

A clinical trial evaluates the effect of pomegranate extract as a mouth wash for gingivitis in type 2 diabetic patients

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Background and Objectives: Type-2 diabetes mellitus (T2DM) is a systemic disease which have higher incidence of gingivitis and periodontitis. Pomegranate peel has higher content of phenolic compounds with potent anti-inflammatory, antioxidant and antibacterial activity. The aim is to evaluate the efficacy of pomegranate peel extract on the clinical and biochemical measures of (IL-1 β) and the enzyme (AST) in saliva of (T2DM) patients suffering from gingivitis in comparison with chlorhexidine.

Patients and Methods: A randomized controlled clinical trial on 45 (T2DM) patients, both sexes with moderate gingivitis, age 40-55 years, (Hb1Ac) < 7%. They were randomly assigned to 3 groups undergo scaling and polishing, (15) for each group, group 1 used chlorhexidine mouthwash (0.12%), group 2 used pomegranate peel extract (6.25%) and group 3 pomegranate peel extract (12.5%). Before and after 14 days, PI, GI, BOP, salivary IL-1 β and AST were evaluated.

Results: All the results for both biochemical and clinical parameters in the three groups showed significant reduction after 14 days of treatment except for the salivary IL-1 β in group 1. No significant difference between group1 and group 2 except for PLI and group1 and group 3 except for IL-1 β , while significant differences between group 2 and 3 after 14days of treatment except for the salivary AST, with no side effects associated with the use of pomegranate peel extract as a mouthwash at both concentrations.

Conclusion: pomegranate peel extract could be considered more effective in treating gingivitis especially at the high concentration with no considerable side effects comparing to chlorhexidine.

Keywords: gingivitis, pomegranate peel, chlorhexidine.

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Introduction

Diabetes mellitus (DM) is a multi-systemic metabolic disorder characterized by abnormal carbohydrate, protein and lipid metabolism and has long been known to have a significant influence on the periodontium and is viewed as a risk factor for gingivitis and periodontitis. The cardinal biochemical feature of this disease is hyperglycemia.^{1,2} Type 2 diabetes mellitus (T2DM) is the more prevalent of the two major categories of diabetes and accounts for about 90-95% of all diabetic cases and mostly has an adult onset.³ It has been shown that uncontrolled T2DM has the greater incidence of severe periodontal diseases compared with those patients who are well controlled or have no T2DM.⁴ Periodontal diseases are one of the most widely spread diseases; they are considered an inflammatory disorder that damages tissues. Chronic gingivitis is one of the most frequent diseases, affecting more than 90% of the population, regardless of age, sex, or race.⁵ IL-1 β is a key pro-inflammatory cytokine that is released after infection, injury, or antigenic challenge. It is present in the saliva of both

healthy and diseased individuals. IL-1 β can serve as a biomarker of periodontal disease⁶ and for the monitoring the response to periodontal therapy.⁷ Aspartate aminotransferase (AST) is one of the enzymes of tissue degradation, changes in this enzymatic activity reflects metabolic changes in the gingiva and periodontium due to the inflammation.^{8,9} Saliva is a complex fluid that influences the oral health. When disruptions in the quality or quantity of saliva occur, there will often be detrimental effects on oral and systemic health. The differences in salivary constituents provide sources for assessment and monitoring of disease states.¹⁰ Pomegranate (*Punica granatum* linn) is a polyphenol rich fruit and contains a diverse range of phytochemicals that has shown a potential anti-inflammatory, antioxidant, antimicrobial and anticancer activity and it used for the prevention and treatment of a wide number of health disorders such as diabetes, diarrhea, and inflammation.¹¹ Pomegranate peel mainly consists of a considerable amount of flavanoids (flavonols, flavanols and anthocyanins), condensed tannins and hydrolysable tannins.^{12,13} It has been approved that the peel of pomegranate has higher content of phenolic compounds than the juice and has more potent antioxidant and anti-inflammatory activity.^{14,15} As a consequence of the side effects of chlorhexidine, the uses of natural products isolated from the plants are considered as good alternatives to synthetic chemicals. The aim of this study was to evaluate the efficacy of pomegranate peel extract as a mouth wash on the clinical and local biochemical measures of pro-inflammatory salivary cytokines (IL-1 β) and the enzyme (AST) in T2DM patients with moderate gingivitis in comparison with chlorhexidine mouthwash.

