

AN EXPERIMENTAL STUDY ON THE SKIN IRRITANT POTENTIAL OF DINH THO PATCH

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*Dinh Tho patches are the transdermal combination of medicinal herbs including *Dracaena cambodiana* Pierre ex Gagnep, *Cinnamomum cassia*, *Kaempferia galanga*, *Homalomena occulta*, *Caesalpinia sappan*, *Illicium verum*, *Artemisia vulgaris* and *Reynoutria japonica* with the intended indication to treat symptoms of swelling, bruising, tension and pain of the musculoskeletal system. This study was carried out to examine the skin irritation of Dinh Tho patches in experimental animals. Each individual rabbit was applied (2.5x2.5cm) Dinh Tho patch on one side of the dorsal area and (2.5x2.5cm) sodium chloride-impregnated gauze in the other size as control. The degree of erythema and edema formation were observed and scored at 1 hour, 24 hours, 48 hours and 72 hours after patch removal. Our results showed that grade 1 or grade 2 erythema only appeared 1 hour after removal of the test product. There were no sign of erythema, edema or other skin injury observed after 24 hours, 48 hours or 72 hours. It can be concluded that Dinh Tho patch did not cause skin irritation in rabbits.*

Keywords: Dinh Tho patch, skin irritation, rabbits, erythema, edema.

I. INTRODUCTION

Traditional medicine refers to health knowledge and practice, that incorporates medicinal plants, animals and minerals, applied singly or in combination to treat and prevent diseases or maintain well-being.¹ A large number of people in developing countries still use traditional medicine for treatment of acute and chronic diseases due to their preference for natural products, their concern over the side effects of modern medicine and the belief that herbal medicines are safe as it has been used for a long time.² Although oral route was the most frequently reported administration

method of traditional medicine, many diseases are treated through the skin route because this route might cause less adverse effects and is more convenient for use.³ Among many topical herbal preparations, the transdermal patches are most frequently prescribed. Different herbs can be dissolved or mixed with the adhesive matrix, and manufactured as a thin patch that might have locally or systemically therapeutic effects.⁴

Dinh Tho patches are the transdermal combination of several medicinal herbs including *Dracaena cambodiana* Pierre ex Gagnep, *Cinnamomum cassia*, *Kaempferia galanga*, *Homalomena occulta*, *Caesalpinia sappan*, *Illicium verum*, *Artemisia vulgaris* and *Reynoutria japonica*. The intended indication of Dinh Tho patches is to treat symptoms of swelling, bruising, tension and pain of the

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musculoskeletal system due to trauma and degeneration. These medicinal herbs have long been used in traditional medicine to treat symptoms of bone and joint diseases.⁵ Some of them have been demonstrated to have anti-inflammatory and analgesic activities. Like many plant-based traditional products, Dinh Tho patches showed promising potential to be an effective product for symptomatic treatment of the musculoskeletal system. However, there has been inadequate scientific evidence on their safety, pharmacological activity and efficacy.

When the transdermal patches are used, the skin is the first organ of the body that is exposed to the active ingredients and acts as a barrier that controls the release and absorption of the ingredients. As such, one of the first steps in a preclinical development program of a skin-applied product is to evaluate the potential for skin irritation. This study was performed to examine the potential skin irritation of Dinh Tho patches in experimental animals.

II. MATERIALS AND METHODS

1. Subjects

The investigational product

Dinh Tho patches were prepared by Dinh Tho Herbal Medicine Research & Application Joint Stock Company. Each patch contains: *Dracaena cambodiana* Pierre ex Gagnep 12mg, *Cinnamomum cassia* 10mg, *Kaempferia galanga* 12mg, *Homalomena occulta* 12mg, *Caesalpinia sappan* 16mg, *Illicium verum* 10mg, *Artemisia vulgaris* 10mg, *Reynoutria japonica* 10mg.

