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Mucus secretion stimulation: A mechanism in gastroprotective effect of *Zingiber officinale*

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ABSTRACT

There are several studies on the gastroprotective effect of *Zingiber officinale*, however, the nature of this effect has not been fully elucidated. In the present study, the gastroprotective activity and the underlying mechanism of the 95% ethanolic extract of *Z. officinale* was investigated. The extract was evaluated against gastric ulceration induced either by hydrochloric acid (HCl) or water immersion restraint stress (WIR) or aspirin (ASP). Pretreatment with the extract (0.1, 0.25, 0.5 or 1.0 g/kg) for 30 minutes before inducing an ulcer by HCl, WIR and ASP decreased gastric lesions with maximal inhibitions of 81.7, 44.1 and 68.2%, respectively. Moreover, the involvement of gastric secretion on this antigastric ulcer activity was determined in a model of histamine–induced secretion in gastric fistulae of rats. The extract significantly increased visible gastric mucus secretion and had a tendency to increase the secretory rate of soluble gastric mucus. Nevertheless, it had no effect on gastric pH, acid and pepsin secretion. These results demonstrate that the *Z. officinale* extract exerts moderate gastric ulcer protection by increasing gastric mucus secretion.

1. Introduction

Gastric ulcers result from an imbalance between aggressive factors such as acid secretion and defensive factors such as bicarbonate and mucus production. Furthermore, the incidence of gastric ulcers is increased by several factors such as stress, smoking, alcohol consumption, *Helicobacter pylori* infection, use of NSAIDs, inadequate dietary habits and nutritional deficiencies. In Thailand, the two primary causes of gastric ulcers are stress and family history of peptic ulcer disease [1] with the use of NSAIDs [2] being a close second. To address the gastric ulcer problem in Thailand, both modern allopathic drugs and herbal extracts have been used. Since pharmaceutical drugs have many side effects, the use of herbal drugs has often been preferred. In particular, the herbal drug is deemed safe if it is derived from a common food or vegetable source. Ginger, *Z. officinale*, is an herb with strong antioxidant properties [3-10] and an ability to prevent the generation of free radicals [11]. It also possesses numerous therapeutic properties including anti-inflammatory [12,13] and anti-microbial activities, the latter especially against *H. pylori*.

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[14,15].

Studies on the effects of the extracts of *Z. officinale* in a variety of gastric ulcer models have yielded conflicting results: either inhibition [15-18] or stimulation [19,20] or an absence of an antiulcer effect [19]. However, isolated and purified preparations of the major constituents of *Z. officinale* such as *6-gingesulfonic acid* [21], *6-gingerol*, *6-shogaol* [18] and *zingiberence* [16] have been shown to have anti-ulcer properties. In addition, ginger powder has been shown to prevent aspirin-induced gastric ulcer formation without affecting gastric juice or acid production [18]. In terms of mechanisms, most of these studies have focused on the effects of ginger extracts and compounds on acid secretion rather than on mucus production. Thus, *6-gingerol* potently reduced gastric acid secretion [22]. There has been no report exploring the effects of the extract of *Z. officinale* on ulcers and gastric pepsin. There is only one report on the ginger extract increasing gastric wall mucus in cold-restraint stress-induced ulcer [23]. Mucus is the major natural defense barrier that protects the gastric mucosa from irritants or corrosive agents, and its role as a mechanism in ulcer protection by drugs should be investigated. The mucus layer can be readily studied: it continuously covers the gastric mucosa and consists of a loosely-adherent layer (soluble mucus) that can be easily removed by suction, leaving a firmly-adherent mucus layer (visible mucus) attached to the epithelium [24].

Therefore, the aim of the present study was to assess the gastroprotective effect of the 95% ethanolic extract of the rhizome of *Z. officinale* as compared to the action of the H_2 antagonist, ranitidine, the commonly used drug for the treatment of peptic ulcer disease. The protective effect was studied in three models of gastric ulcer induction: hydrochloric acid (HCl), water immersion restraint stress (WIR) and aspirin (ASP). Finally, the gastric fistula model was used to define the effect of the extract on gastric secretions of acid, pepsin and mucus.

