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Gastroprotective Effect of Propolis Against Male White Mice Gastric Ulcers Induced by Aspirin

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ABSTRACT
A peptic ulcer is an inflammation of the stomach wall that can cause wounds in the gastric mucosa. Consumption of aspirin over therapeutic doses can cause injury to the stomach. Propolis is known to have an effect to protect stomach damage. This study aims to find out the effect of propolis on male white mice-induced peptic ulcers. A total of 20 mice were divided into 4 experimental groups. The first group was negative control, the second group was positive control (aspirin 350 mg/kg bodyweight for 5 days), the third group was given propolis 500 mg/kg bodyweight for 7 days, and the last group was given propolis then induced with aspirin. The preparations were administered orally. After the treatment, mice were sacrificed to obtain gastric tissue. The tissue was observed macroscopically and microscopically. The data were analyzed descriptively using the average of gastric ulcer index and histopathological analysis. Based on the results of the study, the administration of propolis followed by aspirin has a gastroprotective effect with an average ulcer index of 1, compared to the aspirin group with an ulcer index of 2.5. It can be concluded that the administration of propolis at a dose of 500 mg/kg body weight has a protective effect against male white mice gastric ulcers induced by aspirin.

Keywords: *Propolis, gastroprotective, gastric ulcer, aspirin*

1. INTRODUCTION

Non-communicable diseases (NCDs) are diseases that are not caused by infection with microorganisms. This type of disease is responsible for at least 70% of deaths in the world [1]. One of the most common NCDs is dyspepsia. Heirdarloo et al, in 2019 performed an endoscopy on 100 dyspeptic patients which showed that 54% had antral gastritis, 20% had peptic ulcer, 43% esophagitis, 18% duodenal ulcer, 15% prepyloric ulcer, and 5% gastric metaplasia [2]. Dyspepsia is defined as discomfort in the upper abdominal area. The discomfort can be in the form of one or more of the following symptoms: nausea, vomiting, epigastric pain, burning in the epigastrium, feeling of fullness after eating, and bloating in the upper gastrointestinal tract [3].

Gastric ulcer is an upper gastrointestinal disorder caused by the excess activity of gastric secretions; pepsin and HCl. In gastric ulcer, the gastric mucosa started to broken and continue to the bottom of the epithelial layer. The cause is an imbalance between aggressive factors and defensive factors that maintain the integrity of the gastric mucosa. Aggressive factors are gastric acid excreted by parietal cells, pepsin produced by zymogen cells, and back diffusion of hydrogen ions. While the defensive factors include the formation and secretion of mucus, bicarbonate secretion, mucosal blood flow, and epithelial regeneration. In addition, gastric ulcers can also be caused by NSAIDs, stress, alcohol, and Helicobacter pylori infection [4].

Non-steroidal anti-inflammatory drugs (NSAIDs) are the most widely used drugs for their analgesic, antipyretic, and anti-inflammatory effects worldwide[5]. This drug is known as an exogenous aggressive factor that can cause gastric mucosal damage, both locally and systemically. These gastric mucosal lesions are known as gastritis and ulcers[5].

Aspirin is one of the most used NSAID drugs with a lot of indications to treat pain, inflammation, and prevent cardiovascular disease due to the antiplatelet mechanism[6]. Further, aspirin is also available over the counter in our country. Long-term use of aspirin can affect the balance of aggressive and defensive factors of gastric mucosa causing dyspepsia to more severe condition like gastric ulcer and severe GI bleeding. Aspirin works by inhibit the Cyclooxygenase-1 (COX-1) and Cyclooxygenase-2 (COX-2) enzyme [4] [7] [5]. Inhibition of the COX-1 enzyme in the digestive track lowering the prostaglandin secretion that can reduced cytoprotective effect on the mucosa following to the mucosal damage [8], [9]. The damage characterized by the decreased integrity

of epithelial cells, hemorrhage in the mucosal lamina propria area, lysis and sloughing of the mucosa epithelial cells, the nucleus enlarges, and coagulated nuclear chromatin. The damage is usually accompanied by the release of epithelial cells in the mucosa. If the inflammatory process occurs continuously, the damage will extend to the muscular mucosae [6].

This is in line with a study conducted by Roosdiana, Yudandi, and Erika in 2018, which said that giving aspirin for five days at a dose of 200 mg/Kg bodyweight in white rats caused peptic ulcers [10]. In addition, in 2019 Suheryani et al also reported that administering aspirin for 14 days at a dose of 240mg/KgBB in white rats caused the gastric mucosa to become inflamed which was characterized by bleeding and inflammatory cell infiltration, as well as the presence of tissue necrosis [11].

