Anti-Plaque and Anti-Inflammatory Effects of Prepared Punica Granatum Mouthwash on Patients with Moderate Gingivitis

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Abstract

Objective: Punica granatum is a herbal product used in the field of dentistry which has many potential effects including bactericidal, antifungal, antiviral, and immune modulation. This study aimed to evaluate the effect of prepared Punica granatum as an antiplaque and anti-inflammatory agent in the treatment of gingival inflammation.

Methods: Sixty patients were enrolled in this study. Fourty five patients with moderate gingivitis were divided randomly into 3 groups; chlorhexidine group included 15 patients received once-daily dose of chlorhexidine mouthwash for 1 day, Punica I group included 15 patients received once-daily dose of Punica mouthwash for 1 day, and Punica II group included 15 patients received once-daily dose of Punica mouthwash for 2 consecutive days. Plaque index, gingival index, and bleeding on probing were assessed before rinsing and after 3 and 7 days of rinsing, also saliva were collected before rinsing and after 1 hour, 2 hrs, 1 and three days of rinsing to estimate the level of interleukine-1β. The rest 15 subjects were with clinically healthy gingiva.

Results: Punica II group resulted in significant reduction of plaque index, gingival index, and bleeding on probing after 3 and 7 days of rinsing ($p \leq 0.05$) but with non-significant differences with chlorhexidine group ($p > 0.05$). It had significant effect in reduction of interleukine-1β which begin after one day of rinsing and continued into three days ($p \leq 0.05$).

Conclusions: Punica like chlorhexidine has a positive effect on reduction of plaque accumulation and improvement of gingival inflammation so can be used as an anti-plaque and anti-inflammatory agent in the treatment of gingival inflammation.

Keywords: *Punica Granatum, Gingivitis, Chlorhexidine, Mouthwash, Interleukine-1β.*

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Introduction

Gingivitis is a chronic inflammatory disease limited to gingiva without either attachment or alveolar bone loss, bacterial plaque is the primary etiological factor in gingival inflammation[1]. The relationship between bacterial plaque and gingival inflammation has frequently been postulated as the cause of gingivitis and its role in the etiology has been confirmed in experimental gingivitis studies on human beings[2]. The host immune system is the barrier against infectious microorganisms that affect the oral cavity[3] and the complex network of cytokines that intervene in the immune response of the host against external attacks include proinflammatory cytokines, anti-inflammatory cytokines and receptor cells for these cytokines[4]. Interleukin-1β (IL-1β) is a key pro-inflammatory cytokine that released after infection, injury, or antigenic challenge, and can serve as a biomarker of periodontal disease[5]. Plaque control is the primary preventive measure to prevent the onset of gingivitis and arrest its progression into periodontitis[6]. Although mechanical plaque control methods are efficient in maintaining adequate levels of oral hygiene, studies have shown that patient compliance in following these methods are not adequate in a large population and in order to overcome the shortcomings of mechanical plaque control methods, various chemotherapeutic agents have been employed and developed to improve the efficacy of daily oral hygiene[6]. Chemotherapeutic agents including systemic antibiotics, antiseptic mouthwashes, local drug delivery of antiseptics and antibiotics, host modulating agents have been used as an adjunct to the conventional periodontal therapy[6,7]. Chlorhexidine (CHX) mouthwash is regarded as the 'gold standard' antiplaque treatment and is particularly effective against gingivitis and this is widely used as an adjunct treatment for periodontitis, however, most practitioners do not recommend the long-term and daily use of CHX as a mouth wash and this mainly because of its side effects, such as objectionable taste, tooth discoloration, desquamation, and soreness of oral mucosa[8]. Furthermore, synthetic antimicrobial agents and antibiotics are known to cause antimicrobial resistance, emergence of previously uncommon infections probably due to the inappropriate or widespread overuse of antimicrobials, so natural phytochemicals have proved to be good alternatives to such synthetic agents[9]. Natural products like Curcuma zedoaria, calendula, aloe vera and other herbs have been used for the same purposes[6]. Herbs are nature's gift to humans, they have various bioactive components which possess enormous medicinal value with least side effects, and Punica Granatum is an effective herbal extract with broad spectrum of action as bactericidal, antifungal, anthelmintic potential and being able to modulate the immune response[10]. In an urge of looking for better antiplaque, anti-gingivitis and anti-inflammatory agents with limited side effects as compared with chlorhexidine, herbal products have been tried with fruitful results, so this study aimed to evaluate the effect of hydro alcoholic extract from Punica Granatum fruits as anti-plaque and an anti-inflammatory agent in the treatment of moderate gingivitis throughout [1] investigate its effect clinically on plaque index, gingival index,and bleeding on probing and compared it with chlorhexidine digluconate [2] investigate its effect on pro-inflammatory cytokine (IL-1β)and compared it with chlorhexidine digluconate.

