The Gastroprotective Effects of the Ethanolic Extraction from
*Musa*(ABB group) ‘Kluai Nam Wa’ Flower in Wistar Rats

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บทคัดย่อ
หัวปลีกล้วยได้รับความสนใจทางด้านวิทยาศาสตร์อย่างกว้างขวาง เนื่องจากมีคุณสมบัติทางเภสัชวิทยาที่หลากหลายและมีประสิทธิภาพในการรักษาสูง ในการศึกษาครั้งนี้ได้ทดสอบประสิทธิภาพของสารสกัดจากหัวปลีกล้วยน้ําว้าด้วยแอลกอฮอล์ (EF) ในการป้องกันการเกิดแผลในกระเพาะอาหารของหนูวิสตาร์ ที่เกิดจากการชักน้ำด้วยแอลกอฮอล์ ยาอินโดเมทาซิน (indomethacin) และความเครียด เมื่อทำการป้อน EF ให้แก่หนูผ่านปากแบบเฉียบพลัน ในขนาดความเข้มข้น 250, 500 และ 1,000 มิลลิกรัม/กิโลกรัม เป็นเวลา 1 ชั่วโมง ก่อนชักน้ำให้เกิดแผลในกระเพาะอาหาร และทำการวัดพารามิเตอร์ได้แก่ ขนาดของแผลในกระเพาะอาหาร (ulcer index), % protection, gastric secretion และ สารเมือก (gastric wall mucus content) จากผลการทดลองพบว่า EF ขนาดความเข้มข้น 250, 500 และ 1,000 มิลลิกรัม/กิโลกรัม สามารถลดขนาดของแผลในกระเพาะอาหารได้ในทุกโมเดล ขณะที่ขนาดความเข้มข้น 250 มิลลิกรัม/กิโลกรัม (p<0.05) เมื่อเปรียบเทียบกับกลุ่มควบคุม นอกจากนี้ยังพบว่า EF ขนาดความเข้มข้น 1,000 มิลลิกรัม/กิโลกรัม มีประสิทธิภาพในการป้องกันการเกิดแผลในกระเพาะอาหาร (% protection) ได้ถึง 78.80, 70.26 และ 68.73 เปอร์เซ็นต์ในโมเดลแอลกอฮอล์ ความเครียด และ ยาอินโดเมตาซิน ตามลำดับ สำหรับประสิทธิภาพในการป้องกันการเกิดแผลในกระเพาะอาหารที่เกี่ยวข้องกับการยับยั้งการหลั่งน้ําย่อยในกระเพาะอาหารพบว่า EF สามารถลดปริมาณกรดในกระเพาะอาหารและสามารถลดปริมาณเอนไซม์เพพซินได้อย่างมีนัยสำคัญทางสถิติ (p<0.05) แต่ไม่สามารถลดค่า gastric volume และโปรตีนรวมได้ เมื่อเปรียบเทียบกับกลุ่มควบคุม นอกจากนี้ยังพบว่า EF ตั้งแต่ระดับความเข้มข้นต่ําสุดมีประสิทธิภาพในการป้องกันการเกิดแผลในกระเพาะอาหารจากสารสกัดหัวปลีกล้วยน้ําว้า (*Musa* (ABB group) ‘Kluai Nam Wa’) ด้วยแอลกอฮอล์ ในหนูวิสตาร์.
ABSTRACT

