Original Article

Skin Irritation and Allergic Testing of Thai Herbal Extracts (Ha–Rak with Turmeric) in Healthy Volunteers

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Abstract

Introduction: Ha-Rak is a well-known Thai remedy orally taken for fever. The powder has been used for topical application against dermatitis. In this study, turmeric powder of equal amount was added to Ha-Rak powder yielding Prasakhaminchan of Ha-Rak remedy. Previous studies of both Ha-Rak remedy and turmeric had established safety profiles on rat and human skin. However, no study has been done on the safety profile of Prasakhaminchan of Ha-Rak remedy on human skin. Therefore, this study was to investigate the skin irritation and allergic testing of Thai herbal extracts (Ha-Rak with turmeric) in healthy volunteers.

Method: Various concentrations (0.25-10% w/w) of Ha-Rak extract, turmeric extract, Prasakhaminchan of Ha-Rak extract in white petrolatum and in cream base were prepared. A patch test was performed on 30 healthy volunteers for 48 hours. The skin reactions were evaluated after 48 hours and also 72 hours.

Results: Only one person had slight (1+) allergic reaction to turmeric extract, Prasakhaminchan of Ha-Rak extract in white petrolatum and turmeric extract in cream base. There was no allergic reaction to Ha-Rak extract and Prasakhaminchan of Ha-Rak extract in cream base. There was no irritation from Ha-Rak, turmeric and Prasakhaminchan of Ha-Rak extracts in white petrolatum and in cream base.

Conclusion: Ha-Rak, turmeric and Prasakhaminchan of Ha-Rak extracts at 0.25-10 % w/w in cream base and in white petrolatum did not cause skin irritation, therefore they are safe to be used as topical medication for skin. However, people who has hypersensitivity to turmeric should avoid these medicaments.

Keywords: Prasakhaminchan of Ha-Rak, Patch test, Irritation, Allergic reaction

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Introduction

Yata Ha-Rak remedy (topical application of Ha-Rak) has long been used as an antipruritic for skin rashes.[1] Prasakhaminchan of Ha-Rak remedy consists of Ha-Rak remedy and turmeric powder in equal amount. In Thai medicinal practice, Ha-Rak remedy (Benjalokawichien) has long been used to treat fever, rash, and itching. It contains roots of five herbal plants: *Capparis micracantha* DC., *Tiliacora triandra* (Colebr.) Diels., *Harrisonia perforata* Merr., *Clerodendrum petasites* S. *Moore* and *Ficus racemosa* Linn. Previously, Ha-Rak remedy has been tested for skin safety on volunteers using closed patch test under occlusion. There were neither allergic reactions nor irritation reaction in volunteers.[2] Turmeric (*Curcuma longa* Linn.) has been used in folk medicine for treatments of skin rash and wounds. However, in a 48 hour skin closure test in 50 volunteers with raw turmeric showed allergic reaction in 14 volunteers.[3]

In pratice Ha-Rak remedy powder mixed with turmeric powder has always been the drug of choice to treat skin rash. In other studies, both Ha-Rak remedy and turmeric have shown anti-allergic,[4] anti-inflammatory,[5] anti-oxidant,[6] anti-bacterial properties[7] and also showed good safety profiles in rats[8] and humans[9] for skin irritation and allergic test.

The above information showed that the Prasakhaminchan of Ha-Rak formula had high potential as a topical medication for inflammatory skin diseases. In this study a topical cream and ointment of this formula were developed to assess their safety profiles. The aim of this study was to investigate the safety of topical Prasakhaminchan of Ha-Rak on human skin by using the skin patch test in healthy volunteers in comparison with their components and vehicles.

Materials and Methods

Study design

This study is a clinical trial in healthy volunteers to test for skin irritation and allergic reactions to cream and ointment of herbal extracts on human skin.

