The Additive Antinociceptive Interactions between Celecoxib and *Derris scandens* in the Mice Visceral Pain Model

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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 celecoxib and the extract of *D. scandens* in a mice model of visceral pain and determined the type of interaction between components. 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 celecoxib with the *D. scandens* extract. The ED_{50} was compared to the theoretical additive ED_{50} calculated from the ED_{50} of celecoxib and of the *D. scandens* extract drug alone. Celecoxib and the *D. scandens* extract drug dose-dependently and significantly reduced the abdominal writhing. The combination was the additive effect, the experimental ED_{50} being smaller than the theoretically calculated ED_{50}. Interaction index of the combination was 0.83. The present study demonstrates the additivity antinociceptive interactions between celecoxib and the *D. scandens* extract drug and may be used as a combination analgesic in the treatment of pain conditions.

Keywords: *Derris scandens, Celecoxib, Analgesic activity, Visceral pain model*

1. Introduction

From the Thailand National List of Essential Medicines (2018), *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 the lower back and knee osteoarthritis. The major adverse events of *D. scandens* are gastrointestinal symptoms, however it is more safer than NSAIDs (Kuptniratsaikul et al., 2011). Phytochemical investigation of the *D. scandens* stems afforded a number of secondary metabolites, providing benzil derivatives, coumarins, flavones, isoflavones, isoflavone glycosides, pterocarpans, steroids, and terpenes. Two isoflavones including genistein and lupalbigenin showed inhibition for cyclooxygenase, the enzyme involved in inflammatory reactions (Deachathai, 2016).

Celecoxib, one of the selective cyclooxygenase (COX)-2 inhibitors that offered the potential to retain efficacy while reducing gastrointestinal adverse effects compared with the non-selective COX inhibitors. Celecoxib may cause an increased risk of serious cardiovascular thrombotic events, myocardial infarction, and stroke, which can be fatal. Recently, it has been extensively used in the treatment of osteoarthritis and rheumatoid arthritis (Gong et al., 2012).

The number of older persons in Thailand has grown rapidly and will continue to do so in future decades. Future population ageing will occur even more rapidly with the number of older persons projected to increase to over 20 million by 2035, at which point they will constitute over 30% of the population (Chittinandana et al., 2017). 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 these 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 of Thai Traditional and Alternative Medicine (2017), and Thailand 4.0 Boosting Thai Economic with Herbal Products and Business, to support the research and development of herbal plants to be processed into high-quality goods and promote the use of Thai herbs. A distinct trend toward the integration of herbal medicine with the modern medicine is occurring.

The *D. scandens* extract drug shows interesting analgesic and anti-inflammatory activities such as NSAIDs, and usage is increasing as a replacement or co-administer with another analgesic. The

combination of analgesics of proven efficacy is a strategy intended to achieve one or more therapeutic goals (Raffa, 2001). In certain cases, the co-administration of antinociceptive agents result in synergistic effects and the doses of the individual drugs are substantially reduced (Miranda & Pinardi, 2004). The combined treatment, with the *D. scandens* extract and paracetamol, produced synergistic antinociceptive effects in the mice acute pain model by acetic acid-induced abdominal constriction (writhing test) method was reported (Punjanon, Yingyoung, & Untharin, 2017). This model is a pain-state model using chemical stimuli, which both central and peripheral analgesics are detected. It has been used by many investigators and can be recommended as a simple screening method (Collier et al., 1968; Milind & Monu, 2013). Because celecoxib and *D. scandens* were both effective in the treatment of osteoarthritis and rheumatoid arthritis and they have different major side effects. This study was therefore undertaken to investigate the analgesic activities of the combination between celecoxib and the *D. scandens* extract in the mice visceral pain model and determined their type of pharmacologic interaction (synergism, additivity, or antagonism).

2. Objectives

The objective of the study was to determine the nature of the analgesic interaction between celecoxib and the *D. scandens* extract 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. Celecoxib was obtained from Pfizer (Thailand) Limited. Analytical grade 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 National Laboratory Animal Center, Mahidol University, Thailand. All mice were housed in the Faculty of Science, Rangsit University, Thailand, under standard environmental conditions of 23±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 (ID RSEC03/2558) 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 (Koster, Anderson, and DeBeer, 1959; Jain & Kulkarni, 1999). 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 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 celecoxib, 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±standard deviation. The degree of antinociception was calculated as the percentage of inhibition of writhing using the rational formula.

The percentage of inhibition=(Vc-Vt)/Vc* 100, where:

VC=Mean number of writhing in control animals, Vt=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 celecoxib 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 celecoxib 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 and Discussion

The results of the analgesic effect of the *D. scandens* extract, celecoxib, and the combination by acetic acid induced writing reflex method is presented in Table 1. The results showed that the extract at the doses used just like the reference drug aspirin significantly (78.3% inhibition at 50 mg/kg BW) ($p<0.05$, n=6) reduced the mean number of abdominal constrictions or writhing in a dose dependent manner when compared to the negative control group. The percentage inhibition of writhing was also dose dependently increased from zero in the negative control group (distilled water) to 89.18, 96.10, and 75.11 % in the group that received 1, 000 mg/kg BW of the *D. scandens* extract, 300 mg/kg BW of celecoxib, and 42.42 mg/kg BW of the combination, respectively.

