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Evaluation of skin-irritancy potential of a skin ointment containing the essential oil of *ocimum sanctum* L (Basil/Thulasi) leaves in Wistar albino rats

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Abstract

Skin irritant potential of a skin ointment containing 4% essential oil extracted from the *Ocimum sanctum* L. (Basil/Thulasi) leaves was evaluated on the hair clipped skin of female Wistar albino rats. The calculated Primary Irritation Index was '0' and it falls under the category of "negligible irritant."

Keywords: *Ocimum sanctum* L, essential oil, Skin irritancy test, primary irritation index, wistar albino rat, Basil / Thulasi

Introduction

Treatment of wounds is one of the important issues in medicine. Wounds affect the normal activities and diminish the quality of life resulting in enormous social and economic implications.

Therapeutic dressings with plant extracts of wounds are gaining importance because of the multifactorial properties of plant extracts (such as anti-bacterial, anti-oxidant, and anti-inflammatory). There are many experimental evidences to suggest that plant products have beneficial properties on wound healing as well on many skin diseases. ^[1]

Ocimum sanctum Linn. (Basil/Thulasi) a commonly available plant, belongs to the class Magnoliopsida and is found to have anti-inflammatory, analgesic, immunostimulatory, free radical scavenging and anti-microbial activity. ^[2,3] it is widely distributed throughout India and different parts of the world.

The wound healing property of *Ocimum sanctum* has been studied in animals by investigators using aqueous suspension or alcoholic extract. Vaibhav K Gupta *et al* have reported that the combined orally administered aqueous solution of *Ocimum sanctum* and local silver sulfadiazine ointment is highly effective in healing the wound. ^[4] Somashekar *et al* have reported that alcoholic and aqueous extract of *Ocimum sanctum* Linn significantly increased wound breaking strength, hydroxy proline, hexuronic acid, hexosamines, superoxide dismutase, catalase, reduced glutathion and significantly decreased the percentage of wound contraction and lipid peroxidation when compared with controls and thus favour faster wound healing in rats. ^[5] Asha *et al* have reported that the oral aqueous extract administration and the topically applied aqueous extract of *Ocimum sanctum* L in glycerine promoted better granulation tissue, early and complete epithelialization and better tensile strength compared to the controls in Albino rats. ^[6] All these studies reveal that *Ocimum sanctum* has great potential for wound healing.

Essential oils are one of the commonly used natural products, with one of their main applications being for their use in dermatology. ^[7, 8] At least 90 essential oils have been identified for dermatologic use, with at least 1500 combinations. Essential oils from plants have many beneficial properties, such as wound healing, anti-bacterial, and anti-inflammatory. For many decades essential oils have been used safely in perfumery, food and sanitation products. However, essential oils or their main components are scarcely used in wound healing.

We evaluated the skin irritation property of *Ocimum sanctum* essential oil (Os EO) ointment in Wistar albino rats as an initial step for future use of the *Ocimum sanctum* essential oil ointment in animal models and subsequently in human volunteers.

Materials and Methods

Extraction of essential oil

200 grams of *Ocimum sanctum* L. (Basil / Thulassi) leaves, washed in clean water were boiled with 400 ml of distilled water in the Clevenger apparatus and the vapor was cooled to collect the essential oil and water. The essential oil was separated from water and stored at 2 to 8 °C in a tightly closed container and kept in dark.

Preparation of 4% (v/w) Os EO containing skin ointment

as per the British Pharmacopoeia.^[9] A simple skin ointment was prepared by fusion method. 4 ml of Os EO was added to Wool fat 5 gm, Cetostearyl alcohol 5 gm, hard paraffin 5 gm, White soft paraffin 85 gm and stirred continuously in a closed condition. The preparation was stored at 2-8 °C.

Preparation of skin ointment base without Osseo: The above ingredients without Essential oil were mixed and stored at 2-8 °C.

OECD guideline 404 for testing of chemicals -Acute Dermal Irritation/Corrosion was followed.^[10] The test protocol was approved by the Institutional Animal Ethics Committee (IAEC) and care of the animals was taken according to the

guidelines of CPSCEA, Ministry of Forests and Environment, Government of India.

The skin irritation test was conducted in adult Wistar Albino female rats weighing between 210 to 230 grams. Two group of rats (each group consisting of three animals) were used. Approximately 24 hours before the test, the hair of the animals were removed by closely clipping the hair on two areas on the dorsal side, 1cm from the midline of the vertebral column on either side of the vertebrae. The animals had intact and healthy skin. Skin was not abraded during the procedure. One side of the hair clipped area of the test animal was used as a control, wherein nothing was applied and on the other side of the hair clipped test animal 0.5 gram of Os EO containing skin ointment was applied over an area of 6 cm x 6 cm of the hair clipped skin, covered with a gauze patch, which was held in place with a non-occlusive bandage. The animals were observed for signs of erythema and edema and the response scored at 60 minutes (Table-1). The animal was observed for 4 hours and then returned to individual cage. After 24 hours, the bandage and gauze were removed and the sites were observed for signs of erythema and edema and the response scored. The observations were repeated again at 48 hours and 72 hours. (Table -1)

Table 1: Grading of skin reactions for erythema and edema

Reaction	Score
Erythema and Escher formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to Escher formation preventing grading of erythema	4
Maximum possible score: 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 mm)	3
Severe edema (raised more than 1 mm and extending beyond area of exposure)	4
Maximum possible score: 4	

The control group of animals was tested in the same way, except that instead of Os EO containing ointment, only 0.5 gm of the preparation containing the ointment base alone was used^[11]. The animals were kept in a room at 20 °C (± 2 °C) and the relative humidity was between 50-60%. For feeding, conventional laboratory diet was used with an unrestricted supply of drinking water.