Plant Materials

The roots of *C. micracantha, T. triandra, H. perforata, C. petasites* and *F. racemosa* were collected from Dan-Chang, Suphanburi, Thailand in 2016, washed and dried at 50°C. Turmeric was collected from Nakhon Pathom, Thailand in 2017. It was steamed and sterilized before drying at 50°C. One hundred grams of Ha-Rak remedy and turmeric were each macerated three times with 95% ethanol (500 ml) for 3 days, then filtered and concentrated by rotary evaporator (under reduced pressure) to obtain the ethanolic extracts. The percentage of yields was calculated. Prasakhaminchan extract consists of Ha-Rak extract, turmeric extract mixture in the ratio of 1:1.

The test samples consisted of Ha-Rak extract, turmeric extract, and Prasakhaminchan extract in white petrolatum and in cream base at the concentrations of 0.25%, 0.5%, 1%, 3%, 5% and 10% w/w. The cause was tested in white petrolatum and cream base to determine if volunteers were allergic to Ha-Rak extract, turmeric extract, and Prasakhaminchan extract or base cream, with white petrolatum as vehicle and negative control.

Ethical consideration

Approval for this human study was obtained from Human Ethics Committee of Thammasat University No.1, Faculty of Medicine, Thammasat University, Pathumthani, Thailand. The guidelines followed were in accordance with the principles set in Declaration of Helsinki. Informed consent was given in writing by the volunteers. Certificate of Approval number was 158/2017. Project number was MTU-EC-TM-6-084/60.

Study population

Thirty healthy human volunteers, aged between 18 and 60 years old, were enrolled in this study. They were examined by a dermatologist to exclude those who had any skin disease or other skin condition, especially on the back. The volunteers who had a history of Band-Aid allergy were also excluded.

All the selected volunteers were tested at the Skin Center, Thammasat University Hospital.

Skin patch test

All extracts were loaded into the clear patch chambers (Figure 1), then the patches were applied onto the backs of volunteers and fixed with micropore tape for 48 hours (Figure 2), the patches were labeled and numbered accordingly.

Figure 1 Patch test of extracts at 0.25%, 0.5%, 1%, 3%, 5%, and 10% (w/w) in white petrolatum and in cream base (sample 1-38).

Patch 1 (Sample 1-10): Ha-Rak remedy extract 0.25%, 0.5%, 1%, 3%, 5%, 10% (w/w) in white petrolatum and 0.25%, 0.5%, 1%, 3% in cream base, respectively.

Patch 2 (Sample 11-20): Ha-Rak remedy extract 5% and 10% (w/w) in cream base. Turmeric extract 0.25%, 0.5%, 1%, 3%, 5%, 10% (w/w) in white petrolatum and 0.25%, 0.5% in cream base, respectively.

Patch 3 (Sample 21-30): Turmeric extract 1%, 3%, 5%, 10% (w/w) in cream base. Prasakhaminchan extract 0.25%, 0.5%, 1%, 3%, 5%, 10% (w/w) in white petrolatum, respectively.

Patch 4 (Sample 31-38): Prasakhaminchan extract 0.25%, 0.5%, 1%, 3%, 5%, 10% (w/w) in cream base. White petrolatum (vehicle and negative control) and cream base (vehicle), respectively.

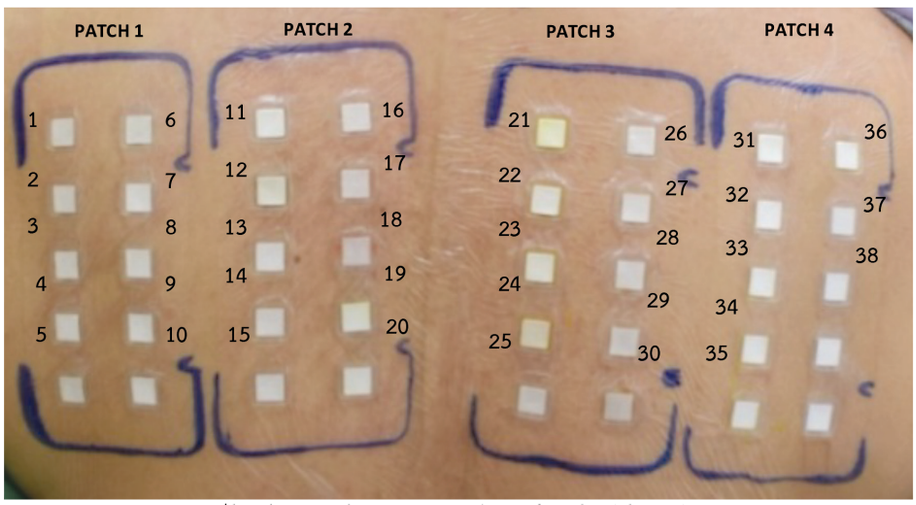

Figure 2 The skin patch test was performed in healthy volunteers.

