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ORIGINAL ARTICLE

The efficacy of a paste containing *Myrtus communis* (Myrtle) in the management of recurrent aphthous stomatitis: a randomized controlled trial

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Abstract Recurrent aphthous stomatitis (RAS) is a common, painful, and ulcerative disorder of the oral cavity with unknown etiology. Treatment is a highly controversial topic. The aim of this study was to evaluate the clinical efficacy of a novel paste containing *Myrtus communis* (Myrtle) in the treatment of recurrent aphthous stomatitis. Myrtle is a particular herb used in some cultures as treatment for mouth ulcers. The study was a randomized, double-blind, controlled before–after clinical trial. Forty-five patients with RAS randomly participated in this study. The subjects were treated with placebo paste and myrtle oral paste in two consecutive episodes. The paste was applied by subjects themselves four times a day for 6 days. Five parameters (size change, pain scale, erythema and exudation level, oral health impact profile, and patient overall assessment of their treatment) were recorded both before (baseline) and during each episodes of treatment (on the morning of days 2, 4, and 6). There were no statistically significant differences between baseline parameters ($p>0.05$). The data indicated a statistically significant reduction of ulcer size ($p<0.001$), pain severity ($p<0.05$), and erythema and exudation level ($p<0.001$). Oral Health Impact Profile improved significantly in the treatment group ($p<0.001$). Patient overall assessment of their treatment improved after applying paste containing myrtle ($p<0.05$). No side effects were reported. This study has shown myrtle to be effective in decreasing the size of ulcers, pain severity and the level of erythema and exudation, and improving the quality of life in patients who suffer from RAS.

Keywords Aphthous stomatitis · *Myrtus communis* · Pain · Quality of life · Therapy

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Introduction

Recurrent aphthous stomatitis (RAS) is a common disease of the oral mucosa affecting 5% to 25% of the general population [1]. Aphthous stomatitis is divided, on morphologic criterion, into three clinical presentation include minor (<1 cm), major (>1 cm), and herpetiform aphthae. Minor recurrent aphthous ulcers are the most prevalent form (80% of all recurrent aphthous ulcers) [2]. Minor ulcers typically involve movable and nonkeratinized mucosa, such as the buccal and labial mucosa and the lateral border of the tongue. For 24–48 h preceding the development of a minor aphthous ulcer, subjects usually describe a prickling or burning sensation in the mucosa. In this prodromal stage, erythema of the surrounding mucosa may be observed or it may appear normal. Within a day or so, an oval or round

ulcer with a gray-white center and erythematous halo develops. During an attack of minor aphthae, a single lesion or up to five concurrent ulcers may occur. These ulcers are self-limiting and resolve within 10–14 days without scarring. The variability in times of recovery can depend on the area affected or on whether superinfection occurred [3–5].

The exact cause of RAS has not been disclosed clearly. However, some etiologic and predisposing factors such as hypersensitivity, immunodeficiency, local trauma, genetic background, nutritional deficiencies, systemic diseases (Crohn's disease, ulcerative colitis, Behcet's syndrome, celiac disease), stress, and infective agents are suggested [5–7].

The ulceration may lead to difficulty in speaking, eating, and swallowing, thus may negatively affect patients' quality of life. Treatment of RAS is symptomatic and based mainly on empirical evidences. It is mainly directed at alleviating pain and decreasing functional disability, inhibition of the acute inflammatory reaction as well as the frequency and the degree of severity of the recurrences. The existence of several therapies, including topical corticosteroids, mouth rinses, antibiotics, local anesthetics, or analgesics agents and immunomodulator agents, supports the fact that no treatment is entirely successful in the management of RAS. The reduction of discomfort and pain in RAS is important for improving patients' quality of life [2, 5, 8, 9].

In view of several drawbacks of synthetic compounds for the human organism, examination of preparations of plant origin for treatment of diseases has received increasing attention.

Myrtle (*Myrtus communis*) is a perennial shrub, widely distributed in the north of Iran. In folk medicine, the leaves are used as a mouthwash for the treatment of candidiasis and it has been over the counter in Persia for a number of years. In fact, myrtle extracts have been reported to possess antihyperglycemic [10] and antibacterial [11, 12] and analgesic [13] properties. Recent reports have described antioxidant activities of different extracts of myrtle, suggesting their therapeutic use for the treatment of diseases related to inflammation and allergy [14].