Patients and methods

Study design. This randomized triple-blind clinical trial was conducted in Erbil city at the outpatient clinics, Departments of Oral Diagnosis and Periodontics' clinics at the College of Dentistry, Hawler Medical University after the approval of the scientific committee. The study included a total number of 45 (25 males and 20 females

T2DM participants who were diagnosed clinically with signs of moderate gingivitis with an age range of 40-55 years, the participants were considered glycemic controlled by the measurement of glycated hemoglobin levels (HbA1c) which was less than 7% and a written consent were obtained from each of the participant who met the eligibility criteria. Type 1 and Type 2 diabetic patients taking insulin therapy, uncontrolled T2DM with blood levels of HbA1c more than 7%, subjects who had undergone periodontal treatment within sixth months prior to this study, patients with systemic disorders, subjects under medication usage over the past two month from the enrollment in this study, smokers, alcoholic, pregnant women, breast feeding women, and persons with fixed or removable orthodontic appliances or removable partial dentures were excluded from the study.

After checking the blood samples levels for HbA1c for the participants, that shall be less than 7%, this level used to reflect that the patient is a diabetic controlled over the last few weeks¹⁶, and before using any mouthwash a baseline recording of the clinical periodontal parameters Plaque index (PLI)¹⁷, Bleeding on probing (BOP%)¹⁸ and Gingival index (GI)¹⁹ were done by the examiner and unstimulated saliva samples were collected for estimating the biochemical salivary levels of IL-1 β and AST. After scaling and polishing, the participants were randomly distributed into the three groups and a dark bottle containing 300ml of chlorhexidine or pomegranate mouthwash at 6.25% or 12.5% concentration was given to each patient, all the mouthwash bottles were in the same size and shape and were coded as A, B and C to ensure concealment of the mouthwash product from the individual users and the examiner. The patients were instructed to use 10 ml of prescribed mouthwashes twice daily (30 minutes after morning and night tooth brushing for 60 s) for 14 days. All the participants were given the same type of tooth brush and toothpaste with standardized brushing technique instructions and they were asked to come back at the end of treatment period for the second examination of the clinical periodontal parameters and

for the collection of saliva like at baseline and a questionnaire regarding the side effects for each mouthwash product was filled by each participant while using the mouthwash during the study period.

Group 1 (n = 15) CHX (0.12%): Diabetic subjects (HbA1c are <7%) diagnosed with moderate gingivitis that received chlorhexidine (CHX) mouthwash (Hexidine by ICPA).

Group 2 (n=15) POM (6.25%): Diabetic subjects (HbA1c are <7%) diagnosed with moderate gingivitis that received twice daily (6.25%) prepared pomegranate peel extract as mouth wash for 14 days.²⁰

Group 3 (n = 15) POM (12.5%): Diabetic subjects (HbA1c are <7%) diagnosed with moderate gingivitis that received twice daily (12.5%) prepared pomegranate peel extract as mouth wash for 14 days.

Preparation of pomegranate extract as a mouthwash: The fruit was obtained from Akre town at the north of Erbil city in Kurdistan. After washing, the fruit, the peel was removed and dried in oven at 40°C; the dried peels were grounded by a mill to a powder. The hydroalcoholic peel extract was prepared in a mixture of 70% ethanol and water by maceration method. The extract was filtered two times by using Whitman paper No.42 and the obtained clear extract was dried at 50°C resulted in dry fine powder the extraction gave a yield of about 52g/ml which was used to prepare Pomegranate mouthwash.²¹ Two concentration (6.25% and 12.5%) of pomegranate peel extract was prepared and used in this study²⁰, and about 300ml of the preparation was poured in to dark plastic sterile bottles (400ml) and given to the patients who were directed about the use as a mouth wash.

Saliva sample collection: From each subject, (5ml) of unstimulated whole saliva

was collected between 9-10 a.m in sterile test tubes after making them rinse with 15ml of water (20 seconds) in order to wash out exfoliated cells. The collected saliva was centrifuged at 4000 rpm for 15 minutes and then the clear supernatant saliva was collected and kept frozen and stored at -20°C for the later estimation of IL- 1 β and AST by ELISA technique.

Biochemical investigation: This included the quantitative measurement of pro-inflammatory mediator IL-1 β (using kit manufactured by Salimetric) and the biochemical enzyme AST (using kit manufactured by Abcam) for the saliva samples that were collected before and after fourteen days from the use of the mouthwashes in the three groups. The salivary IL-1 β levels in each sample was expressed in pg/ml and AST levels in IU/L.

Statistical analysis: Results were presented as mean \pm standard deviation (SD). The data were analyzed using Student's t test (paired and unpaired) and fisher's test (via SPSS 19 software) for the comparisons within and between the groups. P value of ≤ 0.05 was considered as statistically significant.

