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Effect of *Commiphora mukul* in Chronic Oxazolone Induced Mouse Dermatitis Model

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Abstract: Psoriasis is a common, immune-mediated, multifactorial disease characterized by phenotypic diversity and genetic heterogeneity. The objective of this study was to evaluate the effect of *Commiphora mukul* on psoriasis. Six female Balb/C mice per group of which, the first three groups served as untreated, disease controls and standard (0.1% dexamethasone) respectively. Animals of groups 4, 5 and 6 were applied 0.5, 1 and 2% of the *C. mukul* extract on both the ears, respectively. Dermatitis was induced in mice by the application of oxazolone 1.5% (100 µL in ethanol) to the abdominal region for a period of six days. Starting seven days following sensitization, 20 µL of oxazolone 1% in a mixture of acetone and olive oil (4:1) was applied to both sides of the mouse ear on days 7, 10, 13 and 16. For detailed time-course analysis of ear swelling reactions, ear thickness was measured before sensitization phase (day 7) and after each elicitation on days 10, 13, 16 and 19. Results: *C. mukul* potently suppressed ear swelling at each time-point. The suppressive rates of *C. mukul* at concentrations of 0.5, 1 and 2% were 47.3, 55.4 and 62.2% on day 16, respectively as compared to the disease control. Microscopic examination revealed a relatively swollen ear in the disease model as compared to the control animals. Whereas condition was gradually improved in treated groups dose-dependently. The results suggest that *C. mukul* improves chronic inflammatory skin disorders.

Key words: *Commiphora mukul* · Dermatitis · Interferon-γ · Oxazolone · TNFα

INTRODUCTION

Psoriasis is a common, immune-mediated, multifactorial disease. It is characterized by its vast phenotypic diversity and genetic heterogeneity. Psoriasis has an unknown etiology affecting 1 to 3% of Caucasians [1]. The most frequently affected than other ethnic groups were the Caucasians [2] the rationality behind these variations are unclear. However, it is likely that both genetic and environmental factors play a role. *Psoriasis vulgaris,* the most common form of psoriasis, is characteristic of sharply demarcated, red and scaly symmetrical plaques on the elbows, knees or scalp [3]. It is a chronic inflammatory disease of the skin characterized by epidermal hyperplasia, dermal angiogenesis, infiltration of activated T cells and increased cytokine levels. T cell-mediated immunity [4-7] in which cytokines play an essential role, is considered to be the key element in the disease process.

Corticosteroids, immunosuppressants and non-steroidal anti-inflammatory drugs that inhibit cyclooxygenase (COX)-2 are being used clinically for the treatment of psoriasis. Systemic therapies with drugs such as acitretin, methotrexate, cyclosporine, hydroxyurea and thioguanine revealed significant systemic toxicity that needed to be followed carefully. Dramatic skin atrophy is characteristic of corticosteroids upon repeated application on the dorsal skin of rats [8-10].

Commiphora mukul is found to grow in the wilder parts of the Indian states, especially Rajasthan, Karnataka, Maharashtra, Gujarat, Assam as well in neighboring countries *viz.,* Afghanistan, Baluchistan,

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Arabia and northeast Africa in rocky dry areas [11-13]. It has been used for nearly 3000 years in Ayurvedic medicine, mainly as a treatment for arthritis. However, no scientific evidence or publications are available to support *C. mukul*'s therapeutic effect towards the psoriasis, though used traditionally throughout the world. The study of the anti-psoriatic effect of *C. mukul* conducted in the oxazolone-induced mouse contact dermatitis model provides a rational scientific proof that the herb indeed has the potential to treat psoriasis.

MATERIALS AND METHODS

Plant Material (Test Material): Commercially available ethanol extract of *Commiphora mukul* (Batch Number: CM/06001) was procured from Natural Remedies private Ltd., Bangalore, India. It was stored in air-tight container at room temperature.

Animals: The study was carried out in accordance with the Protocol N° 07/IAEC-03/TOX/2005, approved by Institutional Animal Ethics Committee (IAEC), Research and Development, Orchid Chemicals and Pharmaceuticals Limited, Chennai.

A total of 36 female Balb/C mice randomized into six groups consisting of six animals per group were used for the study. Group 1 animals were used as untreated control, which did not underwent any sensitization or elicitation procedures and treatment during the study. Dermatitis was induced to the animals of groups 2 to 6 with oxazolone. Group 2 animals served as the disease control not receiving any treatment with the *C. mukul* extract. Group 3 animals were treated by ear application, with 0.1% dexamethasone. Animals of groups 4, 5 and 6 received 0.5, 1 and 2% of the *C. mukul* extract on both the ears, respectively. The dose volume was maintained at 20 µL uniformly.

