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EFFECTVENESS OF ADJUNCTIVE APPLICATION OF PUNICA GRANATUM EXTRACT GEL IN THE NON-SURGICAL MANAGEMENT OF STAGE 2 PERIODONTITIS (RANDOMIZED CONTROLLED CLINICAL TRIAL)

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ABSTRACT

BACKGROUND: Mechanical removal of dental plaque is the gold standard treatment approach in stage 2 periodontitis. Local drug delivery as an adjunctive to periodontal pocket debridement is commonly used to eliminate microorganisms and control the inflammatory response of the host.

STUDY OBJECTIVE: To evaluate the effect of adjunctive use of *Punica granatum* extract gel in the non-surgical treatment of stage 2 periodontitis primarily, with respect to clinical attachment loss (CAL), secondarily probing pocket depth (PPD), and bleeding on probing (BOP).

MATERIALS AND METHOD: 32 sites in stage 2 periodontitis patients were included in this randomised controlled clinical trial, divided equally into two groups. Periodontal pocket debridement was used to treat Group I (Test group), along with intra-pocket application of pomegranate extract gel. Periodontal pocket debridement alone was used to treat Group II (Control group). CAL, PPD, and BOP were evaluated for the 2 groups at baseline prior to treatment and at twelve weeks after treatment.

RESULTS: The evaluated parameters demonstrated improvement by the end of the study, compared to baseline, in terms of reducing CAL, PPD, and BOP.

CONCLUSION: Pomegranate extract gel is inexpensive, easy to prepare with no adverse effects, and can improve the clinical parameters of stage 2 periodontitis.

KEYWORDS: Non-surgical management, *Punica granatum*, Stage 2 periodontitis.

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INTRODUCTION

Periodontitis is inflammation of the supporting tissues surrounding the teeth. Dental plaque dysbiosis is the main etiological agent for initiation and progression of the disease, it holds bacterial species whose virulence components cause reversible or irreversible damage of the teeth supporting connective tissue fibers and alveolar bone (1). Gingivitis is a reversible condition upon removal of irritating elements; however, infection may spread to the surrounding periodontal tissues causing periodontitis, which is an irreversible destructive condition (2).

According to Tonetti et al in 2018 (3), periodontitis can be classified into stages and grades. The four *stages* depend on severity and complications of the condition. The three *grades* determine the potential for rapid advancement and projected response to therapy (3).

Adequate diagnosis can determine the extent, progress, and activity of disease which is critical to get the proper treatment approach. There are several diagnostic measurements such as probing pocket depth (PPD), bleeding on probing (BOP), mobility evaluation, plaque index (PI) that can detect the inflammatory condition, and radiographs to locate level of interdental bone. These methods are simple, and inexpensive. However, they could not assess the current disease

condition and display only the history and prognosis of the disease (3).

The presence of pathogenic periodontal species in the gingival crevice causes inflammation to commence (4). An important element in the beginning and progression of periodontal disease is bacterial products which can initiate the inflammatory reaction by stimulating host cells, releasing a sequence of inflammatory mediators, enzymes, and cytokines that are able to destroy host tissues and/or inhibit local host defence function (5).

Periodontitis can be treated using a variety of protocols. Nonsurgical therapy includes mechanical instrumentation alone or in conjunction with antimicrobial therapy or host immune modulation (6). Reduction of bacterial plaque biofilm, subgingival calculus, and bacterial pathogens from root surfaces with periodontal infection is the primary objective of periodontal therapy. Mechanical disinfection is considered the gold standard method to diminish the bacterial density in the area and therefore, reducing the host response resulting in improving the clinical manifestations of inflammation and thereby reducing the CAL, PPD, and BOP (7).

Thus, antimicrobial therapy and systemic host modulation are used as adjuncts to nonsurgical treatment to reduce the number of bacteria and their toxins (8). Although systemic antibiotics can control and eliminate many pathogens, they cause many adverse effects on patients, such as the development of bacterial resistance (9).