The flower of *Musa* spp. has interested the scientific community due to its numerous pharmacological properties and potential therapeutic applications. The present study aimed to investigate the gastroprotective effects of the ethanolic extraction from *Musa* (ABB group) ‘Kluai Nam Wa’ flower (EF) on experimental models against gastric ulcer induced by absolute alcohol, indomethacin and swimming stress in Wistar rats. The EF was orally administered at dosages of 250, 500 and 1,000 mg/kg for 1 h prior to induction of gastric ulcer. The common parameters determined were ulcer index (UI), protection percentage (% protection), gastric secretion and gastric wall mucus content. The results demonstrated that pre-treatment with EF at doses of 250, 500 and 1,000 mg/kg showed a potent ulcerprotective effect in a dose-dependent manner which significantly (*p*<0.05) reduced ulcer index at the lowest dose (250 mg/kg) in all models as compared to the control group. Pre-treatment with EF at a dose of 1,000 mg/kg showed potential gastroprotection 78.80%, 70.26% and 68.73% in absolute alcohol, swimming stress, and indomethacin models respectively. The gastroprotective activity of EF involved an anti-secretory effect as it significantly (*p*<0.05) decreased gastric acidity and pepsin without any effect on gastric volume and total proteins when compared to the control group. Pre-treatment with EF promoted gastroprotective effect by significantly (*p*<0.05) increasing gastric wall mucus content and gastric pH when compared to the control group. The results suggest that EF presents anti-ulcer activities against absolute alcohol, indomethacin and swimming stress in Wistar rats. The gastroprotective activity of EF could involve promoting mucus secretion.

**Keywords:** Gastric ulcer, Gastroprotective effect, Banana flowers, *Musa* (ABB group) ‘Kluai Nam Wa’

**INTRODUCTION**

Gastric ulcer is a major public health problem that affects a considerable number of people globally. The pathogenesis of gastric ulcers is often depicted as an imbalance between aggressive factors (acid and pepsin)
and local mucosal defensive factors (secretion of bicarbonate, mucus and prostaglandins). Increased incidence of gastric ulcers is associated with *Helicobacter pylori*, frequent ingestion of anti-inflammatory drugs (NSAIDs: aspirin and indomethacin), stress factors, excess stomach acid, alcohol consumption and cigarette smoking (Amaral et al., 2013). Other factors implicated in the pathogenesis of gastric ulcers include increased gastric acid and pepsin secretion, decreased gastric blood flow, suppression of endogenous generation of prostaglandins, inhibition of mucosal growth and cell proliferation, and alteration of gastric mobility (Toma et al., 2005). While a number of drugs including antacids, proton pump inhibitors, anticholinergics and histamine H2-antagonists are available for the treatment of gastric ulcer, their effectiveness is still debatable and several can result in adverse reactions (Hiruma-Lima et al., 2006). This has been the rationale for the development of new anti-ulcer drug therapies that are more effective, have fewer side effects and can be safely consumed.

The banana belonging to the family Musaceae, is widely distributed in Thailand and has been used in traditional medicine to alleviate various diseases (Kumar et al., 2012). Banana fruit pulp has been explored by a number of researchers and found to have highly anti-ulcerogenic properties (Agarwal et al., 2009). The bioactive compound of fruit pulp has been identified as containing leucocyanidin, a flavonoid that has been found to protect the gastric mucosa by increasing the mucus thickness (Lewis and Shaw, 2001). Although the anti-ulcer properties of banana fruit pulp are widely recognized, the protective action of banana flowers has not yet been identified.

*Musa* (ABB group) ‘Kluai Nam Wa’ is one in the group of *Musa × paradisiaca* (Silayoi, 2002). The *M. × paradisiaca* flower mainly contains flavonoids, saponins, tannins, glycosides, terpenoids and steroids (Mahmood et al., 2011). These compounds are well recognized as the active ingredients for gastroprotection and anti-oxidative stress (Vasconcelos et al., 2010).

Therefore, the present investigation was undertaken to evaluate the possible protective effects of the *Musa* (ABB group) ‘Kluai Nam Wa’ flower against experimental models of gastric ulcers in rats.

**RESEARCH METHODOLOGY**

1. **Vegetal materials and extract preparation**

*Musa* (ABB group) ‘Kluai Nam Wa’ flowers weighing about 20 kg from Pathum Thani Province, Thailand were dried at room temperature and ground before extraction. Dried powder was extracted with 95% alcohol by soxhlet extraction for 8 hours and then
was evaporated to dryness and kept in the refrigerator at 4 °C.

2. Animal preparation

Male Wistar rats (150-290 g) from the International Animal Research Center, Salaya, Mahidol University, Thailand were used in this study. The rats were acclimatized for at least 7 days in individual cages and maintained under standard laboratory conditions (12 h light/dark cycle, 25±3 °C). The animals were maintained on a standard pellet diet and given water *ad libitum*. All animals received humane care in compliance with the ethics in the use of animals policy 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.

