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A protective effect of the methanol extract of *Shelliguea feei METT.* roots on gastric ulcers in mice and rats

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SUMMARY

A protective effect of the methanol extract of *Shelliguea feei METT.* roots on gastric lesions induced by stress in mice and HCl/ethanol in rats has been investigated. Animals were randomly divided into control and test groups and given the methanol extract orally at doses of 0.5, 1.0, and 2.0 g/kg of body weight. This investigation indicated that the methanol extract at doses of 1.0 and 2.0 g/kg significantly reduced stress-induced gastric lesions in mice at the percent protection of 38% and 62%, respectively, and decreased the number of HCl/ethanol-induced ulcers in rats significantly at the percent inhibition of 21.50% and 90.65%, respectively, and severity of ulcers at the score of 3.6 and 1.0 significantly different from the control. These results suggest that the methanol extract of *S. feei* may have a beneficial protective effect on the gastric ulcers.

Key words: *Shelliguea feei;* Ulcer; Anti-ulcerogenik; *Shelegueain A*

INTRODUCTION

Shelliguea feei METT (Polypodiaceae) is an Indonesian plant growing abundantly around the crater of the Tangkuban Parahu Mountain in West of Java of Indonesia. The roots of this plant are traditionally used for the treatment of rheumatism and hypertension, in addition to having an aphrodisiac effect.

Our studies on naturally occurring active compounds have been focused on those having potential antirheumatic activity, as a discovery of new types of antirheumatism is expected to be clinically beneficial. Our previous work on the roots of *S. feei* led to the isolation of a trimeric proanthocyanidin, *Shelegueain A*, which has analgesic and anti-inflammatory activity examined by acetic acid-induced writhing and carrageenan-induced paw edema methods, respectively, and inhibit cyclooxygenase activity (Subarnas and Wagner, 2000). This compound was firstly isolated and identified as a sweet constituent by Baek *et al.* (1993).

The discovery of analgesic and anti-inflammatory activity of the proanthocyanidine isolated from the roots of *S. feei* has prompted us to investigate the effect of the methanol extract of this plant on gastric ulceration in mice and rats, as it is generally known that analgesic and anti-inflammatory drugs have some unwanted effects, and the most common effect is a propensity to induce gastric and intestinal ulceration. The anti-ulcerogenic activity of the extract was examined by measuring the ulcer index and the condition of tissue damage of stomach mucosa in mice and rats.

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MATERIALS AND METHODS

Plant materials

The roots of *Shelliguea feei* used in this experiment were collected around the crater of the Tangkuban Parahu Mountain in Bandung, West of Java. The roots were dried on an open air free of the direct sun's rays.

Animals

Animals used for a stress-induced ulcers experiment were white male mice of ddy strains weighing 25-30 g, whereas for a HCl/ethanol-induced ulcer experiment were white male rats of Wistar strains weighing 200-250 g. The animals were kept in an air-conditioned room and were allowed food and water.

Extract preparation

Dried roots of *Shelliguea feei* (240 g) were powdered and extracted with 3 L of methanol 95% (3 × 24 hr) at a room temperature, and the solvent was evaporated under reduced pressure at 50℃ to yield a concentrated extract. The extract was dispersed in 1% Arabic gum solution just before use. The flow chart of preparing the extract is shown in the figure below.

Powdered roots of S. feel(240g)

↓ Extracted with MeOH 95%, 3 × 24 h, at room temp

MeOH extract

↓ Evaporated, 50℃

Concentrated extract

Fig. 1. Flow chart of extraction of the *Shelliguea feei* roots.

Stress-induced gastric ulcers in mice (Hikino, 1985)

Mice were divided into control and test groups, each group consist of 6 mice. After 24 hours fasting with free access to water, mice were orally administered vehicle in the control group and the methanol extract at doses of 0.5, 1.0 and 2.0 g/kg of body weight in the test groups. After 60 min, mice were put in stress cages and immersed vertically to the level of the mice neck in a water bath (25℃) for 18 hr. After that, mice were sacrificed and their stomachs were removed and inflated with 10% formalin for 10 min. The stomachs were opened along the greater curvature and washed carefully with saline. The number of gastric ulcers in each stomach was calculated. The stress cages used consists of wooden board provided with holes to which mice are tied in vertical position.