All volunteers were advised to avoid water, heavy exercise and scratching. The patch chambers were removed after 48 hours. Thirty minutes after removing the patch, the patched sites were observed and graded for irritation and allergic reactions (total 48 hours of test) and observed again after 24 hours (total 72 hours of test). The test results were subsequently be determined by a dermatologist using the grading criteria of skin reactions according to Cosmetic, Toiletry, and Fragrance Association (CTFA) Guideline, as shown in Table 1.

Table 1 Grading criteria of skin reactions according to Cosmetic, Toiletry, and Fragrance Association (CTFA) Guideline[10]

Symbol	Grade	Clinical description
-	0	Negative reaction
+	1	Slight erythema, either spotty or diffuse
++	2	Moderate uniform erythema
+++	3	Intense erythema with edema
++++	4	Intense erythema with edema and vesicles

Data analysis

Skin response to patch test was evaluated twice and scores were calculated according to the following formula[11]:

$$\text{Z-score} = \frac{(\Sigma(\text{grade} \times \text{n})}{(4 \times N)} \times 100 \times 1/2$$

Note: Z-score = Score for the results response calculated in each case was compared with the safety of the product.

Σ = standard deviation

grade = grade 0-4 of skin reactions

n = number of responders

4 = maximum grade

N = total subjects

This equation gives the ratio of response maximum possible responses. The results response calculated in each case was compared with the safety of the product. A Z≤1.0 was determined as a safety for primary skin irritation of cream or for the leave-on product.[11]

Results

Ha-Rak remedy and turmeric were macerated with 95% ethanol, then filtered and concentrated by rotary evaporator. The percentage yield (w/w) of turmeric extract was higher than Ha-Rak remedy extract, 10.57% and 3.59% respectively. The extract of cream containing Prasakhaminchan of Ha-Rak was analysed by HPLC revealed the content of pectolinarigenin, which was the active substance in Ha-Rak, to be 0.58 ± 0.04 mg/1g and curcumin, the active substance in turmeric was 326.45 ± 21.34 mg/1g. Both substances contain anti-allergic and anti-inflammatory activities.[6]

Thirty healthy volunteers who were selected for skin patch test consisted of 1 male and 29 females aged 23-58 years (mean 36.43 ± 9.07 years). No volunteers dropped out or failed on follow up visits throughout the study period. The assessments at 48 hours patch test and 24 hours later after patch removal were shown in Table 2.

The extract of Ha-Rak remedy at all concentrations (0.25 – 10%) in white petrolatum (WP) did not give any skin reaction to 22 volunteers. Changing the vehicle from WP to cream base gave similar results but there were individuals (volunteers No. 7 and 29) that developed faint erythema outside the area of test blocks (doubtful reaction in Table 2).

The turmeric extract in WP did not give any skin reaction to 23 volunteers but at low concentrations (0.25% and 0.50%) gave doubtful reaction in one volunteer. Changing the vehicle from WP to cream base gave similar results without any doubtful reaction (Table 2).

The extract of Ha-Rak with turmeric (PSK) at all concentrations (0.25 – 10%) in white petrolatum (WP) did not give any skin reaction to 23 volunteers. Changing the vehicle from WP to cream base increased the number of volunteers with reactive skin (angry back) from 7 to 9 persons.

There were 21 volunteers who did not have reaction with WP and 20 volunteers who did not have reaction with the cream base.

Interestingly, there were 7-9 volunteers who developed rashes all over their back area that patches were adhered to (angry back in Table 2), either patches containing tested samples or those with vehicle (WP and cream base). However, these rashes disappeared in 24 hours after the removal of patches without any treatment (Table 2).

