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RESEARCH ARTICLE

Anti-Psoriatic Effect of *Tribulus Terrestris* Extract by Topical Application in Mouse Model of Contact Dermatitis

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ABSTRACT

Tribulus terrestris L (TT) is used in the Chinese medicine to treat various dermal disorders. The aim of this study was to investigate the anti psoriatic effect of TT. The mouse model of psoriasis was developed by sensitization with 100 µl of 1.5% oxazolone application on dorsal lumbar region for six days followed by elicitation with 20 µl of 1% oxazolone applying on both sides of ear on day 7, 10 and 13. The first three groups of mice comprised as vehicle (mixture of acetone and olive oil (4:1)) control, positive control (dermatitis induced by oxazolone) and treatment with dexamethazone at the dose level of 0.1% topical application on upper surface of the ear. Animals of another three groups were applied 0.5%, 1% and 2% of the *T. terrestris* extract on both the ears after induction of dermatitis by oxazolone. The ear of the positive control mice showed erythema, edema and/or indurations and occasionally abrasion. Oxazolone treatment of sensitized animals produced a significant increase in ear weight as compared normal control animals. A dose dependent decrease in ear weight was observed in TT treated groups. Gross macroscopic examination revealed a relatively swollen ear in the disease model as compared to the control animals. Histopathological examination of the ear belonging to the disease control revealed prominent epidermal hyperplasia and marked infiltration of inflammatory cells like monocytes, granulocytes, macrophages mainly into the dermis and some into epidermis. This investigation suggests that the protective effect of TT for oxazolone induced contact dermatitis.

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INTRODUCTION

Psoriasis is a chronic inflammatory disease of the skin characterized by epidermal hyperplasia, dermal angiogenesis, infiltration of activated T cells, and increased cytokine levels (Christophers, 2001). An increase in mitotic activity in the stratum basale, abnormal keratinization and elongation of the dermal papillae toward the skin surface result in a thicker-than-normal stratum corneum that desquamates to produce large, silvery scales (Griffiths and Voorhees, 1996; Barker, 1991; Krueger and Callis, 2003). Psoriasis patients have been shown to have a bias of interferon (IFN) - γ producing Th1 and cyclooxygenase (COX) - induced macrophage lesions in skin and peripheral blood (Austin *et al*., 1999; Ovigne *et al*., 2002; Hernandez *et al*., 2001). Cyclooxygenase (COX)-2 inhibiting non-steroidal anti-inflammatory drugs, corticosteroids, immunosuppressants like FK-506 and cyclosporine A for Th1 cells have been used clinically for psoriasis. Repeated application of corticosteroids on the dorsal skin of rats causes dramatic skin atrophy. FK-506 and cyclosporine A exhibits side effects, such as severe nephrotoxicity and neurotoxicity (Schafer *et al*., 1996; Sakuma *et al*., 2001; Friedman *et al*., 2002). Systemic therapies such as acitretin, methotrexate, cyclosporine, hydroxyurea and thioguanine are all associated with significant systemic toxicity and have to be closely monitored.

T. terrestris has a long history of uses throughout the world. It has been used in China for more than 400 years to treat conditions such as psoriasis, eczema, premature ejaculation and liver disease (Nadkarni, 1976). Other

ancient Eastern cultures used *T. terrestris* for its diuretic properties and to treat infections. However, no scientific proof or publications are available to support *T. terrestris's* therapeutic effect towards psoriasis, though used traditionally throughout the world. The study of the anti-psoriatic effect of *T. terrestris* conducted in the oxazolone-induced mouse contact dermatitis model provides a rational scientific proof that the herb indeed has the potential to cure psoriasis.

MATERIALS AND METHODS

Drugs and chemicals

T. terrestris extract (Ashwathilakhsmi Mansion) was obtained from Natural Remedies Private Ltd. Bangalore, India. Oxazolone and dexamethasone were purchased from Sigma Co., St. Louis, MO, U.S.A. All other chemicals and solvents used were of analytical grade.