2. Materials and Methods

Chemicals: Histamine, hemoglobin, pepsin, chondroitin sulfate, and Alcian blue 8 GX were purchased from Sigma (Sigma-Aldrich, St. Louis, Missouri, USA); ranitidine hydrochloride from SMS Pharmaceuticals Ltd.,(Hyderabad, India), urethane from Fluka, (Buchs, Switzerland) and sodium hydroxide from Merck, (Darmstadt, Germany). All other reagents were of analytical grade and were obtained from Sigma.

Preparation of the ginger extract: Rhizomes of *Z. officinale* Roscoe (voucher number SKP206261501) were obtained from the Applied Thai Traditional Medicine Center, Faculty of Medicine, Thammasat University, Thailand. The rhizomes were washed, chopped into small pieces and dried in a hot air oven at 50˚C for 24 hours. The rhizomes were then macerated in 95% ethanol for 3 days at room temperature (28 ± 3 ˚C), filtered and concentrated to dryness under reduced pressure. The amount of 6-gingerol in the extracts was analyzed using high performance liquid chomatography (HPLC) [25]. One gram of crude ginger yielded 0.1217 g of 95% extract. One gram of extract yielded 0.0934 g of 6-gingerol. The extract was stored at 4˚C and freshly suspended in 1% sodium carboxy-methyl cellulose solution (CMC) on the day of the experiment to provide a homogenous suspension for prior to the administration to the animals.

Animal preparation: Male Wistar rats, 200-250 g each, from the National Laboratory Animal Center at Salaya, Mahidol University, were used. The animals were housed in polycarbonate cages in a controlled temperature of 22 ± 2 ˚C under a 12 hours light-dark cycle. The rats were fed a commercial diet (C.P. Mice Feed; SWT.Co., Ltd.) and tap water ad libitum. The experimental protocol was approved by the Institutional Animal Care and Use Committee of Faculty of Pharmacy, Mahidol University, in accordance with Ethical Principles and Guidelines for the Use of Animals for Scientific purposes by The National Research Council of Thailand. The Ethical Clearance of Animal Protocol number is PYT011/2552.

Experiment on gastric ulceration: Rats were fasted for 24-hour prior to the experimental protocol and were subsequently given, orally, one of the following test solutions: 1% CMC (control) or ginger extract (0.1, 0.25, 0.5, 1.0 g/kg) or ranitidine (50 mg/kg). All test solutions were prepared such that 5 ml/kg could be administered. After 30 minutes, gastric lesions were induced by intragastric administration of HCl (0.6 N 6 ml/kg,[26] for 4 h), water immersion restrain stress (WIR, restraint in stainless steel cage and immersed up to their xiphoid in a water bath at 16 ± 2˚C, [27] for 5 h) or intragastric administration of aspirin (ASP, 200 mg/kg,[28] for 6 h), based on previously established protocols for gastric lesion induction. At the end of the lesion induction period, rats were euthanized with an overdose of 95% carbon dioxide gas. The abdomen was surgically opened and the esophagus and pyloro-duodenal junctions were exposed and ligated. The stomach was infused with 10 ml of 0.5% formalin, then excised and fixed with 0.5% formalin for 10 min. The stomach was cut along the greater curvature and rinsed with normal saline to help keep the tissue flexible for examination. The glandular portion of the stomach was examined under a magnifying lens for gastric lesions. The total length of lesions was measured as the lesion index (mm/animal) for the HCl and WIR models. For the ASP model, the severity scores (0 = no remarkable lesion; 1 = mucosal edema and petechial hemorrhage; 2 = 1-5 small ulcers (1-2 mm); 3 = > 5 small ulcers or 1 medium ulcer (3-4 mm); 4 = > 2 medium ulcers or 1 large ulcer (> 4 mm); 5 = perforated ulcer) were measured as the lesion indices (severity score/animal).

