

ฤทธิ์ของผงขมิ้นในการรักษาแผลในกระเพาะอาหารของหนูขาว

พัชรวิวัลย์ ปั้นแห่งเพชร¹, จินตนาภรณ์ วัฒนธร², อรพิน ผาสุริย์วงษ์²

วิจิตรา ทศนียกุล¹, บุญเกิด คงยิ่งยศ¹

¹ภาควิชาเภสัชวิทยา ²ภาควิชาสรีรวิทยา คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น

Antigastric Ulcer Effect of Turmeric in Rats

Patchareewan Pannangpetch¹, Jintanaporn Watanathorn², Orapin Phasuriwong²,
Wichitra Tassaneeyakul¹, Bunkerd Kongyingyoes¹

¹Department of Pharmacology, ²Department of Physiology

Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand.

บทคัดย่อ

หลักการและเหตุผล: ขมิ้นชัน (*Curcuma longa* Linn.) ผงแห้งของรากขมิ้นชัน (Turmeric) เป็นสมุนไพรที่มีการกล่าวอ้างว่ามีฤทธิ์รักษาแผลในกระเพาะอาหาร อย่างไรก็ตามผลการศึกษาทางวิทยาศาสตร์เกี่ยวกับฤทธิ์รักษาแผลในกระเพาะอาหารของขมิ้นยังมีความขัดแย้งกันอยู่ ดังนั้น คณะผู้วิจัยจึงทำการศึกษาดูว่าผลของขมิ้นชันในการป้องกันและรักษาแผลในกระเพาะอาหารของสัตว์ทดลองเพื่อให้ได้ข้อมูลเพิ่มเติมและชัดเจนขึ้น อันจะเป็นประโยชน์ในการประยุกต์นำไปใช้ด้านการแพทย์สมัยใหม่ต่อไป

วิธีการ: สัตว์ทดลองที่นำมาศึกษาคือ หนูขาวเพศผู้ น้ำหนัก 180-220 กรัม แผลในกระเพาะอาหารจะถูกเหนี่ยวนำให้เกิดขึ้นโดยการฉีด indomethacin 12 มิลลิกรัมต่อน้ำหนักตัว 1 กิโลกรัม และโดยภาวะเครียดอันเนื่องจากอุณหภูมิต่ำและถูกจำกัดการเคลื่อนไหวในการศึกษาฤทธิ์ป้องกัน จะป้อนหนูด้วยผงขมิ้น ขนาด 0.25, 0.5 และ 0.75 กรัมต่อน้ำหนักตัว 1 กิโลกรัม เป็นเวลา 3 วัน ก่อนที่จะถูกเหนี่ยวนำให้เกิดแผล และในการศึกษาฤทธิ์เสริมการสมานตัวของแผล จะป้อนผงขมิ้นในขนาดดังกล่าว หรือ curcuminoids ขนาด 5 มิลลิกรัมต่อน้ำหนักตัว 1 กิโลกรัม เป็นเวลา 3 วัน หลังจากหนูขาวถูกเหนี่ยวนำให้เกิดแผลแล้ว ความยาวของแผลในกระเพาะอาหารจะตรวจวัดด้วยกล้อง stereomicroscope สำหรับการตรวจวัดปริมาณทั้งของ soluble และ insoluble mucus ใช้วิธี spectrophotometry

ผลการทดลอง: ผงขมิ้นขนาด 0.5 กรัมต่อน้ำหนักตัว 1 กิโลกรัม มีฤทธิ์ทั้งป้องกันและเสริมการสมานตัวของแผลในกระเพาะอาหารอันเนื่องมาจาก indomethacin เป็นที่น่าสนใจอย่างยิ่งว่า ผงขมิ้นใน ขนาด 0.75 กรัมต่อน้ำหนักตัว 1 กิโลกรัม ไม่มีฤทธิ์ป้องกันหรือเสริมการสมานของแผลแต่อย่างใด ในทางตรงกันข้าม กลับมีแนวโน้มว่าจะทำให้เกิดแผลรุนแรงมากกว่ากลุ่มควบคุม สารสกัด

Abstract

Background: Dry powdered rhizome of *Curcuma longa*, turmeric or Kha-min (in Thai word), was claimed to have antiulcerogenic effect. However, the scientific evidences showing the effectiveness of turmeric in the treatment of gastric ulcer are still controversy. In order to elucidate the anti-peptic activity of turmeric, therefore, we investigated the antiulcerogenic effect of turmeric powder and curcuminoid against indomethacin and hypothermic-restraint stress induced gastric ulcer in rats.