Rats were randomly divided into 6 groups each of which comprised five animals (*n*=5). Group 1 (normal group) was given a 0.5 ml 1% carboxymethylcellulose (CMC)-water solution orally, group 2 served as a control group (0.5 ml 1% CMC-water solution) with induction by different damaging agents, groups 3 through 5 had pre-treatment with the ethanolic extraction of *Musa* (ABB group) ‘Kluai Nam Wa’ flower (EF) at doses of 250, 500 and 1,000 mg/kg dissolved in 0.5 ml % CMC-water solution respectively and group 6 was a reference drug for ulcer protection (cimetidine 100 mg/kg). All animals were fasted but had access to water for at least 24 h prior to experiments.

After oral feeding experiments for 1 h, five group of rats (Group 2-6) were taken out for induction of gastric ulcer by three methods. First, rats were administered 1.0 ml absolute alcohol orally for 1 h (Lima et al., 2006). Second, indomethacin (Sigma chemical Co.) was suspended in 0.5 ml 1% CMC-water solution and then each rat exposed by intraperitoneal injection of 30 ml/kg for 5 h (Gurbuz and Yesilada, 2007). In the last method, the rats were placed inside a vertical cylinder filled with 20 cm height of water for 5 h. The temperature of the water was maintained at 25 °C (Grover et al., 2001). The rats were sacrificed after induction of ulcer under anesthesia with diethyl ether.

3. Determination of ulcer index and percentage protection

After the rats were sacrificed, the stomachs were rapidly removed. Each stomach was opened along the greater curvature. The glandular portion of the stomach was examined under a dissecting microscope. The gastric lesion area (mm²) was determined as length x width of lesion and the sum of the length of all lesions was designated as the ulcer index and the percentage protection.
4. Determination of gastric secretion

The assay was performed in pylorus ligation as described by Shay et al. (1945). The rats were divided into 6 groups each of which comprised five animals (n=5). Group 1 served as a normal group (0.5 ml 1% CMC-water solution), group 2 served as a control group (0.5 ml 1% CMC-water solution) with induced gastric secretion by the pylorus ligation method, groups 3 through 5 were pre-treated with EF at doses of 250, 500 and 1,000 mg/kg dissolved in 0.5 ml % CMC-water solution respectively and group 6 had cimetidine 100 mg/kg. All animals were fasted but had access to water for at least 36 h prior to experiments. After oral feeding experiments for 1 h, five group of rats (Group 2-6) were taken out for inducing gastric secretion by the pylorus ligation method. After 5 h, the rats were sacrificed, their stomachs were opened and the gastric secretion was collected. The volume of gastric content and its pH were recorded. The acidity was determined by titration with NaOH (0.01 N) using phenolphthalein as an indicator. Total proteins of gastric secretions was determined according to the procedure of Lowry et al., 1951. The total proteins 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. Pepsin content was estimated by measuring the amount of liberated tyrosine by action of pepsin on bovine serum albumin as substrate and expressed in terms of μg/ml of gastric juice (Rungruangsa-Torrissen et al., 2006).

5. Gastric wall mucus content

The gastric wall mucus content was used as an indicator for gastric mucus secretion and estimated following the method of Corne et al., 1974. After collection of the gastric juice, the stomachs were immersed in a solution of Alcian blue (Sigma Chemical Co.) to quantify the mucus. The optical density of the aqueous phase was measured at 605 nm in a spectrophotometer and the results expressed as μg of Alcian blue/g wet stomach.

6. Statistical analysis

Data were expressed as mean±SEM and analyzed using the SPSS for WIN 17.0 computer program. ANOVA and Duncan’s test were regarded as statistically significant when p<0.05.

