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Original Article

The efficacy of *Punica granatum* extract in the management of recurrent aphthous stomatitis

Parichehr Ghalayani[1], Behzad Zolfaghary[2], Ali Reza Farhad[3], Atefeh Tavangar[1], Bahram Soleymani[4]

[1]Department of Oral Medicine and Torabinejad Dental Research Center, Isfahan University of Medical Science, Isfahan, Iran

[2]Depatment of Pharmacognosy, Isfahan University of Medical Science, Isfahan, Iran

[3]Department of Endodontics and Torabinejad Dental Research Center, Isfahan University of Medical Science, Isfahan, Iran

[4]Department of Health, Islamic Azad University, Najafabad Branch, Isfahan, Iran

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Corresponding author:
Dr. Atefeh Tavangar,
E-mail: tavangar@dnt.mui.ac.ir

ABSTRACT

Objective: Recurrent aphthous stomatitis (RAS) is a common, painful ulcerative disorder of the oral cavity with unknown etiology. No documented cure exists and topical application of medications aims to reduce pain associated with this condition. The aim of this study was to evaluate the efficacy of *Punica granatum* (PG) extract on the clinical management of RAS.
Methods: A total of 40 patients with RAS participated in this randomized, double-blind, and placebo-controlled study. During three episodes of RAS, the efficacy of topical PG gel (10%) was evaluated. Patients were randomly assigned to use placebo gel or PG gel daily. The time of pain elimination and the time of complete healing were recorded and the pain degree was assessed and recorded by each patients in different time intervals including: Before using the oral gel (day 0), and on days 1, 3, 5, 7 after using the product. Data were analyzed using the repeated measures ANOVA, paired and independent *t*-test.
Findings: Mean time of pain elimination showed a significant difference ($P < 0.001$) between PG group (3.4 ± 1.09) and placebo group (5.9 ± 0.6). The mean duration of complete healing also showed a significant difference ($P < 0.001$) between PG group (5.3 ± 0.81) and placebo group (8.6 ± 0.99). The visual analog scale score in PG group was significantly less than the placebo group in all time intervals (day 1 to day 7) ($P < 0.001$).
Conclusion: The findings of this study revealed that PG extract in the form of oral gel (10%) may be beneficial in reducing RAS pain and has a positive effect in reducing the overall time period of complete healing. It was concluded that PG is an effective herbal medicine for the management of RAS.

Keywords: Aphthous; extract; pain management; *Punica granatum*; stomatitis

INTRODUCTION

Recurrent aphthous stomatitis (RAS) is a prevalent inflammatory disorder of unknown etiology. Several predisposing and risk factors have been implicated in the pathogenesis of RAS. RAS is a multifactorial process. Genetic pre-disposition, hematologic abnormalities, microbial or immunologic factors, trauma, stress and hormonal state are the important predisposing factors for this disorder.[1-4] Due to this multifactorial etiology, there is no definitive treatment for RAS. The primary goals of therapy are palliative, prevention of recurrence and promotion of ulcer healing.[1,5,6] Several topical agents (antibacterial, anti-inflammatory and anti-histaminic agents), analgesics, local anesthetics and glucocorticoids are available for symptom relief.[1,5-8] In severe forms of RAS, systemic agents such as colchicine, dapsone and corticosteroids may be administered to control the symptoms.[5] Most of these therapies are associated with side-effects or unwanted reactions.[1,2]

Medicinal plant preparations with antibacterial, antifungal, anti-inflammatory, and antioxidant activities have been used for pain reduction and shortening of healing time of oral aphthous ulcers.[9-14] *Punica granatum* has been introduced as a natural medicine for prevention and treatment of inflammation and cancer.[15-17] PG is a

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flavonoid-containing food supplement. Flavonoids of PG have demonstrated antimicrobial activity, free radical scavenging ability, immune system activation, and numerous antioxidant properties.[18-20] Although there is not any study about the treatment effect of PG in RAS, but this study was based on the therapeutic effects of the plant. The anti-inflammatory,[15,16,21] antimicrobial,[22,23] antiviral[24] and anti-candida[25] characteristics of PG may be advantageous for the treatment of RAS. PG has been assessed in the treatment of chronic periodontitis.[26] Anthocyanin dyes, the most important phenolic compounds of PG, have been shown to possess anti-inflammatory effects.[19] Furthermore, polyphenols may protect the host against oxidative stress and pathologic conditions such as cancer, chronic heart disease, and vascular disease.[15,27] In addition, it has been known as astringent, wound healing, anti-inflammatory in Iranian traditional medicine and has been used for healing the aphthous ulcer in Iranian folk medicine especially in Fars province.[28] Considering the positive effects of PG, this study was designed to investigate the efficacy of PG extract in the form of oral gel on the clinical management of RAS.

