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# Therapeutic effect of bromelain and papain on intestinal injury induced by indomethacin in male rats

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## ABSTRACT

**Objectives:** Inflammatory bowel diseases (IBDs) are a multiple inflammatory status in small intestines and colon. Bromelain and Papain were cysteine proteases enzymes extracted from pineapple and papaya, and possess antioxidant and anti-inflammatory characteristics. Therefore, this comparative work aimed to examine the anti-inflammatory and antioxidant effect of bromelain and papain in intestinal inflammation of rats and to evaluate the most potent effect of both types of enzymes.

**Methods:** Forty rats were used in this study (8 rats/group), G1: control group, G2: (Indo group) intestinal inflammation was induced by two doses of Indomethacin (7.5 mg/kg body weight) apart 24 h. G3: (Indomethacin + Bromelain) intestinal inflamed rats treated by oral dose of bromelain (1000 mg/kg/day). G4: (Indomethacin + Papain) intestinal inflamed rats treated by oral dose of papain (800 mg/kg/day). G5: (Indomethacin + Sulfasalazine) intestinal inflamed rats treated by oral dose of sulfasalazine (500 mg/kg/day). Oxidative stress and inflammatory markers were measured along with histological assessment.

**Results:** Indomethacin-induced intestinal inflammation (in both Jejunum and Ileum) characterized by increased oxidative stress biomarkers: Xanthine oxidase, Catalase, Glutathione reductase, and Protein carbonyl and Inflammatory biomarkers: Tumor necrosis factor-α, Interleukin-10, Monocyte chemoattractant protein-1, Nuclear factor-kappa β, C-reactive protein, and Prostaglandin E2, as compared to control rats. On the other hand, administering either bromelain or Papain would effectively decrease symptoms of intestinal inflammation and modulate biomarkers of oxidative stress and pro-inflammatory cytokines.

**Conclusion:** Comparing results revealed that bromelain showed the most potent protective effect and possesses an apparent role in protection against the development of intestinal inflammation.

**Keywords:** Intestinal injury, bromelain, papain, indomethacin, sulfasalazine

## Introduction

The small intestine was considered as the main source of pro-inflammatory mediators, that affect the Systemic Inflammatory Response Syndrome. The breakdown of the intestinal wall leads to injury and intestinal inflammations.[1] Inflammation is a self-protective response against injury by modulating pro-inflammatory and anti-inflammatory components. Imbalances between pro- and anti-inflammatory components are among the key causes of intestinal inflammation like inflammatory bowel diseases (IBDs).[2] Normal inflammation benefits the body and may be quickly eliminated, but uncontrolled inflammatory reactions can harm tissue excessively or permanently, which can lead to acute or chronic inflammatory illnesses.[3]

IBDs is a multiple inflammatory status in small intestines and colon.[4] Among IBD are Crohn's disease (CD) and ulcerative colitis (UC). They have some properties and differ in clinical and genetic causes. CD frequently disturbs the intestinal wall and extends to various parts of the gastrointestinal (GI) tract, while UC mainly disturbs the lining mucosa of the colon and rectum.[5] UC and CD are multifactorial immune-mediated disorders characterized by chronic GI tract (GIT) inflammation.[6]

Increasing creation of pro-inflammatory cytokines in intestines was correlated with intestinal inflammation. The produced inflammatory cytokines considered as pathogenic reasons that harm intestinal permeability and epithelial barrier function.[7]

Indomethacin has been used as a non-steroidal anti-inflammatory drug (NSAID) that inhibits the biosynthesis of prostaglandin by cyclooxygenase-2 inhibitors. NSAIDs are one of the most frequently mentioned drugs in the world to

use against Inflammation and pain, possessing analgesic, anti-febrile, and anti-aggregatory activity.[8] Indomethacin is also used in treating rheumatic disease and acute attacks of gout.[9]

NSAIDs cause a risk of a serious GIT adverse side such as, cause serious mucosal injury to the upper GIT can limit the use. This adverse side may be less likely to occur if Indomethacin is taken over a comparatively short period. In rats, Indomethacin was used for induction of GI damage.[10]

