

Efficacy of *Satureja khuzistanica* extract and its essential oil preparations in the management of recurrent aphthous stomatitis

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ABSTRACT

Background: The purpose of this study was to evaluate the efficacy of *Satureja khuzistanica* Jamzad extract and its essential oil preparations in the treatment of recurrent aphthous stomatitis (RAS) to explain its folkloric use as analgesic and wound healer.

Materials and Method: 60 patients with minor aphthae were selected and randomly divided into three groups. Groups A and group B received topical preparations of *S. khuzistanica* extract and *S. khuzistanica* essential oil, respectively. Group C (control group) received hydroalcoholic solution as a placebo. The date of pain elimination and the duration of complete healing were recorded.

Results: Mean time of pain elimination showed significant differences ($p = 0.0001$) between groups A (3.40 ± 0.50 days) and group B (3.20 ± 0.41 days) with group C (5.70 ± 1.12 days). The mean duration of complete healing also showed significant differences ($p = 0.0001$) between group A (5.90 ± 1.24 days) and group B (6.85 ± 1.30 days) in comparison to group C (10.40 ± 1.66 days). No significant differences were found between groups A and group B regarding both mean of the duration of complete healing of lesions and mean time of pain elimination ($p = 0.10$ and 0.085 respectively).

Conclusion: The results obtained for *S. khuzistanica* extract (group A) was similar to group B (received *S. khuzistanica* essential oil). The findings of this study revealed that *S. khuzistanica* extracts and *S. khuzistanica* essential oil preparation showed better effects in treatment of RAS than placebo. It was concluded that the *S. khuzistanica* is effective herbal medicine for the management of minor aphthae. Results of the present study may confirm the folkloric use of the plant.

Keywords: *Satureja khuzistanica*, essential oil, extract, aphthous, healing, pain

INTRODUCTION

Recurrent aphthous stomatitis (RAS) is one of the most common oral mucosal diseases worldwide (1). Despite much clinical attention, the exact etiology of this disease remains poorly understood (2-3). RAS is multi factorial process and several initialing factors such as trauma, stress, hormonal state, family history, food hypersensitivity, and microbial or immunologic factors are the most important predisposing factors (1-3). Several of topical and systemic preparations have been used for palliation or prevention of this ailment (4-5). In severe form of RAS, systemic agents such as colchicine, dapsone, corticosteroids are administered to control sign and symptoms of RAS (6). All therapies are palliative, and none result in permanent remission (7).

Medicinal plants preparations with antibacterial, antifungal, anti-inflammatory and antioxidant activities have been used for reduction of pain and shortening of healing time of oral aphthous ulcers (8-14). Essential oils and extracts of *Z. multiflora*, *A. nobelis* and *M. communis* have been reported to have promising activity against RAS or other oral cavity disorders (12-14).

Satureja khuzistanica Jamzad (labiatae), (marzhe-khuzistani in Persian), a recently identified plant, is native of western and southern part of Iran (15). It has been used as an analgesic and antiseptic in folk medicine of the region. The unique chemical composition of essential oil of *S. khuzistanica* and phytochemical analysis of extract of this plant has been reported recently (16-17). Extract and essential oil of this plant as

well as carvacrol, the main constituent of its essential oil, have shown propitious antimicrobial, analgesic and antioxidant activity (17-21). On the basis of the use of this plant in folkloric medicine in southern part of Iran for relief of toothache, strengthening the gum, healing the wound, as well as reported antimicrobial (18), anti-inflammatory activities (20) and antioxidant properties of this plant (21), in the present study a randomized, double blind clinical trial was designed to investigate the efficacy of *S. khuzistanica* extract and its essential oil preparations in comparison with hydroalcoholic vehicle as placebo in the management of the recurrent aphthous lesions.

PATIENT AND METHODS

Plant material

Aerial parts of cultivated *S. khuzistanica* in flowering stage from open field were prepared by Khorraman Company in industrial village of Khoramabad, Lorestan Province, Iran. The plant was authenticated by herbal museum of the Faculty of Pharmacy, Tehran University of Medical Science, Tehran, Iran and a voucher specimen (No. 6650-THE) is deposited. The essential oil of *S. khuzistanica* used in this study was obtained by steam distillation of the fresh leaves using a Clevenger-type apparatus for 5 h, which gave yellow oil in 0.8% yield (?). Hydroalcoholic extracts (25 %) of *S. khuzistanica* were prepared in Faculty of Pharmacy, Tehran University of Medical Sciences (13). Dried and powdered aerial part of *S. khuzistanica* (500 g) was extracted 3 times with 2 L of ethanol (50 %; v/v) by percolation at room temperature. The volume of extracts were adjusted to 2 L with the ethanol (50 %; v/v) and labeled A for *S. khuzistanica* extract preparation. A 0.2 % (v/v) hydroalcoholic solution of *S. khuzistanica* essential oil was prepared in an ethanol/water mixture (50 %; v/v) and labeled B. Same hydroalcoholic solution without any active material was used as a placebo and labeled C.

