Comparing pomegranate extract and chlorhexidine mouthwashes in treatment of recurrent intraoral herpes

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Background/aim
Herpes simplex virus is a frequently encountered contagious infectivity causing several diseases. The aim of the present study was to compare the effect of pomegranate peel extract and chlorhexidine (CHX) mouthwashes in the treatment of recurrent intraoral herpes (RIOH), a clinical form of herpes simplex virus infection.
Patients and methods
A total of 12 immunocompetent patients aged from 18 to 35 years with RIOH lesions affecting their keratinized mucosa were selected. The participants were randomly divided into two groups: group I (pomegranate group, *n*=6) was treated using pharmacologically prepared pomegranate peel extract mouthwash and group II (CHX group, *n*=6) was treated using CHX mouthwash. Both treatment modalities were used three times per /day for 10 days. Pain and erythema scores were recorded at 2, 4, 6, and 8 days from baseline in both study groups to assess the effect of mouthwashes used.
Results
Pain and erythema scores showed statistically significant decrease by time in both groups. A higher mean score was recorded in group II, with statistically significant difference in pain scores, but this difference did not reach the level of significance as regarding the erythema score. The percentage of change in both pain and erythema scores revealed better and superior improvement in group I than group II.
Conclusion
Pomegranate peel extract mouthwash was more effective in reducing pain and accelerating wound healing when compared with CHX as topical treatment for RIOH.

Keywords:
chlorhexidine, erythema, pain, pomegranate, recurrent intraoral herpes

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Introduction

Herpes simplex virus (HSV) is the most frequently encountered contagious infection in humans causing several clinical disorders [1]. HSV belongs to Herpesviridae family and has two distinct serotypes: HSV type 1, or HSV-1, which is mainly related to oral and perioral lesions, and HSV type 2, or HSV-2, which is regularly linked to genital lesions. Researchers reported that the preference of a certain viral serotype to an anatomic location is altering, to some extent owing to varying practices. Thus, HSV infection in humans depends on the antigenic nature of the virus, location of inoculation, and response of the host immune system [2].

Primary HSV-1 infection in oral or perioral locations is recognized as primary herpetic gingivostomatitis and transmitted directly by contacting the mucous membranes or abraded skin through mucosal secretions from an active primary or recurrent infection. HSV invades the epithelial cells and replicates intracellular at the location of the initial exposure. After primary infection, HSV migrates to the trigeminal nerve ganglion of the host nervous system. The virus then replicates and stays in an immunologically protected state until reactivation is triggered impulsively occasionally and sporadically by various stimuli, for example, exposure to ultraviolet light, mechanical trauma, cosmetic deformity, psychological anguish, fever, nutritional factors, and suppressed immunity [3,4].

Reactivation of the virus in the sensory ganglia results in cutaneous and mucocutaneous occurrence of recurrent herpes including appearance of two ordinary forms [5]. These are recurrent herpes labialis, in which the lesion classically occurs on the mucocutaneous junction usually on the lips, and recurrent intraoral herpes (RIOH), which affects

mainly keratinized mucosal surface of the oral cavity and is observed mainly in immunocompromised individuals and rarely in immuocompetent patients [6,7].

RIOH starts with prodromal symptoms, such as tingling or a burning sensation followed by vesicle formation within an area of a sensory nerve distribution followed by consequent ulceration. Ragged ulcers with surrounding erythema are observed. Compared with primary infection, recurrent episodes are shorter in duration, less severe, and there is reduced systemic involvement including fever and lymphadenopathy. However, they are still accompanied by pain, mainly of a burning character and still represent a transmission risk through direct contact with the infected lesion and saliva of individuals with active primary or recurrent infection [8,9].

The current management of HSV infection is prevention of transmission, repression of recurrence, attenuation of the clinical route, avoidance of viral shedding complications, palliation, evasion of the trigger factors, and endorsement of healing [10].