Experiment on gastric secretion: The 24-hour fasted rats were anesthetized with urethane (1.2 g/kg, intraperitoneal injection). The esophagus was ligated at the neck region and the trachea was cannulated using polyethylene tube No. 206. The abdomen was opened and the pyloro-duodenal junction was ligated. The polyethylene tube No. 206 gastric fistula was inserted at

the non-glandular part and ligated to prevent slippage. After perforation, the stomach was lavaged with normal saline (*pH* 5.0). The sample was collected through the gastric fistula. The body core temperature was maintained at 37˚C. The sample collection was carried out at 1-h interval for 5 h (h1-h5). The first sample was referred to as the basal secretion (h1). After collecting the secretion at h1, histamine (10 mg/kg,) was administered intramuscularly and the test solutions (1% CMC or extract 0.1-1.0 g/kg or ranitidine 50 mg/kg diluted with 1% CMC to 1 ml/animal) were administered intraduodenally. At the end of the experiment, the stomach was removed and opened down the lesser curvature. The opened stomach was rinsed with normal saline *pH* 7.4 and dried with filter paper. Then the glandular portion was excised in order to determine the visible mucus.

Gastric visible mucus analysis: The visible mucus was determined according to the method described by Corne *et al*., 1974 [29]. After removing the stomach from the body, the glandular part of the stomach was excised, weighed, and immersed in 0.1% Alcian blue 8GX solution for 2 hours. Then the excess of uncomplexed dye was washed twice with 0.25 M sucrose. The stained dye on the gastric mucus was eluted by immersion in 0.5 M $MgCl_2$ for 2 hours. The amount of dye dissolved in the $MgCl_2$ solution was quantified spectrophotometrically µg by measuring absorbance at 605 nm, using Alcian blue 8GX dissolved in $MgCl_2$ as a standard. The amount of gastric visible mucus was expressed as Alcian blue/g stomach.

Gastric sample analysis: The pH of the samples collected at h1–h5 was determined by pH meter (Orion model 420, Vernon Hills, USA). The sample was then centrifuged at 2,500 x g for 5 min (Universal Centrifuge 16A, Hettich, Germany). The supernate was separated and used for the determination of gastric acid, pepsin activity and soluble mucus. Gastric acid was determined by titration with sodium hydroxide to the end point at pH 5.0. The gastric acid-secretory rate was calculated from the total gastric acid divided by the weight of the glandular part of the stomach and expressed as µEq HCl/g stomach/h. The proteolytic activity of pepsin in the gastric sample was measured using hemoglobin as a substrate and purified pepsin as a standard. The pepsin secretory rate was expressed as units/g stomach/h. Gastric soluble mucus was determined, using chondroitin sulfate as a standard to form complex with Alcian blue 8GX. The gastric soluble mucus-secretory rate was calculated from the amount of soluble mucus and expressed as µg chondroitin sulfate/g stomach/h.

Statistical analysis: The data were expressed as mean ± SEM (standard error of the mean). One way analysis of variance (ANOVA) and Tukey's honesty significant difference (HSD) test were used to compare the difference in the values among various experimental groups. ANOVA was used to compare the gastric secretion and gastric pH of each treatment group and then paired sample t-test was used to compare the secretory rates at h2, h3 h4 and h5 with h1 (the basal secretion). A p-value of less than 0.05 ($p<0.05$) was considered statistically significant.

3. Results

Under HCl-induced gastric ulceration, the ginger extract at 0.1, 0.25, 0.5, and 1.0 g/kg remarkably decreased the lesion index of the control group (130 ± 7 mm/animal) with the % inhibition of 55.9 ± 7.5, 77.6 ± 3.5, 79.7 ± 3.4 and 81.7 ± 3.4 %, respectively (Table 1). The inhibitory effects of 0.25, 0.5 and 1.0 g/kg were significantly higher than that 0.1 g/kg treated group. Ranitidine inhibited gastric ulcer by 68 ± 7.6% (from 130 ± 7 to 41 ± 10 mm/animal); the inhibitory effect was greater than 0.1 g/kg

Table 1 Effect of the 1% sodium carboxy-methyl cellulose (control), the 95% ethanolic extract of *Z. officinale* (E) 0.1, 0.25, 0.5, 1.0 g/kg and the ranitidine 50 mg/kg on gastric ulcer induced by hydrochloric acid (HCl) in rats (n=6).

