The Study of Human Skin Irritation of a Novel Herbal Skin Care Product and Ingredients by Human Single closed Patch Testing

Penpun Wattanakrai MD*, Suthida Suwanachote MD*,
Sutinee Kulkollakarn MD*, Natta Rajatanavin MD*

** Division of Dermatology, Department of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok*

Background: *The Government Pharmaceutical Organization of Thailand (GPO) has developed many products using liposome nanotechnology and Thai herbal extracts.*
Objective: *Evaluate the irritation potential of GPO products on human skin using the single application closed patch test under occlusion. The authors also studied the moisturizing efficacy of a commercial curmin extract cream (GPO curmin cream).*
Material and Method: *Thirty-six female volunteers were tested with 12 test materials developed by GPO including liposome, curmin extract: tetrahydrocurcuminoids (THC), and commercial curmin cream. Two and a half percent sodium dodecyl sulfate (SDS) was used as positive control. Standard Finn chambers on Scanpor tape with webril cotton were used as occlusive patch test devices. Cutaneous irritation responses were graded after patch removal and the incidence of irritation compared to the positive control was used for evaluation. Corneometer was used to measure skin hydration before and after application of curmin cream.*
Results: *All volunteers completed the present study. The skin irritation effects from the test materials were significantly lower (p-value < 0.001, McNemar statistic test) than the positive control. Measurement of skin hydration after twice daily application of GPO curmin cream was significantly higher (p-value < 0.001, paired t-test) than the control skin.*
Conclusion: *The test materials and finished products developed by the GPO are not likely to induce skin irritation under normal conditions of use. Furthermore, twice-daily application of the commercial GPO curmin cream can significantly increase skin hydration.*

Keywords: *Herbal extracts, Curmin, Tetrahydrocurcuminoids, Irritation, Hydration*

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At present, the Government Pharmaceutical Organization (GPO) of Thailand is capable of producing its own liposomes to serve the expanding industrial production scale. The GPO has recently extracted from a traditional Thai herb, Turmeric (Curcuma longa Linn), a yellow substance known as curcuminoids with known antioxidant property. Following this achievement, researchers from the Research and Development Institute of the GPO continued to develop curcuminoids into a white extract known as tetrahydrocurcuminoids (THC) with 2 times higher antioxidant properties[1,2]. Thereafter, the researchers introduced nanotechnology in the development of herbal extract through encapsulation of THC into liposome, an invisible particle of nanometer size derived from a natural substance called phospholipids. GPO has applied these innovations into the development of new THC cream formula (GPO curmin cream). This is the first time in Thailand where liposome nanotechnology has been implemented with Thai traditional herb adding value to today's traditional herbal products.

Before new skin care products and ingredients are introduced into the market, the testing for

Correspondence to : Wattanakrai P, Division of Dermatology, Department of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok 10400, Thailand.

potential adverse skin effects (irritation and allergy) is essential[3-5]. This dermatological test for irritation effect on humans was therefore performed to ensure consumer safety for these novel, GPO researched and developed products.

Objective

To assess the skin irritation potential and tolerance of human skin to these novel cosmetic ingredients and finished product, the authors conducted the human single closed patch test, using sodium dodecyl sulfate (SDS) as reference material. Another objective of the present study was to assess the efficacy of the finished product THC cream to increase skin moisture after application.

Material and Method

The protocol was reviewed and approved by the Ethical Review Committee of the Faculty of Medicine, Ramathibodi Hospital, Mahidol University. The present study was conducted at the Division of Dermatology, Ramathibodi Hospital for 2 weeks in March 2005. The temperature during the test period varied from a low temperature of 19-25°C to a high temperature of 34-37°C.

Subjects

The authors recruited 36 healthy, adult volunteers with no underlying skin disease or skin lesion on the test area. All subjects were older than 15 years old, non-pregnant, and not breastfeeding. Patients with a history of allergies or with known allergy to the test products and patients receiving immunosuppressive therapy, corticosteroids, antihistamines, and/or chemotherapy in the previous two weeks were excluded. The volunteers must be able to attend all four days of the test period and must be able to comply with the restrictions during the test period, which included to not participate in any activity or exercise that will cause sweating and to keep the test area dry until the last reading. All volunteers were informed of objectives, test procedures, and possible adverse effects, and were rewarded for their time of participation. They were included into the present study only after signing the consent form.