Methods: Male Wistar rats weighing 180-220 g were used. Gastric lesion was induced by administration of indomethacin 12 mg kg⁻¹ subcutaneously and also by hypothermic-restraint stress. In the study of protective effect, the rats were pretreated with turmeric suspension in 10% propylene glycol orally at doses of 0.25, 0.5 or 0.75 g kg⁻¹ for 3 days. In the investigation of healing effect, the gastric ulcer was induced and followed by administration of turmeric suspension or curcuminoid. Total length of gastric lesion was measured under the stereomicroscope and used as gastric ulcer indicator. Determinations of soluble and insoluble gastric mucus were performed by spectrophotometry method.

Results: Turmeric at dose of 0.5 g kg⁻¹ was not only significantly effective in protecting but also enhancing healing of gastric ulcer induced by indomethacin 12 mg kg⁻¹. Interestingly, turmeric at 0.75 g kg⁻¹ had no antiulcerative effect, and showed a tendency to increase the severity of lesion as compared to control. The curcuminoid 5 mg kg⁻¹ did not show any healing effect in indomethacin-induced ulcer but could significantly inhibit the gastric secretion of soluble mucus at 3.5 hr after curcuminoid administration.

Conclusions: It may be concluded that *Curcuma longa*

จากผงขมิ้น คือสาร curcuminoid 5 มิลลิกรัมต่อน้ำหนักตัว 1 กิโลกรัม ไม่มีฤทธิ์เสริมการสมานตัวของแผลและสาร curcuminoid ในขนาดเดียวกันนี้ มีผลลดการหลั่งของ soluble mucus หลังจากที่ได้รับ curcuminoid ไปแล้วเป็นเวลา 3.5 ชั่วโมง

สรุป : อาจสรุปได้ว่า ผงขมิ้นชันมีฤทธิ์ในการป้องกันและเสริมการสมานตัวของแผลในกระเพาะอาหาร เมื่อได้รับในขนาดที่เหมาะสม และในทางตรงกันข้าม หากได้รับในปริมาณมากเกินไป อาจทำให้เกิดแผลในกระเพาะอาหารมากขึ้นได้ ซึ่งอาจเนื่องจากสาร curcuminoid มีผลยับยั้งการหลั่ง insoluble mucus

is effective in protecting indomethacin induced gastric ulcer and enhancing the healing of ulcer. This effect is dose dependent, and can be achieved only with proper dose.

Key words : *Curcuma longa*, Turmeric, Gastric Ulcer, Kha-min

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Introduction

Curcuma longa L. (Zingiberaceae) is widely cultivated in tropical areas of Asia, including Thailand. Dry powdered rhizome of *Curcuma longa*, called turmeric or Kha-min (in Thai word), is commonly used as a spice and herbal medicine. It is claimed to be effective in treatment of peptic ulcer, flatulence, diarrhea, inflammation and itching¹. In addition, turmeric is one of a recommended herbal medicine for primary health care system in Thailand¹. Use of medicinal plants in modern medicine would be more accepted if it is supported by scientific verification of their efficiency and safety. However, scientific data showing the effectiveness of turmeric in the treatment of gastric ulcer is still controversy². Rafatullah et al³ reported the protective effect of ethanolic extract of *Curcuma longa* against hypothermic-restraint stress, pyloric ligation, indomethacin and reserpine-induced ulcer in rats. But, Bhatia et al⁴ did not find any protective action of curcumin against histamine-induced gastric ulceration in guinea-pig. Moreover, high dose of curcumin was reported to be ulcerogenic⁵ and hepatotoxic⁶. In order to elucidate those contradicting results, and to obtain further scientific evidence to justify the use of turmeric as an antigestive ulcer, therefore, we investigated the antiulcerogenic effect of turmeric powder and curcuminoid against indomethacin and hypothermic-restraint stress induced gastric ulcer in rats.

Methods

Preparation of turmeric powder and curcuminoid.