Local intra-pocket drug delivery systems can minimize systemic adverse actions. Local medication delivery methods can have a controlled release for prolonged intervals of time. In addition, they supply active substances at high levels at the aimed site. Nevertheless, the use of local antibiotic did not get rid of resistance to antimicrobials (10). Thus, other therapies were developed as an alternative to antibiotics to minimize adverse actions.

Plants have phytochemicals that have pharmaceutical action against pathogens, collagenase, and inflammation. They can also act as antioxidants, and exhibit antiseptic features (11).
Natural herbs are considered a long-standing source of medicines, and nearly half of all medications come from natural herbs (12). Oriental remedies have been tested for their influence on diseases of the periodontium, they have an impact on periodontal tissue regeneration, antimicrobial, and anti-inflammatory properties (13).

These materials include Punica granatum that is known as pomegranate (14). Pomegranate has antibacterial, anti-inflammatory, antioxidant, anti-fungal, and anti-carcinogenic effects (15). It has been used to prevent and treat bacterial infections, diabetes, erectile dysfunction, cardiovascular disease, and cancer (16).

Only a few studies have demonstrated the beneficial impact of application of pomegranate locally on periodontal conditions (14,16). In comparison to chlorhexidine and ornidazole gel, pomegranate gel showed better anti-inflammatory and anti-gingivitis benefits (17). Since pomegranate extract gel is risk-free, non-invasive, and has no side effects, it has been found to substitute synthetic agents.

The aim of the study was primarily to evaluate CAL, and secondarily to evaluate PPD, and BOP in stage 2 periodontitis after the local application of pomegranate extract gel.
The null hypothesis of this study was that there will be no change in the before mentioned variables in periodontitis treated by periodontal pocket debridement and *Punica granatum* gel in comparison to that by periodontal pocket debridement alone.

MATERIALS AND METHODS

I. Materials

This research was accepted via the Research Ethics Committee of the faculty of Dentistry Alexandria university (IRB NO:00010556 - IORG 0008839). It also followed the principles of modified Helsinki guideline for human clinical studies (2013) (18) and CONSORT 2010 reporting standards for clinical trials (19).

Sample size

This study was a RCCT conducted from August 2021 to December 2021 in the department of Periodontology, Oral Medicine, Diagnosis and Oral Radiology Faculty of Dentistry, Alexandria University. 32 sites from stage 2 periodontitis patients were chosen for the study (number of groups=2). All patients approved to contribute in the clinical trial and a signed agreement was obtained.

Sample size was calculated assuming 5% alpha error and 80% study power. Sastravaha et al (17). reported mean difference ± standard deviation (SD) = 13.88 ± 8.87, when *Punica granatum* extract gel was used, and 76.21 ± 38.87 when scaling and root planning was used alone (20). Based on comparison of means, the minimum sample size was calculated to be 7 increased to 8 to make up for loss to follow up and for 2 groups = 16. The total sample size for stratification by arch type (maxillary and mandibular) =32 (20).

i. Study design

This study had a CONSORT-compliant randomized controlled design (19).

The PICO questions: were patients with stage 2 (P) treated non-surgically together with the adjunctive application of pomegranate extract gel (I), compared to those treated with non-surgical management alone (C), showed the same improvement in CAL, PPD, and BOP (O)?

Thirty-two sites from stage 2 periodontitis patients having an age range from 30-60 years old were divided equally into two groups. Group I (test

group): were treated with periodontal pocket debridement and intra-pocket pomegranate extract gel. Group II (control group): Only periodontal pocket debridement was used.

Inclusion criteria: Patients with stage 2 periodontitis of both sexes were included, with 3-4mm clinical attachment loss, 3-5 mm PPD, and horizontal bone loss not exceeding the coronal third as seen on radiographic X-rays (21).

Exclusion criteria: Patients who had previously experienced a negative impact from the products (or comparable products) used in this trial were excluded, as were those with Grade C periodontitis, which had a quick rate of development.

a. *Punica granatum* (pomegranate) gel

Preparation of the extract and the gel was at the Department of Pharmacognosy; faculty of pharmacy, Alexandria University.