Sensitization and Elicitation (Challenge Application) Procedure: The animals were sensitized by applying 100 µL of 1.5% oxazolone in ethanol to the abdominal region of the animals for a period of six days. Seven days after sensitization, 20 µL of 1% oxazolone in a mixture of acetone and olive oil (4:1) was applied to both sides of the mouse ear on days 7, 10, 13 and 16. Sensitization and elicitation (challenge) treatments were carried out to induce dermatitis in the animals.

Measurements: During the study, ear thickness was measured with digital Vernier Calipers (Mitutoyo, Japan) at various time points. Ear thickness was measured before sensitization phase (Day 7) and after each elicitation on days 10, 13, 16 and 19 in order to evaluate ear swelling reactions.

Animals were euthanized and mouse ears were excised, fixed in 10%-buffered formalin solution, embedded in paraffin, cut into 5 µm sections and stained with hematoxylin-eosin, 72 hours after the last application of oxazolone, by standard methods. During histopathological evaluation, after the microscopic fields were photographed, the epidermal thickness was measured as the distance from the bottom of the stratum corneum to the basement membrane in the interfollicular epidermis (Reynolds *et al*., 1998).

Inhibition of ear swelling (%), ear weight and epidermal thickness were calculated according to the following equation:

$$\text{Inhibition (\%)} = \frac{\text{Scores of the disease model - Scores of standard (Animals treated with 0.1\% dexamethazone) and C. mukul treated animals}}{\text{Scores of the disease model - Untreated control}}$$

The statistical significance ($P \leq 0.05$) was determined using Student's *t*-test using the statistical software GraphPad Prism 4. The data are represented as mean ± standard deviation (SD).

RESULTS AND DISCUSSION

Topical administration of *C. mukul* in an oxazolone-induced dermatitis mouse model to the ear of the disease model group (Group 2) revealed erythema (reddening of the skin), edema and abrasion of the skin occasionally. The positive agent (dexamethasone 0.1%) potently suppressed oxazolone-induced ear swelling ($P \leq 0.01$) at the rate of 76% on day 16 (Table 1). It is widely recognized that the secretion of cytokines by keratinocytes in response to injury, particularly TNF-α and IL-1α are key mediators of the cutaneous inflammatory response [14-15]. *C. mukul* at the concentrations of 0.5 and 1% potently suppressed ($P \leq 0.05$) ear swelling at each time-point (Table 2) whereas *C. mukul* at the concentration of 2% potently suppressed ($P \leq 0.01$) ear swelling at each time point (Table 2). The rate of suppression by *C. mukul* 0.5, 1 and 2% were 47.3, 55.4% and 62.2% on day 16, respectively as compared to the disease control (Table 1).

C. mukul treatment has been shown to reduce cytokine-induced activation of a number of pro-inflammatory genes in endothelial cells and macrophages, including vascular cell adhesion molecule-1, cyclo-oxygenase-2 and IL-6.

Table 1: Effect of *C. mukul* and dexamethasone on the inhibition of thickness, weight and epidermal thickness of mouse ear induced by repeated application of oxazolone

	Inhibition (%)	*C. mukul* (%)		
Parameters	Dexamethasone	0.5	1	2
Ear thickness	75.7	47.3	55.4	62.2
Ear weight	74.5	42.2	54.0	69.2
Epidermal thickness	77.0	16.0	43.3	65.5

Table 2: Effect of *C. mukul* on the thickness (mm) of mouse ear induced by repeated application of oxazolone

		Days			
Group	Treatment	7	10	13	16
1	Vehicle	0.31±0.03	0.31±0.03	0.32±0.03	0.32±0.02
2	Oxazolone	0.32±0.02	0.54±0.02	0.63±0.08	1.06±0.08
3	Dexamethasone	0.31±0.02	0.47±0.03	0.48±0.07	0.50±0.40**
4	*C. mukul* 0.5%	0.32±0.03	0.45±0.07	0.52±0.10	0.71±0.09*
5	*C. mukul* 1 %	0.31±0.02	0.44±0.04	0.51±0.07	0.65±0.08*
6	*C. mukul* 2 %	0.33±0.02	0.43±0.08	0.59±0.05	0.60±0.06**

* p< 0.05 or ** p< 0.01 significantly lower than disease control (oxazolone). Values are mean ± S

Table 3: Effect of *C. mukul* on the change in weight (g) and epidermal thickness (µm) of mouse ear induced by repeated application of oxazolone

				C. mukul (%)		
Parameters	Vehicle	Oxazolone	Dexamethasone	0.5	1	2
Ear weight (g)	71.5±0.7	242±5.7	115±7.1**	170±14.1*	150±4.1*	124±2.8**
Epidermal thickness (µm)	13.5±0.4	89.7±0.3	31.0±11.7**	77.5±10.8*	56.7±8.1*	39.8±7.4**

* P<0.05 or ** P<0.01 significantly lower than disease control (oxazolone). Values are mean ± SDFig1. Effect of *C. mukul* extract and dexamethasone on ear epidermal thickness of mice treated with oxazolone.

