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

B. Navin Rajesh¹, Albin Fleming¹, Shilpesh Devada²*, Ramchandra Ranvir² and Rajesh Sundar²

¹PG and Research Department of Advanced Zoology and Biotechnology, Loyola College, Chennai; ²Departments of Pharmacology and Toxicology, Zydus Research Center, Sarkhej-Bavla N.H. No. 8A, Moraiya, Ahmedabad-382210 Gujarat, India

**ARTICLE INFO**

Received: January 02, 2013  
Revised: January 04, 2013  
Accepted: January 11, 2013

**Key words:** Contact dermatitis  
Mice  
Oxazolone  
Psoriasis  
*Tribulus terrestris*  
Topical

*Corresponding Author*  
Shilpesh Devada  
shilpeshdevda@yahoo.com


**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).

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

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

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

**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-1α in the allergic contact dermatitis models of inflammation thereby decreasing the proliferation of inflammatory cells. *T. terrestris* 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 and thus the anti-inflammatory effects of \textit{T. terrestris} activation could occur at both the induction of TNF-\alpha and IL-1 and the downstream effects of these cytokines on other cells in the skin (Staels \textit{et al.}, 1998; Delerive \textit{et al.}, 1999; Marks, 1990). The results suggest that \textit{T. terrestris} improves chronic inflammatory skin disorders by the inhibition of TNF\alpha produced by macrophage cells and interferon-\gamma produced by the Th1 cells.

REFERENCES