The volunteers recruited were females aged between 20-60 years (mean age 35.8). Three of the volunteers were taking other medications that were considered not to interfere with the present study including antihypertensive drugs, thyroid hormone, and oral contraceptive pills.

Test Method

The human single closed patch test under occlusion was performed. The test materials were occluded on the upper outer arms in two sets. The first set was occluded 4 hours if no signs of irritation occurred the second set would be further occluded for a total of 24 hours. After the required periods of skin contact, the patches were opened and observed for any signs of skin irritation 1 hour after patch removal, then 24 and 48 hours afterwards.

Test Devices

Standard Finn Chambers; round aluminum disks 8 mm diameter on Scanpor tape (Finn Chamber , Epitest Ltd., Finland) usually used for epicutaneous testing were used. A webril cotton size 1 cm x 1 cm was placed on to each aluminum disk and saturated with a sufficient amount of the test material to cover the surface of the pad. The patches were secured in place to the upper outer arms with non-allergic porous, surgical tape (Micropore , 3M).

Test Materials

The test materials were supplied by the GPO. The 12 materials tested for skin irritancy are shown listed in Table 1. For evaluating the moisturizing effect of the finished product only one commercial product was tested, the THC liposome cream with perfume (GPO curmin cream).

Reference Materials

For positive control, the authors used 2.5% sodium dodecyl sulfate (SDS) prepared by the Ramathibodi Hospital Pharmacy. The pH of 2.5% SDS was 8.21. For negative control, sterile distilled water was used.

Table 1. List of Thai GPO supplied test materials

Blank liposome
Liposome encapsulating tetrahydro-curcuminoids (THC)
THC liposome cream with perfume (THC-P or GPO curmin cream)
THC liposome cream without perfume (THC-N)
Eye gel
Vitamin E liposome cream
Aftersun cream with perfume
Aftersun cream without perfume
Undiluted, cleansing gel formula 1
8% dilution, cleansing gel formula 1
Undiluted, cleansing gel formula 2
8% dilution, cleansing gel formula 2

Table 2. Assessment of reactions for irritant patch test

Grading	Description of skin response
0	No reaction
+/- (1/2)	Doubtful reaction : Glazed appearance of site or barely perceptible erythema
1	Weak reaction : Slight erythema or dryness across most of treatment site
2	Moderate reaction : moderate erythema, possibly spreading with barely perceptible edema at the margin; papules may be present
3	Strong reaction : moderate erythema with generalized edema
4	Severe reaction : severe erythema with severe edema, with or without vesicles, pustule or ulcer

Removal of Test Material

After the appropriate period of exposure, the test materials were removed in the hospital. The test materials were gently removed from application sites without rubbing to avoid cross contamination. The test sites were marked with a permanent marker for further evaluation.

Evaluation

The application sites were assessed at baseline before the first application of test material, and after time periods defined above. The test site reactions were scored for irritation throughout the test, by the same assessor, in increasing severity using a grading scale from grade 0-4 as described in Table 2. Photographs of reaction were taken using a digital camera (Olympus C-730 Ultrazoom).

Moisturizing Effect

For evaluation of the moisturizing effect of THC-P, the skin just above both antecubital fossae was used as the test sites. Corneometer (CM 820, Courage-Khazake, Germany) was used to measure skin hydration. One arm was used as a control site and the other arm received THC-P self-applications by the same group of volunteers twice daily. The first application was demonstrated by the investigators in the hospital. Corneometer measurements were done at baseline before application, then at 30 minutes, 4 hours, 24 hours, and 48 hours after baseline. Each side was measured with the corneometer three times and the mean score value after the three readings was used for statistical analysis to compare the moisture of the side not receiving the THC-P cream with the side applying the test product.

Statistical analysis

Differences of irritation between the test materials and 2.5% SDS were tested for statistical significance using the McNemar test. Analysis of variance with repeated measurement or paired t-test was used to evaluate moisturizing efficacy of the finished product THC-P cream. A p-value of less than 0.05 was considered statistical significant difference.