Patients and methods

Setting and time

The present study was carried out at Hawler Medical University / College of Dentistry at the periodontic, oral diagnosis, and clinical biochemistry laboratories of basic science departments in Erbil city. The study period started from January 2016 to July 2017.

Patients grouping

This comparative clinical study was conducted on 60 patients (30 male and 30 female) attending the periodontic department between 9-10 a.m.with the age (20-35) years old, and all the subjects were systemically healthy with no history of clinical periodontal treatment or drug intake for the last 6 months, no smoker, no alcoholic, and no pregnant women. Fourty five patients with moderate gingivitis (patients with mean gingival index scores of 1.1-2) were divided randomly into three main groups; chlorehexidine (CHX) group consist of 15 patients received once daily dose of CHX (0.12%) as a mouth wash for 1day only, Punica I group consist of 15 patients received once daily dose of prepared Punica Granatum extract as a mouth wash for 1 day only and finally Punica II group included

15 patients who received once daily dose of prepared Punica granatum extract as a mouth wash for 2 consecutive days. In all three groups the doses of mouthwash were 10 ml and used for only one minute, and the patients refrained from mechanical debridement during seven days of the study and also refrained from eating, rinsing or drinking for at least 2-3 hrs after mouth rinsing. The last 15 patients with no any clinical sign of gingival inflammation, they do not receive any mouthwash, and just saliva was collected from them to investigate the normal mean value of salivary IL-1 β.

Intraoral clinical examination

Was performed for all patients with moderate gingivitis by two specialized periodontists. The intra oral clinical examination include; measurement the thickness of plaque according to plaque index PLI (Silness and Löe)[11] by using a straight sharp explorer and measure the amount of bacterial plaque for four surfaces of all the examined teeth and given a score from 0-3, a PLI grade 0 indicates no plaque, grade 2 indicate thin layer of plaque at the gingival margin which only detectable by scraping with a probe, grade 2 indicates moderate layer of plaque along gingival margin which visible by naked eye, grade 3 indicate abundant plaque along gingival margin and interdental spaces filled with plaque, while measurement the extent and severity of gingival inflammation according gingival index GI by (Löe and Silness)[12] and (Löe)[13], inspection by naked eyes and by gentle probing through using periodontal probe for four gingival surfaces of the examined tooth (facial, lingual, mesial, and distal) and given a score from 0-3, grade 0 indicate normal gingiva, grade 1 indicate mild gingival inflammation, grade 2 indicates moderate gingival inflammation, and grade 3 indicates sever gingival inflammation, then the scores of the 4 areas of the tooth was totaled and divided by four to give a tooth score, then by adding the tooth scores together and dividing by the number of teeth examined and individual's GI score was obtained[12,13,14].

An individual mean GI score of 0.1-1.0 indicates mild gingival inflammation, 1.1-2.0 indicates moderate gingival inflammation, and 2.1 to 3 indicates severe gingival inflammation[12,13,14] and finally measurement bleeding on probing BOP according to (Ainamo and Bay,1975)[15] by using Wiliams periodontal probe and pass it gently along the inner surface wall of gingival sulcus and bleeding was noted after 30s as (-) absent or (+) present of bleeding. The selected teeth for clinical examination were Ramforjord's index teeth (16, 21, 24, 36, 41 and 44), all those clinical parameters were assessed before mouth rinse at baseline and after 3 and 7 days of rinsing with chlorhexidine or Punica I and II.

