Gastroprotective Effects of Propolis Against Ibuprofen-induced Gastric Injury: Ulcer Index and Histopathological Evaluation in Wistar Rat Model

Mulyati Sri Rahayu[1*], Nora Maulina[2], Cut Sidrah Nadira[2], Sri Wahyuni[3]

[1] Department of Anatomical Pathology, Faculty of Medicine, Universitas Malikussaleh, North Aceh, Indonesia
[2] Department of Physiology, Faculty of Medicine, Universitas Malikussaleh, North Aceh, Indonesia
[3] Department of Biochemistry, Faculty of Medicine, Universitas Malikussaleh, North Aceh, Indonesia
mulyati.srirahayu@unimal.ac.id

Abstract. Non-steroidal anti-inflammatory drugs (NSAIDs), particularly ibuprofen, commonly induce gastric mucosal injury through cyclooxygenase inhibition, oxidative stress, and inflammation. Propolis, a natural product rich in polyphenols, possesses antioxidant and anti-inflammatory properties that may protect against gastric damage. This study investigated the gastroprotective effects of propolis in male Wistar rats with ibuprofen-induced gastric ulcers. Thirty rats were randomly divided into five groups (n = 6): normal control, ibuprofen-treated control (400 mg/kg/day), and three propolis pretreatment groups receiving 0.13, 0.26, or 0.52 mL/day before ibuprofen administration. Gastric injury was evaluated using the ulcer index and histopathological examination of gastric mucosal damage. Data were analyzed using the Kruskal–Wallis test, followed by Bonferroni-adjusted post hoc comparisons. Ibuprofen caused severe gastric injury, characterized by the highest ulcer index (16.50), epithelial erosion, and inflammatory cell infiltration. Propolis pretreatment reduced the ulcer index in a dose-dependent manner, with the lowest value observed in the 0.52 mL group (15.60). Histopathological analysis showed significantly lower gastric mucosal injury scores in the 0.26 and 0.52 mL groups than in the ibuprofen-treated control ($p < 0.05$). However, no significant differences were observed among propolis-treated groups. Propolis provided partial gastroprotection, although the tested doses did not achieve optimal efficacy.

Keywords: Propolis; Ibuprofen; Ulcer index; Gastroprotection; Histopathology; Wistar rats.

1 Introduction

Peptic ulcer disease (PUD), also referred to as a gastric or peptic ulcer, is characterized by disruption of the gastrointestinal mucosa that extends through the mucosal and submucosal layers and, in more severe cases, into the muscularis propria, resulting from

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exposure to gastric acid and pepsin [1]. Histopathologically, peptic ulcers are defined by erosion of the gastrointestinal mucosa caused by the corrosive effects of gastric acid and pepsin, with progressive extension beyond the epithelial layer into the muscularis propria of the stomach. PUD most commonly affects the stomach and proximal duodenum, although lesions may also develop in the distal esophagus, distal duodenum, or jejunum [2]. The prevalence of gastric ulcers varies worldwide depending on socioeconomic and demographic factors. In the United Kingdom, the prevalence has been reported to range from 6% to 20%, with an estimated prevalence of 2–4% among individuals aged ≥55 years. In the United States, the prevalence of gastric ulcers is estimated to be 12% in men and 10% in women. This condition is responsible for approximately 15,000 deaths each year and imposes an annual healthcare cost of up to US$10 billion. In Indonesia, the mortality rate associated with gastric ulcers was reported to be 0.8% in 2018 but increased substantially to 14.95% in 2020 [3].

PUD often remains asymptomatic, leading to delayed diagnosis and potentially life-threatening complications, such as perforation. Mortality rates for perforated peptic ulcers vary widely, from 1.3% to 20%, with risk factors including nonsteroidal anti-inflammatory drug (NSAID) use, Helicobacter pylori infection, smoking, and corticosteroid use [4]. Ibuprofen is among the most commonly used NSAIDs because of its availability and efficacy as an antipyretic, analgesic, and anti-inflammatory agent. However, it increases the risk of peptic ulceration by non-selectively inhibiting COX-1 and COX-2. While COX-2 inhibition mediates its therapeutic effects, COX-1 suppression reduces gastroprotective prostaglandin synthesis, impairing mucosal defense and increasing gastrointestinal injury [5].