RESULTS

1. Effect of EF on gastric ulcers in rats

The effect of EF treatment in three doses (250, 500 and 1,000 mg/kg) on gastric...
ulcers induced by absolute alcohol, indomethacin and swimming stress was investigated in rats. The results are shown in Figure 1. The rats receiving 0.5 ml 1% CMC only (normal group) showed no lesions (data not shown). Treatment of rats with absolute alcohol, indomethacin and swimming stress (control group) produced acute mucosal lesions mostly in the glandular portion, measured in terms of the ulcer index. Pre-treatment with EF (250, 500 and 1,000 mg/kg) showed a significant (p<0.05) decrease in the ulcer index at the low dose (250 mg/kg) in a dose-dependent manner in all three models when compared with the control group (Figure 2). Pre-treatment with EF at a dose of 1,000 mg/kg showed a potential gastroprotective effect at 78.80%, 70.26% and 68.73% for the absolute alcohol, swimming stress, and indomethacin models respectively, with the potent action similar to that of cimetidine in all models except for swimming stress (Figure 3).

Figure 1  Gross appearance of gastric ulcers in stomach tissues (A-E), absolute alcohol model (F-J), indomethacin model and (K-O) swimming stress model. A, F and K were the control group. B, G and L were the group that had pre-treatment with EF at 250 mg/kg. C, H and M were the group that had pre-treatment with EF at 500 mg/kg. D, I and N were the group that had oral feeding of EF at 1,000 mg/kg. E, J and O were the group that received cimetidine at 100 mg/kg.
2. Effect of EF on gastric secretion

A comparison of the gastric juice parameters of rats that had EF orally administered in one of three doses (250, 500 and 1,000 mg/kg) demonstrated that EF exhibited significant ($p<0.05$) anti-secretory activity by increasing the pH level of gastric juice and reducing acidity and pepsin. However, it did not decrease the volume of gastric juice and total proteins when compared with the control group (Table 1).

3. Effect of EF on gastric wall mucus content.

The gastric wall mucus content on the gastric mucosal surface was significantly ($p<0.05$) increased after treatment with EF when compared to the control group and was not different from the cimetidine group. The results demonstrate that the low dose of EF (250 mg/kg) could protect from loss of gastric wall mucus content (Figure 4).

Figure 2  Effects of the ethanolic extraction from Musa (ABB group) ‘Kluai Nam Wa’ flowers on gastric ulcer induced by absolute alcohol, indomethacin and swimming. Data expressed as means ± SEM ($n=5$). Significant differences were at the 95% level of confidence by Duncan’s test.
Figure 3  Effects of the ethanolic extraction from Musa (ABB group) ‘Kluai Nam Wa’ flowers on percentage protection induced by absolute alcohol, indomethacin and swimming stress. Data expressed as means ± SEM (n=5). Significant differences were at the 95% level of confidence by Duncan’s test.

Table 1  Effects of the ethanolic extraction from Musa (ABB group) ‘Kluai Nam Wa’ flowers on gastric secretion indicating gastric volume, gastric pH, acidity, total proteins and pepsin.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Gastric volume (ml)</th>
<th>Gastric pH (mEq/L)</th>
<th>Acidity (μg/ml)</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>250 mg/kg</td>
<td>4.20±1.07</td>
<td>3.22±0.26</td>
<td>52.8±12.56</td>
<td>397.07±72.20</td>
<td>127.07±14.97</td>
</tr>
<tr>
<td>500 mg/kg</td>
<td>4.34±1.19</td>
<td>3.69±1.24</td>
<td>47.6±16.47</td>
<td>375.40±78.79</td>
<td>115.98±17.96</td>
</tr>
<tr>
<td>1,000 mg/kg</td>
<td>4.12±0.83</td>
<td>3.78±0.51</td>
<td>43.8±5.85</td>
<td>360.67±93.19</td>
<td>115.07±24.37</td>
</tr>
<tr>
<td>Cimetidine</td>
<td>4.00±1.00</td>
<td>4.81±1.47</td>
<td>34.0±16.72</td>
<td>325.13±27.13</td>
<td>96.58±24.37</td>
</tr>
</tbody>
</table>

Data expressed as mean ± SEM (n=5). Significant differences were at the 95% level of confidence by Duncan’s test.
DISCUSSION

The results showed the anti-gastric ulcer activity of EF as evaluated in the most commonly utilized experimental models, including absolute alcohol, indomethacin, and swimming stress induced gastric ulcer in rats. The mechanism of the ulcer protective effect is variable due to factors affecting ulcerogenesis in different models. Basically, ulcers are caused by the imbalance between aggressive and defensive factors.