HCl/ethanol-induced gastric ulcers in rats (Mosaddik and Alam, 2000)

Rats were divided into control and test groups, each group consist of 5 rats. After 24 hours fasting with free access to water, rats were orally administered vehicle in the control group and the methanol extract at doses of 0.5, 1.0 and 2.0 g/kg of body weight in the test groups. After 60 min, rats were administered 0.15 M HCl/60% ethanol (0.1 ml/10 g), and 60 min later all rats were sacrificed and their stomachs were removed and inflated with 10% formalin for 10 min. The stomachs were opened along the greater curvature and washed carefully with saline. The number and severity of gastric ulcers in each stomach were calculated and converted to scores.

The scoring used is as follows (Wattimena, 1982).

- For ulcer number:

	Score
- Normal	1
- Small spots of ulcers, Φ< 0.5 mm	2
- Number of ulcers 1 - 3	3
4 - 6	4
7 - 9	5
> 9 or perforation	6

For severity of ulcers

	Score
- Normal	1
- Ulcers, < 0.5 mm	2
- Ulcers, 0.5 1.5 mm	3
- Ulcers, 1.6 4.0 mm	4
- Ulcers, > 4.0 mm	5
- Perforation	6

Calculation of ulcer index (UI) (Goel, 1985):

$UI = N + S + \% I/10$

UI = ulcer index
N = number of ulcers
S = severity score of ulcers
%I = percent number of animals suffering Gastric ulcers

Calculation of % inhibition:

$$PI = 100 - \left[\frac{\text{UI of test drug}}{\text{UI of control}} \times 100 \right]$$

PI = percent inhibition
UI = ulcer index

Statistical analysis

Data were analyzed by analysis of variance (ANAVA), and the significance of difference was calculated according to Student's *t*-test.

RESULTS

Anti-ulcerogenic effect in stress-induced gastric ulcers in mice

Table 1 shows an effect of the *S. feei* extract on gastric ulcers induced by stress in mice. The extract, at doses of 0.5, 1.0, and 2.0 g/kg of body weight, decreased the number of gastric ulcers in a dose-dependent manner. The significant effect was shown by the doses of 1.0 and 2.0 g/kg with the percent inhibition of 38.3% and 62.5%, respectively.

Table 1. Effect of the methanol extract of *S. feei* roots on stress-induced gastric ulcers in mice

Drug	Dose (g/kg, p.o.)	Number of gastric ulcers (Mean ± S.E)	Inhibition (%)
Control	-	31.2 ± 3.3	-
S. feei Extract	0.5	20.8 ± 2.3	33.0
	1.0	19.3 ± 2.4*	38.3
	2.0	12.0 ± 1.2**	62.5

*: Significantly different from the control according to t-test ($P < 0.05$).
**: Significantly different from the control according to *t*-test ($P < 0.01$).

Anti-ulcerogenic effect in HCl/ethanol-induced gastric ulcers in rats

An effect of the *S. feei* extract on gastric ulcers induced by HCl/ethanol in rats is shown in Table 2, and the ulcer index is indicated in Fig. 2. The extract, at doses of 1.0 and 2.0 g/kg of body weight, decreased significantly the number of gastric ulcers in a dose-dependent manner as compared with the control group, with the percent inhibition of 21% and 91%, respectively.

Table 2. Effect of the methanol extract of *S. feei* roots on HCl/EtOH-induced gastric ulcers in rats.