Topical and systemic antiviral agents have been used in the treatment of HSV infections with the risk of development of resistance especially in immuno-compromised patients and presence of multiple systemic adverse effects [11,12].

Chlorhexidine (CHX) is an effective antimicrobial agent used for prevention of dental plaque formation. It is capable of inhibiting a variety of microorganism such as gram-positive and gram-negative facultative aerobic and anaerobic bacteria and also fungi. There are several in-vitro and in-vivo studies on CHX's antiviral activity against HSV-1 reporting that CHX not only inhibited the replication of HSV-1 *in vitro* but also prevented the development of the virally induced cutaneous lesions. Some adverse effects of the prolonged utilization of CHX are calculus formation, permanent dental discoloration, taste changes, and cell toxicity, which limit its safety and wide usage as a routine mouthwash [13].

As a consequence of the reported adverse effects of CHX, various natural herbal extracts have been introduced. Among these is *Punica granatum* which belongs to family Punicaceae, mostly known as 'Pomegranate'. Pomegranates have been known for hundreds of years for their multiple health benefits, including their antimicrobial activity, antifungal, anti-inflammatory, and antioxidant properties. It has been noted that pomegranates and their extracts may serve as natural alternatives owing to their potency against a wide range of viral pathogens including influenza virus, herpes virus, poxviruses, and HIV-1 [14]. The recent surge in multidrug-resistant bacteria and the possibility of widespread global virus pandemics necessitate the need for additional preventative and therapeutic options to conventional drugs.

So the aim of the present contemplate was to compare the effect of pomegranate peel extract and CHX mouthwashes in the treatment of RIOH.

Patients and methods

Sample size calculation

As no previous studies regarding the effect of pomegranate peel extract mouthwash on RIOH were available in the literature, the sample size of each group was obtained from the available cases data. After recording the results of the current contemplate, the power was calculated based on pain and erythema scores mean and SD values of the studied groups. The power was 0.82, indicating that the sample size (n=6) for each study group was adequate. The used formula for sample size calculation was as follows:

$$1-\beta = \Phi\left(z - z_{1-\alpha/2}\right) + \Phi\left(-z - z_{1-\alpha/2}\right), z = \frac{\mu_A - \mu_B}{\sigma\sqrt{\frac{1}{n_A} + \frac{1}{n_B}}}$$

where k=n_A/n_B is the matching ratio; σ is SD; Φ is the standard normal distribution function; Φ^{-1} is the standard normal quantile function; α is type I error; β is type II error, meaning 1–β is power.

Pomegranate mouthwash preparation

The dried fruit peels of *Punica granatum*, Pomegranate (*Punicaceae*), were crushed into small pieces and grinded into powder. The powder was then dissolved in a mixture of ethanol and double-distilled water (1 : 1 v/v). The obtained material was filtered and then evaporated at 60°C to one-third of its original volume under reduced pressure and freeze-dried to yield a dry powder. The mouthwash was prepared by adding double-distilled water to the dry powder, and 1-ml sample was completely evaporated in the oven, to get the insoluble residues per milliliter which was further used to adjust the concentration of the mouthwash. A very small amount of methylparaben (2 mg) was added as a preservative to the pomegranate mouthwash. The preparation of the mouthwash in a 4 g % (40 µg/ml) concentration was according to Bhadbhade *et al.* [15].

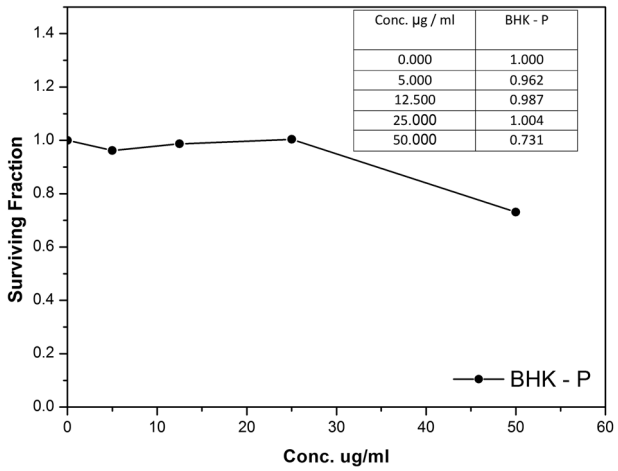
Measurement of potential cytotoxicity of pomegranate peel extract

