Evaluation of Anti-Gastric Ulcer Activity of Ethanolic Extract from Pseudostem of *Musa × paradisiaca* in Experimental Animals

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Abstract

*Musa × paradisiaca* belongs to the family Musaceae. It is one of the most popular fruit crops in Thailand and is traditionally used for various ailments. The present study was carried out on the anti-gastric ulcer activity of *M. × paradisiaca* pseudostem in experimental animals. Experimentally induced gastric ulceration was created using absolute alcohol, indomethacin and swimming stress. Pretreatment with the ethanolic extract of pseudostem at doses of 250, 500 and 1,000 mg/kg for 1 h prior to induction of gastric ulcer showed a significant (p<0.05) dose-dependent reduction to the ulcer index from the low dose (250 mg/kg) in all experimental models when compared to the control group. The protection percentage of ethanolic extract of pseudostem at a dose of 1,000 mg/kg for absolute alcohol, indomethacin and swimming stress models was 75.06, 66.49 and 67.11 %, respectively. For anti-secretion effects, the ethanolic extract of pseudostem caused a significantly (p<0.05) decrease in gastric acidity with no effect on gastric volume, total protein and pepsin. Furthermore, pre-treatment with ethanolic extract of pseudostem in different doses promoted gastroprotective effects by significantly (p<0.01) increasing gastric wall mucus content from the lowest dose (250 mg/kg) and increasing gastric pH at the highest dose (1,000 mg/kg) when compared to the control group. The findings therefore indicate that pseudostem of *M. × paradisiaca* possess anti-ulcer activities in experimental animals induced by absolute alcohol, indomethacin and swimming stress, which might be due to promoting mucus secretion.

Keywords: gastric ulcer; anti-gastric ulcer activity; *Musa × paradisiaca* pseudostem

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1. Introduction

Gastric ulcer is a common gastrointestinal disorder that affects many people around the world. It occurs due to an imbalance between the aggressive (gastric acid secretion) and defensive (gastric mucosal integrity) factors. *Helicobacter pylori* infection, frequent ingestion of nonsteroidal anti-inflammatory drugs (NSAIDs), alcohol, cigarette smoking and stress are involved in the pathogenesis of gastric ulcer, including increased gastric acid and pepsin secretion, decreased gastric blood flow, the suppression of endogenous generation of prostaglandins, inhibition of mucosal growth and cell proliferation and alteration of gastric mobility (Toma *et al*., 2005). Epidemiological studies suggest that gastric ulcer patients have a high risk of gastric cancer (Lee *et al*., 2004). There are two main approaches to treat gastric ulcer. The first is to reduce the production of gastric acid secretion and the second is to modulate gastric mucosal protection. Most commonly used drugs for gastric ulcers include antacids, proton pump inhibitors and histamine H2-antagonists etc., which act by reducing the aggressive factors. Reports on clinical evaluation of these drugs show that there are adverse effects and increased incidence of relapses during ulcer therapy (Gupta *et al*., 2012).

In traditional medicine, several plants have been used for gastric ulcer diseases such as *Aloe vera*, *Croton stellatopilosus*, *Cissus quadrangularis*, *Hibiscus sabdariffa*, *Mangifera indica*, *Mimusops elengi*, *Ocimum tenuiflorum*, *Solanum nigrum*, *Zingiber officinale* and the fruit pulp of *Musa* spp. (Awaad *et al*., 2013).

*Musa* species are one of most popular fruit crops in Thailand. This food plant possesses a number of biological activities including antiulcerogenic, antioxidant, antihyperglycaemic, against hypotension, diarrhea, diabetes, diuretic, cardiac depressant, antitumoral and antimicrobial effects (Kumar *et al*., 2012; Kirtida *et al*., 2013). A search of the phytochemical literature on this plant indicates that the anti-ulcerative properties have been highlighted in unripe plantain bananas that have the natural flavonoid, leucocyanidin. The active agent operates by stimulation of mucus secretion (Lewis and Shaw, 2001). Previous reports of the pseudostem pharmacological properties indicate that it possesses beneficial activity as an analgesic (Gangwar *et al*., 2012-13). Furthermore, the fresh juice collecting from inside the pseudostem is a good treatment for bleeding and wound healing (Klotoe’ *et al*., 2012). Recently, Gopinathan (2013) has reported that the raw material of *Aloe vera* fresh juice in combination with the pseudostem fresh juice of *M. paradisiaca* has gastroprotective activity against alcohol-induced gastric ulcer in rats, which may involve flavonoid ingredients.

