Preliminary Studies on Gastroprotective Effect and Acute Toxicity of Methanolic Extract from *Tacca integrifolia* Rhizome in Rats

การศึกษาเบื้องต้นเกี่ยวกับฤทธิ์ต้านการเกิดแผลในกระเพาะอาหารและความเป็นพิษเฉียบพลันของสารสกัดเมทานอลจากเหง้าของว่านพังพอนในหนูขาว

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ABSTRACT

Methanolic extract of *Tacca integrifolia* rhizomes was evaluated for gastroprotective activity using ethanol induced gastric ulcers in rats model. Acute oral toxicity of this extract was also evaluated. Pretreatment with this extract in doses of 100, 200 and 400 mg/kg could decrease ulcer indexes when compared with the control group. Acute oral toxicity study of this extract showed no symptom of toxicity at the dose as high as 2 g/kg. These results suggest that methanolic extract of *Tacca integrifolia* rhizomes is effective for defending against ethanol induced gastric ulcers without acute oral toxicity in rats.

บทคัดย่อ

ฤทธิ์ต้านการเกิดแผลในกระเพาะอาหารของสารสกัดเมทานอลจากเหง้าของว่านพังพอนถูกประเมินโดยใช้แบบจำลองในหนูขาวที่ถูกเหนี่ยวนำให้เกิดแผลในกระเพาะอาหารโดยใช้เอทานอล ความเป็นพิษแบบเฉียบพลันทางปากของสารสกัดนี้ถูกประเมินด้วยเช่นกัน การป้องกันด้วยสารสกัดนี้ในขนาด 100, 200 และ 400 มิลลิกรัมต่อกิโลกรัม สามารถลดตัวบ่งชี้การเกิดแผลในกระเพาะอาหารเมื่อเปรียบเทียบกับกลุ่มควบคุม การศึกษาความเป็นพิษแบบเฉียบพลันจากทางปากของสารสกัดนี้ในขนาดสูงถึง 2 กรัมต่อกิโลกรัมไม่พบอาการของความเป็นพิษ ผลการศึกษานี้แสดงว่าสารสกัดเมทานอลจากเหง้าของว่านพังพอนมีประสิทธิผลในการต้านการเกิดแผลในกระเพาะอาหารในหนูขาวที่ถูกเหนี่ยวนำให้เกิดแผลในกระเพาะอาหารโดยใช้เอทานอลโดยไม่มีความเป็นพิษแบบเฉียบพลันทางปาก

Key Words: *Tacca integrifolia* Rhizome, Gastroprotective effect, Acute oral toxicity

คำสำคัญ: เหง้าของว่านพังพอน ฤทธิ์ต้านการเกิดแผลในกระเพาะอาหาร ความเป็นพิษเฉียบพลันทางปาก

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Introduction

The term peptic ulcer disease (PUD) is defined as a group of ulcerative disorder of the upper gastrointestinal tract that requires acid and pepsin for their ulcers formation (DiPiro et al., 2008). This disease is an important cause of morbidity and health care costs. Pharmacological interventions like antacids, H_2-receptor antagonists, proton pump inhibitors (PPIs), and prostaglandin analogues, have proved benefits in the treatment of PUD. However, their adverse effects are still the disadvantages of these conventional drugs (Aihara et al., 2003). Therefore, using herbs for preventing PUD is an alternative way in some countries due to intolerance to side effects, failure, or denial of conventional therapy (Gilani et al, 2005; Weldegerima, 2009).

Tacca integrifolia (*T. integrifolia*; family Dioscoreaceae) is a tropical herb with cylindrical rhizome. It has beautiful large leaf blades with greenish to dark purple flower (Phengklai, 1993). The rhizome of this plant has been used in traditional medicine for the treatment of gastrointestinal disorder such as gastric ulcer, enteritis, hepatitis, abdominal pain and diarrhea (Jiang, 1977; Srivastava, 2010). However, there is no scientific research to confirm its pharmacological effects.

Objectives of the study

To provide primary data on the anti-gastric ulcer activity and safety profile of the methanolic extract from the rhizomes of *T. integrifolia*.

Methodology

Preparation of plant extract

Methanolic extract from the rhizomes of *T. integrifolia* has been prepared and kindly provided by Professor Dr. Vichai Reutrakul, the Director of the Center for Innovation in Chemistry (PERCH-CIC). The brief preparation was as followed, dried *T. integrifolia* rhizomes powder was macerated with 95% methanol for 2 days. After filtration, the filtrate was evaporated under reduced pressure in a vacuum rotary evaporator. The extract was lyophilized and stored at 4-5 ºC until used.

Animals

Male Sprague-Dawley rats weighing between 200-300 g were purchased from the National Laboratory Animal Center, Mahidol University, Nakhon Pathom, Thailand. The animals were acclimated in the standard environmental conditions, temperature 24 ± 1 °C and 12 h light-12 h dark cycle, for 1 week before starting the experiments. All experiments were approved by The Animal Ethics Committee of Faculty of Medicine, Chiang Mai University, Thailand.

Gastroprotective study

The rats were divided into five groups (3 males/group). They were starved for 48 h with free access to water till 2 h before the experiment. The control group received 5% Tween 80. The reference group received 10 mg/kg of omeprazole in 5% Tween 80. The test groups received *T. integrifolia* extract in 5% Tween 80 at doses of 100, 200 and 400 mg/kg. All rats were orally received 1 ml absolute ethanol to induce gastric ulcer, 1 h after oral administration of each test agent (Sairam et al., 2001). One hour later, they were sacrificed by an overdose of ether, and their stomachs were immediately excised out. The stomach of each rat was opened along the greater curvature, rinsed with isotonic saline and pinned out on the

paraffin wax plate. The gastric ulcer lesion was measured in length of ulcer lesion under a binocular magnifier (10X). The ulcer index (UI) and percent inhibition of gastric ulcer were assessed.

