# ฤทธิ์ระงับการเกิดแผลในกระเพาะอาหารของเนระพูสีไทย

# Anti-gastric ulcer effects of *Tacca chantrieri* Andre

[1]นาย ไชยยง รุจจนเวท [2]นางดวงพร อมรเลิศพิศานต์
[1]สำนักวิชาวิทยาศาสตร์สุขภาพ มหาวิทยาลัยแม่ฟ้าหลวง เบอร์โทรศัพท์ 053 946824
E-mail chaiyong@mfu.ac.th
[2]คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่

**บทคัดย่อ**

เนระพูสีไทย เป็นพืชล้มลุกอายุหลายปี มีถิ่นกำเนิดในเขตร้อน ในประเทศไทยอาจเรียกว่า ดีงูหว้า หรือ ว่านค้างคาว การแพทย์พื้นบ้านใช้เหง้าหรือใบของพืชชนิดนี้ต้มน้ำกินเพื่อบรรเทาอาการปวดเมื่อยตามเนื้อตัว ปวดท้องและแก้ไขอาการอาหารเป็นพิษ การศึกษาครั้งนี้มีวัตถุประสงค์เพื่อทดสอบฤทธิ์ยับยั้งการเกิดแผลในกระเพาะอาหารโดยกลไกต่างๆในสัตว์ทดลอง ได้สกัดเหง้าของเนระพูสีไทยด้วยเอทานอล นำสารสกัดที่ได้ไปทดสอบในหนูขาวที่ชักนำให้เกิดแผลในกระเพาะอาหารด้วย อินโดเมธาซิน เอทานอล และความเครียด นอกจากนี้ยังได้ทดสอบฤทธิ์ของสารสกัดดังกล่าวในการยับยั้งการหลั่งกรดในกระเพาะอาหารและต่อปริมาณสารเมือกและเฮกโซซามีนในกระเพาะอาหาร ผลการทดสอบพบว่าสารสกัดด้วยเอทานอลของเนระพูสีไทยในขนาด 125 และ 500 มก./กก. สามารถยับยั้งการเกิดแผลในกระเพาะอาหารในทุกการทดสอบได้อย่างมีนัยสำคัญ ($p < 0.01$) และสามารถยังยั้งการหลั่งกรดได้บางส่วน นอกจากนี้ยังพบว่า สารสกัดดังกล่าวยังช่วยรักษาปริมาณสารเมือกและเฮกโซซามีนในกระเพาะอาหารไม่ให้ถูกทำลายโดยแอลกอฮอล์อีกด้วย ผลการศึกษาครั้งนี้ได้ให้หลักฐานที่สนับสนุนการใช้เนระพูสีไทยบรรเทาอาการปวดท้องเนื่องจากแผลในกระเพาะอาหารตามที่ระบุไว้ในทางการแพทย์พื้นบ้าน โดยกลไกการออกฤทธิ์น่าจะมาจากการปกป้องเนื้อเยื่อของกระเพาะอาหารเป็นหลัก ขณะนี้งานวิจัยเพื่อสกัดแยกสารออกฤทธิ์เพื่อพัฒนาเป็นเภสัชภัณฑ์ กำลังดำเนินการอยู่และให้ผลในเบื้องต้นเป็นที่น่าพอใจอย่างมาก

**คำสำคัญ:** เนระพูสีไทย แผลในกระเพาะอาหาร

**Abstract**

*Tacca chantrieri* Andre (Taccaceae), commonly known as bat flower, is a perennial herbaceous plant indigenous to tropical countries. Thai folk medicine mentioned that a decoction of *T. chantrieri* rhizomes or leaves can be used to relieve pains of the body and stomach, and as an anti-dote for food poisoning. The aim of this study was to investigate the claimed anti-gastric ulcer effect of *T. chantrieri* using conventional screening methods. Ethanolic extract of *T. chantrieri* rhizomes was tested in experimental gastric ulcers induced by indomethacin, HCl/EtOH and water immersion stress and also in pylorus-ligated rats. It was found that the extract at doses of 125 and 250 mg/kg significantly ( $p < 0.05$) inhibited ulcers formation in rats and partly inhibited gastric secretion. Furthermore, in HCl/EtOH-induced ulcerated rats, gastric wall mucus and hexosamine content were markedly preserved by the extract pretreatment. These findings indicated that the extract possessed gastroprotective potential and thus substantiate the use of *T. chantrieri* in traditional medicine. The findings also shed a light for further researches to isolate the pharmaco-active fraction(s) or compound(s).

