Analgesic Synergy between Paracetamol and *Derris scandens* in Mice

Tadsanee Punjanon[1*], Wanlapa Yingyong[2] and Natnon Untharin[2]

[1*]Pharmacology and Toxicology Unit, Faculty of Science, Rangsit University, Pathum-thani 12000, Thailand
[2]Biomedical Sciences Program, Faculty of Science, Rangsit University, Pathum-thani 12000, Thailand
*Corresponding author, E-mail: tadsanee@rsu.ac.th

Abstract

Combination therapy is a valid approach in pain treatment, in which a reduction of doses could reduce side effects and still achieve optimal analgesia. The objective was to determine the effects of coadministered paracetamol and the extract of *D. scandens* in a mouse model of visceral pain and determined the type of interaction between components. The effective dose that produced 50% antinociception (ED_{50}) was calculated from the log dose–response curves of fixed ratio combinations of paracetamol with the *D. scandens* extract. The ED_{50} was compared to the theoretical additive ED_{50} calculated from the ED_{50} of paracetamol and of the *D. scandens* extract alone obtained from ED_{50} dose–response curves. The combination was synergistic, the experimental ED_{50} being significantly smaller than the theoretically calculated ED_{50}. The results of this study demonstrate potent interactions between paracetamol and the *D. scandens* extract and validate the clinical use of combination of these drugs in the treatment of pain conditions.

Keywords: *Derris scandens, paracetamol, analgesic synergism, mouse*

บทคัดย่อ

วิธีการให้ยาแก้ปวดร่วมกันในการบรรเทาปวดจะช่วยลดขนาดยาแต่ละชนิดลง ทำให้ผลข้างเคียงของยาลดลงโดยที่ยายังคงมีฤทธิ์ในการแก้ปวดเต็มที่ วัตถุประสงค์ของงานวิจัยนี้เพื่อประเมินฤทธิ์แก้ปวดของยาพาราเซตามอลร่วมกับสารสกัดเถาวัลย์เปรียงในหนูเมาส์ที่ถูกเหนี่ยวนำให้เกิดความเจ็บปวดต่ออวัยวะภายในเพื่อประเมินรูปแบบของการใช้ยาร่วมกัน โดยประเมินจากขนาดยาที่ใช้ในการลดความปวดลงครึ่งหนึ่ง (ED_{50}) โดยคำนวณจากกราฟระหว่างขนาดยากับผลการระงับปวดของการใช้ยาสองชนิดร่วมกันในสัดส่วนต่างๆ เปรียบเทียบกับค่า ED_{50} ที่ได้จากการทดลองกับค่า ED_{50} ทางทฤษฎีที่คำนวณจากค่า ED_{50} ของยาพาราเซตามอลและค่า ED_{50} ของสารสกัดเถาวัลย์เปรียงเพียงชนิดเดียว ผลการวิจัยพบว่าการรวมยาสองชนิดเข้าด้วยกันมีผลเสริมฤทธิ์กัน โดยค่า ED_{50} ที่ได้จากการวิจัยมีค่าต่ำกว่าค่า ED_{50} ทางทฤษฎี ผลจากการวิจัยชี้ให้เห็นถึงการเสริมฤทธิ์ระหว่างยาพาราเซตามอลและสารสกัดเถาวัลย์เปรียง ซึ่งอาจเป็นประโยชน์ในการใช้ยาสองชนิดนี้ร่วมกันในการบรรเทาปวดทางคลินิก

คำสำคัญ: *เถาวัลย์เปรียง ยาพาราเซตามอล การเสริมฤทธิ์ระงับปวด หนูเม้าส์*

1. Introduction

From Thailand National List of Essential Medicines (2015), *D. scandens* Benth is a drug developed from Thai medicinal plants. 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 (Kuptniratsaikul et al., 2011). Low back pain and knee osteoarthritis are frequently found in the elderly. 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 (The Government Public Relations Department, 2015). Therefore, the treatment effect of *D. scandens* is to replace or coadminister with other analgesic drugs by physicians and patients themselves.