Therefore, the aim of this study was to evaluate anti-gastric ulcer activities of the ethanolic extract from the pseudostem of *M. × paradisiaca* in different experimental ulcer models including absolute alcohol, stress and indomethacin in rats.
2. Materials and Methods

2.1 Animal preparation

Male Wistar rats (150-290 g) were from the International Animal Research Center, Salaya, Mahidol University, Thailand. They were acclimatized for at least 7 days in individual cage and maintained under standard laboratory conditions (12 h light/dark cycle, 25±3 °C). The animals were maintained on a standard pellet food and water ad libitum. All animals received humane care in compliance with the ethical use of animals issued by the National Research Council of Thailand, 1999. All efforts were made to minimize animal suffering and to reduce the number of animals used in the experiments.

2.2 Vegetal materials and extract preparation

Approximately 20 kg of M. × paradisiaca pseudostem from Pathum Thani Province, Thailand, were dried at room temperature and ground before extraction. 715 g of dry powder was extracted with 95 % alcohol by a soxhlet apparatus for 8 hours and then was evaporated to dryness and kept in a refrigerator at 4 °C. The net product of the extraction procedure was 61.49 g.

2.3 Ant-ulcerogenic activity

The rats were divided into 6 groups each of which comprised five animals (n = 5). Group 1 served as a normal group and was given 0.5 ml 1 % carboxymethylcellulose (CMC)-water solution orally Group 2 served as a control group with induced gastric ulcer (0.5 ml 1 % CMC-water solution) Group 3 through Group 5 were administered the ethanolic extraction from banana pseudostem at doses of 250, 500 and 1,000 mg/kg, respectively and group 6 served as a reference drug (cimetidine 100 mg/kg) group. All animals were deprived of food but had access to water for at least 24 h prior to the experiment.

2.3.1 Absolute alcohol-induced gastric ulcer

The method of ethanol-induced gastric ulcer was performed following the procedure of Lima et al. (2006). The fasted rats were given extraction samples and vehicles orally for 1 h prior to induction of gastric ulcer with 1.0 ml absolute alcohol. The animals were sacrificed after induction for 1 h under anesthesia with diethyl ether and the stomachs were collected and examined for gastric ulcers.

2.3.2 Indomethacin-induced gastric ulcer

The induction of gastric ulcer by indomethacin was performed according to the method of Gurbuz and Yesilada (2007). The fasted rats were given extraction samples and vehicles orally for 1 h prior to induction of gastric ulcer by intraperitoneal injection of 30 ml/kg indomethacin (Sigma Chemical Co.) suspended in 0.5 ml 1 % CMC-water solution and then the rats were sacrificed and examined for gastric ulcers 5 h later.

2.3.3 Swimming stress-induced gastric ulcer

For the induction of gastric ulcer by stress, the method described by Grover et al. (2001) was employed. All fasted rats were
given extraction samples and vehicles orally for 1 h before induction. After 1 h, the rats were placed inside a vertical cylinder filled with water up to a height of 20 cm. The temperature of the water was maintained at 25±3 °C. The rats were removed from the cylinders after 5 h and then the rats were sacrificed under anesthesia with diethyl ether and the stomachs were collected and examined for gastric ulcers.

