The efficacy of whey associated with dodder seed extract on moderate-to-severe atopic dermatitis in adults: A randomized, double-blind, placebo-controlled clinical trial

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A B S T R A C T

Ethnopharmacological relevance: Atopic dermatitis is a common chronic inflammatory skin condition that is on the rise and adversely affects quality of life of the affected individual. Dry skin and pruritus, major characteristics of this disease, are associated with the dysfunction of the skin barrier. Though mild cases of the disease can be controlled with antihistamines and topical corticosteroids, moderate-to-severe cases often require treatment with immunomodulatory drugs, which have many side effects. It is now more common to use complementary and alternative medicines in the treatment of atopic dermatitis. In traditional Iranian medicine, the use of whey with the aqueous extract of field dodder (Cuscuta campestris Yunch.) seeds in severe and refractory cases of atopic dermatitis is common and has no side effects.

The aim of this study was to assess the efficacy and safety of whey associated with dodder seed extract in the treatment of moderate-to-severe atopic dermatitis in adults.

Materials and methods: The study was a randomized, double-blind placebo control trial that was conducted on 52 patients with moderate-to-severe atopic dermatitis for 30 days. In this study patients received freeze dried whey powder with spray dried water extract of field dodder or the placebo for 15 days. At baseline (week zero), after the end of the 15 day treatment period (week three) and 15 days after stopping the drug or placebo (follow-up/week five), patients were evaluated in terms of skin moisture, elasticity, pigmentation, surface pH and sebum content on the forearm with Multi Skin Test Center® MC1000 (Courage & Khazaka, Germany) and the degree of pruritus and sleep disturbance in patients were also recorded.

Results: 42 patients completed 30 days of treatment with the medicine and the follow-up period. At the end of the follow-up period a significant increase in skin moisture and elasticity in the group receiving whey with dodder was observed compared with the placebo group (p < 0.001). There was a significant difference between the two groups regarding the pruritus at 15 days of receiving treatment or the placebo (p < 0.05), and at the end of the 30-day study period the difference was clearly significant (p < 0.001). Sleep disturbance showed significant changes at the end of follow-up period (p < 0.05). There was no significant difference between the two groups concerning changes in skin pigmentation, however, a significant decrease was observed in the group receiving whey associated with dodder seed extract over time (p < 0.001). There were no significant alterations in skin surface pH and the amount of sebum between the two groups. Temporary side effects were reported including anorexia and mild gastrointestinal problems in drug use.

It is noteworthy that in this study despite the fact that patients received whey with dodder for just 15 days, moisture and elasticity of the skin continued to increase in the second half of the study (follow-up period). This shows that the effect of whey with dodder is not transient and this drug really helped skin barrier reconstruction and accelerated the healing process of skin. This positively influenced the skin parameters and consequently the improvement of pruritus and sleep disturbance.

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1. Introduction

Atopic dermatitis (AD) is a common chronic inflammatory and pruritic skin disease, often associated with a positive family history of allergic diseases, such as allergic rhinitis and asthma (Leung et al., 2004). The disease has a devastating effect on quality of life. The eczematous skin lesions with severe pruritus lead to disrupted sleep and decreased daily performance and social activities of patients in addition to significant financial expenses for both the families of patients and society, as well (Fivenson et al., 2002). AD affects 15–30% of children and 2–10% of adults (Bieber, 2008).

Prevalence in developing countries is more than 10% in the general population and is increasing day by day, while in developed countries prevalence has reached a level plateau of approximately 20% (Kim, 2013). Atopic dermatitis occurs in those who are genetically predisposed. Mutations in the filaggrin gene, which is a key protein in formation of the skin barrier and skin moistening, lead to skin barrier dysfunction. Skin barrier damage increases transepidermal water loss and permits the entry of environmental antigens and allergens from the epidermis leading to inflammatory responses (Howell et al., 2009). Topical corticosteroids, emollients and oral antihistamines are used in mild disease without too many side effects, but many patients with moderate-to-severe atopic dermatitis require systemic immunomodulating treatment (e.g., cyclosporine, azathioprine, systemic corticosteroids and methotrexate) with unfavourable side effects (Hong et al., 2011; Roekvisch et al., 2014).

Since effective treatments for the disease are limited, there is a tendency toward finding better and safer therapies. Among them, the use of complementary and alternative medicines (CAM) is growing in the treatment of inflammatory skin diseases, particularly AD (Boneberger et al., 2010). Traditional Iranian Medicine (TIM), with a long history of several thousand years, offers effective treatments in this field with diet and lifestyle modification and drugs of a plant or animalistic origin. TIM recommends the use of topical emollients and wet compression in mild AD. In TIM, one of the most common treatments for chronic and severe cases of atopic dermatitis is the use of whey with the aqueous extract of field dodder seeds (Avicenna, 2005; Rhazes, 1990).