Drug	Dose (g/kg, p.o.)	Number of gastric ulcers (Mean ± S.E)	Inhibition (%)	Severity score of ulcers (Mean ± S.E.)
Control	-	6.0 ± 0.25	-	5.4 ± 0.55
S. feei Extract	0.5	5.2 ± 0.48	7.5	4.6 ± 1.34
	1.0	3.2 ± 0.45*	21.5	3.6 ± 0.54*
	2.0	1.0 ± 0.02**	90.7	1.0 ± 0.02**

*: Significantly different from the control according to t-test($P < 0.05$).
**: Significantly different from the control according to t-test($P < 0.01$).

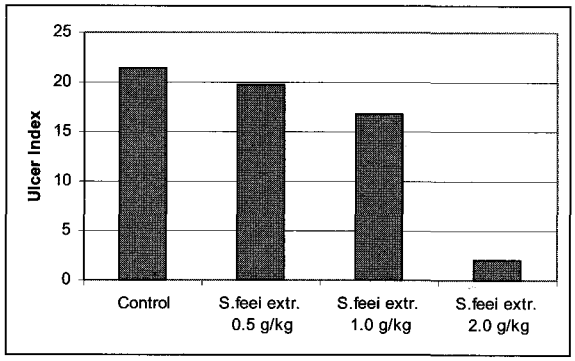

Fig. 2. Ulcer index of the gastric lesions

DISCUSSION

Stress and non-steroidal anti-inflammatory drugs (NSAIDs) are known to have an inducing effect on the occurrence of gastrointestinal lesions, because these factors can stimulate the secretion of gastric acid (Gilman *et al.*, 1991). Acid secretion induced by stress is due to stimulation of nerve vagus, whereas that induced by NSAIDs is because of the inhibition of prostaglandin biosynthesis (Gilman *et al.*, 1991). An anti-ulcerogenic effect of a drug can be evaluated using stress or NSAIDs as a gastrointestinal lesions-inducing factor. In addition, administration of hydrochloric acid directly into the stomach is usually used to cause damage of gastrointestinal mucosa.

In this study, ulceration was induced by immersing mice into water in stress cages and by intragastric instillation of 0.1 ml/10 g of 0.15 MHCl/60% ethanol in rats. Our results (Table 1) revealed that stress caused gastric lesions in mice, and the control group appeared to be the most affected. The extract groups displayed a lesser degree of ulcerative damage, indicating that the extract of *S. feei* had a protective effect against incidences of ulcers. As stress can stimulate acid secretion in the stomach, the extract was supposed to have inhibitory activity on acid secretion induced by stress. These evidences were supported by the results of anti-ulcerogenic test using HCl/ethanol as an inducer.

In the HCl/ethanol-induced test, the HCl in ethanol induced mucosal damage of the stomach of rats. The degree of ulcerative damage was decreased significantly by application of the extract of *S. feei* roots. This means that the extract has a locally protective effect in the stomach against acid influences. That was clearly shown that an increasing dose increased the activity.

The findings indicating that the extract of *S. feei* roots to have anti-ulcerogenic activity were not in line with the previous evidence that *proanthocyanidin Shelegueain A* isolated from this plant has analgesic and anti-inflammatory activity (Subarnas and Wagner, 2000), the activity of which commonly relates to the occurrence of gastric ulcers (Gilman *et al.*, 1991). This may indicate that the extract contains other components having anti-ulcerogenic activity. The mechanism of action may be one or more of the following possibilities. The extract may inhibit gastric acid secretion evoked by nerve vagus stimulation through an anticholinergic action. It is also possible that the extract enhances production of mucous which can strengthen the mucosal barrier, as the extract decreased severity of ulcers induced by HCl in ethanol. Further investigation is needed to clarify the mechanism of action of the extract using NSAIDs as the gastric lesions-inducing agent and to search for an active compound responsible for this activity.

Our results suggest that the methanol extract of *S. feei* roots may have a beneficial preventive effect on gastric ulcers. This evidence indicates that the roots of this plant traditionally used as a remedy for rheumatism might be safe from the side effect on gastric ulcers.

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