Experimental animals

Thirty six female Balb/C mice obtained from the Animal House of Orchid Chemicals & Pharmaceuticals Ltd., Chennai, were randomized into six groups consisting of six animals/ group. Group 1 served as vehicle control. Dermatitis was induced to the animals of groups 2 to 6. Group 1 animals were treated with the vehicle (mixture of acetone and olive oil (4:1)) alone. Group 2 animals did not receive any treatment with the extract and hence served as the disease control. Group 3 animals were treated with dexamethasone at the dose level of 0.1% by applying on to the upper surface of the ear. Animals of groups 4, 5 and 6 were applied 0.5%, 1% and 2% of the *T. terrestris* extract on both the ears respectively. The dose volume was maintained at 20µl for all the groups. The protocol of the study was approved by Institutional Animal Ethics Committee (IAEC).

Model development

Sensitization and elicitation (challenge application) was carried out to induce dermatitis in the animals. The animals were sensitized by the application of 100 µl of 1.5% oxazolone in ethanol to the dorsal lumbar region for a period of 6 days (Roberts *et al.,* 1985; Kitagaki, 1995; Kitagaki *et al*., 1997). Starting 7 days following sensitization, the animals were challenged with 20 µl of 1% oxazolone in a mixture of acetone and olive oil (4:1) by applying on both sides of the mouse ear (Roberts *et al*., 1985; Kitagaki *et al*., 1997) on days 7, 10 and 13.

Parameters observed

Ear thickness was measured using vernier calipers (Mitutoyo Corporation, Japan) at various time points during the course of the experiment. 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 and 16.

The ear weight, evaluation of histopathology and epidermal thickness of the ear were done after animal euthanasia. Seventy two hours after the last application of oxazolone, animals were sacrificed, ears were excised, weighed and fixed in 10%-buffered formalin solution, embedded in paraffin by standard methods, cut into 5 µm sections and stained with hematoxylin-eosin.

Histopathological evaluations were carried out under light microscopy. 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 inter follicular epidermis (Reynolds *et al*., 1998).

$$\% \text{ Inhibition} = \frac{\text{scores obtained from the disease model} - \text{scores of Oxazolone plus } T. \textit{ terrestris} \text{ or dexamethasone treated animals}}{\text{scores obtained from the disease model} - \text{vehicle control}} \times 100$$

Percent of inhibition of ear swelling, ear weight and epidermal thickness was calculated according to the following equation:

Statistical analysis

The data are represented as mean ± standard deviation (SD). The statistical significance was determined using Student's *t*-test.

RESULTS

The effect of *T. terrestris* was measured in an oxazolone-induced dermatitis mouse model by topical administration. The ear of the disease model group (Group 2) caused erythema (reddening of the skin), edema and/or indurations, and occasionally abrasion. Dexamethasone used as the positive agent at the concentration of 0.1% potently suppressed oxazolone-induced ear swelling with a suppressive rate of 79.8% on day 16. *T. terrestris* potently suppressed ear swelling at each time-point (Tables 1 & 3, Figures 1 & 4). The suppressive rates of *T. terrestris* at concentrations of 0.5%, 1% and 2% were 49.1%, 62.3% and 73.7% on day 16 respectively as compared to the disease control.

Oxazolone treatment of sensitized animals produced a significant increase in ear weight as compared normal control animals. A dose dependent ($p < 0.05$ & $p < 0.01$) decrease in ear weight (Tables 2 & 3, Figures 2 & 4) was observed. Topical treatment of *T. terrestris* reduced oxazolone induced inflammation of ear weight by 78.3, 49.3, 60.9 and 73.6 % in 0.1% of dexamethasone, 0.5, 1 % and 2 % respectively as compared to the disease control.

Gross macroscopic examination revealed a relatively swollen ear in the disease model as compared to the control animals. Histopathological examination of the ear belonging to the disease control revealed prominent epidermal hyperplasia and marked infiltration of inflammatory cells (Figure No: 5), consisting of monocytes, granulocytes, and macrophages, mainly into the dermis and some into epidermis. The ear of the vehicle control animals exhibited a thin epidermal layer.