Treatment	Lesion Index (mm/animal)	% Inhibition
Control	130 ± 7	-
E 0.1 g/kg	57 ± 10[a]	55.9 + 7.5[a]
E 0.25 g/kg	29 ± 5[a]	77.6 + 3.5[ab]
E 0.5 g/kg	26 ± 4[ab]	79.7 + 3.4[ab]
E 0.5 g/kg	24 ± 5[ab]	81.7 + 3.5[ab]
Ranitidine	41 ± 10[ab]	68.7 + 7.6[ab]

All values were expressed as mean ± SEM.
[a]$p< 0.05$: significantly lower than the control group
[b]$p< 0.05$: significantly lower than the extract 0.1 g/kg treated group

The effects of ginger extract on WIR-induced gastric ulceration, in contrast to HCl-induced ulcers, was less effective in decreasing WIR-induced gastric ulcer index. Thus, the extract, at 0.1, 0.25, 0.5 and 1.0 g/kg, decreased the ulcer index of the control group (104 ± 9 mm/animal) (Table 2) by only 13.6 ± 8.1, 15.2 ± 4.4, 35.4 ± 10.7 and 44.1 ± 9.0%, respectively. The ulcer of 0.5 and 1.0 g/kg treated groups were significantly less than the control. The inhibition of 1.0 g/kg treated group was also significantly higher than the 0.1g/kg treated group. Ranitidine reduced the lesion index by 86.4 ± 4.4% (from 104 ± 9 to 14 ± 5 mm/animal); the reduction was significantly greater than all of the extract-treated groups.

Effects of ginger extract on ASP-induced gastric ulceration, while there was no significant difference in the severity of the gastric lesion between the control and the 0.1 g/kg treated group (Table 3), all other concentrations of the extract attenuated the lesion significantly. Thus, at the doses of 0.25 and 0.5 g/kg, the extract exhibited a significant 50.1% inhibition of the gastric ulcers and at 1.0 g/kg, a maximal inhibition (68.2%). The strongest inhibition was observed in the ranitidine treatment, at 95.5 ± 4.5%; the inhibition was significantly greater than

Table 2 Effect of the 1% sodium carboxy-methyl cellulose (control), the 95% ethanolic extract of *Z. officinale* (E) 0.1, 0.25, 0.5, 1.0 g/kg and the ranitidine 50 mg/kg on gastric ulcer induced by water immersion restraint stress (WIR) in rats (n=6).

Treatment	Lesion Index (mm/animal	% Inhibition
Control	104 ± 9	-
E 0.1 g/kg	90 ± 8	13.6 ± 8.1
E 0.25 g/kg	88 ± 5	15.2 ± 4.4
E 0.5 g/kg	67 ± 11[a]	35.4 ± 10.7[a]
E 0.5 g/kg	58 ± 9[a]	44.1 ± 9.0[ab]
Ranitidine	14 ± 5[abcde]	86.4 ± 4.4[abcde]

All values were expressed as mean ± SEM.
[a]p< 0.05: significantly lower than the control group
[b]p< 0.05: significantly lower than the extract 0.1 g/kg treated group
[c]p< 0.05: significantly lower than the extract 0.25 g/kg treated group
[d]p< 0.05: significantly lower than the extract 0.5 g/kg treated group
[e]p< 0.05: significantly lower than the extract 1.0 g/kg treated group

those of all of extract-treated groups except the 1.0 g/kg extract-treated group.Under effects of ginger extract on histamine-induced gastric secretory *pH* secretion, histamine significantly decreased gastric pH of the control group between h2 and h4, with the *pH* returning to the basal level at h5 (Table 4). The ginger extract, at every concentration, did not alter the histamine-induced gastric pH pattern. However, ranitidine significantly prevented the histamine-induced *pH* changes from h2 to h5.

Table 3 Effect of the 1% sodium carboxy-methyl cellulose (control), the 95% ethanolic extract of *Z.officinale* (E) 0.1, 0.25, 0.5, 1.0 g/kg and the ranitidine 50 mg/kg on gastric ulcer induced by aspirin (ASP) in rats (n=6).