Experimental studies with NSAIDs confirm the clinical data that NSAIDs not only cause damage to the upper GIT but also affect the lower GIT. However, in experimental studies gastric and intestinal injury continue to be investigated separately. In preclinical studies, Indomethacin is a widespread drug for producing both gastric and intestinal injury, but the experimental models used are different (regarding dose, experimental design, including fed or fasting animals).[11]

Bromelain is a proteolytic enzyme derived from the stem of the pineapple plant with different biological properties as well as anti-inflammatory action and platelet aggregation prevention.[12] It has a medical usage as an anti-inflammatory agent in colonic inflammation, arthritis, soft tissue injuries, chronic pain, and asthma.[13] The primary component of bromelain is a sulfhydryl proteolytic fraction. It is rich in protease inhibitors, peroxidase, acid phosphatase, several protease inhibitors, and calcium. The anti-inflammatory action of bromelain depends on the proteolytic activity.[13]

Papain, a cysteine protease isolated from the latex of papaya fruit, was used in the medical field. It is a proteolytic enzyme that has anti-bacterial, anti-inflammatory, and anti-oxidant properties.[14] In addition, it was used in wound healing and removing injured tissues.[15] The breakdown of difficult protein fibers into short-chain peptides and free amino acids, which are the building blocks for protein synthesis in all organs and tissues, is one of the digestive processes which papain plays a crucial part.[16]

This comparative study was designed to examine the anti-inflammatory and antioxidant effect of Bromelain and Papain in intestinal inflammation of rats and to evaluate the most potent effect of both types of enzymes.

## Materials and Methods

### Chemicals

Bromelain and Papain capsules were purchased from (NOW FOODS company, Glen Ellyn Rd. Bloomingdale, IL60188, USA). Sulfasalazine tablets were purchased from Suliman Faqeeh hospital pharmacy, Jeddah, Saudi Arabia.

### Diet formula

A standard nutritionally balanced diet was used in this study. The diet consists of the following ingredients: crude protein 20.0%, crude fat 4.0%, crude fiber 3.50%, vitamins mixture 1.0%, minerals mixture 3.50%, choline chloride 0.25%, and the diet energy equals 2850 kcal/kg. The diet is manufactured by Grain Silos and Flour Mills Organization, Jeddah, KSA.

### Experimental animals

Forty adult male Westar albino rats weighing 200–240 g obtain from the Animal House Colony of King Fahd Medical Research Center, Jeddah. Animals were kept in special cages at 20–22°C with 12 h light/dark cycle and humidity (60%) at King Fahd Medical Research Center Animal Facility Breeding Colony. A commercially balanced diet and tap water were given *ad libitum*.

### Experimental design

Rats were divided into five groups (eight/group):

Group 1: Healthy control (Negative control received normal saline daily 3 weeks).

Group 2 (Positive group): Intestinal inflammation was induced according to the procedure described by Tawfik *et al*.[17] Briefly, rats were administered two doses of Indomethacin (7.5 mg/kg body weight) apart 24 h.

Group 3 (Intestinal inflammation + Bromelain): Bromelain-treated rats, intestinal inflammation was induced as in group 2, then rats were treated by oral dose of bromelain at a concentration of 1000 mg/kg/day according to Hale *et al*.[18] for 3 weeks.

Group 4 (Intestinal inflammation + Papain): Papain-treated rats, intestinal inflammation was induced as in group 2, rats were treated by oral dose of papain at a concentration of 800 mg/kg/day for 3 weeks.

Group 5 (Intestinal inflammation + Sulfaslazine): Sulfasalazine treated rats, intestinal inflammation was induced as in group 2, rats were treated by oral dose of sulphaslazine at a concentration of 500 mg/kg/day according to Tawfik *et al*.[17] for 3 weeks.

### Anesthesia and collection of blood samples

At the end of the experimental period (3 weeks), rats were fasted overnight, anesthetized with diethyl ether, and blood was collected through retro-orbital venous and heart. Serum was separated by allowing the blood samples to stand for 30 min. at room temperature then centrifuged at 3000 rpm for 20 min. Serum samples were divided into several aliquots and stored at −20°C until analysis was performed. The small intestinal was carefully removed, washed with saline, and weighed.

### Histological assessment

Small intestines samples were set in 10% formalin solution. Then, sectioned and prepared using paraffin blocks. Small

intestines sections were stained with hematoxylin and eosin (H&E) and evaluated by a pathologist at magnification power (×40).