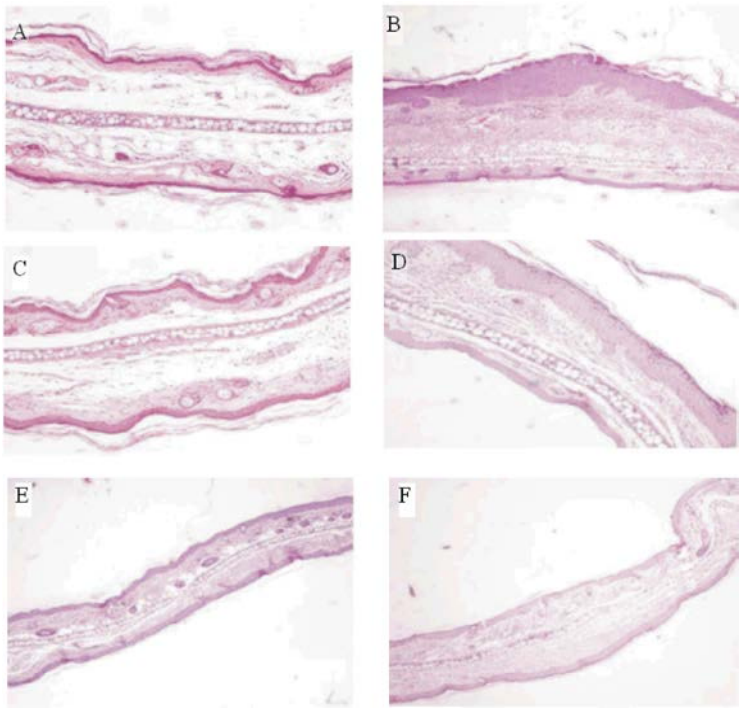

Fig. 1: Effect of C. mukul extract and dexamethasone on ear epidermal thickness of mice treated with oxazolone. (A) Untreated control exhibit a thin epidermal layer; (B) A two to three fold in the ear epidermal thickness in disease control as compared to the untreated control; (C) Decreased ear epidermal thickness in animals treated with dexamethasone (reference drug) at 0.5 %; (D, E and F) Epidermal thickness of the disease induced animals treated with *C. mukul* at concentrations of 0.5, 1 and 2% revealing a significantly reduced epidermal thickness (16.0, 43.3 and 65.5%, respectively). Hematoxylin and eosin stained sections of skin. Magnification x 10.

Thus, the anti-inflammatory effects of *C. mukul* activation could occur at both the induction of TNF-α and IL-1 and the downstream effects of these cytokines on other cells in the skin [16, 17].

Oxazolone treatment of sensitized animals produced a significant increase in ear weight ($P \leq 0.05$) as compared to normal control animals. A dose-dependent ($P<0.05$ and $P<0.01$) decrease in ear weight (Table 3) was observed. Topical treatment of *C. mukul* at 0.5, 1 and 2% reduced oxazolone induced inflammation of ear weight by 42.2, 54 and 69.2% respectively, as compared to the disease control (Table 1). Dexamethasone 0.1%, used as reference drug, also exhibited inhibition (74.5%).

Histopathological Evaluation and Measurement of Epidermal Thickness in the Ear: Histopathological evaluation of mouse ear revealed prominent epidermal hyperplasia and marked infiltration of inflammatory cells (Fig. 1), consisting of monocytes, granulocytes and macrophages, mainly into the dermis and some into epidermis. Microscopic examination showed a relatively swollen ear in the disease model as compared to the control animals. The ear of the untreated control animals exhibited a thin epidermal layer. The severity of the epidermal hyperplasia was assessed by measuring the epidermal thickness induced by oxazolone application. Epidermal thickness (Table 3) was significantly increased in the disease model (two to three folds) as compared to the untreated control. Epidermal thickness of animals treated with *C. mukul* at concentrations of 0.5, 1 and 2 % revealed a significantly reduced epidermal thickness by 16.0, 43.3 and 65.5%, respectively (Table 3), as compared to the untreated control animals. Animals treated with dexamethasone 0.1 % decreased ear epidermal thickness by 77%.

CONCLUSION

The results obtained from the study suggest that *C. mukul* improves chronic inflammatory skin disorders probably through the inhibition of TNFα produced by macrophage cells and interferon-γ produced by the Th1 cells.

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