Results

All the 45 T2DM patients (20 females and 25 males) diagnosed with moderate gingivitis completed the clinical trial. No one of the participants in group 2 or group 3 using pomegranate mouthwash at both studied concentrations (6.25% or 12.5%) reported any side effects during the 14 days period of treatment except one patient in group 3 who used pomegranate at 12.5% concentration complained from unpleasant taste while some participants in group 1 using CHX 0.12% mouthwash (n=11, 73.33%) suffered from variable side effects,

Table 1: Side effects associated with the use of mouthwashes in the three groups.

Groups	Gum Irritation %	Unpleasant taste %	Tooth stain %	No side effects %
Group1 (no=15)	n=4 (26.66%)	n=5 (33.33%)	n=2 (13.33%)	n=4 (26.66%)
Group 2 (no=15)	n=0 (0.0%)	n=0 (0.0%)	n=0 (0.0%)	n=15 (100%)
Group 3 (no=15)	n=0 (0.0%)	n=1 (6.66%)	n=0 (0.0%)	n=14 (93.33%)

Table 2: Comparison of the side effects between the groups.

Groups	P value			
	Gum Irritation	Unpleasant taste	Tooth stain	No side effects
Group 1 vs. Group 2	0.1	0.042*	0.48	0.00005**
Group 1 vs. Group 3	0.1	0.16	0.48	0.000048**
Group 2 vs. Group 3	1	1	1	1

(*) significant difference at p value ≤ 0.05 , (**) highly significant difference at p value < 0.01 .

and as a total side effects associated with the use of pomegranate mouthwash both group 2 and 3 showed high statistical significance comparing to group 3 as shown in Tables 1 and 2.

The analysis of the biochemical (IL-1 β and AST) and the clinical periodontal parameters (BOP, GI, PLI) before and after 14 days from using the mouthwash in

groups 1, 2 and 3 are shown in Table 3. All the results for both biochemical and clinical parameters in the three groups showed significant reduction after 14 days from treatment with either chlorhexidine or pomegranate mouthwash at 6.25% and 12.5% concentration except for the salivary IL-1 β in group 1, the use of chlorhexidine 0.12% didn't show statistically significant

Table 3: Comparisons of biochemical and clinical results between before and after 14days treatment with mouthwash for each group.

Groups		IL-1 β	AST	BOP	GI	PLI
Group 1 (n=15)	Before (mean \pm SD)	35.44 \pm 4.68	41.86 \pm 9.00	53.5 \pm 11.16	1.64 \pm 0.252	1.88 \pm 0.21
	After 14D (mean \pm SD)	33.04 \pm 3.46	34.07 \pm 7.86	32.1 \pm 8.874	1.25 \pm 0.391	1.13 \pm 0.31
	P value	0.122	0.017*	0.00**	0.003**	0.00**
Group 2 (n=15)	Before (mean \pm SD)	36.22 \pm 2.808	40.32 \pm 9.189	54.88 \pm 9.466	1.55 \pm 0.201	1.78 \pm 0.372
	After 14D (mean \pm SD)	29.62 \pm 8.43	29.01 \pm 6.55	35.4 \pm 10.39	1.38 \pm 0.257	1.41 \pm 0.38
	P value	0.007**	0.00**	0.00**	0.05*	0.01*
Group 3 (n=15)	Before (mean \pm SD)	32.15 \pm 6.14	40.11 \pm 11.25	55.92 \pm 8.35	1.58 \pm 0.315	1.60 \pm 0.181
	After 14D (mean \pm SD)	21.82 \pm 8.0	28.39 \pm 8.44	26.67 \pm 6.72	1.13 \pm 0.153	0.97 \pm 0.38
	P value	0.001**	0.001**	0.001**	0.001**	0.001**

(*) significant difference at p value ≤ 0.05 , (**) highly significant difference at p value < 0.01 .

reduction comparing with pretreatment levels.

The inter comparison of results between groups 1, 2 and 3 regarding the biochemical and clinical parameters are shown in Table 4. There was no statistically significant difference between group 2 (POM 6.25%) and group 1 (CHX) regarding all biochemical and clinical parameters after 14 days treatment except for PLI results, in which group 2 was not as effective as group 1 in reducing PLI and was statistically significant at p value ≤ 0.05 . There was more reduction regarding all biochemical and clinical measurements' after 14 days treatment in group 3 (POM 12.5%) but

statistically was not significant when compared to group 1 except for IL-1 β results, group 3 achieved more reduction and it was statistically significant.

