

## Propolis of *Trigona spp.* Protects Mucosa from Aspirin-Induced Gastric Mucosal Damage in Rats

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### Abstract

**Background:** *Helicobacter pylori* and non-steroid anti-inflammatory drugs are the major causes of peptic ulcer in the world. Indonesian native stingless bee species, *Trigona spp.*, produces propolis that might be effective to protect mucosal damage. The aim of the study was to determine the protective effect of *Trigona spp.* propolis on aspirin-induced gastric mucosal damage in rats.

**Methods:** This experimental study was conducted from September–November 2013 at Animal Laboratory of Department of Pharmacology and Therapy Faculty of Medicine Universitas Padjadjaran. Healthy male Wistar rats (n=24) aged 2–3 months old and weighed 200–250 grams were randomly divided into three groups. The first group was control negative, the second group was given 100 mg/kg body weight of aspirin, and the third group was given 200 mg/kg body weight of *Trigona spp.* propolis, one hour before administration of 100 mg/kg body weight of aspirin. After two weeks of treatment, rats were sacrificed by laparotomy to obtain gastric tissues, followed by processing for the paraffin section for histopathological analysis. The grade of gastric mucosal damage was determined under a light microscope. Data were then compared between groups using the Mann-Whitney test.

**Results:** Oral administration of aspirin-induced gastric mucosal damage ranging from grade 0 to grade IV; whereas administration of propolis showed a reduction of gastric mucosal damage's grade when compared to the aspirin group (p<0.05).

**Conclusions:** *Trigona spp.* propolis has a protective effect on aspirin-induced gastric mucosal damage. Further study is encouraged to study an optimal dose of aspirin after propolis administration.

**Keywords:** Aspirin, gastric mucosal damage, propolis, *Trigona spp*

### Introduction

About 10% of the population in western countries are affected by peptic ulcer.<sup>1</sup> A peptic ulcer is predominantly caused by *Helicobacter pylori* and non-steroid anti-inflammatory drugs (NSAID).<sup>2</sup> Aspirin is a member of NSAID that is known as anti-inflammatory, antiplatelet, analgesic, antipyretic, and cardiovascular protection drug. Because of the broad use of aspirin, it becomes the most widely used drug.<sup>3</sup> However, aspirin causes an imbalance between protective factors and aggressive factors, resulting in gastric mucosal damage.<sup>4</sup>

There has been a tendency to go back to nature, seeking new treatment for various diseases. In Indonesia, various natural products have been used to treat diseases, among others honey and propolis. Propolis is a sticky resin substance collected by honeybees from several plants that have been used as one of the natural remedies since thousand years ago. Indonesia has a native stingless bee species *Trigona spp* that produces propolis. Propolis produced by *Trigona spp.* has a higher flavonoid level than *Apis mellifera* propolis.<sup>5</sup> Research shows that propolis has antimicrobial activity, antiviral, antifungal, antiprotozoal, antiparasitic, anti-inflammatory, and antiulcer.<sup>6</sup> The aim of this

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**Table 1 The Distribution of Severity Grade of Gastric Mucosal Damage**

Group	Grade					Total field examined*
	0	I	II	III	IV	
Control	120	-	-	-	-	120
Aspirin	4	34	60	20	2	120
Propolis + Aspirin	27	64	25	4	-	120

Note: \*There were 15 fields examined each Wistar rat (n=8) per group

study was to determine the protective effect of *Trigona spp.* propolis against aspirin-induced gastric mucosal damage in rats.

## Methods

This experimental study was conducted from September–November 2013 at the Animal Laboratory of Department of Pharmacology and Therapy Faculty of Medicine Universitas Padjadjaran. All experiments performed in the animal laboratory in this study were approved by the Health Research Ethics Committee Faculty of Medicine Universitas Padjadjaran. Aspirin was purchased from a drugstore. Aspirin weighed using digital by the dosage of each treatment group and dissolved in 4 mL of aquadest. *Trigona spp.* propolis was purchased from Faculty of Agriculture Industrial Technology Universitas Padjadjaran Jatinangor. In brief, twenty-four healthy male Wistar rats aged 2–3 months old and weighed 200–250 grams were placed in a homogenous temperature and dark-light cycle with ad libitum access to food and drink. Before initiating treatments, rats were allowed to acclimatize to a new environment conditions for seven days. Then, rats were randomly divided into three groups where four rats were placed in one cage. The first group served as a control group that was given only food and drink. The second group was given 100 mg/kg body weight of aspirin, dissolved in 4 ml of distilled water.<sup>7</sup> The third group was given a 200 mg/kg body weight of *Trigona spp.* propolis one hour before administration of 100 mg/kg body weight of aspirin.<sup>7,8</sup> All treatments were given orally every day for two weeks.