Patients

A total of 60 outpatients, 32 (53.3 %) women and 28 (46.7 %) men, mean age 28.48 years (10-64 years), with a history of RAS and currently suffering from ulceration located on the oral mucosa were selected from the outpatients attending the Oral Medicine Clinic, Faculty of Dentistry, Tehran University of Medical Sciences. All patients were interviewed and those with systemic diseases or special syndrome of which aphthous ulcer is one of its symptoms (Behcet's syndrome); those with aphthous lesions older than

2 days, those having smoking habits, patients subjected to any other treatment for at least 4 weeks before the start of the study and those without inclination for entering the study were excluded from the study. Diagnosis of minor aphthae was made based on the patient's health history and the presence of a well-demarcated painful ulcer on the smooth unattached oral mucosa, surrounded by a light red areola (1, 2). All patients were asked to grant the informed consent as required by the local Ethics committee. At the initial appointment, patients were asked to read and sign a letter of informed consent and to complete a medication history questionnaire.

Study design

Patients under the study assigned randomly to one of the three treatment groups named A, B, and C. Patients of each groups received 20 ml of anonymous herbal preparations and instructed to apply the medication four times daily by putting a small sterile cotton pad impregnated with 5 drops of each preparation (unknown to clinicians and patients) on the lesions for one minute and fasted at least for 30 min. They were also instructed to record the date of pain elimination and healing of lesion. The investigators remained in contact with the patients to insure the correct use of the drug, recording the results in the questionnaire and determining any adverse reactions. Patients were re-examined one week later and questionnaires were collected.

Statistical analysis

The values are reported as mean \pm SD. Statistical analysis of data was carried out using SPSS software (Ver. 11.5). One-way ANOVA followed by Dunnett's post test was used to test the differences between groups. Values of $p < 0.05$ were considered significant.

RESULTS

The main constituent of *S. khuzistanica* essential oil used in this study which has previously been reported (16) was carvacrol (92.5 %), thymol (1.1 %), *p*-cymene (0.9 %) and eugenol (0.8 %). Sixty out of 65 patients (28 males and 32 females) with RAS participated in the study. The mean age of the patients was 28.48 years (range 10-64). Twenty patients (33.3 %) received *S. khuzistanica* extract (group A); 20 patients (33.3 %) received *S. khuzistanica* essential oil (group B) and 20 patients (33.3 %) received 50/50 % mixture of ethanol/water solution (group C; control group) as placebo.

The duration of complete healing and dates of pain elimination were recorded. The mean duration of through healing of lesions in the

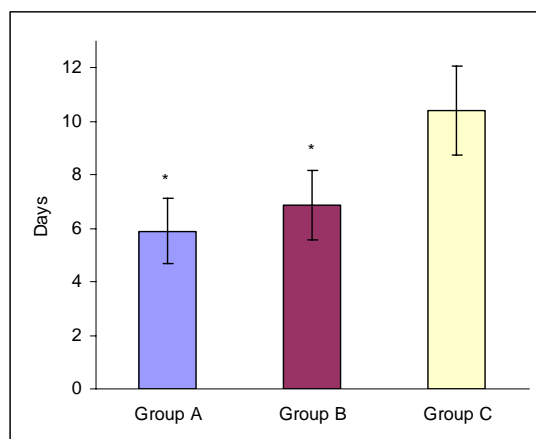


Figure 1. Average time for complete healing of lesions after receiving *S. khuzistanica* extract (group A); *S. khuzistanica* essential oil (group B) and placebo (group C). The bars indicate the standard error of the mean for each group (n=20). *Statistical analysis shows that group A and group B are significantly different from group C ($P = 0.0001$), but there is no significant differences between group A and group B ($p = 0.10$).

studied groups are presented in Fig. 1. Statistical comparisons of findings showed significant differences ($p = 0.0001$) between group A (5.90 ± 1.24 days), group B (6.85 ± 1.3 days) with control group C (10.40 ± 1.66 days). No significant differences were observed between groups A and B ($p = 0.10$) regarding duration of healing of lesions. The average time of pain elimination in the same groups are displayed in Fig. 2. Mean time of pain elimination showed significant differences ($p = 0.0001$) between groups A (3.40 ± 0.50 days) and B (3.20 ± 0.41 days) with group C (5.70 ± 1.12 days). No significant differences were found ($p = 0.085$) between groups A and B regarding mean time of pain elimination. Two patients in-group B reported slight burning sensation after application of medication for the first time.

DISCUSSION

RAS is a common oral disorder of uncertain etiopathogenesis that its management is largely directed toward symptomatic treatment (1-3). Different classes of chemical and biochemical products have been reported to be of some benefit in the management of RAS, but still no definitive treatment is available (2).