Potential cytotoxicity of pomegranate peel extract mouthwash prepared was attested using the method of Skehan *et al.* [16] in the Clinical Pharmacy Department at the National Cancer Institute, Egypt, to obtain the IC_{50} value which is the half maximal inhibitory concentration to measure of the potency of a substance in inhibiting a specific biological or biochemical function. Normal cell line (baby hamster kidney cells, BHK-21, Sigma-aldrich, Germany) was plated in 96-multiwell plate (104 cells/well) for 24 h before application of the tested material to allow attachment of the cells to the wall of the plate. Different concentrations of the attested material under test (0, 1, 2.5, 5, and 10 µg/ml) were added to the cell monolayer triplicate wells prepared for each individual dose. Monolayer cells were incubated with the material for 24 h at 37°C and in atmosphere of 5% CO_2. After 48 h, cells were fixed, washed, and stained with sulforhodamine B stain. Excess stain was washed with acetic acid, and attached stain was removed with Tris-EDTA buffer. Color intensity was measured in an enzyme-linked immunosorbent assay reader (ELISA reader, BioTek, USA). The relation between surviving fraction and material concentration is plotted to get the survival curve of each cell line after specified compound concentration as revealed in Fig. 1.

Patients' selection

A total of 12 immunocompetent patients aged from 18 to 35 years with RIOH lesions affecting their keratinized mucosa were recruited from the outpatient clinic of Oral Medicine and Periodontology Department, Faculty of Dentistry, Cairo University, and included in the present study. The included participants have signs and symptoms affecting the keratinized mucosa appearing during the first 24 h with a history of at least two recurrences during the past 2 years.

Figure 1



Potential cytotoxicity results revealing the safety of the pomegranate mouthwash (P) concentration used in this study.

The medical data were collected from the patients according to the questionnaire of Modified Cornel Medical Index [17]. Diagnosis of RIOH was based on history and clinical examination [18].

Patients recognized with a history of immunodeficiency or other systemic condition related to it (e.g., malignancies, history of organ transplants, HIV infection, chronic infections, systemic immunosuppressive treatment, or corticosteroid therapy) were excluded. In addition, smokers, pregnant, or lactating females were not allowed to participate. Patients using oral antiviral medications in the previous 2 weeks were omitted from the study. Patients with a known history of allergic reaction to the currently used herbal product were also excluded. Participants were instructed to evade accidental trauma on soft tissues and advised to use soft bristles toothbrush. Acidic, spicy, hard, hot food, and beverages should also be avoided.

Ethical approval

The study was carried out according to the ethical guidelines of the World Medical Association (Declaration of Helsinki) for studies involving human participants, and the study protocol was approved by the Ethical Committee of the Faculty of Dentistry, Cairo University and registered in code no 17 12 20. All the patients signed informed consent before treatment, documenting their approval after the study procedures were explained to them.

Study design

The participants were randomly enrolled in the study groups before treatment. The patients were equally divided into two groups: group I (pomegranate group) included six patients who were treated using pomegranates mouthwash and group II (CHX group) included six patients who were treated using CHX mouthwash.

Overall, 4 g% (40 µg/ml) pomegranate peel extract concentration according to Bhadbhade *et al.* [15] and CHX hydrochloride 125 mg/100 ml (Hexitol; Arab Drug Company for Pharmaceutical and Chemical Industries, Cairo, Egypt) mouthwashes were used. Both treatment modalities were repeated three times per day for 10 days 15 ml per time for 30 s. Patients were advised to continue the oral hygiene measures regularly and were followed up at 2, 4, 6, and 8 days from baseline (B) visits in both study groups.