Immunological investigation

This includes quantitative measurements of salivary IL-1β in patients with normal healthy gingiva, and in the three main groups of patients with moderate gingivitis which collected at baseline before mouth rinsing and after 1 hour, 2 hrs, 1 and 3 days of rinsing with Punica (I or II) or chlorhexidine, by using enzyme-linked immunosorbent assay (Elisa technique) supplied by specific kit (KOMA BIOTECH INC, USA).

Saliva sample collection

five ml of un-stimulated saliva samples were collected from all patients at 9-10 a.m. to minimize the effect of diurnal variation on flow and composition, spitting method was used for collection of un-stimulated saliva for patients with moderate gingivitis at base line before rinsing and after 1 hour (1H), 2 hrs (2H), 1 day (1D), 3 days (3D) of rinsing and for patients with healthy gingiva. The samples were collected in sterilized plastic test tubes, then centrifuged for 20 minutes at 4000 rpm to obtain clear supernatant, and finally stored at -20ºc for later estimation of IL-1 β.

Preparation of Punica Granatum extract mouthwash

The Punica Granatum fruit was obtained from Heran at the north of Erbil city. The hydroalcoholic extract (HAE) was prepared from whole fresh fruits (4-5 fruits) that were cut into small pieces and then the small pieces blended with equivalent volume mixture of ethanol and distilled water (1:1, v/v). Then the material was filtered through several layers of gauze and evaporated at 60ºc to one-third of its original volume (to allow ethanol to be

evaporated), then the volume was restored with distilled water according to the required volume of the sample and then 1 ml of the sample was completely evaporated in the oven[1], then the prepared Punica juice poured into dark plastic bottles and given to the patients who directed to the method of using Punica granatum as a mouthwash.

Statistical analysis

The SPSS (version 19) was used; paired T-test and F-test (one-way ANOVA) were used for the study groups, P value ≤ 0.05was regarded as statistically significant.

Results

A total of 60 subjects comprised of 30 females and 30 males with an age of 20-35 years were enrolled in this study, and the mean ages for female and male were 25.5±3.64 and 24.43±3.54 years, respectively.

Clinical results

1- Plaque index: Table 1, revealed a highly significant reduction in the mean value of plaque score in chlorhexidine group after 3 and 7 days of rinsing as compared to its mean value at the baseline before rinsing (P≤0.05). The mean decrease in the mean value of plaque score in CHX group was from (1.886±0.331) at base line to (1.265±0.072) at 3 days and (0.995±0.175) at 7 days and for Punica I group, non significant difference in the mean value of plaque score was seen after 3 and 7 days of rinsing (1.120±0.440) (1.095±0.433) respectively as compared to its mean value at base line (1.210±0.529) before rinsing (P >0.05), while for Punica II group, the result showed a highly significant reduction in the mean values of plaque score after 3 and 7 days of rinsing (1.096±0.315) (1.055±0.283) respectively as compared to its mean value before rinsing (1.726±0.370) (P≤0.05). For comparison between CHX and Punica groups in relation to their effect on plaque index score after 3 and 7 days of rinsing, non-significant differences were found between chlorhexidine and Punica I, and between chlorhexidine and Punica II groups (P >0.05), as showed in Table 4, also non-significant differences were found between the different 3 main groups in relation to their effect on reduction of plaque after 3 and 7 days of rinsing) (P >0.05), as shown in Table 5.

