

Prevention of Indomethacin - Induced Gastric Ulcers in Rats by Extract from Leaves of *Centella asiatica*

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ABSTRACT

Objective: The aim of this study is to investigate the protective effect of *Centella asiatica* extract on gastric ulcers caused by indomethacin in rats.

Methods: Male rats were orally given extract of *C.asiatica* prior to the administration of indomethacin. The stomach was dissected and the number of bleeding spots and histological features were determined.

Results: Giving extract of *C.asiatica* at doses of 10 and 20 mg/kg for 30 minutes prior to oral administration of indomethacin decreased the number of bleeding spots are about 11 times lower than those given indomethacin alone. Histological findings of indomethacin-treated group showed the depth of injury of gastric mucosa which is about half the thickness of the mucosal layer. Treatment with *C.asiatica* decreased the depth of tissue erosion, down to one-third of the thickness of the mucosa.

Conclusion: The results showed protective effect of *C.asiatica* on gastric ulceration caused by the anti-inflammatory drug, indomethacin.

Keywords: *Centella asiatica*; gastric ulcer; indomethacin

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C*entella asiatica* (Linn.) is known as Indian Pennywort or Buobok in Thai. It is a perennial herbaceous creeper of the Apiaceae family that is commonly found in abundance on moist, sandy or clay soils. It has been used for centuries as medicinal herb for the treatment of several conditions including wounds and injuries.¹⁻² Chemically, *C.asiatica* contains triterpenoids and their glycosides include asiaticoside, asiatic acid, madecassoside, madecassic acid, brahmoside, brahminoside and centelloside.³⁻⁴ previous studies reported anti-gastric ulcer, antitumor, antioxidant, immunomodulatory activities of *C.asiatica*.⁵⁻⁸ However, previous studies of the plant's effect on gastric ulceration were done by using various inducers but not indomethacin.^{5,9-11} So this study aims to investigate the effect of the plant extract on gastric injury caused by indomethacin according to its common use as anti-inflammatory drug.

MATERIALS AND METHODS

Chemicals

Indomethacin (Fluka, Switzerland); eosin, hematoxylin (Merck, Germany); Pentothal sodium (Abbott, Italy); sodium chloride injection (Thainakornpatana Co, Thailand); Tween 80 (Sigma, USA).

Plant extract

Aqueous extract of *C.asiatica* leaves was prepared by the Center for Research and Development of Herbal Health Products, Khon Kaen University. Briefly, fresh leaves of *C.asiatica* were blended in water, filtered and dried by freeze dryer (Renown Tech Co, Model 102161). The yield was 1.8%.

Animals and experimental design

Sprague-Dawley rats (250-300g weight) were obtained from the Animal Center, Faculty of Medicine, Khon Kaen University. The animals were kept in dark-light cycle for 12 hours at 25°C for 7 days. Before starting the experiments,

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the animals were fast for 12 hours and divided into 4 groups (n=8), then they were orally administered as follows: Group 1, indomethacin (20 mg/kg) in 0.1% Tween 80; Group 2 and 3, extract of *C.asiatica* in water at the doses of 10 and 20 mg/kg 30 minutes prior to giving indomethacin; Group 4, 0.5 ml of 0.1% Tween 80.

Six hours after the treatment, the animal was killed by intraperitoneally injected with 60 mg/kg pentothal sodium and the abdomen was opened. The stomach was removed, cut along the greater curvature and washed with normal saline solution. The opened stomach was fixed on a paraffin plate and measured for the bleeding spots. The ratio and % of the bleeding area were determined by image analyzer (UTHSCA image tool for version 3.00) and % of bleeding area was determined. Histological examination of the stomach was performed by paraffin section at 5 μ thickness and stained with hematoxylin and eosin.¹²⁻¹³ All data on tissue erosion were obtained from the photographs taken from microscope with open study method.

Statistical analysis

The data were expressed as mean \pm SD. One-way analysis of variance followed by multiple comparison tests were used to access statistical significance of differences between groups.