Clinical assessment

All cases in the study groups were assessed at the follow-up periods for grade of pain score according to Garnick *et al.* [19] as follows: grade 0 (no discomfort), grade 1 (mild discomfort), grade 2 (moderate discomfort), grade 3 (severe discomfort), and grade 4 (intolerable pain). Erythema score was also recorded according to Epstein *et al.* [20] as follows: grade 1 (no erythema), grade 2 (mild erythema), grade 3 (moderate erythema), and grade 4 (severe erythema).

Statistical analysis

Statistical analysis was performed using a commercially available software program (SPSS 19; SPSS Inc., Chicago, Illinois, USA). Pain and erythema scores revealed a nonparametric distribution, and a comparison between the groups using Mann–Whitney *U*-test was conducted. Friedman test for dependent samples was used to study the effect of time on pain and erythema within the same group. The level of significance was set at *P* value less than 0.05.

Results

Potential cytotoxicity results of pomegranate extract mouthwash

Cytotoxicity results revealed the relation between surviving cell fraction and the pomegranate mouthwash concentration proving that the 4 g% (40 µg/ml) used in this study can be used safely, as no value of IC_{50} was obtained (Fig. 1).

Table 1 Demographic characterization of the study groups

Age (mean±SD)		Sex [*n* (%)]			
		Group I		Group II	
Group I	Group II	Male	Female	Male	Female
22.4±2.07	22.6±2.7	3 (50)	3 (50)	3 (50)	3 (50)
t-Value=0.1314		χ^2=0.4			
P (*t*-test)=0.899 (ns)		*P* (χ^2)=0.527 (ns)			

Clinical results

After 10 days, complete healing of RIOH lesions was observed in both groups. No reported adverse effects or allergic reactions were reported in the pomegranate group. Only two patients revealed alteration of the taste sensation with tongue discoloration in the CHX group.

The results revealed that in group I, the patients' age ranged from 20 to 25 years, whereas in group II, it ranged from 19 to 26 years. Six females and six males were included in the study. No statistically significant difference was shown between mean age values (*P*=0.899) and sex distributions (*P*=0.527) in both study groups, as seen in Table 1.

Pain score results

Pain score recordings gradually decreased by time, recording the least mean value after 8 days. Friedman test revealed that the difference in pain scores was statistically significant in group I (*P*=0.001) and in group II (*P*=0.001). Comparison between both groups revealed a higher mean score in group II after 2, 4, 6, and 8 days, with a significant difference after 4 days (*P*=0.031) and 6 days (*P*=0.422; Table 2).

Erythema score results

Erythema score values also gradually decreased by time, recording the least mean value after 8 days. Friedman test revealed that the difference in erythema scores was statistically significant in group I (*P*=0.001) and in group II (*P*=0.002). Comparing both groups revealed a higher mean score also in group II after 2, 4, 6, and 8 days; however, this difference did not reach the level of statistical significance (Table 3).

Calculation of the percentage change of mean pain and erythema scores between the two groups revealed better improvement of both scores in group I (pomegranate group) than group II (CHX group), as shown in Table 4. Cases are presented in Figs 2 and 3.

Table 2 Pain score values in both groups along with the different observation times

	B	After 2 days	After 4 days	After 6 days	After 8 days	*P* value (time) (Friedman test)
Group I (mean±SD)	3.6±0.55	2.4±0.55	1.4±0.55	0.4±0.55	0.2±0.44	0.001*
Group II (mean±SD)	3.4±0.55	2.8±0.45	2.4±0.55	1.2±0.45	0.4±0.55	0.001*
P value (for both groups)	0.549	0.221	0.031*	0.042*	0.513	

*Significant difference than baseline values at $P<0.05$.