The comparison of results after 14 days treatment with pomegranate extract mouthwash at (6.25%) concentration used by group 2 and (12.5%) concentration used by group 3 showed statistically significant differences regarding the reduction of all the biochemical and clinical parameters after the treatment period except for the mean value of salivary AST levels there were no statistical differences between the two groups as shown in Table 5.

Table 4. Comparisons of biochemical and clinical results between the groups.

Groups	IL-1 β		AST		BOP		GI		PLI	
	Mean diff.	P value	Mean diff.	P value	Mean diff.	P value	Mean diff.	P value	Mean diff.	P value
Group1 vs. Group2	3.426	0.15	5.061	0.06	-3.26	0.36	-0.127	0.30	-0.275	0.03*
Group1 vs. Group3	11.22	0.00**	5.677	0.06	5.467	0.06	0.124	0.26	0.165	0.20
Group2 vs. Group3	7.794	0.01*	0.616	0.82	8.727	0.01*	0.251	0.003**	0.44	0.003*

(*) significant difference at p value ≤ 0.05 , (**) highly significant difference at p value < 0.01 .

Table 5. Comparisons of results between the two concentrations of pomegranate mouthwash.

Parameters	Group 2 (POM 6.25%) mean \pm SD	Group 3 (POM 12.5%) mean \pm SD	t-test	p-value
IL-1 β	29.62 \pm 8.430	21.826 \pm 8.003	2.596	0.01*
AST	29.013 \pm 6.554	28.397 \pm 8.447	0.223	0.825
BOP	35.4 \pm 10.394	26.673 \pm 6.725	2.73	0.01*
GI	1.385 \pm 0.257	1.134 \pm 0.153	3.236	0.003**
PLI	1.412 \pm 0.383	0.972 \pm 0.380	-3.15	0.003**

(*) significant difference at p value ≤ 0.05 , (**) highly significant difference at p value < 0.01 .

Discussion

The effectiveness of natural remedies as pomegranate and its extracts as an alternative for the prevention and treatment of periodontal disease need to be explored extensively and it is important to specify their efficacy at the safest concentrations. Pomegranate has been used for hundreds of years in folk medicine, and its pharmacological activity has been evaluated especially for its anti-inflammatory,²² antioxidant,²³ antimicrobial^{24,25} and anti-cariogenic properties.²⁶ This study is a randomized clinical trial that compared the effectiveness and the side effects of two different concentrations (6.25% and 12.5%) of pomegranate peel extract, as a mouthwash, with each other and with the standard chlorhexidine mouthwash (0.12%) in treating gingivitis in type 2 diabetic

patients through the determination of the biochemical levels of IL-1 β and AST enzyme in saliva and the measurement of clinical periodontal parameters (BOP, GI and PLI) all these parameters were measured to assess the inflammation of gingiva and the response to the intervention treatment. Although chlorhexidine has been regarded as the standard for the control of gingivitis and periodontal disease in dentistry due to its proven effectiveness as an antibacterial and antiplaque agent^{27,28} but it is unpleasant taste and staining effect on the teeth are the main side effects that urges the search for new alternatives.²⁹ The findings of this clinical study reported that there were no side effects associated with the use of pomegranate peel extract as a mouthwash at both concentrations and both were totally well tolerated by all the patients

and statistically there was a highly significant differences when compared to the total side effects that associated with the use of chlorhexidine as shown in Table 2. Only one patient reported unpleasant taste on using the 12.5% concentration while the use of chlorhexidine (0.12%) mouthwash was associated with its common unpleasant side effects of irritation, bad taste and tooth stain as shown in Table 1. Similar to our results a study done by Prakash et al 2017 showed that using pomegranate seed extract as a gel for gingivitis experimental model did not show any side effects over 14 days while chlorhexidine 1% gel caused metallic taste in the mouth.³⁰ Also, Rahimabadi study indicated that the use of pomegranate flower extract as mouthwash for 4 weeks did not cause tooth stain while chlorhexidine did.³¹ Comparing with the pretreatment levels, the results of this study indicates that the use of pomegranate peel extract, as a mouthwash, at both concentrations (6.25% and 12.5%) were significantly effective in lowering all the clinical (BOP, GI and PLI) and the salivary biochemical parameters (IL-1 β and AST) related to gingivitis in T2DM after 14 days treatment. A study done by Rahimabadi et al 2017 showed that the use of pomegranate flower extract (mixed with vinegar) or using chlorhexidine 0.12% as mouth wash once daily over 2 weeks was significantly effective in reducing the clinical parameters in diabetic gingivitis (PLI, GBI and MGI) compared to pretreatment levels³¹ but no laboratory investigation was done by Rahimabadi or other studies to assess the response of gingivitis to therapy by pomegranate peel extracts. As many previous studies that showed the effectiveness of using chlorhexidine in reducing the clinical indices of gingivitis in patients with or without diabetes,³¹⁻³³ also the results of our study indicate that the mouth wash with CHX (0.12%) was effective in reducing all the measured clinical parameters and the salivary AST levels after 14days treatment and it was statistically significant (p value <0.01). but with no statistical significant reduction on the pro inflammatory mediator IL-1 β levels, this could be due to that chlorhexidine doesn't have a direct anti-inflammatory activity to reduce IL-1 β , that

