ANALGESIC EFFECTS OF ICH PHU DAN CAPSULES IN EXPERIMENTAL ANIMALS

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Ich phu dan capsules are intended to treat menstrual pain. The study aimed to assess the analgesic effects of Ich phu dan capsules in animal models. Three animal models were used: a hot plate, tail flick and acetic acid-induced writhing test. Mice were divided into four groups and were given orally: group 1 distilled water; group 2 control drug (codeine phosphate in hot plate and tail flick tests or aspirin in writhing test); groups 3 and 4: Ich phu dan at the doses of 1.44 capsules (approx 521.1 mg dried extract) or 2.88 capsules (approx 1042.2 mg dried extract)/kg b.w/day, respectively. The results showed that Ich phu dan capsules at both doses increased the mice's reaction time in hot plate and tail flick models and decreased the number of acetic acid-induced writhing movements in mice. There was no statistically significant difference between the two doses of Ich phu dan capsules. In conclusions, Ich phu dan capsules at the doses of 1.44 capsules (approx 521.1 mg dried extract) and 2.88 capsules (approx 1042.2 mg dried extract)/kg b.w/day showed significant analgesic effect in animal models.

Keywords: Ich phu dan capsules, analgesic, mice, hot plate, tail flick, writhing test.

I. INTRODUCTION

Vietnam is located in a tropical climate, with a very rich source of medicinal plants. People have a lot of experience in using traditional plants for health care. The study of traditional plants in medical treatment is encouraged because this is the right direction towardincreasing the supply of good medicine to the community in terms of effectiveness, safety, cost and availability.

Ich phu dan is a traditional remedy used to treat menstrual pain for decades. According to traditional medicine, the remedy has the effect of tonic blood, activating the blood and regulating the menstrual cycle. To provide a more convenient treatment for patients, we prepared the remedy in a hard capsule form.

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Ich phu dan capsule was a herbal mixture of *Leonurus cardiaca, Cyperus rotundus L., Artemisia vulgaris, Angelica sinensis, Corydalis yanhusuo, Paeonia lactiflora, Astragalus membranaceus, Ligusticum striatum, Glycyrrhiza uralensis*. To have more scientific basis to prove the effect of Ich phu dan, we conducted this study to assess the analgesic effects of Ich phu dan capsules in animal models.

II. SUBJECTS AND METHODS

1. The preparation of Ich phu dan capsules

Ich phu dan capsules were prepared by Bach Thao Duoc Company Ltd.

Each capsule contains 361.9 mg of dried extract from the mixture of ingredients (9.09:1), including:

+ *Leonurus cardiaca* 800 mg
+ *Cyperus rotundus* L. 600 mg
+ *Artemisia vulgaris* 500 mg

+ *Angelica sinensis* 500 mg
+ *Corydalis ambigua* 400 mg
+ *Paeonia lactiflora* 200 mg
+ *Astragalus membranaceus* 140 mg
+ *Ligusticum striatum* 100 mg
+ *Glycyrrhiza uralensis* 50 mg

Right before administering to mice, the gelatin capsule shells were removed. The capsule contents were mixed well with distilled water. Then the mixture were given orally by an oral gavage feeding needle for mice.

2. Experimental animals

Healthy white adult *Swiss* mice of both genders, weighed 18 - 22 g provided by the National Institute of Hygiene and Epidemiology. The animals were housed in cages and given a standard rodent diet and water *ad libitum*. They were acclimated seven days before investigation at the laboratory of the Department of Pharmacology, Hanoi Medical University.

3. Methods

Hot plate test

Mice were divided into four groups: Group 1 was given orally distilled water at the volume of 0.2 mL/10 g b.w/day, Group 2 was given codeine phosphate orally at the dose of 20 mg/kg b.w/day, Group 3 and group 4 were given Ich phu dan capsules orally at the dose of 1.44 capsules (approx. 521.1 mg)/kg b.w/day and 2.88 capsules (approx. 1042.2 mg)/kg b.w/day, respectively . Mice were treated once a day for five days.