Propolis is a substance produced by honey bees, containing resin and beeswax, which is sticky and collected from plants, especially on leaves and flowers to be mixed with bee saliva [12]. Propolis is a natural product from honey bees that has the high antioxidant potential [13]. Propolis is known to contain flavonoids which have been known as antioxidants to ward off free radicals that can cause cell damage. Propolis has the strongest antioxidant activity against free radicals compared to other bee products. In addition, propolis has benefits as an antibacterial, anti-inflammatory, antiviral, hepatoprotective effect, antitumor, vasodilator, and prevents ulcers [14]. Based on the study of Barros et al in 2007, reported on the gastric protective effect of rats from the hydroalcoholic extract of Brazilian green propolis. The results showed that green propolis extract displayed antiulcerogenic effects associated with a cytoprotective activity.

Based on the description above, this study was conducted to determine the effect of propolis on gastric ulcers in male white mice induced by aspirin. The parameters observed were the condition of the stomach which was marked by protection against the formation of gastric ulcers and the histopathological picture of the stomach of male white mice.

2. METHODS

This research was an experimental study and conducted in February - April 2021 at the Pharmacology Laboratory of the Faculty of Pharmacy and Laboratory of Pathology and Anatomy of the Faculty of Medicine, Andalas University.

2.1. Tools and Materials

The tools used were animal cages, animal scales, analytical scales, sonde, surgical instruments, glass measuring and stirrers, microtome, containers, water baths, and Olympus Bx51 microscopes.

The material used were propolis, Aspirin, aqua dest, animal feed and drink, NaCl 0.9%, chloroform, xylol, paraffin, alcohol, albumin, formalin10%, hematoxylin solution, and Eosin solution, male white mice.

2.2. Sample and Animal Preparation

The sample was propolis with the brand "Melia Propolis" that contains 150 mg of propolis every 1 ml. The experimental animal used in this study was 20 male white mice weighed 20-30 g and aged 2-3 months old. Mice were divided into four groups. Each group consists of five mice. Before the study was conducted, the mice were acclimatized for 7 days.

2.3. The Dosage of Aspirin and Propolis

The dosage of aspirin to be given to cause gastric ulcers is 350 mg/kg bodyweight. The dosage is determined based on the preliminary tests that have been done before. Meanwhile, the dosage of propolis used for the treatment of peptic ulcers in this study is 500 mg/kg bb [15].

2.4. The Treatment of Test Animals

The test animals were divided into four groups, where every five mice were placed in one cage. The first group was a control group that was only given food and distilled water for seven days. The second group was given aspirin 350 mg/kg body weight for five days. The third group was given propolis 500 mg/kg body weight for seven days. And the last group was given propolis for seven days and followed by aspirin for five days. The treatments were given orally once a day. Before the administration of aspirin, mice were fasted for 12 hours, not fed but still got water. The volume of the test compound given to 20 g mice was based on the calculation of the volume of drug administration (VAO) where VAO = 1% body weight of Mice.

2.5. Macroscopic Observation of Gastric Ulcer

The mice were sacrificed by using the midline laparotomy method. The stomach of the mice was isolated while gently rinsed with 0.9% NaCl solution and then spread on a flat surface and observed for ulcer lesions formed [16]. The ulcer area will look redder in color than the normal

gastric area. The condition of gastric mucosal was observed by scoring based on the severity of the ulcers: normal stomach = 1; redness in stomach = 1.5; bleeding spots or ulcers up to 0.5 mm in diameter = 2; ulcers with a diameter/length of 0.5-1.5 mm = 3; Ulcers with a diameter/length of 1.6-4 mm = 4; Ulcer with diameter > 4 mm = 5; Perforations with a diameter of 2-7 mm = 6; Perforations with a diameter of 8-13 mm = 7; Perforation with diameter > 13 mm = 8. Calculate the ulcer index by adding up the scores obtained.

The protection ratio was obtained from the gastric ulcer index data which was calculated by the formula:

$$\%\ Protection = 100\% - [\frac{UI\ Treatment\ Group}{UI\ Control\ Group} x\ 100\%]$$

UI: Ulcer index

2.6. Microscopic Observation of Gastric Ulcer through Histological Observation

The microscopic observation of gastric ulcers was done using the gastric histological observations. To make the preparat, the gastric tissue from each group was taken and fixed in 10% NBF until it was completely fixed. The samples were cleared with xylol and embedded using paraffin that had been melted at 56-60ºC for 2 hours. The gastric tissue preparations of mice were stained with the Hematoxylin Eosin method. Observations and readings of preparations were carried out by researchers and experts. The gastric preparations of mice were observed with a microscope with 10, 20, and 40 magnifications [17].