Table 3 Erythema score values in both groups along with the different observation times

	B	After 2 days	After 4 days	After 6 days	After 8 days	*P* value (time) (Friedman test)
Group I (mean±SD)	3.4±0.55	2.4±0.55	2±0	1.2±0.45	1±0	0.001*
Group II (mean±SD)	3.4±0.55	3±0	2.4±0.55	1.8±0.45	1.4±0.45	0.002*
P value (for both groups)	1.00	0.050	0.134	0.072	0.134	–

*Significant difference than baseline values at $P<0.05$.

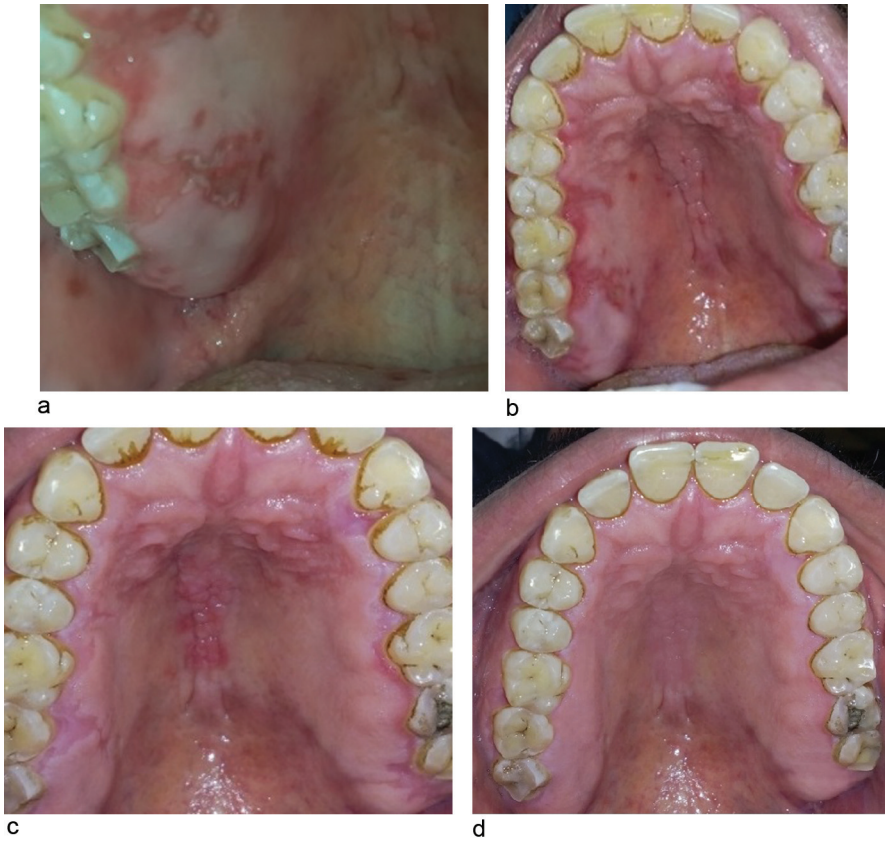
Table 4 Percentage of change in pain and erythema scores in both study groups at different observation times

	Mean % change of pain scores		Mean % change of erythema scores	
Comparison between	Group I	Group II	Group I	Group II
B and after 2 days	33.32	17.65	29.41	11.76
B and after 4 days	61.11	29.41	41.18	29.41
B and after 6 days	88.93	64.71	64.70	47.06
B and after 8 days	94.42	88.24	70.62	58.82