### Biochemical analyses

Oxidative biomarkers including Catalase (CAT), Reduced glutathione (RG), Protein Carbonyl (PC), and Xanthine oxidase (XO) together with inflammatory biomarkers including: Interleukin-10 (IL-10), Prostaglandin E2 (PGE2), Tumor Necrosis Factor-α (TNF-α), Nuclear factor-kappa β (NF-kβ), C-reactive protein (CRP), Monocyte Chemoattractant Protein-1 (MCP-1), and Myeloperoxidase (MPO) were assessed measured using enzyme-linked immunosorbent assay kit according to the manufacturer instructions.

### Statistical analysis

Data were statistically analyzed using SPSS computer Program. The results were presented as mean ± standard error and percentage change versus control. The differences between mean values were determined by analysis of variance (ANOVA test), ($P \leq 0.05$).

## Results

This study underlined the medicinal effect of bromelain and papain on intestinal injury caused by indomethacin in experimental animals, through several biochemical investigations as oxidative stress biomarkers: CAT, RG, PC, XO together with inflammatory biomarkers including: IL-10, PGE2, TNF-α, NF-kβ, CRP, Monocyte Chemotactic protein (MCP) and MPO.

As indicated in Table 1, indomethacin-induced intestinal inflammation manifested as significant ($P \leq 0.05$) elevation in XO, CAT, RG, and PC activities by 200%, 72.2%, 124%, and 308.4%, respectively, compared to the healthy control group. While, treating rats with either Bromelain (Indo+Bro) or Papain (Indo+Pap) reduced the levels of oxidative stress biomarkers XO, CAT, RG, and PC when compared to Indomethacin-treated group (Indo). Moreover, comparing treatment groups: Bromelain (Indo+Bro) or Papain (Indo+Pap), show a significant ($P \leq 0.05$) change in XO, CAT, and PC between these two groups. While no significant change was recorded paring results of RG between Bromelain (Indo+Bro) or Papain (Indo+Pap) groups. Moreover, treating rats with Sulfasalazine (Indo+Sulf), induced a significant ($P \leq 0.05$) change when compared to the control group in results of XO, CAT, and PC by 43.5%, 33.2%, and 108.5%, respectively, but no significant change was observed in (Indo+Sulf) group regarding RG results when compared to healthy control group by 0.4%.

Table 2 represents the action of bromelain, papain, and sulfasalazine on inflammatory cytokines (IL-10, TNF-α), Monocyte Chemotactic Protein, and PGE in all experimental groups. Indomethacin-induced intestinal inflammation manifested as significant ($P \leq 0.05$) elevation in IL-10, TNF-α, MCP-1, and PGE2 activities by 100%, 452%, 85%, and 2.4%, respectively, compared to healthy rat group. While, treating rats with either Bromelain (Indo+Bro) or Papain (Indo+Pap) reduced the levels of inflammation markers cytokines in IL-10, TNF-α, MCP-1, and PGE2 when compared to Indomethacin-treated group (Indo). Moreover, comparing treatment groups Bromelain (Indo+Bro) or Papain (Indo+Pap) show a significant change in IL-10, TNF-α, and PG between these two groups. Treating with sulfasalazine (Indo+Sulf) induced a significant ($P \leq 0.05$) change when compared to the control group in case of TNF-α, MCP-1, and PGE2 by 197%, 39.9%, and 165.7%, respectively.

The effect of bromelain, papain, and sulfasalazine on inflammation markers (Nuclear Factor-kappa β, CRP, and MPO) in all experimental groups was given in Table 3. Indomethacin induced an increase in NF-kβ, CRP, and MPO activities by 116%, 646.25%, and 106.3% respectively compared to the control group. While using either Bromelain (Indo+Bro) or Papain (Indo+Pap) in treating, reduced levels of NF-kβ, CRP, and MPO as compared to indomethacin-treated group (Indo). Furthermore, comparing treatment groups Bromelain (Indo+Bro) or Papain (Indo+Pap) show a significant ($P \leq 0.05$) change in NF-kβ, CRP, and MPO between the two groups. Treating with Sulfasalazine (Indo+Sulf) induced a ($P \leq 0.05$) change when compared to the control group in results of NF-kβ and CRP by 3.6% and 127.3%, respectively, but no significant ($P \leq 0.05$) change in (Indo+Sulf) group regarding MPO results when compared to the healthy control group by −3.8%.