2.3.4 Evaluation of the ulcer index and the protection percentage.

After the rats were sacrificed, the stomachs were rapidly removed. Each stomach was opened along the greater curvature and rinsed with normal saline to remove the gastric contents and blood clots in order to assess the extent of gastric damage. The glandular portion of the stomach was examined under a dissecting microscope. The gastric lesion area (mm$^2$) was determined as length × width of lesion and the sum of the length of all lesions was designated as the ulcer index and the protection percentage.

\[
\text{Ulcer Index} = \frac{\text{Ulcer area (mm}^2\text{)}}{\text{Stomach area (mm}^2\text{)}} \times 100
\]

\[
\% \text{ protection} = \frac{\text{UI control} - \text{UI treated}}{\text{UI control}} \times 100
\]

2.4 Assay of gastric secretion with pylorus ligation

The evaluation of gastric secretion was performed by pyloric ligation, which was carried out in anaesthetized rats to induce gastric ligation as described by Shay et al. (1945). Rats were divided into 6 groups containing 5 animals (n = 5), Group 1 served as a normal group, Group 2 served as control, Groups 3 through 5 served as groups given the ethanolic extract of pseudostem at doses of 250, 500 and 1,000 mg/kg, and group 6 served as a cimetidine 100 mg/kg group. After rats were fasted for 36 h, the extracts were administered orally 1 h before pyloric ligation. At 5 h after pyloric ligation, the rats were sacrificed. The stomachs were removed and opened along the greater curvature to collect the gastric secretion. The volume of gastric content and its pH were recorded, and then it was centrifuged at 1,000 rpm for 10 min and the acidity of the supernatant was determined by titration with 0.01 N sodium hydroxide using phenolphthalein as indicator. Acidity was calculated using the formula:

\[
\text{Acidity} = \frac{\text{Volume of NaOH} \times \text{Normality of NaOH} \times 100}{0.1 \text{ Meq/L per 100g}}
\]

2.4.1 Total proteins

Proteins of gastric secretion were determined according to the procedure of Lowry et al. (1951). Briefly the method precipitated dissolved proteins of gastric juice in 90 % alcohol with gastric juice at a 9:1 ratio. Then 0.1 ml of alcoholic precipitation of gastric juice was dissolved in 1 ml of 0.1 N NaOH and from this 0.05 ml was taken to put in another test tube, then added with 4 ml of alkaline mixture and kept for 10 min. Then 0.5 ml of Folin’s phenol reagent was added and left for
10 min to allow color development. Reading were taken at 640 nm against a blank prepared with distilled water. The protein content was calculated from a standard curve prepared using bovine serum albumin (Sigma Chemical Co.) and expressed in terms of µg/ml of gastric juice.

2.4.2 Pepsin

Pepsin secretion was estimated following the method of Rungruangsak-Torrisen et al. (2006). The supernatant 200 µl of the gastric sample was added to 200 µl of 1% bovine serum albumin in 60 mM HCl. The mixture was incubated at 37 °C for 10 min. The reaction was stopped after exactly 10 min incubation by adding 1 ml of 5 % TCA, mixed thoroughly, letting stand at room temperature for 30 min, and centrifuging at 5000×g for 20 min. One ml of 0.5 M NaOH was added to 0.5 ml of the supernatant, followed by 0.3 ml of Folin-Ciocalteu reagent (1:3 dilutions). After standing for 10 min at room temperature, the absorbance was measured at 720 nm, compared with the L-tyrosine standard curve. The pepsin content was expressed in terms of µg/ml of gastric juice.

2.5 Gastric wall mucus content

Gastric wall mucus content was estimated following the method of Corne et al. (1974). After the collection of the gastric juice, the stomachs were soaked for 2 h in 0.1 % Alcian blue 8 GX (Sigma Chemical Co.) dissolved in 0.16 M sucrose buffered with 0.05 M sodium acetate adjusted to pH 5.8 with HCl. Unbound dye was removed by two successive washes of 15 and 45 min in 0.25 M sucrose solution. Dye complex with mucus was diluted by immersion in 10 ml aliquots of 0.5 M MgCl₂ for 2 h. The resulting blue solutions were shaken briefly with an equal volume of diethyl ether and the optical density of the aqueous phase was measured at 605 nm. Alcian blue was used as it stains only the barrier mucus and does not penetrate the mucosal tissue. The dye complex with barrier mucus was recoverable. The amount of gastric wall mucus was determined from the standard curve of Alcians blue dissolved in MgCl₂, which has been expressed as µg Alcian blue/g wet stomach.