Experimental animals

The animals used in this study are adult healthy *New Zealand White* rabbits. The weight range of rabbits was 2.0 - 2.2kg. Each rabbit was

housed in a cage and fed in standard conditions one week before and throughout the study period at the Department of Pharmacology, Hanoi Medical University.

2. Methods

The skin irritation study of Dinh Tho patches was conducted according to OECD and ISO 10993-10 guidelines for skin irritation assessment of topical products.^{6,7}

The back area of three rabbits was shaved to select animals with clean and healthy skin. Twenty four hours before administration, the dorsal area was shaved [approximately (10×15) cm²]. Each individual was treated as follows: one side of the skin was applied (2.5×2.5cm) Dinh Tho patch and the other side was applied (2.5×2.5cm) sodium chloride-impregnated gauze. The administration site was fixed using a non-stimulating tape and an elastic bandage. The patch or gauze was removed 4 hours later and the processed reagents remaining on the skin was cleaned using sterilized distilled water.

All animals were observed once a day for 72 hours after administration of the test product. The general symptoms, and skin irritation toxicity were examined. The degree of erythema and edema formation were studied and evaluated according to Table 1 at 1 hour, 24 hours, 48 hours and 72 hours after removal of the patch. In addition, any other lesion would be described in detail. Further observations may be performed to establish reversible ability if there was any lesion.

The primary irritation index (P.I.I.) was calculated based on the degree of erythema and edema at 24 hours, 48 hours and 72 hours. Skin irritation toxicity was determined according to Table 2.

Table 1. Evaluation of skin reaction^{6,7}

Signs	Score
<i>Erythema</i>	
No erythema	0
Very slight (barely perceptible)	1
Well defined	2
Moderate to severe	3
Severe	4
<i>Maximum possible</i>	4
<i>Edema</i>	
No edema	0
Very slight (barely perceptible)	1
Well defined (edges of area raised)	2
Moderate to severe (edges of area raised ~ 1mm)	3
Severe (edges of area raised more than 1mm and extending outside the application area)	4
<i>Maximum possible</i>	4

Table 2. Rating table of skin irritation test^{6,7}

Response category	Mean PII
Negligible	0 - 0.4
Slight	0.5 - 1.9
Moderate	2 - 4.9
Severe	5 - 8

III. RESULTS

1. General observation (food consumption and body weight)

In this experiment, no animal fatality was observed. There were no change in food consumption, body weight gain and clinical signs in all rabbits.

2. Skin irritation evaluation

As shown in Table 3, there were no sign of

erythema or edema on the control area in all rabbits at all time points. On Dinh Tho patch-applied area, a barely noticeable erythema appeared in one rabbit, a noticeable erythema appeared in one rabbit, and there was no erythema in the remaining one. At 24 hours, 48 hours and 72 hours, there were no sign of erythema or edema observed in all rabbits. No edema was observed in all rabbits at all time points. As a result, the primary irritation index of

Dinh Tho patch calculated based on the degree of erythema and edema at 24 hours, 48 hours

and 72 hours was 0. According to Table 2, Dinh Tho patch did not cause skin irritation in rabbits.

Table 3. Evaluation of erythema and edema formation in Dinh Tho patch-applied rabbits

Rabbit	Erythema								Edema							
	1h		24h		48h		72h		1h		24h		48h		72h	
	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C
1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

