Original Article

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

Parichehr Ghalayani¹, Behzad Zolfaghary², Ali Reza Farhad³, Atefeh Tavangar¹, Bahram Soleymani⁴

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 patient 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.¹⁻⁵,⁶ In severe forms of RAS, systemic agents such as colchicine, dapsone and corticosteroids may be administered to control the symptoms.⁷ 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.⁸⁻¹⁴ *Punica granatum* has been introduced as a natural medicine for prevention and treatment of inflammation and cancer.¹⁵⁻¹⁷ PG is a
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, and anti-candida 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 aphiuthus 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-ciocalteu methods. Twenty microliters of sample, 1.58 mL of deionized water and 100 µL of folin-ciocalteu were mixed. The mixture was incubated for 8.5 min at room temperature and then 300 µL of NaHCO, 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 gallic 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 aphiuthus lesions and aphiuthus lesions older than 2 days were excluded from the study. Diagnosis of minor aphiathae 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 questionnaire 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 the 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 $3.4 \pm 1.09$ days in PG group and $5.9 \pm 0.6$ 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 $5.3 \pm 0.81$ days in PG group and $8.6 \pm 0.99$ 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. 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.

Amanlou et al., examined the effect of *Satureja khuzistanica* extract in the management of RAS pain. 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.

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. 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. 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. Braqa et al., showed that PG extract can be a potential antibacterial therapeutic agent due to its ability to inhibit enterotoxin...
production.[23] 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.

ACKNOWLEDGMENTS

This work was supported by the Vice Chancellor for research of Isfahan University of Medical Sciences (No: 386229).

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.

REFERENCES

1. Greenberg MS, Glick M. Burket’s oral medicine diagnosis and treatment. 11th ed, Hamilton: BC Decker; 2008. p. 63-5.
2. Ship JA. Recurrent aphthous stomatitis. An update. Oral Surg Oral Med Oral Pathol Oral Radial Endod 1996;81:141-7.
3. Porter SR, Hegarty A, Kaliakatsou F, Hodgson TA, Scully C. Recurrent aphthous stomatitis. Clin Dermatol 2000;18:569-78.
4. Porter SR, Scully C, Pedersen A. Recurrent aphthous stomatitis. Crit Rev Oral Biol Med 1998;9:306-21.
5. Scully C, Gorsky M, Lozada-Nur F. The diagnosis and management of recurrent aphthous stomatitis: A consensus approach. J Am Dent Assoc 2003;134:200-7.
6. Barrons RW. Treatment strategies for recurrent oral ulcers. Am J Health Syst Pharm 2001;58:41-50.
7. MacPhail L. Topical and systemic therapy for recurrent aphthous stomatitis. Semin Cutan Med Surg 1997;16:301-7.
8. Saxen MA, Ambrosius WT, Rehemtulla al-KF, Russell AL, Eckert GJ. Sustained relief of oral aphthous ulcer pain from topical diclofenac in hyaluronan: A randomized, double-blind clinical trial. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1997;84:356-61.
9. Badiee B. The efficacy of myrtle in the management of aphtholus oral ulcers. [Thesis]. Qazvin: School of Dentistry, Qazvin University of Medical Sciences; 1998.
10. Samet N, Laurent C, Susarla SM, Samet-Rubinstein N. The effect of bee propolis on recurrent aphthous stomatitis: A pilot study. Clin Oral Investig 2007;11:143-7.
11. Mansoor P, Hadjiakhondi A, Ghavami R, Shafiee A. Clinical evaluation of zataria multiflora essential oil mouthwash in the management of recurrent aphthous stomatitis. DARU 2002;10:74-7.
12. Jafari SH, Amanlou M, Borhan-Moja K, Farsam H. Comparative study of zataria multiflora and Anthemis nobelis extracts with mythus communis preparation in the treatment of recurrent aphthous stomatitis. DARU 2003;11:23-7.
13. Motallebnejad M, Moghadamnia A, Talei M. The efficacy of Hypericum perforatum extracts on recurrent aphthous ulcers. J Med Sci 2006;8:39-43.
14. Amanlou M, Babae N, Saheb-Jamee M, Salehinia A, Farsam H, Tohidast Z. Efficacy of Satureja khuzistanica extract and its essential oil preparations in the management of recurrent aphthous stomatitis. DARU 2007;15:231-5.
15. Lansky EP, Newman RA. Punica granatum (pomegranate) and its potential for prevention and treatment of inflammation and cancer. J Ethnopharmacol 2007;109:177-206.
16. Wang R, Wei Wang, Wang L, Liu R, Yi Ding, Du L. Constituents of the flowers of Punica granatum. Fitoterapia 2006;77:534-7.
17. Jung KH, Kim MJ, Ha E, Uhm YK, Kim HK, Chung JH, et al. Suppressive effect of Punica granatum on the production of tumor necrosis factor (Tnf) in BV2 microglial cells. Biol Pharm Bull 2006;29:1258-61.
18. Sudheesh S, Vijayalakshmi NR. Flavonoids from Punica granatum – Potential antiperoxidative agents. Fitoterapia 2005;76:181-6.
19. Ricci D, Giampieri L, Bucchi I, Fraternelle D. Antioxidant activity of Punica granatum fruits. Fitoterapia 2006;77:310-2.
20. Murthy KN, Reddy VK, Veigas JM, Murthy UD. Study on wound healing activity of Punica granatum peal. J Med Food 2004;7:256-9.
21. Noda Y, Kaneyuki T, Mori A, Packer L. Antioxidant activities of pomegranate fruit extract and its anthocyanidins: Delphinidin, cyanidin, and pelargonidin. J Agric Food Chem 2002;50:166-71.
22. Braga LC, Shupp JW, Cummings C, Jett M, Takahashi JA, Carmo LS, et al. Pomegranate extract inhibits staphylococcus aureus growth and subsequent enterotoxin production. J Ethnopharmacol 2005;96:335-9.
23. Beil W, Birkholz C, Sewing KF. Effects of flavonoids on parietal cell acid secretion, gastric mucosal prostaglandin production and Helicobacter pylori growth. Arzneimittelforschung 1995;45:697-700.
24. Li Y, Ooi LS, Wang H, But PP, Ooi VE. Antiviral activities of medicinal herbs traditionally used in southern mainland China. Phytother Res 2004;18:718-22.
25. Nair R, Chanda S. Anti candidial activity of Punica granatum exhibited in different solvents. Pharm Biol 2005;43:21-5.
26. Sastravahla G, Yotruengnit P, Booncong P, Sangtherapitikul P. Adjunctive periodontal treatment with Centella asiatica and
Ghalayani, et al.: Punica granatum for the management of apthous stomatitis