Propolis is a natural resin that is widely used in traditional medicine. It contains polyphenols, particularly flavonoids and phenolic acids, which have strong antioxidant and anti-inflammatory properties. Some studies have reported that propolis has significant anti-inflammatory and gastroprotective activities. Experimental studies have demonstrated that propolis can attenuate NSAID-induced gastric mucosal injury, including ulcers associated with analgesic and anti-inflammatory agents, such as paracetamol and mefenamic acid [6–8]. These findings suggest that propolis is a promising natural therapeutic agent for preventing and managing NSAID-induced peptic ulcers.

Although previous studies have shown that propolis administration reduces the number and severity of ulcers caused by ibuprofen induction [9], its protective effects on gastric mucosal integrity have not been comprehensively characterized. In addition, its effects on gastric mucosal integrity have not been fully evaluated using the ulcer index and microscopic tissue damage using histopathological assessment. Therefore, this study aimed to evaluate the gastroprotective effect of propolis against ibuprofen-induced gastric ulcers by assessing the ulcer index and histopathological changes in the gastric mucosa.

2 Methods

2.1 Experimental design

A true experimental laboratory study was conducted using a post-test-only control group design. The animals were randomly assigned to five groups: normal control, negative control, and three treatment groups receiving propolis at different doses.

2.2 Animal treatment

Animal studies were conducted in the Laboratory of Pharmacology at the Faculty of Pharmacy, Universitas Sumatera Utara, Indonesia. The inclusion criteria required male rats to be healthy and weigh 150–200 g on average. Thirty adult male rats were assigned to five groups of six animals each; group I served as the normal control and received standard pellet feed for 14 days. Group II served as the negative control and received ibuprofen at 400 mg/kg body weight/day for 7 days to induce gastric ulcer formation. Groups III, IV, and V received propolis at doses of 0.13 mL, 0.26 mL, and 0.52 mL, respectively, for 7 consecutive days, followed by ibuprofen at 400 mg/kg body weight/day for the subsequent 7 days. On the fifteenth day, all rats were euthanized by cervical dislocation under anesthesia, and gastric organs were collected and prepared for macroscopic and histological examination. The sample size for each group was determined using the Federer formula. Animals were randomly allocated to the experimental groups using a simple randomization method.

2.3 Preparation of Propolis

The propolis branded ‘MP’, with a concentration of 150 mg/mL, was purchased from the market and used in this study. The standard propolis dose was 200 mg/kg body weight, and the propolis preparation was diluted with carboxymethylcellulose (CMC). The dose calculations for the experimental animals were based on a standard dose of 200 mg/kg BW. Thus, a 200 g rat requires 40 mg of propolis. At a concentration of 150 mg/mL, the administration volume was adjusted according to the treatment group: 0.13 mL for the P1 group (50% of standard dose), 0.26 mL for the P2 group (100% of standard dose), and 0.52 mL for the P3 group (200% of standard dose). Propolis was administered orally via gastric gavage.

2.4 Gastric ulcer index examination

The gastric ulcer index was evaluated by macroscopic examination of the gastric mucosa. Ulcer formation was assessed based on the number and severity of gastric lesions using the modified Gerhard scoring system, and the scores were used to calculate the gastric ulcer index. Gastric ulcer number score: 1 = normal gastric mucosa; 2 = hemorrhagic spots or one ulcer; 3 = 2–4 ulcers; 4 = 5–7 ulcers; 5 = 8–10 ulcers; 6 = >10 ulcers or perforation. Gastric ulcer severity score: 1 = normal gastric mucosa; 2 = hemorrhagic spots or ulcers with a diameter of 0.5 mm; 3 = ulcers with a diameter of 0.5–1.0 mm; 4