The seeds of fresh pomegranates were extracted, sorted, and finely ground using an electric grinder. Filtering the obtained juice by direct percolation through a glass funnel* using filter paper to achieve clear pomegranate concentrated extract. At this point, the gel was prepared by dissolving 5 g of carboxymethyl cellulose in 100 ml of pomegranate extract and mixing it gently for 15 mins and heated until a gel of consistency convenient for usage, as the orabase gel, is obtained. As a preservative, very little methyl paraben (2 mg) was applied (22).

II. Methods

Grouping and Randomization

Using a computer-generated list of random numbers, the selected thirty-two sites were distributed at random and divided into two groups (19):

Group I: 16 patients were treated with periodontal pocket debridement and intra-pocket application of pomegranate gel

Group II: 16 patients were treated with periodontal pocket debridement alone.

Non-surgical Phase

Patients in both groups completed a thorough medical and dental history prior to start the therapy.

BOP was recorded 15 seconds after probing from the selected sites. Score 0 = normal gingiva, score 1 = mild inflammation (slight change in color with no BOP), score 2 = moderate inflammation (redness, edema, glazing, and BOP), and Score 3 = severe inflammation (marked redness, edema, and tendency to bleed spontaneously) (23).

*Boro 3.3 Glass from HEQI GLASS company, Shanghai, China

PPD was measured from the free gingival margin to the deepest point of the pocket at the chosen sites by using Michigan-O Probe (24). CAL was measured from cementoenamel junction to the deepest point of the sulcus (24). Figure (1) Measurements were made at six points on each tooth: mesio-buccal, disto-buccal, mid-buccal, mesio-palatal/lingual, mid-palatal, and disto-palatal.

Patients were given oral hygiene instructions, which included twice-daily teeth brushing with the appropriate technique.

To eliminate supra- and subgingival calculus, scaling was done using an ultrasonic scaler*. Subgingival curettage of internal pocket wall and root planning was performed to remove necrotic cementum by universal curettes**.

If there was occlusal trauma, coronoplasty was performed.

Pomegranate gel was injected using a syringe with a curved, blunt-end needle that had a diameter of 0.9 mm. In an effort to completely fill the periodontal pocket, the gel was gently probed into the test sites and the needle was delicately pushed into the pocket. Up to the gingival margin, the gel was administered, and any extra gel was dabbed away with sterile gauze. Figure (2)

After the gel was applied in place, patients were told to practice careful dental hygiene and avoid chewing anything sticky or hard for the next few days. After 12 weeks, The BOP, PPD and CAL were recorded at the selected sites by using Michigan-O Probe (24). Figure (3)

*Woodpecker Company, China

**Kohler. Medizintechnik GmbH & Co. KG, Dorsten, Germany

Statistical Analysis

With the help of descriptive statistics, graphs, and normality tests, the normality of all the variables was examined. For all quantitative variables, means and standard deviation (SD) were determined, whereas for qualitative variables, frequencies and percentages were calculated. Comparisons between the two study groups were done using independent samples Mann-Whitney U test and t-test depending on the normality of the variable.

Comparisons between different timepoints within each group were done using paired t-test. Significance was set at p value <0.05. Data were analyzed by IBM SPSS for Windows (Version 23.0).

Figure (1): Probing pocket depth at baseline **(A)** study group **(B)** control group

Figure (2): Application of pomegranate extract gel intra-pocket in study group

Figure (3): Probing pocket depth 12 weeks **(A)** study group **(B)** control group

RESULTS

In the present study, table (1) and figure (4) represent the change in CAL in both groups. There was no significant difference in average CAL between both groups at baseline (P =0.41). Regarding the inter group comparisons, both groups showed a significant decrease in average CAL after 12 weeks compared to baseline values, (P <0.001 and P =0.001 in the test and control groups, respectively) By the end of study, CAL decreased from (3.28±0.45mm) to (2.67±0.31mm) in the test group, and from (3.37±0.44mm) to (3.00±0.35mm) in the control group. After 12 weeks, the test group showed significantly lower average CAL than the control group (difference= -0.33±0.12, P= 0.049).