2.6 Statistical analysis

Results were expressed as means ± standard error of the mean (SEM) and statistical significance was determined using one-way analysis of variance (ANOVA), followed by Dunnett’s test. Data were considered significantly different at a level of p<0.05.

3. Results

3.1 Anti-ulcer effect of ethanolic extraction from Pseudostems of M. × paradisiaca

The ulcerogen treated animals were seen to have extensive gastric lesions in the glandular portion of stomach in all experimental models. The absolute alcohol models produced more damage lesions than the others, with severe hemorrhagic lesions, erosion, necrosis and hyperemia (Figure 1). Oral pre-treatment
with ethanolic extract from pseudostem of *M. × paradisiaca* at doses of 250, 500 and 1,000 mg/kg showed significant (p<0.05) dose-dependent reduction of gastric lesions from the lowest dose (250 mg/kg) in all experimental models when compared to the control group (Table 1). In comparison with cimetidine, the ethanolic extract of pseudostem exhibited significant (p<0.001) anti-gastric ulcer activity similar to cimetidine at the lowest dose (250 mg/kg) in the absolute alcohol model, at the moderate dose (500 mg/kg) in the indomethacin models and at the highest dose (1,000 mg/kg) in the swimming stress model.

Pre-treatment with the ethanolic extract of pseudostem at the highest dose effectively protected the gastric mucosa against ulcer induced by absolute alcohol, indomethacin and swimming stress with the % protection at 75.06, 66.49 and 67.11 %, respectively. Remarkably, the gastroprotection of pseudostem extract at the highest dose were not different from cimetidine 100 mg/kg in all models except the swimming stress model.

**Figure 1** Macroscopic pictures of gastric ulcers in stomach tissues (A) absolute alcohol model (B) indomethacin model and (C) swimming stress model. Aa, Ba and Ca were the control group. Ab-Ad, Bb-Bd and Cb-Cd were groups that had oral feeding of the ethanolic extraction of pseudostem at doses of 250, 500 and 1,000 mg/kg, respectively. Ae, Be and Ce were groups that received a reference drug (cimetidine 100 mg/kg). The results showed that pre-treatment with the ethanolic extraction of pseudostem could reduce ulcer lesions similarly to cimetidine when compared to the control group, the normal group which was treated with vehicle alone (0.5 ml 1 % CMC) showed no ulcer lesion (not shown).
Table 1  Effects of the ethanolic extract from pseudostem of *M. × paradisiaca* on gastric ulcer induced by absolute alcohol, indomethacin and swimming stress

<table>
<thead>
<tr>
<th>Groups (mg/kg)</th>
<th>Absolute alcohol</th>
<th>Indomethacin</th>
<th>Swimming stress</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ulcer index</td>
<td>% Protection</td>
<td>Ulcer index</td>
</tr>
<tr>
<td>Control</td>
<td>51.93±5.03</td>
<td>-</td>
<td>11.10±1.50</td>
</tr>
<tr>
<td>Extract 250</td>
<td>30.34±7.48***</td>
<td>41.57***</td>
<td>9.00±1.01**</td>
</tr>
<tr>
<td>Extract 500</td>
<td>20.13±2.40***</td>
<td>61.23***</td>
<td>6.37±1.14***</td>
</tr>
<tr>
<td>Extract 1,000</td>
<td>12.95±2.13***</td>
<td>75.06ns</td>
<td>3.72±0.73***</td>
</tr>
<tr>
<td>Cimetidine</td>
<td>8.09±1.80***</td>
<td>84.42</td>
<td>2.67±0.78***</td>
</tr>
</tbody>
</table>

Data expressed in terms of means ± SEM (n = 5), significant difference of Ulcer Index compared with the control group and % protection compared with the cimetidine group: *p<0.05, **p<0.01, ***p<0.001.

