

THE ADDITIVITY ANTINOCICEPTIVE INTERACTIONS BETWEEN DICLOFENAC AND THE *DERRIS SCANDENS* EXTRACT DRUG IN MICE

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ABSTRACT

Objective: 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 diclofenac and the *Derris scandens* extract drug.

Methods: Acetic acid-induced abdominal constriction test in mice was used to determine the type of interaction between components. The effective dose that produced 50% antinociception (ED₅₀) was calculated from the log dose-response curves of fixed ratio combinations of diclofenac with the *D. scandens* extract drug. The ED₅₀ was compared to the theoretical additive ED₅₀ calculated from the ED₅₀ of diclofenac and of the *D. scandens* extract drug alone.

Results: Diclofenac and the *D. scandens* extract drug dose-dependently and significantly reduced the abdominal writhing. The combination was the additive effect, the experimental ED₅₀ being smaller than the theoretically calculated ED₅₀. Interaction index of the combination was 0.89.

Conclusion: The present study demonstrates the additivity antinociceptive interactions between diclofenac and the *D. scandens* extract drug and may be used as a combination analgesic in the treatment of pain conditions.

Keywords: *Derris scandens*, Diclofenac, Analgesic effect, Mouse, Abdominal constriction test.

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INTRODUCTION

Derris scandens (Roxb.) Benth, or Jewel Vine, known as Tao-Wan-Priang in Thai, is well-known the Asian medicinal plant. The stem of *D. scandens* has been used as active ingredient in the Thai traditional medicine recipes for pain treatment, for example, of osteoarthritis, joint diseases, musculoskeletal diseases, rheumatic diseases, and muscle tension [1]. 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 [2].

From the Thailand National List of Essential Medicines, the *D. scandens* extract is a drug developed from Thai medicinal plants. One formulated capsule contains 400 mg of the 50% ethanol extract from the stem of *D. scandens*. Its indications are relieving pain in lower back pain and knee osteoarthritis [3-5]. 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 nonsteroidal anti-inflammatory drugs (NSAIDs), are given to treat patients. However, the adverse effects of anti-inflammatory drugs are reported such as irritation and ulcers of the gastric and intestine system. The Thai Ministry of Public Health has the policy to support the research and development of herbal plants to be processed into high-quality goods and promote the use of the Thai herbs [6]. 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 [7]. Among the currently recommended analgesic products for arthritis-related pain, fixed-low-dose combination products hold promise for pain control because such products allow lower doses of individual agents resulting in decreased toxicity and acceptable efficacy due to the synergy between the

individual drugs. Better evidence and recommendations are required to improve treatment of chronic arthritis-related pain [8].

The *D. scandens* extract drug shows interesting analgesic and anti-inflammatory activities such as NSAIDs, and usage is increasing as replace or coadminister with another analgesic. Diclofenac is some of the most frequently used NSAIDs in clinical practice. There is no available information about the effect of the *D. scandens* extract drug on the analgesic interaction with diclofenac. In this study, we emphasized to study the analgesic interaction of the combination between diclofenac and the *D. scandens* extract drug in acetic acid-induced abdominal constriction test in mice and determine its type of analgesic interactions (synergism, additivity, or antagonism). The results from this study will provide the information of the combination between analgesic and alternative medicine. It is possible to suggest that the combinations of diclofenac and the *D. scandens* extract drug may be effective for the clinical treatment of pain.

METHODS

Drugs and chemical reagents

The commercial "GPO Thao-Wan-Priang Capsules," 50% ethanolic extract from the stem of *D. scandens* produced by the Government Pharmaceutical Organization of Thailand was used. Aspirin was obtained from Merck, AG, Darmstadt, Germany. Diclofenac was obtained from the Community Pharmacy Public Company Limited, Thailand. Analytical grades of sodium chloride and acetic acid (Sigma, St. Louis, USA) were purchased locally. All other chemicals and solvents were of the highest grade commercially available.

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 $22\pm 1^\circ\text{C}$, 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 and acclimatized for at least 1 week before use. 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. The experiment was conducted in accordance with the Care of Laboratory Animals and Ethical Guidelines of National Research Council of Thailand. The experiment protocol was submitted and approved for ethical considerations by the Rangsit University Animal Ethics Committee (ID RSEC03/2558).

Acetic acid-induced abdominal constriction (writhing) test in mice

This study was carried out using acetic acid-induced abdominal writhing reflex pain model [9,10]. Thirty-six mice were randomly divided into 6 groups (1-6, six animals per group, per treatment), fasted for 12 h, and treated as follows: Group 1 (negative control group) received 0.1 ml/10 g body weight (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 four doses of the *D. scandens* extract drug or diclofenac, respectively, using gastric gavage. Thirty minutes after the *D. scandens* extract drug 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 the back, development of tension in abdominal muscles, and elongation of the body in hind limb) for 30 min.