Absolute alcohol is the main factor that leads to intense damage of the gastric mucosa by inducing multiple hemorrhagic red bands (patches) of different sizes along the long axis of the glandular stomach. The pathogenesis of ethanol induced gastric mucosal damage was considered including decreasing mucus production, disturbance of mucosal microcirculation, formation of oxygen-derived free radicals, decreasing cell proliferation and an exacerbated inflammatory response (Gupta et al., 2012; Amaral et al., 2013).

Indomethacin, a nonsteroidal anti-inflammatory drug (NSAID) causes gastric mucosal damage by interfering with prostaglandin synthesis and bicarbonate secretion by inhibition of gastric mucosal cyclooxygenase (COX) activity and by producing mucosal oxidative stress through...
directing generation of reactive oxygen species (ROS) (Wallace, 2008; Heeba et al., 2009).

Gastric ulcers induced by swimming stress were mediated by stimulation of the vagal nerve to increase gastric acid secretion impairment of mucosal defense system such as disturbance of gastric mucosal blood flow, reduction of endogenous defense mediators, decrease in mucus content and promotion of gastric oxidative stress (Rujjanawate et al., 2005; Silva and Sousa, 2011).

This study pharmacologically evaluated the anti-gastric ulcer activity of EF at orally administered doses of 250, 500 and 1,000 mg/kg. These were shown to have equipotent action by decreasing the ulcer index and increasing the % protection approximating the cimetidine group in all models, when compared to the control group. The protective effect of EF suggests a possible gastroprotective mechanism of action.

The mechanisms of gastroprotection from the banana flowers could involve the bioactive constituents of this plant. The phytochemical composition of M. × paradisiaca flowers was reported by Mahmood et al. (2011) as containing alkaloids, saponins, glycosides, tannins, flavonoids, terpenoids and steroids. These compounds, especially flavonoids and tannins, were found to have a preventive action and antioxidative stress on gastric injury in rats (Vasconcelos et al., 2010).

From the results of the gastric secretion parameters, pre-treatment with EF could significantly decrease acidity and pepsin. This finding indicates that pre-treatment with EF could prevent gastric ulcers by decreasing aggressive factors.

Gastric wall mucus plays an important role as a defensive factor against gastrointestinal damage. Mucus-type glycoproteins can be detected by the amount of alcian blue binding (Howiriny et al., 2005). Pre-treatment with EF significantly increases the gastric mucus content at low dose of EF (250 mg/kg) when compared to the control group, and the effect was not different from cimetidine. However, the effects of EF and cimetidine were not different within the normal group, whereas the control group clearly significantly differed from the normal group. The results show that pre-treatment with EF had a gastroprotective effect. This could be the formation of protective complexes between EF and mucus, which may act as a barrier against several agents introduced into the stomach (Hiruma-Lima et al., 2006). Moreover, the promoting of mucus secretion may involve gastroprotective ingredients. The flavonoid leucocyanidin in banana fruit pulp has been found to stimulate mucus secretion (Lewis and Shaw, 2001).
Therefore, the gastroprotective mechanism of banana flowers may be similar to banana fruit pulp. The increase of mucus secretion could neutralize acid secretion from the lumen. This effect might reduce the size of the ulcer area. The finding indicates that the gastroprotective effect of the banana flower may involve the bioactive constituents such as flavonoids by promoting mucus secretion.

CONCLUSION

The present study established that EF has significant gastroprotective properties against absolute alcohol, indomethacin and swimming stress in rats. The action of EF may involve modulation of mucus secretion. This observation indicates that the extract of *Musa* (ABB group) ‘Kluai Nam Wa’ flower can be a potential source for gastric ulcer treatment.

REFERENCES