### 3.2 Effect of ethanolic extract from pseudostem of *M. × paradisiaca* on gastric secretion with pylorus ligation in rats indicating gastric volume, pH, acidity, total proteins and pepsin

Pre-treatment with the ethanolic extract of pseudostem at doses of 250, 500 and 1,000 mg/kg showed a significant (p<0.01) reduction in acid secretion, whereas there were insignificant reductions on gastric volume, total proteins and pepsin. On the other hand, the extract showed a significant (p<0.05) marked increase in the gastric pH at the highest dose (1,000 mg/kg). The results are expressed in Table 2.

Table 2  Effects of the ethanolic extract from pseudostem of *M. × paradisiaca* on gastric volume, gastric pH, acidity, total proteins and pepsin.

<table>
<thead>
<tr>
<th>Groups (mg/kg)</th>
<th>Gastric volume</th>
<th>Gastric pH</th>
<th>Acidity (mEq/L)</th>
<th>Total proteins (µg/ml)</th>
<th>Pepsin (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.52±1.18</td>
<td>2.12±0.67</td>
<td>83.0±13.88</td>
<td>488.17±92.21</td>
<td>157.56±8.56</td>
</tr>
<tr>
<td>Extract 250</td>
<td>4.10±0.82</td>
<td>3.41±1.51ns</td>
<td>51.00±13.67**</td>
<td>408.73±99.74ns</td>
<td>121.96±20.44ns</td>
</tr>
<tr>
<td>Extract 500</td>
<td>4.16±1.45</td>
<td>4.13±1.77ns</td>
<td>46.00±15.35**</td>
<td>395.13±55.11ns</td>
<td>117.62±9.31ns</td>
</tr>
<tr>
<td>Extract 1,000</td>
<td>3.70±0.77</td>
<td>4.20±0.95*</td>
<td>45.80±9.55**</td>
<td>393.27±47.70ns</td>
<td>117.00±30.56ns</td>
</tr>
<tr>
<td>Cimetidine</td>
<td>4.00±1.00</td>
<td>4.81±1.47**</td>
<td>34.00±16.72***</td>
<td>325.13±27.13*</td>
<td>96.58±35.90**</td>
</tr>
</tbody>
</table>

Data expressed as means ± SEM (n=5). Significant difference from the control group: *p<0.05, **p<0.01 and ***p<0.001.
3.3 Effect of ethanolic extract from pseudostem of *M. × paradisiaca* on gastric wall mucus content.

The mean value of gastric wall mucus content was significantly (p<0.01) increased in the ethanolic extract of pseudostem at the lowest dose (250 mg/kg) when compared to the control group. Results demonstrated that the lowest dose of the extract could protect against loss of gastric wall mucus content. When compared to the normal group, the effect of pseudostem extract and cimetidine was not different from the normal group, whereas the control group clearly differed significantly from the normal group (p<0.01, Table 3).

Table 3 Effects of the ethanolic extract from pseudostem of *M. × paradisiaca* on gastric wall mucus content.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Gastric wall mucus (µg Alcian blue/g wet stomach)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>149.42±17.67**</td>
</tr>
<tr>
<td>Extract 250</td>
<td>223.13±32.83**</td>
</tr>
<tr>
<td>Extract 500</td>
<td>231.78±17.16**</td>
</tr>
<tr>
<td>Extract 1,000</td>
<td>233.44±40.53**</td>
</tr>
<tr>
<td>Cimetidine</td>
<td>255.53±36.04***</td>
</tr>
<tr>
<td>Normal</td>
<td>294.47±40.17***</td>
</tr>
</tbody>
</table>

Data expressed as means ± SEM (n=5). Significant difference from the control group: *p<0.05, **p<0.01 and ***p<0.001. Significant difference from the normal group: #p<0.05, ##p<0.01.

4. Discussion

In the present study we have investigated the anti-ulcer activity, anti-secretory property and mucoprotective effects of the ethanolic extract from the pseudostem of *M. × paradisiaca* at doses of 250, 500 and 1,000 mg/kg on gastric ulcer models induced by absolute alcohol, indomethacin and swimming stress. The mechanisms of induced-gastric ulcer in different models are mostly similar, such as impairment of the mucosal integrity and increased gastric acid secretion.

