



Efficacy of Pomegranate Peel Extract Mouthwash on Gingivitis and C-reactive protein

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Abstract

Background and objective: Gingivitis is characterized as the world's most predominant inflammatory periodontal disease. Healthy mouth and teeth are obtained by brushing and uses mouthwash. And because of the side effects of chlorhexidine, pomegranate peel extract mouthwash used alternative which are thought to have anti-inflammatory effect. The aim of this study is to prepare a herbal mouthwash and to evaluate the efficacy of pomegranate peel extract mouthwash on plaque index, gingival index, and C-reactive protein on moderate gingivitis patients.

Method: this study is a clinical crossover design study, which consists of 25 volunteers with moderate gingivitis, who visited Duhok Dental Training Center. This study started from December 2022 to March 2023. Plaque and gingival index with a sample of saliva for C-reactive protein measuring were taken as a baseline before each mouth wash and after that, the pomegranate peel extract mouthwash was used for two weeks, then three weeks wash out between them, then chlorhexidine mouth wash was used for two weeks (crossover method). All saliva samples were analyzed using (CRP ELISA Test).

Results: Twenty-five patients who used pomegranate peel extract mouthwash showed a significant reduction after two weeks used (CRP=162.28, GI=0.54, PI=0.82) and p value (<0.0001) in mean C-reactive protein, gingival index, and plaque index from baseline which was (CRP=669.78, GI=1.88, PI=2.11).

Conclusion: Pomegranate peel extract mouthwash had been shown effective against moderate gingivitis in adult patients with no systemic diseases through lowering in mean salivary C-reactive protein, gingival index, and plaque index.

Keywords: Chlorhexidine, C-Reactive protein, Gingivitis, Pomegranate Saliva.

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Introduction

Gingivitis is inflammations of the gingival tissues that help the teeth with a wide extensive unfold prevalence.¹ The occurrence of gingivitis is related to the natural formation of dental biofilm of large densities of endogenous oral organisms which lead to the buildup of metabolites and by-products with inflammatory potential.¹⁻² Dental plaque is mostly a faded yellow biofilm that is evidently developed at the teeth. Like all biofilm dental biofilm is fashioned through colonizing microorganism seeking to connect themselves to the tooth's clean surface. The mouthwashes are beneficial for discounting microbial plaque, inhibiting the formation of plaque polysaccharide matrix.³ Gingivitis has an effect on all ages and genders.⁴ The acknowledged inadequacies of the mechanical plaque management practices of many people have fueled a year-length look for chemical sellers to manipulate plaque.⁵ Pomegranate, (*Punica granatum*) is one of the oldest eating fruits. The peel and arils are an excellent source of bioactive substances like polyphenols, flavonoids, tannins, minerals, and vitamins.⁶⁻⁷ It has been used as an astringent, hemostatic agent, and as a medicine for diabetic control.⁸ Pomegranate display sensitivity toward the *Streptococcus pyogenes*, *Lactobacillus bulgaricus*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli*. The pomegranate has active polyphenolic flavonoids like punicalagin and ellagic acids which enables to save gingivitis.⁹ Pomegranate has been shown to have anti-inflammatory, antimicrobial, and anti-mutagenic properties. Ellagic acid, ellagitannins, punicalagin, Punic acid, anthocyanidins, anthocyanins, flavonoids, and estrogenic flavones are among the compounds that have medicinal benefits.¹⁰⁻¹¹ Pomegranate additives' properties may decrease the possibility of gingivitis and promote general dental health.¹² In one of the