Treatment	Severity scores/animal	% Inhibition
Control	3.67 ± 0.33	-
E 0.1 g/kg	3.67 ± 0.33	0
E 0.25 g/kg	1.83 ± 0.31[ab]	50.1 ± 8.4[ab]
E 0.5 g/kg	1.83 ± 0.31[ab]	50.1 ± 8.4[ab]
E 0.5 g/kg	1.17 ± 0.40[ab]	68.2 ± 10.9[ab]
Ranitidine	0.17 ± 0.17[abcd]	95.5 ± .54[abcd]

All values were expressed as mean ± SEM.
[a]p< 0.05: significantly lower than the control group
[b]p< 0.05: significantly lower than the extract 0.1 g/kg treated group
[c]p< 0.05: significantly lower than the extract 0.25 g/kg treated group
[d]p< 0.05: significantly lower than the extract 0.5 g/kg treated group

The *pH* between h2 to h4 of ranitidine treated group was significantly higher than the rest of experimental groups. During the effects of ginger extract on histamine-induced gastric acid secretory rate, the gastric acid secretory rate of the control group was significant higher than the basal point with a maximum at h2, and gradually decreased from h3 to h5 (Table 4).

Table 4 Effects of the 95% ethanolic extract of *Z. officinale* (E) on histamine-induced gastric secretory pH, gastric acid secretory rate (μEq/g stomach/h), gastric pepsin secretory rate (unit/g stomach/h) and gastric soluble mucus secretory rate (μg AB/g stomach/h) at h1, h2, h3, h4, and h5 (R; ranitidine) (n=6).

Treatment		Time (h)				
		1	2	3	4	5
pH	Control[##]	3.87 ± 0.47	1.56 ± 0.02[a**]	1.70 ± 0.06[a**]	2.23 ± 0.11[a**]	2.98 ± 0.16
	E 0.1 g/kg[##]	3.97 ± 0.44	1.54 ± 0.04[b**]	1.69 ± 0.08[b**]	2.38 ± 0.20[b*]	3.02 ± 0.31
	E 0.5 g/kg[##]	3.83 ± 0.34	1.56 ± 0.04[b**]	1.75 ± 0.04[b**]	2.29 ± 0.08[b**]	3.34 ± 0.35
	E 1.0 g/kg[##]	3.94 ± 0.15	1.50 ± 0.03[b**]	1.64 ± 0.05[b**]	2.04 ± 0.07[b**]	2.79 ± 0.11**
	R 50 mg/kg[#]	3.81 ± 0.33	2.78 ± 0.28*	3.03 ± 0.30	3.36 ± 0.27	3.49 ± 0.28
Acid	Control[##]	1.24 ± 0.31	106.33 ± 10.59[a**]	57.02 ± 14.01a**	12.56 ± 2.87[a**]	3.58 ± 0.60**
	E 0.1 g/kg[##]	1.23 ± 0.53	117.35 ± 24.80[b**]	57.24 ± 13.64b**	10.27 ± 2.63**	5.11 ± 2.22
	E 0.5 g/kg[##]	1.32 ± 0.59	112.32 ± 16.90b**	39.14 ± 4.73**	10.13 ± 1.58**	2.42 ± 0.56
	E 1.0 g/kg[##]	0.91 ± 0.23	103.66 ± 14.93[b**]	57.90 ± 11.19b**	16.95 ± 2.42b**	3.72 ± 0.99*
	R 50 mg/kg[#]	1.03 ± 0.45	6.64 ± 1.12**	3.95 ± 0.64**	2.40 ± 0.57	1.89 ± 0.58
Pepsin	Control[##]	275.21 ± 67.73	1201.25 ± 106.31[a**]	549.48 ± 79.07[a*]	311.84 ± 50.70	206.11 ± 52.05
	E 0.1 g/kg[##]	261.85 ± 47.09	1165.25 ± 69.01b**	589.24 ± 65.48[b**]	207.78 ± 56.23	188.96 ± 60.25
	E 0.5 g/kg[##]	243.80 ± 54.27	1351.16 ± 230.43b**	607.63 ± 44.70[b**]	278.44 ± 64.87	219.32 ± 78.91
	E 1.0 g/kg[##]	217.00 ± 40.38	1387.35 ± 195.32b**	727.63 ± 127.66[b**]	484.13 ± 60.41b**	410.44 ± 69.60*
	R 50 mg/kg[#]	172.29 ± 40.22	321.62 ± 52.24*	203.70 ± 42.47	224.90 ± 56.82	234.57 ± 43.68
Soluble mucus	Control[##]	6.29 ± 2.36	66.27 ± 17.86*	21.62 ± 4.59**	7.36 ± 2.36	8.14 ± 2.47
	E 0.1 g/kg[##]	11.42 ± 4.63	128.65 ± 36.53b*	52.80 ± 11.86b*	19.74 ± 2.45[b]	12.26 ± 4.05
	E 0.5 g/kg[##]	4.97 ± 1.99	67.88 ± 14.63**	24.21 ± 6.94*	6.81 ± 1.22	8.61 ± 3.58
	E 1.0 g/kg[##]	3.71 ± 1.09	82.94 ± 19.50**	42.11 ± 9.65**	9.57 ± 1.25*	6.99 ± 1.77
	R 50 mg/kg[#]	7.36 ± 2.69	9.84 ± 3.74	8.23 ± 3.40	3.38 ± 1.46	5.22 ± 1.48