RESULTS

Bleeding of stomach

An administration of indomethacin (20 mg/kg dissolved in 0.1% Tween 80) induced 12.13 \pm 5.84 bleeding spots of gastric mucosa with area of bleeding of 0.15 \pm 0.12 mm², whereas the control group (giving 0.5 ml of 0.1% Tween 80) showed 0.63 \pm 0.74 bleeding spots. Receiving the extract of *C.asiatica* leaves 30 minutes prior to indomethacin can significantly decrease the number of bleeding spots down to 0.63 \pm 0.52 and 1.00 \pm 1.20 spots for 10 and 20 mg/kg doses, respectively. The % of bleeding areas of these animals were close to the value of control animals. (Table 1, Fig 1).

Histological examination of stomach

The stomach of indomethacin-treated animals showed ulceration in the mucosa. By measuring from the photographs, the depth of mucosal erosion was about half of the thickness (Fig 2A). Some epithelial cells did not have nucleus, which suggested cell death. Red blood cells and polymorphonuclear cells were found, which represented the tissue inflammation (Fig 2B). These inflammatory features were not seen in the control group (receiving 0.1% Tween 80). Receiving 10 and 20 mg/kg of *C.saiatica* extracts can prevent bleeding and inflammation of the stomach. The tissue erosion observed was no more than 1/3 of the mucosal thickness (Fig 3).

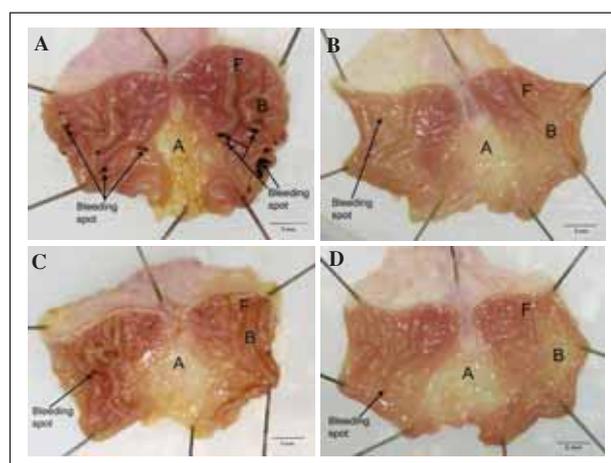


Fig 1. Stomach of rats receiving indomethacin (A), *C.asiatica* extract at 10 and 20 mg/kg doses 30 minutes prior to indomethacin (B-C), and 0.1% Tween 80 (D). (arrow indicated bleeding spots, A= antrum, B= body, F= fundus)

DISCUSSION

The present study demonstrated a prevention of indomethacin-induced gastric ulceration by *C.asiatica* extract. The bleeding spots were about 11 times lower when giving the plant extract prior to indomethacin administration. Our results supported the earlier reports that *C.asiatica* significantly inhibited gastric ulceration induced by cold and restraint stress, ethanol, acetic acid and aspirin.^{5,9-11} The findings that the doses of *C.asiatica* used in our study were in between the earlier studies in rats^{5,10} suggested the differences in plant extract preparations. Terpenoids such as asiatic acid, asiatic acid and madecassic acid as reported

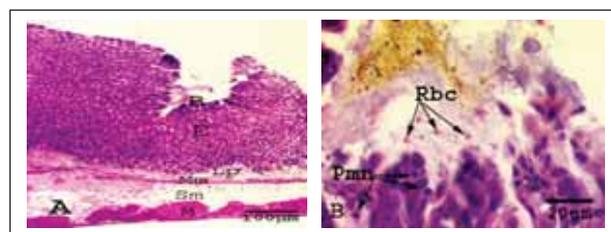


Fig 2. Histological features of rat stomach receiving indomethacin at dose of 20 mg/kg. A demonstrated the epithelial erosion about half of the thickness. B represented the high power magnification of area B in A, which showed the red blood cells and polymorphonuclear cells infiltration. (M = muscular layer, Sm = submucosal layer, Mm = muscularis mucosae, Lp = lamina propria, E = epithelium, Pmn = polymorphonuclear cell, Rbc = red blood cell)

TABLE 1. Number and area of bleeding spots in rat stomach receiving indomethacin and *C.asiatica* extract

Treatment	Animal weight (g)	Number of bleeding spots	Area of bleeding (mm ²)	% of bleeding area
Indomethacin (20 mg/kg)	306.00 \pm 33.66	12.13 \pm 5.84	0.15 \pm 0.12	0.029 \pm 0.024
<i>C.asiatica</i> (10 mg/kg)	298.59 \pm 50.33	0.63 \pm 0.52*	0.02 \pm 0.02	0.003 \pm 0.003*
<i>C.asiatica</i> (20 mg/kg)	282.10 \pm 53.39	1.00 \pm 1.20*	0.02 \pm 0.02	0.005 \pm 0.005*
Control (0.1% Tween 80)	292.55 \pm 51.82	0.63 \pm 0.74*	0.03 \pm 0.03	0.004 \pm 0.005*

values expressed in term of mean \pm SD of eight animals.