2.7. Data Analysis

The data obtained from the study were analyzed descriptively using the average gastric ulcer index and standard deviation data.

3. RESULTS AND DISCUSSION

Propolis is a natural product from honey bees contain flavonoids which have been known as antioxidants to ward off free radicals that can cause cell damage [13]. In this study, the gastroprotective effect of propolis against aspirin-induced gastric ulcers in mice was determined from macroscopic and microscopic observations.

Macroscopic observation of the mice stomach was carried out by calculating the Ulcers Index (UI) from each treatment group to get the degrees of the ulcer [18]. The ulcers index of each treatment group is shown in Figure 1 and Table 1. The percentage of protection obtained from the average of treatment group ulcer index against the average of positive control ulcer index (aspirin) is 60 %.

Table 1. The average index of gastric ulcer

Group of Treatment	Ulcer Index					Average ± SD
	M1	M2	M3	M4	M5	
Control	1	1	1	1	1	1.00 ± 0.00
Aspirin	3.5	3.5	1.5	13.5	1.5	2.5 ± 1.41
Propolis	1	1	1	1	1	1.00 ± 0.00
Propolis+Aspirin	1	1	1	1	1	1.00 ± 0.00

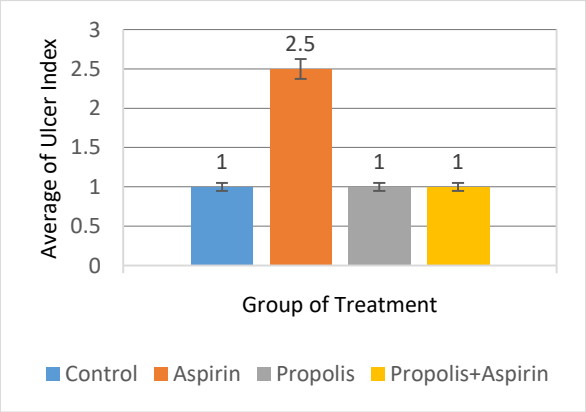

Figure 1 Graph of the average index of gastric ulcer

Based on the macroscopic observations, in the control group, the stomach looked normal, there were no visible signs of gastritis or ulcers with ulcer index score of 1. The same results were also seen in the propolis and propolis + aspirin treatment groups. Meanwhile, in the aspirin treatment group, the ulcer index score of the mice was 2.5. It can be concluded that aspirin has an aggressive factor for gastric mucosa that resulted in gastric damage. Aspirin can cause damage to the gastric mucosal through epithelial and microvascular mechanism. Aspirin is involve in the changes of gastric mucosal permeability, and also cause prostaglandin depletion through COX-1 inhibition. [5] [19].

Prostaglandin has a vital role to protect the integrity of the gastric mucosa, through the rise of local blood flow and stimulate the secretion of mucus and bicarbonate. So, decreased of prostaglandin synthesis leads to decreased epithelial mucus and bicarbonate secretion, mucosal blood flow, and epithelial proliferation. In addition to having prostaglandin inhibiting properties, aspirin can also increase the production of free radicals and superoxide and can interact with adenylyl cyclase to alter cellular cAMP concentrations [4]. The use of NSAIDs can also cause microvascular damage that causes decreased blood flow. In addition, NSAIDs also increase the expression of adhesion molecules and neutrophil attachment to the vascular epithelium in gastric microcirculation. Obstruction of blood flow that occurs in these blood vessels causes microvascular ischemia and the formation of free radicals[5].

Histological examination of gastric tissue was done for the microscopic observation of the treatment group. The stomach was stored in a sample pot containing 10% Neutral Formalin Buffer (BNF) solution. It aims to prevent tissue damage, stop metabolic processes, and preserve histological components.

Based on the results of histological images, there were differences found between the control group, the aspirin group, and the propolis+ aspirin group. Meanwhile, the propolis group had the same histological results with the control group. From the histological results, it can be seen how the mucosa (Mc), Submucosa (Sm), Muscularis (Mu), and serosa (Sr) gastric preparations were stained with hematoxylin-eosin as shown in Figure 2.