= ulcers with a diameter of 1.0–1.5 mm; 5 = ulcers with a diameter of 1.5–2.0 mm; 6 = ulcers with a diameter of >2.0 mm or perforation. Furthermore, the degree of ulcer formation was assessed using the ulcer index (UI) with the following equation [10]:

$$U = U\,N + U\,S + (U\,P \times 10 - 1)$$

Note :

U = Ulcer Index

U N = average number of ulcers per animal

U S = average ulcer severity score

U P = percentage of animals with ulcers in a group

2.5 Histopathological Examination of the Gastric

Gastric tissue samples were opened along the greater curvature and cross-sectioned, then fixed in 10% buffered neutral formalin (BNF) for at least 24 hours, and dehydrated in graded ethanol solutions of 70%, 80%, and 90%. Subsequently, clearing was performed using xylene thrice. The tissue was then infiltrated with paraffin, embedded, and cooled at room temperature. The resulting paraffin blocks were sectioned at 3-4 µm thickness using a microtome. Staining with hematoxylin was carried out for 8 minutes, then the slides were rinsed with running water, washed with lithium carbonate for 15-30 s, and rinsed with running water. Ultimately, the samples were stained with eosin for 2-3 min, washed with running water, and then dried. The degree of gastric epithelial damage in mice was assessed at 200× magnification using a light microscope, using semi-quantitative grading according to histopathological criteria for gastric mucosal injury [11].

Gastric ulcer index and histopathological assessments were performed using a double-blind protocol and subsequently validated by two independent pathologists. Inter-rater agreement was assessed to ensure scoring reliability, and the final dataset comprised the average scores derived from double-blind evaluations of gastric tissue damage.

Table 1. Histopathological criteria for gastric mucosal injury

0	Normal gastric mucosa with intact epithelial architecture and no evidence of epithelial erosion, degeneration, inflammatory cell infiltration, or other pathological alterations.
1	Mild injury: Focal epithelial damage involving a single site, characterized by mild epithelial erosion and/or epithelial degeneration with minimal inflammatory cell infiltration.
2	Moderate injury: Multifocal epithelial damage involving several sites, characterized by moderate epithelial erosion, degeneration, and inflammatory cell infiltration, affecting multiple areas of the gastric mucosa.

3	Severe injury: Diffuse gastric mucosal damage characterized by extensive epithelial erosion, widespread epithelial degeneration, and marked inflammatory cell infiltration throughout the gastric mucosa.

2.6 Ethical Approval

The Ethics Research Commission of the Faculty of Medicine, Universitas Malikussaleh approved all experimental procedures in this study (reference number: 13/UN.45.2.6/KEPK/2024, June 28, 2024).

2.7 Statistical Analysis

Data were analyzed using the Kruskal–Wallis test to assess differences in gastric mucosal injury scores among the groups, followed by a Bonferroni-corrected post hoc test for pairwise comparisons. Statistical significance was set at $p < 0.05$.

3 Results

The gastric ulcer index was assessed across all experimental groups based on ulcer formation. The normal group had the lowest ulcer index (2.00), indicating no or minimal ulcerative lesions. In contrast, the ibuprofen-induced negative control group had the highest ulcer index (16.50), indicating severe gastric injury. Propolis pretreatment showed a dose-dependent decrease in the ulcer index, from 16.00 at 0.13 mL to 15.83 at 0.26 mL and 15.60 at 0.52 mL (Figure 1).