Whey is a protein complex derived from milk and considered a functional food. Whey has several properties as an antioxidant, anti-inflammatory, and immunomodulating component, which makes it effective in the treatment of inflammatory and autoimmune diseases (Lee et al., 2011).

The aim of this study was to assess the efficacy and safety of whey associated with dodder seed extract (WaDSE) in the treatment of moderate-to-severe atopic dermatitis in adults that was conducted as a randomized, double-blind, placebo-controlled trial.

2. Materials and methods

2.1. Patients

52 patients with moderate-to-severe atopic dermatitis referred to the Dermatology Clinic of Afzalipour Hospital, Kerman University of Medical Sciences, Iran, have entered into the trial after the confirmation of diagnostic criteria for atopic dermatitis (Hanifin and Rajka, 1980). Inclusion criteria were as follows:

- Hanifin and Rajka criteria approved for atopic dermatitis.
- Moderate-to-severe atopic dermatitis (SCORAD > 25).
- Aged 18 and older.
- Poor response to conventional treatment for atopic dermatitis (topical steroids and antihistamines).
- AD consistently symptomatic for at least six months.
- Lack of exudates or infection.
- Lack of pregnancy and lactation.
- The absence of concomitant systemic disease (except asthma and allergic rhinitis).

Patients with abnormalities in blood cell count, liver enzymes and renal function tests, secondary bacterial infections, who were receiving systemic corticosteroids and other immunosuppressant drugs and phototherapy during the study or who demonstrated drug intolerance symptoms were excluded. Prior to intervention, informed written consent was obtained from all patients and patients in both groups were asked to continue previously received topical steroids with the same strength and frequency. This study was approved by the Medical Research Ethics Committee, Shahid Beheshti University of Medical Sciences.

2.2. Drug and placebo preparation

In traditional medicine, field dodder seed is used in the treatment of chronic atopic dermatitis as a decoction associated with whey. In this study, dosage form change was necessary for uniformity and ease of use. Thus, a powder form of whey was prepared, and as the dodder seed decoction has a bitter taste, dried aqueous extract was prepared and administered in capsule form.

2.2.1. Whey

In traditional medicine, whey is produced using three methods:
(1) the use of rennet, (2) the use of vinegar, (3) the use of oxymelt (Aghili Khorasani, 2011b). The second method was used in this study as it is quick and easy. For this purpose, whole cow milk was boiled and 18 g of pure vinegar per kilogram of milk was added. After the curds appeared, they were isolated using fine filters. Casein and whey, major milk proteins with different properties, account for approximately 80% and 20% of milk proteins, respectively (Shah, 2000). The curds consist mainly of casein while whey remains in the aqueous environment. Unlike casein, whey proteins are not coagulated in the acidic environment of the stomach and are quickly absorbed when they reach the intestines (Boirie et al., 1997). As there are heat-sensitive protein structures in the liquid whey, the freeze drying technique was used to convert it into a powder form. In order to standardize, the amount of total protein was measured by the biuret method (Goyal and Gandhi, 2009) and found to be equal to 33 ± 1% (w/w) dry weight. As the therapeutic dose of liquid whey in atopic dermatitis is 450 ml per day (Aghili Khorasani, 2011b), its average dry weight was determined as equal to 30 ± 1.5 g (mean ± SD, n=3). Thus, the daily intake of whey powder was defined as 30 g. The produced powder was packed into 15 g vacuum-sealed bags.

2.2.2. Dodder seed extract

*C. campestris* Yunck. plants (field dodger) grown on *Alhagi persarum* (camel thorn) were collected from the unutilized lands around the city of Kerman, Iran (located at latitude 30°15′N and longitude 57°01′E) in August 2013. The sample was authorized and kept at the Faculty of Pharmacy herbarium, Kerman University of Medical Sciences, by Dr. Mitra Mehrbani (Voucher specimen No. 2002). Plant material was separated from camel thorn, cleaned, dried under shade and pulverized to pass a 40 mesh sieve. Aqueous field dodder seed extract was prepared according to traditional methods (Aghili Khorasani, 2011a). The plant seeds were poured into boiling water and put on medium heat for 20–30 minutes to be decocted and were then filtered. The aqueous extract obtained was dried using the spray drying method. Standardization was done using the rutin flavonoid (quercetin-3-rutinoside) measurement by ultraviolet–visible spectrophotometry, which was equivalent to 11.5 ± 1.5% (w/w) (Harborne, 1998). The spray dried extract was filled in 500 mg capsules. As the average daily use of dodder seeds is 12 g (Aghili Khorasani, 2011a), dry weight of the aqueous extract was measured; the mean value was found to be equal to 33 ± 1% (SD, n=3). Thus, the daily intake of whey powder was defined as 30 g. The produced powder was packed into 15 g vacuum-sealed bags.