Data analysis

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

The percentage of inhibition = $(V_c - V_t) / V_c \times 100$, where:

V_c = Mean number of writhing in control animals

V_t = Mean number of writhing in test animals

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 performed using one-way analysis of variance (ANOVA), and the difference between the means was tested using *post hoc* least significant difference test. The value of $p < 0.05$ was considered statistically significant.

A dose-response curve was also obtained by the oral coadministration of diclofenac with the *D. scandens* extract drug in fixed ratio combinations of fractions of their respective ED_{50} values: 1/2, 1/4, 1/8, and 1/16 (ratio value given in Table 1). A dose-response curve and experimental ED_{50} for the combination of diclofenac and the *D. scandens* extract drug 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 subadditive or antagonistic interactions [11].

RESULTS

The result of the acetic acid-induced abdominal constriction test was summarized in Table 1. The *D. scandens* extract drug, diclofenac, and the combination produced a significantly dose-dependent antinociceptive effect in the chemical viscerosomatic assay of the acetic acid abdominal constriction test compared with the control group. The *D. scandens* extract drug produced 5.5, 32.5, 68.4, and 90.2% of inhibition at the dose of 1, 10, 100, and 1000 mg/kg bw, respectively, which was comparable to reference analgesic drug, aspirin (78.3% of inhibition at 50 mg/kg bw). Diclofenac produced 3.7, 36.3, 49.5, 62.7, and 80.6% of inhibition at the dose of 1, 10, 50, 100, and 150 mg/kg bw, respectively

($p < 0.05$, $n = 6$). The combination of diclofenac with the *D. scandens* extract drug produced 11.6, 34.7, 45.6, and 79.9% of inhibition at the dose of 8.2 (1/16), 16.5 (1/8), 32.9 (1/4), and 65.9 (1/2) mg/kg bw, respectively.

The log dose-response curves for the antinociceptive effect of the *D. scandens* extract drug, diclofenac, and the combination were obtained using at least six animals at each of at least four doses as shown in Fig. 1. A least-squares linear regression analysis of the log dose-response curves allowed the calculation of the dose that produced 50% of antinociception effective dose 50% (ED_{50}) which were 35.5, 30.4, and 29.2 mg/kg bw, respectively.

The antinociceptive activity of the oral coadministration of fixed ratio combinations of ED_{50} fractions of diclofenac with the *D. scandens* extract drug was assessed by calculating the ED_{50} of the mixtures from the corresponding dose-response curves. The additive 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 32.9 and 29.2 mg/kg bw, respectively. The interaction index of the combination was 0.89.

DISCUSSION

As the *D. scandens* extract drug shows interesting analgesic and anti-inflammatory activities such as NSAIDs and usage is increasing as replace or coadminister with another analgesic. In accordance with the medical uses of the *D. scandens* extract, the present study was carried out to evaluate the interaction of antinociceptive effect between diclofenac and the *D. scandens* extract drug. The major finding of the current study was the demonstration, for the first time to best of our knowledge, that the combination showed the additivity analgesic interaction in the chemical viscerosomatic assay of the acetic acid abdominal constriction test. This model is a pain-state model using chemical stimuli, which both central and peripheral analgesics are detected. A good relationship exists between the potencies of analgesics in writhing assays and their clinical potencies in this model. It has been used by many investigators and can be recommended as a simple screening method [12,13].

The combination tested showed an additivity interaction with interaction index at 0.89. The reduction of doses at 5.2 and 6.0% of diclofenac and the *D. scandens* extract drug in combination, respectively, compared with ED_{50} of the single drugs. However, in the previous study,

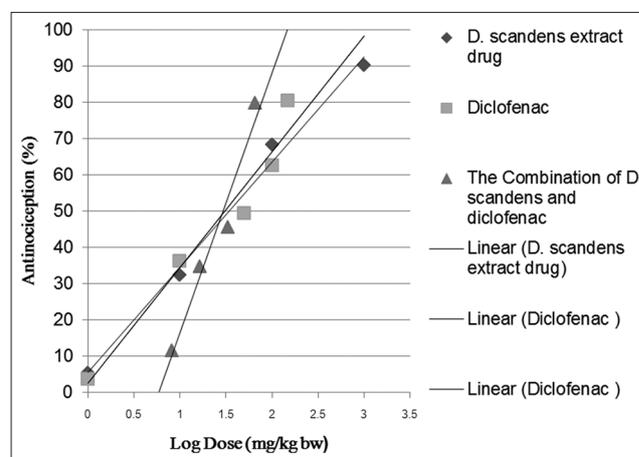


Fig. 1: Log dose-response curves for the antinociception induced by the oral administration of the *Derris scandens* extract drug, diclofenac, and the combination determined from acetic acid-induced abdominal constriction in mice. The antinociception is the percentage of inhibition which calculated from $(V_c - V_t) / V_c \times 100$, Where: V_c = Mean number of writhing in control animals, V_t = Mean number of writhing in test animals ($n = 6$ animals per group)

