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Pharmacological Evaluation of Liquorice for Various Dermatological Disorders in Mice

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Abstract
Objective of present study was to evaluate the anti- psoriatic and anti-acne activity of liquorice in mice. **Background:** Liquorice (*Glycyrrhiza* sp.) is one of the oldest and most frequently prescribed herbs in traditional Chinese medicine. **Methods:** The anti-psoriatic effect of liquorice was assessed in mouse tail model for psoriasis was developed by exposed mice tail to UV light for 14days. The anti-acne activity of liquorice was assessed in oxazolone induced activity on sebaceous glands of rats' model. The animals were dividing into four groups treated as group-1, group-2, group-3, and group-4, mice received normal ointment base, salicylic acid ointment, 1% liquorice and 2% liquorice of liquorice respectively up one week on tail region. **Results**: On all four groups after exposing to U.V light animals produce psoriatic lesions with characterised by itching and scaling. Epidermal thickness of disease induced animals (0.775****±0.0703), epidermal thickness of 1% liquorice treated is (2.9038****±0.064) 2% liquorice treated is (3.105****±0.0629). Histopathological examination of tail belonging to the disease control revealed prominent epidermal hyperplasia and marked infiltration of inflammatory cells consisting of monocytes, granulocytes and macrophages, mainly into the dermis and some into epidermis. **Conclusion:** 2% liquorice significantly increased epidermal thicknesses with respectively as compared to the remaining group animals. 2% liquorice having significant anti-psoriatic activity against UV induced psoriasis in mice. Volume of sebaceous gland and thickness was found to be significantly, increased thickness and decreased sebaceous gland secretions volume in the disease model as compared to the control animal.

Keywords
Skin, psoriasis, epidermis, acne and liquorice

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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 Psoriasis is a chronic inflammatory disease of the skin characterized by epidermal hyperplasia, dermal angiogenesis, infiltration of activated T cells, and increased

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Psoriasis is a chronic life-long inflammatory disease that primarily affects the skin, musculoskeletal system, the gastrointestinal system and the eye [1]. It is a chronic inflammatory disease of the skin characterized by epidermal hyperplasia, dermal angiogenesis, infiltration of activated T cells, and increased cytokine levels [2]. These are typically itchy, red and scaly patches. Psoriasis in a non-contagious disease involves the mechanism of immune system reacting to skin cells. An increase in mitotic activity in the start umbasale, abnormal keratinisation and elongation of the dermal papillae towards the skin surface result in a thicker stratum corneum that desquamates to produce large scales [3]. Acne vulgaris is a chronic, inflammatory disease of the pilosebaceous unit, that affects seborrhoeic areas like face, back, and chest and characterized by comedones, papules, pustules, nodules, cysts, and scars. Almost every individual has some degree of acne during puberty with spontaneous resolution occurring in early adult life. Occasionally, the disease persists into the fourth decade or even remains a lifelong problem. Because of the involvement of the face with considerable cosmetic problems, acne is a major psychosocial problem for many teenagers and young adults [4].

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. 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. Systemic therapies such as acitretin, methotrexate, cyclosporine, hydroxyurea and thioguanineare all associated with significant systemic toxicity and have to be closely monitored [5]. Histologically, psoriasis is characterized by epidermal hyperplasia (acanthosis), dermal infiltration of immune cells, and hypervascularity. Psoriatic pathological features also include excessive proliferation and impaired differentiation of epidermal keratinocytes, likely mediated by a dysregulated immune system [5].

Some people may develop skin diseases that affect the skin, including cancer, herpes and cellulitis. Some wild plants and their parts are frequently used to treat these diseases. The use of plants is as old as the mankind. Natural treatment is cheap and claimed to be safe [6]. Various Studies indicate that *Glycyrrhiza glabra Linn* possesses antibacterial, antioxidant, antimalarial, antispasmodic, anti-inflammatory and anti-hyper glycemic properties. Various other effects like antiulcer, antiviral, antihepatotoxic, antifungal and herpes simplex have also been studies. These results are very encouraging and indicate this herb should be studies more extensively to confirm these results and reveal other potential therapeutic effects. Liquorice has various pharmaceutical activities including anti-inflammatory, anti-ulcer, anti-cancer, anti-virus, anti-allergy, and hepatoprotective activities. Based on above activities we are tried to evaluate liquorice for anti-psoriatic and anti-acne activity [7].

MATERIAL AND METHODS

Chemical reagents, instruments and equipment's

- Fresh powder of liquorice was Obtained from the local market in the Guntur, Salicylic acid ointment and UV chamber (used to induce psoriasis).

