RESEARCH ARTICLES

GASTROPROTECTIVE EFFECT OF THAI PROPOLIS

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ABSTRACT

Propolis is a resinous substance collected by honeybees from plant exudates. The present study was undertaken to evaluate Thai propolis for its anti-gastric ulcer activity by experimental models. Oral administration of the propolis at 100 and 200 mg/kg significantly inhibited gastric ulcer formation induced by indomethacin, HCl/EtOH, and water immersion restraint-stress in rats. In pylorus ligated rats, pretreatment with propolis had no effect on gastric volume and pH thus indicating the lack of antisecretory effect of propolis. In ethanol-induced ulcerated rats, gastric wall mucus and hexosamine content were markedly preserved by the propolis pretreatment. The findings indicated that Thai propolis possessed gastroprotective potential related to preservation of gastric mucus synthesis and secretion.

Key words : bee, propolis, gastric ulcer, gastroprotective

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บทคัดย่อ

พรอพอลิสเป็นสารเหนียวที่ผึ้งสร้างจากสารคัดหลั่งของพืชที่ผึ้งเก็บสะสมมา ได้ทำการศึกษาเพื่อประเมินฤทธิ์ของพรอพอลิสไทยในการต้านการเกิดแผลกระเพาะอาหารโดยใช้รูปแบบการทดลองต่างๆ พบว่าเมื่อให้พรอพอลิสทางปากในขนาด 100 และ 200 มก./กก. สามารถป้องกันการเกิดแผลในกระเพาะอาหารที่เกิดจากอินโดเมทาซิน กรดเกลือ/เอทานอล และความเครียดจากการแช่น้ำได้อย่างมีนัยสำคัญ เมื่อให้พรอพอลิสแก่หนูที่ถูกผูกกระเพาะส่วนไพลอรัส พบว่าพรอพอลิสไม่มีผลลดปริมาณของเหลวในกระเพาะอาหารและความเป็นกรด ซึ่งชี้ให้เห็นว่าพรอพอลิสไม่ได้ออกฤทธิ์ต้านการหลั่งกรด พรอพอลิสสามารถรักษาปริมาณมิวคัสและเฮกโซซามีนในกระเพาะอาหารของหนูที่ได้รับกรด/เอทานอลได้ ทั้งหมดนี้ชี้ให้เห็นว่าพรอพอลิสของไทยมีฤทธิ์ปกป้องกระเพาะอาหารได้โดยผ่านทางการสังเคราะห์และการหลั่งมิวคัสของกระเพาะอาหาร

คำสำคัญ : ผึ้ง, พรอพอลิส, แผลกระเพาะอาหาร, การปกป้องกระเพาะอาหาร

INTRODUCTION

Traditional bee keeping in Thailand was started about 100 years ago with *Apis cerana* in coconut plantations on Samui Island. Modern bee keeping with *Apis mellifera* was started in the early 1940's but did not increase markedly until the early 1970's[1]. It has been estimated by the Northern Beekeeper Association of Thailand that by 1998 the number of bee colonies in Thailand had increased to about 250,000. Bee products are honey, wax, royal jelly, pollen and propolis.

Honey, the principal bee product, has been reported to have an antibacterial effect on *Helicobacter pylori*[2], a pathogenic bacteria causing peptic ulcer, and a protective effect on acute gastric mucosal lesions induced by 50% ethanol[3]. It was demonstrated that a natural mixture of higher aliphatic primary alcohols isolated from beewax possessed anti-gastric ulcer effect when tested in different experimental models[4]. The anti-ulcerogenic property of the mixture was suggested to be related to a cytoprotective mechanism via enhancement of the quantity and quality of the gastric mucus[5,6]. The mixture was also reported to have a protective effect on the pre-ulcerative phase of carrageenan-induced colonic ulceration in the guinea-pig[7].