As two of the most emphasized pathophysiological mechanisms for RAS are inflammatory reactions and hypersensitivities, in order to determine both the efficacy and safety of the aqueous extract from leaves of myrtle when applied topically for the treatment of RAS, a novel paste containing 5% myrtle was developed and a randomized, double blind, before–after, placebo controlled clinical trial was performed.

To our knowledge, no study has been carried out on RAS treated with paste containing myrtle and the present study is the first one addressing this subject. The purpose of this study is to assess the clinical efficacy and safety of myrtle oral paste in treating RAS.

Materials and methods

M. communis was collected from the National Park of Sisangan situated in the north of Iran in November 2005. Identification was carried out by Dr. A. Moghadamnia (Department of Pharmacology, Faculty of Medicine, Babol University of Medical Sciences). A voucher specimen has been kept in our laboratory for future reference. The leaves were shade dried, powdered, and stored in a tightly closed container for future use. The powdered leaves were extracted with boiling water for 15–20 min. After filtration, the extracts were filtered and lyophilized. The residues were dissolved in water and filter-sterilized through a 45-μm Millipore filter. Of the topical agents that are available for the treatment of RAS, Amlexanox paste is the most extensively studied. Amelexanox paste contains 2-amino-7isopropyl-5oxo-5H-(1) benzopyrano-(2,3-b)-pyridine-3-carboxylic acid as its active component benzyl alcohol, gelatin, glyceryl monostearat, mineral oil, pectin, petrolatum, and sodium carboxymethylcellulose [15]. The placebo paste contained above ingredients except the active component. Myrtle was added to placebo paste to produce myrtle oral paste of 5%.

Subjects and study design

The study was a randomized, double blind, controlled before–after clinical trial. All studies recruited subjects from the clinical patients of the participation centers and had identical inclusion and exclusion criteria. For inclusion criteria, all subjects (1) were 18 years of age or older, (2) had a clear history of RAS occurring at least twice a year, (3) presenting with one to three aphthous ulcers (less than 48 h duration), measuring no more than 10 mm in diameter, (4) had an expectation that their ulcers normally takes more than 5 days to resolve without treatment, and (5) had no underlying medical or hematological cause for their ulceration. All the patients with systemic diseases, a history of an immunologic problem, a present or recent history of drug or alcohol abuse and subjects who were unable to understand consent form and who to use a visual analog scale (VAS) for pain measurement and patients who taking systemic nonsteroidal anti-inflammatory drugs or immunomodulatory agents within 1 month prior to the study were excluded.

Between September 2005 and October 2006, a total of 58 randomly selected subjects were evaluated for inclusion to the study. Based on the criteria, 45 subjects were enrolled in this study. There were 26 subjects who were enrolled at center 1 (Oral Medicine Department, Faculty of Dentistry, Tehran University of Medical Sciences) and 19 subjects at center 2 (Oral Medicine Department, Faculty of Dentistry, Babol University of Medical Sciences).

The protocol for the study had received approval from the Institutional Review Board of Tehran University of

Table 1 Erythema and exudation level

	0	1	2	3
Erythema	No erythema	Light red/pink	Red but not dark in color	Dark in color
Exudation	No exudation	Light exudation	Moderate exudation	Heavy exudation with pseudomembrane

Medical Sciences, and each subject signed a detailed informed consent form. All steps of the study were planned and according to the principals outlined in the Declaration of Helsinki [16] and ethical codes provided by Undersecretary of Research, Iran Ministry of Health.

Our study has been designed in such way that subjects are actually comparing the pain between two aphthous stomatitis episodes, avoiding interindividual variation in pain assessment. Thus, variations in pain threshold had no effects on the outcome. The subjects were treated with placebo paste and myrtle oral paste in two consecutive episodes.

After providing written informed consent, each subject was allocated a study number; a pharmacist then dispensed the study drug to each participant. The study subjects were blinded to the treatment agents, as were the physicians involved with pain assessment and clinical examinations. During the conduct of study, only the dispensing pharmacist had knowledge of the study codes, demonstrating which subject receives in which episode placebo or treatment agent. Treatment assignment codes were not available to the investigators until all subjects completed the study.

