The Effect of Punica Granatum Gel as An Adjunctive Therapy in Patients with chronic Periodontitis: A Clinical, Microbiological and histological Study

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Abstract: Background: Periodontitis is defined as an inflammatory disease of the supporting tissues of the teeth caused by groups of specific microorganisms, resulting in progressive destruction of the periodontal ligaments and alveolar bone with increased probing depth formation, recession, or both. Herbal medicine has been widely used all over the world since ancient times for their better therapeutic value and fewer adverse effects. Punica granatum (Pomegranate) is one of the oldest edible fruit which has a long history as a medicinal fruit that has been reported to promote tissue healing and modulate host responses. The purpose of this research was to evaluate the effectiveness of Punica Granatum gel as an adjunctive to non-surgical periodontal therapy in patients with–moderate to severe chronic periodontitis. **Methodology**: Twenty periodontal sites in ten patients with moderate to severe chronic periodontitis aged between 20 and 45 years with pocket depth of 5-7 mm were included. Supportive periodontal therapy (SPT) was provided and the target teeth in the test quadrant received subgingival delivery of the medicated gel while the control quadrant received placebo gel. The clinical parameters, which include bleeding index (BI), gingival index (GI) and plaque index (PI) were recorded at baseline and after 15 days. The antimicrobial activity of the test gel was evaluated microbiologically and the anti-inflammatory effect was evaluated both clinically and histologically. **Results:** Significant reduction in mean bleeding index (BI), gingival index (GI) and plaque index (PI) was observed from baseline to 15 days' post gel application. In–test sites compared to control sites. The microbiological results revealed that oral gel of Punica granatum was potentially effective in suppressing microbial growth. Histological results revealed mild perivascular inflammatory reaction and increased collagen fibers in test specimens compared to dense infiltration of inflammatory cells with destruction of collagen fibers in control sites. **Conclusion:** Local delivery of Punica granatum gel plus scaling and root planing significantly reduced the clinical signs of chronic periodontitis and improve the gingival health.

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1. Introduction:

Periodontitis is a complex disease in which disease expression involves intricate interactions of the biofilm with the host inflammatory response and subsequent alterations in bone and connective tissue metabolism. [(1)] Loss of connective tissue attachment to the teeth is the clinical feature which differentiates periodontitis from gingivitis. [(2)]Scaling and root planing (SRP) is the gold standard, but this mechanical debridement alone may fail to eliminate the putative pathogens from the pockets completely because of the invasion of these organisms within the gingival tissue or in deeper areas inaccessible to periodontal instrumentations thus, results in recurrence of periodontal disease. [(3)] Therefore, the selective removal or inhibition of pathogenic microbes with systemic or locally delivered antimicrobial and host modulating agents, in combination with SRP, is often considered as an effective approach at specific disease active sites. [(4)] Periodontal diseases are associated with bacterial infections; therefore, antibacterial treatment seems to be an appropriate method of improving the condition of the inflamed tissues. But the problem of systemic administration of antibiotics is the distribution of drug throughout the body, which may give rise to toxicity problems. [(5)] One method of minimizing the distribution of therapeutic agents in the body is through the use of local drug delivery system. Periodontal local delivery devices that have been used for the targeted delivery of antimicrobial agents include: fibers, strips and compacts, films, gels and nanoparticles. [(6)]

There are natural agents that are effective and economically viable alternatives, [(7)] have wide popular acceptance, being used to fight diseases at low cost [(8)] and contribute to improving people's access to

prevention and treatment of periodontal disease. [9] WHO strategy in the year of 2000 recognized the need to incorporate the principles, the resources and the techniques of Natural Medicine in the public health. [10]

Punica granatum (family Punicaceae), generally known as "pomegranate," is a shrub or small tree native to Asia where several of its parts have been used as astringent, and for hemostatic as well as diabetic control. The fruit of this tree is used for the treatment of throat infections, coughs, and fever due to its anti-inflammatory properties. [11]The most therapeutically beneficial pomegranate constituents are ellagic acid, ellagitannins (including punicalagins), punicic acid, flavonoids, anthocyanidins, anthocyanins, and estrogenic flavonols and flavones. [12]A 10% topical pomegranate gel was efficient in reducing recurrent aphthous stomatitis pain and time for complete healing of ulcers. This was attributed to its anti-inflammatory, antioxidant and antimicrobial properties of pomegranate. [13] A gel based Punica granatum bark extract was effective in treating denture stomatitis as effectively as miconazole. [14]

Pomegranate fruit extract gel was active against S. sanguis, S. mutans and Streptococcus mitis by controlling their adhesion on to glass surface and that it could be used to prevent adherence of various microorganisms in the oral cavity. [15] In a recent in vitro study, pomegranate pulp extract gel showed highly significant inhibitory effect at 5%, 25%, 50% and 100% against S. mutans when compared to aloe vera and sorbitol. This action is probably due to the antimicrobial property of tannins. [16] Pomegranate could be a potential anticariogenic agent due to its property to inhibit S. mutans.