earliest medical books, the pomegranate's healing properties were described, the Eber's Papyrus from ancient Egypt (1500 BC).¹³ Chlorhexidine mouthwash is the most advanced anti-plaque asset and is taken into consideration to be the gold standard among chemical plaque measures however there is the life of facet impacts which include brownish discoloration of teeth and longtime use of this mouthwash may also impair flavor sensation, dry mouth, unpleasant taste in mouth.^{14,15} For this reason, natural mouthwash has been brought on every day to preserve oral hygiene and to deal with plaque-induced gingivitis as correctly as chlorhexidine.¹⁶ Certainly considered one of the typically used markers of systemic inflammation is C-reactive protein which is automatically measured in serum samples. However, salivary patterns provide anon-invasive and without problems reachable which could enhance factor of care (POC) testing of inflammation.¹⁷ Dependable detection of CRP in saliva as opposed to blood could provide a blessing concerning sampling methods and availability however the use of saliva as a diagnostic body fluid comes with challenges.¹⁸ The compounds of pomegranate ellagic polyphenols inhibited the expression of CRP genes in addition to polyphenols in pomegranate lessen CRP ranges with the aid of using suppressing the hobby of the cyclooxygenase-2 enzyme and interference with regulating a few seasoned inflammatory indicators such as tumor necrosis factor.^{19,20} The aim of this study is to prepare a herbal mouth wash which is a pomegranate peel extract mouth wash and to compare the efficacy of pomegranate peel extract mouthwash on plaque index, gingival index, and C-reactive protein.

Patients and methods

Following the approval from the institutional ethical committee, Duhok Directorate General of Health The clinical study was



conducted in the Periodontics Department, College of Dentistry/ University of Duhok/UOD and Duhok dental training center, Kurdistan region of Iraq. The study started from December 2022 to March 2023. This study included case history and clinical and lab examination of 25 patients who were clinically diagnosed with grade (2) moderate gingivitis according to (Leo and Sliness,1963)³⁷ with an age range between (20-30) years. Pomegranate peel extract were previously prepared in the Collage of Agriculture, University of Duhok under the supervision of assistant teacher, according to scientific references.²¹ Pomegranates variety was collected from the local market of Duhok, Kurdistan, Iraq. The chemical (ethanol) were of analytical grade. Pomegranate peels were separated and cleaned with tap water, and then dried in an oven (OUmai) at 50 °C. Using a grinder, the dried peels were ground, and 20 g of the ground pomegranate peels were then separately steeped in 100 ml of 95% ethanol. Following evaporation, samples were filtered using Whatman filter paper number 1 and the filtrate was kept at 4 °C in the incubator. To remove the most components from the pomegranate peels, the extraction process was carried out three times. The phenolics, antioxidant, and bactericidal properties of the pooled extract were analyzed. The last step we collected crude oil. We prepared about 100ml of pomegranate peels extract. For (2%) of pomegranate peels extract solution used as a mouth rinse was prepared by adding 2ml of the extract completed to 100ml of distilled water and stored in a labeled glass container until used by the patient.³⁸ In this study, 25 volunteers (male and female) with special criteria were clinically identified as having grade 2 moderate gingivitis, with ages ranging from 20 to 30 years. This study excluded (physical, medically compromised patients, pregnant women, alcoholics, and smokers). Ethical committee consent was

given by the General Directorate of Health in Duhok. A special consent form was obtained from each patient after explaining the procedure and the purpose of the study. General information; including name, gender, age, phone number, and date of saliva sample taken was obtained from each patient. Each patient before using any of mouth wash (PPE mouthwash 2%, Chlorhexidine mouthwash 0.12%), A baseline recording of clinical gingival parameters, plaque index (PI), Gingival index (GI) was done by examiner through using of periodontal probe and probing of mesial, distal, buccal, palatal surface of all teeth and summing the values obtained for each tooth and calculating the averages(plaque index 0=no plaque, 1= thin film plaque on the gingival margin, 2=visible plaque on gingival margin and sulcus,3=dense plaque on the gingival margin and sulcus)(gingival index 0=healthy gingiva,1=mild discoloration no bleeding on probing, 2=red shiny gingiva and bleeding on probing, 3=red ulcerated gingiva with spontaneous bleeding)²²⁻³⁷ and un stimulated saliva sample were collected from the patients for estimating the CRP. Mechanical treatment (scaling and polishing) was done for the patients at the dental clinic. Firstly PPE mouth wash(300ml) was given to each patient and instructed to use 10ml of PPE mouthwash for 30sec after tooth brushing twice daily and not eat anything 30 min after that and should use it for 14 days.⁶⁻²³⁻²⁸ At the end of the treatment period, the second examination (PI, GI) and collection of saliva was performed, a three-week washout according to the crossover design study was instructed to each patient (clear the effect of first mouthwash) and they were asked to come back for second mouth wash use which was CHX (300ml 0.12%)and (PI, GI) was recorded from each patient as well as collected saliva sample baseline and scaling and polishing done for each patient after that using the mouth wash same as a first mouth



