Evaluation of Antinociception Effect of *Derris scandens* Using Acetic Acid-induced Abdominal Constriction Test in Mice

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Abstract

The objective of this research is to determine the antinociceptive effect induced by the 50% ethanolic extract of *Derris scandens* using the acetic acid-induced abdominal constriction test (writhing test) in mice. The methodology included the extract was orally administered at the doses of 1, 10, 100 and 1,000 mg/kg BW, while aspirin (50 mg/kg BW) was used as the standard reference drug. The results indicate that the doses of the extract used at 10, 100 and 1,000 mg/kg BW and the reference drug showed a potent antinociceptive effect by significantly ($p \leq 0.05$, n = 6) decreasing the number of abdominal constrictions and stretching of hind limb induced by the intraperitoneal injection of acetic acid (0.75% v/v), and also increased the percentage inhibition of writhing in a dose dependent manner. The value of the median effective dose of suppression of abdominal constrictions (ED_{50}) calculated from the log dose–response curve was 35.5 mg/kg BW. The data obtained suggest that the extract of *D. scandens* appears to have antinociceptive effect. Further study needs to assess the nature of the analgesic interaction of coadministration between the extract of *D. scandens* and other analgesic drugs by using this model.

Keywords: *Derris scandens, antinociceptive effect, abdominal constriction test, mice*

บทคัดย่อ

วัตถุประสงค์ ของงานวิจัยนี้เพื่อประเมินฤทธิ์ระงับปวดของสารสกัดเถาวัลย์เปรียงที่สกัดด้วย 50% เอทานอลในหนูเม้าส์ที่ถูกเหนี่ยวนำให้เกิดความปวดในช่องท้องด้วยกรดอะซิติก (Writhing test) ดำเนินการวิจัย โดยการป้อนสารสกัดเถาวัลย์เปรียงขนาด 1, 10, 100 และ 1,000 มิลลิกรัมต่อกิโลกรัมให้หนูเม้าส์ ใช้ยาแอสไพริน (ขนาด 50 มิลลิกรัมต่อกิโลกรัม) เป็นยาระงับปวดมาตรฐาน ผลการวิจัยพบว่าหนูกลุ่มที่ได้รับสารสกัดจากเถาวัลย์เปรียงขนาด 10, 100 และ 1,000 มิลลิกรัมต่อกิโลกรัม และกลุ่มที่ได้รับยามาตรฐานแสดงผลการระงับปวดอย่างมีนัยสำคัญทางสถิติ ($p \leq 0.05$, n = 6) โดยลดจำนวนการเกิด Writhing ซึ่งเป็นอาการที่สัตว์ทดลองแอ่นตัวพร้อมกับบิดลำตัวและเหยียดเกร็งขาหลังเมื่อเกิดความปวดในช่องท้อง จากการเหนี่ยวนำด้วยการฉีดกรดอะซิติก (0.75% v/v) ทางช่องท้อง และค่าร้อยละของการยับยั้งการเกิด Writhing เพิ่มขึ้นโดยสัมพันธ์กับขนาดสารสกัดที่เพิ่มขึ้น ขนาดของสารสกัดที่ลดการเกิด Writhing ลง 50% (ED_{50}) ซึ่งคำนวณจากกราฟระหว่างขนาดสารสกัด (log scale) กับร้อยละของการยับยั้งการเกิด Writhing เท่ากับ 35.5 มิลลิกรัมต่อกิโลกรัม ผลจากการวิจัยนี้แสดงว่าสารสกัดเถาวัลย์เปรียงมีฤทธิ์ระงับปวด ควรศึกษาเพื่อประเมินการเปลี่ยนแปลงฤทธิ์ระงับปวดเมื่อใช้สารสกัดเถาวัลย์เปรียงร่วมกับยาระงับปวดชนิดอื่นๆ ด้วยโมเดลนี้ต่อไป