### Histological examination

Examination of H&E-stained sections of the jejunum of rats of group I [Figure 1] showed that the wall of the jejunum is formed of four layers: mucosa, submucosa, muscularis externa, and serosa. The mucosa showed regularly arranged closely packed intestinal villi crypts. While in rats on group II [Figure 2] showed loss of the intestinal mucosal architecture in the form of large ulcer. There was a complete loss of the intestinal villi with their lining epithelium (columnar and goblet cells) together with the presence of a large area of loose connective tissue containing mononuclear inflammatory cells covering the heavily inflamed submucosa. On the other hand, rats in group III [Figure 3] exhibited better intestinal epithelial tissue similar to the control group. The presented area appeared with apparent decrease in the inflammatory cells' infiltrations. The lining epithelium exhibited the presence of goblet cells with their preserved mucin content. The improvement was evidenced by a significant reduction in the ulcerated areas. In addition, sections of rats in group IV [Figure 4] showed restoration of the histological architecture of the four layers of the jejunum. The mucosa showed intact architecture with

**Table 1:** The effect of bromelain, papain, and sulfasalazine on oxidative stress biomarkers in experimental groups

| Groups | Xanthine oxidase (ng/mL) | Catalase (ng/mL) | Reduced glutathione (ng/mL) | Protein Carbonyl (ng/mL) |
|---|---|---|---|---|
| Healthy control | 2.78±0.09 | 5.41±0.12 | 2.08±0.10 | 10.41±0.46 |
| Indo group | 8.34±0.21[a] | 9.32±0.17[a] | 4.66±0.10[a] | 42.52±1.60[a] |
| Percent change | 200% | 72.2% | 124% | 308.4% |
| Indo+Bro | 4.49±0.14[ab] | 6.97±0.13[ab] | 2.98±0.15[ab] | 25.21±1.0[ab] |
| Percent change | 62% | 28.8% | 43.2% | 142.2% |
| Indo+Pap | 5.52±0.32[abc] | 8.10±0.21[abc] | 3.17±0.14[ab] | 31.98±0.58[abc] |
| Percent change | 98% | 49.7% | 52.4% | 207% |
| Indo+Sulf | 3.99±0.19[abd] | 7.21±0.15[abd] | 2.09±0.11[bcd] | 21.71±0.48[abcd] |
| Percent change | 43.5% | 33.2% | 0.4% | 108.5% |

Indo: Indomethacin, Bro: Bromelain, Pap: Papain, Sulf: Sulfasalazine. [a]Significance versus Control, [b]Significance versus Indo, [c]Significance versus (Indo+Bro), [d]Significance versus (Indo+Pap)

**Table 2:** The effect of Bromelain, Papain and Sulfasalazine on inflammation markers cytokines in experimental group

| Groups | Interleukin-10 (pg/mL) | Tumor necrosis factor-α (ng/mL) | MCP-1 (ng/L) | Prostaglandin E2 (ng/mL) |
|---|---|---|---|---|
| Healthy control | 85.57±2.44 | 23.00±0.92 | 94.700.±0.76 | 0.76±0.03 |
| Indo group | 171.16±5.81[a] | 126.98±2.88[a] | 176.13±2.19[a] | 2.57±0.10[a] |
| Percent change | 100% | 452% | 85% | 2.4% |
| Indo+Bro | 105.01±2.39[ab] | 86.21±1.06[ab] | 140.05±2.31[ab] | 1.62±0.12[ab] |
| Percent change | 22% | 274% | 47% | 113.2% |
| Indo+Pap | 136.16±1.81[abc] | 93.55±1.21[abc] | 155.35±2.22[ab] | 1.87±0.12[abc] |
| Percent change | 59% | 306% | 64% | 146.1% |
| Indo+Sulf | 91.37±2.19[bcd] | 68.41±1.96[abcd] | 132.50±0.56[abcd] | 1.26±0.09[abcd] |
| Percent change | 7% | 197% | 39.91% | 65.7% |