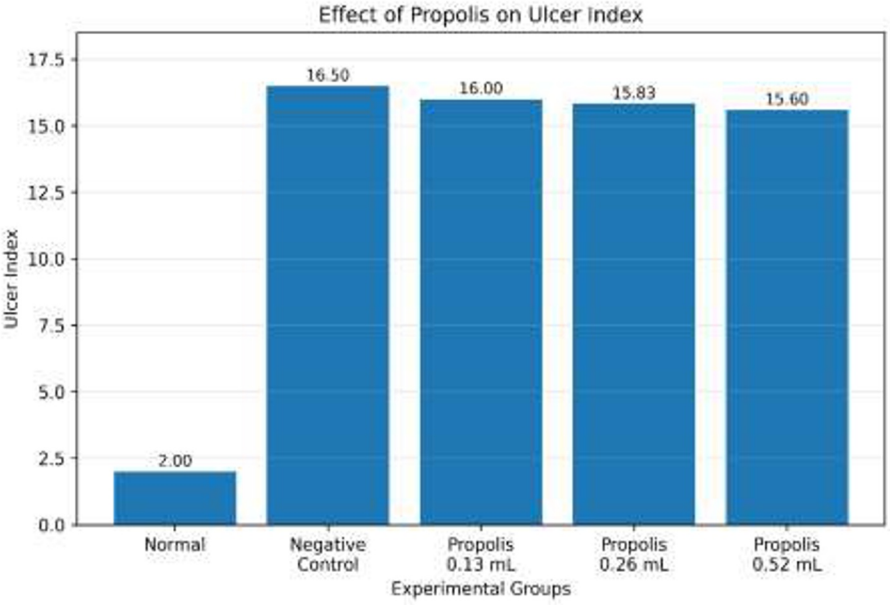


Fig. 1. The ulcer index between groups.

The analysis of the gastric mucosal injury score showed that differences among the groups were statistically significant (Kruskal–Wallis test, $p = 0.000$). Post hoc Bonferroni analysis revealed significant differences between the normal group and all other groups ($p < 0.05$). In addition, the 0.26 mL and 0.52 mL propolis groups had significantly lower gastric damage scores than the negative control group ($p = 0.038$ and $p = 0.022$, respectively). In contrast, no significant differences were observed between the negative control and the 0.13 mL group ($p = 0.664$) or among the propolis-treated groups ($p > 0.05$). These findings indicate that higher propolis doses were associated with reduced gastric histopathological damage, although the differences among the treatment groups were not statistically significant (Table 3).

Table 2. Differences in gastric damage score between groups.

Group	Median (IQR)	*p*-value
I (Normal)	0 (0)	
II (negative control)	2.00 (1.00)	
III (0.13 mL)	2.00 (0.25)	0.000*
IV (0.26 mL)	1.50 (1.00)	
V (0.52 mL)	1.00 (1.00)	

Notes: The Kruskal-Wallis test was used for comparisons between groups ($p = 0.001$); IQR = interquartile range.*Statistical significance was set at $p < 0.05$.

Table 3. Comparison of gastric damage scores across treatment groups.

Group	Comparison	S.E	*p*-value	95% CI	
				Lower Bound	Upper Bound
I	II	0.259	0.000*	-3.136	-1.529
	III	0.259	0.000*	-2.636	-1.029
	IV	0.259	0.000*	-2.303	-0.696
	V	0.272	0.000*	-2.242	-0.557
II	I	0.259	0.000*	1.529	3.136
	III	0.259	0.664	-0.303	1.303
	IV	0.259	0.038*	0.029	1.636
	V	0.272	0.022*	0.090	1.776
III	I	0.259	0.000	1.029	2.636
	II	0.259	0.664	-1.303	0.303
	IV	0.259	1.000	-0.470	1.136
	V	0.272	1.000	-0.409	1.276
IV	I	0.259	0.000	0.696	2.303
	II	0.259	0.038	-1.636	-0.029
	III	0.259	1.000	-1.136	0.471
	V	0.272	1.000	-0.742	0.942
V	I	0.272	0.000	0.557	2.242
	II	0.272	0.022	-1.776	-0.091
	III	0.272	1.000	-1.276	0.409
	IV	0.272	1.000	-0.942	0.742

Notes: A Bonferroni post-test was used to assess significant differences within each group; I = normal group; II = negative control; III = 0.13 mL; IV = 0.26 mL; V = 0.52 mL; CI = 95% confidence interval; * indicates a statistically significant difference (p-value < 0.05); S.E.: Standard Error.