The hot plate (Hot plate model DS37, Ugo Basile, Italy) was maintained at 56^{o}C. The mice were placed in the beaker (on the hot plate) to observe their response to electrical heat-induced pain. One hour after the last dose, the reaction time (in seconds) was recorded when the mice licked their hind paws or jumped as an indicator of the response to heat-induced pain.[1,2]

Tail flick test

Mice were divided into four groups and treated once a day for five days as mentioned above. The Tail flick model 37360 (Ugo-Basile, Italy) was used. An intense light beam was focused on the mouse's tail and a timer started. When the mouse flicked its tail, the timer stopped and the recorded time (latency) measured the pain threshold. One hour after the last dose, tail flick latency was recorded.[3]

Acetic acid-induced writhing test

Mice were divided into four groups: Group 1 was given orally distilled water at the volume of 0.2 mL/10 g b.w/day, Group 2 was given aspirin orally at the dose of 150 mg/kg b.w/day, Group 3 and group 4 were given Ich phu dan capsules orally at the dose of 1.44 capsules (approx. 521.1 mg)/kg b.w/day and 2.88 capsules (approx. 1042.2 mg)/kg b.w/day, respectively. Mice were treated once a day for five days.

One hour after the last dose, abdominal constriction was induced in mice by intraperitoneal injection of 0.2 mL 1% acetic acid.[1,2] The number of abdominal constrictions was cumulatively counted over a period of 5 minutes within 30 minutes.

4. Statistical analysis

Data were analyzed by the T-test using Microsoft Excel software version 2010. Data were presented as a mean ± standard deviation. A p-value of less than 0.05 is statically significant.

III. RESULTS

1. Hot plate test

The reaction time to thermal stimulation on the hot plate of the mice treated with codeine phosphate and Ich phu dan capsules increased significantly compared with untreated mice in group 1. There was no statistically significant difference between codeine-treated mice and Ich phu dan-treated mice and between 2 doses of Ich phu dan capsules (Table 1).

Table 1. Effects of Ich phu dan capsules on reaction time to thermal stimulation in hot plate test

Group	n	Reaction time ($\overline{X}$ ± SD) (s)
Control	10	12.45 ± 3.97
Codeine phosphate 20 mg/kg b.w/day	10	19.05 ± 5.66**
Ich phu dan 1.44 capsules (approx 521.1 mg dried extract)	10	21.14 ± 3.59***
Ich phu dan 2.88 capsules (approx 1042.2 mg dried extract)	**10**	18.78 ± 4.64**

*** p < 0.01: compared with control*

**** p < 0.001: compared with control*

2. Tail flick test

The tail-flick latency of the mice treated with codeine phosphate and Ich phu dan capsules increased significantly compared with untreated mice in group 1. There was no statistically significant difference between codeine-treated mice and Ich phu dan-treated mice and between 2 doses of Ich phu dan capsules (Table 2).

Table 2. Effects of Ich phu dan capsules on tail flick latency in tail flick test

Group	**n**	**Tail flick latency ($\overline{X}$ ± SD) (s)**
Control	10	8.26 ± 3.02
Codeine phosphate 20 mg/kg b.w/day	10	11.36 ± 1.30**
Ich phu dan 1.44 capsules (approx 521.1 mg dried extract)	10	10.61 ± 1.11*
Ich phu dan 2.88 capsules (approx 1042.2 mg dried extract)	**10**	11.08 ± 2.62*

** p < 0.05: compared with control,*

*** p < 0.01: compared with control*

3. Acetic acid-induced writhing test

The number of acetic acid-induced writhing movements every 5 minutes from 5 minutes to 30 minutes after acetic acid injection of the mice treated with Ich phu dan capsules decreased significantly compared with untreated mice in group 1. There was no statistically significant difference between the two doses of Ich phu dan capsules. There was no statistically significant difference between aspirin-treated mice and Phong thap dan-treated mice (except in the first 5 minutes) (Table 3).