Epidermal thickness was measured to assess the severity of the epidermal hyperplasia induced by oxazolone application. Epidermal thickness (Tables 2 & 3, Figures 3 & 4) was found to be significantly increased (two to three folds) in the disease model as compared to the vehicle control. Epidermal thickness of the disease induced animals treated with *T. terrestris* at concentrations of 0.5, 1 and 2 % revealed a significantly decreased epidermal thickness by 35.6, 54.8 and 73.0% respectively, as compared to the vehicle control animals. Animals treated with dexamethasone at the concentration of 0.1 % decreased ear epidermal thickness by 80.4%.

Table 1: Effect of *T. terrestris* on the thickness (mm) of mouse ear induced by repeated application of Oxazolone

Group N°	Days			
	7	10	13	16
Vehicle Control (G1)	0.31 ± 0.02	0.31 ± 0.02	0.32 ± 0.03	0.32 ± 0.02
Disease Control (G2)	0.31 ± 0.03	0.56 ± 0.04	0.68 ± 0.09	1.46 ± 0.06
Dexamethasone 0.1% (G3)	0.31 ± 0.01	0.46 ± 0.02	0.50 ± 0.08	0.55 ± 0.04
T. terrestris 0.5% (G4)	0.32 ± 0.03	0.40 ± 0.05	0.50 ± 0.12	0.90 ± 0.07
T. terrestris 1 % (G5)	0.31 ± 0.02	0.39 ± 0.05	0.45 ± 0.09	0.75 ± 0.09
T. terrestris 2 % (G6)	0.32 ± 0.03	0.36 ± 0.06	0.42 ± 0.08	0.62 ± 0.05

Note: Values are Mean ± S.D.

Table 2: Effect of *T. terrestris* on the change in weight and epidermal thickness of mouse ear induced by repeated application of Oxazolone

Parameters	Vehicle control	Oxazolone	*Dexametha-sone (0.1%)*	*T. terrestris* 0.5%	*T. terrestris* 1 %	*T. terrestris* 2 %
Ear weight (mg)	72.5±0 .6	245±5.7	110±7.1↓↓	160±13.2↓	140±2.8↓	118±3.8↓↓
Epidermal thickness (µm)	14.5±9.8	93.7±11.2	30.0±10.3↓↓	65.5±8.8↓	50.3±7.2↓	35.9±5.2↓↓

Note: Values are Mean ± S.D; Key: ↓- significantly lower than disease control ($p< 0.05$), ↓↓- significantly lower than disease control ($P<0.01$)

Table 3: Effect of *T. terrestris* and dexamethasone on percent (%) inhibition of thickness, weight and epidermal thickness of mouse ear induced by repeated application of Oxazolone

Parameters	*Dexamethasone 0.1%*	*T. terrestris* 0.5%	*T. terrestris* 1%	*T. terrestris* 2%
Ear thickness (Day 16)	79.8	49.1	62.3	73.7
Ear weight	78.3	49.3	60.9	73.6
Epidermal Thickness	80.4	35.6	54.8	73.0

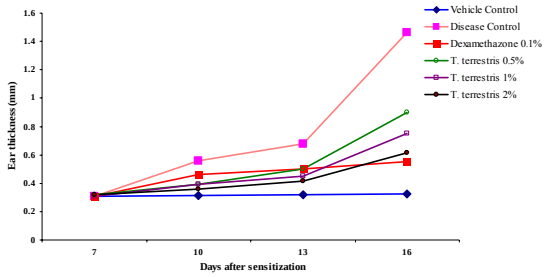

Fig. 1: Effect of *T. terrestris* on the change in thickness of mouse ear induced by repeated application of Oxazolone