* significantly differences from the indomethacin treated group (p<0.001).

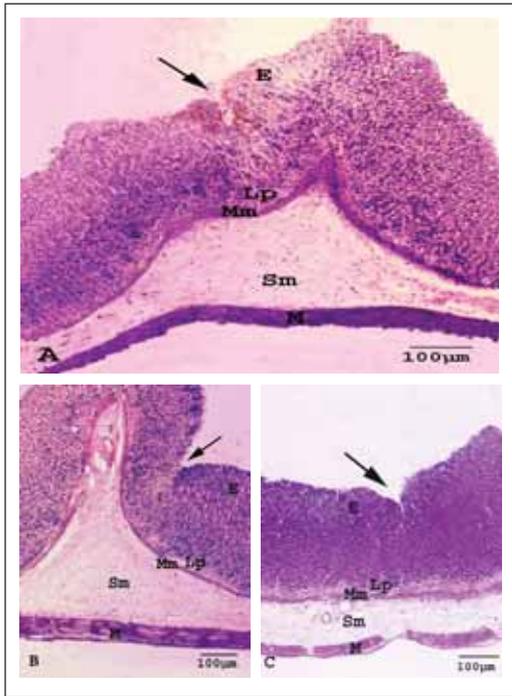


Fig 3. Histological features of rat stomach receiving *C.asiatica* extracts at dose of 10 and 20 mg/kg (A and B) prior to indomethacin administration and 0.1% Tween 80 (C). Arrows showed the erosion of epithelium layers. (M = muscular layer, Sm = submucosa layer, Mm = muscularis mucosae, Lp = lamina propria, E = epithelial layer)

to play roles in wound healing may contribute to the antiulcerative effect of *C.asiatica* on indomethacin-induced gastric damages.¹⁴⁻¹⁵ Therefore, *C.asiatica* extract has a potential in future development as pharmaceuticals or nutraceutical products for gastric ulcer prevention, according to its safety and traditional consumption.

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บทคัดย่อ

การป้องกันการเกิดแผลในกระเพาะอาหารจากยาอินโดเมธาซิน โดยสารสกัดจากใบบัวบกในหนูขาว

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วัตถุประสงค์: งานวิจัยนี้มีจุดมุ่งหมายเพื่อศึกษาฤทธิ์ของสารสกัดใบบัวบกต่อการเกิดแผลในกระเพาะอาหารที่เกิดจากการใช้ยาอินโดเมธาซินในหนูขาว

วิธีการ: หนูขาวเพศผู้ได้รับการป้อนสารสกัดใบบัวบกก่อนการให้ยาอินโดเมธาซิน จากนั้นตรวจสอบหาจุดที่มีเลือดออกในกระเพาะอาหาร และศึกษาลักษณะทางเนื้อเยื่อวิทยาของกระเพาะอาหาร

ผลการศึกษา: การให้สารสกัดใบบัวบกขนาด 10 และ 20 มก./กก. เป็นเวลา 30 นาที ก่อนให้อินโดเมธาซิน สามารถลดจำนวนจุดที่มีเลือดออกลงได้ประมาณ 11 เท่าของแผลที่เกิดจากการได้รับยาอินโดเมธาซิน ลักษณะทางเนื้อเยื่อวิทยาของกระเพาะอาหารของสัตว์ทดลองที่ได้รับยาอินโดเมธาซินเป็นแผลลึกประมาณครึ่งหนึ่งของชั้นเยื่อเมือก เมื่อให้ใบบัวบกสามารถลดการเกิดแผลได้เหลือรอยแผลเพียง 1/3 ของชั้นเยื่อเมือก

สรุป: ผลการศึกษาครั้งนี้แสดงให้เห็นว่าใบบัวบกสามารถป้องกันการเกิดแผลที่กระเพาะอาหารที่เกิดจากด้านการอักเสบอินโดเมธาซินได้