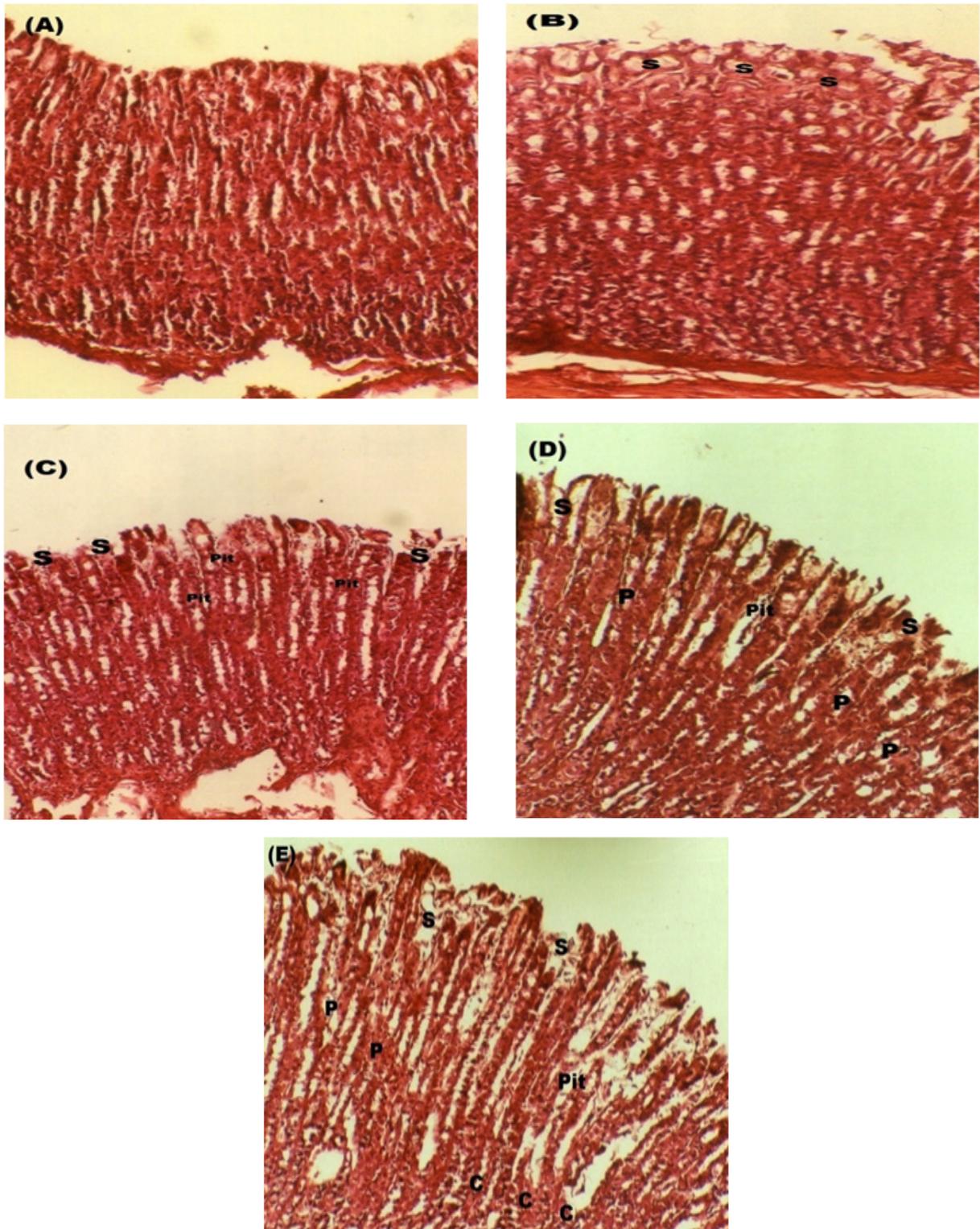
In the fifteenth day, rats were sacrificed by using the midline laparotomy method under ketamine anesthesia. The stomach of the rats was cut open along the lesser curvature and gently rinsed with 0.9% NaCl solution. Gastric tissue samples from each group were fixed in

