

## Anti-Gastric Ulcer Activity of the Water Extract from Payawanorn (*Pseuderanthemum Palatiferum*)

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### Abstract:

**Objective:** To examine the anti-gastric ulcer activity of *Payawanorn* (*Pseuderanthemum palatiferum*) water extract (PPE) in rats.

**Material and Methods:** Evaluation of anti-gastric ulcer activity of PPE was performed using three induced gastric ulcers models: (1) ethanol/hydrochloric acid (EtOH/HCl), (2) restraint water immersion stress and (3) indomethacin. Ulcer indices were determined by microscopic examination (10X). Pylorus ligation and gastric-wall mucus determination were used to investigate the mechanism of anti-gastric ulcer activity of PPE.

**Results:** Oral administration of PPE (150, 300 and 600 mg/kg) significantly inhibited gastric ulcer formation induced by EtOH/HCl, by water immersion restraint stress, and by indomethacin in rats ( $p$ -value<0.05). PPE at 600 mg/kg showed a high percent inhibition of gastric ulcer formation in all models. In the pylorus-ligated model, pretreatment with PPE had no effect on pH, acidity output or the gastric acid secretion rate, demonstrating that PPE lacks an anti-secretory effect. Gastric wall mucus was markedly preserved by pretreatment PPE at 600 mg/kg ( $18.91 \pm 2.57$   $\mu$ g alcian blue/g wet stomach) after ethanol-induced ulcer when compared to that the control group ( $12.38 \pm 1.02$   $\mu$ g alcian blue/g wet stomach).

**Conclusion:** PPE possesses an anti-gastric ulcer effect related to the preservation of gastric mucus, supporting the traditional use of *P. palatiferum* to treat gastric ulcers.

**Keywords:** anti-gastric ulcer, gastric-wall mucus, *Pseuderanthemum palatiferum*, pylorus ligation

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## Introduction

*Pseuderanthemum palatiferum* (*P. palatiferum*) (family Acanthaceae) originated in Vietnam; it is used as a traditional medicine in Thailand. Its vernacular names include *Hoangoc* or *Xuan-hoa* in Vietnamese and *Payawanorn* in Thai.<sup>1</sup> It is used to treat or prevent several symptoms and diseases such as wounds, hemorrhoids, trauma, colitis, hypertension, diarrhea, arthritis, rheumatoid, pharyngitis, inflammatory bowel diseases, dysentery, constipation, influenza or colds, hepatitis, peptic ulcer, tumor, nephritis, and diabetes among others.<sup>2-5</sup>

Triterpenoid saponin, flavonoid and several compounds from the leaves of *P. palatiferum* have been reported such as  $\beta$ -sitosterol, stigmasterol, kaempferol, apiginin, salicylic acid, phytol, essential amino acids (lysine, methionine, threonine), minerals (calcium, potassium, magnesium, iron), and volatile substances (2-pyrrolidinone, 2-piperidinone, 4-ethyl-2-methoxyphenol, megastigmatrienone 4, 1,2,4-triethyl-5-methyl-benzene, hexadecanoic acid, octadecatrienoic acid methyl ester, and phytol).<sup>3,4,6,7</sup> Of these, flavonoids have been demonstrated to have gastroprotective properties.<sup>8</sup> Supporting data for the anti-gastric ulcer activity of *P. palatiferum*, however, are limited. The objective of this study was to examine the anti-gastric ulcer activity of *P. palatiferum* water extract (PPE) in rats.

## Material and Methods

### Plant material and extract preparation

Fresh *P. palatiferum* leaves were collected in Chiang Mai province. The plant voucher specimen was located in the Queen Sirikit Botanic Garden Herbarium (BQG 42335). The fresh leaves were washed, minced and immersed in distilled water for 24 hour (hr). The crude extract of *P. palatiferum* was collected by filtration, evaporated at 60–70 °C and then lyophilized. The yield was approximately 6% (w/w).

### Experimental animals

Male Sprague-Dawley rats, 200–250 g, were purchased from the National Laboratory Animal Center, Mahidol University, Salaya, Nakorn Pathom Province, Thailand. The rats were kept in an animal room at controlled conditions of 24±1 °C, 50.0±10.0% relative humidity and a 12 hr light–12 hr dark cycle for at least one week before starting the experiments. The rats had free access to food as well as to water. All animal protocols were approved by The Animal Ethics Committee of the Faculty of Medicine, Chiang Mai University, Thailand (20/2557).