Intervention and assessment

The baseline parameters were taken and recorded on the day of first visit. All subjects were assessed four times during the treatment course (visits 1–4). In all evaluations, the size of ulcers were measured by two blind and independent investigators in each center at initial visit (day 0) and days 2, 4, and 6. Ulcer size was measured using a sterile calibrated dental probe with millimeter marking and the longest diameter was used as measurement. Any disagreement in assessor-related measurements was resolved by consensus between them. For subject who has more than one ulcer, the most accessible ulcer was selected. Pain was evaluated by subjects at the initial visit and at each evaluation thereafter (days 2, 4, and 6). Subjects ranked the severity of pain and burning sensation on 100-mm VAS, a 100-mm line labeled at one end "no soreness" and the other end "worst possible soreness". The subjects were given a pencil and asked to mark the VAS scale at the point which best represented the present pain level of the ulcer [17]. The level of erythema and exudation were evaluated by the investigators on a four-point scale ranging from 0 to 3 (Table 1) based on the methods of Liu et al [18]. Moreover, patients' quality of life was measured by means of the Oral Health Impact Profile (OHIP; 14-item questionnaire) on day 4, which measures patients' perceptions of the impact of oral conditions on their well-being [19].

At first visit, subjects were instructed to apply paste on the oral lesion and written instruction on how to apply the drug was given to them. Subjects were advised to dry the ulcer by patting it with soft clean gauze, squeeze 1 cm of the drug onto a wet fingertip, and dab the paste onto the ulcer but not rub it into the ulcer, as this would cause irritation. Subjects were advised to apply the drug four times a day, preferably after oral hygiene. Subjects were observed for 30 min for any possible signs of acute hypersensitivity reactions or other adverse events.

Efficacy evaluations were made on the morning of days 2, 4, and 6. In each visit, the subjects were asked about any unwanted side effects such as burning sensation or taste sense malfunction and were examined for any abnormal alteration in the appearance of mucosa such as hypersensitivity and infection. The investigator also examined the ulcer area for any associated mucosal changes inconsistent with a healing aphthous ulcer. In addition, all subjects were requested to immediately inform investigators at any time for any side effect. At the last appointment in each treatment, subjects were asked to make an overall assessment of the gel on three-point description scale (poor, moderate, and good).

Table 2 Comparison of baseline values of VAS, size, and erythema and exudation level at study entrance

	Mean rank	Sum of ranks	Myrtle oral paste (mean ± SD)	Placebo paste (mean ± SD)	*p*
Pain, VAS (mm)	12.88	206.00			>0.05[a]
Erythema and exudation level	8.00	40.00			>0.05[a]
Size (mm)			3.05±1.450	2.82±0.51	>0.05[b]

VAS visual analog scale

[a] Wilcoxon signed ranks test

[b] Paired *t* test

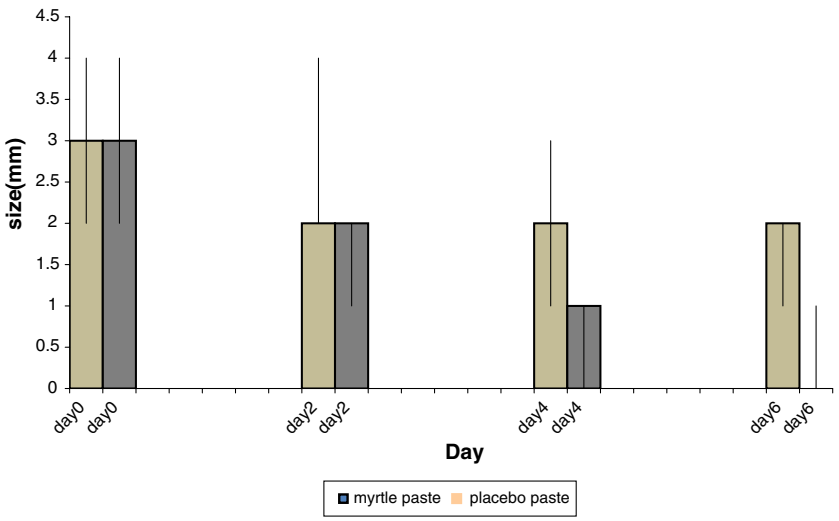

Fig. 1 Median changes in ulcer size. Median ulcer size in the myrtle oral paste group matched well with that of the placebo group ($p>0.05$). Median ulcer sizes in the myrtle oral paste group on days 2, 4, and 6 were significantly smaller compared with that of the placebo group ($p<0.001$)

Outcomes measure

Demographic data were collected. The baseline parameters of VAS, ulcer size, and level of erythema and exudation were compared between two groups for any significant differences. Subjective outcome measures were pain score (days 2, 4, and 6) measured by VAS during treatment with myrtle oral paste over placebo control group, OHIP score on day 4 for each group, and subject overall assessment score at the end of each treatment on three-point description scale (poor, moderate, and good). In addition, subjects were asked to report any side effects.

