

RESEARCH ARTICLES

GASTROPROTECTIVE EFFECT OF ROSELLE MUCILAGE

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ABSTRACT

The present study was undertaken to evaluate roselle (*Hibiscus sabdariffa*) for its anti-ulcer activity. Roselle mucilage fraction (RMF) was isolated and examined in experimentally ulcerated rats. Oral administration of the RMF from 125-500 mg/kg significantly inhibited gastric ulcer formation induced by indomethacin, ethanol and water immersion restraint stress. Anti-gastric secretory effect of the RMF in pylorus-ligated rats was not clearly demonstrated since the gastric volume, pH and acidity output were not affected by the RMF pretreatment in a dose-related manner. In acidified ethanol-induced ulcerated rats, gastric wall mucus but not hexosamine content, was markedly preserved by the RMF pretreatment. The findings indicate that roselle mucilage possesses gastroprotective potential which is related to a cytoprotective mechanism via preservation of gastric mucus content.

Key words : roselle, *Hibiscus sabdariffa*, anti-ulcer activity, mucilage

ฤทธิ์ปกป้องกระเพาะอาหารของมิวลิเลจจากกระเจี๊ยบแดง

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ห้องปฏิบัติการผลิตภัณฑ์ธรรมชาติ สถาบันวิจัยจุฬาภรณ์ ศูนย์วิจัย ณ มหาวิทยาลัยเชียงใหม่ คณะแพทยศาสตร์
มหาวิทยาลัยเชียงใหม่

บทคัดย่อ

ได้ทำการศึกษาเพื่อประเมินฤทธิ์ของกระเจี๊ยบแดงในการต้านการเกิดแผลกระเพาะอาหาร โดยใช้ส่วนสกัดมิวลิเลจจากกระเจี๊ยบแดงทดสอบในสัตว์ทดลอง พบว่าเมื่อให้ส่วนสกัดมิวลิเลจทางปากขนาด 125-500 มก./กก. สามารถป้องกันการเกิดแผลในกระเพาะอาหารที่เกิดจากอินโดเมทาซิน กรด/เอทานอล และความเครียดจากการแช่น้ำได้อย่างมีนัยสำคัญ เมื่อให้ส่วนสกัดมิวลิเลจแก่หนูที่ถูกผูกกระเพาะส่วนไพโลรัส พบว่าส่วนสกัดมิวลิเลจไม่มีผลต่อปริมาณของเหลวในกระเพาะอาหาร ความเป็นกรด และปริมาณกรดที่หลั่งออกมา ซึ่งชี้ให้เห็นว่าส่วนสกัดมิวลิเลจไม่ออกฤทธิ์ต้านการหลั่งกรด ส่วนสกัดมิวลิเลจสามารถรักษาปริมาณมิวคัสในกระเพาะอาหารของหนูที่ได้รับกรด/เอทานอลไว้ได้ ในขณะที่ไม่มีผลต่อปริมาณเฮกโซซามีน ทั้งหมดนี้ชี้ให้เห็นว่าส่วนสกัดมิวลิเลจจากกระเจี๊ยบแดงมีฤทธิ์ปกป้องกระเพาะอาหารได้โดยไม่เพิ่มการสังเคราะห์ แต่รักษาปริมาณสารเมือกในกระเพาะอาหารไว้ได้

คำสำคัญ: กระเจี๊ยบแดง, ฤทธิ์ต้านการเกิดแผลกระเพาะอาหาร, มิวลิเลจ

INTRODUCTION

In Thai traditional medicine, roselle (*Hibiscus sabdariffa* L.) is used for the treatment of various diseases and disorders including hypertension, hyperlipidemia, bladder stone and gastric ulceration¹. It was claimed that roselle could be used as a fragrant, antispasmodic drink, anti-hypertensive, anthelmintic in taeniasis, and anti-bacterial agent². An aqueous extract of roselle was reported to be an effective diuretic in patients with urologic disorders³. We have already found (in our preliminary study) that an aqueous extract of roselle exerts an anti-gastric ulcer effect when tested in indomethacin, HCl/EtOH, and water immersion stress-induced ulceration in rats; and that a purified, mucilage fraction maintained the activity in the stress-induced ulcer model. The aim of the present study was to evaluate the anti-ulcer activity of the mucilage fraction.