Rhizomes of *Curcuma longa* L. obtained from a local market were dried and finely ground. Following the extraction of volatile oil from turmeric powder by n-hexane, the residual powder was macerated with ethanol for 2 days. After filtering, alcohol was evaporated from the filtrate under low temperature and pressure. The residual viscous liquid was crystallized in the mixture of methanol and ether (1:1).

The obtained yellow crystal is the curcuminoid, a mixture of curcumin, monodesmethoxycurcumin and didesmethoxycurcumin, which was confirmed by thin layer chromatographic technique.

Anti-ulcer studies

The anti-ulcerogenic effect was studied in male Wistar rats, weighing between 180-220g, obtained from the Experimental Animal Unit, Faculty of Medicine, Khon Kaen University.

A. Preventive test:

Indomethacin-induced gastric lesion.

Turmeric powder was suspended in 10% propylene glycol. Turmeric suspension at doses of 0.25, 0.5 and 0.75 g kg⁻¹ d⁻¹ or 10% propylene glycol solution were given orally via gastric tube twice daily at 9.00 and 16.00 for three consecutive days. The rats were then deprived of food for 24 hr but allowed free access to water. Subsequently, indomethacin (Sigma Chemical, St Louis, Co.) 12 mg kg⁻¹ suspended in 80% propylene glycol was given subcutaneously⁷. The animals were sacrificed 7 hr later. The stomach was removed and incised along the greater curvature. The length (mm) of each lesion was measured under a stereomicroscope with a reference scale, and the sum of lesion length per stomach was used as the lesion indicator.

Hypothermic-restraint stress induced gastric lesion

Turmeric powder suspension was given as described above. The rats were then deprived of food for 24 hr but allowed free access to water prior to the experiment. Movement of the rat was restricted by placing each individual in a small cage (5x5x18 cm), and they were kept in a cold chamber (4 °C) for 2 hours⁸. Thereafter, they were sacrificed and the length (mm) of lesion was measured as described above.

B. Ulcer healing tests**Indomethacin-induced ulcer**

The rats were fasted overnight and given indomethacin 12 mg kg⁻¹ subcutaneously. Turmeric suspension at dose of 0.25, 0.5 and 0.75 g kg⁻¹ d⁻¹ or 10% propylene glycol were given orally 7 hr after indomethacin injection and twice daily at 9.00 and 16.00 for the next 3 consecutive days. The rats were maintained under uniform diet during this period. They were sacrificed 6 hr after the final administration of turmeric, and the length (mm) of each lesion was measured. In the investigation of the effect of curcuminoid on the healing process, curcuminoid was dissolved in 10 % alcohol and given at the dose of 5 mg kg⁻¹ d⁻¹ for 3 days following the same schedule as that of turmeric. The vehicle control group was administered 10 % alcohol.

The determination of gastric mucus

The rats were pretreated with curcuminoid 5 mg kg⁻¹ d⁻¹ or 10% alcohol for three days and fasted for 16 hr before the experiment. After the animals were anesthetized, their stomachs were exposed and ligated at the pyloduodenal junction. The gastric contents were then collected at 1.5, 2.5 and 3.5 hr after the last administration of curcuminoid or 10% alcohol. The measurement of soluble mucus was made according to the method of Whiteman⁹: the soluble mucus in gastric content was interacted with Alcian blue solution resulting in a complex which was dissolved in sodium lauryl sulfate and then read the optical density at 620 nm. The soluble mucus was expressed as microgram of chondroitin sulfate (determined from standard curve) per gram of stomach.

Using the same protocol, the glandular part of stomach was removed to determine the amount of surface mucus gel (insoluble mucus) at 0.5 and 1.5 hours after the last administration of curcuminoid. Determination was made according to the method of Corne et al¹⁰: the surface mucus gel is allowed to form complex with Alcian blue solution, then eluted by magnesium chloride, and finally determined spectrophotometrically at 605 nm. The amount of mucus was expressed as optical density (OD) per 100 g stomach.

Statistical analysis

Results were given as mean \pm SEM. Statistical significance was determined by Student's t test, and the level of significance was set at $p < 0.05$.