Discussion

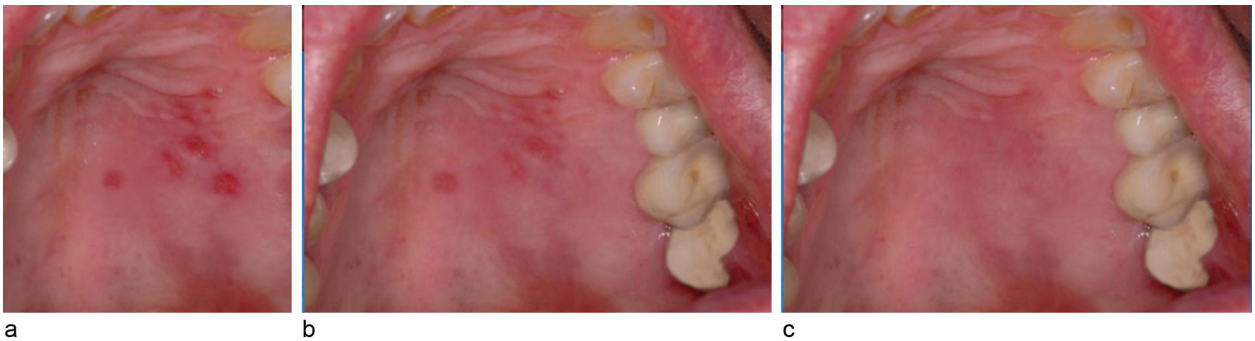
The infectious nature of HSV makes it a common risk in the dental practice. Suitable infection control measures can assist limit the dangers of disease transmission related to HSV for both the practitioners and the patients [8]. Mouthwashes are generally utilized for the prevention and therapy in a variety of oral and dental diseases. RIOH is one of the

Figure 2



A case of recurrent intraoral herpes (RIOH) before and after using pomegranate mouthwash. (a) RIOH lesions presented with multiple painful ulcers at the palatal mucosa (baseline visit). (b) RIOH lesions in the same patient showing presence of the multiple ulcerations also at the midpalatal mucosa (baseline visit). (c) RIOH lesions at the fourth day follow-up visit. (d) After 10-day follow-up, complete healing of RIOH lesions was observed.

Figure 3



A case of recurrent intraoral herpes (RIOH) before and after using chlorhexidine mouthwash. (a) RIOH lesions presented with multiple painful ulcers at the palatal mucosa (baseline visit). (b) RIOH lesions at the fourth day follow-up visit. (c) After 10-day follow-up, complete healing of RIOH lesions was observed.

less commonly occurring contagious oral disease caused by HSV-1 [13]. There is no standardized topical treatment for managing RIOH that can prevent or completely cure this type of infection as it is still self-limiting. The treatment is mainly targeting control of accompanied pain and managing the present ulcerations and viral shedding [21]. To the authors' knowledge, no studies have been conducted using pomegranates in the treatment of RIOH or comparing the used mouthwashes, so results of the present study cannot be compared directly with previous studies and is originally focused on the present results. A meaningful power analysis for sample size calculation was difficult to perform before beginning the study because no randomized control trials were previously conducted using pomegranate or comparing both mouthwashes in treatment of RIOH. In this contemplate, pomegranate peel extract mouthwash was pharmaceutically prepared as a natural herbal extract and CHX was commercially available.

Some mouthwashes, in adding to their antiseptic effectiveness, may also be toxic for epithelium and connective tissue cells with limited safety and accompanied adverse effects. CHX is one of the commercially available mouthwashes with various documented adverse effects associated with its prolonged use [13]. Pomegranate peel extract mouth wash was found safe at 4 g% (40 μg/ml) concentration after testing its potential cytotoxicity, which was previously prepared by Bhadbhade *et al.* [15] using the same concentration.

CHX and pomegranates share many important beneficial effects, mainly their antimicrobial and anti-inflammatory properties in addition to the antiviral effects [13].

In the current study, the results showed a gradual decrease in pain score values by time, recording the least mean value after 8 days. The difference in pain score was statistically significant in both groups. Comparing both groups revealed a higher mean pain score in group II after 2, 4, 6, and 8 days, with a significant difference after 4 and 6 days. The results revealed in group II are attributed to the direct virucidal effect and inhibition of viral replication of CHX as previously reported by several studies [13,22]. The antiviral role of pomegranate (group I) is based on the prevention of virus binding to the host cell by blocking its surface receptors or the virus surface ligands. The acidity and polyphenols high content in pomegranates can result in structural damage to the virus or virus capsid as revealed by the virucidal properties against influenza virus. The researchers on HIV infections showed the potential of producing anti HIV-1 microbicides from naturally safe food sources [23].