All values were expressed as mean ± SEM.
[#]p<0.05, [##] p<0.001 : ANOVA analysis of pH or secretory rates within group
*p<0.05, **p<0.01: significantly different from the basal secretion within group
Comparison at the same time point;
[ap]<0.05, [bp]<0.01: significant difference with the ranitidine treated group

The patterns of the extract-treated groups were similar to the control groupwith the exception that the secretory rate of the 0.1 and 0.5 g/kg groups had returned to their own basal levels at h5. In contrast, ranitidine reduced the gastric acid secretory rate to 95% at h2 and the rate declined further at h3 and returned to basal levels at h4 and h5. Gastric secretion in the ranitidine treated rats was significantly less than the extract-treated group at h2 (doses of 0.1, 0.5 and 1.0 g/kg), h3 (doses of 0.1 and 1.0 g/kg) and h4 (dose of 1.0 g/kg).

Under effects of ginger extract on histamine-induced gastric pepsin secretory rate, the pepsin secretory rate of the control group showed the peak at h2, substantially decreased at h3 and then gradually returned to the basal level at h4 and h5 (Table 4). The extract-treated groups showed a similar pattern to the control group with the exception at the dose of 1.0 g/kg, of which the values at h4 and h5 were still high and did not return to the basal levels. As in the case of *pH* and acid secretion, ranitidine showed the strongest effect, decreasing the peak of pepsin secretory rate (h2) by 84%, to the levels indistinguishable from basal at h3, h4 and h5. Therefore, pepsin levels in the ranitidine-treated animals were significantly lower than those of the extract-treated groups at any time points examined.

Effects of ginger extract on histamine-induced gastric soluble mucus secretory rate. The pattern of the soluble mucus secretory rates of the control group and the extract-treated groups were similar, with a peak at h2 and a steady decline thereafter (Table 4). There were no significant differences in gastric soluble secretory rates between control and all of the extract-treated groups at any time point. In contrast, the ranitidine-treated group showed no significant increases in soluble mucus secretory rates as compared to its own basal secretion. The secretory rate of the 0.1 g/kg treated group at h2 to h4 was significantly higher than the ranitidine treated group.

Effects of ginger extract on histamine-induced gastric visible mucus secretory rate. The visible mucus content of the control was 418.40 ± 39.56 µg AB/g stomach (Fig. 1). These rates were not altered by either the lower two doses (0.1 g/kg and 0.5 g/kg) of the extract or by ranitidine. However, the 1.0 g/kg of extract caused a significant 65% increase in the secretory rate of visible mucus over the control (688.31 ± 74.51 µg AB/g stomach). This value was also 86% higher than that of the ranitidine-treated group (371.04 ± 56.63 µg AB/g stomach).

