The treatment of atopic dermatitis with licorice gel

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***Glycyrrhiza glabra* L. has been used in herbal medicine for skin eruptions, including dermatitis, eczema, pruritus and cysts. The effect of licorice extract as topical preparation was evaluated on atopic dermatitis. The plant was collected and extracted by percolation with suitable solvent. The extract was standardized, based on Glycyrrhizinic acid by using a titrimetry method. Different topical gels were formulated by using different co-solvents. After standardizing of topical preparations, the best formulations (1% and 2%) were studied in a double–blind clinical trial in comparison with base gel on atopic dermatitis over two weeks (30 patients in each group). Propylene glycol was the best co-solvent for the extract and Carbopol 934P as gelling agent showed the best results in final formulations. The quantity of glycyrrhizinic acid was determined 20.3% in the extract and 19.6% in the topical preparation. Two percent licorice topical gel was more effective than 1% in reducing the scores for erythema, oedema and itching over two weeks ($p<0.05$). The results showed that licorice extract could be considered as an effective agent for treatment of atopic dermatitis.** (*J Dermatol Treat* (2003) **14:** 000–000)

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Introduction

Atopic dermatitis is the cutaneous expression of the atopic state, characterized by a family history of asthma, hay fever, or dermatitis in up to 70% of patients.[1] Hanifin summarized four current hypotheses concerning the cause of atopic dermatitis in his review: cyclic nucleotide dysfunction, the role of superantigen, Ig-E mediated allergy to airborne and food allergens, and autoimmunity to self-antigen .[2] Topical corticosteroid creams or ointments are the mainstay of therapy to control the acute flares of atopic dermatitis, probably due to their broad immunomodulatory effects.[3,4] As these and other therapies have side effects it is necessary to evaluate new therapeutic methods. A traditional Chinese medication consisting of a mixture of several herbs, especially *Glycyrrhiza uralensis* (a Chinese species of licorice) has provided a therapeutic option for children with extensive atopic dermatitis.[5,6] Chinese licorice root (*G. Uralensis*) which is a staple herb for skin disease in east Asia, contains steroid-like substances, which, when taken internally, or even applied topically, rapidly provide relief.[7]

Glycyrrhiza glabra L. is native to Eurasia and cultivated in Europe (e.g. Spain, Italy, France), the Middle East (e.g. Syria, Iran, Turkey, Iraq), and Asia. Those parts used are the dried roots collected in the autumn.[8] *G. glabra* contains substantial amounts of flavones, such as liquiritigenin and liquiritin, and triterpenoids, such as glycyrrhetinic acid and glycyrrhizin. Liquiritigenin, disodium glycyrrhetinic acid and glycyrrhizin have been shown to have anti-inflammatory activity.[9–11] Glycyrrhiza *glabra* has significant anti-inflammatory and anti-allergic activity. Glycyrrhizin reinforces cortisol's inhibition of antibody formation, stress reaction, and inflammation. Alcohol extract of *G. glabra* has displayed anti-microbial activity *in vitro* against *Staphylococcus aureus*, *Streptococcus mutans*, *Mycobacterium smegmatis*, and *Candida albicans*. The majority of the antimicrobial effects are due to soflavenoid components, with the saponins having a lesser antibacterial effect.[12] This study was designed to compare the clinical efficacy of licorice gel (1% and 2%) with that of placebo, in patients with atopic dermatitis.

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Materials and methods

Materials

The following chemicals were used as received from the suppliers. Methyl and propyl paraben, propylene glycol, PEG 200, PEG 300, PEG 400, isopropyl alcohol, glycerin, triethanolamine, methanol, acetone, ethanol, NaOH, NH_3, formalin (Merck), Carbopol 940 (BF Goodrich).

Plant material

Glycyrrhiza glabra L. roots were collected from Shiraz, in the south of Iran, in spring 2001. The dried roots were powdered so that all the material could be passed through a mesh size no larger than 0.5 mm.

Extraction procedure

Powdered roots (600 g) were macerated in 1 l of methanol for one day, and the step was repeated twice, following by filtration through filter paper. The filtrate was evaporated to dryness under reduced pressure and weighed (122 g, 20.33%).