wash which was PPE mouth wash for about 14 days. Recording (PI, GI) parameters as well as saliva was collected in plain tubes. In this study crossover method used for the first in Duhok city as well as to make the patients factor constant, as the mouth wash will be used by the same patient, so we have controlled the point that it is the same patient in terms of quality of food and the of brushing teeth and lifestyle. All saliva samples centrifuged at 4000rpm for 5min and stored in an Eppendorf tub and frozen at -20c in refrigerator until required for analyzed.²⁴ Each Eppendorf tub labeled in English letters (A, B, C....and so on) for each patient (25 no.) . However, each A for example has 4 samples of saliva, A1 before PPE mouth wash, A2 after PPE mouth wash, A3 before CHX mouth wash, A4 after mouth wash and so on. Salivary CRP had been measured by an Enzyme-linked Immunosorbent Assay (ELISA) device. All frozen saliva samples were wet at room temperature (20-25°) before use, and the CRP ELISA kit (ELK1040) was used to measure the level of CRP. Diluted the 25sample wash buffer in to working concentration added 1ml of standard diluent in standard vial then kept for 10 min at room temperature with shaking gently. The concentration of standard in the stock was 4000pg/ml prepared 7 tubes containing 0.5ml of standard dilution and used diluted standard to produce a double dilution series mixed each tube thoroughly before the next transfer. Concentration of standard was (4000, 2000, 1000, 500, 250, 125, 62-5). Biotin antibody and streptavidin-HRP reagent diluted to the working concentration 100-fold with biotin and HRP reagent diluent. Added 100ml of each standard working and 100ml of samples in to appropriate wells covered with plate sealer then incubated for 80 min at 37°. Washed the plate with wash solution for three time added CHX mouthwash showed a significant decrease after use of the mouthwash for two

100ml of biotinylated antibody working to each well. Covered the plate with plate sealer and incubated for 50min at 37° then wash three time and added 100ml of streptavidin – HRP working to each well and covered the plate with plate sealer and incubated for 50min at 37°. Wash 5times then added 90ml of TMP substrate solution to each well covered with new plate sealer and incubated for 20min at 37° protected from light. Added 50ml stop solution to each well then removed any drop of water and fingerprinted on the plate and read and conducted measurement at 450nm. At a wavelength of 450 nm, the color change was quantified spectrophotometrically. The optical density (OD) of the sample was then compared to the standard curve to determine the concentration of CRP in the sample.²⁵ Levels of CRP, GI, and PI were presented as mean and Standard deviation. The measurement of CRP, GI, and PI of pomegranate and CHX mouthwashes from baseline and two weeks of use were taken The Pomegranate group was used first for two weeks, then wash out for three weeks, and the CHX group start to use for two weeks. A p-value of < 0.05 was determined as the significant level of difference. The statistical calculations were performed by JMP pro-14.3.0.²⁶

Results

All the patients (25; male and female) who have grade 2 moderate gingivitis finished the clinical trial shown in tables and figures (1.2.3) the evaluation of CRP.GI and PI of PEE and CHX mouthwashes at baseline and after two weeks from uses. In Table (1) the study group mean slandered deviation (SD) of CRP, GI, and PI parameters of PPE mouthwash was close to that of the CHX mouthwash of p-value of (CRP=0,0642, GI=0,0623, PI=0,0506). In Table (2) the PI parameter of the study group mean (SD) of weeks from baseline different to PPE mouth wash which was p value of (0,0071).