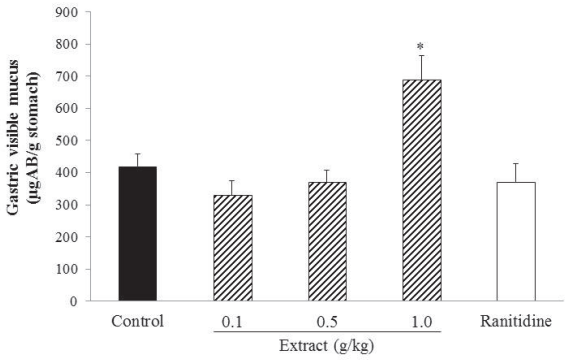

Figure 1 Effects of the 95% ethanolic extract of *Z. officinale* on histamine-induced gastric visible mucus secretory rate (µg AB/g stomach/h) (R; ranitidine) (n=6). All values were expressed as mean ± SEM.
*p<0.05: significantly higher than other groups

4. Discussion

In the present study, oral administration of ginger extract reduced gastric lesions in three different models of gastric ulcers, by 81.7% (HCl), 44.1% (WIR) and 68.2% (ASP). In contrast, ranitidine inhibited ulcer formation in these models by 68.5% (HCl), 86.5% (WIR) and 95.4% (ASP). Since gastric ulcers can be caused by several factors, three animal models developed to mimiculcer formation under different underlying causes [30], were selected for testing the extract. First, the HCl model was used to reveal the cytoprotective mechanism. HCl directly destroys the gastric mucosa barrier and causes massive localized erosion of the mucosa leading to severe localized inflammation and hemorrhage. Second, the WIR model was used to investigate the proton pump stimulatory mechanism. It reproduces the stress situation aroused by physical and psychological discomfort. Immobilization reduces the motor activity and the cold water interferes with the core body temperature of the animals. Cold-restraint increases histamine release in the stomach under vagal influence [31] and this in turn activates H_2 receptors to stimulate gastric acid secretion in the stomach. Moreover, WIR causes inflammation by increasing several proinflammatory cytokines [32]. Third, the ASP model was used to probe the mucosal protective mechanism. Aspirin inhibits prostaglandins synthesis which protects the gastric mucosa by producing leukotrienes and bicarbonate ions. Aspirin inhibits gastric peroxidase and increases levels of mucosal hydrogen peroxide and hydroxyl ions to cause oxidative mucosal damage [33].

This study agrees with the report of al-Yahya *et al*., that ginger reduces the gastric ulcer induced by both irritant (80% ethanol, 0.6 M HCl, 0.2 M NaOH, 25% NaCl and NSAIDs) and non-irritant (hypothermic restrain stress) [34] stimuli. The present study however, is different in that it provides a mechanistic basis for this action. Yamahara *et al*., reported that the acetone extract of ginger and ginger-based compounds such as zingiberene (main terpenoid of acetone extract) and 6-gingerol (the pungent principle) significantly inhibited HCl/ethanol-induced gastric ulcer [16] Siddaraju *et al*., revealed that the aqueous extract (0.2 g/kg) protected swim stress-induced ulcers by 86%.8 Khushtar et al. reported that ginger oil (0.5, 1.0 g/kg p.o.) had a protective action on aspirin plus pylorus ligation model [17] Wang *et al*., showed that the ginger powder 0.2 g/kg in 1% CMC reduced aspirin-induced gastric hemorrhagic ulcer area [18]. On the other hand,

Wu *et al*., presented that roasted ginger (4.5 g/kg) and dry ginger did not inhibit indomethacin (other NSAIDs)-induced gastric ulcer [19]. While it is clear that ginger and its extracts most likely influence gastric ulcers, a meaningful conclusion based on the various reports will depend on a careful comparative analyses of the methods of extract preparation, the dose, frequency, duration and route of treatment and the ulcer models tested. Another confounding factor is that the plant contains various ingredients, some of which may be inhibitory and others stimulatory; thus some factors may contribute to the failure of host defense systems and others may enhance mucosal healing.

The present results strongly suggest that the 95% ethanolic extract of *Z. officinale* could be beneficial for the prevention of gastric mucosa injury, and especially to counteract irritants such as found in spicy, acidic or sour meals. The prevalence of gastroduodenal mucosal injuries (erosion or ulcer) in NSAIDs users is 63.5% in Thailand [35]. Since ginger extract caused a 68% reduction in ASP-induced lesions, taking ginger should be of benefit. Ginger extract can also be used in stress-inducing ulcers, although the effects were much more moderate in this model.

