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Clinical trial of a herbal topical cream in treatment of Acne vulgaris

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ABSTRACT

The main anti-acne medicines including retinoids, systemic and topical antibiotics and hormones have several severe side effects. According to the literature, it is expected that *Calendula officinalis*, *Rosa canina*, *Zataria multiflora* and *Trigonella foenum graecum* which have antibacterial, anti-inflammatory and anti-oxidant effects, and *Glycin max* that have phytosterogenic property, heal the inflamed lesions of the disease. The purpose of the present study was to formulate and clinically evaluate an anti-acne cream using the extract of the above mentioned plants. A general formula of a cleansing cream containing bees wax, spermaceti, borax, liquid paraffin and water was considered and then corrected. The best formula was chosen according to its physicochemical properties. The stability of the formulation was evaluated. After diagnosis of *acne vulgaris*, the number of inflamed lesions and severity of the disease were determined. After two weeks of administration of the coded formulations, the process of improvement and possible complications were reviewed. If necessary, the treatment was continued for another two weeks. Finally, the patients were examined for the number of lesions and the severity again. The significance of data was analyzed statistically using SPSS. The final product was water in oil cream with suitable appearance and acceptable physico-chemical stability. Clinical trial results were significantly illustrated the effectiveness of the formulation mainly against papular and pustular lesions; disease severity and acne-induced inflammation, when compared to placebo. Previous researches have shown the presence of steroid saponins in *Trigonella foenum graecum* that make it a good anti-inflammatory agent. Also, anti-oxidant and anti-microbial effect of the other above mentioned plants are reported. Considering the results of the present study, when compared with placebo, a formulation containing the mixed extract of the plants has a good efficacy in the treatment of acne vulgaris by reduction of the number of lesions and inflamed area.

Key words: Acne vulgaris, Herbal extract, Cream

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INTRODUCTION:

Acne is a common skin disease that affects nearly 80% of adolescents and young adults aged 11 to 30 years. The development of inflammatory lesions often drives acne patients to seek treatment.

Topical medicaments used in acne treatment, e.g. tretinoin may cause primarily mild dermatitis. Bleaching of skin and hair is the common side effect of benzoyl peroxide. Although there is no proved documentation regarding systemic absorption of retinoids through skin, they should be avoided in pregnancy and lactation¹. Cosmeceuticals and pharmaceuticals are growing areas of interest and controversy in acne therapy and developing new active botanical extracts and compounds to provide them as anti-acne agents is still a field with great potential².

Calendula officinalis is a short-lived aromatic perennial plant in the family Asteraceae that is used for the treatment of skin disorders and pain, and as a bactericide, antiseptic and anti-inflammatory agent. Its main components are carotenoids, flavenoids, triterpene saponins and essential oil with anti-inflammatory, antibacterial and antiviral effects. It has been demonstrated that a formulation containing the plant extract stimulates epithelization and renewing of wounded skin^{3,4}.

Trigonella foenum (Fenugreek) is a plant in the family Fabaceae containing saponins, mucilage and alkaloids. The mucilage component of the plant is responsible for its protecting, emollient and healing effects. The saponin component has antimicrobial and anti-inflammatory activities.^{3,4}

Rosa canina is a rose species normally ranging in height from 1-5 m which is high in certain antioxidants. It mainly contains fatty components, vit-E and, essential oil and protein substances. It has been shown that galactolipid component of the plant, with anti-inflammatory effect may prevent chemotatic reactions in peripheral circulation⁵.

Glycin max is a species of legume native to East Asia and mainly consisting of phospholipids and phytoesters with anti-tumor and phytoestrogenic properties^{3,4}.

Zataria multiflora Boiss. is a plant belonging to the Lamiaceae family that geographically grows in Iran, Pakistan and Afghanistan⁶. Its main compositions are flavenoids, essential oil, tannin and polyphenols with anti-inflammatory, antiallergic and angioprotective effects. The main constituents of the essential oil of this plant are phenolic compounds such as carvacrol and thymol⁷. Its antifungal, and antihelminthic effects are well demonstrated⁸. The essential oil of *Z. multiflora* has been traditionally used for its antiseptic, anti spasm and local anesthetic effects^{6,9}. The present article includes formulation of a topical cream using above mentioned herb extracts and a controlled clinical trial using this multicomponent dermatological cream.

MATERIALS AND METHODS

Plant materials

Calendula officinalis was collected from Kolah-Ghazi Mountain, Isfahan, and identified in the department of botany Isfahan University, Iran. Soy beans were purchased from Behshahr, Iran. The other plant materials were kindly donated by research and natural resources center, Isfahan, Iran. Aerial parts of *C. officinalis* and *Z. multiflora*, hips of *R. canina* and soybeans were dried in the shadow at room temperature for 72 hours, then weighed and stored in dark, cool and dry place. Then, they were grounded to fine powder and separately suspended in methanol in a beaker to avoid solvent evaporation, the container was closed using parafilm and stored in shadow for 48 hours. The extracts were then filtered and concentrated in rotary evaporator to a completely dried film. *Z. multiflora* essential oil was extracted from 250 g powder in a BP extraction apparatus for 2 hours.