METHODS

PG wild flowers were collected in the Arsanjan area (Fars province, Iran) in Jun 2007 and were identified by the Pharmacognosy Department of Isfahan University of Medical Sciences. A voucher specimen (No. 2026) has been deposited at the pharmacognosy department. Flowers were air-dried under a controlled temperature (25°C) in shadow. They were powdered and extracted by percolation with ethanol 75% V/V.[29] Hydroalcoholic extract of flowers concentrated in the freeze dryer afforded a crude dried extract (400 g). Phenolic compounds were evaluated according to the folin-ciocaltea methods. Twenty microliters of sample, 1.58 mL of deionized water and 100 μL of folin-ciocalted were mixed. The mixture was incubated for 8.5 min at room temperature and then 300 μL of $NaHCO_3$ was added. The mixture was incubated for 2 h at 20°C. Absorption at 765 nm was measured. The total phenolic contents were expressed as galic acid equivalents.[30] Methyl cellulose was used for the preparation of 10% oral gel. Placebo gel contained ferric oxide.

A total of 40 patients (18 women and 22 men) with a mean age of 28.45 years (10-64 years) with a current history of RAS suffering from ulcerations located on the oral mucosa were selected from the out-patients visiting the Oral Medicine clinic, School of Dentistry, Isfahan University of Medical Sciences. The protocol of this study was approved by the institution review board and Ethics committee of Isfahan University of Medical Sciences and each patient signed a consent form. The IRCT trial number of this research is 201112273251N2. All patients were interviewed and those suffering from any systemic disorders such as Behcet's disease, Reiter's disease, bowel disease, celiac disease or any immunosuppressive conditions, those taking anti-inflammatory or analgesic drugs, those with major or herpetiform aphthous lesions and aphthous lesions older than 2 days were excluded from the study. Diagnosis of minor aphthae was made on the basis of the patient's medical history, clinical examination, and the presence of well-demarcated painful ulcer with a diameter less than 1 cm, which was surrounded by light red areola.[1,2]

Patients were randomly divided into two subgroups. The experimental group (n = 20) received PG oral gel and the placebo group (n = 20) received placebo gel. This study was designed as a double-blind investigation in which the tube of the product was coded (A, B) by a third person and was given to the patients randomly. The patients were instructed to apply the gel three times daily by placing a small sterile cotton pad impregnated with gel on the lesions for 1 min. They were asked not to eat for at least 30 min after administering the preparations. Each patient was instructed and informed about completing the questioner and the pain scale (number 0 to 10). The degree of pain was recorded by patients on days 0, 1, 3, 5 and 7 using a visual analog scale (VAS). In addition, they were instructed to record the data of pain elimination and duration of complete healing on a provided questionnaire. Complete healing was confirmed by a clinical examiner. The investigators kept in contact with patients to insure the proper use of drug and the correct recording of the results in the questionnaire. Patients were re-examined after 1 week and the questionnaires were collected. Patients and researchers were unaware of the results until the end of the study. At the end, codes were revealed and the result of each group was determined separately.

Statistical analysis was carried out using the SPSS software (V.11.5). Repeated Measures ANOVA was used to compare the VAS score in different times. Paired t-test was used to compare VAS score in every two consecutive times in each group, Independent t-test was applied to comparison the time of pain elimination, duration of complete healing also VAS score of mentioned days (days 1, 3, 5, 7) between placebo and PG groups.

RESULTS

The mean VAS score decreased from day 0 to day 7 for both groups ($P < 0.001$) and there was a significant

difference between two groups in this regard ($P < 0.001$). Paired *t*-test showed that reduction in every two consecutive time periods were significant ($P < 0.001$). Independent *t*-test revealed that the difference of VAS score was significant between two groups for each time period ($P < 0.001$) [Table 1 and Figure 1].

The mean time of pain elimination means the duration of time from day 0 (onset of product usage) until the day which the pain of aphthous ulcer was eliminated completely and VAS score turned to 0 for each patient. This time period was 3.4 ± 1.09 days in PG group and 5.9 ± 0.6 days in the placebo group. The statistical analysis showed a significant difference between two groups regarding the mean time of pain elimination ($P < 0.001$).

The mean time of complete healing means the duration of time from day 0 (onset of product usage) until the day, which the ulcer healed completely without scar and the fibrinopurulent membrane disappeared. This time period was 5.3 ± 0.81 days in PG group and 8.6 ± 0.99 days in the placebo group. This difference was statistically significant ($P < 0.001$).