Results

All 36 volunteers recruited remained until the present study was completed. None of the volunteers developed irritation after the 4 hour occlusion period. For the extended 24 hour patch test occlusion, one subject developed allergic reaction to the Micropore surgical tape on the test areas. Another subject developed a faint erythema (grade 0.5) at all test materials including the negative control site at the 1 hour reading. All of which disappeared at the 24 and 48 hour reading. This may have been due to the irritation from the skin occlusion itself not from any specific test material. These two subjects were excluded from further evaluation. The 24 hour patch test results of the remaining 34 subjects are shown in Table 3. The positive control 2.5% SDS showed a cutaneous irritant response in 23 subjects (67.6%) at 1 hour, 25 subjects (73.5%) at 24 hours and 24 subjects (70.6%) at 48 hours reading. The mean severity grades of reaction were 0.7 ± 0.25, 0.74 ± 0.25, and 0.71 ± 0.25 respectively. Only four of the other test materials (THC-P, THC-N, 100% cleansing gel 1, Vitamin E liposome) showed doubtful reactions (grade 0.5) in at least one subject. THC-P, THC-N, 100% cleansing gel 1, and Vitamin E liposome caused reaction in four subjects, three subjects, one subject, and one subject respectively. These reactions were seen only at the 1 hour reading and disappeared at the 24 and 48 hour reading. Comparison of the skin irritation effect between the positive control chemical, 2.5% SDS and the other test materials at 1 hour reading is shown in Table 4. The incidence of positive irritant reactions to THC-P cream, THC-N cream, the undiluted cleansing gel 1, Vitamin E liposome cream were significantly lower

Table 3. Number (percent) of subjects and grade of irritant response to test material*

Test material	Time (hr)	N (%) Grade of skin reaction (n = 34)					Mean ± SD of severity grade
		Neg	Pos	0.5	1+	2+	
2.5% SDS	1	11 (32.4)	23 (67.6)	14 (41.2)	9 (26.5)	-	0.70 ± 0.25
	24	9 (26.5)	25 (73.5)	13 (38.2)	12 (35.3)	-	0.74 ± 0.25
	48	10 (29.4)	24 (70.6)	14 (41.2)	10 (29.4)	-	0.71 ± 0.25
Distilled water	1	34 (100)	-	-	-	-	-
THC-P cream	1	30 (88.2)	4 (11.8)	4 (11.8)	-	-	0.50
THC-N cream	1	31 (91.2)	3 (8.8)	3 (8.8)	-	-	0.50
100% cleansing gel 1	1	33 (97.1)	1 (2.9)	1 (2.9)	-	-	0.50
Vit E liposome cream	1	33 (97.1)	1 (2.9)	1 (2.9)	-	-	0.50
THC liposome	1	34 (100)	-	-	-	-	-
Blank liposome	1	34 (100)	-	-	-	-	-
8% cleansing gel 1	1	34 (100)	-	-	-	-	-
100% cleansing gel 2	1	34 (100)	-	-	-	-	-
8% cleansing gel 2	1	34 (100)	-	-	-	-	-
Aftersun P cream	1	34 (100)	-	-	-	-	-
Eye gel	1	34 (100)	-	-	-	-	-
Aftersun N cream	1	34 (100)	-	-	-	-	-

* The test materials with no skin reactions at the 24 hour and 48 hour reading are not shown

Table 4. Number of subjects (%) with any grade of irritant reactions at 1 hour reading

Test material		2.5% SDS (n = 34)		p-value*
		irritation	no irritation	
THC-P cream	irritation	4 (11.8)	-	<0.001
	no irritation	19 (55.9)	11 (32.4)	
THC-N cream	irritation	3 (8.8)	-	<0.001
	no irritation	20 (58.8)	11 (32.4)	
100% cleansing gel 1	irritation	-	1 (2.9)	<0.001
	no irritation	23 (67.6)	10 (29.4)	
VitE liposome cream	irritation	-	1 (2.9)	<0.001
	no irritation	23 (67.6)	10 (29.4)	

* McNemar test

Table 5. Mean ± SD corneometer measurement of skin moisture and mean difference of skin applying and not applying THC-P cream