2.2.3. Placebo

To prepare a placebo similar to whey in terms of colour, shape, smell and taste, corn starch, cornmeal and lactose in the ratio 6:1:1 were used. The placebo 500 mg capsules were filled with corn starch. Packaging was the same as the original drug. This placebo does not have a known effect on atopic dermatitis.

The drug and placebo were supplied in the School of Traditional Medicine, Kerman, Iran and all products were controlled in the Microbial Control Laboratory of the faculty for possible microbial contamination before use. In order to fingerprint every batch produced, the whey powder was measured using the biuret method for total protein, and in the case of the dried dodder extract, TLC (thin-layer chromatography) was used (Goyal and Gandhi, 2009; Harborne, 1998).

Patients were instructed to prepare the medicine by mixing a 15 g packet of powder in 200 ml of warm water and consuming it with two capsules twice a day 30–60 min. before eating breakfast and dinner.

2.3. Assessment

Patients were randomized into two treatment and placebo groups and received WaDSE or a placebo for 15 days. Five variables were analysed, including skin moisture, elasticity, pigmentation, sebum and surface pH using Multi Skin Test Center® MC1000 (Courage & Khazaka, Germany) with Complete Skin Investigation (CSI) software. The device uses specific probes for measuring skin parameters and makes it possible to assess, compare and quantitatively analyse changes of biophysical skin conditions related to atopic dermatitis during the treatment process. The Corneometer probe measures electrical capacitance of the skin surface for evaluating moisture. The Sebometer probe estimates sebum secretion on skin with a photometric method which is independent of moisture. The Pigmentation measurement probe works based on the absorption principle. This probe emits light of a defined wavelength that is absorbed by the melanin, then the receiver measures the amount of light reflected from the skin. Recorded values of moisture, sebum and skin pigmentation are expressed as arbitrary units (AU) on a scale from 0 to 99. In order to measure the elasticity of the skin by the suction method, the skin is sucked by a special probe under a negative pressure of 400 mbar for three seconds and, after stopping the negative pressure, the skin is released by the probe within the next three seconds. With this method, the amount of skin resistance to pressure in relation to its ability to return to its original position is expressed as a percentage, where a higher percentage represents healthier skin. The pH meter measures skin surface pH using a glass electrode. The advantage of this method is to minimize the possibility of human error in estimating skin barrier function and the severity of skin changes, using non-invasive bioengineering methods (Knor et al., 2011; Sator et al., 2003).

In this study, the primary outcome variables were measured using the device mentioned above by a physician at three points of the anterior forearm and the average was calculated. The patients were also asked to report pruritus and sleep disturbance due to itching over the preceding three days depending on the severity of symptoms from zero to 10 (zero indicates absence and 10 represents the maximum value) to be included as the secondary outcome variables (The European Task Force on Atopic Dermatitis, 1993). These assessments were carried out in all patients upon admission (week zero), 15 days after receipt of the drug (week three) and 15 days after the end of the treatment for follow-up (week five).

2.4. Sample size

Based on statistical calculations with the default power (1–β) 0.95, type 1 error (α) 0.05, 20% improvement in drug efficacy variables ($X_1 - X_2$) and the group variance ($S_1$ and $S_2$) about 25%, and with the overall sample size formula

$$n = \frac{(S_1^2 + S_2^2)(Z_{1-\alpha/2} + Z_{1-\beta/2})^2}{(X_1 - X_2)^2}$$

the estimated sample size was 40 subjects. With a 30% possibility of withdrawal rate, a sample size of 52 patients (26 patients in the medicine group and 26 patients in the placebo group) was considered.

2.5. Randomization and blinding

Using the randomization table designed by an independent statistician, random numbers successively were assigned to the patients and they were equally randomized into two groups. All
patients were informed they may be allocated to the drug or placebo group. In this study, the evaluating physicians had not been in contact with the statistician and clinical pharmacist and were kept blinded to allocation. Patients were also unaware until the study was completed that they had received the drug or a placebo.

2.6. Statistical analysis

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS), version 16. Continuous variables were expressed as mean ± SEM.

In the case of each variable, a t-test was conducted in order to determine whether there is a significant difference between the drug and placebo groups. Repeated measures ANOVA was used for analysis between and within the groups at weeks zero, three and five. The level of significance was established at 0.05.