MATERIALS AND METHODS

Extraction of roselle and preparation of roselle mucilage

The lyophilized aqueous extract of roselle was prepared by maceration of dried roselle calyces with hot distilled water at 75 °C for 5 hr. The extract was filtered through layers of gauze, evaporated and dried under reduced pressure. A mucilage fraction was separated from the lyophilized aqueous extract by the method described by Kalyansundaram *et al.* (1980)⁴. Briefly, the lyophilized aqueous extract was put into ten times volume of boiling 0.1 N HCl. The solution was then filtered using Whatman No. 4 filter paper. The filtrate was mixed with 3 times volume of 95% ethanol, stirred and allowed to stand overnight. The supernatant liquid was separated out by filtration through Whatman No. 4 filter paper and the precipitated roselle mucilage fraction (RMF) was freeze-dried. The RMF yield was 12% w/w of the dry starting material. In the experiments, the RMF was dissolved in distilled water 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.

Experimental gastric ulcers

Roselle mucilage fraction (RMF) was administered orally to 48 hr fasted rats 60 min prior to induction of gastric ulcers by indomethacin⁵, HCl/EtOH⁶ or water immersion restraint stress⁷. Later (1 hr after HCl/EtOH or 5 hr after indomethacin and water immersion stress) the rats were sacrificed and examined for gastric ulcers. Each 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

RMF was administered orally to 48 hr fasted rats. One hour later, the rat's pylorus was ligated⁸. The animals were killed 5 hr later by an overdose of ether. The stomach was removed and its content was subjected to measurement of volume and pH and assay for titratable acidity.

Determination of gastric wall mucus content

Gastric wall mucus was determined by the Alcian blue method⁹. Briefly, the RMF was administered orally to 48 h 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.⁶. 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 MgCl₂ 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 Glick¹⁰. Briefly, the RMF was administered orally to 48 h 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.⁶. 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 95% confidence limits was considered to be significant.

RESULTS

Oral administration of the RMF from 125-500 mg/kg significantly inhibited gastric ulcer formation induced by indomethacin, ethanol and water immersion restraint stress (Table 1). In pylorus ligated rats, the mean gastric volume of the RMF treated group did not significantly differ from that of the control group while the pH and acidity output in some RMF treated group did, but not in a dose-related manner (Table 2).

The determined gastric mucus contents in 250 and 500 but not 125 mg/kg RMF treated groups were found to be significantly higher than that of the control ulcerated group (Table 3). Conversely, RMF at 125 but not 250 or 500 mg/kg significantly increased gastric hexosamine content compared with that of the ulcerated group (Table 4).

Table 1. Effects of roselle mucilage fraction (RMF) 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	12.1 ± 1.6		116.4 ± 8.4		8.4 ± 1.4	
RMF 125 mg/kg	2.7 ± 0.5*	77.7	78.5 ± 16.0	32.5	5.9 ± 1.3	29.8
RMF 250 mg/kg	1.9 ± 0.6*	84.3	61.9 ± 18.6*	46.8	0.7 ± 0.3*	91.7
RMF 500 mg/kg	1.1 ± 0.4*	90.9	64.3 ± 11.3*	44.8	0.6 ± 0.3*	92.8

Note : data expressed as mean ± S.E.M. (n = 8), * significantly different from the control group ($p < 0.05$), I (%) = inhibition of ulcer formation expressed as percentage.