Paracetamol (acetaminophen) is one of the most widely used analgesics. Paracetamol has a different mode of action from other classes of analgesics, and hence a different profile of effects on body systems. It is currently considered to be a selective COX-3 inhibitor and also shows weak anti-inflammatory activity. A previous study has shown orally administered paracetamol to have profound analgesic effects on bone cancer pain in mice when administered alone and when administered with a subanalgesic dose of morphine (Saito, Aoe & Yamamoto, 2005). The significant synergistic interaction between paracetamol and oxcarbazepine in a mouse model of visceral pain was reported (Tomic et al., 2010). A combination of paracetamol and NSAIDs provides superior efficacy in the treatment of acute postoperative pain to either drug alone (Ong et al., 2010).

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 results 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 combinations of drugs in a rodent model are required. 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). Because paracetamol and *D. scandens* were both effective in visceral animal pain model (Punjanon, Phumsuay & Wongsawat, 2016) so we examined the effects of their combination in these pain models, and determined their types of pharmacologic interactions (synergism, additivity, or antagonism).

2. Objectives

The objectives of the study was to determine the nature of the analgesic interaction between the *D. scandens* extract and paracetamol using the acetic acid-induced abdominal constriction test in mice (writhing test).

3. Materials and methods

3.1 The extract, drug and chemical reagents

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

3.2 Experimental animals

Adult male albino ICR mice (30-35 g) were obtained from the National Laboratory Animal Center, Mahidol University, Thailand. All mice were housed in the 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.

3.3 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/10 g BW., p.o. of distilled water, group 2 (positive control group) received 50 mg/kg BW. p.o. of aspirin, and groups 3, 4, 5 and 6 received at each of at four doses of *D. scandens* extract or paracetamol, respectively using gastric gavage. Thirty minutes after the *D. scandens* 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.

3.4 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}) for each drug. 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 $p < 0.05$ was considered statistically significant.

A dose–response curve was also obtained by the oral coadministration of paracetamol with the *D. scandens* extract in fixed ratio combinations of fractions of their respective ED_{50} values: 1/2, 1/4, 1/8, 1/16 (ratio value given in Table 1). A dose–response curve and experimental ED_{50} for the combination of paracetamol and the *D. scandens* extract administered orally by gavage was also obtained with the same scheme. The interaction index was calculated as experimental ED_{50}/theoretical ED_{50}. If the value is close to 1, the interaction is additive. Values lower than 1 are an indication of the magnitude of supra-additive or synergistic interactions and values higher than 1 correspond to sub-additive or antagonistic interactions (Tallarida, 2001).

4. Results

The result of the acetic acid-induced abdominal constriction test was shown in Table 1. The 50% ethanolic extract of *D. scandens*, paracetamol, and the combination 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 *D. scandens* 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 ($p < 0.05$, n = 6) which was comparable to reference analgesic drug, aspirin (78.3% inhibition at 50 mg/kg BW). Paracetamol produced 17.6, 39.6, 61.3, and 86.6 % inhibition at the dose of 50, 100, 250, and 750 mg/kg BW, respectively ($p < 0.05$, n = 6). The combination of paracetamol with the *D. scandens* extract produced 21.1, 42.4, 61.8, and 86.8 % inhibition at the dose of 24.2 (1/16), 48.4 (1/8), 96.8 (1/4), and 193.5 (1/2) mg/kg BW, respectively ($p < 0.05$, n = 6).

Table 1 The effect of the *D. scandens* extract, paracetamol, and the combination on acetic acid-induced abdominal constriction in mice

Treatment	Dose (mg/kg BW)	No. of writhes in 30 min	Inhibition (%)
Control (DW)	0.1 ml/kg BW	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
Paracetamol	50	59.3 ± 3.4*	17.6
	100	43.5 ± 2.6 *	39.6
	250	27.8 ± 3.7*	61.3
	750	9.7 ± 1.1*	86.6
Paracetamol/	24.2	56.8 ± 2.0*	21.1
D. scandens extract	48.4	41.5 ± 3.9*	42.4
(1/0.22)	96.8	27.5 ± 2.9*	61.8
	193.5	9.5 ± 2.3*	86.8

-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); *$p < 0.05$ was significantly different from control group.