**Table (1):** Comparison of CRP, GI, and PI between pomegranate and CHX mouthwashes at baseline

Parameters	Study groups mean (SD)		p-value (two-sided)
	CHX mouthwash (n=25) +	Pomegranate mouthwash (n=25)	
CRP	468.61 (272.70)	669.78 (353.10)	0.0642
GI	1.68 (0.38)	1.88 (0.21)	0.0623
PI	1.94 (0.09)	2.11 (0.23)	0.0506
An independent t-test was performed for statistical analyses.			

Table (2): Comparison of CRP, GI, and PI between pomegranate and CHX mouthwashes at two weeks

Parameters	Study groups mean (SD)		p-value (two-sided)
	CHX mouthwash (n=25)	Pomegranate mouthwash (n=25)	
CRP	202.44 (151.24)	162.28 (172.54)	0.4627
GI	0.33 (0.33)	0.54 (0.35)	0.1130
PI	0.47 (0.37)	0.82 (0.23)	0.0071

An independent t-test was performed for statistical analyses.

In table (3), There was a significant reduction ($P < 0.0001$) observed in CRP, GI, and PI patients who was used PPE mouth wash from baseline and after two weeks. As well as after giving three wash out period (cross over method) to the patients to clear the effect of

PPE mouth wash, and after use the next mouth wash which was CHX mouth wash there was also significant declined ($P < 0.0001$) in CRP, GI and PI between pre and posttest of CHX mouth wash.

Table (3): Comparison of CRP, GI, and PI between pre and post-test of pomegranate and CHX mouthwashes

Parameters	CHX mean		Mean diff (95% CI)	p-value
	Baseline	Two weeks		
CRP	468.61	202.444	-266.17 (-371.81 to -160.52)	<0.0001
GI	1.68	0.33	-1.34 (-1.65 to -1.03)	<0.0001
PI	1.94	0.47	-1.48 (-1.78 to -1.17)	<0.0001
	Pomegranate mouthwash		Mean diff (95% CI)	p-value
	Baseline	Two weeks		
CRP	669.78	162.28	-507.5 (-648.49 to -366.51)	<0.0001
GI	1.88	0.54	-1.34 (-1.48 to -1.20)	<0.0001
PI	2.11	0.82	-1.29 (-1.51 to -1.08)	<0.0001

Paired t-test was performed for statistical analyses.

Discussion

Gingivitis is a common problem that affects all individuals regardless of age, sex or race. Maintaining strict oral hygiene and using

adjuncts like chemotherapeutic drugs can help avoid it. These substances prevent the growth of dental plaque, but they nevertheless have a number of undesirable



side effects, such as CHX, tooth discoloration, changed taste perception, gingival desquamation, and sore mucosa.²⁷ Natural items with beneficial antioxidant and antibacterial characteristics, such as pomegranates, were thought to be risk-free, without adverse effects.³⁵ It had been demonstrated that pomegranates contain a substance with pharmacological action that may be beneficial for maintaining good dental health, particularly in preventing the onset of gingivitis. The mechanism behind this property is an anti-inflammatory effect, anti-bacterial activity, of polyphenol rich continent of pomegranate peel extract which is anthocyanins and hydrolysable tannins lead to direct removal of plaque from teeth.²⁷ This clinical trial used to provide the effectiveness of pomegranate peel extract mouthwash in treated moderate gingivitis patients through measurement of salivary C-reactive protein level and (plaque, gingival) indices for assessing gingival inflammation and response to the mouthwash. The presence of CRP in gingival crevicular fluid could be result of systemic inflammation induced by inflammation of periodontal tissue so salivary CRP elevated in patients with periodontal diseases.³⁹ Chlorhexidine mouthwash has been regarded as the standard mouthwash for controlling of dental plaque caused by gingivitis in the Dentistry due to its proven effectiveness as antibacterial and antiplaque agent.²⁸⁻²⁹ but because of its disadvantages like staining of teeth and an unpleasant taste, PPE mouthwash showed effective for treatment of moderate gingivitis patients after using for 14 days that reported in this study also done by other researchers who searching for alternative choices.³⁰ The results showed that all 25 patients who using of pomegranate peel extract as a mouthwash of 2% were significantly ($p < 0.0001$) effective in lowering the clinical gingival and plaque indices and C-reactive protein related to gingivitis after two weeks of treatment. The