Table 1 The effect of the *D. scandens* extract, celecoxib, 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	19.8 ± 4.36*	74.24
D. scandens extract	1	66.3 ± 2.66	13.85
	10	43.3 ± 2.07*	43.72
	100	23.0 ± 9.96*	70.13
	1,000	8.3 ± 1.51*	89.18
Celecoxib	5	53.7 ± 9.52*	30.30
	25	38.7 ± 3.78 *	49.78
	100	22.5 ± 3.73*	70.78
	300	3.0 ± 2.28*	96.10
D. scandens extract/ Celecoxib	5.30	65.3 ± 15.50*	15.15
	10.61	46.7 ± 1.97*	39.39
	21.21	33.3 ± 9.61*	56.71
	42.42	19.17 ± 13.08*	75.11

-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±SD (n = No. of writhes in 30 mins of 6 mice in each group); *$p<0.05$ was significantly different from control group.

Log dose–response curves for the antinociceptive effect of the 50% ethanolic extract of *D. scandens,* celecoxib, 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 = 0.98-0.99) of the log dose–response curves allowed the calculation of the dose that produced 50% of antinociception (ED_{50}) which were 21.66, 20.76, and 16.91 mg/kg BW, respectively.

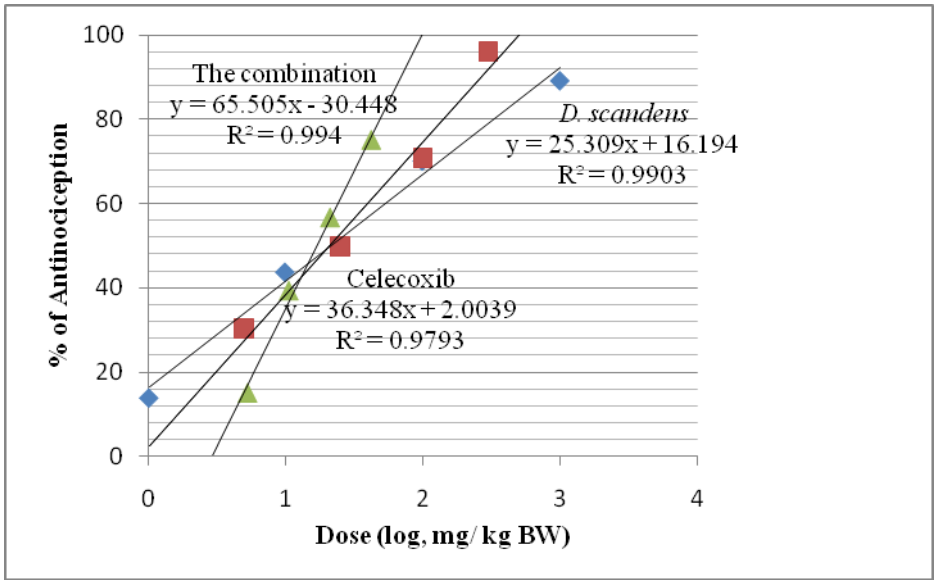

Figure 1 Dose–response curves for the antinociception induced by the oral administration of D. *scandens* extract (◆.) celecoxib (■), and the combination (▲). Each point is the mean ±SD of 6 animals.

The antinociceptive activity of the oral coadministration of fixed ratio combinations of ED_{50} fractions of celecoxib with the *D. scandens* extract was assessed by calculating the ED_{50} of the mixtures from the corresponding dose–response curves. The additivity 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 21.21 and 16.91 mg/kg BW, respectively. The interaction index of the combination was 0.83.

Table 2 Theoretical and experimental ED_{50} values and interaction index for combinations of celecoxib 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	-	21.66	-
Celecoxib	-	20.76	-
Celecoxib/ *D. scandens* extract	21.21	16.91	16.91/21.21 = 0.83

Acetic acid-induced abdominal constriction test (writhing test) is a Pain-state model using chemical stimuli, which both central and peripheral analgesics are detected. Acetic acid induced writhing reflex is a sensitive method for screening peripherally acting analgesics and the response in thought to involve local peritoneal cells and mediated through the prostaglandin pathway (Collier et al., 1968). A good relationship exists between the potencies of analgesics in writhing assays and their clinical potencies in this

model (Milind & Monu, 2013). The result showed that the *D. scandens* extract reduced the mean number of writhing in a dose dependent manner. This suggests that the analgesic effect of this extract seen in this study may be mediated through peripheral pain mechanism and or may be through inhibition of the activities or synthesis of prostaglandins. This finding agrees to the previous report that two isoflavones isolated from *D. scandens* including genistein and lupalbigenin showed inhibition for cyclooxygenase (Deachathai, 2016).