Phytochemical evaluation:

1. Identification Tests for saponin glycosides: -

A) Foam test: -

10 mg of liquorice powder was taken in 50ml measuring cylinder, water was added up to 25ml of the measuring cylinder and mark it as point 'A. Shake vigorously for 5 minutes. Allow it to stand for 30minutes. Now mark the final volume as point 'B'. Measured the distance from 'A to B'. It showed the formation of foam with increasing its volume. Therefore, the sample is "Saponin glycoside."

B) By using H_2SO_4:

1gm of liquorice was taken and add 80% of H_2SO_4, yellow colour was observed, therefore the sample is liquorice

Experimental animals:

All the experiments were carried out on albino mice's in Chalapathi Institute of Pharmaceutical Sciences. The mice were divided into groups and each group of animals were kept in separate cages for acclimatization at a temperature 25±2°C and relative humidity of 52-55%with 12 Hr light/dark cycle one week before and before commencement of the experiment.They were kept on standard pellet diet ad libitum and drinking water throughout the study period. The protocol of experimental study was approved by Institutional Animal Ethics Committee (IAEC) with 20/IAEC/CLPT/2017-18, dt-29/11/2017.

That paragraph should be shifted to lift column along with table:
The ointment was prepared by using two concentrations of liquorice (1% w/w) and following ingredients.

Ingredients	Quantities required
Hard paraffin	0.12gms
White bees wax	0.18gms
Cetostearyl alcohol	0.3gms
White soft paraffin	5.4gms
Drug (liquorice)	0.1gm

By using ingredients ointment base was prepared.

Experimental Design for psoriasis: -

- The animals were dividing randomly in to four groups and each group contain six mice which are having same age and weight.
- All four groups of animals were exposed to UV light for 14days in order to inducing psoriasis (tail part).
- Those four groups treated as group-1(control animal), group-2 (standard drug treated animals), group-3 (1% liquorice), group-4 (2% liquorice)
- Salicylic acid ointment should be applied to mouse tail for standard group
- Test group animals should be applied with liquorice ointment on their tails. This process is continued for 7days.
- Later mice were sacrificed; tails were collected and send to histopathological studies.

RESULTS

The effect of liquorice at two concentrations was measured in an U. V induced mouse tail model by exposure of U.V rays. The mouse tail of all groups caused erythema, itching and scaling. Salicylic acid ointment used as the positive agent at concentration of 0.1% potentially suppressed U.V rays induced tail swelling. On all four groups after exposing to U.V, animals produce psoriatic lesions with characterised by itching and scaling (Fig-1). 2% liquorice treated animals increase in tail thickness was observed. Topical treatment of liquorice reduces U.V induced inflammation of tail thickness increases is compare ($p<0.001$) with treatment of 0.1%of salicylic acid ointment (Table-1, Fig-2). Gross microscopic examination revealed a relatively swollen tail in the mouse tail model as compared to the control animals. Histopathological examination of tail belonging to the disease control revealed prominent epidermal hyperplasia and marked infiltration of inflammatory cells consisting of monocytes, granulocytes and macrophages, mainly into the dermis and some into epidermis. The tail of control animals exhibits a thin epidermal layer is showed in (Fig-3). Epidermal thickens was measured to assess the severity of epidermal hyperplasia induced by U.V exposure. Epidermal thickness was found to be significantly increased in the both test groups when compared to control group is showed in (Table-1). Epidermal thickness of disease induced animals treated with extracts of liquorice at concentration of 2% revealed a significantly increased epidermal thickness by respectively (3.105****±0.0629) as compared to the control animals.

ACNE: -

Experimental Design for Acne: -

All four groups of mice are treated with oxazolone ointment to induce acne.

The oxazolone ointment was applied for ears of mice for 7days.

Those four groups are treated as control, standard, 2% liquorice groups.

Later liquorice ointment was applied for 7days.

After completion of treatments, mice were sacrificed, and ears were collected and send to histopathological studies.