Indo: Indomethacin, Bro: Bromelain, Pap: Papain, Sulf: Sulfasalazine. [a]Significance versus control, [b]Significance versus Indo, [c]Significance versus (Indo+Bro), [d]Significance versus (Indo+Pap)

**Table 3:** The effect of bromelain, papain, and sulfasalazine on inflammation markers in the experimental group

| Groups | Nuclear factor-kappa β (ng/mL) | C-reactive protein (ng/ml) | Myeloperoxidase (ng/mL) |
|---|---|---|---|
| Healthy control | 1.37±0.03 | 6.40±0.42 | 13.67±0.65 |
| Indo group | 2.96±0.30[a] | 47.76±2.4[a] | 28.20±0.21[a] |
| Percent change | 116% | 646.25% | 106.3% |
| Indo+Bro | 1.57±0.31[ab] | 15.25±0.82[ab] | 14.28±0.74[b] |
| Percent change | 14.6% | 138.3% | 4.5% |
| Indo+Pap | 1.61±0.31[abc] | 26.12±1.6[abc] | 16.53±0.63[abc] |
| Percent change | 17.5% | 308.1% | 20.9% |
| Indo+Sulf | 1.42±0. 29[bcd] | 14.55±0.64[ab] | 13.15±0.34[bd] |
| Percent change | 3.6% | 127.3% | −3.8% |

Indo: Indomethacin, Bro: Bromelain, Pap: Papain, Sulf: Sulfasalazine. [a]Significance versus control, [b]Significance versus Indo, [c]Significance versus (Indo+Bro), [d]Significance versus (Indo+Pap). ($P \leq 0.05$)

intact regularly arranged closely packed long villi and crypts that were lined by continuous surface columnar epithelium as compared with the sulfasalazine group. Intraepithelial neutrophils were also seen and many mitotic figures. Few inflammatory cells were seen in the lamina propria. The submucosa appeared nearly normal. The muscularis externa was seen clear with its inner circular and outer longitudinal smooth muscle fibers. There was an apparent significant recovery of intestinal histology. Similarly, stained sections of jejunum of rats in group V [Figure 5] showed almost all the mucosal architecture was regenerated with regularly arranged intestinal villi and crypts except few small areas that showed irregular or loss of their crypts and their lining epithelium (columnar and goblet cells). The lamina propria underlying the epithelium in between the crypts still showed infiltration with inflammatory cells, mainly lymphocytes. Submucosa showed few inflammatory cell infiltration. Muscularis Externa appeared nearly normal.

## Discussion

IBD is a chronic and progressive inflammatory condition of the colon and small intestine.[19] NSAIDs, such as an Indomethacin, have damaged the GIT as a serious adverse effect. Recent advances in diagnostic methods have shown that mucosal breaks of the small intestine are high prevalence

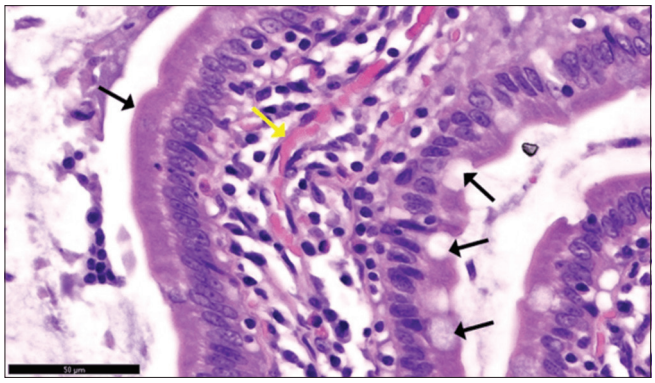

**Figure 1:** Control rat group, (a): Photomicrograph of a section of higher magnification of a finger-like projection of the corium of the jejunum and the covering intestinal epithelium. Villi are covered with a simple columnar epithelium composed of absorptive enterocytes (red↑) and goblet cells (black↑). The core of the villous is formed of lamina propria (LP) that contains central lacteals (yellow ↑)