Table (2), and Figure (5) represent PPD in both groups over time. There was no significant difference in average PPD between both groups at baseline (P =0.54). Regarding the inter group comparisons, both groups showed a significant decrease in PPD after 12 weeks. Compared to baseline values, (P <0.001 and P =0.001 in the test and control groups, respectively) By the end of study, PPD decreased from (3.93±0.58mm) to (2.88±0.35mm) in the test group, and from (3.83±0.43mm) to (3.30±0.54mm) in the control group. Also, at 12 weeks the test group showed significantly lower PPD than the control group (difference=-0.42±0.92, P= 0.04).

Regarding BOP, there were no significant differences between both groups at baseline (P=1.00), and after 12 weeks (P=0.13). However, intragroup comparisons revealed that both groups showed a significant decrease in BOP after 12 weeks compared to baseline scores values (P <0.001 and P =0.001 in the test and control groups, respectively). At baseline, all cases in both groups showed score 3 BOP, while at 12 weeks 60% of the test group and 33.3% of the control group showed score 0, 33.3% of the test group and 40% of the control group showed score 1, 6.7% of the test group and 20% of the control group showed score 2, and only one case (6.7%) in the control group having score 3 as presented in table (3)

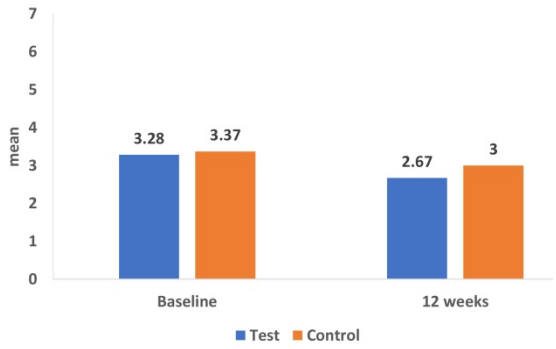


Figure (4): Average attachment loss in the two study groups at different timepoints

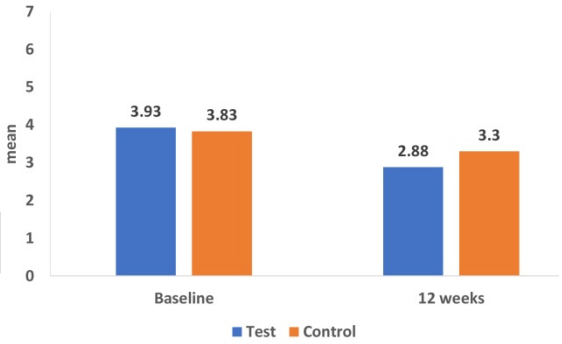


Figure (5): Average probing depth in the two study groups at different timepoints

Table (1): Attachment loss in the two study groups at different timepoints

In mm		Test	Control	Difference	95 % CI	P value
		Mean (SD)				
Baseline a	Mesial	3.20 (0.56)	3.30 (0.59)	-0.10 (1.15)	-0.53, 0.33	0.60
	Distal	3.37 (0.48)	3.43 (0.46)	-0.07 (0.94)	-0.42, 0.28	0.35
	Average	3.28 (0.45)	3.37 (0.44)	-0.08 (0.89)	-0.42, 0.25	0.41
12 weeks a	Mesial	2.73 (0.32)	2.93 (0.42)	-0.20 (0.74)	-0.48, 0.08	0.23
	Distal	2.60 (0.43)	3.07 (0.50)	-0.47 (0.93)	-0.82, -0.12	0.02*
	Aver	2.67	3.00	-0.33	-	0.04

	age	(0.31)	(0.35)	(0.12)	0.58, -0.09	9*
Percent change b	Mesial	-13.40 (10.43)	-10.01 (11.01)	-3.38 (21.45)	-11.41, 4.64	0.57
	Distal	-21.27 (16.91)	-10.24 (12.25)	-11.03 (29.53)	-22.08, 0.01	0.27
	Average	-17.82 (11.69)	-10.29 (9.62)	-7.53 (21.42)	-15.54, 0.48	0.25
P value c 12 weeks vs. baseline	Mesial	<0.001*	0.003*			
	Distal	<0.001*	0.006*			
	Average	<0.001*	0.001*			

a: Independent samples t-test, b: Mann-Whitney U test, c: Paired samples t-test.
*statistically significant at p value <0.05