After 48 hours only one volunteer (No. 16) developed hypersensitivity to turmeric showing a slight skin reaction (1+) to 3%, 5%, 10% turmeric extract in WP and to 0.25-10% turmeric extract in cream base. This individual also showed slight skin reaction (1+) to 0.25 -10% PSK extract in WP but not with the cream preparation (Table 2 and Figure 3).

Table 2 The results of skin patch test from 30 healthy volunteers

Samples	Patch on 48 hours Assessment 30 minute after patch removal.							72 hours fallow up Assessment 24 hrs. after patch removal.							Z-Score
	CTFA grade							CTFA grade							
	0	1	2	3	4	±/?	Angry back	0	1	2	3	4	±/?	Angry back	
1. HaRak 0.25%+ WP	22						8^{x}	30							0
2. HaRak 0.5%+ WP	22						8^{x}	30							0
3. HaRak 1%+ WP	22						8^{x}	30							0
4. HaRak 3%+ WP	22						8^{x}	30							0
5. HaRak 5%+ WP	22						8^{x}	30							0
6. HaRak 10%+ WP	22						8^{x}	30							0
7. Ha-Rak 0.25% +CB	22						8^{x}	30							0
8. Ha-Rak 0.5% + CB	21					1^{29}	8^{x}	30							0
9. Ha-Rak 1% + CB	21					1^{29}	8^{x}	30							0
10. Ha-Rak 3% + CB	21					1^{29}	8^{x}	30							0
11. Ha-Rak 5 %+ CB	22					1^{7}	7^{y}	30							0
12. Ha-Rak 10%+ CB	22					1^{7}	7^{y}	30							0
13. Turmeric 0.25%+WP	22					1^{7}	7^{y}	30							0
14. Turmeric 0.5%+WP	22					1^{7}	7^{y}	30							0
15. Turmeric 1 % +WP	23						7^{y}	30							0
16. Turmeric 3% +WP	23						7^{y}	29	1^{16}						0
17. Turmeric 5%+WP	23						7^{y}	29	1^{16}						0

Table 2 The results of skin patch test from 30 healthy volunteers

Samples	Patch on 48 hours Assessment 30 minute after patch removal.							72 hours fallow up Assessment 24 hrs. after patch removal.							Z-Score
	CTFA grade							CTFA grade							
	0	1	2	3	4	±/?	Angry back	0	1	2	3	4	±/?	Angry back	
18. Turmeric 10% +WP	23						7^{y}	29	1^{16}						0
19. Turmeric 0.25%+CB	23						7^{y}	29	1^{16}						0
20. Turmeric 0.5%+CB	23						7^{y}	29	1^{16}						0
21. Turmeric 1%+CB	23						7^{y}	29	1^{16}						0
22. Turmeric 3%+CB	23						7^{y}	29	1^{16}						0
23. Turmeric 5%+CB	23						7^{y}	29	1^{16}						0
24. Turmeric 10%+CB	23						7^{y}	29	1^{16}						0
25. PSK 0.25% +WP	23						7^{y}	29	1^{16}						0
26. PSK 0.5% +WP	23						7^{y}	29	1^{16}						0
27. PSK 1% +WP	23						7^{y}	29	1^{16}						0
28. PSK 3% +WP	23						7^{y}	29	1^{16}						0
29. PSK 5% +WP	23						7^{y}	29	1^{16}						0
30. PSK 10% +WP	23						7^{y}	29	1^{16}						0
31. PSK 0.25%+CB	21						9^{z}	30							0
32. PSK 0.5%+CB	21						9^{z}	30							0
33. PSK 1%+CB	21						9^{z}	30							0
34. PSK 3%+CB	21						9^{z}	30							0
35. PSK 5%+CB	21						9^{z}	30							0
36. PSK 10%+CB	21						9^{z}	30							0
37. White petrolatum	21						9^{z}	30							0
38. Cream base	20					1^{8}	9^{z}	30							0

