ANALGESIC ACTIVITY OF PHONG THAP DAN TABLETS IN ANIMAL MODELS

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Phong thap dan tablets are intended to treat low back pain. This study was carried out to evaluate the analgesic effects of Phong thap dan tablets in experimental animals. The analgesic effects were evaluated in three animal models: hot plate, mechanical stimulation and acetic acid-induced writhing test. Mice were divided into 4 groups given oral water, control drug (codein phosphate in hot plate and mechanical stimulation tests or aspirin in writhing test), Phong thap dan at 2.88 tablets (1.44 g) or 8.64 tablets (4.32 g)/kg b.w/day, respectively. Our results showed that Phong thap dan tablets at both doses increased the reaction time to thermal stimulation, increased the paw withdrawal latency and the force required to elicit a paw withdrawal and decreased the number of acetic acid-induced writhing movements in mice. There was no statistically significant difference between 2 doses of Phong thap dan tablets in three animal models. We conclude that Phong thap dan tablets at the doses of 2.88 tablets and 8.64 tablets/kg b.w/day showed significant analgesic effect in animal models.

Keywords: Phong thap dan tablets, analgesic, mice, hot plate, mechanical stimulation, writhing test.

I. INTRODUCTION

Low back pain is the leading cause of disability worldwide both in developed and developing countries.[1] It is usually defined as pain, muscle tension, or stiffness localised below the costal margin and above the inferior gluteal folds, with or without leg pain (sciatica).[2] There are several classes of drugs used to treat low back pain, including paracetamol, non-steroidal anti-inflammatory drugs, muscle relaxants, and opioid. Although these drugs are effective in relieving pain, they still come with side effects, especially when taken for a long time.

There has always been a tendency to search for natural analgesic constituents from herbal medicine which is considered to be safer and cheaper alternative. Phong thap dan remedy has been used to treat low back pain in Traditional Medicine Department, Hanoi Medical University Hospital since 2016 and showed efficacy and safety in low back pain treatment. To improve the patient's convenience, Phong thap dan remedy were prepared in tablets form. Phong thap dan tablets include *Rhizoma seu Radix Notopterygii, Radix Angeliace pubescentis, Cinnamomum loureirii Nees, Radix Gentianae macrophyllae, Radix Angenicae sinensis, Rhizoma Ligustici wallichii, Radix Glycyrrhixae, Piper futokadsura Sieb et Zucc, Ramulus Mori Albae, Gummi resina Olibanum, Myrrha, Radix Archiranthis bidentae, Herba Siegesbeckiae, Semen Strychni, Vitex negundo L., Rhizoma Atractylodis* and *Cotex Eucommiae.* To supply non-clinical evidence for future clinical trials, this study was carried out to evaluate the analgesic activity of Phong thap dan tablets in animal models.

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II. METHODS

1. The preparation of Phong thap dan tablets

Phong thap dan 500 mg tablets (prepared by Department of Pharmacy, National Hospital of Traditional Medicine and Traditional Medicine Department, Hanoi Medical University Hospital).

Ingredients	Raw material strength (per 1 tablet)
- *Rhizoma seu Radix Notopterygii*	266.67 mg
- *Radix Angeliace pubescentis*	266.67 mg
- *Cinnamomum loureirii Nees*	133.33 mg
- *Radix Gentianae macrophyllae*	266.67 mg
- *Radix Angenicae sinensis*	533.33 mg
- *Rhizoma Ligustici wallichii*	222.22 mg
- *Radix Glycyrrhixae*	111.11 mg
- *Piper futokadsura Sieb et Zucc*	533.33 mg
- *Ramulus Mori Albae*	800 mg
- *Gummi resina Olibanum*	222.22 mg
- *Myrrha*	222.22 mg
- *Radix Archiranthis bidentae*	333.33 mg
- *Herba Siegesbeckiae*	666.67 mg
- *Semen Strychni*	25 mg
- *Vitex negundo L.*	533.33 mg
- *Rhizoma Atractylodis*	333.33 mg
- *Cotex Eucommiae*	333.33 mg

2. Experimental animals

Healthy white adult *Swiss* male and female mice, weighed 18 - 22g provided by National Institute of Hygiene and Epidemiology were used in this study. The animals were housed in cages with access to a standard certified rodent diet and water *ad libitum*. They were acclimated to housing one week before investigation at the laboratory of the Department of Pharmacology, Hanoi Medical University.