2- Gingival index: Table 2, shows that in CHX group a non-significant difference in the mean value of GI after 3 and 7 days of rinsing (1.341±0.149) (0.975±0.050) respectively when compared to its mean value at base line before rinsing (0.931±1.075) (P>0.05) and for Punica I group, the result showed a significant reduction in the mean value of GI after 3 and 7 days of rinsing (1.243±0.237) (1.215±0.215) respectively as compared to its mean value at base line before rinsing (1.600±0.391) (P≤0.05), while for Punica II group, the result showed a highly significant reduction in the mean values of GI after 3 and 7 days of rinsing (1.199±0.122) (1.150±0.173) respectively as compared to its mean value at base line before rinsing (1.556±0.173) (P≤0.05). Table 4 showed a comparison between CHX and Punica groups about their effect on the gingival index after 3, and seven days of rinsing, which revealed non-significant differences between CHX and Punica I and also between CHX and Punica II groups (P>0.05). Also, non-significant differences were found between the different three main groups about their effect on reduction of gingival inflammation after 3 and seven days of rinsing (P>0.05), as shown in Table 5.

3- Bleeding on probing: Table 3, showed that in in CHX group a significant reduction in the mean value of BOP from (0.773± 0.144) at base line to (0.262±0.262) after 3 days of chlorehexidine rinsing and this reduction continued highly with no bleeding on probing after 7 days of rinsing (P≤0.05), and for Punica I group, the result showed a significant reduction in the mean value of BOP after 3 and 7 days of rinsing (0.223±0.382) (0.223±0.382), respectively as compared to its mean value at base line before rinsing (0.583±0.382) (P≤0.05), while for Punica II group, the result showed a highly significant reduction in the mean values of BOP after 3 and 7 days of rinsing (0.186 ± 0.143) (0.186± 0.143) respectively as compared to its mean value at base line before rinsing (0.807± 0.016) (P≤0.05). For comparison between CHX and Punica groups about their effect on reduction of bleeding on probing, the results showed non-significant differences between CHX and Punica I, and between CHX and Punica II groups after 3 and seven days of rinsing (P>0.05) as shown in Table 4. Also, non-significant differences were found between the three different main groups about their effect on bleeding on

probing after 3 and 7 days of rinsing (P>0.05) as shown in Table 3.

Immunological results

Table 6, showed the comparison between the main value of salivary IL-1β at base line before rinsing and after different times of rinsing in CHX and Punica I or II groups by using paired sample T-test, and revealed that in CHX group the main value of IL-1β was highly decreasing after 1 hour of rinsing (10.48±3.10) and significant difference was found after 1 hr of rinsing as compared to its mean value at base line before rinsing (14.70±5.27) (P≤0.05), then slight increase in non-significant difference after 2 hrs of rinsing (13.93±2.37) (P>0.05), then highly decrease with significant difference after 1 day of rinsing (9.95±2.79) (P≤0.05) and non-significant difference after 3 days of rinsing (15.07±8.94) as compared to its mean value at base line before rinsing (P>0.05), while in Punica I group, non-significant difference was found after 1 hr of rinsing (22.67±3.83) as compared to its mean value at base line before rinsing (18.20±0.17) (P>0.05), and then significantly decrease with significant differences were found after 2 hrs of rinsing (9.70±6.45) (P≤0.05), then non-significant differences were found after 1 and 3 days of rinsing (14.50±12.30) (16.93±8.40) respectively when compared to its mean value at base line before rinsing (18.20±0.17) (P>0.05), finally in Punica II group non-significant difference was found after 1 hr of rinsing (15.87±3.84) as compared to its mean value at base line before rinsing (17.07±2.10) (P>0.05) then highly increase with significant differences after 2 hrs of rinsing (24.67±7.72) (P≤0.05) and then significantly decrease after 1 and 3 days of rinsing (13.23±3.87) (13.10±0.81) respectively with significant differences as compared to its value at base line before rinsing (17.07±2.10) (P≤0.05). Table 7, showed the comparison between CHX and Punica groups (I and II) in relation to IL-1 β after 1h, 2hrs,1 and 3 days of rinsing , the results showed significant differences between CHX and Punica II after 1hr and 2hrs of rinsing (P≤0.05), non-significant differences between CHX and PunicaI after 1hr of rinsing, (P>0.05), while significant differences were found between CHX and Punica I after 2hrs of rinsing (P≤0.05), finally non-significant differences were found between chlohexidine and Punica 1 or II after 1and 3 days of rinsing (P>0.05). Table 8, showed that there were significant differences between the 3 main groups after 1 hr and 2hrs of rinsing (P≤0.05) and non-significant differences were found after 1 and 3 days of rinsing (P>0.05).