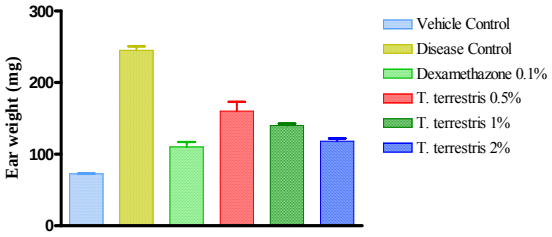

Fig. 2: Effect of *T. terrestris* on the reduction of oxazolone-induced ear weight

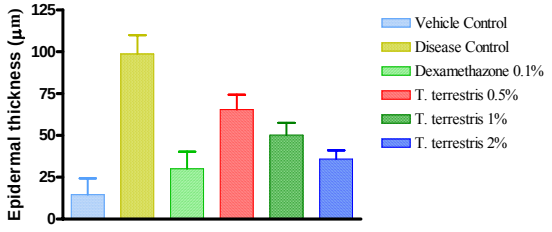

Fig. 3: Effect of *T. terrestris* reduce oxazolone-induced epidermal thickness

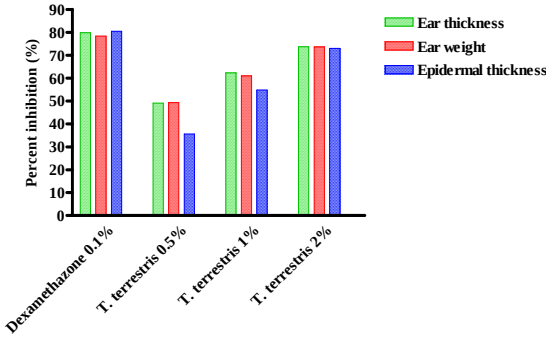

Fig. 4: Percent (%) inhibition of oxazolone-induced ear thickness, weight and epidermal thickness with *T. terrestris* treatment

DISCUSSION

Chronic contact dermatitis was induced in the ear of Balb/C mice by repeatedly applying Oxazolone. The dermatitis thus induced was accompanied by sustained ear swelling, prominent epidermal hyperplasia and marked infiltration of inflammatory cells consisting of monocytes, granulocytes and macrophages. Interferon-γ and Tumor necrosis factor –α play significant role in activating various types of cells, resulting in inflammatory events (Issekutz *et al*., 1988), and to induce thickened epidermis due to the increase in keratinocyte proliferation (Carroll *et al*., 1997). 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 (Piguet, 1993; Murphy *et al*, 2000). In this study, topical treatment with *T. terrestris* extract inhibits the secretion of TNF-α and IL-1 in the allergic contact dermatitis models of inflammation thereby decreasing the proliferation of inflammatory cells. *T. terrestris* treatment has been shown to reduce cytokine-

