

Evaluation of antioxidant efficacy of Purslane extract in Patients with Recurrent Aphthous Stomatitis: a randomized, placebo-controlled, triple-blinded, clinical trial

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Abstract

Background: This herbal medicine is considered a rich source of antioxidants with anti-inflammatory effects. The purpose of this study was to evaluate the effectiveness of purslane in treatment of recurrent aphthous stomatitis (RAS) and also its effect on antioxidant level. **Materials and methods:** 50 patients were selected for this randomized triple-blind placebo-controlled trial. All subjects were randomly divided in to two groups, one group received purslane (n=25) and another group, placebo (n=25) for 3 month. Superoxide dismutase (SOD), glutathione peroxidase (GSHPx) and total antioxidant status (TAS) was measured in plasma at baseline and after 3 month of treatment. Also pain intensity based on the visual analogue scale (VAS), the mean interval between lesion, number of lesions and the mean duration of complete healing at baseline and in month 1, 2 and 3 were recorded. Statistical analysis was performed by using Mann-Whitney and T-test. **Results:** A significant decrease in pain intensity in VAS scores was seen after treatment in intervention group ($p < 0.001$). The mean duration of complete healing showed significant differences ($P < 0.001$) between the two groups. The mean interval between lesions also showed significant differences ($P < 0.001$) among the intervention group (33.12 days) compared with the placebo group (17.88 days). No significant differences were found regarding the number of lesions, level of erythrocyte GSHPx, TAS and SOD. No serious side-effects occurred in either of groups. **Conclusions:** According to this study, purslane is clinically effective in treatment of RAS (number of lesions, pain intensity and duration of healing) although it is unable to change the level of antioxidants.

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Introduction

One of the most common oral inflammatory ulcerative diseases, involving more than 20% of population worldwide, is recurrent aphthous stomatitis (RAS)¹. The disease is

characterized by painful, round or ovoid ulcers with circumscribed margins, white-gray pseudomembrane and erythematous haloes in non-keratinized mucosa specially the lips, the wall of the cheeks, soft palate and ventral surface of the tongue and floor of the mouth². Three clinical forms of the disease has been recognized: minor (MiRAS), major (MaRAS) and herpetic form ulcers. MiRAS accounts for 80% of RAS patients³.

The etiology of RAS is unknown, but several immunologic, hematologic, allergic and psychologic disorders have been proposed as causative agents. Such malnutritions as iron, folic acid and B12 vitamin deficiencies, local trauma, emotional stress, hormonal changes, and infectious agents have also been suggested as etiologic factors^{2,4}. Recently it has been proposed that free radicals may lead to RAS through oxidative stress pathway⁵. Decreasing antioxidant defenses in the body or increasing free radicals level, known as oxidative stress, has several destructive effects and often leads to tissue breakdown, which is proposed to play a significant role in development and progression of RAS⁵.

Purslane or *Portulaca oleracea* is from the Portulacaceae family, which possesses antioxidant and anti-inflammatory properties⁶, and it has been clinically effective in the treatment of oral mucosal disorders such as oral lichen planus⁷. The aims of this study were to evaluate the efficacy of purslane in the treatment of RAS, and its antioxidant efficacy in the serum of patients with RAS.

Materials and methods

Subjects and study design

The present study was a randomized, triple blinded, placebo-controlled clinical trial. Fifty patients with MiRAS comprised of 22 male and 28 female (range from 19 to 55 years) from the Department of Oral Medicine, School of Dentistry, Tehran University of Medical Sciences were enrolled in this study based on the inclusion and exclusion criteria (Table 1).

Table 1 - Selection Criteria

Inclusion criteria
1. History of presenting at least three recurrences per year
2. Clinically being in active phase
3. Educated enough to understand the method and sign the informed consent form (older than 18)
Exclusion criteria
1. History of any local or systemic non-aphthous diseases such as diabetes, hepatitis, HIV, blood pressure, cardiovascular or neurological or respiratory disorders
2. Treatment with immunosuppressive drugs 1 month prior to the study
3. Treatment with iron or vitamins 3 months prior to the study
4. Patients with Behcet disease and any other active inflammatory bullous diseases

All the patients were randomly divided into two groups (balance block randomized): 25 patients in intervention group and 25 patients in placebo group. Gender, age and medical history of patients were recorded. Research Ethics Committee of Tehran University of Medical Sciences approved the study. Written informed consent was obtained from all subjects.

Plant collection and extraction

We collected plants from the southern part of Tehran, capital city of Iran. It was authenticated in faculty of pharmacy, Tehran University of Medical Sciences. Aerial parts of the plant and some of the seeds were stabilized in boiling alcohol and were extracted by continuous extractor in environmental temperature with 96°C ethanol. This method effectively prevents any enzymatic destruction. The resulting extract was filtered by filter and centrifuge. Then a vacume-distiller was used to produce a very viscose residue.

A dosage form was prepared and the extraction was granulated with an appropriate amount of lactose. The granules were filled in hard capsules, each containing 235mg extract, based on previous studies⁷. The capsules had the same color, with the same shape and size and were coded.