The objective outcome measures were size of the ulcers (millimeters) and level of erythema and exudation on days 2, 4, and 6. Moreover, site of ulcer were monitored for any unwanted side effects such as hypersensitivity and infection.

Statistical analysis

The analyzer was blind to the study. Data were presented as mean ± standard deviation (SD) for quantitative variables with normal distribution and ranks for the others. Paired *t* test was used for the comparison of baseline size of ulcers and OHIP before and after the treatment with myrtle oral paste. Nonparametric Wilcoxon signed ranks test was used for the comparison of baseline values of VAS and level of erythema and exudation. Also, Wilcoxon was used for comparison of the level of erythema and exudation, size, VAS, and patient overall assessment of their treatments between two groups.

All data were analyzed using SPSS software (version 13.0 for Windows; SPSS Inc., Chicago, IL, USA). The level of significance was established at p values less than 0.05.

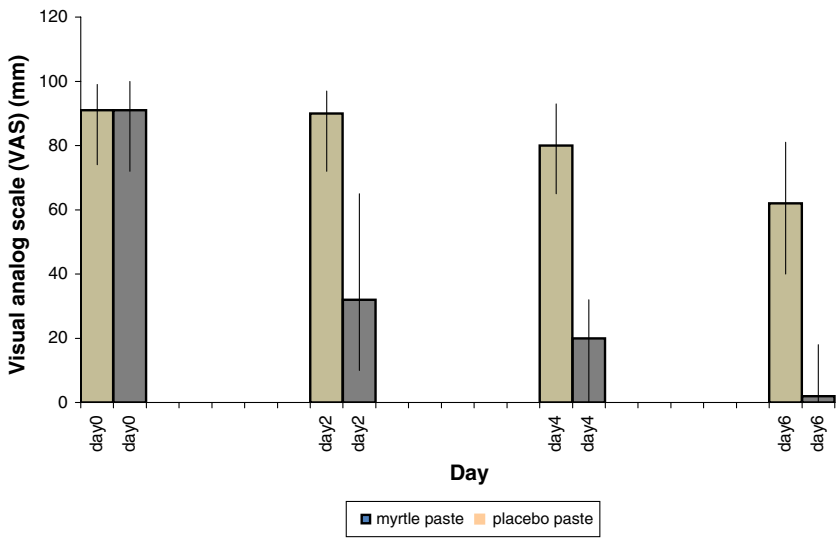

Fig. 2 Median changes in VAS. Median VAS score in the myrtle oral paste group matched well with that of the placebo group ($p>0.05$). Median VAS scores in the myrtle oral paste group on days 2, 4, and 6 were significantly smaller compared with that of the placebo group ($p<0.05$)

Table 3 Level of erythema and exudation in treatment and placebo groups

Degree	Visit			
	First (day 0)	Second (day 2)	Third (day 4)	Forth (day 6)
Treatment group				
0	0.0	10 (25.6%)	20 (51.3%)	31 (79.5%)
1	5 (12.8%)	21 (53.8%)	17 (43.6%)	8 (20.5%)
2	23 (59%)	7 (17.9%)	2 (5.1%)	0.0
3	11 (28.2%)	1 (2.6%)	0.0	0.0
Placebo group				
0	0.0	0.0	1 (2.6%)	3 (7.7%)
1	1 (2.6%)	6 (15.4%)	8 (20.5%)	22 (56.4%)
2	26 (66.7%)	22 (56.4%)	29 (74.4%)	14 (35.9%)
3	12 (30.8%)	11 (28.2%)	1 (2.6%)	0.0

$p<0.001$

Results

All subjects were recruited to the study from September 18, 2005 to November 23, 2007. Forty-five subjects consented and were enrolled. Five subjects were dropped from the study (four subjects discontinued the treatment and one subject stopped taking the treatment without any reason). As our treatment and control group were the same, the demographic characteristics were identical. Forty subjects completed the study. They were 17 (42.5%) males and 23 (57.5%) females. Their mean age was 29.5±10.3 years (range 18–58 years). On entry to the study, no statistically significant differences were found between the treatment group and control group in terms of size, pain severity (VAS score), and erythema and exudation level of ulcers (Table 2).

