Originial Article

Comparative Study of *Punica granatum* Gel and Triadent Oral Paste Effect on Recurrent Aphthous Stomatitis (a Double Blind Clinical Trial)

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ABSTRACT

Statement of the Problem: Recurrent aphthous stomatitis is a common oral lesion, and the use of herbal remedies containing tannin and flavonoid has been reported to be effective in treating aphthous stomatitis.

Purpose: This study was aimed to evaluate the effect of *Punica granatum* muco-adhesive gel on controlling the oral recurrent aphthous stomatitis and its comparison with Triadent oral paste.

Materials and Method: In this double-blind clinical trial, 60 patients with minor aphthous stomatitis were enrolled. These patients had no systemic diseases and were not on any medications. The patients were randomly treated with *Punica granatum* formulated gel, Triadent oral paste and placebo. The time of pain elimination and the time of complete healing were recorded and the pain degree was assessed and recorded by each patient. The data were analyzed using survival analysis and ANOVA test.

Results: The Kaplan-Meier survival analysis demonstrated that pain relief time in *Punica granatum* group was lower than placebo group (p= 0.002), even so, it caused no significant difference with the Triadent group in comparison with the placebo group (p= 0.08). The survival analysis also indicated that the wound healing time in *Punica granatum* group was significantly lower than the other two groups (p< 0.05).

Conclusion: *Punica granatum* gel has a successful effect in controlling and treating recurrent aphthous stomatitis. It can be considered as an affordable and inexpensive treatment.

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Introduction

Aphthous stomatitis is one of the most common mucosal diseases in the world that is manifested in the form of numerous symmetrical painful ulcers, apart from each other, with a specific amount in the necrotic center by the erythematous halo around the ulcer. [1-2]

Recurrent aphthous stomatitis (RAS) is a multifactorial process. Three major factors, including genetic factor, hematologic and immunologic abnormalities, and local factors are determined to cause this disorder. [2-3] Due to the multi factorial etiology, there is no definitive treatment for RAS. Topical and systemic agents such as analgesics, anti-bacterial and anti-inflammatory agents, and glucocorticoids are used to decrease the pain and reliefs the symptoms. [4-5]

Concerning the side effects of chemical drugs, especially their systemic consumption, more attention has been paid to herbal medicines. Extensive research has shown that herbal compounds, which contain tannins and flavonoids, are effective in controlling the symp-

toms of aphthous stomatitis. [6-14] A preliminary study has demonstrated that a 5% solution of *Myrtus Communis* and *Melisa officinalis* extracts can treat aphthous ulcer. [11] *Anthemisnobelis* is another herbal treatment in aphthous ulcer that was evaluated in Jafari *et al.*'s study. [12] Motallebnejad *et al.*'s study [13] showed that *Hypericum perforatum* mouthwash might also reduce pain and time of healing in aphthous ulcer.

Another suggested herb is pomegranate flowers of *Punica granatum* (PG) from the punicacea family. PG or pomegranate is an important medicinal plant in the northern regions of Iran. Different parts of the pomegranate, including flowers, leaves, fruits, and pomegranate stems contain tannins such as ellagicacid, gallic acid, and flavonoids. With astringent effects on the tissues of the body, tannin compounds in pomegranate are commonly used in the treatment of burns, and wound healing. [15]

Flavonoid compounds in pomegranate have strong antioxidant effects and help regulate the immune system. They can also be effective in the treatment of cancers, chronic cardiovascular diseases, inflammations and chronic periodontitis. [16-19] Due to its ellagitannin and punicalagin, pomegranate has antibacterial effects [17] and its antiviral properties against the herpes virus have been proven. [20] In general, pomegranate is highly effective in accelerating the process of wound healing, especially aphthous stomatitis due to its anti-inflammatory, anti-oxidant, anti-microbial, anti-viral and antifungal properties. [17-22]

The aim of this study was to prepare a mucus gel from pomegranate flower, check its effect on RAS, and compare it with the triamcinolone muco-adhesive paste (Triadent).

