



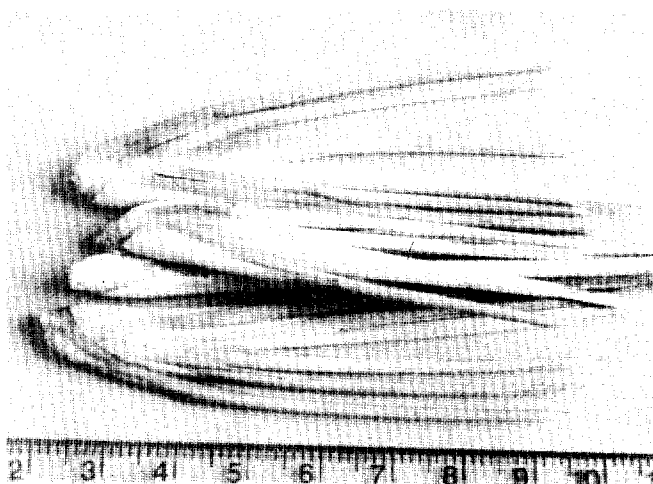
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PRIMARY SKIN IRRITATION STUDIES OF ANTIFUNGAL LEMON GRASS OIL CREAM IN RABBITS

THAILAND INSTITUTE OF SCIENTIFIC AND TECHNOLOGICAL RESEARCH



BY

JAKKRAPONG LIMPANUSSORN
PRAPAIPAT KLUNGSUPYA
PATTAMA SOONTORNSARATUNE
TUANTA SEMATONG

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THAILAND INSTITUTE OF SCIENTIFIC AND TECHNOLOGICAL RESEARCH

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THE STUDY OF ANTIFUNGAL ACTIVITY OF TAKHRAI
(*Cymbopogon citratus* Stapf) AND TAKHRAI HOM (*Cymbopogon nardus* Rendle)

REPORT NO. 3

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IN RABBITS

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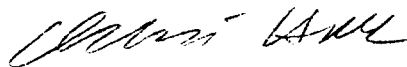
JAKKRAPONG	LIMPANUSSORN
PRAPAIPAT *	KLUNGSUPYA
PATTAMA	SOONTORNSARATUNE
TUANTA	SEMATONG

EDITORS

VALYALADA HONGTHONG
NARUMOL RUENWAI

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(Mr. Chalermchai Honark)

Governor

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การศึกษาความระคายเคืองเบื้องต้นต่อผิวหนังของครีมน้ำมันตะไคร้ต้านเชื้อราในกระต่าย
โดย จักรพงษ์ ลิ้มปุ่นสุวรรณ, ประไพภัทร คลังทรัพย์, ปัทมา สุพรรณคารทูล และ
เตียนตา เสมาทอง

บทคัดย่อ

จากการศึกษาความระคายเคืองเบื้องต้นต่อผิวหนังของครีมน้ำมันตะไคร้ต้านเชื้อราในกระต่าย ซึ่งผลิตโดยสาขาวิจัยอุตสาหกรรมเภสัชและผลิตภัณฑ์ธรรมชาติ (สวภ.) สถาบันวิจัย-วิทยาศาสตร์และเทคโนโลยีแห่งประเทศไทย (วท.) โดยศึกษาตามวิธีของ Draize และคณะ (1944) พบว่าครีมน้ำมันตะไคร้เข้มข้น 2.5% ให้ผลการทดสอบไม่แตกต่างจากยารักษาโรคผิวหนัง Travogen^(R), Fungisil^(R) และ Tonaf^(R).

PRIMARY SKIN IRRITATION STUDIES OF ANTIFUNGAL
LEMON GRASS OIL CREAM IN RABBITS

By Jakkrapong Limpanussorn*, Prapaipat Klungsupya*,
Pattama Soontornsaratune* and Tuanta Sematong*

ABSTRACT

The primary skin irritation studies of antifungal lemon grass oil creams were conducted in healthy adult rabbits, New Zealand White hybrid strain. The antifungal lemon grass oil creams were prepared by Pharmaceuticals and Natural Products Department (PNPD), Thailand Institute of Scientific and Technological Research (TISTR). The experiments were conducted according to Draize et al. (1944). It was found that 2.5% antifungal lemon grass oil creams showed no different results from Travogen^(R), Fungisil^(R) and Tonaf^(R).