is released in saliva due to inflamed gingiva, also it is well known that CHX itself has the side effect of being irritant to the tissues which may elicit inflammation even at therapeutic concentrations and only through its antibacterial activity CHX may modify the inflammatory process of gingiva.^{34,35} Comparing POM (6.25%) concentration with the standard CHX (0.12%), both mouthwashes showed similar effects on reducing the clinical and biochemical markers of gingivitis and there were no significant differences between the two groups except that CHX showed superior activity over POM (6.25%) in reducing the plaque index (at p value ≤ 0.05). While POM (12.5%) mouthwash showed superior activity to CHX in lowering all the clinical and biochemical parameters of gingivitis but statistically it was not that significant difference, and POM(12.5%) showed a highly significant reduction on salivary IL-1 β than CHX this is mainly due to the combination of anti-inflammatory, anti-oxidant and antibacterial activity of polyphenol rich constituent of pomegranate peel extract which is mainly attributed to the peel contents of anthocyanins and hydrolysable tannins.^{15,22,23} Similar to our results a study done by Sastrava 2005 on patients with periodontitis but they were not diabetics found that there was statistically significant reduction in the levels of IL-1 β in gingival crevicular fluid (GCF) after 3months of using herbal biodegradable chips of punica granatum extracts.³⁶ Prakash et al 2017 showed that the use of chlorhexidine (1%) gel for experimental model gingivitis increased the levels of IL-1 β in GCF after 2 weeks treatment while the topical application of 10% punica seed extract as a gel produced less elevation in the levels of IL-1 β 30. The results of Turkoglu 2009 study after using chlorhexidine mouthwash over 4weeks did not show any reduction in IL-1 β in GCF³⁷. Comparing the two different concentrations of pomegranate mouthwash with each other, the results showed that the higher concentration of POM (12.5%) used in this study has more inhibitory effect, on the salivary IL-1 β and on all the clinical parameters of gingivitis among diabetic patients, and was statistically significant reduction comparing

to POM (6.25%) at p-value (≤ 0.05) which may indicate to a concentration dependent effect of pomegranate peel extract but both concentration had the same effect in reducing AST levels in saliva. Until the time of completing our study, no clinical study was found to compare two concentrations of pomegranate peel extract as a mouthwash for treating gingivitis in T2DM patients. T2DM is considered an important predisposing factor for the development of periodontal disease through alteration of the host inflammatory response to oral biofilm which might exacerbate to gingivitis and if not treated can progress to periodontitis causing loss of tooth³⁸ and although chlorhexidine prescribed as the standard treatment for gingivitis but pomegranate peel extract used in this study showed comparable and even superior effectiveness in treating gingivitis and this is not only due to its antibacterial activity but also it has anti-inflammatory and anti-oxidant activity by which it can decrease the inflammation of gingiva and the pharmacological basis of these actions is the polyphenolic rich constituents of the peel extract which has a potent inhibitory effects on cyclo-oxygenase (COX1 and COX2) enzymes activity and the immunomodulatory action over IL-1 β that result consequently in suppressing the production of prostaglandine E2 (PGE2) and nitrous oxide in a dose dependent manner and by all these mechanisms pomegranate extract can result in alleviation of inflammation^{11,22,39,40} especially when pomegranate extract used at the higher concentration.

Conclusion

This clinical study showed that the use of Pomegranate peel extract as a mouthwash was effective at both low (6.25%) and high (12.5%) concentrations on lowering all the clinical and the biochemical markers related to gingivitis among diabetic patients, and the higher concentration showed more activity over all the studied parameters. Within the limitations of our study the use of high concentration of pomegranate peel extract as a mouthwash could be safe, with no considerable side effects and have similar and even superior activity compared to the conventional chlorhexidine therapy

and might replace it in the future. However, further clinical studies are required to be conducted over large number of subjects and for longer durations for treating other periodontal diseases.

Conflicts of interest

The authors reported no conflict of interests.

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