10% formalin for 24 hours. Paraffin sections were prepared and stained with hematoxylin and eosin.<sup>9</sup> For each rat, there were 15 fields to examine using a light microscope, thus, per group (n=8) there were 120 fields to examine. These fields were graded using the grading system to evaluate the severity of mucosal damage based on its depth which categorized into grade 0 to grade IV. The grade 0 was normal gastric mucosal; grade I was characterized by a damage of surface mucosal cell on luminal surface and partly exfoliated, but there is no damage to gastric pit cells; grade II was characterized by an extensive luminal surface cell damage and damage to the cells lining the gastric pits, but there was no damage to gastric gland cells; grade III was characterized by damage on surface and pit cells, cellular damage in the upper part of the gastric glands (parietal cell area), numerous exfoliated cells and a whole layer of the necrotic superficial epithelium; and grade IV was characterized by severe damage of grade III and extending into the lower part of the gastric glands (chief cell area), and submucosal edema.<sup>9</sup>

Data were analyzed with Statistical Product and Service Solutions (SPSS) software, and a Mann-Whitney test was performed. Data difference was considered statistically significant if the p-value was less than 0.05.

## Results

Histological examination revealed that there were various grades of gastric mucosal damage in each group as shown in Figure 1. As expected, all rats from the control group had normal tissues (grade 0) as presented in Table1. Furthermore, aspirin administration had shown higher grades, whereas extra propolis before aspirin administration had shown a shift into lower grades (p<0.05, the Mann Whitney test), suggesting that propolis *Trigona spp.* had protective effects against aspirin-induced gastric mucosal damage.



**Figure 1** Grade 0 with normal mucosa appearance (A). Exfoliation on surface mucosal cell (S) in grade I (B) and damage extended to cell lining gastric pit in grade II(C). Grade 3 showed damage in surface mucosa cell (S), pit, and parietal cells (P) (D). Extensive necrosis and damage were extended to the chief cell (C) in grade IV (E). (H&E, 400X)<sup>9</sup>

## Discussion

Aspirin is one of the most widely used drugs. It has been reported that aspirin is associated with gastric mucosal damage. This study has shown that administration of aspirin 100 mg/kg body weight has induced changes in mucosal gastric; including cellular changes, interstitial hemorrhage and cellular damage in the gastric gland. Furthermore, exfoliated cells are frequently noted in gastric lumen. Moreover, grade II is the most frequent in the group with aspirin administration; and this grade has been reduced to grade I by the administration of propolis prior to aspirin (Table 1). In grade II, damage cells are from the luminal surface to cells lining in the gastric pit, whereas the damage only happens in mucosal cells of the luminal surface in grade I.<sup>9</sup> This indicates that aspirin might have an impact on local and systemic effects. In an acidic environment, aspirin is dissolved in fat, allowing aspirin to enter mucosa and dissociate inside the gastric mucosa. The dissociation causes uncoupled oxidative phosphorylation and release reactive oxygen species (ROS).<sup>4,10</sup>

Aspirin has a systemic effect that contributes to the gastric mucosa damage. Aspirin inhibits prostaglandin synthesis through inhibition of cyclooxygenase enzyme. Prostaglandin plays an important role as a defensive factor of the gastric mucosa. Cyclooxygenase-1 produces prostaglandin that regulates mucus secretion, bicarbonate secretion, and maintains the mucosal blood flow. Furthermore, cyclooxygenase-2 produces prostaglandin that regulates the proliferation of epithelial cells and maintains the vascular endothelium to intact by preventing attachment of neutrophil. Inhibition of prostaglandin synthesis will disrupt those functions and make gastric mucosa becomes vulnerable to aggressive factor such as acid juice and pepsin.<sup>4,10</sup>

Propolis is believed to have a polyphenol component flavonoids that has many forms; those are flavonol, flavonon, flavon, flavanolol, flavan, and isoflavon. Flavonoid acts as an antioxidant, therefore, the decreased grading of gastric mucosal damage in the propolis group may be partially due to its antioxidant activity.<sup>11,12</sup> Flavonoid level of *Trigona spp.* propolis is 4%, greater than of *Apis mellifera* which is 1.5%.<sup>13</sup>