* Pharmaceuticals and Natural Products Department,
Thailand Institute of Scientific and Technological Research

INTRODUCTION

According to the research project "Modern pharmaceuticals and natural products based on Thai traditional pharmacopoeia", of Pharmaceuticals and Natural Products Department (PNPD), Thailand Institute of Scientific and Technological Research (TISTR), the antifungal activities of various Thai medicinal plants have been screened. Lemon grass oil was proved to possess superior quality to the others and the results agreed with previous works (Moleyer and Narasimham 1988; Onawunmi 1989). Then, the cream containing lemon grass oil as an active principle was prepared for *in vitro* study. The cream demonstrated promising activity for further studies (Wannisorn et al. 1994). The acute oral and acute dermal toxicity studies of the cream showed no toxic signs and no mortalities at the dose of 2,000 mg/kg (Limpanussorn et al. 1995).

The aim of the present study was to investigate primary skin irritation of the antifungal lemon grass oil cream for subsequent clinical study.

MATERIALS AND METHODS

Material

Antifungal lemon grass oil creams	TISTR formula
Travogen ^(R)	Schering, Germany
Fungisil ^(R)	Silom Medical Corporate, Thailand
Tonaf ^(R)	Song Thai Medical Co., Ltd., Thailand
Rabbits, New Zealand White hybrid strain	Department of Animal Science, Faculty of Agriculture, Kasetsart University

Rabbit feed	Starfeed, Krungtappokphand Co, Ltd.
Drinking water	Filtered water
Patch (10 layers of sterilized gauze cut in squares, 2.5 cm x 2.5 cm)	
Autoclave, model SS-320	Tomy Limited, Japan
Electric clipper	Wahl Clipper Corp., Sterling, Illinois, U.S.A.
Adhesive tape	Leukoplast porous. BDF Intanin Co., Ltd., Thailand.
Elastic bandage	Pack and Grand, CCN Co., Ltd. Thailand
Cotton wool, 1 ml glass syringe, disposable needle; size 21Gx1"	

Methods

Primary skin irritation test was conducted according to Draize et al. (1994).

Rabbits were acclimatized to the laboratory environment for one week. Six animals were used for each test material. One day before experimentation, an area of skin approximately 10 cm x 10 cm on the dorso-lumbar region of each rabbit was clipped free of hair. Two areas of the skin approximately 2.5 cm x 2.5 cm, one on each flank, were selected. The area on one flank was abraded using a sterilized needle.

Each test material of 0.5 g was introduced under 2.5 cm x 2.5 cm gauze patch. The patches were applied to intact and abraded skin sites on each rabbit. The patches were then secured to the skin by adhesive tape. The entire trunk of the rabbit was wrapped with elastic cloth to avoid dislocation of the patches for

an exposure period of 24 hours. The animals were not restrained. At the end of the exposure period, the dressing was removed and the skin was wiped with a moistened cotton wool to remove any residual test material. The animals were assessed for the degree of erythema and oedema evidence at each site at 30 to 60 minutes (0 hour) and approximately 48 hours following removal of the dressings. The following numerical scoring system was used.

Erythema and eschar formation:

No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4

Oedema formation:

No oedema	0
Very slight oedema (barely perceptible)	1
Slight oedema (edges of area well-defined by definite raising)	2
Moderate oedema (raised approximately 1 millimeter)	3
Severe oedema (raised more than 1 millimeter and extending beyond the area of exposure)	4

Classification of irritation

The numerical scores for erythema and oedema at the intact and abraded skin sites of all six rabbits, at both 0 and 48 hours readings, were totalled. The sum was divided by 24 and the value obtained termed the Primary Irritation Index (PII). The test material was classified as follows (Drazie J.H. 1959):

<u>Primary irritation index</u>	<u>Classification</u>
0.0 - 0.4	Non - irritant
0.5 - 1.9	Slightly irritant
2.0 - 4.9	Moderately irritant
5.0 - 8.0	Severe irritant

RESULTS

The scores and primary irritation index (PII) for skin reactions are shown in Tables 1-5.

