

Therapeutic Efficacy of Different Concentrations of *Myrtus communis* (Essential oil of common myrtle) in the Treatment of Recurrent Aphthous Stomatitis: A Randomized Controlled Clinical Trial

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Abstract

Background and objectives: Recurrent aphthous stomatitis (RAS) is a painful ulcerative lesion and its incidence is 20% in the society. *Myrtus communis* (myrtex) has been effective in the treatment of RAS. In this study, two different concentrations of myrtex extract were evaluated to discover the most effective concentration for the treatment of RAS.

Methods: In this randomized, double-blind clinical trial, 60 patients with RAS were evaluated. Thirty patients used myrtex extract 5% and thirty patients used myrtex extract 2.5% (10 drops on lesion for 20 seconds 5 times per day). The severity of pain and burning sensation experienced by patients were measured by visual analogue scale (VAS) and the size of the lesion was estimated by transparent calibrated grid Data analysis was done by running t-test and repeated measures statistical test.

Results: The mean of the largest RAS diameter before treatment was decreased in both groups 1 day and 7 days after treatment (P-value=0.000); however, these differences were not significant in between groups (P-value =0.401). Furthermore, the severities of pain and burning sensation were decreased in both groups (p-value = 0.000). Nevertheless, this decrease was similar in both groups (P-value = 964).

Conclusion: Treatment with different concentrations of myrtex extract is effective in decreasing RAS diameter, pain, and burning sensation. In addition, the therapeutic efficacy of 2.5% and 5% concentrations of myrtex extract was similar in this regard.

Keywords: Recurrent Aphthous Stomatitis, Myrtle extract, *Myrtus communis*

Introduction

Recurrent aphthous stomatitis (RAS) is one of the most prevalent oral diseases. Its prevalence in the community is 5% to 50% and the mean of its incidence is 20% (1). RAS occurs more often in the second decade of life and is more prevalent in women than in men. RAS has a multifactorial etiopathogenesis, but the exact etiology of RAS remains elusive (2). Heredity, malnutrition, allergies to some drug categories, stress, local trauma, hormonal disorders, infection, poor oral health, and underlying diseases such as folate-deficiency anemia and iron deficiency anemia, vitamin B 12 malabsorption, cyclic neutropenia, and celiac disease have been considered as risk factor of RAS in specific subgroups of patients(3). RAS ulcers are extremely painful, presenting as round ulcers with well-defined margin, covered with yellowish-gray pseudomembranous, and surrounded by erythematous haloes (3). RAS lesions involve the non-keratinized mucosa, and in particular the labial and buccal mucosa, cheeks, the floor of the mouth and the ventral or lateral surface of the tongue (4). Symptoms such as tingling, itching, and burning sensation appear before the appearance of the ulcer. RAS ulcers heal within 10 to 14 days without leaving any scars (5). There is no definite laboratory procedure available for RAS diagnosis (6). Since there is no laboratory test to diagnose RAS, its diagnosis is based on history and clinical manifestations. Currently, the treatment of this disease is nonspecific and is based on empirical data in most cases (7) and none of these treatments leads to permanent disease remission (8). The primarily goals of RAS treatment is pain alleviation, restoration of oral functions, suppression of inflammatory responses, and reduction in the frequency of the recurrences (9). RAS

treatment should be initiated as soon as possible. Identifying and eliminating predisposing factors for RAS lessens the frequency of recurrence (10). Current topical treatment modalities consist of antibiotics, anti-inflammatory drugs, anesthetics, steroids, sucralfate, tetracycline suspensions, and silver nitrate (10), also the use of other systemic therapies affecting immune system is useful in patients with treatment-resistant RAS or other types of RAS associated with systemic involvement (9). In general, the prognosis of RAS is desirable and spontaneous remission can be achieved after several days (10).