Propolis is a resinous substance collected by honeybees from plant exudates. Bees use propolis to seal holes in their honeycombs and protect the entrance against intruders but more importantly it appears to act as an antiseptic to prevent microbial infection of larvae, honey stores and the combs[8]. Propolis is reputed to have antiseptic, antimycotic, bacteriostatic, astringent, choleric, spasmolytic, anti-inflammatory, anaesthetic and anti-oxidant properties[8-10]. Propolis is also used by herbalists to treat duodenal ulcers and in homeopathic medicine[8]. Propolis from South America was found to have antibacterial activity against Gram positive bacteria[11]. It was reported that phenolic compounds isolated from Brazilian propolis showed activity against *Trypanosoma cruzi* and bacteria, and a relaxant effect in guinea-pig isolated trachea[12].

The present study was aimed to investigate the anti-gastric ulcer effect of Thai propolis in experimental models.

MATERIALS AND METHODS

Propolis

Propolis in tablet form (25 mg/tab) was kindly provided by the Bee Products Industry Co., Ltd., Thailand. It was ground and suspended in 0.5% carboxymethylcellulose (CMC) to desired concentrations.

Animals

Male Sprague-Dawley rats weighing 150-200 g were purchased from the National Laboratory Animal Center, Salaya Mahidol University, Thailand. They were acclimatized for at least 7 days in an animal room where the temperature was maintained at 22 ± 3 °C and there was a 12 hours light-dark cycle. The food was supplied by Pokphan Animal Feed Co., Ltd., Bangkok. The bedding was autoclaved. The rats had free access to food and water unless stated otherwise. All animals received humane care in compliance with the ethics in the use of animals issued by the National Research Council of Thailand 1999.

Indomethacin-induced gastric ulcers

Propolis was administered orally to 48 hr. fasted rats 60 min prior to induction of gastric ulcers by indomethacin suspended in 0.5% carboxymethylcellulose at a single i.p. dose of 30 mg/kg[13]. After 5 hr the rats were sacrificed and examined for gastric ulcers.

HCl/EtOH-induced gastric ulcers

Propolis was administered orally to 48 hr fasted rats 60 min prior to induction of gastric ulcers by 1.0 ml HCl-EtOH (60 ml ethanol + 1.7 ml HCl + 38.3 ml water) p.o.[14]. The animals were sacrificed and examined for gastric ulcers 60 min later.

Restraint water immersion stress-induced gastric ulcers

Propolis was administered orally to 48 hr fasted rats. Sixty minutes later, rats were restrained individually in stainless steel cages and immersed up to their xiphoid in a water bath maintained at 22 ± 2 °C, according to the method of Takagi et al[15]. After 5 hr of this exposure, the rats were sacrificed and examined for gastric ulcers.

Evaluation of the gastric ulcers

After each rat had been sacrificed, the stomach was removed, opened along the greater curvature and the glandular portion of the stomach was examined. The length in mm

of each lesion was measured under a dissecting microscope and the sum of the length of all lesions was designated as the ulcer index.

Pylorus ligation

Propolis was administered orally to 48 hr fasted rats. One hour later, pylorus ligation as described by Shay et al[16] was performed. Briefly, rats were lightly anesthetized by ether. The abdomen was opened and the pylorus was ligated. The abdomen was closed by suturing. The animals were killed 5 hr later by an over dose of ether. The stomach was removed and its content was subjected to measurement of volume and pH and assayed for titratable acidity.

Determination of gastric wall mucus content

Gastric wall mucus was determined by the Alcian blue method[17]. Briefly, propolis was administered orally to 48 hr fasted rats 60 min prior to induction of gastric ulcers by 1.0 ml HCl/EtOH (60 ml ethanol + 1.7 ml HCl + 38.3 ml water) p.o.[14]. Sixty minutes later, the animals were sacrificed and the stomach was excised and opened along the lesser curvature, weighed and immersed in 0.1% w/v Alcian blue solution for 2 hours. The excessive dye was then removed by two successive rinses in 0.25 M sucrose solution. Dye complexed with gastric wall mucus was extracted with 0.5 M $MgCl_2$ for 2 hours. The blue extract was then shaken vigorously with an equal volume of diethyl ether and the resulting emulsion was centrifuged. The optical density of Alcian blue in the aqueous layer was read against a buffer blank at 580 nm using a spectrophotometer. The quantity of Alcian blue extracted per gram wet stomach was then calculated from a standard curve.