### Drugs

Drugs used in the study were indomethacin (Sigma, St. Louis, USA), cimetidine (Sigma, St. Louis, USA), misoprostol (Pfizer, NY, USA), pentobarbital sodium (Sante Animale, Liboune, France). All test drugs except pentobarbital sodium were dissolved in distilled water. Drugs were orally administered in a volume of 0.5 mL/100 g of body weight.

### Experimental protocol

#### Investigation of anti-gastric ulcer activity

Rats in the anti-gastric ulcer activity study were fasted for 48 hr but allowed water ad libitum. Water was withdrawn 1 hr before starting the experiment. The rats were divided into 5 groups of 6. Each group received an oral dose of distilled water, cimetidine (100 mg/kg) or PPE (150, 300, 600 mg/kg) 1 hr before the induction of gastric ulcers.

Gastric ulcers were induced by one of three methods. The first method involved ethanol/hydrochloric acid (EtOH/HCl) to induce ulcers.<sup>9,10</sup> Each of the 6 rats in this group was orally administered 1 mL of EtOH/HCl. The rats were sacrificed 1 hr later and the extent of gastric ulcers was determined. The second method involved restraint water immersion stress-induced gastric ulcers.<sup>11,12</sup> Each of the 6 rats in this group was placed in separate

compartments of a stainless-steel cage which was immersed up to the level of the xiphoid in a water-bath maintained at  $22 \pm 2$  °C for 5 hr to induce stress ulcers. After 5 hr the rats were sacrificed and the extent of gastric ulcers was determined. The third method involved indomethacin-induced gastric ulcers.<sup>13,14</sup> In this group, indomethacin at a dose of 100 mg/kg was orally administered to each of the 6 rats in this group. Five hours later the rats were sacrificed and the extent of gastric ulcers was determined.

The stomachs of all the sacrificed rats from each of the three groups were removed and opened along the greater curvature to determine the extent of gastric ulcers using a dissecting microscope (10X). The extent of the gastric ulcers was evaluated by measuring the length of the lesion in millimeters (mm) then the ulcer index and percent inhibition of gastric ulcer were calculated as the following formulae:

$$\text{Ulcer index} = \frac{\sum \text{Total lengths of lesion in each group}}{\text{Numbers of rats in that group}}$$

$$\% \text{ Inhibition} = \frac{\text{Ulcer index}_{\text{control}} - \text{Ulcer index}_{\text{treated}}}{\text{Ulcer index control}} \times 100$$

### Investigation of the anti-gastric ulcer activity mechanism

The mechanism of anti-gastric ulcer activity was investigated using two investigative methods, pylorus ligation<sup>15,16</sup> and gastric-wall mucus determination.<sup>17,18</sup>

With pylorus ligation, 18 rats were divided into 3 groups of 6 rats each and orally given distilled water, cimetidine (100 mg/kg) or PPE (600 mg/kg) 1 hr before pylorus ligation. Five hours after ligation, the rats were sacrificed and the gastric contents were collected, centrifuged at 2,500 rpm for 5 min and the volume measured. The total acidity of the supernatant was determined by titration with 0.1 N NaOH to an end point of pH 7.4 using phenolphthalein as an indicator.

For gastric-wall mucus determination, another group of 42 rats were divided into 7 groups of 6 rats each. Rats in groups 1 (distilled water) and 2 (PPE, 600 mg/kg) were not subjected to gastric ulcer induction, while rats in groups 3–7 were given 1 mL of EtOH/HCl. Rats in groups 3–7 were orally pretreated with either distilled water, misoprostol (100 µg/kg), PPE (150, 300 or 600 mg/kg) 1 hr before gastric ulcer induction. One hour after gastric ulcer induction, all rats were sacrificed and the stomachs were removed, opened, rinsed with normal saline and weighed, then immersed in alcian blue solution (0.1% w/v) for 2 hr. The excessive dye was removed with 0.25 M sucrose solution. The dye complex, including gastric wall mucus, was extracted by immersion in 10 mL of 0.5 M magnesium chloride for 2 hr. An equal volume of diethyl ether was then added to the blue extract and the emulsion was centrifuged at 2,500 rpm for 15 min. The optical density of alcian blue in the aqueous layer was determined using a spectrophotometer at a wavelength of 580 nm. The quantity of alcian blue extract per unit weight (g) of wet stomach was calculated using a standard curve for concentration and absorbance of alcian blue.

### Statistical analysis

Statistical evaluation of the differences in means between the groups was conducted using one-way analysis of variance and the post hoc least-significant difference test. P-values less than 0.05 were considered significant. Data are expressed as mean  $\pm$  standard error of the mean (S.E.M).