Considering that herbal medicines are more favorable and induce less complication than chemical drugs, the use of a herbal medicine with therapeutic properties and at specific concentration can be of great help to the physician in relieving pain and burning sensation induced by RAS. Myrtus communis (myrtex) is a herbal medicine that has been used in the treatment of RAS is holy in Zoroastrianism and it is the symbol of Ahura Mazda (11). Myrtus communis, known as myrtex, is from Myrtaceae family. Myrtex leaves constitute 1.5% to 2% of myrtex extract, containing terpinolene, cineol, linalool, and teripneol and linaline acetate. The extract of myrtex leaves contain tannins, flavonoids, and vitamin C (82 mg / 100 g in dry leaves) (12). Myrtex has a variety of therapeutic effects, including anti-herpes simplex virus type 1 activity (13), antioxidant (14), anti-inflammatory (15), and anti-diarrheal (16). Myrtex has antiparasitic effect against Trichomonas vaginalis (17) and giardia and it is used for treating respiratory infections (18). However, different effects have been reported for various concentrations of myrtex extract regarding RAS treatment, indicating that myrtex extract at the concentration of 5% was more effective than

myrtex extract at the concentration of 10% (19). Moreover, the antimicrobial effect of myrtex extract 2.5% was higher than myrtex extract 5% (20). Considering that myrtex extract 5% is currently available in the market, it is important to discover whether the effective myrtex extract concentration for the treatment of aphthous stomatitis is 5% or lower concentrations of this extract can exert this therapeutic effect efficiently? Therefore, in this study, the treatment efficacy of two different concentrations myrtex extract (5% and 2.5%) was compared with respect to RAS management.

Materials and methods

This randomized, double-blind clinical trial was registered at the Iranian registry of clinical trials (IRCTID: 2015053020903N). This research was presented to the ethics committee of Shahid Sadoughi University of medical sciences and approved the study with number 17/1/223594. Before initiating the study, informed consent was obtained from each patient. Sixty patients who referred to department of oral and maxillofacial medicine in Dentistry school, Yazd, were selected from November 2014 to March 2015. With respect to clinical features (1), RAS was diagnosed in patients and then confirmed by an oral and maxillofacial medicine specialist. According to medical history, patients had no history of smoking or tobacco use and did not have any systemic disease associated with RAS, HIV, cyclic neutropenia, celiac disease, hormonal disorders, and anemia. Then, information on patients' age, sex, size of the lesion, as well as the severity of pain and burning sensation experienced by each patient was recorded. The size of the lesions was measured by placing a transparent calibrated grid on the

RAS, delimiting RAS boundary, and replacing transparent calibrated grid on a 1 mm graph paper, which is a reliable method for measuring the size of the lesions (21, 22). The severity of pain and burning sensation was evaluated by the Visual Analogue Scale (VAS), which is a horizontal line, 100 mm in length (23). Zero means no pain and 100 indicates the highest level of pain experienced by the patient over his/her lifetime. Sixty patients were selected randomly using random number table (24). Patients were randomly assigned into two groups of A and B (30 patients in each group). Group A received myrtex extract at 5% concentration and group B received myrtex extract at 2.5% concentration. Myrtex extract was obtained from Kashan Barij Essence Pharmaceutical Company (ingredients: Essential oil of common myrtle (*Myrtus communis*)). Due to the fact that the present study was in the second phase of clinical trial and also the availability of previous similar studies using a control group and higher concentrations of myrtex extract and revealing the absence of placebo effect on the control group (19), this study merely focused on lower concentrations of myrtex extract without considering control group. Since the healing process of RAS lesions is recognized and it can be treated, the use of placebo in the control group is ethically unacceptable. RAS heals spontaneously within 10-14 days without leaving the scar (5). Myrtex extract 2.5% was prepared by diluting myrtex extract 5% with 1: 1 ratio using ethyl alcohol 80%. The preparation of solutions was carried out in the pharmacology laboratory of pharmacy department, Yazd University of Medical Sciences. The pre-made myrtex extract was used due to its availability and cost-effectiveness. The use of ethyl alcohol 80% could not make any

difference in terms of drug content because it was used in both solutions, including A and B for the purpose of dilution. Ethyl alcohol, even if as a placebo, cannot alter the pain, burning sensation, size of RAS, and the disease remission (19). Patients were instructed to drop 10 drops of the solution onto a small piece of cotton and then place it on RAS for 20 seconds. They should use the solution 5 times a day. The duration of the prescription was based on the manufacturer's instructions. Patients were told to continue taking medication until one day after achieving lesion remission. These patients do not allow to use other medications such as antibiotics or NSAIDs during this study. It should be noted that the patient and physician were unaware of the type of the drug and two different concentrations of myrtex extract were coded into A and B by a pharmacist. Patients were examined one day and seven days after taking the medication. Patient's remarks regarding the time of pain and burning sensation relieve as well as oral lesions remission in terms of ulcer size were recorded using the questionnaire. In patients with multiple ulcers, size was measured based on the largest wound but treatment was applied to all wounds. Data were collected and analyzed using SPSS (ver.17), and the required tables and indexes/variables were completed and running T-test and repeated measures statistical test.