Test area	Mean ± SD (n = 34)				
	Baseline	30 min	4 hr	24 hr	48 hr
with THC-P	48.3 ± 10.7	61.6 ± 10.3	59.6 ± 10.0	60.7 ± 10.2	59.2 ± 7.4
without THC-P	47.8 ± 9.1	50.3 ± 7.0	48.0 ± 9.7	48.0 ± 10.8	49.7 ± 8.0
Mean difference	0.44	11.28*	11.56*	12.66*	9.50*

* Paired t-test Significant (p-value < 0.001)

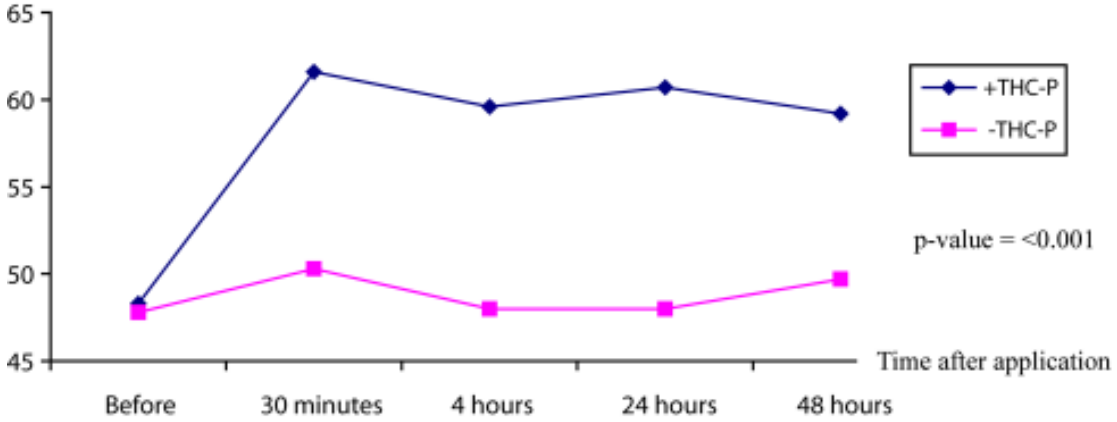

Fig. 1 Skin moisture of area applying THC-P cream and control area not applying THC-P Cream

than the skin reaction to 2.5% SDS (p- value < 0.001). The other eight test materials and negative control showed negative results throughout the testing period.

The moisturizing effect of the THC- P cream compared with that of the control sites not applying the cream measured by corneometer are shown in Table 5 and Fig. 1.

Statistical analysis using ANOVA with repeated measurement showed the corneometer measurement of moisture in skin applying THC-P cream to be significantly (p-value < 0.001) higher than control skin not applying the cream at every time follow up measurement up to 48 hours (Table 5).

Discussion

Many procedures for skin safety testing of newly developed chemicals and finished products exist. In 1944, Draize published a method for assessing skin corrosion and acute irritation in rabbits that has served as the basis for classification of skin corrosion and irritation hazard to human[3-5]. However, the Draize test has come under increasing criticism for two main reasons, animal welfare concerns, and the poor predictive validity for human response[6-8]. This has led to the emergence of the development of new ethical testing and risk assessment strategies in human subjects[9].

In the mid-1990s, Basketter et al developed the human-4-hour patch test for acute "chemical" irritation classification[10,11] but cosmetic products and ingredients do not usually cause acute irritation to the consumer. In 1996 a Task Force of COLIPA, the European Cosmetic, Toiletry, and Perfumery Association, published test guidelines for assessment of skin compatibility of cosmetic ingredients and finished products in man. The aim of the test(s) published was not to classify the ingredient for its effect on the skin but to determine whether it is likely to induce skin irritation under normal condition and reasonably foreseeable misuse. Examples of direct ingredient/formulation application on human tests include single/repeat application open epicutaneous test, and single/repeat application closed patch epicutaneous test under occlusion or semi-occlusion[12,13].

This human single closed patch test the authors conducted to assess the skin irritation potential of Thai GPO cosmetic ingredients and finished products in volunteers was valid and ethical. It can be done utilizing test devices available in Thailand. The positive control used 2.5% SDS produced doubtful to weak tolerable reactions between 67.6-73.5% of the subjects (average severity score ranging from 0.70 $\pm$ 0.25 to 0.74 $\pm$ 0.25) depending on the time of reading. The variation in severity and grade of irritation between the subjects from none to 0.5, 1+, and 2+ is normal due to wide inter-individual variability in human skin irritation responses and due to other environmental conditions[14,15]. For this reason, skin irritation responses in humans are usually comparative.