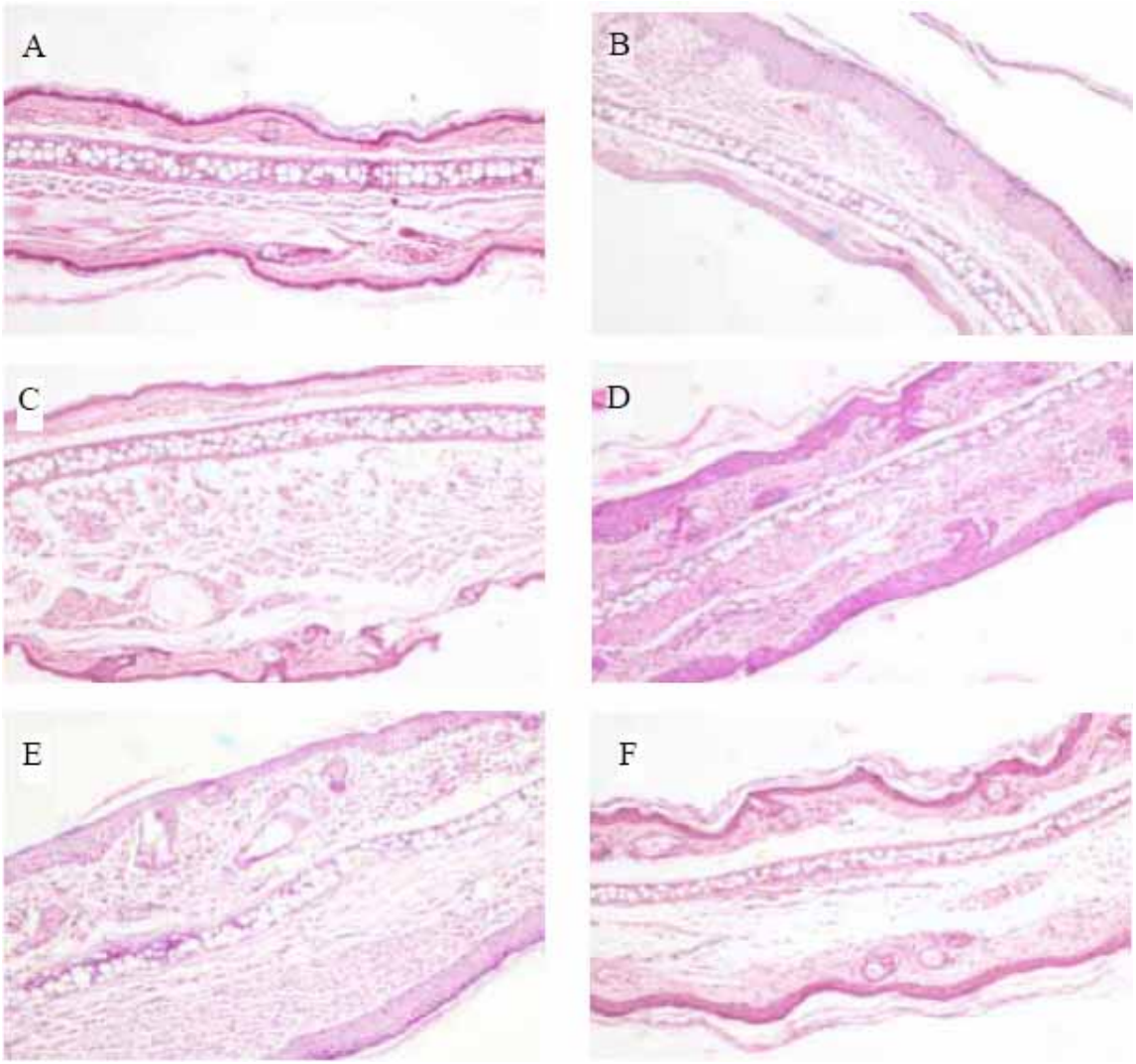

Fig. 5: Hematoxylin and Eosin (H&E) stained sections of skin (magnification x10)
A: Negative control mouse sensitized with oxazolone at 1.5%; **B**: Disease induced skin by oxazolone challenge at 1%; **C**: Skin treated with dexamethasone at 0.1 %; **D**: Skin treated with *T. terrestris* at 0.5 %; **E**: Skin treated with *T. terrestris* at 1 %; **F**: Skin treated with *T. terrestris* at 2%

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 and thus the anti-inflammatory effects of *T. terrestris* 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 (Staels *et al.*, 1998; Delerive *et al.*, 1999; Marks, 1990). The results suggest that *T. terrestris* improves chronic inflammatory skin disorders by the inhibition of TNFα produced by macrophage cells and interferon-γ produced by the Th1 cells.

REFERENCES

Austin LM, Ozawa M, Kikuchi T, Walters IB and Krueger JG, 1999. Invest. Dermatol., 113: 752-759.

Barker JN, 1991. The pathophysiology of psoriasis. Lancet, 338: 227-30.

Carroll JM, Crompton T, Seery JP and Watt F M, 1997. Transgenic mice expressing IFN-γ in the epidermis have eczema, hair hypopigmentation, and hair loss. J Invest Dermatol, 108: 412- 422.