Note: PSK = Prasakhaminchan of Ha-Rak, WP = White petrolatum, CB = Cream base,
±/? = Doubtful reaction (Faint erythema outside the area of test blocks)
Angry back = Red rash on area of the back covered by patches
8^{X} = volunteer No. 4, 9, 11, 12, 15, 17, 25, 26
7^{y} = volunteer No. 4, 9, 11, 12, 15, 17, 26
9^{z} = volunteer No. 4, 9, 11, 12, 15, 17, 25, 26, 29
1^{7}, 1^{9}, 1^{16}, 1^{29} = volunteer No. 7, 9, 16 and 29

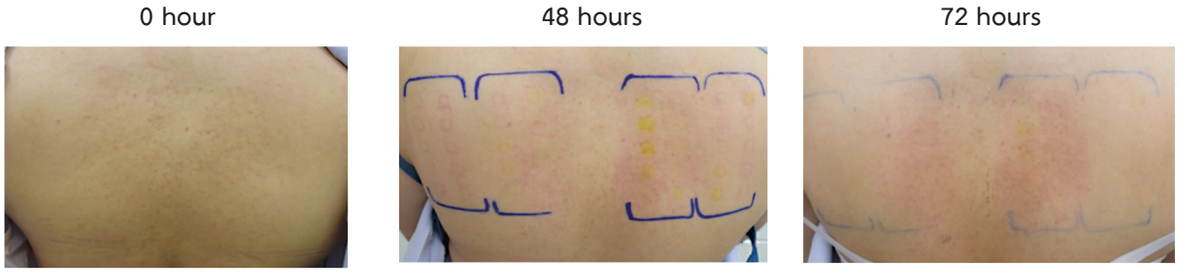

Figure 3 Allergic skin reaction of a volunteer to the test samples containing turmeric.

Discussion

The results of the skin patch test showed that the extracts of Ha-Rak remedy, turmeric, and Prasakhaminchan of Ha-rak remedy in white petrolatum and in cream base at concentrations of 0.25-10% w/w were safe for human skin due to the absence of skin irritation. The skin responses were classified into four levels based on the intensity of the skin reaction as determined by the z-score irritation index shown in Table 3.

According to the study on setting a primary irritation index and safety zone for cosmetic products, the safety zone for the leave-on product was 1.12 for lotions and creams.[15, 16] From this study, Ha-Rak extract, turmeric extract, Prasakhaminchan of Ha-rak extract in white petrolatum and in cream base had Z value = 0. These results confirm the safety profile of these extracts to human skin.

In this study, there was a volunteer who developed a slight allergic reaction to turmeric extract in white petrolatum, in cream base and in PSK extract in white petrolatum. This volunteer could have been allergic to turmeric. The allergy or hypersensitivity is an abnormal reaction by the immune system of an individual to a substance that normally does not cause reaction in most other people. Allergic contact dermatitis occurs when skin becomes sensitive to a certain substance (allergen), comes in contact with that substance again. This is a delayed skin reaction that typically develops in 12 to 72 hours after exposure. But irritant contact dermatitis occurs when the skin is repeatedly exposed to a mild irritant over a long period of time. If skin is exposed to a strong irritant, skin damage can be immediate. The allergic response to an externally applied agent is less common than irritant reaction.[12]

Therefore, the cream containing Prasakhaminchan of Ha-Rak remedy is safe for normal human skin and has high potential to be developed as a topical medication for skin inflammation treatment in the future. However, further study on its efficacy for specific skin diseases and its safety for various skin conditions needs to be investigated. It should however be avoided in person who has a history of turmeric hypersensitivity.

Table 3 Human primary irritation index for cosmetic products by human patch test[10]

Range of z-score	Range of Response	Criteria
0.0 ≤ Z ≤ 0.5	0.0 ≤ R ≤ 0.87	Slight
0.5 ≤ Z ≤ 2.0	0.87 ≤ R ≤ 2.42	Mild
2.0 ≤ Z ≤ 3.0	2.42 ≤ R ≤ 3.44	Moderate
3.0 ≤ Z	3.44 ≤ R	Severe

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Conflicts of interest

The authors declare no conflict of interest.