Discussion

Over the decades, very few studies have been conducted to show the clinical efficacy of Punica granatum on PLI, GI, and BOP [16,17,18,19,20,21] and also on IL-1 β[22,23,24,25,26], so clinically in our study it has been demonstrated that hydroalcoholic extract from Punica granatum fruit exerted a significant reduction in clinical parameters (PLI, GI, and BOP) and acted as anti-inflammatory agent throughout its great anti-bacterial plaque activity and its significant effect on the reduction of pro-inflammatory cytokine IL-1 β for long duration reach to 3 days after rising when has been used as mouth wash once daily for two days in Punica II group. According to plaque index values, our study revealed that Punica II group had a significant effect in reduction of plaque index scores to a great extent after 3 and 7 days of rinsing as CHX group, indicating its strong anti-plaque effects despite the usage of Punica once time daily for two consecutive days, while Punica I group had no significant effect on reduction of plaque index after 3 and 7 days, this may be due to short usage of Punica I, once time daily for one day. In contrary to our results, another study[16,17,18] found that chlorhexidine reduced plaque to a great extent as compared with Punica granatum mouthwash, the differences in the results may be because of difference in the method employed and the difference in the duration of the study, another microbiological study[19] among 60 healthy patients showed that after 1 min of mouth rinsing, more reduction in plaque was observed with Punica granatum as compared with chlorhexidine and concluded that Punica granatum might be a possible alternate for the prevention of formation and treatment of dental plaque, the differences in the results may be because of difference in the methodology employed, i.e., instead of plaque index, colony-forming units were taken into consideration, another reason could be that the duration of their study was different and very short, also another study[20] on thirty periodontally healthy volunteers which randomly divided into three groups and refrained from mechanical oral hygiene measures for 4 days and they used one of the randomly assigned mouthrinses; pomegranate,

chlorhexidine and distilled water twice daily, then plaque index was assessed at days 0 and 5 to evaluate the effect of a pomegranate mouth rinse on plaque and to evaluate its antibacterial

Table 1: comparison between the mean value of PLI at the baseline before rinsing and after 3 and 7 days of rinsing in Chlorhexidine, punica I, and punica II groups.

Index	Groups	Time	Mean± Std. deviation	N	t-test	df	P-Value
PL I	CHX	before 3D 7D	1.886±0.331 1.265±0.072 0.995±0.175	15 15 15			
					3.208	3	0.049(S)
					6.788	3	0.007(HS)
	Punica I	before 3D 7D	1.210±0.529 1.120±0.440 1.095±0.433	15 15 15			
					0.996	3	0.393(NS)
					1.289	3	0.288(NS)
	Punica II	before 3D 7D	1.726±0.370 1.096±0.315 1.055±0.283	15 15 15			
					21.300	3	0.000(HS)
					15.428	3	0.001(HS)

S: Significant, HS: Highly significant, NS: Non-significant.

Table 2: Comparison between the mean value of GI before rinsing and after 3 and 7days of rinsing in chlorhexidine, Punica I, and Punica II groups.

Index	Groups	Time	Mean± Std. deviation	N	t-test	df	P-Value
GI	CHX	before 3D 7D	0.931±1.075 1.341±0.149 0.975±0.050	15 15 15			
					-0.858	3	0.454(NS)
					-0.858	3	0.454(NS)
	Punica I	before 3D 7D	1.600±0.391 1.243±0.237 1.215±0.215	15 15 15			
					4.626	3	0.019(S)
					4.372	*3*	0.022(S)
	Punica II	before 3D 7D	1.556±0.173 1.199±0.122 1.150±0.173	15 15 15			
					13.960	3	0.001(HS)
					196.696	3	0.000(HS)

Table 3: Comparison between the mean value of BOP before rinsing and after 3 and 7 days of rinsing in chlorhexidine, Punica I, and Punica II groups.