Table 3. Effects of Ich phu dan capsules on the number acetic acid–induced writhing response

Group	n	Number of writhing movements					
		0 - 5 minute	> 5 - 10 minute	> 10 - 15 minute	> 15 - 20 minute	> 20 - 25 minute	> 25 - 30 minute
Control	10	6.90 ± 2.13	21.00 ± 5.83	16.30 ± 4.27	13.90 ± 4.36	11.,90 ± 3.73	7.70 ± 2.95
Aspirin 150 mg/kg b.w/day	10	2.90 ± 2.13**	14.00 ± 5.64*	11.40 ± 4.55*	9.70 ± 2.45*	8.00 ± 1.25**	5.40 ± 1.07*
Ich phu dan 1.44 capsules (approx 521.1 mg dried extract)	10	5.90 ± 2.02#	12.80 ± 4.42**	10.80 ± 3.97**	8.20 ± 3.12**	6.40 ± 3.03**	4.00 ± 2.26**
Ich phu dan 2.88 capsules (approx 1042.2 mg dried extract)	10	6.20 ± 3.43#	13.90 ± 4.33**	10.70 ± 4.69*	8.40 ± 3.60**	6.50 ± 2.84**	4.40 ± 2.12*

** p < 0.05: compared with control*

*** p < 0.01: compared with control*

 # p < 0.05: compared with aspirin-treated mice

IV. DISCUSSION

In this study, the analgesic activity of Ich phu dan capsules was assessed in different well-accepted animal models, including a hot plate test, tail-flick test and acetic acid-induced writhing test. Three models in this study permitted the investigation of peripheral and central analgesic acitvities.[1,2,3]

The hot-plate test and tail-flick test have been used to evaluate the centrally mediated analgesic activity, which focuses mainly on changes above the spinal cord level. A centrally acting analgesic, codeine, used in these two models increased the reaction time of mice. Ich phu dan capsules at the doses 1.44 capsules (approx 521.1 mg dried extract) or 2.88 capsules (approx 1042.2 mg dried extract)/kg b.w/day) showed significant analgesic effects in hot-plate and tail-flick tests. This indicated the central analgesic effect of the Ich phu dan capsules. Mechanism studies needed to be carried out to demonstrate whether the capsules acted via spinal cord level or higher centers of central nervous system.

The writhing test is a chemical induced pain indicating peripheral mechanisms. Acetic acid was injected to mice to activate the prostaglandin synthesis which induce abdominal constrictions. A non-steroidal anti-inflammatory drug, aspirin, used in this model reduced the level of prostaglandin and reduced the sensitivity of mice to pain inducing agents. Ich phu dan capsules at the doses

1.44 capsules (approx 521.1 mg dried extract) or 2.88 capsules (approx 1042.2 mg dried extract)/kg b.w/day) reduced the number of acetic acid-induced abdominal constrictions in mice. This indicated the peripheral analgesic effect of the Ich phu dan capsules. This effect may work via reducing the level of prostaglandin synthesis or other inflammatory mediators.

These results indicated that Ich phu dan capsules might possess centrally and peripherally mediated analgesic acitivity.

Ich phu dan capsules were prepared from a herbal mixture of *Leonurus cardiaca, Cyperus rotundus L., Artemisia vulgaris, Angelica sinensis, Corydalis yanhusuo, Paeonia lactiflora, Astragalus membranaceus, Ligusticum striatum, Glycyrrhiza uralensis*. We haven't found any study investigating the analgesic activity of the mixture of these herbal medicines. However, some ingredients of Ich phu dan capsules were investigated in other studies and were demonstrated to have analgesic properties in animal models separately.

The alcoholic extract of aerial part of *Leonurus cardiaca* was investigated the nociceptive response using tail flick and hot plate tests in mice. The results showed an increase in the antinociceptive effect atdose of 500 mg/kg in both that hot plate and tail flick tests.[4]

The methanolic extract of *Leonurus sibiricus aerial* parts was injected intraperitoneally to mice at doses of 250 and 500 mg/kg. The study showed a significant analgesic effect in acetic acid-induced writhing in mice.[5]

Researchers also evaluated the antinociceptive activity of the hydromethanol extract of *Cyperus rotundus* in mice. The extract significantly increased the latency period to the thermal stimuli at all the tested doses (50, 100 and 200 mg/kg) in the hot-plate tests.[6]

The analgesic activity of Aerial Parts of *Artemisia vulgaris* L. was evaluated in experimental animal models. The analgesic activity was tested by tail-flick and hot plate methods in albino rats and mice. In the hot plate and tail flick model, the methanolic extract of *Ar. vulgaris* increased the pain threshold significantly.[7]