3. Results

3.1. Patient enrolment and/or exclusion

A total of 52 patients participated in the study from February to July 2014, 26 patients were randomized in the placebo group and 26 patients in the treatment group. During the 30-day study, 10 patients (two patients in the treatment group and eight patients in the placebo group) were excluded from the study population for the following reasons: within the first 15 days of study, one in the treatment group and three in the placebo group were excluded because of poor compliance and one in the treatment group due to use of prohibited drugs. During the second half of the study period, five patients in the placebo group (one due to disease progression and four due to being busy) refused to continue with the follow-up and were excluded.

The number of patients who completed the study and were analysed was 42 (87%), 24 and 18 patients in the treatment and placebo groups, respectively. This information is summarised in the flowchart shown in Fig. 1.

Table 1 shows the baseline characteristics of the groups. There was no significant difference between the treatment and placebo groups in terms of age, sex, AD family history and duration of disease. In addition, there was no significant difference between the treatment and placebo groups at baseline in terms of skin moisture, elasticity, pigmentation, pH and sebum content.

3.2. Efficacy

The efficacy outcomes are shown in Table 2 and Fig. 2. At the end of the 30-day period of study, in terms of the primary outcome, the value of skin moisture and elasticity variables in the group receiving WaDSE was clearly significant compared to that of
4. Discussion

The current study indicated the prescription of whey associated with dodder seed extract to cause a clear and considerable increase in skin moisture and elasticity, decrease of pigmentation, reduced itching and improvement of sleep disturbance in patients with atopic dermatitis. Comparisons of mean ± SEM of patients receiving WaDSE revealed degrees of skin moisture and elasticity to be enhanced by approximately 100% and 40%, respectively.

An increase in elasticity, decrease in pigmentation, reduced itching and improvement of sleep disturbance occurring subsequent to reduced itching may be considered as a direct result of skin moisture enhancement. Notably, it can be said that although the patients received WaDSE only within the first 15 days of the study, skin moisture and elasticity continued to increase during the second half of the study (follow-up period). This result highlights that the effect of WaDSE is not transient; moreover, the drug actually contributes to the reconstruction of the skin barrier and is therefore effective in terms of the skin parameters by accelerating the trend of skin repair. Additionally, a decrease of skin pigmentation in patients consuming WaDSE proposes the efficacy of this drug in the improvement of post-inflammatory hyperpigmentation.

Skin barrier malfunctions caused by genetic and environmental factors, as well as immune system dysregulation is of the main pathophysiological aspects of atopic dermatitis (Leung et al., 2004). Ceramide deficiency as the major component of stratum corneum lipids and the main retaining factor of extracellular space water leads to epidermal water loss. Consequently, skin dryness occurs, which involves numerous pores and cracks, and enables the penetration of pathogens, antigens and irritants, subsequently leading to infection and inflammation (Cork et al., 2006).

A wide range of essential and non-essential amino acids, minerals, lipids and biologically active proteins such as lactoferrin, beta-lactoglobulin, alpha-lactalbumin, glycomacropeptide and immunoglobulins exist in whey, giving it a high nutritional value (Walzem et al., 2002). Due to its rich protein content, whey provides the possibility for better skin repair and proper skin barrier function (MacKay and Miller, 2003). On the other hand, lactoferrin present in whey is involved in bacteriostatic, bactericidal and anti-fungal activities (Farnaud and Evans, 2003; Orsi, 2004), and indicates that whey consumption in AD patients can reduce secondary bacterial and fungal infections in skin lesions.

Studies demonstrated that the consumption of whey is associated with an increase in serotonin level, leading to an increased ability for coping with stress (Markus et al., 2002). This in turn can influence the promotion of psychological health among AD patients whose quality of life has been negatively impacted as a result of the long-term tendency of the disease and its complications