Fig. 1: U.V. light induced tail swelling in mice

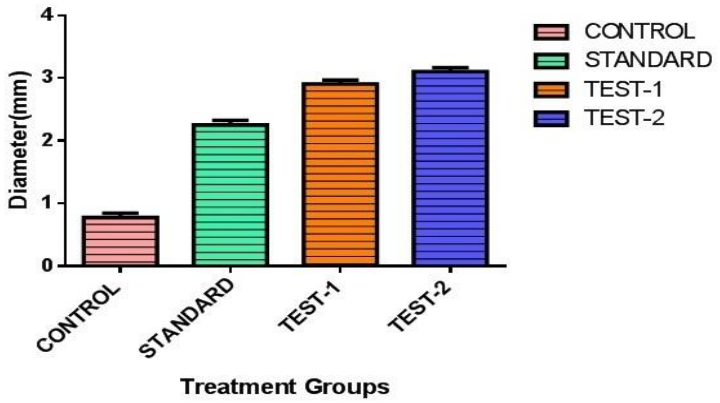

Fig. 2: Effect of liquorice on UV rays induced epidermal thickness

Table 1: Effect of liquorice on the thickness (mm) of mouse tail after repeated exposure of U.V rays

Group	Dose	Diameter(mm)
Control	-	0.775****± 0.0703
Standard	0.1%Salicylic Acid Ointment	2.25****± 0.0763
Test-I	1%liquorice ointment	2.9038****± 0.064
Test-II	2%liquorice ointment	3.105****± 0.0629

Note: Values are Mean ± SEM; epidermal thickness was significantly increased than the control group n=5, (p< 0.001).

Table 2: - Effect of liquorice on the thickness (mm) of mouse ear after repeated application of oxazolone

Group	Dose	Diameter (mm)
Control	-	0.7916****± 0.0382
Standard	0.1%Salicylic Acid Ointment	1.783****± 0.0379
Test-I	1%Liquorice Ointment	2.48****± 0.0809
Test-II	2%Liquorice Ointment	2.58****± 0.0903

Note: Values are Mean ± SEM; ear thickness of mice significantly increased than the control group n=5, (p< 0.001).

RESULTS:

The effect of liquorice extracts was measured in an oxazolone induced activity on sebaceous glands of mice model group caused reddening of the skin and itching and sebum formation and occasionally Abrasion is showed in Fig -4.

For treatment of acne salicylic acid used as the standard agent at the concentration of 0.1% potency suppressed oxazolone induced ear come done formation and increase volume of sebaceous glands. Oxazolne treatment of sensitized animals produced a significant increase in ear thickness and also the

weight of ear as compared normal control animals is showed in (Table 2). Histopathological examination of the ear belonging to the disease is controlled it indicates increasing the thickness of the outer layer and visually it indentified by decreasing of the formed redness and come done formation on the ear skin. It indicates the decreasing of the volume of animals exhibited a thin epidermal layer is showed in (Fig 6)

Epidermal thickness was measured to assess the severity of the increased sebaceous gland volume and the thickness induced by oxazolone application. volume of sebaceous gland and thickness was found to be significantly increased thickness and decreased sebaceous gland secretions volume in the disease model as compared to the control animal.