Index	Groups	Time	Mean± Std. Deviation	N	t-test	df	P-Value
BOP	CHX	before	0.773±0.144	15			
		3D	0.262±0.262	15	3.545	3	0.038(S)
		7D	0.000±0.000	15	10.771	3	0.002 (HS)
	Punica I	before	0.583±0.382	15			
		3D	0.223±0.231	15	4.734	3	0.018(S)
		7D	0.223±0.231	15	4.734	3	0.018(S)
	Punica II	before	0.807±0.016	15			
		3D	0.186±0.143	15	9.129	3	0.003(HS)
		7D	0.186±0.143	15	9.129	3	0.003(HS)

Table 4: Comparison between chlorhexidine and Punica (I and II) groups about PLI, GI and BOP before rinsing and after 3 and 7 days of rinsing.

Index	Times	Dependent Variable groups		Mean Difference (I-J)	P-Value
PLI	before	CHX	Punica I	.676250*	.048(S)
			Punica II	0.160	.602(NS)
	3D	CHX	Punica I	0.145	.531(NS)
			Punica II	0.169	.467(NS)
	7D	CHX	Punica I	-0.100	.664(NS)
			Punica II	-0.060	.794(NS)
GI	before	CHX	Punica I	-0.669	.190(NS)
			Punica II	-0.625	.219(NS)
	3D	CHX	Punica I	0.098	.452(NS)
			Punica II	0.142	.285(NS)
	7D	CHX	Punica I	-0.240	.066(NS)
			Punica II	-0.175	.161(NS)
BOP	before	CHX	Punica I	0.190	.283(NS)
			Punica II	-0.034	.843(NS)
	3D	CHX	Punica I	0.040	.765(NS)
			Punica II	0.076	.567(NS)
	7D	CHX	Punica I	-0.223	.075(NS)
			Punica II	-0.186	.127(NS)

Table 5: Comparison between the three main groups about PLI, GI, and BOP at the baseline before rinsing and after 3 and 7 days of rinsing.

Index	Times	Groups	Sum of Squares	df	Mean Square	F-test (One way ANOVA)	P-Value
PLI	base line	Between groups	.999	2	.500	2.852	.110$^{(NS)}$
		Within groups	1.577	9	.175		
	3D	Between groups	.067	2	.033	.338	.722$^{(NS)}$
		Within groups	.892	9	.099		
	7D	Between groups	.020	2	.010	.102	.904$^{(NS)}$
		Within groups	.895	9	.099		
GI	base line	Between groups	1.119	2	.559	1.254	.331$^{(NS)}$
		Within groups	4.015	9	.446		
	3D	Between groups	.042	2	.021	.678	.532$^{(NS)}$
		Within Groups	.280	9	.031		
	7D	Between Groups	.123	2	.062	2.348	.151$^{(NS)}$
		Within Groups	.236	9	.026		
BOP	base line	Between groups	.117	2	.058	1.053	.388$^{(NS)}$
		Within groups	.500	9	.056		
	3D	Between groups	.012	2	.006	.176	.841$^{(NS)}$
		Within groups	.295	9	.033		
	7D	Between groups	.114	2	.057	2.323	.154$^{(NS)}$
		Within groups	.221	9	.025		

Table 6: Shows the mean value of IL-1 β in normal healthy patients, and the comparison between the mean value of IL-1 β at the baseline before rinsing and after 1 hr, 2 hrs, 1 day and 3 days of rinsing in CHX, Punica and PunicaII groups using 2-sided t-test.