**Keywords:** *Tacca chantrieri*, gastric ulcer

## Introduction

*Tacca chantrieri* Andre (Taccaceae), commonly known as bat flower, is a perennial herbaceous plant that is indigenous to tropical countries. The plant can be ornamental due to its queer looking flower that shapes like a flying bat. Every parts of the plant are edible. Thai folk medicine mentioned that a decoction of *T. chantrieri* roots or leaves can be used to relieve pains of the body and stomach, and as an anti-dote for food poisoning ( Chuakul *et al*., 1996). The aim of this study was to investigate the claimed anti-gastric ulcer effect of *T. chantrieri*.

**Materials and methods**

*Plant material*

The rhizomes of *Tacca chantrieri* were collected from Payao province in April 2003. The plant was identified and found to be identical with the voucher specimen no. 18010 which is deposited at the Herbarium of the Department of Biology, ChiangMai University. The rhizomes were cleaned, air-dried and chopped into small pieces, powdered and stored in air-tightened, light protected containers.

*Extraction*

*T. chantrieri* rhizome powder was macerated with 95% ethanol overnight and filtered. The filtrate was concentrated in vacuo at 55 $^{O}$C and lyophilized to obtain a dry ethanolic extract ( 15.5 % yield) which was then subsequently reconstituted in water at appropriate concentrations for the experiments.

*Animals*

Male Sprague-Dawley rats weighing 150 - 200 g and Swiss albino mice weighing 25 - 30 g were purchased from the National Laboratory Animal Center, Salaya Mahidol University, Thailand. They were acclimatized for at least 7 days in an animal room where the temperature was maintained at 22 $\pm$ 3 $^{O}$C and there was a 12 hour light-dark cycle. The food was supplied by Pokphan Animal Feed Co. Ltd. Bangkok. The bedding was autoclaved. The rats had free access to food and water unless stated otherwise. All animals received humane care in compliance with the ethics in the use of animals issued by the National Research Council of Thailand 1999.

*Indomethacin-induced gastric ulcers*

The *T. chantrieri* ethanolic extract (TCE) was administered orally to 48 hr fasted rats 60 min prior to induction of gastric ulcers by indomethacin suspended in 0.5 % carboxymethylcellulose at a single i.p. dose of 30 mg/kg (Djahanguiri, 1966). After 5 hr the rats were sacrificed and examined for gastric ulcers.

*HCl/EtOH-induced gastric ulcers*

The TCE was administered orally to 48 h fasted rats 60 min prior to induction of gastric ulcers by 1.0 ml HCl/EtOH (60 ml ethanol + 1.7 ml HCl +38.3 ml water) p.o. (Mizui and Doteuchi, 1988). The animals were sacrificed and examined for gastric ulcers 60 min later.

*Restraint water immersion stress-induced gastric ulcers*

The TCE was administered orally to 48 hr fasted rats. Sixty minutes later, rats were restrained individually in stainless steel cages and immersed up to their xiphoid in a water bath maintained at 22 $\pm$ 2$^{O}$ C, according to the method of Takagi *et al*. (1963). After 5 hr of this exposure, the rats were sacrificed and examined for gastric ulcers

*Evaluation of the gastric ulcers*

After each rat was sacrificed, the stomach was removed, opened along the greater curvature and the glandular portion of the stomach was examined. The length in mm of each lesion was measured under a dissecting microscope and the sum of the length of all lesions was designated as the ulcer index.

*Pylorus ligation*

The TCE was administered orally to 48 hr fasted rats. One hour later, pylorus ligation as described by Shay *et al*. (1945) was performed. Briefly, rats were lightly anesthetized by ether. The abdomen was opened and the pylorus was ligated. The abdomen was closed by suturing. The animals were killed 5 hr later by an over dose of ether. The stomach was removed and its content was subjected to measurement of volume and pH and assay for titratable acidity.

