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ANALGESIC EFFECT OF "DINH THONG PHONG" CAPSULES ON EXPERIMENTAL ANIMALS

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ABSTRACT

Purpose: *Evaluate the analgesic effect of "Dinh Thong Phong" capsules on experimental animals.*

Subjects and methods: *"Dinh Thong Phong" capsules, meeting the standard criteria, were evaluated for analgesic effect on white mice. In particular, the central analgesic effect was assessed using the hot plate model, peripheral analgesic effect was assessed using the writhing pain model.*

Results: *In the hot plate model, "Dinh Thong Phong" capsules at a dosage of 860 mg/kg and 2,580 mg/kg significantly increased the reaction time to heat ($p < 0.001$) compared to the control group. The difference in reaction time to heat between the groups using "Dinh Thong Phong" capsules and the group using codeine phosphate at a dose of 20 mg/kg was not statistically significant ($p > 0.05$). In the writhing model, "Dinh Thong Phong" capsules at a dose of 860 mg/kg and 2,580 mg/kg significantly reduced the number of writhing episodes ($p < 0.001$) compared to the control group. The difference in the number of writhing episodes between the groups using "Dinh Thong Phong" capsules and the group using aspirin at a dose of 150 mg/kg was not statistically significant ($p > 0.05$).*

Keywords: Analgesic effect, experimental animals, Dinh Thong Phong capsules.

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1. INTRODUCTIONS

"Dinh Thong Phong" (DTP) capsules are a product prepared from traditional medical remedies that have been used for treatment by many generations of physicians. The remedy included the following medicinal herbs: Nguu Tat (*Achyranthes bidentata*), Ty giai (*Dioscorea tokoro Makino*), Tho Phuc Linh (*Smilax glabra* Roxb), Hoang Ky (*Astragalus membranaceus),* Ich Mau (*Leonurus japonicus Houtt)*, Thuong Truat *(Atractylodes lanceae Asteraceae*), Thien Nien kien (*Homalomena occulta*), Tran Bi *(Pericarpium Citri Reticulatae),* Pha Co Chi (*Cullen corylifolium*), Ke Huyet dang (*Sargentodoxaceae*) Ha Thu o do (*Fallopia multiflora*), Ban Ha che (Tiphonium t*rilobatum*), Hoat thach (*Talcum*), Day gam (*Gnetum montanum*) and Hy Thiem thao (*Sigesbeckia orientalis)*

Although prepared from traditional medicine, the DTP products still have undergone the prescribed testing stages before being used on humans [2].

We conducted this study to evaluate the analgesic effect of DTP hard capsules on experimental animals (with the hot plate model and the cramping pain model on white mice).

2. SUBJECTS AND METHODS

2.1. Subjects

DTP hard capsules meet basic standards; each capsule weighs 600 mg contains 530 mg of a mixture of DTP dry extract powder and sodium hydrocarbonate.

The recipe for one DTP hard capsule included a mixture of DTP dry extract powder and sodium hydrocarbonate (530 mg); excipients (lactose, avicel, magnesium stearate...) just enough 600 mg.

Experimental animals: healthy white mice, Swiss strain, weight 20 ± 2 g/mouse. Animals were provided by the National Institute of Hygiene and Epidemiology. Animals were raised according to a standard regimen at the laboratory of the Military Institute of testing, Pharmaceutical research and Medical equipment for 5-7 days before the study. During the study period, mice were fed standard food and were drank clean water.

Research equipment and chemicals: hot plate model - DS37 (Ugo Basile, Italy); codeine phosphate provided by the Central Institute of Drug Testing; Acetic acid, PA (Merck, Germany); Aspirin tablets 100 mg (Traphaco Joint Stock Company, Vietnam); medical needle with blunt tip; Other laboratory equipment (1 ml syringe, scissors, Kocher forceps, mortar and pestle, rubber gloves, medical mask...).

2.2. Methods

- Study on the central analgesic effect of DTP hard capsules on the hot plate model described by Woolfe Gand and colleagues with appropriate modifications [5], [13]: randomly divided white mice that meet experimental standards into 4 groups, 10 mice each.

+ Group 1a (control group): mice received distilled water.

+ Group 2a (reference drug group): mice administered codeine phosphate at a dose of 20 mg/kg.

+ Group 3a (DTP dose 1 group): mice received DTP capsules at a dose of 860 mg/kg/day (equivalent to the human dose).

+ Group 4a (DTP dose 2 group): mice received DTP capsules at a dose of 2,580 mg/kg/day (three times the human dose).

The white mice were administered water, reference drug, or DTP capsules once daily in the morning, with a volume of 0.2 ml/10g body weight/day continuously for 5 days.