3. Methods

Hot plate test

Mice were divided into 4 groups: Group 1 was given orally distilled water at the volume of 0.2 mL/10 g b.w/day, Group 2 was given orally codeine phosphate at the dose of 20 mg/kg b.w/day, Group 3 was given orally Phong thap dan tablets at the dose of 2.88 tablets (1.44 g)/kg b.w/day. Group 4 was given orally Phong thap dan tablets at the dose of 8.64 tablets (4.32 g)/kg b.w/day. Mice were treated once a day for 5 days.

The hot plate (Hot plate model DS37, Ugo Basile, Italy) was maintained at 56°C. The mice of each group were placed in the beaker (on the hot plate) to observe their response to electrical heat induced pain. 1 hour after the last dose, the reaction time (in seconds) was recorded when the animals licked their hind paws or jumped as an indicator of the response to heat-induced pain.[3,4]

Mechanical stimulation test

Similarly to hot plate test, mice were divided into 4 groups and treated once a day for 5 days.

Mice were divided into 4 groups: Group 1 was given orally distilled water at the volume of 0.2 mL/10 g b.w/day, Group 2 was given orally aspirin at the dose of 150 mg/kg b.w/day, Group 3 was given orally Phong thap dan tablets at the dose of 2.88 tablets (1.44 g)/kg b.w/day. Group 4 was given orally Phong thap dan tablets at the dose of 8.64 tablets (4.32 g)/kg b.w/day.

The mechanical stimultion test was carried out, using The Dynamic Plantar Aesthesiometer (Ugo Baslle, Italy). Mice were housed in an enclosure with a mesh screen floor, under which a movable touch-stimulator unit was placed. Under the direction of the researcher, the apparatus applied a von Frey (0.5 mm) filament to the plantar surface, increasing the force incrementally until the paw withdrawal threshold was reached.[5,6] 1 hour after the last dose, the paw withdrawal latency and the force required to elicit a paw withdrawal reflex was recorded automatically.

Acetic acid-induced writhing test

Mice were divided into 4 groups: Group 1 was given orally distilled water at the volume of 0.2 mL/10 g b.w/day, Group 2 was given orally aspirin at the dose of 150 mg/kg b.w/day, Group 3 was given orally Phong thap dan tablets at the dose of 2.88 tablets (1.44 g)/kg b.w/day. Group 4 was given orally Phong thap dan tablets at the dose of 8.64 tablets (4.32 g)/kg b.w/day. Mice were treated once a day for 5 days.

1 hour after the last dose, the abdominal constriction was induced in mice by intraperitoneal injection of 0.2 mL 1% acetic acid.[7] 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

As shown in table 1, the reaction time to thermal stimulation on hot plate of the mice treated with codeine phosphate and Phong thap dan tablets increased significantly compared with untreated mice in group 1. There was no statistically significant difference between codeine-treated mice and Phong thap dan-treated mice and between 2 doses of Phong thap dan tablets.

Table 1. Effects of Phong thap dan tablets on reaction time to thermal stimulation

Group	n	Reaction time ($\bar{X}$ ± SD) (s)
Control	10	12.89 ± 3.39
Codeine phosphate 20 mg/kg b.w/day	10	21.06 ± 7.32**
Phong thap dan 2.88 tablets/kg b.w/day (1.44 g/kg b.w/day)	10	26.50 ± 12.43**
Phong thap dan 8.64 tablets/kg b.w/day (4.32 g/kg b.w/day)	10	23.08 ± 9.77**

***p < 0.01: compared with control*

2. Mechanical stimulation test

As shown in table 2, the paw withdrawal latency and the force required to elicit a paw withdrawal of the mice treated with codeine phosphate and Phong thap dan tablets increased significantly compared with untreated mice in group 1. There was no statistically significant difference between codeine-treated mice and Phong thap dan-treated mice and between 2 doses of Phong thap dan tablets.

Table 2. Effects of Phong thap dan tablets on paw withdrawal latency and the force required to elicit a paw withdrawal

Group	n	Force (gram)	Paw withdrawal latency (s)
Control	10	6.70 ± 1.15	3.78 ± 0.69
Codeine phosphate 20 mg/kg b.w/day	10	8.96 ± 1.33***	5.16 ± 0.81***
Phong thap dan 2.88 tablets/kg b.w/day (1.44 g/kg b.w/day)	10	9.24 ± 3.34*	5.40 ± 2.02*
Phong thap dan 8.64 tablets/kg b.w/day (4.32 g/kg b.w/day)	10	9.26 ± 1.65***	5.65 ± 1.53**

**p < 0.05: compared with control,*
***p < 0.01: compared with control*
****p < 0.001: compared with control*

3. Acetic acid-induced writhing test

As shown in table 3, the number of acetic acid-induced writhing movements of the mice treated with aspirin and Phong thap dan tablets decreased significantly compared with untreated mice in group 1. There was no statistically significant difference between 2 doses of Phong thap dan tablets. There was no statistically significant difference between aspirin-treated mice and Phong thap dan-treated mice (except the first 5 minutes).