Earlier studies had reported that while ginger powder, at 0.2 g/kg, did not affect gastric juice or acid production [18] the oral administration of *6-gingerol* (1.5-50 mg/kg) significantly and dose-dependently inhibited gastric acid secretion [22]. However, these studies did not examine the effect of ginger on other gastric secretions such as pepsin or mucus. To understand whether the extract protects the gastric mucosa by altering the rates of gastric secretion, the rat gastric fistula model was used in the present study. Normally, endogenous histamine, secreted from the ECL-cells, stimulates gastric acid secretion via H_2 receptor on parietal cells in gastric glands. In this model, gastric secretion was stimulated by intramuscular injection of histamine. The efficacy of various test solutions (varying concentrations of the extract or ranitidine), administered intraduodenally to avoid interference by gastric luminal contents, on histamine-stimulated secretions was examined. Overall, this study showed that rats treated with the extract did not change the time course profile of gastric secretion induced by histamine. Only the dose of 1.0 g/kg prolonged the induction of gastric pepsin secretory rate above that of the control. Thus, the extract did not decrease the aggressive factors, suggesting that the gastric protection of ginger possibly comes from increasing defensive factors such as mucus secretion.

Gastric mucus functions as a barrier and its release is often triggered by chemical irritants. The mucus layer continuously covers the gastric mucosa and as mentioned earlier consists of a loosely adherent, soluble mucus, layer that can be easily removed by suction, and a firmly adherent, visible mucus layer attached to the epithelium [24]. The effect of ginger extract on both layers was investigated. The extract had no effect on the gastric soluble mucus secretion. However, at a dose of 1.0 g/kg, the extract increased the visible mucus content significantly, up to 65% over control. Thus, the extract is highly effective in blocking gastric ulceration without blocking gastric acid and pepsin secretion. It appears to do so by increasing the adherent, gastric visible mucus secretion. Since the extract was administered intraduodenally, it did not have a direct contact with the gastric mucosa, so it is unlikely that this mucus stimulating effect was caused by irritation. Although the exact mechanism of increasing visible mucus of ginger extract is not known, ginger, the high calcium containing food36 may induce visible mucus secretion by Ca2-dependent exocytosis [37]. This result was similar to Singh and Kaur which showed that the ginger extract by supercritical carbondioxide extraction increased gastric wall mucus in cold-restraint stress induced ulcer [23]. Since this extract, the other ginger extract [23] and ginger oil [17] were shown to compose of 6-gingerol and increase visible mucus, 6-gingerol was presumed to be one of the active compounds that contributed to the ulcer protective activity of the extract by increasing soluble mucus. The gastroprotective effect of the extract should be caused by several mechanisms. Ginger has shown strong antioxidant, 5,10 anti-inflammatory [12,13] and anti-microbial activities, especially against *H. pylori* [14,15]. Stimulation of visible mucus secretion is another mucosal protective mechanism.

Based on the present results, ginger extract is highly effective in attenuating gastric ulceration caused by HCl and has a less dramatic, albeit protective, effect on ulcers caused by stress and ASP. Although the extract cannot inhibit the secretion of gastric acid and peptic juice, it can stimulate the production of visible mucus. Therefore it can be suggested as an ulcer preventive agent. As compared to ranitidine, the ginger extract showed a higher protective effect against irritant agents such as HCl but was less effective than ranitidine in protecting against stress and NSAIDs induced ulcers. This is not unexpected because ranitidine, an H_2 antagonist, inhibits gastric acid secretion, the offensive factors induced by stress [30] and NSAIDs [38,39]. So ginger may help to reduce, partially, risk of gastric ulcer disease and it is possible that a combination of the extract with ranitidine will enhance the protective activity. Partaking of ginger as a foodstuff or alternative herbal medicine has the potential of reducing irritation from a meal and for reducing ulcer development as a consequence of NSAIDs consumption or behavioral stress. In Thailand, ginger is widely available and used as an edible vegetable; therefore it can also be useful as a therapy for a variety of diseases including gastric ulcers.

5. Conclusion

The *Z. Officinale* extract exerts moderate gastric ulcer protection by increasing gastric mucus secretion. The ginger extract has a higher protective effect than ranitidine against irritant agents but is less effective than ranitidine in protecting against stress and NSAIDs induced ulcers.

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