Punica granatum extracts. A preliminary study. J Int Acad Periodontol 2003;5:106-15.

27. Middleton E Jr, Kandaswami C, Theoharides TC. The effects of plant flavonoids on mammalian cells: Implications for inflammation, heart disease, and cancer. Pharmacol Rev 2000;52:673-751.

28. Razi M. Al-hawi fi al-tibb. Translated by Afsharipour S. Tehran: Academy of Medical Science of Islamic Republic of Iran; 2004. p. 300-3.

29. Ghasemi N. Iranian herbal pharmacopeia. Tehran: Ministry of Health and Medical education; 2002. p. 99-107.

30. Singleton VL, Orthofer R, Lumuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocaltu reagent. Methods Enzymol 1999;299:152-78.

How to cite this article: Ghalayani P, Zolfaghary B, Farhad AR, Tavangar A, Soleymani B. The efficacy of Punica granatum extract in the management of recurrent aphthous stomatitis. J Res Pharm Pract 2013;2:88‑92.

Source of Support: This work was supported by the Vice Chancellor for research of Isfahan University of Medical Sciences (No: 386229).

Conflict of Interest: None declared.

Announcement

iPhone App

A free application to browse and search the journal’s content is now available for iPhone/iPad. The application provides “Table of Contents” of the latest issues, which are stored on the device for future offline browsing. Internet connection is required to access the back issues and search facility. The application is Compatible with iPhone, iPod touch, and iPad and Requires iOS 3.1 or later. The application can be downloaded from http://itunes.apple.com/us/app/medknow-journals/id458064375?ls=1&mt=8. For suggestions and comments do write back to us.