TABLE 1. SCORES FOR SKIN REACTIONS OF PRIMARY SKIN IRRITATION STUDY OF 2.5% ANTIFUNGAL LEMON GRASS OIL CREAM A

Rabbit No.	Intact skin				Abraded skin			
	0 hr		48 hr		0 hr		48 hr	
	Erythema	Oedema	Erythema	Oedema	Erythema	Oedema	Erythema	Oedema
1	2	0	0	0	2	0	0	0
2	1	0	0.5	0	1	0	0	0
3	1	0	1	0	1	0	1	0
4	2	0	2	0	2	0	2	0
5	2	0	1.5	0	2	0	1.5	0
6	2	0	0	0	2	0	0	0

Primary irritation index = 1.23

TABLE 2. SCORES FOR SKIN REACTIONS OF PRIMARY SKIN IRRITATION STUDY
OF 2.5% ANTIFUNGAL LEMON GRASS OIL CREAM G

Rabbit No.	Intact skin				Abraded skin			
	0 hr		48 hr		0 hr		48 hr	
	Erythema	Oedema	Erythema	Oedema	Erythema	Oedema	Erythema	Oedema
1	1	0	0	0	1	0	0	0
2	2	0	1	0	2	0	1	0
3	2	0	1	0	2	0	1	0
4	2	0	1.5	0	2	0	1.5	0
5	2	0	2	0	2	0	2	0
6	2	0	1.5	0	2	0	1.5	0

Primary irritation index = 1.50

TABLE 3. SCORES FOR SKIN REACTIONS OF PRIMARY SKIN IRRITATION STUDY
OF TRAVOGEN^(R)

Rabbit No.	Intact skin				Abraded skin			
	0 hr		48 hr		0 hr		48 hr	
	Erythema	Oedema	Erythema	Oedema	Erythema	Oedema	Erythema	Oedema
1	2	0	0	0	1	0	0	0
2	0	0	0	0	0	0	0	0
3	2	0	0.5	0	2	0	0.5	0
4	2	0	0	0	2	0	0	0
5	2	0	2	0	2	0	2	0
6	1	0	0	0	1.5	0	0	0

Primary irritation index = 0.94

TABLE 4. SCORES FOR SKIN REACTIONS OF PRIMARY SKIN IRRITATION STUDY
OF FUNGISIL^(R)

Rabbit No.	Intact skin				Abraded skin			
	0 hr		48 hr		0 hr		48 hr	
	Erythema	Oedema	Erythema	Oedema	Erythema	Oedema	Erythema	Oedema
1	0	0	0	0	0	0	0	0
2	2	0	0.5	0	2	0	0	0
3	2	0	1	0	2	0	1	0
4	2	0	2	0	2	0	2	0
5	2	0	2	0	2	0	2	0
6	1.5	0	1	0	1.5	0	0.5	0

Primary irritation index = 1.29

TABLE 5. SCORES FOR SKIN REACTIONS OF PRIMARY SKIN IRRITATION STUDY
OF TONAF^(R)

Rabbit No.	Intact skin				Abraded skin			
	0 hr		48 hr		0 hr		48 hr	
	Erythema	Oedema	Erythema	Oedema	Erythema	Oedema	Erythema	Oedema
1	1	0	1	0	1	0	1	0
2	2	0	1	0	2	0	2	0
3	2	0	2	0	2	0	2	0
4	1	0	0	0	1	0	0	0
5	1.5	0	0.5	0	1.5	0	1	0
6	2	0	0.5	0	2	0	0	0

Primary irritation index = 1.25

CONCLUSIONS AND DISCUSSION

Primary skin irritation studies of antifungal lemon grass oil creams were conducted according to Draize et al. (1994).

The test materials were applied to the intact and the abraded skin sites on each rabbit under semioclusive condition for an exposure period of 24 hours.

The primary irritation index (PII) of 2.5% antifungal lemon grass oil cream A, 2.5% antifungal lemon grass oil cream G, Travogen^(R), Fungisil^(R) and Tonaf^(R) were 1.23, 1.50, 0.94, 1.29 and 1.25, respectively. Therefore, the values of PII of 2.5% antifungal lemon grass oil cream A and 2.5% antifungal lemon grass oil cream G were in the same range as the drugs available in the market, Travogen^(R), Fungisil^(R) and Tonaf^(R).

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