Table 3. Effects of Phong thap dan tablets 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	13.10 ± 5.43	26.00 ± 9.57	20.50 ± 6.20	17.10 ± 6.45	11.10 ± 5.04	8.20 ± 3.88
Aspirin 150 mg/kg b.w/day	10	0.90 ± 0.88***	12.10 ± 6.40**	13.60 ± 6.42*	9.50 ± 4.40**	6.90 ± 2.42*	4.60 ± 2.12*
Phong thap dan 2.88 tablets/kg b.w/day (1.44 g/kg b.w/day)	10	3.60 ± 3.03***,#	16.30 ± 6.58*	11.60 ± 5.10**	10.90 ± 5.99*	7.10 ± 1.73*	4.80 ± 2.30*

Group	n	Number of writhing movements					
		0 - 5 minute	> 5 - 10 minute	> 10 - 15 minute	> 15 - 20 minute	> 20 - 25 minute	> 25 - 30 minute
Phong thap dan 8.64 tablets/kg b.w/day (4.32 g/ kg b.w/day)	10	3.30 ± 2.63***,#	14.90 ± 5.93**	12.00 ± 5.14**	9.40 ± 3.95**	6.70 ± 4.11*	4.50 ± 3.60*

**p < 0.05: compared with control*
***p < 0.01: compared with control*
****p < 0.001: compared with control*
#p < 0.05: compared with aspirin treated mice

IV. DISCUSSION

In this study, the analgesic activity of Phong thap dan tablets was assessed in different well accepted animal models, including hot plate test, mechanical stimulation test and acetic acid-induced writhing test.

The hot plate test was performed as a thermal pain model which is known useful for study of the central mechanism of analgesic activity.[3,4] The analgesic activity was assessed by writhing test which has been reported to be useful for investigation of peripheral antinociceptive activity and performed as a chemical pain model.[7] The mechanical stimulation test was considered to investigate both peripheral and centrally analgesic activities.

Phong thap dan tablets at the doses of 2.88 tablets/kg b.w/day (1.44 g/kg b.w/day) and 8.64 tablets/kg b.w/day (4.32 g/kg b.w/day) showed significant analgesic effects in all of the animal models in this study. This result indicated that Phong thap dan tablets might possess centrally and peripherally mediated analgesic activities.

Phong thap dan tablets were prepared from herbal medicine as mentioned above. Some of them was demonstrated the analgesic properties in animal models. Zhang Z et al. (2010) examined the analgesic and anti-inflammatory actions of cis-mulberroside A isolated from *Ramulus mori* in several models of inflammatory pain in mice, including acetic acid-induced pain and carrageenan-induced mouse paw edema. The results showed that cis-mulberroside A showed high analgesic and anti-inflammatory activities.[8]

Manirujjaman et al. (2013) investigated the analgesic activity of Methanolic and Petroleum Ether Extracts of *Vitex Negundo* Leaves using acetic acid-induced writhing test in mice. The methanolic and petroleum ether extracts, at the dose of 200 mg/kg body weight, displayed 82.60% & 74.66% pain inhibition which was significant ($p < 0.001$) compared to control. These results indicate that the extracts possess strong analgesic activity.[9]

Li X et al. (2013) studied the anti-inflammatory and analgesic effects of *Radix Angelicae Pubescentis* (R.A.P) ethanol extracts in three classic anti-inflammatory models and two analgesic models. In anti-inflammatory tests, all the extracts had a certain inhibition on the acute inflammation induced by xylene, ethanol extract significantly inhibited the inflammation in the three models. In analgesic experiment, compared with the blank control group, the comparisons between R.A.P. groups and control group had significant difference ($p < 0.01$).[10]

Overall, the above results might be the supporting evidence for the potential analgesic activity of Phong thap dan tablets in traditional medicine.

V. CONCLUSION

Phong thap dan tablets at the doses of 2.88 tablets/kg b.w/day (1.44 g/kg b.w/day) and 8.64 tablets/kg b.w/day (4.32 g/kg b.w/day) showed significant analgesic effect in animal models: increased the reaction time to thermal stimulation in hot plate test, increased the paw withdrawal latency and the force required to elicit a paw withdrawal in mechanical stimulation test and decreased the number of writhing movements in acetic acid-induced writhing test in mice.

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