Determination of gastric wall mucus content

Gastric wall mucus was determined by the Alcian blue method (Corne *et al*., 1974). Briefly, the TCE was administered orally to 48 h fasted rats 60 min prior to induction of gastric ulcers by 1.0 ml HCl/EtOH (60 ml ethanol + 1.7 ml HCl +38.3 ml water) p.o. (Mizui and Doteuchi, 1988). Sixty minutes later, the animals were sacrificed and the stomach was excised and opened along the lesser curvature, weighed and immersed in 0.1 % w/v Alcian blue solution for 2 hours. The excessive dye was then removed by two successive rinses in 0.25 M sucrose solution. Dye complexed with gastric wall mucus was extracted with 0.5 M $MgCl_2$ for 2 hours. The blue extract was then shaken vigorously with an equal volume of diethyl ether and the resulting emulsion was centrifuged. The optical density of Alcian blue in the aqueous layer was read against a buffer blank at 580 nm using a spectrophotometer. The quantity of Alcian blue extracted per gram wet stomach was then calculated from a standard curve.

Measurement of gastric hexosamine content

Hexosamine content in gastric tissue was assayed by the method of Glick (1967). Briefly, the TCE was administered orally to 48 h fasted rats 60 min prior to induction of gastric ulcers by 1.0 ml HCl/EtOH (60 ml ethanol + 1.7 ml HCl +38.3 ml water) p.o. (Mizui and Doteuchi, 1988). Sixty minutes later, the animals were sacrificed and the antral part of the stomach was hydrolyzed with 6 N HCl overnight. The tissue was neutralized with 6 N NaOH and incubated with acetylacetone at 100$^{O}$ C for 15 min. The mixture was then coupled with Ehrlich's reagent and allowed to stand at room temperature for 40 min. The optical density of the sample was measured spectrophotometrically at 530 nm using glucosamine as a standard.

*Statistical analysis*

Data were subjected to statistical analysis using ANOVA and statistical comparison was done using Duncan Multiple Range Test. The value exceeding 95 % confidence limits was considered to be significant.

**Results**

The TCE at doses 250 and 500 mg/kg significantly inhibited ulcer formation induced by indomethacin, acid ethanol and water immersion stress as shown in Table 1. and the inhibition was dose related.

**Table 1.** Effects of the *Tacca chantrieri* ethanolic extract (TCE) on gastric ulcers in rats

| Group | Gastric ulcer inducer | | | | | |
|---|---|---|---|---|---|---|
| | Indomethacin | | HCl/EtOH | | Stress | |
| | Ulcer index (mm) | I (%) | Ulcer index (mm) | I (%) | Ulcer index (mm) | I (%) |
| Control | 7.5 ± 1.3 | | 101.0 ± 11.9 | | 9.9 ± 0.9 | |
| TCE 250 mg/kg | 1.5 ± 0.6** | 80 | 29.0 ± 7.5** | 71 | 4.7 ± 0.8** | 52 |
| TCE 500 mg/kg | 0.3 ± 0.2** | 96 | 6.9 ± 2.2** | 93 | 1.8 ± 0.7** | 82 |

Note : data expressed as mean ± S.E.M. ( n = 8 )
** $p < 0.01$ significantly different from the control group
I (%) = inhibition of ulcer formation expressed as percentage

In the pylorus ligated rats, the mean gastric volume, pH and acidity were not affected by the TCE pretreatment at a dose of 250 mg/kg (Table 2). However at a dose of 500 mg/kg, the TCE could significantly increased the gastric pH and decreased the acidity output.

**Table 2.** Effects of the *Tacca chantrieri* ethanolic extract (TCE) on gastric secretion in rats

| Group | Gastric vol. (ml) | Gastric pH | Acidity mEq/L |
|---|---|---|---|
| **Control** | 9.5 ± 0.8 | 1.62 ± 0.08 | 126 ± 5 |
| TCE 250 mg/kg | 8.0 ± 1.0 | 2.00 ± 0.16 | 125 ± 7 |
| TCE 500 mg/kg | 7.2 ± 0.7 | 2.44 ± 0.39* | 60 ± 11** |

Note : data expressed as mean ± S.E.M. ( n = 8 )
* $p < 0.05$ ; ** $p < 0.01$ significantly different from the control group

Table 3 shows that the mean value of the gastric mucus contents in HCl/EtOH induced ulcerated rats was significantly lower than that of the control group. The TCE at doses 250 and 500 mg/kg significantly increased the mucus content even more than that of the nonulcerated rats. The mean gastric hexosamine content in control ulcerated rats was significantly less than that in the normal nonulcerated group. Pretreatment with the TCE at 250 and 500 mg/kg significantly increased the hexosamine content.