Table (2): Probing depth in the two study groups at different timepoints

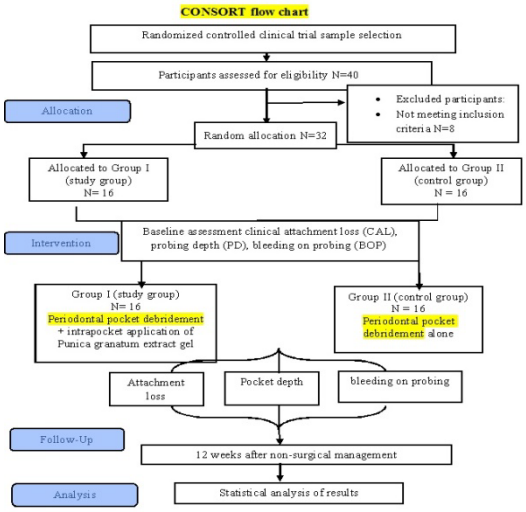
In mm		Test	Control	Difference	95% CI	P value
		Mean (SD)				
Baseline a	Mesial	3.80 (0.82)	3.90 (0.43)	-0.10 (1.31)	-0.60, 0.40	0.28
	Distal	4.07 (0.68)	3.77 (0.65)	0.30 (1.33)	-0.20, 0.80	0.76
	Average	3.93 (0.58)	3.83 (0.43)	0.10 (1.02)	-0.28, 0.48	0.54
12 weeks a	Mesial	2.80 (0.32)	3.30 (0.49)	-0.50 (0.83)	-0.81, -0.19	0.21
	Distal	2.98 (0.52)	3.30 (0.62)	-0.32 (1.14)	-0.76, 0.09	0.03*
	Average	2.88 (0.35)	3.30 (0.54)	-0.42 (0.92)	-0.76, -0.07	0.04*
Percent change b	Mesial	-24.21 (12.35)	-14.66 (13.76)	-9.55 (26.14)	-19.33, -0.23	0.01*
	Distal	-26.38 (11.35)	-11.35 (13.56)	-15.03 (25.01)	-24.38, -5.68	0.04*
	Average	-26.09 (7.57)	-13.67 (11.24)	-12.42 (19.16)	-19.59, -5.26	0.009*
P value c 12 weeks vs. baseline	Mesial	<0.001*	0.001*			
	Distal	<0.001*	0.008*			
	Average	<0.001*	0.001*			

a: Independent samples t-test, b: Mann-Whitney U test, c: Paired samples t-test.
*statistically significant at p value <0.05

Table (3): Bleeding on probing in the two study groups at different timepoints

		Test	Control	P_1 value
		N (%)		
Baseline	Score 0	0 (0%)	0 (0%)	1.00
	Score 1	0 (0%)	0 (0%)	
	Score 2	0 (0%)	0 (0%)	
	Score 3	15 (100%)	15 (100%)	
	Median (IQR)	3.00 (3.00, 3.00)	3.00 (3.00, 3.00)	
12 weeks	Score 0	9 (60%)	5 (33.3%)	0.13
	Score 1	5 (33.3%)	6 (40%)	
	Score 2	1 (6.7%)	3 (20%)	
	Score 3	0 (0%)	1 (6.7%)	
	Median (IQR)	0.00 (0.00, 1.00)	1.00 (0.00, 2.00)	
P_2 value 12 weeks vs. baseline		<0.001*	0.001*	

P_1:Mann-Whitney U test was used, : P_2: Wilcoxon signed rank test was used
*statistically significant at p value <0.05



DISCUSSION

The non-surgical periodontal management aims to remove or reduce populations of pathogenic microorganisms, arrest inflammatory progression, and help in gaining new attachment level (8). Mechanical periodontal pocket debridement is the common treatment for stage 2 periodontitis (6, 21, 25). It involves carefully using power-driven and hand scalers to remove plaque, endotoxin, calculus, and other retentive regional elements from the roots (7).