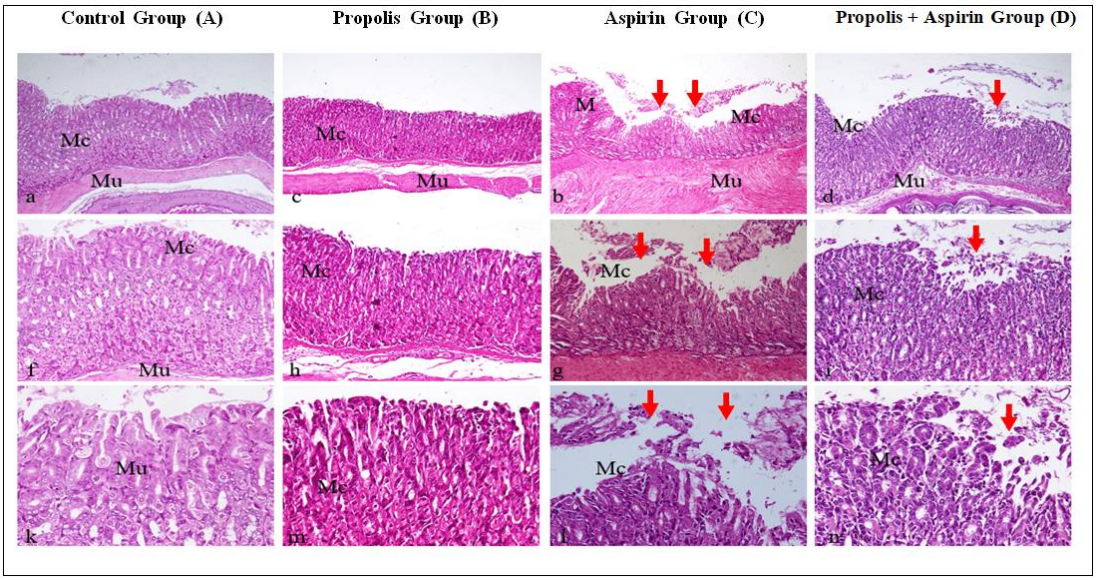

Figure 2 Gastric tissue histology of control (A), Propolis (B), Aspirin (C), and Propolis + Aspirin (D) group of treatment, showing mucosa (Mc), submucosa (Sm), muscularis (Mu), and serosa (Sr) in 10x (1st row), 20x (2nd row), and 40x (3rd row) magnifications.

Based on the histological image, in the control group, the mucosa contains tubular glands with normal epithelium, the mucosal surface appears intact, the stroma contains capillaries, neither hyperemia nor hemorrhage is seen. Administration of propolis did not show any changes in the histological image or histological abnormalities compared to the control.

In the aspirin administration group, a gastric mucosa with ulcerated area, degenerated and necrotic cells were seen, hyperemic in blood vessels, the presence of hemorrhage in the mucosa, and inflammatory cell appeared in the stroma. In the propolis + aspirin group of treatment shown a mucosal damage but it was lighter than the aspirin group, there were foci of mucosal damage showing minimal damage, with some mucosal surfaces containing degenerated epithelium.

Based on the research, it can be seen that the administration of propolis can prevent the gastric mucosal damage in male white mice induced by aspirin. Propolis is contain flavonoids that have antioxidants mechanism to ward off the free radicals that can cause cell damage. Propolis has the strongest antioxidant activity against free radicals compared to other bee products.

The mechanism of the gastroprotective effect of propolis is thought to be due to several compounds in propolis including caffeic, ferulic, p-coumaric and cinnamic acid that had gastroprotective activity and the ability of propolis to reduce gastric juice volume, total acidity, and gastric pH and increase antioxidant activity[15], [20], [21].

Treatment of peptic ulcers is based on the use of antisecretory drugs, including histamine type-2 receptor antagonists (H2-RAs) and proton pump inhibitors (PPIs), as well as antibiotics used for H. pylori infection. However, these therapeutic agents are usually associated with many adverse side effects, such as hypersensitivity, vitamin B12, and iron deficiency, arrhythmias, increased susceptibility to pneumonia, impotence, gynecomastia, fractures, hematopoietic changes, hypergastrinemia, and gastric cancer [22]. In this context, natural products are considered an attractive resource for new antiulcer treatments. Among them, propolis has been used in traditional medicine to treat gastric ulcers and this has prompted researchers to investigate and validate its use as an antiulcer agent.

4. CONCLUSION

There is a gastroprotective effect of propolis against aspirin-induced gastric ulcers. This can be seen from the histological results of the treatment group which showed a lower damage effect by aspirin. Administration of propolis did not show histological side effects at the given dose, this gave the impression that the dose and interval given were still within safe limits.

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