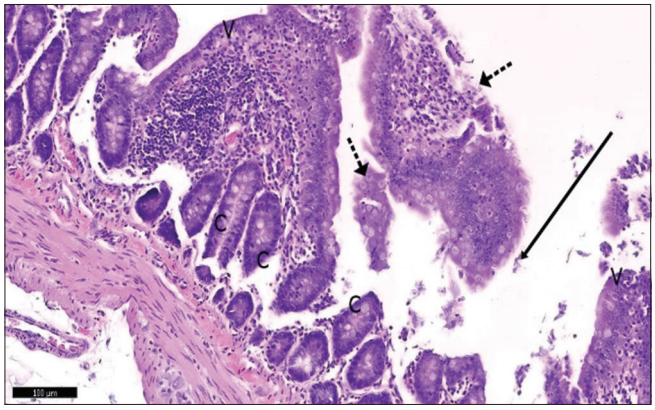


**Figure 2:** Positive Control Indo group, Higher magnification of the previous section of the of the jejunum nearby the ulcer showing diffuse inflammatory cell infiltrates (*) in the core of the villous with flattened epithelium and villous (v) blunting with multiple vacuolated areas (*). Notice absent goblet cells only columnar epithelium with pyknotic nuclei (↑) dilated congested blood vessels (BV) in the submucosa

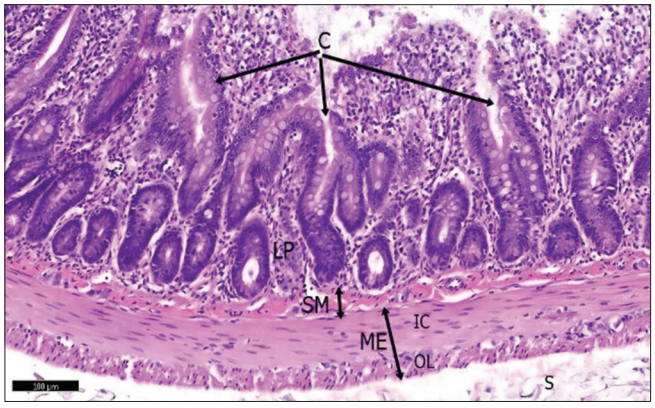


**Figure 3:** Indomethacin + Bromelain rat group, Photomicrograph of a section in the jejunum of a male albino rat of bromelain treated group (group III), showing healthy normal intestinal, also there is a regularly arranged closely packed villi (v) lined by surface columnar cells with basal oval nuclei (black ↑) and goblet cells (red ↑). Notice many mitotic figures (Dot ↑)

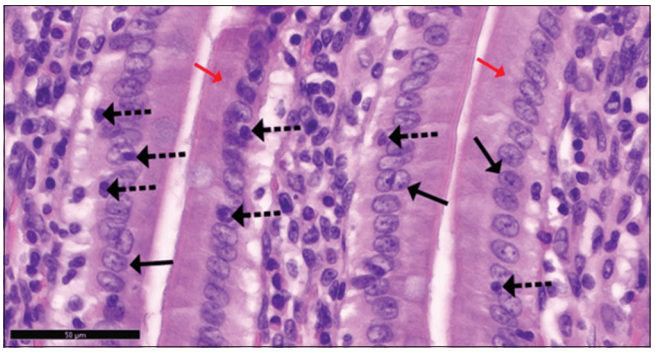

**Figure 4:** Indomethacin + Papain rat group, Photomicrograph of a section in the jejunum showing regularly arranged closely packed villi lined by surface columnar cells with basal oval nuclei (red↑). Notice intraepithelial neutrophils (Dot ↑) are also seen and many mitotic figures (black ↑)

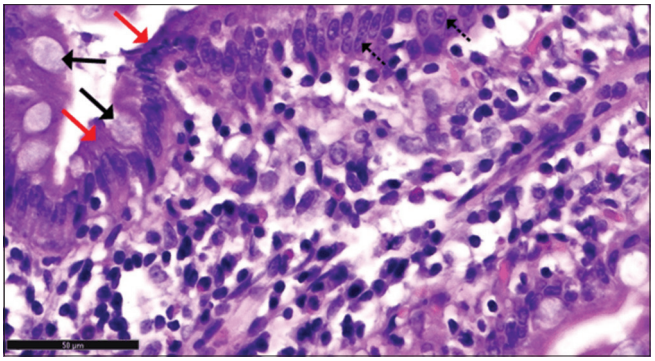

**Figure 5:** Indomethacin + Sulfasalazine rat group, (a): photomicrograph of a section in the jejunum of a male albino rat showing regularly arranged closely packed villi lined by surface columnar cells (black ↑) with basal oval nuclei and numerous goblet cells (red↑). Villi and few goblet cells (↑) H&E (×40)

in patients receiving NSAIDs.[20] In addition, Cheung *et al*.[21] reported that, a small intestinal injury was induced at a dose of Indomethacin 200 mg/kg of body weight.