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บทคัดย่อ

การทดสอบการระคายเคืองและการแพ้ต่อผิวหนังของสารสกัดสมุนไพรไทยยาทาห้ารากประสะขมิ้นชันในอาสาสมัครสุขภาพดี

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**** ศูนย์แห่งความเป็นเลิศทางด้านการแพทย์แผนไทยประยุกต์ คณะแพทยศาสตร์ มหาวิทยาลัยธรรมศาสตร์

ภูมิหลัง: ยาห้ารากในตำรับยาไทยใช้สำหรับแก้ไข้ ผงของยาห้ารากถูกนำมาใช้รักษาผิวหนังอักเสบ ในการศึกษาครั้งนี้ได้เติมผงขมิ้นในยาห้ารากในอัตราส่วนที่เท่ากัน เพื่อให้ได้ยาประสะขมิ้นชัน ประสะขมิ้นชันคือยาห้ารากผสมขมิ้นชันพัฒนาขึ้นเพื่อใช้เป็นยาทาในการรักษาผิวหนังอักเสบ ซึ่งยาห้ารากและขมิ้นชันได้มีการศึกษาความปลอดภัยในผิวหนังของหนูและคนแล้ว อย่างไรก็ตามยังไม่มีการศึกษาความปลอดภัยของครีมผสมสารสกัดยาทาห้ารากประสะขมิ้นชันต่อผิวหนังคน ดังนั้นการศึกษานี้มีวัตถุประสงค์เพื่อศึกษาการระคายเคืองและการแพ้ต่อสารสกัดประสะขมิ้นชันในผิวหนังของอาสาสมัครสุขภาพดี

วัสดุและวิธีการ: ทำการศึกษาด้วยวิธีการแปะแผ่นบรรจุสารทดสอบบนผิวหนังของอาสาสมัครสุขภาพดี นาน 48 ชั่วโมง ได้แก่ สารสกัดยาห้าราก สารสกัดขมิ้นชัน สารสกัดยาห้ารากประสะขมิ้นชัน ผสมในวาสลีนขาว และยาพื้นชนิดครีม ที่ระดับความเข้มข้น ร้อยละ 0.25, 0.5, 1, 3, 5 และ 10 โดยน้ำหนัก และประเมินปฏิกิริยาต่อผิวหนังที่ระยะเวลา 48 และ 72 ชั่วโมง

ผลการศึกษา: ในบรรดาอาสาสมัครสุขภาพดีจำนวน 30 คน พบเพียง 1 รายที่มีปฏิกิริยาภูมิแพ้แบบไม่รุนแรง (1+) ต่อสารสกัดขมิ้นชันกับสารสกัดยาห้ารากประสะขมิ้นชัน ที่ผสมในวาสลีนขาว และสารสกัดขมิ้นชันในยาพื้นชนิดครีม แต่ไม่พบปฏิกิริยาภูมิแพ้ต่อสารสกัดห้ารากและสารสกัดประสะขมิ้นชันในยาพื้นชนิดครีม นอกจากนั้นยังไม่พบการระคายเคืองจากสารสกัดห้าราก สารสกัดขมิ้นชัน และสารสกัดห้ารากประสะขมิ้นชัน ทั้งในแบบผสมวาสลีนขาวและยาพื้นชนิดครีม

สรุป: สารสกัดยาห้าราก สารสกัดขมิ้นชัน และสารสกัดยาห้ารากประสะขมิ้นชัน ที่ระดับความเข้มข้นร้อยละ 0.25-10 โดยน้ำหนัก ผสมในยาพื้นชนิดครีม และในวาสลีนขาวไม่ก่อให้เกิดการแพ้ต่อผิวหนัง มีความปลอดภัยที่จะนำมาใช้เตรียมเป็นผลิตภัณฑ์สำหรับทาผิวหนัง แต่ควรระวังการใช้ในผู้ที่มีปฏิกิริยาภูมิแพ้ต่อขมิ้นชัน

คำสำคัญ : ประสะขมิ้นชัน, การทดสอบด้วยแผ่นแปะ, การระคายเคือง, ปฏิกิริยาภูมิแพ้