Measurement of gastric hexosamine content

Hexosamine content in gastric tissue was assayed by the method of Glick[18]. Briefly, propolis was administered orally to 48 hr fasted rats 60 min prior to induction of gastric ulcers by 1.0 ml HCl/EtOH (60 ml ethanol + 1.7 ml HCl + 38.3 ml water) p.o.[14]. Sixty minutes later, the animals were sacrificed and the antral part of the stomach was hydrolyzed with 6 N HCl overnight. The tissue was neutralized with 6 N NaOH and incubated with acetylacetone at 100 °C for 15 min. The mixture was then coupled with Ehrlich's reagent and allowed to stand at room temperature for 40 min. The optical density of the sample was measured spectrophotometrically at 530 nm using glucosamine as a standard.

Statistical analysis

Data were subjected to statistical analysis using ANOVA and statistical comparison was done using Duncan Multiple Range Test. The value exceeding 99% confidence limits was considered to be significant.

Table 1. Effects of propolis on gastric ulcers in rats

Group	GASTRIC ULCER INDUCER					
	Indomethacin		HCl/EtOH		Stress	
	Ulcer index (mm)	I (%)	Ulcer index (mm)	I (%)	Ulcer index (mm)	I (%)
Control	7.5 ± 1.3		101.0 ± 11.9		9.9 ± 0.9	
Propolis 100 mg/kg	2.2 ± 0.6*	71	9.4 ± 4.5*	91	2.7 ± 0.6*	73
Propolis 200 mg/kg	0.5 ± 0.2*	93	1.4 ± 0.9*	99	0.1 ± 0.0*	99

Note : data expressed as mean ± S.E.M. (n = 8)
* significantly different from the control group ($p < 0.01$)
I (%) = inhibition of ulcer formation expressed as percentage

RESULTS

Propolis at doses of 100 and 200 mg/kg significantly ($p<0.01$) inhibited ulcer formation induced by indomethacin, ethanol and water immersion stress as shown in Table 1. The inhibition was dose related. In the pylorus ligated rats, the mean gastric volume and pH were not affected by propolis pretreatment. Only the acidity output in the propolis 200 mg/kg treated group was significantly ($p<0.01$) decreased from that of the control group (Table 2). Table 3 shows that the mean value of the gastric mucus content in HCl/EtOH induced ulcerated rats was significantly lower than that of the control group. Propolis at doses of 100 and 200 mg/kg significantly ($p<0.01$) restored the mucus

content back to the level comparable to that of the non-ulcerated rats. The mean gastric hexosamine content in control ulcerated rats was significantly less than that in the normal non-ulcerated group as shown in Table 4. Pretreatment with propolis at 100 and 200 mg/kg significantly incerased the hexosamine content. The effect of propolis on gastric wall mucus content and gastric hexosamine content was not dose related.

Table 2. Effects of propolis on gastric secretion in rats

Group	Gastric vol. (ml)	Gastric pH	Acidity mEq/L
Control	9.5 ± 0.8	1.62 ± 0.08	126 ± 5
Propolis 100 mg/kg	8.4 ± 1.0	1.87 ± 0.42	100 ± 9
Propolis 200 mg/kg	8.6 ± 0.8	1.64 ± 0.07	51 ± 4*

Note : data expressed as mean ± S.E.M. (n = 8)
* significantly different from the control group ($p < 0.01$)

Table 3. Effects of propolis on gastric wall mucus content in rats

Group	Gastric wall mucus (µg Alcian blue/g wet stomach)
Control HCl/EtOH ulcerated rats	804 ± 29
Propolis 100 mg/kg	1597 ± 72*
Propolis 200 mg/kg	1072 ± 70*
Non-ulcerated rats	1167 ± 16*