Formulation design.

Due to occlusion characteristics of fatty bases, the minimum amounts of fats were used. Also, lactic acid was added to adjust the cream pH to 5.3. To enhance wash ability of the final product, polyethylene glycol 2000 was added. Since creams and other water containing dosage forms should be preserved from microbial contamination, methyl and propyl paraben (3:1 ratio) were added to the formulation. Finally, herbal material was incorporated to cream base by levigation. The amounts of herbal materials are listed in Table 1

Table 1. The amount of herbal material used in formulation design.

Plant Material	Amount
<i>Z. multiflora</i> Extract	1g
<i>R. canina</i> Extract	1g
<i>C. officinalis</i> Extract	2g
<i>T. foenum</i> Extract	2g
<i>G. max</i> Extract	1g
<i>Z. multiflora</i> Essential oil	0.3ml

Clinical trial

A clinical trial was initiated to evaluate the efficacy of final formulation in comparison to placebo in patients with *acne vulgaris*. Sixty patients with mild to moderate *acne vulgaris* were selected and inspected for the disease. After identification by dermatologist, informed consent was obtained from patients. The purpose of the study, nature of the herbal preparation, the procedures to be carried out and the potential risks and benefits were explained to the study subjects in detail. The Ahvaz Jundishapur University of Medical Sciences Ethical committee permission was obtained before performing the experiment. Patients who had systemic

complications, menopausal disorders and patients with drug induced acne were excluded from the study. Also, pregnant and lactating patients and some who had systemic topical treatment during last 3 month were not included. The final population of each group was about 30 persons. During the initial visit, the patients were assigned to two treatment groups. The first group was contained patients with less than 10 papulo-pustular lesions which were considered as mild. The second was contained patients with more than 10 papulo-pustular lesions that were considered as moderate¹⁰.

Then, patients were assigned to two herbal cream and placebo control groups and were recommended to apply once daily. Also they were advised to come for follow up 2 and 4 weeks after treatment. Inflammation index and the total number of lesions were recorded at every follow up. The severity index was determined by dermatologist according to the lesion counts, the diameter and the depth of lesions for each patient.

Statistical Method

After data collection, labels were decoded and results analyzed using SPSS software program. All the results were analyzed statistically using Student-t test for paired data of different levels of significance. The results were presented as mean± standard error.

RESULTS AND DISCUSSION

The mean age of patients for treatment and control group were 21.97 and 22.13 years respectively. The pretreatment records are shown in Table 2.

Table 2. Comparison of *acne vulgaris* indices in treatment and control groups before treatment (Mean± SE, n=30)

Index	The number of papules	The number of pustules	The number of nodules	Inflammation	Severity index
Control	19.03±2.26	1.13±0.36	0.40±0.28	17.93±2.95	1.73±0.11
Test	16.40±3.05	2.51±0.57	0.68±0.36	22.24±0.12	1.73±0.08
P value	0.49	0.098	0.589	0.27	1.00

The results indicated that the incidence of papular lesions before treatment in treatment and control group were 16.40 ± 3.05 and 19.03 ± 2.26 respectively and There was no significant difference between them ($P= 0.49$). Also, there were no significant differences regarding the other disease indices between the two groups ($p > 0.1$)

The results indicated that the inflammatory acne index and total counts were significantly reduced after 2-4 weeks of treatment with the herbal cream (Figure 2). It was showed that the rate of healing of papular and pustular lesions in test and control groups were significantly different ($p < 0.01$, and $p < 0.05$, respectively). Although, there was no significant difference between the number of nodular lesions in treatment and control groups ($p = 0.240$), the

inflammation index in the treatment group was significantly reduced ($p < 0.0001$). The results of administration of herbal and placebo cream are tabulated in table 3 and plotted in Figure 1.

Table 3. Comparison of *acne vulgaris* indices in treatment and control groups after treatment (Mean \pm SE, n=30)

Index	The number of papules	The number of pustules	The number of nodules	Inflammation	Severity index
Control	13.46 \pm 2.80	0.60 \pm 0.22	0.13 \pm 0.08	14.20 \pm 2.76	1.6 \pm 0.12
Test	4.51 \pm 0.90	0.62 \pm 0.25	0.1 \pm 0.07	5.03 \pm 1.03	1.2 \pm 0.07
P value	<0.0001	0.104	0.66	<0.0001	<0.0001

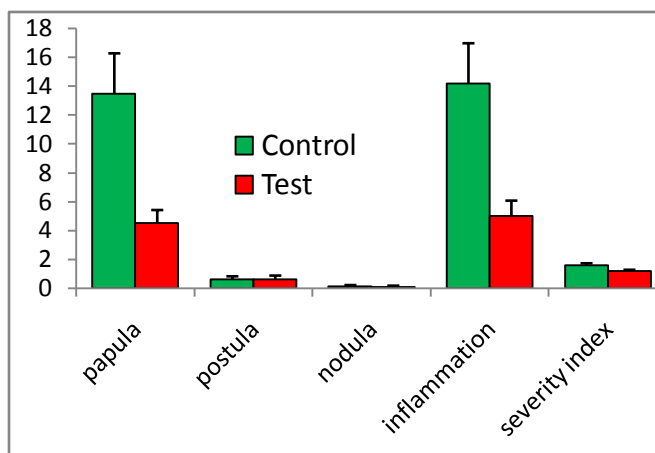


Figure 1. Comparison of the number of acne lesions, inflammation and severity indices in test and control groups (Mean \pm SE, n=30).