Results from this study showed the oral co-administration of celecoxib 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 showed the additive interaction with interaction index at 0.83. Celecoxib and the *D. scandens* extract exert the similar analgesic action by inhibiting prostaglandin synthesis catalyzed by COX-2 isozymes. Because the gastrointestinal symptoms are the similar major side effects of *D. scandens* and NSAIDs, so the concomitant use of them should be avoided (Puttarak, Sawangjit, & Chaiyakunapruk, 2016). Celecoxib, a specific COX-2 inhibitor, has been linked with an increased risk of cardio-renal effects and minimized gastrointestinal symptoms (Gong et al., 2012). Because of the oral fixed drug combination analgesics have potential advantages over monotherapy, so the main reasons for developing combination analgesics are to gain efficacy and to reduce toxicity (Raffa, 2001). The components of the celecoxib and the *D. scandens* extract displayed additive analgesia with their different major toxic effects. This interaction could allow lower doses of each substance to be used in combination, resulting in an improved safety profile. 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 additive interaction between celecoxib 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. However, there are some limitations of the application in humans, such as the different mechanism of host response to the drug, and the different dose of drug to use between mice and human due to the higher dose of drug use and the adverse reactions in humans.

5. Conclusion

In conclusion, the data of the present study demonstrated that celecoxib combined with the *D. scandens* extract produces an additive analgesic effect. It is possible to suggest that the combinations of celecoxib and the extract will be effective for the clinical treatment of pain. In addition, it is demonstrated that the effect of the combinations celecoxib/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.

6. Acknowledgements

The author would like to acknowledge the Research Institute and the Faculty of Science, Rangsit University, Thailand, for providing grants and facilities to carry out this study.

7. References

Chittinandana, D., Kulnartsiri, N., Pinthong, J., & Sawaengsuksant, P. (2017). Aging population: Global perspectives. Bank of Thailand. Retrieved Feb 1, 2019, from https://www.bot.or.th/Thai/MonetaryPolicy/EconomicConditions/AAA/2AgingPopulation_GlobalPerspectives.pdf

Collier, H. O. J., Dinneen, L. C., Johnson, C. A., & Schneider, C. (1968). The abdominal constriction response and its suppression by analgesic drugs in the mouse. *British journal of pharmacology and chemotherapy*, *32*. 295-310.

Deachathai, S. (2016). Chemical constituents and biological activities of *Derris scandens* Benth. *KKU Science Journal, 44*(3), 435-457.

Gong, L., Thorn, C. F., Grosser, M. M., Altman, R. B., & Klein, T. E. (2012). Celecoxib pathways: pharmacokinetics and pharmacodynamics. *Pharmacogenetics and genomics, 22*(4), 310–318. doi: 10.1097/FPC.0b013e32834f94cb

Jain, N. K., & Kulkarni, S. K. (1999). Antinociceptive effects of *Tanacetum parthenium* L. extract in mice and rats. *Journal of ethnopharmacology*, *68(1-3)*, 251-259.

Koster, P., Anderson, M., & DeBeer, E. J. (1959). Acetic acid analgesic screening. *Federation Proceedings, 18*, 412-418.

Kuptniratsaikul, V., Pinthong, T., Bunjob,M., Thanakhumtorn, S., Chinswangwatanakul, P., & Thamlikitkul, V. (2011). Efficacy and safety of *Derris scandens* Benth extracts in patients with knee osteoarthritis. *The Journal of Alternative and Complementary Medicine, 17*(2), 147-153.

Milind, P., & Monu, Y. (2013). Laboratory models for screening analgesics. *International Research Journal of Pharmacy, 4*(1), 16-19.

Miranda, H. F., & Pinardi, G. (2004). Isobolographic analysis of the antinociceptive interactions of clonidine with nonsteroidal anti-inflammatory drugs. *Pharmacological research, 50*, 273–278.

Punjanon, T., Yingyoung, W., & Untharin, N. (2017). Analgesic synergy between paracetamol and *Derris scandens* in mice. *Proceeding of RSU International Research Conference 2017*, 20-24.

Puttarak, P., Sawangjit, R., & Chaiyakunapruk, N. (2016). Efficacy and safety of *Derris scandens* (Roxb.) Benth. for musculoskeletal pain treatment: A systematic review and meta-analysis of randomized controlled trials. *Journal of ethnopharmacology, 194,* 316-323.

Raffa, R. B. (2001). Pharmacology of oral combination analgesics: rational therapy for pain. *Journal of clinical pharmacy and therapeutics*, *26*, 257–264.

Thai Traditional and Alternative Medicine. (2017). Thailand 4.0 Boosting Thai economic with herbal products and business. Retrieved Feb 1, 2019, from http://bps.moph.go.th/new_bps/sites/default/files/1.%20thai%20traditional%20medicine_210260.pdf

Thailand National List of Essential Medicines 2018. Retrieved Feb 1, 2019, from http://drug.fda.moph.go.th:81/nlem.in.th/