The main problem with aphthous ulcers is accompanying pain. If pain could be controlled, more complicated treatments could be avoided. Topical anti-inflammatory corticosteroids reduce pain severity and ulcer frequency (4). Local anesthetics work well but they should be repeated

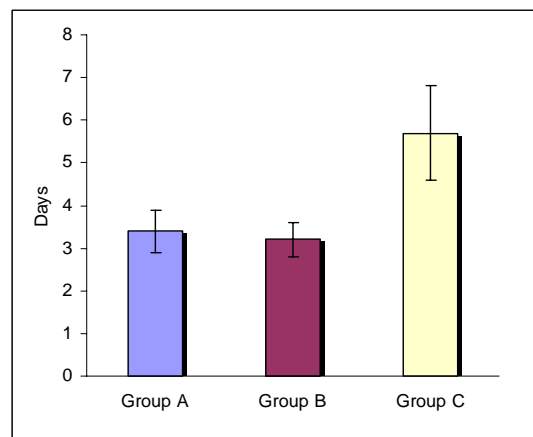


Figure 2. Average time for elimination of pain among three groups after receiving *S. khuzistanica* extract (group A); *S. khuzistanica* essential oil (group B) and placebo (group C). The bars indicate the standard error of the mean for each group (n=20). *Statistical analysis shows that group A and group B are significantly different from group C ($p = 0.0001$), but there is no significant differences between groups A and group B ($P = 0.085$).

many times (7). Management of pain in RAS with various herbal preparations have also been reported (8, 9, 14). Simple non-synthetic, natural antimicrobial agents including in the commercial mouthwash Listerine, whose active ingredients are a mixture of essential oils have been used to decrease the pain, duration and severity of RAS and possibly reduce the occurrence of ulceration in susceptible patient (22).

As shown in Fig. 2, in addition to the pain-attenuating effect of both preparations used in this study from 5.70 ± 1.12 days in control group to 3.40 ± 0.50 and 3.20 ± 0.41 days following therapy in group A and B, there was a reduction in the mean healing time from 10.40 ± 1.66 days in control group to 5.90 ± 1.24 and 6.85 ± 1.30 days following the therapy in group A and group B (Fig. 1). Previous studies have shown that healing time in placebo group is about 9 days (1, 2, 6, 12, 14) which is similar to findings of this experiment (10.40 ± 1.66 days). The results of this study regarding complete healing time and elimination of pain are consistent with the results reported for other plants (Table 1) (12-14).

The reported antioxidant activity (21) as well as remarkable antibacterial, analgesic and anti-inflammatory effects of the essential oil and extract of this species (18,20) may be considered as a value in management of minor type of RAS. These effects may be either attributed to antibacterial effects of essential oil, which was reported for *S. khuzistanica* and other species (18-19), altering the microbial flora which results in

Table 1. Comparison of mean time of pain elimination and duration of healing of ulcer in minor aphthae for selected herbal preparations

Plant name and	Type of preparation and concentration (mg/ml)	Mean time for pain elimination (days)*	Mean duration of healing of ulcer (days)
<i>M. communis</i> ^a	EO (50)	5.70	5.40
<i>Z. multiflora</i> ^b	EO (2)	2-7	4.50
<i>Z. multiflora</i> ^c	EX (250)	3.00 ± 1.14	6.00 ± 2.80
<i>A. nobilis</i> ^c	EX (250)	5.20 ± 3.11	8.7 ± 3.90
<i>M. communis</i> ^c	EO (50)	4.30 ± 2.12	7.60 ± 3.10
<i>S. khuzistanica</i> ^d	EO (2)	3.20 ± 0.41	6.85 ± 1.30
<i>S. khuzistanica</i> ^d	EX (250)	3.40 ± 0.50	5.90 ± 1.24

^a. See Ref. 12, ^b. Ref. 13, ^c. Ref. 14, ^d. Present study. EO= essential oil, EX= extract

*. Values represent the mean ± SD.

less immunologic damage as reported for the effect of some antibacterial mouthwash against RAS (13, 22). On the other hand, the beneficial effects of this herb in management of minor type of RAS could be in part due to presence of sitostrols or flavonoids in extract of the aerial parts of *S. khuzistanica*, which may act as antioxidant and anti-inflammatory agents (21, 23-24).

The *S. khuzistanica* essential oil, presented good results as a single preparation (group B), but it causes slight burning at first time of application. The clinical improvement including pain reduction and shortening the duration of RAS in patient as well as patient acceptance, ease of use, minimal side effects compared to *S. khuzistanica*

essential oil preparation support that *S. khuzistanica* extract could act as a potent herbal preparation for treatment RAS. The effectiveness of preparations of this plant may be attributed to the protective effect of these preparations in reducing irritation, inflammation and prevention of the probable secondary infections of the ulcers due to anti-inflammatory, anti-bacterial activity and anti-oxidant capability of these products or other mechanisms that should be elucidated through further investigation.

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