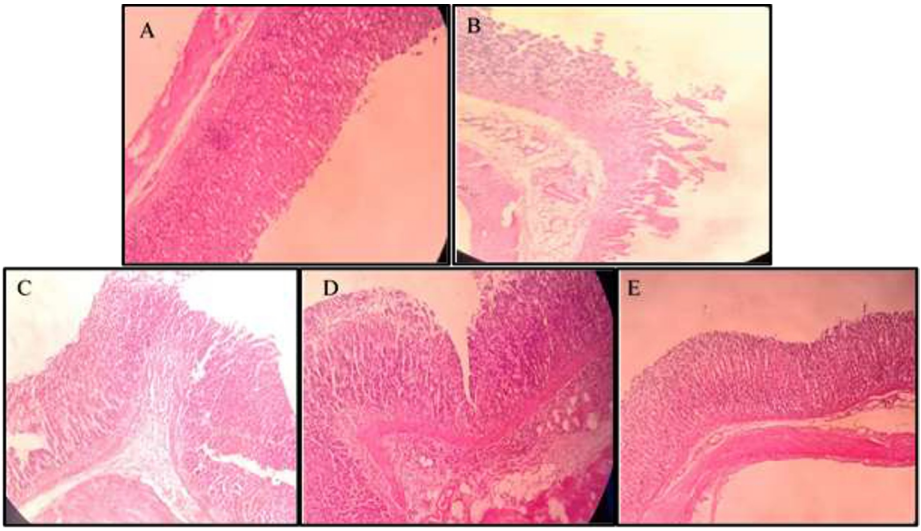


Fig. 2. (A) Gastric from the control group showed normal histological structure of gastric tissue; (B) the negative group showed extensive epithelial erosion accompanied by expansion of the submucosa with edema; (C) propolis at a dose of 0.13 mL showed multifocal areas of epithelial erosion and degeneration; (D) propolis at a dose of 0.26 mL showed focal epithelial erosion accompanied by minimal inflammatory cell infiltration; (E) propolis at a dose of 0.52 mL showed apparently normal gastric tissue with few inflammatory cells and mild submucosal edema.

4 Discussion

The present study demonstrated that prophylactic administration of propolis attenuated ibuprofen-induced gastric injury in male Wistar rats. Ibuprofen alone produced the highest ulcer index and severe histopathological damage, characterized by extensive epithelial erosion, submucosal edema, and inflammation cell infiltration. In contrast, pretreatment with propolis progressively reduced both macroscopic ulcer severity and microscopic mucosal injury with increasing administered doses. Histopathological improvement was statistically significant at 0.26 mL and 0.52 mL compared with the ibuprofen-treated group, whereas no significant differences were observed among the propolis-treated groups. These findings suggest that propolis protects the gastric mucosa; however, the doses used in the present study may not be within the optimal therapeutic range.

Ibuprofen is a non-selective cyclooxygenase inhibitor that suppresses COX-1 and COX-2 activity. While COX-2 inhibition contributes to its anti-inflammatory effects, COX-1 inhibition markedly reduces the synthesis of gastroprotective prostaglandins, particularly prostaglandin E2 (PGE2). The resulting decrease in prostaglandin production reduces mucus and bicarbonate secretion, impairs mucosal blood flow, delays epithelial restitution, and increases the susceptibility of the gastric mucosa to acid-mediated injury [5,12]. In addition, ibuprofen promotes excessive generation of reactive oxygen species (ROS), mitochondrial dysfunction, neutrophil infiltration, and activation

of proinflammatory cytokines, ultimately resulting in epithelial erosion, edema, and inflammatory cell infiltration [11]. These mechanisms are consistent with the severe epithelial erosion and submucosal edema observed in the negative control group in the present study.