Results

Sixty patients with RAS were investigated in the current study. The patients were divided into groups of A and B, which were matched together regarding age and gender. Group A was treated with myrtex extract 5% and group B with myrtex extract 2.5%. There were 18 men (60%) and 12 women (40%) in group A and 21 men (70%) and 9 women (30%) in

group B. The mean age of patients was 28.22 ± 6.04 ranging from 20 to 40 years old. The mean age of patients was 27.56 ± 6.04 in group A and 28.86 ± 6.47 in group B. There was no significant difference between the two groups regarding age and gender (respectively p. value= 0.409 and p. value= 0.471). The mean of the largest RAS was 3.21 ± 1.53 in Group B ranging from 1.5 to 7 mm and 3.77 ± 1.67 mm in group A ranging from 2 to 7 mm before initiating the treatment. According to results of t-test, two groups did not differ significantly in this regard (p. value= 0.178). The mean of burning sensation severity was 53 ± 21.41 ranging from 9 to 88 in group B and 54.8 ± 19.24 ranging from 27 to 91 in group A before undergoing treatment. The difference between group A and group B was not significant in terms of severity of burning sensation (p-value=0.733). Table 1 demonstrates the mean of RAS diameter in both groups during the treatment.

In order to determine the efficacy of myrtex extract 2.5% and myrtex extract 5% and duration of treatment (one day to seven days) in reducing RAS diameter and the effect of the drug over time repeated measures analysis of statistical test was run. As shown in Table 2, the efficacy of drug in reducing ulcer diameter over time was significant (p-value = 0.000). However, the effect of myrtex extract 2.5% was not significantly different from myrtex extract 5% considering RAS diameter reduction (p-value = 0.401). Furthermore, the interaction between concentration of myrtex extract and duration of treatment was not significant (p-value = 0.058). Table 3 demonstrates the means of severity of pain and burning sensation, induced by RAS, based on the duration of treatment.

Table1. Comparison of RAS diameter in two groups in terms of time

Group	Myrtex extract 2.5%		Myrtex extract 5%		p-value
	Mean (mm)	Standard deviation	Mean (mm)	Standard deviation	
Time of investigating RAS					
Before treatment	3.21	1.53	3.77	1.67	0.111
One day after treatment	3.15	0.27	3.37	0.27	0.402
Seven days after treatment	0.63	0.24	0.78	0.24	0.759
p-value	0.000		0.000		

Table2. Effects of time, concentration, and interaction between time and concentration on RAS diameter

The effect of treatment	p-value
Time	0.000
Concentration	0.401
The interaction between time and concentration	0.058

Repeated measures analysis of statistical test was run to find the efficacy of myrtex extract 2.5% and myrtex extract 5% and duration of treatment (one day to seven days) in reducing the severity of burning sensation and the effect of the drug over time. As it is clear from Table 4, the reduction in the severity of burning sensation during RAS treatment was significant ($p = 0.000$). However, the effect of myrtex extract 2.5% was not significantly

different from myrtex extract 5% considering burning sensation reduction ($p\text{-value}=0.964$). In addition, the interaction between concentration of myrtex extract and duration of treatment was not significant ($p\text{-value} = 0.057$).