คำสำคัญ: *เถาวัลย์เปรียง ฤทธิ์ระงับปวด การทดสอบฤทธิ์ระงับปวดโดยการเหนี่ยวนำให้เกิดความปวดในช่องท้อง หนูเม้าส์*

1. Introduction

Derris scandens (Roxb.) Benth, known as Tao-Wan-Priang in Thai, is well-known Asian medicinal plant. Its dried stem has been used in Thai traditional medicine for the treatment of bone and joint pain, osteoarthritis, expectorant, antitussive, and diuretic (Laupattarakasem, el al., 2003). The major active constituents of *D. scandens* stem extracts are benzyls and isoflavones, including genistein, coumarins, scandinone, scandenin, prenylated isoflavones, and isoflavone (Mahabusarakam et al., 2004; Rao et al., 2007). The leaf and root extracts of *D. scandens* as well as the isolated flavonoids, ovaliflavanone and lupinifolin showed anti-inflammatory activity on carrageenan-induced paw edema in rats (Ganapaty, Josaphine, and Thomas, 2006).

A clinical trial was carried out to compare the efficacy and side effects between the 50% ethanol extract of *D. scandens* (Roxb.) Benth and diclofenac, a nonsteroidal anti-inflammatory drug for the

treatment of two groups of patients with lower back pain. The results of pain alleviation were not different significantly between the two treatment groups (Srimongkol et al., 2007). The result from a phase III clinical trial showed that the volunteers with knee osteoarthritis who received the *D. scandens* extract resulted in better effective treatment, fewer side effects, and non-toxic effects when compared with naproxen, an anti-inflammatory drug. The gastrointestinal irritation and dyspepsia were observed more often in the naproxen than in the Derris group (Kuptniratsaikul et al., 2011).

From Thailand National List of Essential Medicines (2015), *D. scandens* Benth is a drug developed from Thai Medicinal Plant. One formulated capsule contains 400 mg of the 50% ethanol extract of *D. scandens.* This drug is intended for relieving pain in lower back and knee osteoarthritis. Low back pain and knee osteoarthritis are frequently found in aging. The increasing of prevalence tendency is also found. Anti-inflammatory drugs, such as NSAIDs, are given to treat patients. However, the adverse effects of anti-inflammatory drugs are reported such as irritation and ulcers of gastric and intestine system. Thai Ministry of Public Health has a policy to support the research and development of herbal plants to be processed into high-quality goods and promote the use of Thai herbs. Therefore, the treatment effect of *D. scandens* is to replace or coadminister with other analgesic drugs by physicians and patients themselves.

The combination of analgesics of proven efficacy is a strategy intended to achieve one or more therapeutic goals (Raffa, 2001). In certain cases, the coadministration of antinociceptive agents result in synergistic effects and the doses of the individual drugs are substantially reduced (Miranda and Pinardi, 2004). The studies to assess the nature of the interaction between the combination of drugs in a rodent model are required. However, no studies have yet provided evidence of the analgesic activity of *D. scandens* in animal model. Acetic acid-induced abdominal constriction test (writhing test) is a Pain-state model using chemical stimuli, which both central and peripheral analgesics are detected. This model has been used by many investigators and can be recommended as a simple screening method (Collier et al., 1968). A good relationship exists between the potencies of analgesics in writhing assays and their clinical potencies in this model (Milind and Monu, 2013). The present study aimed to evaluate the possible antinociceptive effect of the 50% ethanolic extract of *D. scandens* using the acetic acid-induced abdominal constriction test in mice. The result from this study is needed to be used in further study to assess the nature of the analgesic interaction between *D. scandens* and other analgesic drugs.

2. Objective

The objective of the study was to determine the antinociceptive effect of the 50% ethanolic extract of *Derris scandens* using the acetic acid-induced abdominal constriction test in mice (writhing test).

