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Short communication

Anti-gastric ulcer effect of *Kaempferia parviflora*

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Abstract

Kaempferia parviflora is a Zingiberaceous plant, which has been reputed for its beneficial medicinal effects. The present study was undertaken to evaluate the *Kaempferia parviflora* ethanolic extract (KPE) for its anti-gastric ulcer activity by experimental models. Oral administration of the KPE at 30, 60 and 120 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 the KPE had no effect on gastric volume, pH and acidity output. In ethanol-induced ulcerated rats, gastric wall mucus was significantly preserved by the KPE pretreatment at doses of 60 and 120 but not at 30 mg/kg. The findings indicate that the ethanolic extract of *Kaempferia parviflora* possesses gastroprotective potential which is related partly to preservation of gastric mucus secretion and unrelated to the inhibition of gastric acid secretion.

Keywords: *Kaempferia parviflora*; Zingiberaceae; Gastric ulcers

1. Introduction

Kaempferia parviflora Wall. ex Baker or Krachai Dam belongs to the Zingiberaceae family. The alcoholic infusion of its rhizome has been used as a tonic for rectifying male impotence, body pains and gastrointestinal disorders (Yenjai et al., 2004). However, there has been no scientific report to support these claims. The present study is, thus, aimed to evaluate anti-gastric ulcer effect of *Kaempferia parviflora* using standard experimental models.

2. Materials and methods

2.1. Plant material and extraction

Kaempferia parviflora rhizomes were collected from Amphoe Na Haeo, Loei, Thailand. The plant was authenticated by the Queen Sirikit Botanical Garden, Chiangmai, Thailand, and a voucher specimen (QSBG C. Maknoi 477) was deposited in its herbarium. The rhizomes were washed thoroughly in tap water, shade-dried and powdered. The rhizome powder was extracted with 95% ethanol, evaporated in vacuo at 55 °C and lyophilized to obtain a dry extract (5.7% yield) which from now on is referred as KPE. Phytochemical screening of the KPE gave positive tests for alkaloids, anthrones, coumarins and, flavonoids. The KPE was suspended in 5.0% Tween-80, to required concentrations and used for the experiments.

2.2. Experimental animals

Male Sprague–Dawley rats weighing 150–200 g were purchased from the National Laboratory Animal Center, Salaya Mahidol University, Thailand. The animals 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-h light:12-h dark cycle. The food was supplied by Perfect Companion Co. Ltd., Samut Prakan. The animals had free access to food and water unless stated otherwise. All animals received humane care in compliance with the ethics in the

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use of animals issued by the National Research Council of Thailand, 1999.

2.3. Indomethacin-induced gastric ulcers

The KPE was administered orally to 48 h 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 (Djahanguiri, 1969). After 5 h the rats were sacrificed and examined for gastric ulcers.

2.4. HCl/EtOH-induced gastric ulcers

The KPE was administered orally to 48 h fasted rats 60 min prior to induction of gastric ulcers by 1.0 ml HCl/EtOH (60 ml EtOH + 1.7 ml HCl + 38.3 ml H_2O) p.o. (Mizui and Doteuchi, 1988). The animals were sacrificed and examined for gastric ulcers 60 min later.

2.5. Restraint water immersion stress-induced gastric ulcers

The KPE was administered orally to 48 h 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. (1963). After 5 h of this exposure, the rats were sacrificed and examined for gastric ulcers.

2.6. Evaluation of the gastric ulcers

After each rat was 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.

2.7. Pylorus ligation

The KPE was administered orally to 48 h fasted rats. One hour later, pylorus ligation as described by Shay et al. (1945) 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 sacrificed 5 h later by an overdose of ether. The stomach was removed and its content was subjected to measurement of volume and pH and assayed for titratable acidity.

2.8. Determination of gastric wall mucus content

Gastric wall mucus was determined by the Alcian blue method (Corne et al., 1974). Briefly, the KPE was administered orally to 48 h fasted rats 60 min prior to induction of gastric ulcers by 1.0 ml HCl/EtOH (60 ml EtOH + 1.7 ml HCl + 38.3 ml H_2O) p.o. (Mizui and Doteuchi, 1988). 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 h. 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 h. 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 extract per gram wet stomach was then calculated from a standard curve.