In the present study, the authors used exaggerated conditions of application that is the extended period for 24 hours and occlusive patch test instead of open or semi-occlusive testing. This is because the test materials are cosmetic ingredients most intended for use as leave on products. In addition, the authors strictly performed readings on skin irritation reactions including even minimal faint or doubtful erythema (0.5 grade) at 1 hour reading as positive reaction. Even so,

all of the Thai GPO supplied materials tested were significantly less irritating than the positive control 2.5% SDS.

In summary, the test materials and finished products developed by the GPO are not likely to induce skin irritation under normal conditions of use. The authors also demonstrated that twice-daily application of the finished product, THC-P cream (the commercial GPO curmin cream) could significantly increase skin hydration.

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การศึกษาฤทธิ์ระคายเคืองต่อผิวหนังมนุษย์ ของ ครีมสมุนไพร ขมิ้นชัน จีพีโอเคอร์มิน และส่วนผสมผลิตภัณฑ์ ขององค์การเภสัชกรรม โดยวิธี Single Closed Patch Testing

เพ็ญพรรณ วัฒนไกร, สุธิดา สุวรรณโชติ, สุธินี กุลกลการ, ณัฏฐา รัชตะนาวิน

ภูมิหลัง: *องค์การเภสัชกรรม ได้ทำการวิจัยและผลิต ไลโปโซม และสารสกัดจากสมุนไพร ขมิ้นชัน มาใช้เป็นส่วนผสมในผลิตภัณฑ์บำรุงผิว และครีมขมิ้นชัน (ครีมจีพีโอเคอร์มิน)*

วัตถุประสงค์: *เพื่อทดสอบผลการระคายเคืองต่อผิวหนังมนุษย์ของผลิตภัณฑ์ ด้วย วิธี single closed patch test และศึกษาผลของครีมขมิ้นชัน จีพีโอเคอร์มิน ต่อความชุ่มชื้นผิว*

วัสดุและวิธีการ: *ทดสอบผิวหนัง อาสาสมัคร 36 ราย ด้วย 12 สารทดสอบ ขององค์การเภสัชกรรม เช่น ไลโปโซม, สารสกัดจากขมิ้นชัน เตตระไฮโดรเคอร์คูมินอยด์, ครีมขมิ้นชันจีพีโอเคอร์มินครีม ใช้ 2.5% sodium dodecyl sulfate (SDS) เป็นตัวชี้วัดบวก แผ่นทดสอบใช้ Finn chambers บนเทป Scanpor ตรงกลางบุสำลี webril ปิดลงบนผิวหนัง หลังเปิดแผ่นทดสอบผู้วิจัยประเมินปฏิกิริยาระคายเคืองต่อผิวหนัง นำอุบัติการณ์การเกิดปฏิกิริยาระคายเคืองผิวหนังของ 2.5% SDS และสารทดสอบมาวิเคราะห์ความแตกต่าง ความชุ่มชื้นผิวหนังใช้เครื่อง corneometer วัดผิวหนังบริเวณที่ทา และไม่ได้ทาครีม จีพีโอเคอร์มิน*

ผลการศึกษา: *อาสาสมัครหญิงทั้ง 36 ราย ร่วมงานวิจัยจนครบ เมื่อเปรียบเทียบการเกิดผลบวกปฏิกิริยาระคายเคืองผิวหนังจาก ตัวชี้วัดบวก กับผลิตภัณฑ์ทดสอบ พบว่า สารทดสอบเกิดการระคายเคืองน้อยกว่าตัวชี้วัดบวกอย่างมีนัยสำคัญทางสถิติ (p-value < 0.001, McNemar test) ส่วนค่าความชุ่มชื้นของผิวหนัง บริเวณที่ทาครีมมีค่าสูงกว่าผิวหนังที่ไม่ได้ทาครีมอย่างมีนัยสำคัญทางสถิติ (p-value < 0.001, paired t test)*

สรุป: *สารทดสอบทำให้ระคายเคืองน้อย และบริเวณทาครีมมีค่าความชุ่มชื้นของผิวหนังสูง*