same results have been obtained to provide the effectiveness of pomegranate peel extract mouthwash in the reduction of periodontal parameters and C-reactive protein.³¹ Ahuja and colleagues conducted a similar study using pomegranate mouthwash, which was shown to be more effective against gingivitis due to styptic action with adequate plaque reduction.²⁹ As well as Sastravaha et al used pomegranate as biological chips for deep pockets after scaling and root planning and found that the incidence of bleeding and plaque was greatly reduced.²³ Pomegranate peel extract mouthwash fights plaque and reduce tarter forming by inhibiting the action of plaque causing microorganisms through inhibiting ability of microorganisms to adhere to tooth surface.⁶ Samira in her randomized, controlled, double-blind clinical trial that used pomegranate-containing mouthwash (twice daily for 14 days) showed a significant reduction of oral microbial plaque which caused gingival inflammation due to their anti-inflammatory and antimicrobial effects.³² This study provided a significant reduction in salivary C-reactive protein ($p\text{-value} < 0.0001$) of patients who used Pomegranate peel extract mouthwash from baseline and after two weeks. In an ohio study, people who rinsed with pomegranate juice experienced a decreased in total salivary protein levels are usually elevated in people with gingivitis.³⁴ The same study showed high sensitivity of C-reactive protein in patient's consumption pomegranate mouthwash.³³ Hassan and his colleagues study showed that, PPE presented a significant decrease in salivary CRP, GI and PI in moderate gingivitis at once after utilization. This could be regarding to the prevalence of polyphenolic flavonoids as an active ingredient in extract.³⁶ Efficacy of pomegranate peel extract mouth wash as anti- gingivitis and anti-plaque on moderate gingivitis patients provided significant improvement in all clinical indices and



suggested that using of PPE mouthwash adjunct to non- surgical treatment of gingivitis as well as concluded that pomegranate containing mouthwash did not show any adverse effects.⁴⁰ Many studies provided the effectiveness of using Chlorhexidine 0.12% mouthwash in decreasing gingivitis.³²⁻³³ The results reported of this clinical study revealed the lowering in the parameters of gingival and plaque index of patients using Chlorhexidine 0.12% mouthwash from baseline and after two weeks. The plaque index parameter of a study group of Chlorhexidine mouth wash showed a significant decrease ($p < 0.0001$) after use of the mouth wash for two weeks from baseline as compared to pomegranate peel extract mouth wash. But several articles beside this study done for improving the efficacy of pomegranate peel extract as a mouth wash provided effective for treatment of gingivitis because of positive effect of pomegranate peel extract as anti-inflammatory and antiplaque, others researchers reported the antibacterial effect of pomegranate peel extract mouthwash in their research.³⁻¹¹⁻³⁰⁻³⁵ This study improved the efficacy of PPE mouthwash on treatment of gingivitis and lowering CRP so it was showed anti-inflammatory effect.

Conclusion

Pomegranate peel extract mouthwash might be considered as a new and safe topical treatment option for treatment of moderate gingivitis. Pomegranate peel extract mouthwash was more effective in reducing salivary CRP, gingival and plaque indices when compared with Chlorhexidine. Further clinical studies with longer sample size a recommended.

Conflict of interest

The author reports no conflicts of interest.

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