The gastroprotective effect observed in this study may be related to the antioxidant and anti-inflammatory properties of propolis reported in previous studies. However, this mechanism remains hypothetical in the present study because NF-κB activity and oxidative stress biomarkers were not directly assessed. The therapeutic properties of propolis are largely associated with its flavonoid- and phenolic-rich composition. These bioactive compounds exert antioxidant effects by neutralizing reactive oxygen species and reducing inflammation through suppression of the NF-κB signaling pathway. [13]. In addition, propolis promotes mucus secretion, improves gastric microcirculation, and supports epithelial regeneration, thereby preserving gastric mucosal integrity and reducing epithelial erosion and inflammatory cell infiltration [10,14]. These mechanisms may explain the reduced gastric epithelial damage observed in the propolis-treated groups.

The present study found that the ulcer index decreased only modestly from 16.50 in the negative control group to 15.60 in the highest-dose propolis group. In contrast, microscopic evaluation showed a substantial reduction in epithelial erosion and inflammatory infiltration. Macroscopic ulcer scoring primarily measures lesion number and surface dimensions. In contrast, histopathological examination detects early cellular degeneration, inflammatory responses, epithelial regeneration, and preservation of tissue architecture before substantial macroscopic healing becomes evident. The histopathological improvement across treatment groups further supports a dose-dependent biological response. The low-dose propolis group continued to show multifocal epithelial erosion and degeneration, comparable to that observed in ibuprofen-treated animals, indicating that this dose was insufficient to adequately counteract NSAID-induced mucosal injury. In contrast, animals receiving 0.26 mL of propolis showed only focal epithelial erosion with minimal inflammatory infiltrates. In contrast, those treated with 0.52 mL exhibited nearly normal gastric architecture with mild edema and sparse inflammatory cells. These findings suggest that increasing concentrations of bioactive polyphenols progressively enhance antioxidant capacity, suppress inflammatory responses, and preserve gastric epithelial integrity.

Despite the apparent dose-dependent trend, no statistically significant differences were observed among the propolis-treated groups. Several factors may explain this finding, including the duration of propolis pretreatment, which may have been insufficient to achieve maximal mucosal protection, and the natural variability in the chemical composition of propolis, which depends on the botanical origin and geographic location and may contribute to variability in biological efficacy [6,15,16]. Although complete protection was not achieved in this study, histopathological improvements at medium and high doses suggest that propolis has considerable potential as a natural adjunctive agent to prevent NSAID-associated gastric mucosal injury.

These findings align with previous studies demonstrating the gastroprotective effects of propolis in various ulcer models, including those induced by ethanol, indomethacin, aspirin, acetic acid, and stress. Most studies have reported reductions in ulcer area, lipid

peroxidation, inflammatory cytokine expression, and histopathological damage after propolis administration [6,8,17]. However, relatively few studies have specifically evaluated ibuprofen-induced gastric injury, despite its widespread clinical use of ibuprofen worldwide. Therefore, the present study extends the existing evidence by demonstrating that propolis protects against ibuprofen-induced gastric injury by reducing the ulcer index and histopathological damage.

5 Conclusion

This study demonstrated that propolis exerts a gastroprotective effect against ibuprofen-induced gastric injury in male Wistar rats. Pretreatment with propolis reduced the gastric ulcer index and significantly improved gastric histopathological architecture, particularly at doses of 0.26 mL and 0.52 mL, as evidenced by reduced epithelial erosion and inflammatory cell infiltration and preserved mucosal integrity. Although the reduction in the macroscopic ulcer index was modest and no significant differences were detected among the propolis-treated groups, microscopic evaluation revealed substantial protection of the gastric mucosa, indicating that histopathological assessment is more sensitive than macroscopic examination for detecting early gastroprotective effects. Nevertheless, further studies are required to characterize the chemical composition of the propolis preparation, investigate molecular mechanisms involving oxidative stress and inflammatory biomarkers, determine the optimal dose, and confirm its effectiveness in clinical studies.

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Disclosure of Interests. The authors declare no conflicts of interest.

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