Score of Primary Irritation (SPI) calculation

The erythema and edema scores were summed at 1 hour, 24 hour, 48 hour, and 72 hour. The summed score was divided by the number of observations. (Table-2)

$$\text{SPI for each animal} = \frac{\sum \text{Erythema and edema score at 1 hr, 24 hr, 48 hr, and 72 hr}}{\text{Number of observations}}$$

The SPI values for the three test animals were summed and divided by the number of animals to get the SPI (test animals). Similarly the SPI value for (base ointment animals) was calculated for the base ointment animals.

Calculation of the Primary Irritation Index (PII)

The difference between the summation of SPI scores of animals from the test group and base ointment group were calculated. The PII was calculated as the arithmetical mean of the SPI values of the number of animals.

$$\text{PII} = \frac{\sum \text{SPI (Test ointment)} - \sum \text{SPI (Base ointment)}}{\text{Number of animals}}$$

Results

Erythema or edema was not observed in all the three test group animals at any of the specified time (at 1 hr, 24 hr, 48 hr, and 72 hr) after application of the Os EO ointment. Similarly erythema and edema were not observed in all the three ointment base group animals at 1 hr, 24 hr, 48 hr, and 72 hr.

The SPI for each animal at the specified times were 0 (Table 2). The Primary Irritation score (PII) for the Os EO ointment was found to be 0, which falls under the category of negligible irritant". (Table 3).

Table 2: Score of Primary Irritation in Wistar albino female rats after application of *Ocimum sanctum* Essential Oil containing ointment or base of the ointment alone over skin.

Rat No.	Reaction	Score of Os EO containing ointment				Score of ointment containing only the base of ointment			
		1 hour	24 hour	48 hour	72 hour	1 hour	24 hour	48 hour	72 hour
1	Erythema	0	0	0	0	0	0	0	0
	Edema	0	0	0	0	0	0	0	0
2	Erythema	0	0	0	0	0	0	0	0
	Edema	0	0	0	0	0	0	0	0
3	Erythema	0	0	0	0	0	0	0	0
	Edema	0	0	0	0	0	0	0	0

Table 3: Skin response (Erythema and edema) categories

Category	Primary Irritation Index (PII)
Negligible	0-0.4
Slight irritation	0.5-1.9
Moderate irritation	2.0-4.9
Severe irritation	5.0-8.0

Discussion

Essential oils are used mostly for the treatment of human skin infections (such as bacterial, fungal and viral), inflammatory skin conditions (such as dermatitis, eczema, and lupus), and general skin manifestations (such as wrinkles, scars, and scabs). Essential oils also have wound healing potentials.^[1]

Some human clinical studies have evaluated the efficacy of essential oils on human skin ulcers. Warnke et al have reported that essential oil of *Eucalyptus globulus* caused a decrease in inflammation, reduction of the odour, and improved wound healing malodorous necrotic ulcers of cancer patients.^[12]

Orafidiya et al have reported that 2% and 5% *Ocimum gratissimum* oils in hydrophilic cetomacrogol base reduced acne lesions faster than standard therapy and the oils were well tolerated.^[13] Bassette et al have reported that 5% *Melaleuca alternifolia* essential oil gel showed improvement in the acne lesions.^[14] Matis et al have reported that the combined essential oils of *Citrus sibensis* and *Ocimum basilicum* resulted in 75% improvement in the rate of acne lesions healing.^[15] Chin and Cordell have reported that *Melaleuca alternifolia* essential oil gel showed an accelerated healing rate of abscessed wounds and cellulitis.^[16]

Blackwood et al have opined that there was a concluding evidence to show that there was definitely a potential for the use of *Melaleuca alternifolia* (tea tree) oil as an additional/alternative treatment to standard wound treatments.^[17] Hartman et al have reported that *L. angustifolia* and *Matricaria recutita* essential oil mixture lead to complete wound healing in four of five patients with chronic leg ulcers.^[18] Gaba, et al have reported a successful essential oil combination of *L. angustifolia*, *Artemisia vulgaris*, and *Salvia officinalis* in treating chronic wounds such as venous ulcers, pressure sores, skin tears, and abrasions. They have speculated that the essential oils had increased the circulation and vascular permeability resulting in accelerated angiogenesis.^[19]

Evaluation of the skin irritation of pharmaceutical and cosmetic products containing natural products is an important requirement of their biocompatibility. In the present study, the 4% essential oil (extracted from the *Ocimum sanctum* L. leaves) skin ointment was applied over the hair clipped skin area of female Wistar albino rats. The calculated Primary Irritation Index was '0' and it falls under the category of "negligible irritation" with respect to erythema and edema induction.

The present study indicates that the 4% *Ocimum sanctum* L. (Basil / Thulassi) essential oil containing ointment is a safe

Conclusion

The present study indicates that the 4% *Ocimum sanctum* L. (Basil / Thulassi) essential oil containing ointment is a safe

skin formulation for female Wistar albino rats. Further animal studies are required to evaluate the safety, wound healing potential and anti-bacterial effect of the 4% *Ocimum sanctum* L. essential oil skin ointment.

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Conflict of Interest

The authors have no conflict of interest regarding this investigation.

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