The reaction time to the temperature of the mice was measured before the first dose and one hour after the final dose. The reaction time to heat was determined from the time the mouse was placed on the hot plate (maintained at a temperature of 56ºC) until the mouse licked its hind paw. Mice with excessively fast reactions (less than 8 seconds) or excessively slow reactions (more than 30 seconds) before drug administration were excluded. The comparison was made between the reaction times to heat stimulation before and after drug administration.

- Study on the peripheral analgesic effect of DTP hard capsules on the writhing model as described by Koster and colleagues [10]. Mice were randomly divided into 4 groups, each consisting of 10 mice:

+ Group 1b (control group): Mice received distilled water.

+ Group 2b (reference drug group): Mice received aspirin at a dose of 150 mg/kg.

+ Group 3b (DTP dose 1 group): Mice received DTP capsules at a dose of 860 mg/kg/day (equivalent to the human dose).

+ Group 4b (DTP dose 2 group): Mice receiving DTP capsules at a dose of 2,580 mg/kg/day (three times the human dose).

The mice were administered the drug or distilled water at the same volume of 0.2 ml/10g body weight continuously for 5 days. On the 5th day, 60 minutes after the last administration, writhing pain was induced by injecting a 0.6% acetic acid solution (0.1 ml/10g body weight) into the peritoneum of mice. The mice showed writhing behavior (manifestations: abdominal contractions, pressing the abdomen against the floor, stretching the body and hind limbs, etc.). The number of writhing episodes was counted every 5 minutes for 20 minutes after the injection of 0.6% acetic acid. The comparison was made between the number of writhing episodes in each group. The percentage of pain inhibition was calculated using the following formula: A(%) = 100 x (Dc - Dt)/Dc, where A(%) is the percentage reduction in the number of writhing episodes of the reference drug group compared to the test drug group; Dc is the number of writhing episodes in the control group; Dt is the number of writhing episodes in the reference drug group and the test drug group.

- Data analysis: The results presented as mean ± SD. Differences in mean values between groups compared using one-way ANOVA and Post Hoc Least - significant differences (LS) test for homogeneous variance; one-way ANOVA and Dunnett's T3 test for heterogeneous variance. Evaluate the differences in mean values within the same group at different time points using paired sample T-test. Statistical analyses performed using IBM SPSS Statistics 20.0. Differences considered statistically significant when $p \leq 0.05$.

3. RESULTS

3.1. Effects on the hot plate model

Table 1. Reaction time of mice on the hot plate model (n = 10)

Study group	Reaction time (seconds)		$p_{before-after}$
	Initial time	After 5 days	
Group 1a(1)	11.94 ± 1.07	12.50 ± 1.48	0.320
Group 2a(2)	13.55 ± 3.59	28.76 ± 2.38	< 0.001
Group 3a(3)	11.30 ± 0.87	25.72 ± 2.70	< 0.001
Group 4a(4)	12.79 ± 1.30	27.73 ± 3.49	< 0.001
p	> 0,05	$p_{1-2,3,4} < 0.001$; $p_{2-3} = 0.013$; $p_{2-4} = 0.385$; $p_{3-4} = 0.094$	

At the initial time before drug administration, the reaction time to heat among the groups showed no significant difference (p > 0.05). After 5 days of drug administration, the reaction time to heat in the reference drug group, DTP dose 1 group, and DTP dose 2 group was significantly higher than that in the control group (p < 0.001). In addition, in the DTP dose 1 group, the reaction time was significantly lower than the reference drug group (p = 0.013). No significant difference was observed between the reference drug group and the DTP dose 2 group, as well as between the DTP dose 1 group and the DTP dose 2 group (p > 0.05).

Comparing the time before and after drug administration, in both the reference drug group and the group using DTP capsules, the reaction time of mice to heat after 5 days of drug administration was significantly higher than the time before drug administration (p < 0.001).

3.2. Effects on the writhing model

Table 2. Results of the number of writhing episodes every 5 minutes (n = 10)

Study group	Number of writhing episodes (minutes)			
	0-5	5-10	10-15	15-20
Group 1b(1)	2.50 ± 0.97	11.60 ± 1.35	12.20 ± 0.79	9.70 ± 1.34
Group 2b(2)	0.80** ± 1.40	8.10*** ± 1.10	8.10*** ± 0.88	6.90*** ± 1.10
Group 3b(3)	0.90** ± 1.29	7.40*** ± 1.35	8.80*** ± 0.92	7.10*** ± 0.99
Group 4b(4)	0.80** ± 1.14	7.50*** ± 0.97	8.20*** ± 1.40	7.30*** ± 1.34
*Note: **: p < 0.01; ***: p < 0.001 compared to the control group in the same column.*				

At each 5-minute interval after acetic acid injection, the number of writhing episodes in the control group was significantly higher than in the reference drug group, DTP dose 1 group, and DTP dose 2 group. There was no significant difference in the number of writhing episodes when comparing the reference drug group with DTP dose 1 group, DTP dose 2 group, and when comparing DTP dose 1 group with DTP dose 2 group (p > 0.05).