3. Materials and methods

The Extract, Drug and Chemical Reagent

The commercial "GPO Thao-Wan-Priang Capsules" 50% ethanolic extract of *D. scandens* was used. Aspirin was obtained from Merck, AG, Darmstadt, Germany. Analytical grade of sodium chloride and acetic acid (Sigma, St. Louis, USA) were purchased locally.

Experimental Animals

Adult male albino ICR mice (30-35 g) were obtained from National Laboratory Animal Center, Mahidol University, Thailand. All mice were housed in Faculty of Science, Rangsit University, Thailand, under standard environmental conditions of 24 $\pm$ 1 °C, 60-70% humidity, and 12 h light and 12 h dark cycle. All animals had free access to water and standard pellet laboratory animal diet. Before experiments began, the animals were deprived of food for 12 h and allowed to adapt to the laboratory for at least 2 h before testing. Each animal was used for one experiment only. All animal experiments were submitted and approved for ethic considerations from the Research Institute of Rangsit University and carried out accordance with current Guidelines for The Care of Laboratory Animals and Ethical Guidelines, National Research Council of Thailand

Acetic Acid-Induced Abdominal Constriction (Writhing) Test in Mice

This study was carried out using acetic acid-induced abdominal writhing reflex pain model (Jain and Kulkarni, 1999; Koster, Anderson, and DeBeer, 1959). Thirty-six mice were randomly divided into 6 groups (1-6, six animals per group, per treatment), fasted for 12 hours and treated as follows: group 1 (negative control group) received 0.1 ml/kg BW., p.o. of normal saline, group 2 (positive control group) received 50 mg/kg BW. p.o. of aspirin, and groups 3, 4, 5 and 6 received 1,10, 100 and 1000 mg/kg BW. p.o. of *D. scandens* extract respectively using gastric gavage. Thirty minutes after the extract or drug administration, 0.75% v/v glacial acetic acid (0.1 ml/10 g BW) was administered intraperitoneally to all mice to induce abdominal contortions or writhings. The analgesic effect was assessed and recorded in each mouse by counting the incidences of writhes (arching of back, development of tension in abdominal muscles, elongation of the body in hind limb) for a period of 30 min.

Data Analysis

Numbers of writhing were presented as mean $\pm$ SEM. The degree of antinociception was calculated as the percentage of inhibition of writhing using the formula

$$= \frac{\text{(Mean of control group-mean of treated group)}}{\text{Mean of control group}} \times 100$$

A least-squares linear regression analysis of the log dose–response curves allowed the calculation of the dose that produced 50% of antinociception (ED_{50}) of the extract. The analysis was done using one way analysis of variance (ANOVA) and the difference between the means tested using Post Hoc LSD test. The value of $p \leq 0.05$ was considered statistically significant.

4. Results and Discussion

The result of the acetic acid-induced abdominal constriction test was shown in Table 1. The 50% ethanolic extract of *D. scandens* produced a dose-dependent antinociceptive effect in the chemical viscero-somatic assay of the acetic acid abdominal constriction test compared with the control group. The extract produced 5.5, 32.5, 68.4 and 90.2 % inhibition at the dose of 1, 10, 100 and 1,000 mg/kg BW, respectively ($p \leq 0.05$, n = 6) which was comparable to reference analgesic drug, aspirin (78.3% inhibition at 50 mg/kg BW)

Log dose–response curves for the antinociceptive effect of the 50% ethanolic extract of *D. scandens* was obtained using at least six animals at each of at least four doses as shown in Figure 1. A least-squares linear regression analysis (R = 0.99) of the log dose–response curves allowed the calculation of the dose that produced 50% of antinociception (ED_{50}) which was 35.5 mg/kg BW.