Although the ulcer size and the ulcer pain of the treatment group and the placebo group matched well at the study entry ($p>0.05$), significant group differences appeared at the later visits ($p<0.001$; Figs. 1 and 2). Also, the myrtle oral paste group had significantly lowered erythema and exudation level than those of the placebo control group at the later visits ($p<0.001$; Table 3). There was significant difference between the treatment group and the placebo group in term of OHIP score ($p<0.001$). Subjects overall assessment of their treatments is shown in Table 4. There was significant difference between Myrtle oral paste group and placebo group in the distribution of scores ($p<0.05$). No adverse side effects such as hypersensitivity, pain, infection, and taste sense malfunction occurred in any of subjects. In this study, the myrtle oral paste demonstrated significant improvement in all efficacies outcome measures in the treatment of RAS.

Discussion

The etiology of RAS is unknown, but it has a strong hereditary component and appears to be related to an immune reaction against the oral mucosa [20]. A possible relation between the inflammatory process and free radical metabolism has been reported in several studies [21]. In one study, Cimen et al. found increased oxidative stress and decreased antioxidant defense in mucosa of subjects with RAS [22]. Several studies suggest that local bacteria may play a role in the pathogenesis of RAS, perhaps by modifying the host's immunological response to secondary infection by such bacteria following early ulcer development. It has been suggested that immunologically driven reactions to heat shock proteins (a group of highly conserved and constitutively produced proteins found in both prokaryotic and eukaryotic cells which plays roles in normal intracellular housekeeping) are important, as subject with RAS have cross reactivity between a streptococcal heat shock protein and the oral mucosa, considerably raised levels of serum antibodies to heat shock protein, and have increased lymphoproliferative responses to a heat shock protein-derived peptide [23, 24]. RAS may therefore represent a T cell-mediated response to antigens of *Streptococcus sanguis* that cross react with mitochondrial heat shock protein and causes damage to the oral mucosa [24].

Myrtle was chosen as a possible treatment for RAS in this study because of its known antibacterial [11, 25], antigenotoxic, and free-radical scavenging activities [26].

Our interpretation of the data is that paste containing myrtle shows promise as a possible therapeutic in the management of RAS though it is not clear whether this

Table 4 Distribution of scores from subjects overall assessment of their treatment

Score	Placebo paste	Myrtle oral paste (5%)
Good	10 (26%)	17 (43%)
Moderate	13 (33%)	15 (38%)
Poor	16 (41%)	7 (18)

$p<0.05$

reflects an antibacterial effect in reducing secondary infection of ulcers, or whether it is related to free-radical scavenging effect or a combination of both.

RAS results in considerable pain and distress for subjects and presents a difficult management challenge for the clinician, as evidenced by the wide range of treatments that have been proposed for these ulcers. Pain reduction is essential not only for subject comfort but also because it can affect oral intake, swallowing, and speaking. If we control the pain, we could avoid using more complicated treatments. Pain is subjective and depends on factors such as personal experience, age, social, ethnic factors, and perceptual abilities [27]. Indeed, it is well recognized among clinicians that some patients with large ulcers seem to complain very little about pain, whereas others with small ulcers experience seemingly much more pain. In the present study, the subject served as his or her own control, thus avoiding variations in pain threshold. In this study, paste containing myrtle (5%) demonstrated improved efficacy in the treatment of RAS over placebo paste, as measured by both the primary "objective" outcome of complete ulcer healing and reducing the level of erythema and the secondary "subjective" outcome of complete pain relief and by means of Oral Health Impact Profile as well as measuring the subjects overall acceptability of the product. In conclusion, the results of this study provide evidence that myrtle oral paste is a well-tolerated effective treatment modality for RAS. Additional studies with higher number of subjects and in the prevention of ulcer development if treatment is commenced at the prodromal stage may be needed to examine the role of myrtle in the management of RAS.

Conflict of interest The authors declare that they have no conflict of interest.

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