Note : Data expressed as mean ± S.E.M. (n = 10)
* significantly different from control ulcerated rats ($p < 0.01$)

Table 4. Effects of propolis on gastric hexosamine content in rats

Group	Hexosamine content (µg /100 mg wet stomach)
Control HCl/EtOH ulcerated rats	21.7 ± 1.6
Propolis 100 mg/kg	30.3 ± 1.2*
Propolis 200 mg/kg	47 ± 1.6*
Non-ulcerated rats	37.0 ± 2.2*

Note : Data expressed as mean ± S.E.M. (n = 8)
* significantly different from control ulcerated rats ($p < 0.01$)

DISCUSSION

It is known that poplar trees (*Populus spp.*) are the major source of propolis in temperate countries and that the chemical constituents of this material fall into four major groups, namely beeswax constituents (fatty acids, esters, long chain alcohols), flavonoids, other phenolic compounds, and volatile compounds (mainly terpenes)[9]. In tropical regions propolis can be expected to have different compositions because of different tree species involved, as has been shown for Brazilian propolis[12,19,20] and for propolis from the Canary Islands[21]. Therefore no comment can be made at present about the constituents of Thai propolis which might be responsible for the observed pharmacological effects.

Results obtained in this study show the anti-gastric ulcer activity of Thai propolis when evaluated in the most commonly utilized experimental models which include indomethacin, HCl/EtOH and restraint water immersion stress - induced gastric lesions in rats[22,23].

The pathogenesis of gastric ulcers is often depicted as an imbalance between mucosal integrity and aggressive factors. Factors that impair mucosal defense are HCl, gastrin, histamine, *Helicobacter pylori,* aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs), ethanol, caffeine and stress while factors that promote mucosal integrity are gastric mucus and bicarbonate, gastric mucosal barrier, prostaglandins (PGs) and mucosal blood flow[24,25].

According to the experimental models used in this study, non-steroidal anti-inflammatory drugs (NSAIDs) like indomethacin induce ulcer formation by depleting cytoprotective prostaglandins, e.g. PGE_2 and

PGI_2 in the cyclooxygenase pathway of arachidonic acid metabolism[26]. PGE_2 and PGI_2 of gastric and duodenal mucosa are responsible for mucus production and maintaining cellular integrity of the gastric mucosa[27]. In the HCl/EtOH induced gastric ulceration model, HCl causes severe damage to gastric mucosa[28] whereas ethanol produces necrotic lesions by direct necrotizing action which in turn reduces defensive factors, the secretion of bicarbonate and production of mucus[29]. The water immersion stress-induced ulcers are mediated by increases in gastric acid secretion[30] and decreases in mucosal microcirculation[31] and mucus content[32]. Since propolis could prevent ulceration in all three models and its anti-ulcer effect was much more pronounced in the ethanol model, it was not likely that inhibition of gastric secretion was the action of propolis. This was in accordance with the finding that in pylorus ligated rats propolis had no effect on gastric volume and pH though the acidity output was significantly decreased.

The gastric wall mucus, obligatory components of which are hexosamines, is thought to play an important role as a defensive factor against gastrointestinal damages[33]. The determined gastric wall mucus was used as an indicator for gastric mucus secretion while the mucosal hexosa-mine content was used as an indicator for gastric wall mucus synthesis[34]. In the present study, the gastric wall mucus and hexosamine contents in HCl/EtOH ulcerated rats were markedly lowered when compared with those of the non-ulcerated group. It was found that pretreatment with propolis significantly incerased both gastric mucus and hexosamine contents in HCl/EtOH ulcerated rats. This finding indicated that propolis preserved both gastric mucus synthesis and secretion in the experimental rats.

In conclusion this study provides evidence that propolis obtained from bee farming in Thailand possesses an anti-gastric ulcer effect which is related to a cytoprotective mechanism via preservation of gastric mucus synthesis and secretion.

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