Table 1 The effect of the 50% ethanolic extract of *D. scandens* on acetic acid-induced abdominal constriction in mice

Treatment	Dose (mg/kg BW)	No. of writhes in 30 min	Inhibition (%)
Control (NSS)	-	72.2 ± 6.8	-
Aspirin	50	15.6 ± 6.2	78.3
D. scandens extract	1	68.0 ± 2.0	5.5
	10	48.6 ± 3.3*	32.5
	100	38.6 ± 2.2*	68.4
	1,000	12.0 ± 0.6*	90.2

-Thirty minute after treatment, mice were injected i.p. with 0.75% (v/v) acetic acid (0.1 ml/10 g BW); the number of induced writhing was counted for 30 min.
-Values are mean ± SEM (n=6); $^{*}p \leq 0.05$ was significantly different from control group.

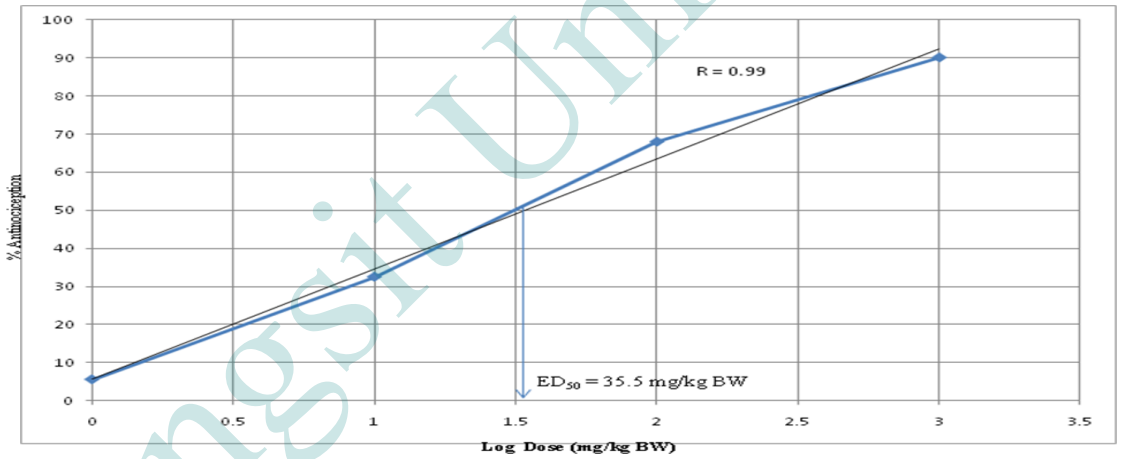

Figure 1 Dose–response curves for the antinociception induced by the oral administration of the extract of D. *scandens*. Each point is the mean ± SEM of 6 animals

5. Discussion

In accordance with the medical uses of the *D. scandens* extract, the present study was carried out to evaluate the antinociceptive effect of the 50% ethanolic extract of this plant. The major finding of the current study was the demonstration, for the first time to our knowledge, that the ethanolic extract of *D. scandens* produced pronounced and dose-related antinociception inacetic acid-induced writhing response model. This method has been used as model of chemonociception and validated for screening of virtually all classes of analgesics (Jain and Kulkarni, 1999; Koster, Anderson, and DeBeer, 1959). The oral administration of paracetamol and diclofenac showed dose-dependent antinociceptive effects with different potencies in the 0.6% acetic acid-induced abdominal constriction test of mice with ED_{50} of 17.9 and 127.2 mg/kg BW, respectively (Miranda et al., 2006). Because of the treatment effect of *D. scandens* is in order to replace or coadminister with other analgesic drugs such as NSAIDs and paracetamol which widely used to treat moderate to mild pain, this obtained data can be used to evaluated different combinations of the *D. scandens* extract with paracetamol and other commonly used NSAIDs by isobolographic analysis in the further study.

6. Conclusion

The present results indicate that the 50% ethanolic extract of *D. scandens* has antinociceptive effect as this significantly reduced the contortions induced by acetic acid in the writhing test. This finding validated the traditional analgesic properties of this plant. The extract will, therefore, be of potential benefit in the management of pain, although further studies are necessary for the identification of the active principles and the elucidation of its mechanism of action.

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