Glycyrrhizic acid determination

The licorice extract (2 g) was stirred with 20 ml of 3% HNO_3 in acetone for 1 hour, then filtered and washed with 10 ml of acetone. The residue was refluxed with 20 ml of acetone, filtered again, and this operation repeated three times. The combined acetone extracts were diluted to 100 ml, and washed down with 40 ml of ethanol; 0.9 ml of 30% NH_3 was added dropwise to the mixture. Ammonium glycyrrhizate was filtered, washed 2–3 time with acetone (50 ml total), and dissolved in 25 ml of water; 20 ml of formalin was added and after 1 min the mixture was titrated with 0.1 N NaOH in the presence of phenolphthalein as indicator.[13] This method was also used for determination of glycyrrhizic acid in licorice preparations. The assay was repeated three times.

Preparation of the formulations

PEG 200, 300, 400, isopropyl alcohol and propylene glycol were used as co-solvent for dried extract and propylene glycol was chosen as the best levigator. Table I shows the constituents of the selected preparations. Carbopol 940 was dispersed in preserved water (methyl paraben 0.18% and propyl paraben 0.02%) and glycerin overnight. The extract was dissolved in propylene glycol and was added to the polymer dispersion and stirred with a double bladed mixer (Ika-werk, Germany) at 500 rpm for 10 min, and neutralized by triethanolamine to pH 6.4 and then mixed at 300 rpm for 10 min. The formulations were stored at 4, 25, and 40°C to ensure physical stability evaluation for two weeks. Final formulations for the clinical trial were controlled microbiologically based on USP XXIV.

Formulation*	Composition % (w/w)			
	Licorice extract	Carbopol	Propylene glycol	Glycerin
F1	1	0.30	10	5
F2	1	0.30	15	5
F3	1	0.30	20	5
F4	1	0.40	15	5
F5	1	0.40	20	5
F6	1	0.50	10	5
F7	1	0.50	15	5
F8	1	0.50	20	–
F9	1	0.50	20	5
F10	1	0.75	10	5
F11	1	0.75	15	5
F12	1	0.75	20	5
F13	1	1.00	10	5
F14	1	1.00	15	5
F15	1	1.00	20	5
F16	2	0.25	20	5
F17	2	0.50	20	5
F18	2	0.75	20	5
F19	2	1.00	20	5
Placebo	–	0.50	20	5

*Each formulation consists of preserved water (propyl paraben 0.02% w/w and methyl paraben 0.18% w/w) to 100 g. The formulations was neutralized by triethanolamine to pH=6.8.

Table I

Formulations composition

Clinical trial and statistical analysis

The study was a randomized (simple-random sampling), double blind, prospective, placebo-controlled trial. The primary endpoint of the clinical trial is severity in oedema, itching and erythema. On the assumption of an overall mean difference of 0.5 units and a standard deviation of 0.5 units, 78 patients (26 in each group) were required to achieve a power of 95% to reject a null hypothesis of equality, applying a two-sided test at the 5% significance level. With an estimated fraction of 35% of the patients being not evaluable, a total of 108 patients, aged over 15 years, with clinically diagnosed mild to moderate degrees of atopic dermatitis (1. pruritus and scratching, 2. course marked by exacerbation and remissions, 3. lesions typical of eczematous dermatitis, 4. personal or family history of atopy, 5. clinical course lasting longer than 6 weeks) were recruited.[1] Before enrollment to the study, written informed consent was obtained from all patients or the parents of those under the age of 18 years. Patients receiving systemic or topical steroids, antibiotics, or

other effective topical therapy within the previous 7 days, pregnant women, nursing mothers, and patients with other skin disorders were excluded. Lesions location was classified as head and neck, trunk, hands, and feet.