Earlier studies indicated that combining local delivery agents with pocket debridement helps in reducing PPD and improving CAL (26). Because of existence pathogens within compound or complex pockets, where the instruments cannot reach, these pathogens cannot be completely removed by mechanical treatment alone (27).

Antimicrobial resistance is known to be induced by synthetic antibiotics and antimicrobials, by evolution of distinct infections (9). Phytochemicals have proved to be a genuine choice to combat such artificial agents (11). Flavonoids in pomegranate have a proven antimicrobial action towards Streptococcus sanguis, which is proven to be the primary colonizer in the production of plaque (28, 29).

Pomegranate mouthwash has antimicrobial effect towards Aggregatibacter actinomycetemcomitans (Aa), Porphyromonas gingivalis (Pg) and Prevotella intermedia (Pi), which are the most destructive colonizers (29). Moreover, *Punica granatum* has antimicrobial effect towards Eikenella corrodens, which is a secondary colonizer in the plaque biofilm formation on the teeth surfaces. CHX had a less drastic effect on E. corrodens when compared to *P. granatum* gel (30).

Several studies have been conducted to examine the effectiveness of P. granatum in treating periodontal pathology (16,17, 28-38).

In the present study, a statistically significant reduction was seen in both groups in CAL at 12 weeks compared to baseline values (P <0.001 and P =0.001 in the test and control groups, respectively). This is in harmony with the study by Sastravaha et al. who reported that gain in clinical attachment was more in pomegranate group. Sastravaha et al claimed that it may be due to punicalagins which have the ability to form connections with collagen fibers, and help in collagen stability (17).

Regarding PPD, both groups revealed statistical decline in PPD at 12 weeks. The decline in test group was more than in the control group (P=0.04). This could be due to the anti-inflammatory effect of pomegranate. This is consistent with the findings by Sastravaha et al. who stated that the greater PPD reduction in the pomegranate group may be due to the astringent properties of the tannin-rich Punica extract gel. Tissue contraction is a frequent addition to the astringent characteristic (17,34). The pomegranate active components including polyphenolic flavonoids (e.g., punicalagins and ellagic acid) can prevent gingival inflammation through a number of mechanisms including reduction of oxidative stress in the oral cavity (35).

Highly statistical reduction in BOP was noted in both groups at twelve weeks, the test group was (P <0.001), and the control group was (P=0.001). The decrease of BOP observed in the test group can be explained by the antibacterial effects of PEG on the periodontal pathogens. As it was reported in vivo study by Aparecida et al. in 2016 that PEG had the ability to inhibit *P.gingivalis* (36). Another invitro study by Armelia et al. in 2018, reported that P. juice effectively inhibited biofilm formation of *P.gingivalis*, *A.actinomycetemcomitans*, and *T.denticola* that were considered the main pathogenic complex in the pathology of periodontitis (37).

The presence or absence of BOP is a good indicator of inflammation. In 2021, Tyagi et al. claimed that pomegranate extracts in form of chip and gel can be beneficial for treating periodontal pockets after non-surgical treatment and found a significant decrease in bleeding and plaque scores (38).

The limitations of our study were the lack of sufficient information about number of times for gel application, Further studies should be done with repeated application of the gel which may result in greater reduction in PPD and CAL. Further microbiological and biochemical studies are also recommended to prove the anti-inflammatory effect of PEG and its mode of action.

CONCLUSION

Based on the obtained results of the present study, it was concluded that pomegranate extract gel can be used successfully as a locally applied adjuvant to conventional therapy in the treatment of stage 2 periodontitis. It is simple to prepare, and easy to use with no adverse reactions or side effects. Further studies are needed to reach more comprehensive outcomes.

CONFLICT OF INTEREST

The authors affirm that they have no competing interests.

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