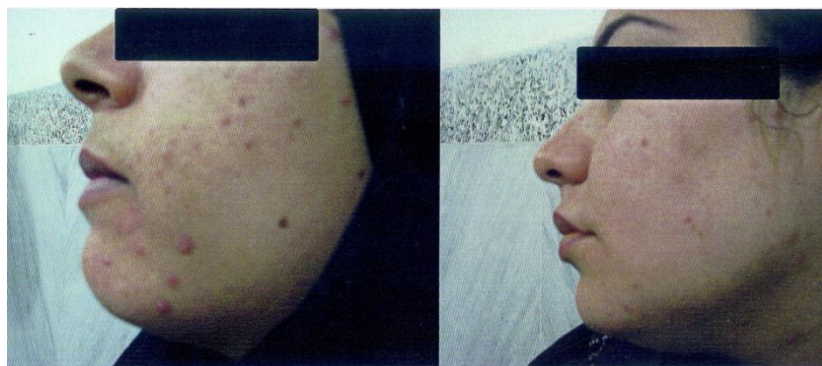


Figure2. A woman with acne vulgaris: before treatment with the herbal cream (left), and 2 weeks after treatment (right). Clinical improvement was observed after treatment with the formulated mixed herbal cream.

Regarding prevalence of acne specifically in teenagers, and side effects of oral systemic medications, it was decided to incorporate a mixture of some medicinal plants with proved antimicrobial, anti-inflammatory, anti-oxidant and phytoestrogenic activities in a proper formula for treatment of acne vulgaris. Extract of *T. foenum* seeds which contains steroid saponins have shown wound healing property¹¹. Also, anti-oxidative, anti-inflammatory and

hepatoprotective properties of the plant are previously reported^{12, 13, 14}. Recent studies suggested NSAID – like mechanism for its anti-inflammatory, analgesic and anti-hyperthermic effects^{15, 16}. It has been reported that the plant is a potent antimicrobial agent against *s. aureus*¹⁷ which is the main cause of skin infections. Hips of *Rosa canina* contain vast amount of ascorbic acid with well known anti oxidant property¹⁸. The plant is also used for its antimicrobial, wound healing and anti-inflammatory properties^{5, 19, 20}. *Calendula officinalis* is a plant with known wound healing effect and have been used in traditional medicine for treatment of burns and other dermal disorders²¹. It has been also used for its anti-inflammatory and anti-oxidant properties²². Soy bean is a phytoestrogenic anti-inflammatory plant with known anti microbial and anti-oxidant effects^{23, 24}. There are many reports regarding antifungal and antibacterial effects of *Z. multiflora*²⁵. The results of the present clinical trial indicated that treatment with the formulated herbal cream resulted in clinical efficacy in comparison with negative control (Figure. 1). A 3 fold smaller reduction in papula number was found after 3 weeks of treatment with the herbal cream and control groups. Also comparing the number of pustules and nodules after 3 weeks, the reduction of inflammation and severity indices in test group were highly significant ($p < 0.0001$). Many herbs with antioxidant, anti-inflammatory and soothing properties have been used as anti-acne therapeutic alone or in combination. Among them, oat (*Avena sativa*), chamomile, turmeric, licorice, *Aloe vera*, curcumin, soybean, coffee, mushrooms, green tea and pine bark extract are more emphasized. Although there is not any crucial evidence of their activity, but theoretically, their healing and anti-inflammatory properties help the reduction of acne-induced erythma. Also their soothing effect is a positive factor for reduction of acne induced dried skin²⁶. However, the result of the study of pocrolimus cream in the treatment of moderate *acne vulgaris* indicated that there was no significant improvement in the number of inflammatory lesions¹⁰. Nevertheless, the results of a controlled phase II clinical trial showed the effectiveness of an Ayurvedic multicomponent dermatological formulation, containing the soft extract of some Indian endemic herbs in the treatment of *acne vulgaris*^{26, 27}.

According to the results of an in vitro study, extracts of rose, duzhong (*Eucummmia ulmodes*) and yerba mate (*ilex paraguarensis*) mate exhibited notable antimicrobial activity against *propionibacterium acne*. They were also able to suppress the secretion of tumor necrosis factor (TNF) and some other inflammation mediators².

CONCLUSION

Although the empiric evidences for the efficacy of herbal medicines in acne is not strongly enough, the results of the present study suggest that the combination of the herbs are useful in the

treatment of *acne vulgaris*. However, long-term clinical trials are still needed to assess the probable adverse effects of the formulation.

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