Log dose–response curves for the antinociceptive effect of the 50% ethanolic extract of *D. scandens*, paracetamol, and the combination were 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 were 35.5, 158.0, and 63.1 mg/kg BW, respectively.

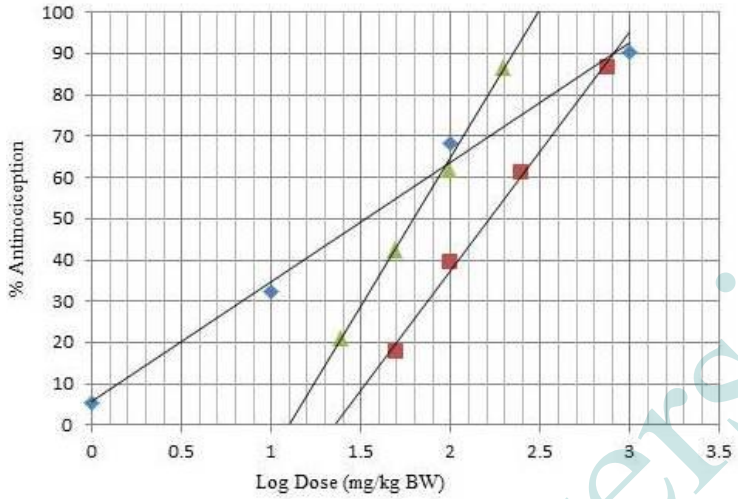

Figure 1 Dose–response curves for the antinociception induced by the oral administration of D. *scandens* extract (◆) paracetamol (■), and the combination (▲). Each point is the mean ± SEM of 6 animals.

The antinociceptive activity of the oral coadministration of fixed ratio combinations of ED_{50} fractions of paracetamol with the *D. scandens* extract was assessed by calculating the ED_{50} of the mixtures from the corresponding dose–response curves. The synergy was present when the drug combination was administered orally. Table 2 represented the theoretical additive and the experimental observed ED_{50} values of the combinations which were 96.8 and 63.1 mg/kg BW, respectively. The interaction index of the combination was 0.65.

Table 2 Theoretical and experimental ED_{50} values and interaction index for combinations of paracetamol with the *D. scandens* extract in the writhing test of mice

Drugs	ED_{50}theoretical (mg/kg BW)	ED_{50}experimental (mg/kg BW)	Interaction index
D. scandens extract	-	35.5	-
Paracetamol	-	158.0	-
Paracetamol/ *D. scandens* extract (1/0.22)	96.8	63.1	63.1/96.8 = 0.65

5. Discussion

The oral coadministration of paracetamol and the *D. scandens* extract produced a dose-dependent antinociceptive effect in the chemical viscero-somatic assay of the acetic acid abdominal constriction test. The combination tested showed a synergic interaction with interaction index at 0.65. The reduction of doses at 35.4 and 38.0% of paracetamol and the *D. scandens* extract in combination, compared with single drugs ED_{50} was found. These results confirm previous findings in which the significant synergistic interaction between paracetamol and other drugs in this algesiometric test ((Tomic et al., 2010; Miranda et al., 2006) and in the patients (Ong et al., 2010). In certain cases, the coadministration of antinociceptive drugs with different mechanisms of action results in synergistic (greater than additive) effects, and the doses of the individual drugs can be substantially reduced thereby minimizing drug-specific adverse effects. A mechanism of action of the *D. scandens* extract is still unknown. The properties of a system that define a pharmacologic interaction between 2 drugs are likely complicated. The type of interaction between 2 drugs may be explained by altering the kinetics of each other or at various levels of drug action. The synergistic interaction between paracetamol and the *D. scandens* extract provides new information about combination pain treatment and should be explored further in patients, especially with somatic and/or visceral pain.

6. Conclusion

In conclusion, the data of the present study demonstrated that paracetamol combined with the *D. scandens* extract produces a supra-additive or synergic analgesic effect. It is possible to suggest that the combinations of paracetamol and the extract will be effective for the clinical treatment of pain. In addition, it is demonstrated that the effect of the combinations paracetamol/the *D. scandens* extract is superior to that of either component alone. Therefore, these mixtures are a viable alternative to clinical pain management, especially because the low doses of the components may be a potential index of lower incidence of adverse effects.

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