The improvement recorded in the pomegranate group, which was more than the CHX group, in pain scores can be owing to the presence of tannins in the pomegranate extract, which have anesthetic properties [24].

Erythema score recordings also reported gradual decrease by time, recording the least mean value after 8 days. Comparing both groups revealed a higher mean erythema score also in group II after 2, 4, 6, and 8 days; however, this difference did not reach the level of statistical significance. The percentage of change in erythema scores also revealed better improvement of both scores in group I (pomegranate group), which was superior to group II (CHX group). The main ingredient of pomegranate fatty acids, punicic acid, is considered a superior anti-inflammatory compound with a property to suppress prostaglandin production essentially involved in the pain pathway. Pomegranate ingredients such as ellagic acid can reduce interleukin-8 and nitric oxide secretion. In addition, pomegranate extract has the capability of inhibiting cyclooxygenase, lipoxygenase, and matrixmetalloproteinases enzymes, which are the key enzymes in the production of multiple inflammatory mediators and induction of tissue destruction. Apart from the aforementioned mechanisms, pomegranates can play an immunoregulatory action on macrophages and T and B lymphocytes. Pomegranate extracts have also the ability to scavenge free radicals and decrease macrophage oxidative stress and lipid peroxidation through its antioxidant activity as previously documented [25]. The insignificant difference recorded in the erythema scores between the two groups may be owing to the self-limitation of the disease which takes about 14 days for complete resolution of the infection and healing, which was reduced in the current study showing complete healing in 10 days [3].

The antibacterial activity of pomegranates acts against secondary infection, which helps in acceleration of infection resolution revealed by the superior results of group II in the current study. This antibacterial effect was owing to the presence of ellagitannin and punicalagin, which can prevent adherence of bacteria, reduce total protein accompanied with presence of plaque forming bacteria, decrease activities related to

cell injury, and increase activity of ceruloplasmin, which have a protective action against oral oxidative stress [26].The better improvement of the assessment values in group I can be attributed to the accelerated wound healing property of pomegranate. Increase of collagen, DNA, and protein synthesis as well as contraction rate and tensile strength when using pomegranates as topical agents was recorded. Another study documented the effect of pomegranate extract on accelerated time of wound healing and postulated that this may be owing to the high polyphenolic and tannin contents of its extract. In addition, a significant decrease of wound size and increased rate of wound contraction and collagen turnover were documented in an experimental study. It was reported that it increased fibroblast migration and proliferation with enhancement of angiogenesis [25].

Conclusion

Extract of pomegranate peel mouthwash might be considered as a new and safe topical treatment option for RIOH. Pomegranate mouthwash was more effective in reducing pain and accelerating wound healing when compared with CHX. Further clinical studies with larger sample size are recommended.

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Conflicts of interest

There are no conflicts of interest.

References

1 Fatahzadeh M, Schwartz RA. Human herpes simplex virus infections: epidemiology, pathogenesis, symptomatology, diagnosis and management. J Am Acad Dermatol 2007; 57:737–763.

2 Kleymann G. Agents and strategies in development for improved management of herpes simplex virus infection and disease. Expert Opin Investig Drugs 2005; 14:135–161.

3 Lugito MDH, Pradono SA. Valacyclovir in the management of recurrent intraoral herpes infection. J Dent Indonesia 2014; 21:27–31.

4 Arduino PG, Porter SR. Oral and perioral herpes simplex virus type I (HSV-I) infection: review of its management. Oral Dis 2006; 12:254–270.

5 Tovaru S, Parlatescu I, Tovaru M, Cionca L. Primary herpetic gingivostomatitis in children and adults. Quintessence Int 2009; 40: 119–124.