The aim of this study was to evaluate the effectiveness of Punica Granatum gel as adjunctive periodontal treatment on controlling moderate to severe chronic periodontitis.

2. Materials and Methods:

Preparation of Pomegranate Extract Gel:

Fresh pomegranates were obtained and their seeds were isolated and ground into fine juice in an electric grinder. The concentrated extract was gotten through direct percolation by filtering the juice in a Buckner funnel through a filter paper. At this stage, a control gel was set up by dissolving 5 g of carboxymethyl cellulose in 100 ml of distilled water and stirring it delicately for 15 min until a gel of consistency (0.05%) advantageous for use, as the orabase gel, is acquired. in the same way, the test gel was set up by dissolving 5 g of carboxymethyl cellulose in 100 ml of the concentrated extract of pomegranate juice. A little measure of methyl paraben (2 mg) was added as a preservative to both test and control gels. The control gel had a similar content with the exception of the pomegranate extract. [17]

Study Design and Sample Selection:

This randomized controlled clinical study (split-mouth design) with ethical committee approval F-5004-17, was carried out at Females' dental clinics, college of dentistry, Qassim University, Saudi Arabia. Informed consent was obtained from all subjects who were willing to participate in this study.

In this study, 20 periodontal sites in 10 female patients with localized or generalized moderate to severe chronic periodontitis aged between 20 and 45 years with pocket depth of 5-7 mm were included. The diagnosis of periodontitis was based on the clinical and radiographic features. [18]

The patient had to fulfill the following criteria: Having moderate to severe chronic periodontitis, a minimum of two periodontal pockets in contralateral quadrants, not pregnant, having no systemic disease, no antibiotic treatment during the 6-month period prior to the study, no smoking, no periodontal treatment during the previous 6 months, no orthodontic treatment during the study, and no history of allergy to the tested drugs. [19]

Study Protocol:

Selected sites were divided randomly into control and experimental sites. In the experimental site scaling and root planing was performed, followed by placement of the Pomegranate gel and periodontal pack application. In the control site, subgingival scaling and root planing was performed, followed by application of control gel and periodontal pack. Parameters were applied prior to treatment which include: Plaque index (PI) (Turesky-Gilmore-Glickman modification of the Quigley-Hein, 1970), [20] gingival index (GI) (Loe and Silness, 1963), [21] and bleeding index (BI) (Ainamo and Bay, 1975). [22] These parameters were recorded on day 0 and 15 using UNC 15 mm probe.

Periodontal Therapy:

After thorough scaling and root planing, cotton roll isolation was done in the both sites. Pomegranate and placebo gels were carried with a 5-ml disposable syringe equipped with a 25-gauge needle. Coe-Pak was used to cover the pocket so as to prevent the ingress of oral fluids as well as to allow the retention of the material within the pocket. Oral hygiene instructions were given. Subjects were recalled after 7 days, and periodontal dressing was removed. Patient recalled after 15 days of the placement of the Pomegranate gel in order to assess the various clinical parameters. The examiner was blinded of which site is the test and control site. [18]

Figure 1:Recording the Clinical Parameters in Both Sides

Figure 2: Scaling and Root Planing in Both Sides.

Figure 3: Irrigation of Pocket with Saline in Both Sides.

Figure 4: Pomegranate Gel Application in Test Side.

Figure 5: Placebo Gel Application in Control Side.

Figure 6: Periodontal Pack Application in Both Sides.

Figure 7: Preparation of Pomegranate Extract Gel:

Preparation of the Culture:

In this method (4-mm) holes were made with glass borer nutrient agar plate. The underside of the petri plate was marked to label the wells. The cotton swabs were dipped into the broth culture of the test organisms and were gently squeezed against the inside of the tube to remove excess fluid. Escherichia coli, Staphylococcus aureus and Candida albicans were swabbed on Agar plates. Swabbing was done in outside diameter of the plates. The plates were allowed to dry for about 5 minutes. Then the samples showed in (table 1) were added in wells of nutrient plates. The pomegranates extracted 10% (20mcg) was used as control whereas Amoxicillin (25mcg) and clotrimazole (10mcg) were used as references for bacterial and fungal species respectively. The plates were incubated at 37ºC for 24 hrs. The zones of inhibition were measured in millimeters, using a ruler on the underside of the plate. The zone size was recorded and all the cultures were discarded in the "to be autoclaved area".