Table 1: The antinociceptive effect of the *D. scandens* extract drug, diclofenac, and the combination determined from acetic acid-induced abdominal constriction in mice

Treatment groups	Dose (mg/kg bw)	Number of writhes in 30 min	Inhibition (%)
Control (distilled water)	0.1 ml/kg bw	72.2±2.8	-
Aspirin	50	15.6±2.5 ^{a*}	78.3
<i>D. scandens</i> extract	1	68.0±2.0	5.5
	10	48.6±3.3 ^{a*,b*}	32.5
	100	38.6±2.2 ^{a*,b*,c*}	68.4
	1000	12.0±0.6 ^{a*,b*,c*,d*}	90.2
Diclofenac	1	69.3±2.2	3.7
	10	45.8±2.2 ^{a*,e*}	36.3
	50	36.3±2.6 ^{a*,e*,f**}	49.5
	100	26.8±2.6 ^{a*,e*,f*,g**}	62.7
	150	14.0±1.4 ^{a*,e*,f*,g*,h**}	80.6
Diclofenac/ <i>D. scandens</i> extract (1/1.16)	8.2	63.7±2.9	11.6
	16.5	47.0±3.2 ^{a*,i**}	34.7
	32.9	39.2±0.7 ^{a*,i*,j**}	45.6
	65.9	14.5±2.7 ^{a*,i*,j*,k*}	79.9

Thirty minutes 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. SEM: Standard error of mean (n=6 animals per each group). Analyzed by one-way ANOVA followed by Tukey's HSD test. *p<0.001, **p<0.05. ^aVersus control group, ^{b,c,d}versus *D. scandens* extract drug at the concentration of 1, 10, and 100 mg/kg bw, respectively, ^{e,f,g,h}versus diclofenac at the concentration of 1, 10, 50, and 100 mg/kg bw, respectively, ^{i,j,k}versus the combination at the concentration of 8.2, 16.5, and 32.9 mg/kg bw, respectively. *D. scandens*: *Derris scandens*, bw: Body weight, HSD: Honestly significantly different

Table 2: Theoretical and experimental ED₅₀ values and the interaction index for the combinations of diclofenac with the *D. scandens* extract drug on acetic acid-induced abdominal constriction in mice

Drugs	ED ₅₀ theoretical (mg/kg bw)	ED ₅₀ experimental (mg/kg bw)	Interaction index
<i>D. scandens</i> extract drug	-	35.5	-
Diclofenac	-	30.4	-
Diclofenac/ <i>D. scandens</i> extract drug (1/1.16)	32.9	29.2	29.2/32.9=0.89

D. scandens: *Derris scandens*, ED₅₀: Effective dose 50%

we found the synergistic analgesic interaction between the *D. scandens* extract drug and paracetamol with interaction index at 0.65 in the similar pain model [14].

The rationale underlying the practice of combining drugs in pain management is based on two considerations. First, combining drugs with different mechanisms may enhance the pain relief. Second, a single drug that provides satisfactory pain relief may cause at the same time, unacceptable side effects. Drug combination allows the reduction in the dose of the single component to achieve the same analgesic effect with reduced incidences of side-effects [15].

All of the NSAIDs appear to share at least one common mechanism, namely, inhibition of cyclooxygenase (COX) enzyme(s) which leads to a decrease in the synthesis of various prostaglandins and thromboxanes [16]. On the other hand, the mechanism of action of paracetamol includes inhibition of COX-3 in central nervous system, interaction with spinal 5-HT₃ receptors, and peripheral β-endorphin receptors [17,18]. Differences in the mechanism of action of paracetamol and NSAIDs make them a viable option for an effective combination.

Although the certain mechanism of action of the *D. scandens* extract drug is still unknown. Our study supports the hypothesis that the mechanism of analgesic action of *D. scandens* is likely to NSAIDs. The isoprenylated compounds, isolated from *D. scandens* stems, showed a high inhibitory effect on eicosanoid production for COX and 5-lipoxygenase [19]. Although the additivity analgesic interaction of the combination between the *D. scandens* extract drug and diclofenac could not significant reduce the dose of each drug alone, it may reduce incidences of side-effects. The major adverse event of *D. scandens* was gastrointestinal symptoms and showed no different relative risk with NSAIDs [20]. Diclofenac causes of high gastric or hepatic and renal toxicity [21].

The properties of a system that define a pharmacologic interaction between 2 drugs are likely complicated. The type of interaction between

two drugs may be explained by altering the kinetics of each other or at various levels of drug action. The additivity interaction between diclofenac and the *D. scandens* extract drug provides new information about combination pain treatment and should be explored further in patients, especially with somatic and/or visceral pain.

CONCLUSION

The data of the present study demonstrated that diclofenac combined with the *D. scandens* extract drug produces an additivity analgesic effect. It is possible to suggest that the combinations of diclofenac and the extract will be effective for the clinical treatment of pain. In addition, it is demonstrated that the effect of the combinations diclofenac/the *D. scandens* extract is superior to that of either component. 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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