Hukkanen RR, Long GG, et al. Use of severity grades to characterize histopathologic changes. Toxicologic pathology. 2018;46(3):256-65.

[13] Campo GM, Avenoso A, Campo S, Ferlazzo AM, Micali C, Zanghì L, et al. Hyaluronic acid and chondroitin-4-sulphate treatment reduces damage in carbon tetrachloride-induced acute rat liver injury. Life sciences. 2004;74(10):1289-305.

[14] Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med. 1967;70(1):158-69.

[15] Bradley PP, Priebat DA, Christensen RD, Rothstein G. Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker. Journal of investigative dermatology. 1982;78(3):206-9.

[16] Sinha K, Sil PC. Targeting oxidative stress and inflammation in NSAIDs induced gastropathy: a plausible therapeutic approach. Inflammation and Cell Signaling. 2015;2(2).

[17] Okkay IF, Okkay U, Karatas O. Effects of Rhodiola rosea on indomethacin-induced gastric injury. Dicle Tıp Dergisi. 2021;48(4):732-9.

[18] Alkushi AGR, Elsawy NAM. Quercetin attenuates, indomethacin-induced acute gastric ulcer in rats. Folia morphologica. 2017;76(2):252-61.

[19] Bhattacharyya A, Chattopadhyay R, Mitra S, Crowe SE. Oxidative stress: an essential factor in the pathogenesis of gastrointestinal mucosal diseases. Physiological reviews. 2014;94(2):329-54.

[20] Agarwal S, Chaudhary B, Bist R. Bacoside A and bromelain relieve dichlorvos induced changes in oxidative responses in mice serum. Chemico-Biological Interactions. 2016;254:173-8.

[21] Taler-Verčič A, Goličnik M, Bavec A. The structure and function of paraoxonase-1 and its comparison to paraoxonase-2 and-3. Molecules. 2020;25(24):5980.

[22] Rahman Z, Dwivedi DK, Jena GB. Ethanol-induced gastric ulcer in rats and intervention of tert-butylhydroquinone: Involvement of Nrf2/HO-1 signalling pathway. Hum Exp Toxicol. 2020;39(4):547-62.

[23] Yu L, Li R, Liu W, Zhou Y, Li Y, Qin Y, et al. Protective Effects of Wheat Peptides against Ethanol-Induced Gastric Mucosal Lesions in Rats: Vasodilation and Anti-Inflammation. Nutrients. 2020;12(8).

[24] Lv Y-p, Teng Y-s, Mao F-y, Peng L-s, Zhang J-y, Cheng P, et al. Helicobacter pylori-induced IL-33 modulates mast cell responses, benefits bacterial growth, and contributes to gastritis. Cell Death & Disease. 2018;9(5):457.

[25] Kobori A, Yagi Y, Imaeda H, Ban H, Bamba S, Tsujikawa T, et al. Interleukin-33 expression is specifically enhanced in inflamed mucosa of ulcerative colitis. Journal of Gastroenterology. 2010;45(10):999-1007.

[26] Boehme MWJ, Autschbach F, Ell C, Raeth U. Prevalence of silent gastric ulcer, erosions or severe acute gastritis in patients with type 2 diabetes mellitus--a cross-sectional study. Hepato-gastroenterology. 2007;54(74):643-8.