T: Dinh Tho patch-applied area C: control area

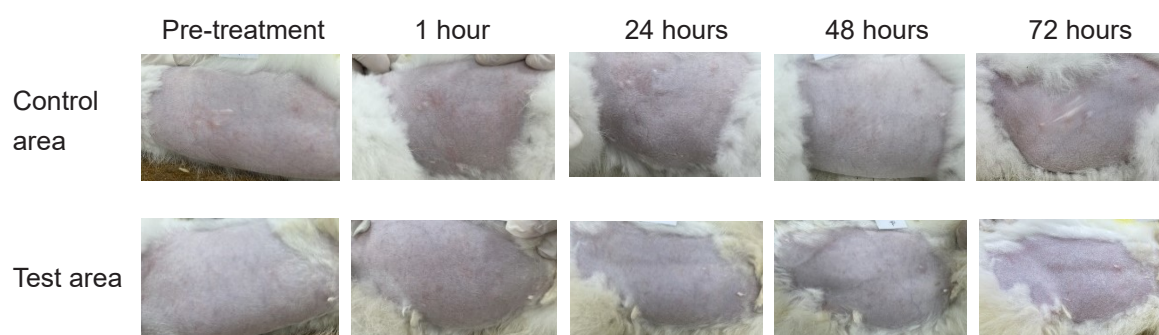


Figure 1. Images of rabbit' skin

IV. DISCUSSION

Dinh Tho patch is a herbal product which consists of two parts. The center of the patch is the herbal combination of herbal medicine including *Dracaena cambodiana* Pierre ex Gagnep, *Cinnamomum cassia*, *Kaempferia galanga*, *Homalomena occulta*, *Caesalpinia sappan*, *Illicium verum*, *Artemisia vulgaris* and *Reynoutria japonica*. The surrounding edge is a non-woven fabric structure that can be applied to the skin, allowing the patch to stay in the correct position for treatment; the patient can still performs normal activities during the treatment period. The combination of those herbal medicines in a transdermal patch has a significant potential symptomatic treatment of swelling, bruising, tension and pain of the

musculoskeletal system.

To ensure the safety of this product, non-clinical studies need to be performed and the first step is to evaluate its skin irritation in experimental animals. This test involves applying the reagents to the animal skin and evaluating the signs and symptoms of skin toxicity.

In this study, the skin irritancy potential of Dinh Tho patch was investigated by applying the product to the skin of rabbits. Dermal irritation is the production of reversible inflammatory changes in the skin following the application of a test product.⁶ Dinh Tho patch was applied to the skin of rabbits, each animal serving as its own control. All signs and symptoms of the skin

were monitored and the degree of erythema and edema was scored. Our results showed that grade 1 or grade 2 erythema only appeared one hour after removal of the test product. There were no sign of erythema, edema or other skin injuries observed after 24 hours, 48 hours or 72 hours. It can be concluded that Dinh Tho patch did not cause skin irritation in rabbits. Rabbits are considered to be very sensitive animals. Therefore, our results suggest a very low potential of skin irritation of Dinh Tho patch when used in humans.

The herbal medicines in this product have been used for a long time to treat the symptoms of bones, joints and muscles. They have also been demonstrated to have anti-inflammatory activity in vitro and in vivo models.⁸⁻¹⁷ Some ingredient was shown to decreased the serum levels of NO, TNF- α , and PGE2 and the expression of iNOS, COX-2, and NF- κ B, that played important roles in the anti-inflammatory mechanism.¹⁰ Besides, some herbs have been demonstrated to have other activities. *Dracaena cambodiana* Pierre ex Gagnep, and *Kaempferia galanga* were shown to reduce pain in animal models.^{9,12} *Caesalpinia sappan* increased fibroblast proliferation, fibroblast migration, and collagen production, that might promote the wound healing process.¹⁴ *Cinnamomum cassia* and *Reynoutria japonica* were evaluated on different models and showed significant anti-arthritic effects.^{11,17}

Consequently, the combination of the above medicinal herbs in Dinh Tho patch is based on both traditional medicine and scientific studies. With a very low risk of causing skin irritation in humans, Dinh Tho patch promises to become a treatment method for musculoskeletal diseases. To have full scientific evidence for the efficacy and safety of the product, the next research direction includes evaluating the

product's dermal repeated-dose toxicity, and the pharmacological effects including analgesic, anti-inflammatory activities and effects on experimental disease models.

V. CONCLUSION

Our studies demonstrated that Dinh Tho patch did not cause skin irritation in rabbits.

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