## Results

### Investigation of anti-gastric ulcer activity

PPE (150, 300, and 600 mg/kg) and cimetidine (100 mg/kg) significantly reduced gastric ulcer formation caused by all inducers compared to those of control group (Table 1). The ulcer index of rats pretreated with PPE at

a dose of 600 mg/kg was similar to that of cimetidine in the EtOH/HCl- and indomethacin-induced gastric ulcer experiments (p-value>0.05). Inhibition of gastric ulcers by PPE was dose-dependent manner in all three models (Figure 1).

**Investigation of the anti-gastric ulcer activity mechanism**

In the pylorus-ligated model, the effect of PPE at a high dose (600 mg/kg) and of cimetidine (100 mg/kg)

on gastric volume, gastric pH, total acidity, and gastric acid secretion are shown in Table 2. Cimetidine, an anti-secretory drug, reduced gastric volume, total acidity, and gastric acid secretion rate while gastric pH was increased. In the PPE-treated group, however, gastric pH, total acidity, and gastric acid secretion rate did not differ from the control group. Pretreatment with PPE significantly decreased only gastric volume compared to that of control group (p-value<0.05).

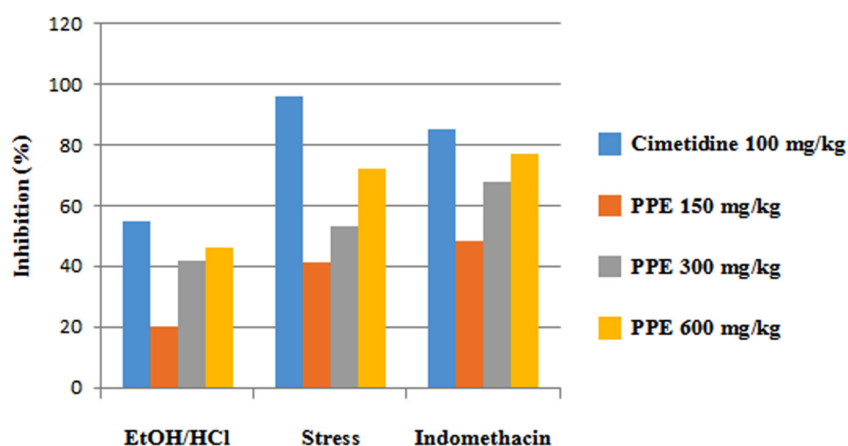
**Table 1** Effects of *Pseuderanthemum palatiferum* extract on gastric ulcer formation in rats in three models

Group	Ulcer index (mm)		
	EtOH/HCl	Stress	Indomethacin
Control	110.6±6.5	15.6±1.2	8.6±0.7
Cimetidine 100 mg/kg	49.8±1.5*	0.6±0.1*	1.3±0.1*
PPE 150 mg/kg	88.2±1.3*	9.2±0.3*	4.4±0.2*
PPE 300 mg/kg	64.2±2.0*	7.3±0.3*	2.8±0.3*
PPE 600 mg/kg	59.5±4.4*	4.4±0.3*	2.0±0.4*

Data are expressed as mean±S.E.M. (n=6)

\*significantly different from control group (p-value<0.05)

EtOH=ethanol, HCl=hydrochloric acid, PPE=*Pseuderanthemum palatiferum* water extract



**Figure 1** *Pseuderanthemum palatiferum* gastric ulcer inhibition in rats in three models

The effect of PPE and misoprostol on the quantity of gastric wall mucus is shown in Figure 2. EtOH/HCl resulted in a significant reduction of gastric wall mucus in the control group compared with the normal group (without EtOH/HCl). Pretreatment with misoprostol (100 µg/kg

a PGE1 analogue) or PPE (600 mg/kg) significantly protected gastric wall mucus from the effect of EtOH/HCl. Additionally, the gastric wall mucus value of rats pretreated with PPE at a dose of 600 mg/kg without gastric ulcer induction was similar to that of the normal rats.