Materials and Method

Preparation of mucoadhesive gels

Percolation method was used for extraction of the mucus gel from pomegranate. The plant powder was moistened with ethanol 75%, and it was streamed into a percolator hopper. The extraction was performed at a speed of 4 to 6 drops per minute (per 100 grams of powder), and was stopped after 48 hours. Then, it was concentrated by rotary machine at 40°C to 70°C for 4 to 8 hours until the desired consistency was obtained. To prepare a Carbomer 934 gel, firstly, sulfur potassium was dissolved in water. Then, a certain amount of Cermoller was slowly spread with the aid of a magnetic stirrer to become completely homogeneous. In order to prepare a gel of sodium CMC (carbonymethylcellulose), a certain amount of sodium CMC was weighed and slowly spread with the aid of a magnetic stirrer to make the powder particles fully wet.

To prepare the gel, the powders were prepared separately by using the mentioned methods and mixed. Finally, the extract was injected into polyethylene glycol 400, and added to the gel to achieve a homogeneous gel with 2% consistency. The viscosity test and muco adhesion test were employed for the prepared PG gel. The mean of strength for this gel was 34.75 dym/cm$^{2.}$ Iron oxide was used to make a placebo due to its similarity to pomegranate flowers in terms of color and smell.

Clinical trial program

In this double-blind clinical trial, 60 patients with oral RAS and the age range of 18 to 50 years were enrolled. Simple random sampling method was performed for the patients referring to the Faculty of Dentistry, Isfahan University of Medical Sciences and some dental clinics in Isfahan. This study was approved by the Ethics Committee of Isfahan University of Medical Sciences, Isfahan, Iran, and registered in the Iranian Registry of Clinical Trials (IRCT201606063251 N6). Written informed consents were obtained from all the participants took part in the survey. All the patients were interviewed and those suffering from any systemic disorders such as Behçet's disease, Reiter's disease, bowel disease, celiac disease or any immunosuppressive conditions, or those taking anti-inflammatory or analgesic drugs, and those with major or herpetiformaphthous lesions and aphthous lesions older than 2 days were excluded from the study. The 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 the light red areola. For data collection, individual biography specifications, such as name, age, gender, lesion characteristics such as the number of ulcers, location, time intervals between the incidence of ulcers, duration of ulcer existence, history of trauma, sensitivity and other systemic diseases were checked and recorded. The patients were divided into 3

groups of 20. Since the study was a double blind study, both the patient and the examiner were blind to the type of medication. Subsequently, the Triadent oral paste was removed from the corresponding tube by a third person and it was transferred to similar tubes of PG and the placebo. The tubes were coded as A, B, C; and they were offered to the patients. The code was written in each patient's form. All patients took the medicine three times a day, and they put the oral gel, approximately 1 cm in length, on the ulcer with a sterile wipe and they have been informed not to eat or drink for at least 30 minutes after taking the medicine. The individual was supposed to use the medicine until the ulcer healed completely.

The patient was instructed and informed about completing the questionnaire and the pain scale. The degree of pain was recorded by visual analog scale (VAS) consisting of a 10 cm horizontal line between extremities with (0) indicating no pain and (10) for unbearable pain. The ulcer size was determined by measuring the distance between the two opposite edges of the ulcer border by using a periodontal probe in millimeters. In addition, they recorded the data on pain elimination and the duration of complete healing.

Statistical Methods

At the end of the study, the results obtained from all groups were collected according to the pharmaceutical codes, and then the codes were revealed. All the statistical analyses were performed using statistical package for the social sciences (SPSS), version 22. The Kaplan-Meier survival analysis and ANOVA test were applied in order to compare the effect of PG gel and Triadent oral paste on RAS. The significance level in all statistical tests was considered to be 0.05.

Results

Among 60 patients participating in this project, 60% were female and 40% were male. The age range of these patients was 18 to 50 years. Kaplan-Meier Survival analysis was used to determine the effect of gels used in different groups for the duration of pain relief and ulcer healing. The Kaplan-Meier survival analysis showed that the pain relief time in PG group was lower than the placebo group (p= 0.002); however, it had no significant difference with the Triadent group in comparison with the placebo group (p= 0.08) (Figure 1). Although the duration of pain relief in the pomegranate group was lower than that of the Trident group, this difference was not statistically significant (p= 0.1) (Figure 1).