Evaluation of gastric ulcer

The sum of the total length of ulcer lesions in each group was divided by the number of the rats in that group and expressed as an ulcer index (Okabe et al., 1978).

$$UI = \frac{\sum (\text{Total length of lesions in each group})}{\text{Sum of the number of rats in that group}}$$

The percentage of inhibition of gastric ulcer was calculated according to the following formula (Sanyal et al., 1982):

$$\% \text{ Inhibition} = \frac{(UI_{control} - UI_{treat})}{UI_{control}} \times 100$$

Acute oral toxicity study

The acute oral toxicity study was performed in accordance with the OECD Guideline (OECD, 2001). The rats (5 males and 5 females for each group) were divided into two groups, the control group and the treatment group. Both groups were kept under observation for 14 days after the oral administration of 5% Tween 80 (the control group) and a single dose (2 g/kg) of *T. integrifolia* extract in 5% Tween 80 (the treatment group). The visual observations included changes in the skin, fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous system, as well as somatomotor activity and behavioral pattern. All rats were sacrificed on the 15th day of the study, and internal organs (heart, lung, kidney, spleen, and liver) were removed for macroscopic examination.

Statistical analysis

An analysis of variance by one-way ANOVA was used. Data were expressed as mean ± S.E.M. and $p < 0.05$ was considered significant difference.

Results

Gastroprotective study

Significant reductions of gastric ulcer sizes were observed in rats pretreated with *T. integrifolia* extract ($p < 0.05$) at all dose levels, compared with those in the control group, in a dose dependent manner (Table 1). Its efficacy was comparable to the reference drug omeprazole given at the dose of 10 mg/kg, with no significant differences at all dose levels. Moreover, *T. integrifolia* extract could dose dependently reduce the severity of gastric ulcers as well (Figure 1 and 2).

Table 1 Effect of the *T. integrifolia* extract on ethanol-induced gastric lesions in comparison with the control and omeprazole groups.

Group	Ulcer index (mm)	Inhibition (%)
Control	105.50 ± 10.45	-
Omeprazole 10 mg/kg	1.87 ± 0.28*	98
***T. integrifolia* 100 mg/kg**	17.80 ± 4.83*	83
***T. integrifolia* 200 mg/kg**	7.57 ± 1.41*	93
***T. integrifolia* 400 mg/kg**	0.67 ± 0.58*	99

Data are represented as mean ± S.E.M. (n = 3).

* Significantly different from the control group, $p < 0.05$.

Acute oral toxicity study

No sign of acute toxicity was observed in all rats receiving the control vehicle and *T. integrifolia* extract at the dose of 2 g/kg body weight during the investigation period.

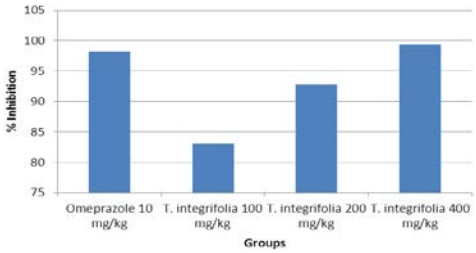

Figure 1 Comparison of anti-ulcer activity (% inhibition of gastric ulcer formation) of the *T. integrifolia* extract and omeprazole compared with the control group.

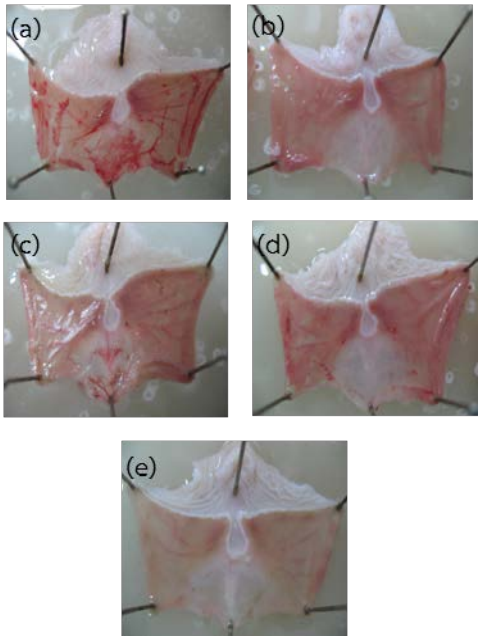

Figure 2 Effect of the *T. integrifolia* extract on ethanol-induced gastric lesions: control (a), omeprazole 10 mg/kg (b), *T. integrifolia* 100 (c), 200 (d) and 400 mg/kg (e).

Discussion and Conclusions

Ethanol causes severe damage to gastric mucosa by its direct toxic effect to reduce defensive factors, the secretion of bicarbonate and the production of mucous (Marhuenda et al, 1993). The *T. integrifolia* extract showed anti-gastric ulcer activity when evaluated in ethanol-induced gastric ulcer model, and the activity was dose related. It is therefore possible that *T. integrifolia* extract protects gastric wall mucous against ethanol damage probably by increasing mucosal resistance or potentiating of defensive factors and/or decreasing aggressive factors.

In terms of acute toxicity, the absence of sign of toxicity and death among rats in all groups throughout the two weeks of experiment support the safety of this plant usage that its oral LD50 is greater than 2,000 mg/kg. Further studies using various models of gastroprotective study, with higher number of experimental animals should be performed to confirm its efficacy and to find out its mechanism of action.

These results suggest that the methanolic extract of *Tacca integrifolia* rhizomes tends to be effective for defending against ethanol induced gastric mucosal injury and safe for using as traditional medicine.

Acknowledgement

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