Results**I. The protective effect of turmeric on the gastric ulcer induced by indomethacin.**

Indomethacin 12 mg kg⁻¹ produced visible multiple lesions in the glandular part of the stomach. The rats were pretreated orally with turmeric 0.25, 0.5 and 0.75 g kg⁻¹ d⁻¹ for 3 days. Turmeric at dose of 0.5 g kg⁻¹ significantly inhibited the indomethacin-induced gastric mucosal lesion (Table 1). However, increasing the dose further to 0.75 g kg⁻¹ did not show any inhibiting effect, but appeared to enhance the ulcerogenic effect of indomethacin.

II. The protective effect of turmeric on the hypothermic-restraint stress induced gastric ulcer.

The hypothermic-restraint stress for 2 hr produced erosion in the glandular part of stomach. Turmeric given orally, at all doses, exerted no protective effect against hypothermic-restraint condition induced stress ulcer (Table 2). Nevertheless, it is noteworthy that the group of rats received turmeric at high dose, 0.75 g kg⁻¹, showed a tendency for increased severity of mucous erosion, even though it was not statistically different to control lesion (Table 2).

III. The healing effect of turmeric and curcuminoid on indomethacin-induced gastric ulcer.

Seven hours after the injection of indomethacin, turmeric was orally administered for 3 consecutive days. Turmeric at a dose of 0.5 g kg⁻¹ enhanced the healing of ulcer induced by indomethacin while the higher or lower doses did not reach a statistical significance. The average length of lesion was statistically reduced from 47.22 \pm 17.22 mm in control group to 11.67 \pm 8.19 mm in turmeric treated group (Table 3).

The oral administration of curcuminoid 5 mg kg⁻¹ for 3 days, using the same procedure as that of turmeric, did not exhibit any improvement in the healing of ulcer as compared to control group (data not shown).

IV. The effect of curcuminoid on gastric mucus secretion.

The oral administration of curcuminoid 5 mg kg⁻¹ had no effect on the production of insoluble mucus (Table 4) as compared to the effect of vehicle (10% alcohol).

Table 1 The protective effect of turmeric on the gastric ulcer induced by indomethacin.

Treatments	Number of animals	Doses (g kg ⁻¹)	The length of lesions (mm) (mean \pm SEM)
Control	15	-	32.90 \pm 11.98
Turmeric	5	0.25	7.14 \pm 3.42
Turmeric	5	0.5	5.57 \pm 3.88*
Turmeric	5	0.75	55.14 \pm 25.67

*: $p < 0.05$, Student's t-test

Table 2 The protective effect of turmeric on the hypothermic-restraint stress induced gastric ulcer

Treatments	Number of animals	Doses (g kg ⁻¹)	The length of lesions (mm) (mean ± SEM)
Control	15	-	67.55 ± 31.23
Turmeric	5	0.25	93.33 ± 28.22
Turmeric	5	0.5	24.17 ± 12.50
Turmeric	5	0.75	106.17 ± 34.85

Table 3 The ulcer healing effect of turmeric on indomethacin induced gastric ulcer.

Treatments	Number of animals	Doses (g kg ⁻¹)	The length of lesions (mm) (mean ± SEM)
Control	15	-	47.22 ± 17.22
Turmeric	5	0.25	13.33 ± 6.79
Turmeric	5	0.5	11.67 ± 8.19*
Turmeric	5	0.75	15.00 ± 7.47

*: p<0.05, Student's t-test

Table 4 The effect of curcuminoid on the production of insoluble mucus.

Time (hr)	Amount of insoluble mucus (OD ₆₆₅ /100g of stomach)	
	Control	Curcuminoid
0.5	2.27 ± 0.53	2.60 ± 0.60
1.5	4.67 ± 1.00	3.27 ± 0.67

The number of animals used in each group was 5. The insoluble mucus was determined at 0.5 and 1.5 hr after the last administration of 5 mg curcuminoid or 10% alcohol for control group.

Nevertheless, this compound significantly decreased the secretion of soluble mucus from 74.57 ± 2.28 to 41.96 ± 3.72 µg g⁻¹ of stomach at 3.5 hours after the administration (Table 5).

Table 5 The effect of curcuminoid on the secretion of soluble mucus.