2.9. 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 statistically significant.

3. Results and discussion

Oral administration of the KPE at doses of 30, 60 and 120 mg/kg as well as cimetidine at a dose of 100 mg/kg significantly inhibited gastric ulcer formation induced by indomethacin, HCl/EtOH and water immersion restraint stress (Table 1). The doses used in these experimental models may be so high that the effect produced nearly reached its peak and the dose-related manner could not be demonstrated clearly. In pylorus-ligated rats, the KPE at the same doses did not decrease the gastric volume and acidity nor increase the gastric pH while cimetidine, a specific H_2-receptor antagonist could (data not shown). It was also found that pretreatment with the KPE at doses of 60 and 120 mg/kg but not 30 mg/kg significantly increased the amount of gastric mucus content in HCl/EtOH-ulcerated rats (Table 2).

According to the experimental models used in this study, NSAIDs like indomethacin induce ulcer formation by depleting cytoprotective PGs. PGE_2 and PGI_2 of gastric and duodenal mucosa are responsible for mucus production and maintaining cellular integrity of the gastric mucosa (Konturek et al., 1984). In the HCl/EtOH induced gastric ulceration model, HCl causes severe damage to gastric mucosa (Yamahara et al., 1988) whereas ethanol produces necrotic lesions by direct necrotizing action which in turn reduces defensive factors, the secretion of bicarbonate and production of mucus (Marhuenda and Martin, 1993). The water immersion stress-induced ulcers are caused by an increase in gastric acid secretion (Kitagawa et al., 1979) and decreases in mucosal microcirculation (Guth, 1972) and mucus content (Koo et al., 1986).

The finding that the KPE failed to increase the gastric pH and decrease the gastric volume and acidity in pylorus-ligated rats suggests that anti-secretory action is unlikely ascribed to the anti-gastric ulcer effect of the KPE.

Table 1
Effects of *Kaempferia parviflora* ethanolic extract (KPE) on gastric ulcers in rats

Group	Gastric ulcer inducer					
	Indomethacin		HCl/EtOH		Water immersion restraint stress	
	Ulcer index (mm)	*I* (%)	Ulcer index (mm)	*I* (%)	Ulcer index (mm)	*I* (%)
Control	10.9 ± 3.2		122.8 ± 11.5		19.2 ± 2.3	
KPE 30 mg/kg	1.1 ± 0.8*	90	26.1 ± 8.0*	79	3.3 ± 0.6*	83
KPE 60 mg/kg	0.3 ± 0.1*	97	3.3 ± 1.1*	97	7.3 ± 0.7*	62
KPE 120 mg/kg	0.5 ± 0.3*	95	0.5 ± 0.4*	99	3.8 ± 0.7*	80
Cimetidine 100 mg/kg	0.2 ± 0.1*	98	71.3 ± 6.9*	42	1.4 ± 0.5*	93

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

Table 2
Effects of *Kaempferia parviflora* ethanolic extract (KPE) on gastric wall mucus content in rats

Group	Gastric wall mucus (μg Alcian blue/g wet stomach)
Control HCl/EtOH ulcerated rats	331 ± 35
KPE 30 mg/kg	438 ± 25
KPE 60 mg/kg	588 ± 53*
KPE 120 mg/kg	663 ± 51*
Cimetidine 100 mg/kg	366 ± 18

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

The gastric wall mucus is thought to play an important role as a defensive factor against gastrointestinal damage (Davenport, 1968). The determined gastric wall mucus was used as an indicator for gastric mucus secretion (Lukie and Forstner, 1972). The finding that pretreatment with the KPE at doses of 60 and 120 mg/kg but not 30 mg/kg significantly increased gastric mucus content in HCl/EtOH ulcerated rats suggests that the gastroprotective effect of the KPE is mediated only partly by preservation of gastric mucus secretion.

In conclusion, this study provides evidence that the ethanolic extract of *Kaempferia parviflora* possesses an anti-gastric ulcer effect, which is related partly to a preservation of gastric mucus secretion and unrelated to the inhibition of gastric acid secretion.

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