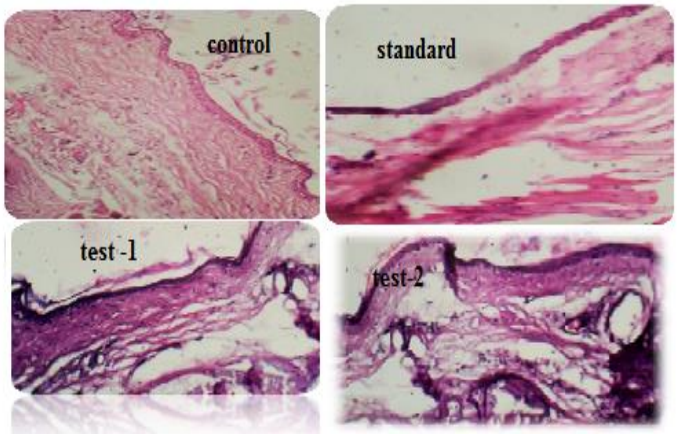

Fig. 3: Histology of tail representing the epidermal thickness

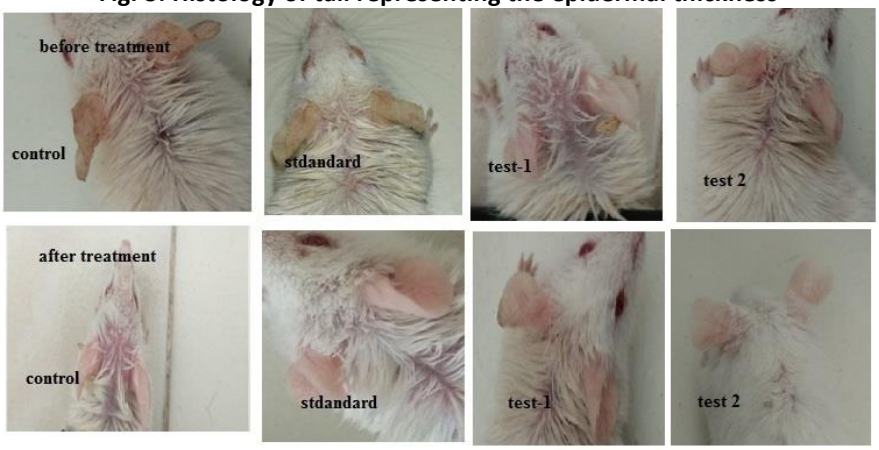

Fig. 4: Oxazolone induced erythema (Acne) in mice

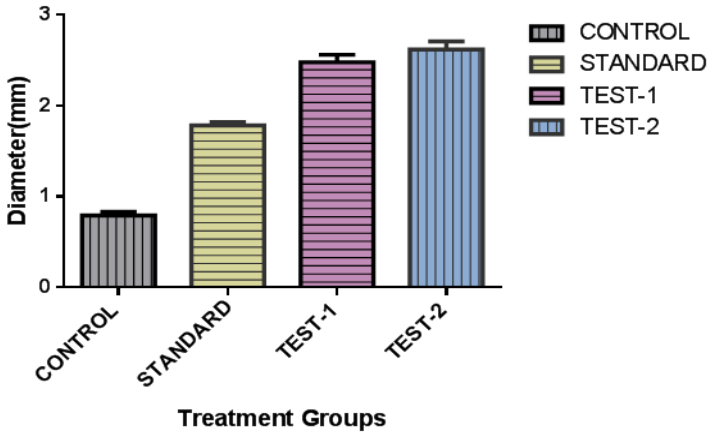

Fig. 5: Effect of liquorice on thickness (mm) of mice ear

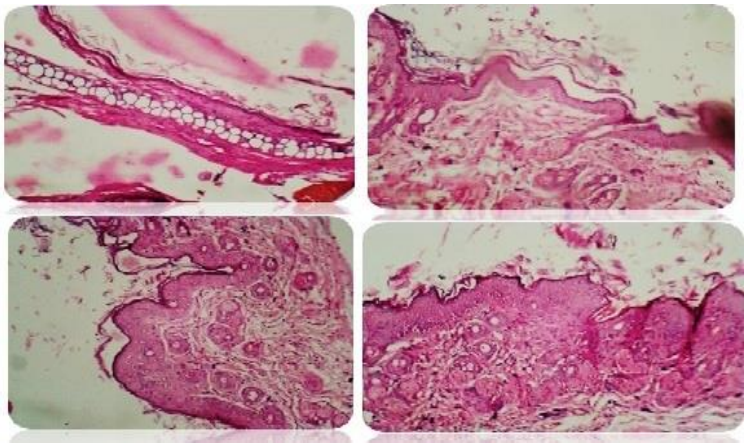

Fig 6: Histology of ear representing epidermal thickness

DISCUSSION

Psoriasis was induced in the tail of albino mice by repeatedly exposure of U.V. The psoriasis induced was accompanied by sustained tail swelling prominent epidermal hyperplasia and marked infiltration of inflammatory cells consisting of monocytes, granulocytes and macrophages. The epidermis is thickened due to increase in keratinocyte proliferation. In this study, topical treatment with liquorice may be inhibits the secretion of TNF-α and IL-1 in the mouse tail models of Psoriasis. There by decreasing the proliferation of inflammatory cells. The results suggested that extracts of liquorice suppress psoriasis by the inhibition of TNF-α produced by macrophage cells and interferon produce by the Th-1cells.

Acne was induced in the ear of albino mice by repeatedly applying of oxazolone. Acne was inducing by formation of erythma, itching, come done formation and marked infiltration of inflammatory cells. various types of cells play significant role by their activation. Drugs like adaplane modulate the keratinisation and inflammatory process. This anti-inflammatory effect is due to inhibition of the lipoxygenase activity and also to oxidative metabolism of arachidonic acid. In this study topical treatment of liquorice extract inhibits the qualitative increase and volume of sebaceous glands secretions decreasing is seen by photo microscopy of skin. The results suggested that liquorice extract improves chronic inflammatory skin disorders like acne.

CONCLUSION

From the results it concludes that, two concentrations of test compounds of *Glycyrrhiza glabra* demonstrate to possess anti-psoriasis and anti-acne activity. The results also revealed that the 1% of *Glycyrrhiza glabra* possess more significant effect on acne. like psoriasis and acne. Thus, it concludes that 2%of *Glycyrrhiza glabra* possess good Anti-psoriatic and Anti-acne activities.

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