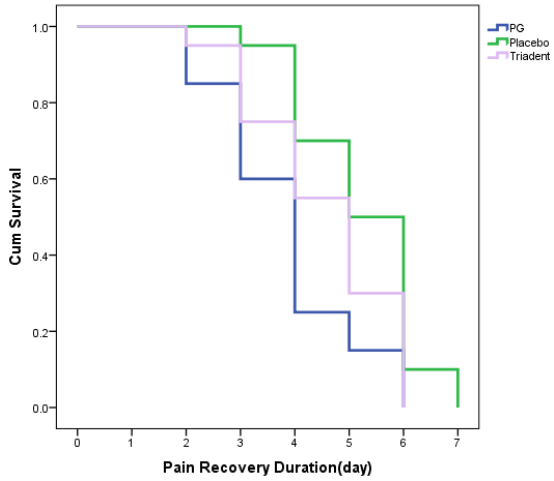

Figure 1: Survival analysis for duration of pain recovery in three groups

According to the table 1, the time of pain elimination in the PG group was the least, Triadent group was at the next stage and the placebo group was the third one (Table 1).

Table 1: Mean time of pain elimination and complete ulcer healing in three groups

Groups / Time (day)	Placebo	Triadent	Punica granatum
Pain elimination	5.25±1.11	4.55±1.27	3.85±1.26
Ulcer Healing	6.5±1.05	5.75±1.08	5.05±0.99

Moreover, in order to evaluate the duration of ulcer healing, Kaplan-Meier survival analysis method was used in the studied groups (Figure 2).

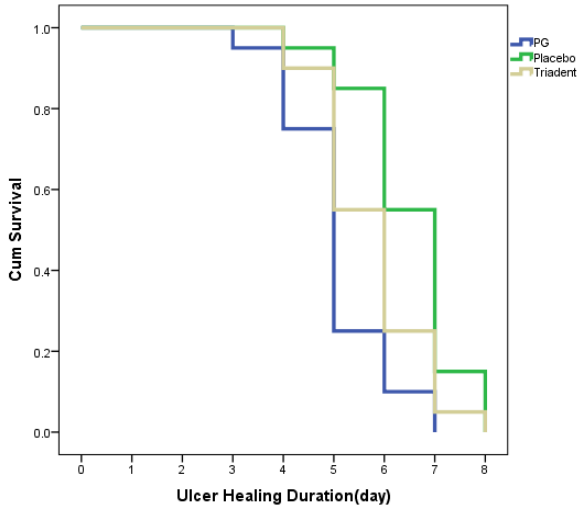

Figure 2: survival analysis for ulcer healing duration in three groups

In this analysis, it was shown that the duration of the ulcer healing in PG group was significantly lower than the placebo group (p= 0.001), also than the Triadent group (p= 0.04). According to the table 1, the time of complete ulcer healing in PG group was the least then the Triadent group, and the placebo group was the third, respectively.

The size of the ulcer was recorded on day 0, 3, 5 to evaluate the process of the ulcer healing (Figure 3). Although on the day 0, the mean of the ulcer size in PG group was greater than the two other groups, but on the day 3 the ulcer sizes measured the least in the PG group (2.6mm^2) followed by 3 mm^2 in Triadent group and 4 mm^2 in the placebo group with a significant difference between the three studied groups (p <0.05).