**Table 3.** Effects of the *Tacca chantrieri* ethanolic extract (TCE) on gastric wall mucus and hexosamine content in rats

| Group | Gastric wall mucus (ug Alcian blue/g wet stomach) | Hexosamine content (ug /100 mg wet stomach weigh) |
|---|---|---|
| Control ulcerated rats | 804 ± 29 | 21.7 ± 1.6 |
| TCE 250 mg/kg | 1972 ± 83* | 29.2 ± 2.2* |
| TCE 500 mg/kg | 1631 ± 98* | 42.7 ± 2.3** |
| Nonulcerated rats | 1167 ± 16* | 37.0 ± 2.2** |

Note : Data expressed as mean ± S.E.M. (n = 10)
* $p < 0.05$ ; ** $p < 0.01$ significantly different from the control group

**Discussion**

Results obtained in this study show the anti-gastric ulcer activity of Thai medicinal plant, *Tacca chantrieri* when evaluated in the most commonly utilized experimental models which include indomethacin, HCl/EtOH and restraint water immersion stress-induced gastric lesions in rats (Robert *et al*., 1979, Murakami *et al*., 1985).

The pathogenesis of gastric ulcers is often depicted as an imbalance between mucosal integrity and aggressive factors. Factors that impair mucosal defense are HCl, gastrin, histamine, *Helicobacter pylori*, aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs), ethanol, caffeine and stress while factors that promote mucosal integrity are gastric mucus and bicarbonate, gastric mucosal barrier, PGs and mucosal blood flow (Brunton, 1996, Friedman and Peterson, 1998).

According to the experimental models used in this study, non-steroidal anti-inflammatory drugs (NSAIDs) like indomethacin induce ulcer formation by depleting cytoprotective prostaglandins e.g. $PGE_2$ and $PGI_2$ in the cyclooxygenase pathway of arachidonic acid metabolism (Sato *et al*.,1981). $PGE_2$ and $PGI_2$ of gastric and duodenal mucosa are responsible for mucus production and maintaining cellular integrity of the gastric mucosa (Konturek *et al*.,1984).

In the HCl/EtOH induced gastric ulceration model, HCl causes severe damage to gastric mucosa (Yamahara *et al*., 1988) whereas ethanol produces necrotic lesions by direct necrotizing action which in turn reduces defensive factors, the secretion of bicarbonate and production of mucus (Marhuenda *et al*., 1993). The water immersion stress-induced ulcers are mediated by increases in gastric acid secretion (Kitagawa *et al*., Fujiwara M. 1979) and motility (Watanabe, 1966) and decreases in mucosal microcirculation (Guth, 1972) and mucus content (Koo *et al*., 1986).

The TCE could prevent ulceration in all three models. In pylorus ligated rats, the TCE had no effect on gastric volume and altered the gastric pH and acidity output only at a dose of 500 mg/kg. This finding signifies that anti-secretory action is not the sole mechanism in the anti-gastric ulcer effect of the TCE.

The gastric wall mucus, obligatory components of which are hexosamines , is thought to play an important role as a defensive factor against gastrointestinal damages (Davenport, 1968). The determined gastric wall mucus was used as an indicator for gastric mucus secretion while the mucosal hexosamine content was used as an indicator for gastric wall mucus synthesis (Lukie and Forstner, 1972).

In the present study, the gastric wall mucus and hexosamine contents in HCl/EtOH ulcerated rats were markedly lowered when compared with those of the nonulcerated group. It was found that pretreatment with the TCE significantly increased both gastric mucus and hexosamine contents in HCl/EtOH ulcerated rats. This finding indicated that the TCE preserved both gastric mucus synthesis and secretion in the experimental rats. It was reported that steroid saponins are presented in *T. chantrieri* (Zhou *et al*., 1983). Studies on the saponin role to the plant's pharmacological activities and investigation on its mechanism of action are being conducted.

In conclusion this study provides evidence that a Thai medicinal plant, *Tacca chantrieri* possesses an anti-gastric ulcer effect which is related to a cytoprotective mechanism via preservation of gastric mucus synthesis and secretion.

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