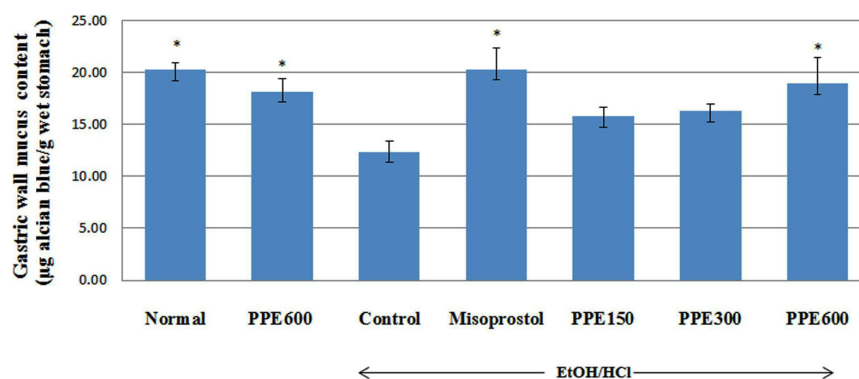
**Table 2** Effects of *Pseuderanthemum palatiferum* extract on gastric secretion in rats

Group	Gastric volume (mL/100 g)	Gastric pH	Total acidity (mEq)	Gastric acid secretion rate (mL/100 g/h)
Control	2.8±0.1	1.2±0.0	120.0±4.5	0.6
Cimetidine 100 mg/kg	1.2±0.1*	7.3±0.3*	18.0±4.0*	0.2
PPE 600 mg/kg	2.4±0.1*	1.6±0.1	107.0±8.0	0.5

Data are expressed as mean±S.E.M. (n=6)

\*significantly different from control group (p-value<0.05)

PPE=*Pseuderanthemum palatiferum* water extract



Data are expressed as mean±S.E.M. (n=6)

\*significantly different from control group (p-value<0.05)

**Figure 2** Effects of *Pseuderanthemum palatiferum* extract on gastric wall mucus content in rats.

## Discussion

Gastric ulcers are caused by an imbalance between damaging forces and mucosal defenses. Gastric ulceration occurs when mucosal defenses fail.<sup>2</sup> In this study, PPE showed anti-gastric ulcer activity in the experimental models, including EtOH/HCl-, indomethacin-, and restraint water immersion stress-induced gastric ulcers in rats.

Ethanol produces necrotic lesions by direct necrotizing action which in turn decreases defensive factors (the production of mucus and secretion of bicarbonate), increases lipid peroxidation, and decreases superoxide dismutase, GSH levels, and catalase. Additionally, HCl causes strong damage to the gastric mucosa.<sup>19,20</sup>

Nonsteroidal anti-inflammatory drugs (NSAIDs) such as indomethacin cause gastric ulcers through their ability to block cyclooxygenase-1 and cyclooxygenase-2, thereby inhibiting PG production. The pathogenesis of the NSAIDs-induced gastric damage also involves decreased secretion of bicarbonate and mucus, decreased mucosal blood flow, increased acid and pepsinogen secretion, neutrophil infiltration and alterations of microvascular structures. Reactive oxygen species, such as hydroxyl radicals and superoxide radical anions, are believed to play a major role in the pathogenesis of NSAIDs-induced gastric damage. Indomethacin-induced ulcers in rats is a standard model for evaluating the protective effects of compounds against NSAIDs-induced gastric damage.<sup>19,21</sup>

Stress is a physiological response caused by the disruption of homeostasis. Stress-induced gastric ulcer is a typical sample of a stress-associated organ injury.<sup>22</sup> Water immersion induces stress and causes gastric ulcers by a stimulation of gastric acid secretion and a reduction in mucosal microcirculation and mucus content.<sup>19</sup> An increase in gastric acid secretion would be expected to be an important factor in ulceration during water immersion.<sup>22</sup>

The present study showed that the PPE-treated group reduced gastric volume but did not affect gastric

pH, total acidity or gastric acid secretion rate. This suggests that anti-secretory action is not likely to be the anti-gastric ulcer activity of PPE.

Gastric mucus plays a major role as a protective factor against gastrointestinal damage. It consists of a viscous, adherent, elastic, and gel formed by glycoproteins and water that protects the entire gastrointestinal mucosa. Gastric mucus acts as an antioxidant, decreasing mucosal damage mediated by oxygen free radicals. The protective effects of the mucus barrier depend on the gel structure and the layer of the mucosal surface. Thus, gastric wall mucus determination is useful for indicating gastric mucus secretion.<sup>19,23</sup> PPE significantly promoted gastric mucus content in the EtOH/HCl model, which suggests that the main gastroprotective activity of PPE is related to the protection of gastric mucus.

## Conclusion

This study demonstrates that the water extract of *Pseuderanthemum palatiferum* possesses an anti-gastric ulcer activity which is related to the preservation of gastric mucus rather than to the inhibition of gastric acid secretion.

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