The topical preparations (1% and 2% licorice gels and placebo) were administered to patients, in three groups, three times a day for two weeks. The overall clinical response was assessed by the investigator based on effect on oedema, itching, erythema and scaling, according to the following 4 -point scale: absent=0, mild=1, moderate=2, and severe=3. Follow up of patients ceased after two weeks. ANOVA, followed by Student-Newman-Keuls test, was used to determine significant differences between groups and $p<0.05$ was considered significant.[14]

	Licorice extract (1%)	Licorice extract (2%)	Placebo
Age			
Mean	32.7	34.1	35.3
Range	16–51	16–49	17–53
Sex			
Male	13	12	10
Female	17	18	20
Duration of eczema (years)			
Mean	3.8	3.5	3.6
Range	0.01–25	0.01–21	0.01–25
Area of eczema			
Head and neck	8	9	7
Trunk	3	2	2
Hands	18	19	19
Feet	1	–	2

Table II

The comparison of patient characteristics

Results and discussion

Licorice contains as its major active ingredient the triterpene glycoside glycyrrhizin (also known as glycyrrhizic or glycyrrhizinic acid) in concentrations ranging from 1% to 24%, depending on sources and methods of assay. Glycyrrhizin on hydrolysis yields glycyrrhetinic (or glycyrrhetic) acid and two molecules of glucoronic acid.[8] Thus the standardization of licorice extract and its preparations were performed by determination of glycyrrhizinic acid. From the glycyrrhizic acid determination conducted according to the previously described method, the quantity of glycyrrhizic acid obtained was 20.3±0.81% and 19.6±0.74% in licorice extract and licorice gel respectively. All investigated formulations in the clinical trial used the criteria of USP microbial limitation.

On observing the clinical features of atopic dermatitis, some of them showed resistance to topical steroids and were even exacerbated by application of steroids, so it is likely that long term topical use of steroids may modulate the barrier-immunity function of the skin. The present study compared the efficacy of up to two weeks treatment with 1% and 2% licorice extract as a herbal gel with placebo in patients with a clinical diagnosis of atopic dermatitis.

A total of 108 patients were recruited in the present study by a single investigator. 18 patients were excluded from the efficacy analyses. Nine patients were given systemic antibiotics, five patients were pregnant, one patient was a nursing mother and three patients were suffering from other skin disorders. All of the 90 evaluable subjects complete two weeks treatment. Table II shows patient characteristics in several groups.

The assessment of the overall clinical responses, relative to baseline, at the end of treatment after one and two weeks is shown in Figure 1. Treatment with 1% and 2% licorice gel resulted in a statistically significant reduction in the scores for erythema after two weeks ($p<0.05$). This effect was not observed for 1% extract gel after one week ($p>0.05$). The 2% licorice gel showed more reduction in the scores for erythema than 1% extract at the end of first and second weeks ($p<0.05$). The licorice extract treatment was significantly more effective than baseline in reducing the scores for oedema and itching after one week ($p<0.05$) and two weeks ($p<0.01$), and 2% licorice gel was more effective than 1% extract at the end of first and second weeks ($p<0.05$). Treatment with licorice extract was not significantly effective in reducing the scores of scaling ($p>0.05$). The effect of 1% and 2% licorice gel in reducing in reducing the scores for erythema, oedema and itching were significantly more than placebo after one and two weeks ($p<0.01$).

Baseline evaluation showed that treatment groups were well balanced in respect of number per group, age and sex distribution, previous eczema treatment and severity of signs and symptoms of eczema.

At the end of the treatments, the reduction of erythema scores was 35.02% for 1% licorice extract and 60.76% for 2% licorice gel. The reduction of oedema scores was 56.64% and 83.76% for 1% and 2% licorice gel treatment after two weeks respectively. At the end of the treatments, the reduction of itching scores was 44.1% and 72.53% for 1% and 2% licorice gel respectively. A study carried out in 1994 reinforced the excellent reputation of *Glycyrrhiza glabra* in atopic dermatitis. Thirty-seven children were given a Chinese herbal medicine containing licorice (and some other plants) orally. After one year, 18 of the children had experienced at least a 90% reduction in their symptoms.[6] Another study showed that topical steroids exacerbated the dermatitis in about one third of the patients[15] but no side effects were observed in treatment with licorice topical gel.