Cytokine	Groups	(I-J) Time	N	Mean± Std. Deviation	Mean Difference (I-J)	Std. Error	P-Value
IL-1β	Normal		15	12.32±2			
	CHX	Base line (I)	15	14.70±5.27			
		1H(J)	15	10.48±3.10	4.21667*	1.87	0.027(S)
		2H(J)	15	13.93±2.37	0.77	1.87	0.683(NS)
		1D(J)	15	9.95±2.79	4.75000*	1.87	0.013(S)
		3D(J)	15	15.07±8.94	-0.37	1.87	0.845(NS)
		Total	75	12.83±5.43			
	Punica I	Base line (I)	15	18.20±0.17			
		1H (J)	15	22.67±3.83	4.46667	2.72	0.106(NS)
		2H(J)	15	9.70±6.45	-8.50000*	2.72	0.003(S)
		1D(J)	15	14.50±12.30	-3.70000	2.72	0.179(NS)
		3D(J)	15	16.93±8.40	-1.26667	2.72	0.643(NS)
		Total	75	16.4±8.43			
	Punica II	Base line (I)	15	17.07±2.10			
		1H(J)	15	15.87±3.84	-1.20000	1.59	0.452(NS)
		2H(J)	15	24.67±7.72	7.60000*	1.59	**0.000**((HS)
		1D(J)	15	13.23±3.87	-3.83333	1.59	0.018(S)
		3D(J)	15	13.10±0.81	-3.96667*	1.59	0.015(S)
		Total	75	16.78±5.99			

Table 7: Comparison between CHX and Punica (I or II) groups about their effect on IL-1 β after 1 h, 2 hrs,1 and 3 days of rinsing (LSD).

Cytokine	Time	Dependent variable groups		Mean Difference (I-J)	Std. Error	P-Value
IL-1β	1H	CHX	Punica I (P)	-12.18333*	1.31750	0.000((HS)
			Punica II (PP)	-5.38333*	1.31750	0.000((HS)
	2H	CHX	Punica I (P)	4.23333	2.17881	0.059(NS)
			Punica II (PP)	-10.73333*	2.17881	0.000((HS)
	1D	CHX	Punica I (P)	-4.55000	2.78123	0.109(NS)
			Punica II (PP)	-3.28333	2.78123	0.244(NS)
	3D	CHX	Punica I (P)	-1.86667	2.59203	0.475(NS)
			Punica II (PP)	1.96667	2.59203	0.452(NS)

Table 8: Comparison between the three main groups (CHX, Punica I and II) about their effect on IL-1 β after the different time of rinsing by using F-test.

Time	Groups	Sum of Squares	df	Mean Square	F	Sig.
1H	Between groups	1118.269	2	559.135	42.949	0.000(HS)
	Within groups	546.775	42	13.018		
	Total	1665.044	44			
2H	Between groups	1785.633	2	892.817	25.076	0.000(HS)
	Within groups	1495.367	42	35.604		
	Total	3281.000	44			
1D	Between groups	165.436	2	82.718	1.426	0.252(NS)
	Within groups	2436.608	42	58.014		
	Total	2602.044	44			
3D	Between groups	110.233	2	55.117	1.094	0.344(NS)
	Within groups	2116.367	42	50.390		
	Total	2226.600	44			

properties against *A. Actinomycetemcomitans, Porphyromonas gingivalis*, and *Prevotella intermedia* in vitro and the results showed that in all 3 groups, PLI was significantly increased from the baseline to day 5 (P = 0.1) with no significant difference between chlorhexidine and pomegranate mouth rinse with respect to the PLI, and pomegranate mouth rinse created no adverse effects on PLI and revealed inhibition of all three strains of periodontal pathogens. In analysis of gingival index, our results revealed that Punica granatum II and I groups were more efficient in reducing of GI index scores to a great extent after 3 and 7 days of rinsing than CHX group indicating their strong anti-gingivitis effect throughout reduction of gingival inflammation, but non-significant differences were found between chlorhexidine and Punica granatum mouthwash group I or II in relation to their effect on reduction of gingival inflammation. In contrary, a study found that both pomegranate and CHX led to significant reduction in gingival scores after 5 and 7 days of rinsing[1], other studies showed that Punica granatum had more significant effect in reducing gingival index score when compared to CHX group, the differences in the result may be due to the difference in the method employed and the difference in the duration of study, also a study[21] found that 10% Punica granatum gel was not efficient in preventing supragingival dental plaque formation and gingival inflammation (gingivitis), in which gel was placed into tooth shield in a non-diluted form; it may be speculated that gel solubilization with saliva would be necessary for its antimicrobial action to take place, while in our study, direct interaction of saliva to Punica granatum led to more acceptable results for reduction of plaque accumulation and gingival inflammation. In analysis of bleeding on probing value, our results revealed that both Punica (I or II group) and chlorhexidine groups were very helpful in reducing bleeding on probing after 3 and 7 days of rinsing, and non-significant differences were found between chlorhexidine and Punica I or II groups in relation to their effect of reduced bleeding on probing which indicate that Punica granatum like CHX have potential significant effect on reduction of bleeding by probing due its strong styptic action[1]. Our study agrees with another study[1], who found that both pomegranate and chlorhexidine led to significant