6 Cunningham A, Griffiths P, Leone P, Mindel A, Patel R, Stanberry L, Whitley R. Current Management and recommendations for access to antiviral therapy of herpes labialis. J Clin Virol 2012; 53:6–11.

7 Martin S, Greenburg M. Burket's oral medicine. 11th ed. Pennsalvania, USA: Bc Deck er Inc.; 2008. 42–49.

8 Lewis MAO. Herpes simplex virus: an occupational hazard in dentistry. Int Dent J 2004 54:103–111.

9 Westley S, Seymour R, Staines K. Recurrent intra-oral herpes simplex 1 infection. Dent Update 2011; 38:368–370.

10 Tovaru S, Parlatescu I, Tovaru M, Cionca L, Arduino P-G. Recurrent intraoral HSV-1 infection: a retrospective study of 58 immunocompetent patients from Eastern Europe. Med Oral Patol Oral Cir Bucal 2011; 16: e163–e169.

11 Bhateja S, Arora G, Mastud SK. Recurrent intraoral herpes (RIH) infection – a case report. Biomed J Sci Tech Res 2017; 1:1–3.

12 Stoopler ET, Alfaris S, Alomar D, Sollecito TP. Recurrent intraoral herpes. J Emerg Med 2016 51:324–325.

13 Pourshahidi S, Rezazadeh F, Motamedifar M, Davarmanesh M, Ebrahimi H, Alipour A. In vitro comparative study on antiherpetic effect of chlorhexidine and persica mouthwashes with acyclovir. J Basic Appl Sci 2012; 8:286–290.

14 Ismail T, Sestili P, Akhtar S. Pomegranate peel and fruit extracts: a review of potential anti-inflammatory and antiinfective effects. J Ethnopharmacol 2012; 143:397–405.

15 Bhadbhade SJ, Acharya AB, Rodrigues SV, Thakur SL. The antiplaque efficacy of pomegranate. Quintessence Int (Berl) 2011; 42:29–36.

16 Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, *et al.* New coloremetric cytotoxicity assay for anti-cancer drug screening. J Natl Cancer Inst 1990; 82:1107–1112.

17 Norton JC, Powell BJ, Penick EC, Sauers CA. Screening alcoholics for medical problems with the Cornell Medical Index. J Stud Alcohol 1977; 38:2193–2196.

18 Westley S, Seymour R, Staines K. Recurrent intra-oral herpes simplex-1 infection. Dent Update 2011; 38:368–374.

19 Garnick JJ, Singh B, Winkley G. Effectiveness of a medicament containing silicon dioxide, aloe, and allantoin on aphthous stomatitis. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1998; 86:550–556.

20 Epstein JB, Sherlock C, Page JL, Spinelli J, Phillips G. Clinical study of herpes simplex virus infection in leukemia. Oral Surg Oral Med Oral Pathol 1990; 70:38–43.

21 Minami M, Kita M, Nakaya T. The inhibitory effect of essential oils on HSV1 replication in vitro. Microbiol Immunol 2003; 47:681–684.

22 Baqui A, Kelley J, Jabra-Rizk MA. In vitro effect of oral antiseptic on HIV1 and HSV1. J Clin Periodontal 2001; 28:610–616.

23 Howell AB, D'Souza DH. The pomegranate: effects on bacteria and viruses that influence human health. Evid Based Complement Alternat Med 2013; 606212:1–11.

24 Hekmatian E, Shadmehr E, Asghari G. Effect of peel extract lozenge on gag reflex in dental patients. J Isfahan Den School 2011; 7:229–235.

25 Prasad D, Kunnaiah R. *Punica granatum*: a review on its potential role in treating periodontal disease. J Indian Soc Periodontol 2014; 18:428–432.

26 Kote S, Kote S, Nagesh L. Effect of pomegranate juice on dental plaque microorganisms (Streptococci and Lactobacilli). Anc Sci Life 2011; 31:49–51.