Table 3. Comparison of the severity of burning sensation in two groups in terms of time

Group	Myrtex extract 2.5%		Myrtex extract 5%		P-value
	Mean	Standard deviation	Mean	Standard deviation	
Time of estimating the severity Burning sensation					
Before treatment	53	3.7	55.65	3.76	0.824
One day after treatment	43.63	3.61	40.97	3.67	0.544
Seven days after treatment	6.97	2.66	7.55	2.71	0.509
P-value		0.000		0.000	

Table 4: Effects of time, concentration, and interaction of concentration and time on severity of pain and burning sensation induced by RAS

The effect of treatment	p-value
Time	0.000
Concentration	0.964
The interaction between time and concentration	0.057

Discussion

Myrtex is used in traditional medicine for its antimicrobial activity and wound healing property. It is supposed that myrtex can prevent the secondary infection of the oral aphthous ulcers. No side effects or toxic

effects have been noted for myrtex extract during this study. Nevertheless, when it was applied on RAS, a burning sensation and then a temporary anesthesia of the area around wound were reported, which is common among herbal essences (25). The mean of RAS diameter and severity of burning sensation

were measured in both groups 1 day and 7 days after the administration of myrtex extract. In this regard, the difference between group A and group B was not significant. In other words, the diameter of the ulcers and the severity of burning sensation were reduced similarly in both groups.

The results of Azimi et al. study showed that myrtex essential oil 5% was more effective than myrtex essential oil 10% and placebo considering the treatment of RAS and reduction of burning sensation (19). They believed that the higher efficacy of myrtex essential oil 5%, in comparison with myrtex essential oil 10%, was due to limited number of drug recipients and the limited ability of the body to response to the drug's effects. They also stated that as each drug has a threshold and with increasing the concentration of the drug less than toxic threshold, body's response enhances to some extent; the response process is disrupted due to saturation of the receptors. In this study, myrtex extracts 2.5% and 5% had a fairly similar therapeutic effect. Prescribing lower dosage of the medication is economically cost-effective, and it is notable since the patient has to tolerate less pain and burning sensation while taking the medication.

Taheri et al. Concluded in their study that myrtex essential oil, even in solution form, has similar therapeutic effects to Adcortyle® concerning the treatment of aphthous stomatitis; whereas, myrtex essential oil does not bring about side effects (i.e. fungal growth) (26). It should be noted that the effect of myrtex essential oil on aphthous lesion was also observed in this study

According to Khazaeli et al. study, it seems that oral paste containing myrtex essential oil

is an ideal formulation for the treatment of RAS because oral paste containing myrtex essential oil has adhesion property along with protective property and could significantly reduce healing duration of burning sensation and the size of the lesions in the experimental group compared to control group (27). The results of Khazaeli's et al. study on the efficacy of myrtex essential oil in reducing lesions and healing burning sensation induced by aphthous ulcers were consistent with the results of this investigation. KHazaeli et al., could prepare myrtex essential oil, extracted from its leaves, and applied it as a myrtex oral paste that could exert different effect in comparison with pre-made myrtex extract solution, revealing higher treatment efficacy. However, the use of myrtex oral paste is more difficult than the use of its solution if RAS is out of reach or if there are multiple RAS. The results of Rad et al. study indicated that myrtex extract 5% was effective in the treatment of RAS and had similar therapeutic effects to triamcinolone acetonide dental paste (25). It should be noted that in the aforementioned study, the results on the effect of myrtex extract 5% on aphthous stomatitis treatment were consistent with our research results. This discrepancy can be due to method of measuring lesions or due to use of a higher concentration of myrtex essential oil as using myrtex essential oil 10% in Azimi's study was less effective than myrtex essential oil 5% (19).

This study had some limitations, including unavailability or lack of access to more samples, lack of patients' cooperation to participate in the study, and patients' non-compliance to take the drug based on the recommendations leading to exclusion of some of patients from the study.

Conclusion

The results of this study suggested that different concentrations of myrtex essential oil could decrease the size of the lesion, pain severity, and burning sensation induced by RAS. In a nutshell, two different concentrations of myrtex extract had similar

effects on the treatment of RAS; however, the therapeutic efficacy of both concentrations enhanced by increasing the duration of treatment. Nevertheless, future studies are recommended to recruit larger sample, consider control group, and extract myrtex essential oil themselves in order to obtain more precise results, so that this plant can be used as an alternative treatment for RAS.

Declarations

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Ethics approvals and consent to participate

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Conflict of interest

None

Authors' contributions

All authors contributed equally to this work

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