Christophers E, 2001. Psoriasis-epidemiology and clinical spectrum Clin Exp Dermatol, 26: 314-320

Delerive P, De Bosscher K and Besnard S, 1999. Peroxixome proliferator-activated receptor-alpha negatively regulates the vascular inflammatory gerne response by negative cross-talk with transciription factors NF-kappaB and AP-1. J Biol Chem, 274: 32048-32054.

Friedman ES, LaNatra N and Stiller MJ, 2002. NSAIDs in dermatologic therapy: review and preview. J Cutan Med Surg, 6: 449-459.

Griffiths CE and Voorhees JJ, 1996. Psoriasis, T cells and autoimmunity. JR Soc Med, 89: 315-319.

Hernandez GL, Volpert OV, Iniguez MA, Lorenzo E, Martinez-Martinez S, Grau R, Fresno M and Redondo JM, 2001. Selective inhibition of vascular endothelial growth factor-mediated angiogenesis by cyclosporin A: roles of the nuclear factor of activated

T cells and cyclooxygenase 2. J Exp Med, 193: 607-620.

Issekutz TB, Stoltz JM and Van der Meide P, 1988. The recruitment of lymphocytes into the skin by T cell lymphokines: the role of γ-interferon. Clin Exp Immunol, 73: 70-75.

Kitagaki H, Fujisawa S, Watanabe K, Hayakawa K and Shiohara T, 1995. Immediate-type hypersensitivity response followed by a late reaction is induced by repeated epicutaneous application of contact sensitizing agents in mice. J Invest Dermatol, 105: 749-755.

Kitagaki H, Ono N, Hayakawa K, Kitazawa T, Watanabe K and Shiohara T, 1997. Repeated elicitation of contact hypersensitivity induces a shift in cutaneous cytokine milieu from a T helper cell type 1 to a T helper cell type 2 profile. J Immunol 159: 2484-2491.

Krueger GG and KP Callis, 2003. Development and use of alefacept to treat psoriasis. J. Am. Acad. Dermatol, 49: 87 - 97.

Marks DB, 1990. Biochemistry. Williams & Wilkins, Baltimore, Md, USA, p: 210-258.

Murphy JE, Robert C and Kupper TS, 2000. Interleukin-1 and cutaneous inflammation a crucial link between innate and acquired immunity. J Invest Dermatol, 114: 602-608.

Nadkarni AK, 1976. Indian Materia Medica. Vol 1: Bombay, India: Popular Prakashan; ppp-1229-32.

Ovigne JM, Baker BS, Brown DW, Powles AV and Fry L, 2002. Epidermal CD8+ T cells reactive with group A streptococcal antigens in chronic plaque psoriasis. Exp Dermatol, 10: 168-174.

Piguet PF, 1993. TNF and the pathology of the skin. Res Immunol, 144: 320-326.

Reynolds NJ, Voorhees JJ and Fisher GJ, 1998. Cyclosporin A inhibits 12-0-tetradecanoyl-phorbol-13-acetate-induced cutaneous inflammation in severe combined immunodeficient mice that lack functional lymphocytes. Br J Dermatol, 139: 16-22.

Roberts LK, Spangrude GJ, Daynes RA and Krueger GG, 1985. Correlation between keratinocyte expression of Ia and the intensity and duration of contact hypersensitivity responses in mice. J Immunol, 135: 2929-36.

Sakuma S, Higashi Y, Sato N, Sasakawa T, Sengoku T, Ohkubo Y, Amaya T and Goto T, 2001. Tacrolimus suppressed the production of cytokines involved in atopic dermatitis by direct stimulation of human PBMC system. (Comparison with steroids). Inter Immunopharmacol, 1: 1219-26.

Schafer-Korting M, Schmid MH and Korting HC, 1996. Topical glucocorticoids with improved risk-benefit ratio. Rationale of a new concept. Drug Safety, 14: 375-385.

Staels B, Koenig W and Habib A, 1998. Activation of human aortic smooth-muscle cells is inhibited by PPAR-alpha but not by PPAR gamma activators. Nature, 393: 790-793.