With the aggressive properties of alcohol, it rapidly penetrates the gastric mucosa apparently causing cell and plasma membrane damages. The ulcers caused by alcohol may be due to stasis in gastric blood flow which contributes to the development of the haemorrhage and necrotic aspects of tissue injury and also promotes gastric oxidative stress (Gupta *et al*., 2012). The ethanolic extract from pseudostem presented anti-ulcer activity by significantly (p<0.001) reducing gastric lesions at the lowest dose (250 mg/kg) similarly to cimetidine. These results indicate a possible gastroprotective activity, since ethanol acts directly on gastric mucosal cell.

The main ulcerogenic mechanism of NSAIDs such as indomethacin, is inhibition of prostaglandin synthesis and bicarbonate secretion by inhibition of gastric mucosal cyclooxygenase (COX), besides provoking damages to the vascular endothelium, reduction of the gastric blood flow, formation of
obstructive micro-thrombi, activation of neutrophils and promoting gastric oxidative stress (Heeba et al., 2009; Moraes et al., 2009). The results for indomethacin reveal that the highest dose (1,000 mg/kg) of ethanolic extract of pseudostem possesses a gastroprotective effect which does not differ from cimetidine treatment. This suggests the possible gastroprotection of pseudostem extract in the indomethacin model.

Stress ulcers are due to both physiological and psychological factors, which are crucial for gastrointestinal defense and increased accumulation of acid and pepsin leading to autodigestion of the gastric mucosa and promoting gastric oxidative stress (Rujjanawate et al., 2005). Accordingly, the ethanolic extracts of pseudostem showed a potent anti-gastric ulcer effect in a dose-dependent manner which significantly (p<0.05) reduced the gastric lesions from the lowest dose (250 mg/kg). This finding indicates that the pseudostem extract also possesses an anti-gastric ulcer effect on the swimming stress model.

With regard to anti-gastric secretion, the rats that had received the ethanolic extract of pseudostem orally showed decreased acid secretion as well as increased gastric pH in the gastric juice but it had no effect on gastric volume, total protein and pepsin. These results indicate that there is an anti-ulcerogenic action by decreased aggressive factor. The action may be correlated with the increase mucus secretion mechanism by neutralizing acid secretion.

Gastric mucus is the first line of defense against acid and adheres together with bicarbonate secreted by the epithelium to serve as a barrier against self-digestion (Moraes et al., 2009). Alcian blue dye was used to detect the mucus-type glycoproteins, as it binds with negatively charged materials. The increase in bound alcian blue suggests the gastroprotective effect of the ethanolic extract of pseudostem (Howiriny et al., 2005). The results obtained in the present work show a significant increase in the amount of adherent mucus in the rats treated with the ethanolic extract of pseudostem at the lowest dose (250 mg/kg). This result indicates a possible mucoprotective activity. It could be the formation of protecting complexes between the extract and mucus, which can act as a barrier against several agents introduced into the stomach (Hiruma-Lima et al., 2006). From the study of Gopinathan (2013), the raw material of the Aloe vera fresh juice combination with fresh pseudostem juices of M. paradisiaca produced an ulcer protective effect against the alcohol model in rats, which may involve with flavonoid ingredients. The flavonoid, leucocyanidin active compound has been found in the banana fruit pulp. It was stimulates gastric secretion (Lewis and Shaw, 2001). Thus, the anti-gastric ulcer mechanism of banana pseudostem may involve phytochemical ingredients to stimulate mucus secretion, similarly to banana fruit pulp. Moreover, the pharmacological properties of pseudostem extract are seen in sap juice from the pseudostem, which has good effect on the
treatment of bleeding and wound healing (Klotoe’ et al., 2012). These findings support that the protective effect of banana pseudostem extract against gastric ulcer might involve the presence of phytoconstituents and their pharmacological properties.

5. Conclusion

From the results of this study, it is clear that the ethanolic extract from pseudostems of M. × paradisiaca has significant anti-ulcer activity in experimental models. Its action might involve mucoprotective activity. This interesting observation indicates that the ethanolic extract from the pseudostem of M. × paradisiaca can be a potential source for the treatment of gastric ulcers.

6. References