In this study, the effect of Indomethacin on oxidative stress parameters was similar to findings achieved previously by Abdel-Hamed *et al*.[22] which reported that, the production of oxidative stress in rat small intestine as a response to the Indomethacin administration. Similarly, Shu *et al*.[23] showed that oxidative stress was induced by administering rats Indomethacin (1.5 mg/kg.b.w) for 14 days consecutive days. Moreover, Basivireddy *et al*.[24] indicated increased activity of xanthine oxidase (XO) following Indomethacin administration. In the same manner, results agreed with previous work study by Basivireddy *et al*.[25] work who reported that the administration of indomethacin 5% w/v for 5 days, to induce UC in male Westar rats, caused a significant elevation in colonic XO content compared to the normal control group.

It was clear that antioxidants suppress excessive oxidative stress by reacting with free radicals and scavenging reactive oxygen species (ROS).[21] Among antioxidant systems in the body is glutathione reductase, which plays a key role in

controlling the redox state of the cell by acting as a scavenger of reactive oxygen.[26]

The study showed that administering bromelain, papain, and sulfasalazine as treatments reduced the elevated levels of oxidative stress biomarkers. Bromelain is a phytotherapeutic drug with efficient characteristics and low side effects.[4] Mekkawy *et al*.[27] proved that the presence of sulfhydryl groups in bromelain, consequently accounting for its antioxidant activity, thus it could act as ROS scavenger. In agreement with the present results, Kalaiselvi *et al*.[28] reported the antioxidant role of ethanolic extract of *Ananas comosus* peels and improvement of oxidative status by quenching and detoxifying the radicals, due to the recognized Bromelain activity in *A. comosus*. Likewise, bromelain has an immense antioxidant effect in ameliorating the renal toxicity induced by dichlorvos.[29]

Papain is known to reduce oxidative stress by scavenging damaged and oxidized proteins and breaking them down.[16] A study by Mehdipour *et al*.[30] showed that ripe papaya juice is considered as efficient scavenger of free radicals that formed during exposing to radiation, furthermore, the green papaya, which is rich in papain, is used for healing inflamed ulcerations. Furthermore, a previous study by Mehdipour *et al*.[31] indicated the neuroprotection role of papain through antioxidant and anti-glutaminergic mechanisms. While, Olagunju *et al*.[32] observed that the aqueous seed extract of the unripe mature fruits of *Carica papaya* has nephro-protective, due to its antioxidant or oxidative free radical scavenging activities.

Results represented that, Indomethacin administration induced small intestinal damage as NSAIDs through inhibiting cyclooxygenase and subsequent deficiency of prostaglandins, which plays an important role in the mucosal defense system.[33] In addition, Numerous mediators, including oxidative stress caused by the generation of reactive oxygen metabolites, neutrophil infiltration, and the release of pro-inflammatory cytokines, were linked to the pathophysiology of IBD.[34]

A histopathological study by Ismail *et al*.[35] proved that increased levels of both TNF-α and PGE2 caused epithelial cell necrosis, edema, and neutrophil infiltration, the increased levels of PGE2 is attributed to its enhanced synthesis rather than reduced catabolism, both of which are mediated by TNF-α.

The study results agreed with Tawfik *et al*.[17] who administered Indomethacin (7.5 mg/kg) by 24 h apart to induce IBD, they showed a significantly increased serum levels of CRP and TNF-α compared to the normal control group. Furthermore, previous findings[36] stated that, Indomethacin administration to fasted rats induced small intestinal damage with an increase in the expression of TNF-α in both rats and mice.[37] Moreover, it agreed with Shu *et al*.[23]who reported that levels of MPO and TNF-α were significantly increased by administering Indomethacin (1.5 mg/kg.b.w) for 14 consecutive days with reduced levels of PGE2 content. Similarly, Nagarjun *et al*.[38] proved that using Indomethacin in a dose of (7.5 mg/kg) induced IBD accompanied by an elevation in MPO content in rat ileum compared to normal control which is reported to be an index of neutrophil infiltration and inflammations. Results agreed with Martinez-Fierro *et al*.[39] who reported increased serum levels of CRP and TNF-α in IBD patients following treatment with NSAIDs.