Some researches also evaluated the antinociceptive properties of *Corydalis yanhusuo* extract (YHS) by testing it in standardized pain assays and investigating its mechanism. The results showed that YHS increased the tail-flick latency in the tail-flick assay without resulting in the development of tolerance.[8]

The *in vivo* analgesic pharmacological effects of white peony root and mucuna pruriens and their combinations were investigated in acetic acid-induced writhing test and hot plate test. It was found that both *Paeonia lactiflora* root and Kapok had analgesic effects.[9]

Our research results are consistent to those of other authors when evaluating the analgesic effects of each medicinal ingredient contained in Ich phu dan capsules. This shows that the combination of these medicinal herbs in the capsules does not cause interactions that make them ineffective.

In conclusion, the present findings indicate the analgesic effect of Ich phu dan capsules and suggest that this effect is mediated by both peripheral and central mechanisms. However, the specific mechanisms of Ich phu dan capsules were not clarified in this study and further research is needed.

When evaluating a product, we not only investigate its effect but also need to consider its safety. In another study, we evaluated the toxicity of Ich phu dan capsules for 30 days in rats. The results showed that Ich phu dan capsules did not influence the weight, general

conditions, hematological and biochemical tests in rats.

These results support the use of Ich phu dan capsules in different painful conditions, including menstrual pain.

V. CONCLUSION

Ich phu dan capsules at the doses 1.44 capsules (approx 521.1 mg dried extract) or 2.88 capsules (approx 1042.2 mg dried extract)/ kg b.w/day) showed significant analgesic effect in animal models: increased the reaction time to thermal stimulation in hot plate test; increased tail flick latency in tail-flick test and decreased the number of writhing movements in acetic acid-induced writhing test in mice.

REFERENCES

1. Deuis JR, Dvorakova LS, Vetter I. Methods Used to Evaluate Pain Behaviors in Rodents. *Front Mol Neurosci*. 2017; 10:284.

2. Qnais E, Bseiso Y, Wedyan M, Alkhateeb H. Evaluation of Analgesic Activity of the Methanol Extract from the Leaves of Arum Palaestinum in Mice and Rats. *Biomed Pharmacol J*. 2017; 10(3).

3. Foroud M, Vesal N. Evaluation of the anti-nociceptive effects of morphine, tramadol, meloxicam and their combinations using the tail-flick test in rats. *Vet Res Forum* 2015 6(4): 313-8.

4. Rezaee-Asl M, Sabour M, Nikoui V, Ostadhadi S, Bakhtiarian A. The Study of Analgesic Effects of *Leonurus cardiaca* L. in Mice by Formalin, Tail Flick and Hot Plate Tests. Int Sch Res Notices. 2014 Sep 1;2014;687-697.

5. Islam MA, Ahmed F, Das AK, Bachar SC. Analgesic and anti-inflammatory activity of *Leonurus sibiricus*. Fitoterapia. 2005 Jun; 76 (3-4); 359-62.

6. Imam MZ, Sumi CD. Evaluation of antinociceptive activity of hydromethanol extract of Cyperus rotundus in mice. *BMC Complement Altern Med*. 2014 Mar 4; 14:83. doi: 10.1186/1472-6882-14-83. PMID: 24589067; PMCID: PMC3946594.

7. Kumar Ashok, Praveen & Upadhyaya, Kumud. (2013). Evaluation of Analgesic and Anti-inflammatory Activities of Aerial Parts of Artemisia vulgaris L. in Experimental Animal Models. *Journal of Biologically Active Products from Nature*. 3. 101-105. 10. 1080/22311866.2013.782761.

8. Wang, Lien & Zhang, Yan & Gong, Nian & Kweon, Tae Dong & Vo, Benjamin & Wang, Chaoran & Chung, Jae & Alachkar, Amal & Liang, Xinmiao & Luo, David & Civelli, Olivier. (2016). The Antinociceptive Properties of the Corydalis yanhusuo Extract. *PloS one*. 11. e0162875. 10.1371/journal.pone.0162875.

9. Xue-Ying Yu , Shi-Bo Zhao , Hui-Man Yang et al. Study on the anti-inflammatory and analgesic effects of white peony root and mucuna pruriens and their combinations in vivo. *Drug Combination Therapy* 2022; 4(2):5.