reduction in bleeding on probing scores after 5 and 7 days of rinsing despite the difference in the method and duration of the study[1]. In contrary to our results studies[16,18] showed that Punica Granatum was more efficient in reducing bleeding on probing than chlorhexidine. In analysis of IL-1β, our results revealed that in Punica II group, Punica had a significant inhibitory effect on salivary IL-1 β which began after 2 hrs of rinsing and continued into even after 1 and 3 days of rinsing, this strong anti-inflammatory effect may be due to its strong effect on reduction of pro-inflammatory IL-1 β production due its immunoregulatory action on macrophages and T-cells while in Punica I group, Punica granatum had a significant effect on the reduction of IL-1 β which began only after 2 hrs of rinsing with non significant difference with CHX group after 1 and 3 days of rinsing and this may be due to the short usage of Punica I once time for 1 day, finally in chlorhexidine group, CHX was very helpful in reducing of IL-1β which began after 1 hr of rinsing as compared to its base line and this reduction continued into even after 1 day after rinsing and had significant differences when compared with Punica II after 1 and 2 hrs of rinsing and pumica I after 1 hr of rinsing indicate its strong anti-inflammatory effect despite its usage once time daily for 1 day. A study revealed that pomegranate fruit extract has a broad inhibitory effect on matrix metalloproteinases expression and IL–1β induced tissue destruction[23], and the anti-inflammatory effect of pomegranate could be due to its immunoregulatory action on macrophages and T and B lymphocytes[22]. Another study revealed that pomegranate extract exhibited anti-inflammatory activity through inhibition of nuclear factor kappa-B activity NF-κB and prevention of Mitogen-activated protein kinase cascades, it also decreased nitric oxide and PGE2 synthesis in human intestinal Caco-2 cells, blocking NF-κB, inflammatory cell signaling pathways that produce various destructive factors may be a potential strategy to prevent inflammation-induced bone resorption and a promising mechanism to treat tissue destruction. Also, another study revealed that pomegranate could produce an anti-gingivitis effect throughout possess direct antioxidant properties and indirect effects by enhancing the free radical scavenging activity of hepatic enzymes catalase, superoxide dismutase, and peroxidase[24].

So the treatment with Punica Granatum can be used due to; easy to prepare, has profound styptic action, and shows acceptable effect throughout significant reduction of plaque index scores, gingival index scores, bleeding on probing scores and IL-1β so can be as an adjunct to mechanical teeth debridement in the treatment of moderate gingivitis. More clinical and immunological studies on a long-term basis are required to know the precise effect of this product.

Conclusions

Punica granatum is a recent herbal product used in field of dentistry, Our study clinically concluded that Punica granatum has the same effect as chlorhexidine with non-significant differences in the reduction of; dental plaque accumulation (antibacterial plaque effect), gingival inflammation (anti-gingivitis effect), and bleeding on probing (profound styptic action). Also, Punica II has an inhibitory effect on pro-inflammatory mediator IL-1 β for long duration reach 3 days after rinsing (anti-inflammatory effect).

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