Next to antioxidant, the flavonoid in propolis is reported to have other gastroprotective effects; those are increasing mucosal blood flow, mucus and amount of prostaglandin in the stomach. Beside flavonoid, Propolis has other

components that have antioxidant activity and thus can protect gastric from aggressive factors, such as caffeic acid phenethyl ester (CAPE). In other research, propolis has been reported to be able to reduce gastric juice volume, total acidity, and pH, and to increase antioxidant activity.<sup>14,15</sup> Another study has reported that 200 mg/kg body weight of Indian propolis has protective effects against NSAID-induced gastric ulcers, similar to propolis *Trigona spp.* that has a protective effect against aspirin-induced gastric mucosal damage in rats.<sup>8</sup> *Trigona spp.* propolis has the same protective effect against aspirin-induced gastric mucosal damage with Indian propolis and Brazilian propolis, suggesting that protective effect due to phytochemicals.<sup>8,16</sup>

The limitation of the study is that the dosage given in this study is only one dose. Studies with a varying dosage of propolis *Trigona spp.* to determine the optimal dosage for protective effects against aspirin-induced gastric mucosal damage is recommended.

In conclusion, *Trigona spp.* propolis has a protective effect against aspirin-induced gastric mucosal damage. Further study is needed to explore the effect of propolis administration on the optimal dose of aspirin.

## References

1. Amin D. A textbook of clinical pharmacology and therapeutics. Ritter JM, Lewis LD, Mant TGK, Ferro A, editors. A textbook of clinical pharmacology and therapeutics. 5<sup>th</sup> Ed. Great Britain: Hodder Arnold; 2008. p. 247.
2. Soll AH, Graham DY. Peptic ulcer disease. Yamada T, Alpers DH, Kalloo AN, Kaplowitz N, Owyang C, Powell DW, editors. Textbook of Gastroenterology. 5<sup>th</sup> Ed. West Sussex: Wiley Blackwell; 2009. p. 936–73.
3. Yeomans ND. Aspirin: old drug, new uses and challenges. J Gastroenterol Hepatol. 2010;26(3):426–31.
4. Musumba C, Pritchard DM, Pirmohamed M. Review article: cellular and molecular mechanisms of NSAID-induced peptic ulcers. Aliment Pharmacol Ther. 2009;30:517–31.
5. Mahani, Karim RA, Nurjanah N, Dahlianti R. Keajaiban Propolis Trigona. 1<sup>st</sup> Ed. Jakarta: Pustaka Bunda; 2011. p. 33–55
6. Lotfy M. Biological activity of bee propolis in health and disease. Asian Pacific J Cancer Prev. 2006;7:22–31.
7. Seleem HS. Effect of aspirin versus aspirin and vitamin C on gastric mucosa (fundus)

- of adult male albino rats. histological and morphometric study. *Egypt J Histol.* 2010;33(2):313–26.
8. Pillai SI, Kandaswamy M, Subramanian S. Antiulcerogenic and ulcer healing effects of Indian propolis in experimental rat ulcer models. *J Api Prod Api Med Sci.* 2010;2(1):21–8.
  9. Morini G, Grandi D. Methods to measure gastric mucosal lesion in the Rat. *Curr Protoc Toxicol.* 2010;43:1–15.
  10. Wallace JL. Prostaglandin, NSAID, and gastric mucosal protection: why doesn't the stomach digest itself. *Physiol Rev.* 2008;88(4):1547–65.
  11. Narayana KR, Reddy MS, Chaluvadi MR. Bioflavonoid classification, pharmacological, biochemical effects and therapeutic potential. *Indian J Pharmacol.* 2001;33(1):2–16.
  12. Mota KS, Dias GE, Pinto ME, Luiz-Ferreira Â, Souza-Brito AR, Hiruma-Lima CA, et al. Flavonoids with gastroprotective activity. *Molecules.* 2009;14(3):979–1012.
  13. Siregar HC, Fuah AM, Octaviany Y. Propolis: madu multikhasiat. 1<sup>st</sup> Ed. Jakarta: Penerbit Swadaya; 2011.p. 35
  14. De Barrosa MP, Sousab JP, Bastosb JK, de Andradea SF. Effect of Brazilian green propolis on experimental gastric ulcers in rats. *J Ethnopharmacol.* 2007;110(3):567–71.
  15. Mohafez OMM, Abdel-Raheem IT, Nafady AM. Antioxidant, lipid peroxidation-inhibitory and antiulcer activities of brown propolis. *Bull Pharm Sci.* 2010;33:169–77.
  16. Lemos M, de Barros MP, Sousa JP, da Silva Filho AA, Bastos JK, de Andrade SF. *Baccharis dracunculifolia*, the main botanical source of brazilian green propolis, displays antiulcer activity. *J Pharm Pharmacol.* 2007;59(4):603–8.