Table 3. Total number of writhing episodes within 20 minutes (n = 10)

Study group	Total number of writhing episodes average within 20 minutes	% Reduction compared to the control group
Group 1b(1)	36.0 ± 2.49	
Group 2b(2)	23.9*** ± 2.60	33.61
Group 3b(3)	24.2*** ± 2.30	32.78
Group 4b(4)	23,8*** ± 2.20	33.89
*Note: ***: p < 0.001 compared to the control group*		

The total average number of writhing episodes within 20 minutes in the control group was 36.0 ± 2.49 episodes, significantly higher than in the reference drug group (23.9 ± 2.60 episodes), DTP dose 1 group (24.2 ± 2.30 episodes), and DTP dose 2 group (23.8 ± 2.20 episodes), with a difference of p < 0.001. When comparing the total average number of writhing episodes within 20 minutes between the reference drug group, DTP dose 1 group, and DTP dose 2 group, no significant difference was observed (p > 0.05). The percentage of reduction in the number of writhing episodes in the reference drug group, DTP dose 1 group, and DTP dose 2 group compared to the control group was 33.61%, 32.78%, and 33.89%, respectively.

4. DISCUSSIONS

Pain is the first, most common and typical symptom of gout. Pain relief is one of the important goals in the treatment of gout. In this study, to evaluate the analgesic effect of DTP hard capsules, we used the hot plate model to evaluate the central analgesic effect and the writhing model to evaluate the peripheral analgesic effect.

- On the hot plate model:

The hot plate method used temperature as a pain-inducing agent. Heat affects nerve endings on the skin and mucous membranes, and the sensation of pain is transmitted to the brain, eliciting an appropriate response from the body. Codeine phosphate has an analgesic effect by increasing the pain threshold and reducing reflex responses to pain by central mechanisms [1]. Mice administered codeine phosphate were used as positive controls in the study. The results showed that DTP hard capsules at both doses of 860 mg/kg and 2,580 mg/kg in white mice demonstrated an analgesic effects by prolonging the reaction time of mice to heat.

- On the writhing model:

To evaluate analgesic effects by peripheral mechanisms, many pharmacological models can be used. We used a common model, the most commonly used was the popular writhing model.

The pain-inducing agent often used was acetic acid and phenylquinone [5] (In this study, we used acetic acid to stimulate macrophages and mast cells present in the peritoneum; thereby releasing pain-inducing substances such as TNF-α, IL-1β, IL-8). Aspirin was chosen as a positive control because aspirin inhibits the enzyme cyclooxygenase (COX), which leads to inhibition of the synthesis of

prostaglandin, thromboxane, and other products such as prostacyclin synthesis by COX, reducing the sensitivity of sensory nerve endings to pain-inducing substances [1]. The research results showed that DTP hard capsules at a dose of 860 mg/kg and 2,580 mg/kg in white mice had analgesic effects, reducing the number of writhing episodes after pain induction by acetic acid.

Thus, DTP hard capsules demonstrated an analgesic effects through both central and peripheral mechanisms. The results of this study were further supported by evidence from previous studies that many components and herbal ingredients in the DTP capsule formula have analgesic and anti-inflammatory effects (such as Nguu Tat had anti-inflammatory effects, often used in bone and joint pain relief remedies [6]; Hy Thiem had analgesic and anti-inflammatory effects [3]; Ich Mau had analgesic effect in the writhing model induced by acetic acid and anti-inflammatory effects [12]; Thuong Truat had anti-inflammatory effects, used to treat bone and joint diseases [8], [9]; Pha Co Chi [14], Ty Giai [11], Tho Phuc Linh [7], and Hoang Ky [4] all have anti-inflammatory effects).

5. CONCLUSIONS

Study on the analgesic effect of DTP hard capsules on experimental white mice (Evaluate the central analgesic effect on the hot plate model and peripheral analgesic effect on the writhing model), results:

- In the hot plate model, DTP hard capsules at a dose of 860 mg/kg and 2,580 mg/kg significantly increased the reaction time to heat ($p < 0.001$) compared to the control group. The difference in reaction time to heat between the groups administered DTP and the group administered codeine phosphate at a dose of 20 mg/kg was not statistically significant ($p > 0.05$).

- On the writhing model, DTP hard capsules at a dose of 860 mg/kg and 2,580 mg/kg significantly reduced the number of writhing episodes ($p < 0.001$) compared to the control group. The difference in the number of writhing episodes between the groups administered DTP and the group administered aspirin at a dose of 150 mg/kg was not statistically significant ($p > 0.05$).

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