### Table 1
Baseline characteristics of patients.

<table>
<thead>
<tr>
<th>Variable</th>
<th>WaDSE</th>
<th>Placebo</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (female)</td>
<td>20 (83.3)</td>
<td>16 (88.8)</td>
<td>0.621</td>
</tr>
<tr>
<td>Age (years)</td>
<td>28.62 ± 2.30</td>
<td>24.33 ± 1.50</td>
<td>0.155</td>
</tr>
<tr>
<td>Duration of AD (years)</td>
<td>13.45 ± 1.82</td>
<td>12.77 ± 2.09</td>
<td>0.760</td>
</tr>
<tr>
<td>Positive familial history of AD</td>
<td>11 (45.8)</td>
<td>8 (44.4)</td>
<td>0.931</td>
</tr>
<tr>
<td>Skin moisture</td>
<td>13.43 ± 2.08</td>
<td>19.72 ± 2.59</td>
<td>0.063</td>
</tr>
<tr>
<td>Skin elasticity</td>
<td>56.04 ± 5.32</td>
<td>61.73 ± 5.76</td>
<td>0.476</td>
</tr>
<tr>
<td>Skin pigmentation</td>
<td>22.66 ± 1.64</td>
<td>23.15 ± 2.42</td>
<td>0.864</td>
</tr>
<tr>
<td>Skin surface pH</td>
<td>5.80 ± 0.10</td>
<td>5.70 ± 0.11</td>
<td>0.546</td>
</tr>
<tr>
<td>Skin sebum</td>
<td>0.87 ± 0.25</td>
<td>1.03 ± 0.41</td>
<td>0.725</td>
</tr>
<tr>
<td>Pruritus</td>
<td>5.70 ± 0.45</td>
<td>4.72 ± 0.39</td>
<td>0.122</td>
</tr>
<tr>
<td>Sleep disturbance</td>
<td>2.41 ± 0.57</td>
<td>1.61 ± 0.24</td>
<td>0.255</td>
</tr>
</tbody>
</table>

Abbreviations: WaDSE, whey associated with dodder seed extract; AD, atopic dermatitis.

### Table 2
Change in variables during the study period.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Week 3 WaDSE Mean ± SEM</th>
<th>Placebo Mean ± SEM</th>
<th>p</th>
<th>Week 5 WaDSE Mean ± SEM</th>
<th>Placebo Mean ± SEM</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin moisture</td>
<td>20.97 ± 2.02</td>
<td>19.44 ± 2.44</td>
<td>0.629</td>
<td>29.91 ± 1.68</td>
<td>17.66 ± 2.20</td>
<td>&lt; 0.001 **</td>
</tr>
<tr>
<td>Skin elasticity</td>
<td>70.97 ± 4.29</td>
<td>63.48 ± 4.95</td>
<td>0.261</td>
<td>79.03 ± 2.96</td>
<td>59.30 ± 4.59</td>
<td>&lt; 0.001 **</td>
</tr>
<tr>
<td>Skin pigmentation</td>
<td>20.75 ± 1.63</td>
<td>23.31 ± 2.47</td>
<td>0.375</td>
<td>19.81 ± 1.54</td>
<td>23.82 ± 2.47</td>
<td>0.158</td>
</tr>
<tr>
<td>Skin surface pH</td>
<td>5.66 ± 0.10</td>
<td>5.74 ± 0.11</td>
<td>0.619</td>
<td>5.62 ± 0.07</td>
<td>5.66 ± 0.09</td>
<td>0.757</td>
</tr>
<tr>
<td>Skin sebum</td>
<td>1.20 ± 0.33</td>
<td>1.03 ± 0.40</td>
<td>0.753</td>
<td>1.22 ± 0.36</td>
<td>1.08 ± 0.41</td>
<td>0.799</td>
</tr>
<tr>
<td>Pruritus</td>
<td>2.75 ± 0.41</td>
<td>4.83 ± 0.45</td>
<td>0.002*</td>
<td>2.04 ± 0.32</td>
<td>5.38 ± 0.45</td>
<td>&lt; 0.001 **</td>
</tr>
<tr>
<td>Sleep disturbance</td>
<td>0.79 ± 0.26</td>
<td>1.50 ± 0.30</td>
<td>0.086</td>
<td>0.60 ± 0.20</td>
<td>1.77 ± 0.32</td>
<td>0.005*</td>
</tr>
</tbody>
</table>

Abbreviations: WaDSE, whey associated with dodder seed extract; SEM, standard error mean.

** p < 0.001.
* p < 0.05.
Fig. 2. Change in all the variables in the treatment (WaDSE) group from baseline (week 0) to weeks 3 and 5, compared to the placebo group. (a). Skin moisture. (b). Skin elasticity. (c). Skin pigmentation. (d). Skin surface pH. (e). Skin sebum content. (f). Pruritus. (g). Sleep disturbance. *p < 0.05; **p < 0.001.
complications and could even be posed as an alternative for systemic and highly morbid treatments. Despite short-term prescription of the mentioned drug in this study, skin moisture and elasticity underwent considerable enhancement.

Since changes in skin pH and sebum content displayed a long-term trend, other controlled trials over longer periods of time and with larger sample sizes should be performed on the parameters related to the assessment of the drug efficacy. Moreover, implementation of other clinical trials with longer follow-up periods will be able to evaluate the effects of this drug on the relevant atopies, such as asthma and allergic rhinitis.

Acknowledgements

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References