Time (hr)	Amount of soluble mucus (µg g ⁻¹ of stomach)	
	Control	Curcuminoid
1.5	47.67 ± 4.66	37.67 ± 4.33
2.5	59.20 ± 10.67	45.33 ± 5.00
3.5	74.57 ± 2.28	41.96 ± 3.72*

The number of animals used in each group was 5. The soluble mucus was determined at 1.5, 2.5 and 3.5 hr after the last administration of 5 mg curcuminoid or 10% alcohol for control group. *: p<0.05, Student's t-test

Discussion

This study provides evidence that *Curcuma longa* has protective and healing enhancing effects against indomethacin but has no effect against stress-induced gastric ulcer in rats. However, in the former case, the effect is dose dependent with the presence of response only with the optimal dose, 0.5g kg⁻¹. Curcuminoid, the major constituent of turmeric powder, could inhibit the gastric soluble mucus secretion.

The precise mechanism of hypothermic-restraint stress induced gastric erosion is not clear, but increased gastric acid secretion¹¹ and decreased gastric mucosa blood flow¹² were postulated. In the present study, we could not demonstrate the gastroprotective effect of turmeric against hypothermic-restraint stress. Thus, it is likely that the turmeric powder may not modify gastric acid secretion or gastric mucosa blood flow.

A deficiency of endogenous prostaglandins is a major factor in the pathogenesis of gastric ulcer caused by indomethacin¹³. Prostaglandins function as a gastroprotector by stimulating the gastric mucus production, increasing gastric mucosal blood flow, and inhibiting gastric acid secretion^{13, 14}. Therefore, it is conceivable that active ingredients of turmeric perform to replenish the impaired gastroprotective mechanism of prostaglandins possibly through the stimulation of mucin production¹⁵. It is interesting that this action occurred only at the optimal dose as the antiulcerative action was absent in the dose 0.75 g kg⁻¹. Besides, at the high dose 0.75 g kg⁻¹, the ulcer length was apparently larger than in the control group but not statistically significant. It seems to be that turmeric could also cause ulcer. We might be able to see the ulcer

effect caused by turmeric more obviously if it could be administered at a dose higher than 0.75 g kg⁻¹. This characteristic of pharmacological action may possibly indicate the antagonistic effects among substances presented in turmeric powder.

Powdered rhizome of *Curcuma longa* contains approximately 0.6% of curcumin¹⁶. Hence, it was assumed that curcuminoid at a dose of 5 mg kg⁻¹ was comparable to turmeric powder at a dose of 0.75 g kg⁻¹. Curcuminoid 5 mg kg⁻¹ did not show a healing promoting effect in indomethacin induced ulcer. On the other hand, it showed an inhibitory effect on the secretion of soluble mucus. The soluble mucus can be washed from the mucosal surface and mixed with the gastric juice. The soluble mucus may act as a lubricant due to its viscous property, and also perform as temporary mucus layers external to the mucus gel that plays role in preventing erosive effect of acid¹². This indicates that the curcuminoid 5 mg kg⁻¹ may have an ulcerogenic effect, instead of antiulcerogenic, by inhibiting the gastric secretion of soluble mucus. In agreement with our finding, Prasad et al.⁵, reported that the administration of curcumin 100 mg kg⁻¹ produced gastric ulcer in albino rats. They also found that pretreatment with an acid secretion inhibitor, metiamide, could prevent the development of gastric lesion and a decrease in mucus secretion¹⁷.

It is important to remind that turmeric may be toxic if received in large amount and improperly used as a medicine. There were reports of certain cytotoxic activities of *Curcuma longa*. High concentrations of curcumin in culture of rat hepatocytes induced LDH-leakage and depletion of glutathione⁶. In the study of anti-inflammatory activity of curcumin, Mukhopadhyay et al.¹⁶ reported both its anti-inflammatory and irritant activities. Recently, Blasiak et al.¹⁸, using Comet assay to detect double and single strand DNA, found that curcumin itself at 10, 25 and 50 μM could damage DNA of human lymphocytes and gastric mucosa cells. In the chronic toxicity test in mice, the number of red blood cells as well as white blood cells were decreased after administration of turmeric extract 100 mg kg⁻¹ for 3 months¹⁹. The observed hematological changes may be attributed to the cytotoxic effect of *Curcuma longa*.

In conclusion, *Curcuma longa* at appropriate amount was effective in protecting indomethacin induced gastric ulcer and also enhancing the healing of ulcer. However if administration in large amount, it may cause an ulcer by inhibiting the secretion of gastric soluble mucus. This may explain the contradicting effect of *Curcuma longa* that has been previously observed, and its application as a drug must be exercised with cautions.

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