Table 2. Effects of roselle mucilage fraction (RMF) on gastric secretion in rats

Group	Gastric vol. (ml)	Gastric pH	Acidity mEq/L
Control	9.1 ± 1.2	1.0 ± 0.1	93.8 ± 12.8
RMF 125 mg/kg	8.9 ± 1.1	2.8 ± 0.2*	72.5 ± 9.2
RMF 250 mg/kg	8.8 ± 1.1	2.1 ± 0.2	51.3 ± 7.4*
RMF 500 mg/kg	7.0 ± 1.4	2.5 ± 0.3*	51.3 ± 5.7*

Note : data expressed as mean ± S.E.M. (n = 8), * significantly different from the control group ($p < 0.05$).

Table 3. Effects of roselle mucilage fraction (RMF) on gastric wall mucus content in rats

Group	Gastric wall mucus (µg Alcian blue/g wet stomach)
Control ulcerated rats	804 ± 29
RMF 125 mg/kg	747 ± 26
RMF 250 mg/kg	1214 ± 63*
RMF 500 mg/kg	1453 ± 64*
Nonulcerated rats	1167 ± 16*

Note : Data expressed as mean ± S.E.M. (n = 8), * significantly different from control ulcerated rats ($p < 0.05$).

Table 4. Effects of roselle mucilage fraction (RMF) on gastric hexosamine content in rats

Group	Hexosamine content ($\mu\text{g}/100\text{ mg wet stomach}$)
Control ulcerated rats	21.7 ± 1.6
RMF 125 mg/kg	$29.2 \pm 1.5^*$
RMF 250 mg/kg	27.3 ± 2.8
RMF 500 mg/kg	24.7 ± 1.5
Nonulcerated rats	$37.0 \pm 2.2^*$

Note : Data expressed as mean \pm S.E.M. (n = 8), * significantly different from control ulcerated rats ($p < 0.05$).

DISCUSSION

Results obtained in this study showed the anti-gastric ulcer activity of RMF when evaluated in the most commonly utilized experimental models which include indomethacin, HCl/EtOH and water immersion restraint stress-induced gastric lesions in rats^{11, 12}.

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^{13, 14}.

According to the experimental models used in this study, non-steroidal anti-inflammatory drugs (NSAIDs) like indomethacin induce ulcer formation by depleting cytoprotective PGs, e.g. PGE₂ and PGI₂ in the cyclooxygenase pathway of arachidonic acid metabolism¹⁵. PGE₂ and PGI₂ of gastric and duodenal mucosa are responsible for mucus production and maintaining cellular integrity of the gastric mucosa¹⁶. In the HCl/EtOH induced gastric ulceration model, HCl causes severe damage to gastric mucosa¹⁷, whereas ethanol produces necrotic lesions by direct necrotizing action which in turn reduces defensive factors, the secretion of bicarbonate and production of mucus¹⁸. The water immersion stress-induced ulcers are mediated by increases in gastric acid secretion¹⁹ and motility²⁰ and decreases in mucosal microcirculation²¹ and mucus content²². Since RMF could prevent ulceration in all three

models and the effect in pylorus ligated rats was not clearly demonstrated, it is not likely that inhibition of gastric secretion is the action of the RMF.

The gastric wall mucus, obligatory components of which are hexosamines, is thought to play an important role as a defensive factor against gastrointestinal damages²³. The determined gastric wall mucus was used as an indicator for gastric mucus secretion while the mucosal hexosamine content was used as an indicator for gastric wall mucus synthesis²⁴. 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 nonulcerated group. It was found that pretreatment with RMF significantly preserved gastric mucus but not hexosamine content in HCl/EtOH ulcerated rats. This finding indicates that RMF may not play a role in gastric mucus synthesis although it does preserve the mucus content.

In conclusion this study provides evidence that roselle mucilage possesses an anti-gastric ulcer effect which is related to a cytoprotective mechanism via preservation of gastric mucus content. Mucilage is believed to be mixtures of polysaccharides of high molecular weight but also may contain proteins and minerals²⁵. Without further study of the roselle mucilage, it is not possible to make any comment on the compounds responsible for the observed activity.

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