Intervention

Patients in intervention group received the capsules containing purslane extract, and patients in control group received the same capsules in shape, size and color but containing placebo. The subjects were told to take 2 capsules each day (in morning and at night) for 3 months. The investigator and subjects and analyzer were blind to the code record.

Outcome measures

The number of lesions, the mean interval between lesions (in days), and the mean duration of complete healing (in days), at baseline and in month 1,2 and 3 were measured. Moreover, the following outcome measures were recorded:

Pain Severity: To evaluate the severity of pain, a visual analogue scale (VAS) ranged from 0, showing no pain; to 10, showing extreme pain⁸ was used at baseline and in month 1, 2 and 3.

Antioxidant Status: In brief, blood samples were collected from subjects based on venous puncture method at baseline and in month 1, 2 and 3 in order to detect the level of total antioxidant status (TAS) and suoe oxide dismutase (SOD) and glutathione peroxidase (GSHPx). The blood was centrifuged at 3000 rpm for 5 minutes at . RANSEL kit was used to measure the activity rate of SOD. The activity rate of SOD was measured based on inhibition of the reaction between superoxide radical and Iodophenyl nitrophenol phenyl tetrazoliu-chloride (INT) by SOD. One unit of enzyme was defined as the amount of enzyme that inhibits 50% of INT reduction rate. RANSEL kit was used to measure the activities of GSHPx, based on reduction in absorption degree at 340 nm (after the oxidation of NADPH). The measurement of TAS was based on spectrophotometry.

Statistical analysis

T-test and Mann-whitney test served for comparisons of pain severity, the mean interval between lesion occurrence, and serum level of antioxidant between the two groups. Statistical significance was set at 0.05.

Results

A significant difference existed in pain severity between the two groups after 3 months, with more pain relief occurring in

intervention group ($p < 0.001$) (Table 2).

Table 2 - Comparison of pain relief between the two groups

Pain relief	Placebo (%)	Intervention (%)
1 (1 degree worsening)	4	0
0 (no change)	48	4
-4 (4 degrees improvement)	28	40
-3 (3 degrees improvement)	8	20
-2 (2 degrees improvement)	8	20
-1 (1 degree improvement)	4	16

Patients in the intervention group experienced significantly longer interval between lesion recurrence compared to the patients in control group at 3 months follow up (33.12 vs. 17.88 days respectively, $p < 0.001$) (Table 3). At the same follow up, the decrease in healing duration was also significantly more among patients in intervention group compared to that in patients in control group (6.56 ± 4.50 vs. 1.52 ± 4.07 day respectively, $p < 0.001$) (Table 3).

The differences in number of lesions (Table 3), TAS levels, GSHPx levels, and SOD levels (Table 4) between the two groups at 3 months follow up remained insignificant ($p > 0.05$).

Table 3 - Comparison of number of lesions, healing duration and interval between lesions recurrences

	Intervention	Placebo	P value
Healing duration	6.56 ± 4.50	1.52 ± 4.07	$P < 0.001^*$
Interval between lesions	33.12	17.88	$P < 0.001^*$
Number of lesions	1.28 ± 1.07	0.8 ± 1.65	$P = 0.23$

Table 4 - Comparison of mean and standard deviation of TAS, SOD and GSHPx between the two groups

	Case N=25	Control N=25	P value
TAS	0.01 ± 0.23	0.04 ± 0.2	0.62
GSHPx	18.87 ± 29.78	19.20 ± 53.31	0.98
SOD	14.6 ± 48.91	42.44 ± 70.53	0.11

Discussion

Studies have shown that RAS prevalence is higher among women than men⁹ especially between the ages of 20 to 30 years⁴. In our study the average age of participants was 35 years, and the majority of them were women (28 women vs. 22 men).

Although Azizi et al.¹⁰ and Gudaz et al.¹¹ found no significant differences in antioxidant levels between RAS patients and control group^{10,11}, disorders in antioxidant system in patients with RAS has been reported by several studies^{1,12-14}.

To the best of our knowledge, the present study is the first triple-blind placebo-controlled clinical trial on efficacy of

antioxidant-rich purslane and its anti-inflammatory effects^{15,16} in treatment of RAS. According to the results, no significant changes in the levels of SOD, GSHPx, and TAS occurred in the purslane-treated patients. However, our study demonstrated a beneficial effect of purslane in healing or controlling symptoms of RAS. This effect might be related to other components of this plant, such as: phosphate, zinc, iron, etc. Efficacy of various drugs in treatment of RAS such as rebamipide, colchicine and petonxyfilline has been studied previously^{9,17}. None of them, however, has been as effective as purslane. However, many adverse side effects were reported for colchicine and talidomide¹⁷⁻¹⁹, none of which occurred in purslane-treated patients up to 3 months of treatment.

In the present study we demonstrated that an oral tablet with purslane can reduce pain severity, healing duration, and can increase the interval between lesions recurrences with no adverse side effects at 3 months follow up. Further studies on larger groups of patients with longer follow-up might be needed to confirm these results.

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Conflict Of Interest

The authors declare that they have no conflict of interests.

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