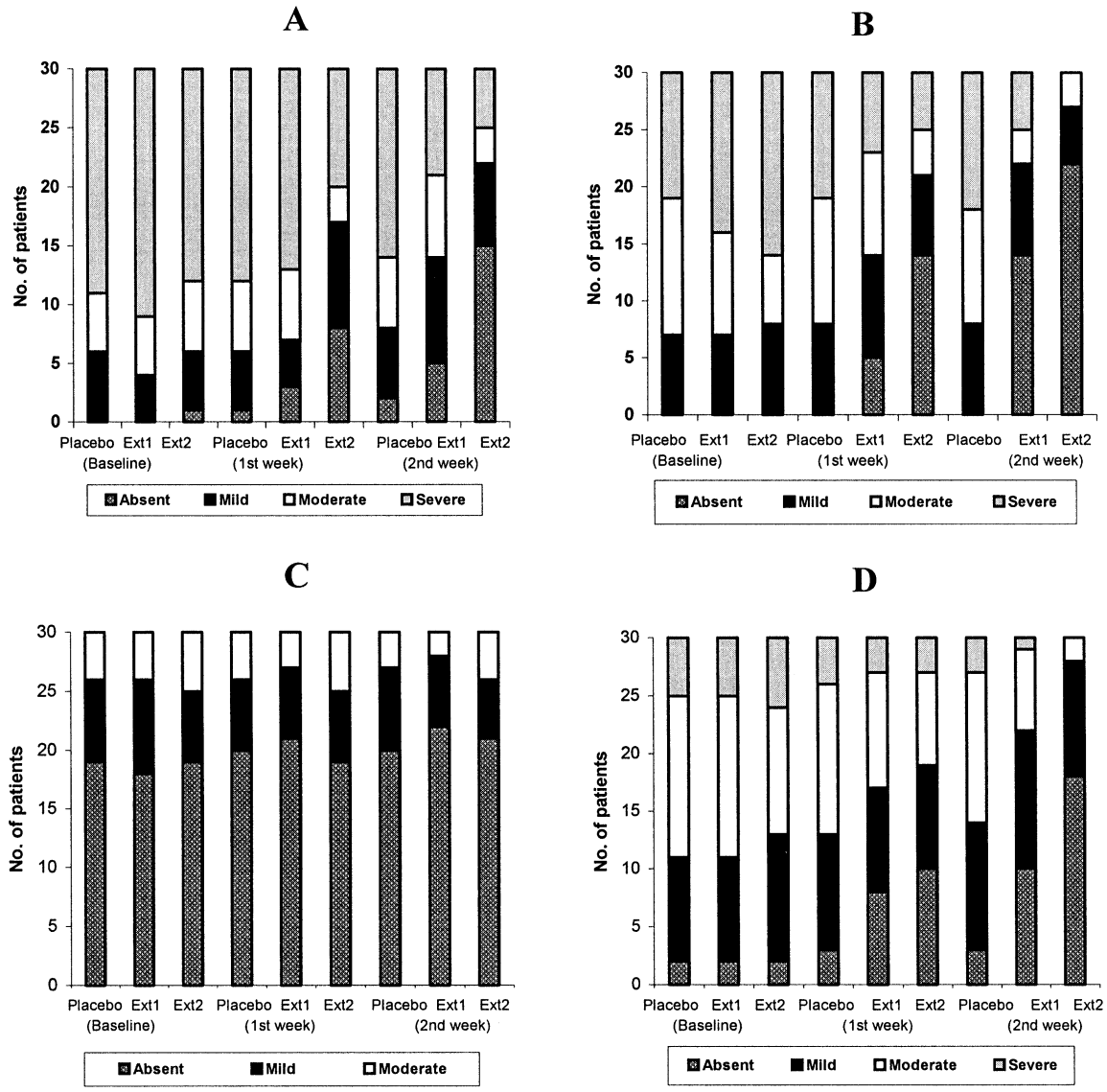

Figure 1

Investigator assessment of overall clinical response, A: Erythema scores, B: Oedema scores, C: Scaling scores, D: Itching scores;

Conclusion

Finally we would like to summarize the current approach with regard to the management of atopic dermatitis. The final goal of the treatment is to give patients the opportunity for improved social activities in their daily life. Skin care, control of pruritus and exclusion of the exacerbating factors are three facets of the therapy and the appropriate use of topical licorice preparations in considering the patients' response to this excellent therapeutic tool. The use of 2% licorice extract gives satisfactory effects in treatment of atopic dermatitis.

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