DISCUSSION

RAS manifests in a variety of ways in patients suffering from this disease. Currently, there is no known etiology for the ulcers, nor is there a treatment that can safely and conclusively decrease the frequency of ulcer outbreaks in patients.[1,2] The main complaint with aphthous ulcers is the accompanying pain. Management of RAS pain with various herbal preparations has been reported. There are not any studies about the effect of PG in the treatment of aphthous ulcer and it was the first time that the treatment effect of PG on recurrent aphthous ulcer was evaluated.

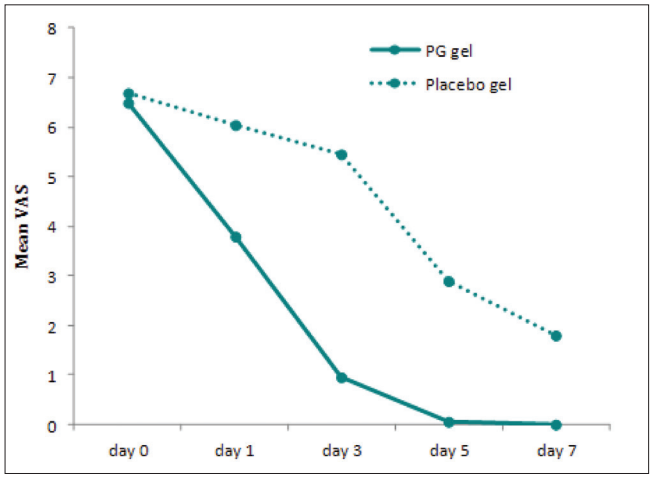

Figure 1: The mean severity of pain according to visual analog scale (VAS) for *Punica granatum* (PG) and placebo groups in different days

Amanlou *et al*., examined the effect of *Satureja khuzistanica* extract in the management of RAS pain.[14] The mean time of pain elimination was 5.7 days in the control group compared to 3.4 days in *S. khuzistanica* extract group. The present study showed the mean time of pain elimination using PG to be 3.4 days for PG group, which was similar to *S. khuzistanica* extract. Furthermore, there was a reduction in mean healing time from 10.4 days in the control group to 5.9 days in *S. khuzistanica* extract group, which was similar to the findings of the present investigation.[14]

Jafari *et al*., showed that complete healing time of RAS following therapy with *Zataria multiflora*, *Anthemis nobelis*, mixture of these two agents and *Myrthus communis* was 6, 8.5, 6.5 and 7.2 days, respectively.[12] The results of the present study suggests that PG with a mean healing time of 5.3 days can be a proper alternative for the clinical management. Jaffari *et al*., showed that mean time for pain elimination after therapy with *Z. multiflora* extract, *A. nobelis* extract, mixture of these two and *M. communis* mouth rinse was 3, 5, 3.1, and 4 days, respectively.[12] PG with mean pain elimination time of 3.4 days was shown to have a comparable effect with other herbal agents in the management of RAS symptoms.

The anti-inflammatory, antioxidant, and antimicrobial characteristics of PG could be advantageous in the treatment of aphthous ulcers. Antioxidant activity of PG neutralizes the oxygen free radicals, which play an important role in the inflammatory process and aphthous ulcer formation. This activity may accelerate the healing process of aphthous ulcers.[15,16,18,19] Braqa *et al*., showed that PG extract can be a potential antibacterial therapeutic agent due to its ability to inhibit enterotoxin

Table 1: Comparison of VAS score for PG and placebo groups in different days

Group	Mean VAS (SD)				
	Day 0	Day 1	Day 3	Day 5	Day 7
PG gel	6.5 (0.65)	3.8 (0.38)	0.95 (0.31)	0.05 (0.001)	0
Placebo gel	6.7 (0.77)	6.05 (0.23)	5.45 (0.43)	2.9 (0.68)	1.8 (0.13)
P value	0.21	<0.001	<0.001	<0.001	<0.001

VAS=Visual analog scale, PG=*Punica granatum*

production.[22] The effectiveness of PG products may be attributed to its protective effects in reducing irritation and inflammation. It can also prevent the possible secondary infection of ulcers due to anti-inflammatory, antibacterial and antioxidant capabilities.

Small sample size and possible inaccuracies in oral gel was used by the patients during the study period are the main limitations of this investigation. Further, studies should be considered to investigate the effect of this product in a larger sample sizes with different demographics.

The results of this study showed that topical application of hydroalcoholic extract of PG may present an effective treatment for minor aphthous ulcer. The clinical improvement including pain reduction and improving the RAS healing period, patient compliance, ease of use and minimal side-effects are the advantages of using PG in RAS management.

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AUTHORS' CONTRIBUTION

Parichehr Ghalayani: Study design and performance, Behzad Zolfaghary: Producing PG gel and manuscript writing, Ali Reza Farhad: Manuscript design and writing, Atefeh Tavangar: Study design and performance, manuscript design and writing, corresponding author, Bahram Soleimani: Statistical analysis of the results.

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