In the same context, the beneficial effects of bromelain, papain, and sulfasalazine were confirmed in counteracting inflammatory biomarkers. Proteolytic enzymes such as bromelain, papain, pancreatin, trypsin, chymotrypsin, and rutin are essential regulators and modulators of the inflammatory response.[16]

Papain was described as anti-inflammatory enzymes that accelerate tissue regeneration and improved recovery and healing of skin wounds and ulcers.[12] According to Amazu *et al*.[40] papaya seeds may act as a potential antioxidant and anti-inflammatory compounds by reducing the production and expression of cytokines and modulation of transcription factors. This is presumably because papaya seed extract is rich in papain, flavonoids, and phenolic acids.[41]

Temporarily, oral administration of bromelain decreases inflammation of the colon in animals with active (IBD). Bromelain supplementation also significantly reduced inflammations in test animals with established IBD.[42] In addition, bromelain has actions involving other enzyme systems in exerting its anti-inflammatory effect on soft tissue injury. Moreover, treatment with bromelain inhibited the growth and proliferation of oral cancer cells. These findings suggest that Bromelain may be applied as anti-inflammatory and a chemotherapeutic reagent of oral cancer.[43]

Sulfasalazine is widely used in the management of IBDs and rheumatoid arthritis in humans. Currently, anti-inflammatory agents such as sulfasalazine is the approved drugs for the treatment of IBD.[34] Sulfasalazine is mainly used for the treatment of IBD, including UC and CD. This effect of sulfasalazine was explained by many previous studies and was attributed to its inhibitory effect on various proinflammatory mediators released by the mucosa, including ROS, IL-1, TNF-α, and decreased immunoglobulin production by plasma cells. Besides, it was found that SFZ has analgesic effects against rheumatoid arthritis and diabetic neuropathic pain. Liu *et al*.[44] reported that treatment by Sulfasalazine repaired UC injury in experimental rats, who received 5% dextran sodium sulfate in distilled water for 5 days, which reduced the levels of malondialdehyde, TNF-α, and IL-10. Our study agreed with previous work[35] which found that sulfasalazine (in a dose of 2 mg/mL) was effective in lowering the oxidative stress, inflammation, and immune response biomarkers, such as MPO, NF-kB, TNF-α, and PGE2.

Then histology associated with IBD includes inflammation of the intestinal mucosa with neutrophil and other inflammatory

cell infiltration. As reported by Klahan and Pimpimol,[17] Intestinal brush border membranes had structural and functional deterioration as a result of oxidative stress related to enteritis brought on by indomethacin, and enterocytes with villus tip cells experienced mitochondrial malfunction. In the meantime, sulfasalazine-treated rats' histological testing demonstrated repair of the distinctive mucosal folds. In the same manner, treatment with sulfasalazine (500 mg/kg) significantly attenuated the extent and severity of the histological features of cell damage. In addition, a previous study by Liu *et al*.[45], Jebur *et al*.[46] showed that Bromelain attenuates the abnormal architecture of testes in aluminum chloride-exposed rats.

## Conclusion

The study revealed that long-term using of Indomethacin in increased concentrations causes Intestinal inflammation (of both Jejunum and ileum) while administering either bromelain or Papain would effectively decrease symptoms of intestinal inflammation and modulate biomarkers of oxidative stress and proinflammatory cytokines. Comparing results revealed that, bromelain showed the most potent protective effect and possesses a promising role in protection against the development of intestinal inflammation.

## Authors Declaration Statements

### Ethics approval and consent to participate

This study was approved by the ethics committee of Faculty of Medicine at King Abdulaziz University (Reference No 185-19) Animal Study.

### Availability of data and materials

The data sets used in this study are available with the corresponding author and will be provided on a reasonable request.

### Competing interest

The authors have no conflict of interest to declare.

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