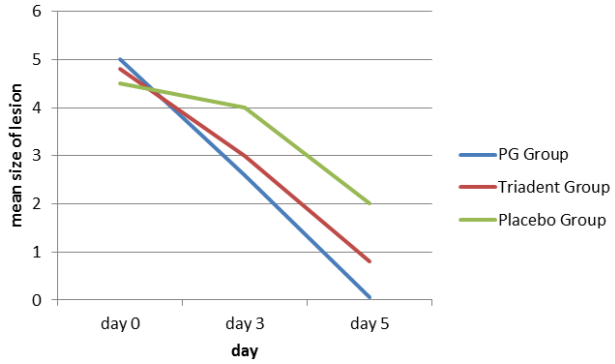

Figure 3: The mean size of lesion on day 0, 3, 5 in three experimental groups

On day 5, the mean ulcer sizes in PG, Trident and placebo groups were 0.05, 0.85, 2 respectively, showing significant differences between the experimental groups and the placebo (p< 0.05)

Discussion

Oral RAS is one of the most common oral mucosal problems, and given the fact that it has a multifactorial etiology, and there is no definite treatment for its improvement. [1-2] Since most of the patient's problems are pain and irritation caused by aphthous stomatitis, most of the treatments are based on pain alleviation and the symptoms of the patient. [1-2]

There are a few studies about the clinical effect of the PG in the treatment of aphthous ulcer. Ghalayani *et al.* [8] evaluated the effect of PG extract on RAS, which was compared with the control group. The present study is in fact a continuation of the research carried out by the same researcher and his colleagues in 2013. [8] In the present study, the PG extract was prepared by a precise formulation and formed as a muco-adhesive gel that would have longer durability on the ulcers. While in Ghalayani *et al.*'s study [8], the extract of the herb was very dilute, and its durability was poor on the ulcers. In addition, in order to investigate the effect of this plant on aphthous stomatitis, along with the placebo group, the Triadent muco-adhesive paste was also used. In Ghalayani *et al.*'s study[8], both the duration of the pain relief and the complete healing of the ulcer in the PG group were significantly less than those in the placebo group (p< 0.05), which is very similar to the present research. In both studies, the PG extract and PG muco-adhesive gel have been shown to be effective in decreasing the pain intensity, reducing the symptoms of the patient and accelerating the ulcer healing process. However, the patients were more satisfied with using muco-adhesive gel with PG extract, due to its durability, lack of dispersion in the mouth and its convenience. Gavanji *et al.* [6] compared the alcohol and water extract of PG varpleni.flora, PG var Sweet Alak and PG varSaveh Black on minor RAS. They found that the alcoholic extract of PG varpleni.flora have the greatest therapeutic effect on minor RAS. In the same line, Braga *et al.* [23] showed that PG extract have antibacterial therapeutic effect and can inhibit production of enterotoxin in *staphylococcus aureus*. Respectively, Pirbalouti *et al.* [24] demonstrated that the pomegranate flower extract had positive effect on shortening the healing period of wound healing in rats. PG extract can lead to the proliferation and synthesis of collagen in tissues and thus have a significant positive effect on the healing process of ulcers. [25]

Polyphenolic compounds in the pomegranate, including anthoyanins has anti-inflammatory effect and prevent the adhesion of leukocytes to endothelial cells, and it decreases the level of IL-2, interferon gamma, and TNF-α. Reducing these inflammatory factors is effective in treating diabetes and oral RAS. The pomegranates also take care of the body against inflammation and oxidative stress by inhibiting free radicals and having antioxidant properties. [17, 26-27]

Since both the antioxidant and the immune system activity disturbances are considered as two important factors in aphthous etiology and since this disease is accompanied by inflammation and pain, PG flower is

very effective in treating RAS, due to its antioxidant, anti-inflammatory, anti-bacterial and wound healing properties. [16-20]

One of the main limitations of this study were barriers to recruiting sufficient number of patients, for many patients refused to take medications on time and during repeated examinations of the study. Finally, it is suggested that studies similar to the present study be conducted with a larger sample size and in different regions, with different demographic conditions. Subsequently, the effect of this drug in different environmental and biological conditions can also be studied and the results can be compared with each other.

Conclusion

The results of this study showed that the PG muco-adhesive gel not only has a significant effect on the treatment and the control of the symptoms of RAS, but it can also compete with Triadent muco-adhesive paste. Moreover, since this drug has herbal origin and bears fewer side effects, it can be considered as an effective auxiliary treatment method for controlling oral RAS.

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Conflict of Interest

The authors declare no conflicts of interest related to this study.

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