Statistical analysis:

All collected data were analyzed using the Statistical program for Social Science (SPSS) Version 20. Independent- T test was used to compare Statistics of different groups with Significance set at P <0.05.

3. Results:

Clinical Result:

In this study, 20 periodontal sites in 10 female patients with moderate to severe chronic periodontitis. In the test site, pomegranate gel was placed after subgingival scaling and root planing. Control sites received placebo gel after subgingival scaling and root planing. Pomegranate gel was found to be biologically acceptable without any complications or allergic reactions.

At base line, no statistically significant difference was recorded for all clinical parameters between test and control sites. However, significant reduction in mean GI, GBI and PI was observed from baseline to 15th day in test site compared to control sites. Positive correlation was observed in GI, GBI, and PI on 15 days in the test group.

Table 1: Comparison between GI, GBI, PI of test and control groups

	Test			**Control**		
	0 day	**15 days**	**P value**	**0 day**	**15 days**	**P value**
GI	10.75	6.7	.016	9.16	9	.852
GBI	4.75	1.5	.041	4.66	3.83	.301
PI	8.9	4.25	.045	9.41	6.66	.141

GI: gingival index GBI: gingival bleeding inde PI: Plaque index

Antimicrobial Results:

The antimicrobial efficacy of both pure juice and oral gel of Punica granatum (pomegranate) against the one strains of Gram positive bacteria (S. aureus), one strains of Gram negative bacteria (E. coli) and fungi (Candida albicans) using were evaluated by the agar well diffusion method via determination of the surrounding zones of inhibition. Test was repeated four times for oral gel per each bacteria and fungi; each plate was sectioned four quadrants and wells were made in three quarter with control disc in the fourth one. Measurements were taken in millimeters after incubation for 24 hours at 37 ^{o}C for E. coli and S. aureus and 72 hours at 25 ^{o}C for C. Albicans.

The descriptive results of antimicrobial efficacy of Punica granatum (pomegranate) extract has been tabulated in (Table 2).

All organisms were sensitive to positive control, zone of inhibition for Aureus, amoxicillin + clavulanic acid was 15.2±1.3 mm while zone of inhibition was 14.6 ±1.14for E. coli and 15.8±0.84 for clotrimazole (Table 2). Oral gel of Punica granatum (pomegranate) showed higher efficacy against bacteria and fungi as compared to positive control.

The results revealed that oral gel of Punica granatum (pomegranate) were potentially effective in suppressing microbial growth with variable potency. The maximum inhibitory effect was found against C.albicans 21.3mm ± 3.69 zone of inhibition as compared to E.coli 18.33 ± 1.1zone of inhibition and S. aureus 16.7± 0.95 zone of inhibition had less inhibitory effect.

Table 2: Antimicrobial activity of Punica granatum (pomegranate) extract and positive control using agar diffusion assay.

Microorganism	*S. aureus*	*E. coli*	*C.albicans*
Inhibition zone in mm			
Pomegranates oral gel 10%			
Mean	16.7	18.33	21.43
Standard deviation	0.95	1.11	3.69
Standard error	0.359	0.42	1.39
Control			
Amoxicillin + clavulanic acid 30 mcg (DISCS)			
Mean	1.04	0.88	
Standard deviation	1.303840481	1.140175425	
Standard error	0.582071643	0.509006886	
P	< 0.5	<0.5	
clotrimazole 10mcg DISCUS			
Mean			0.64
Standard deviation			0.836660027
Standard error			0.37350894
P			<0.1

Histological result:

Control

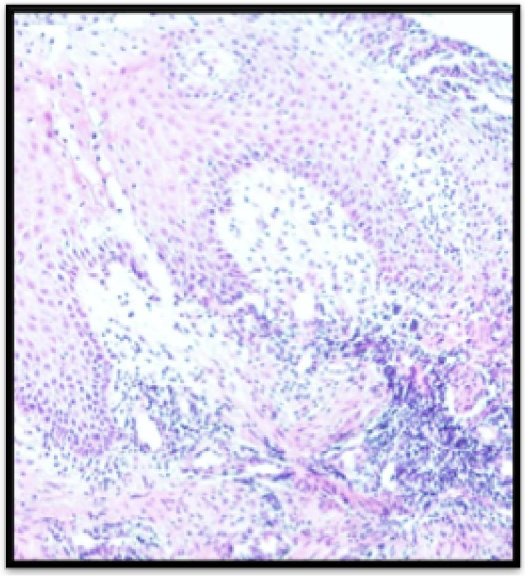

Figure 8 H and E stained soft tissue section from the interdental papilla of the control group displaying epithelial hyperplasia and moderate degree of chronic inflammatory. cell infiltration (×160)

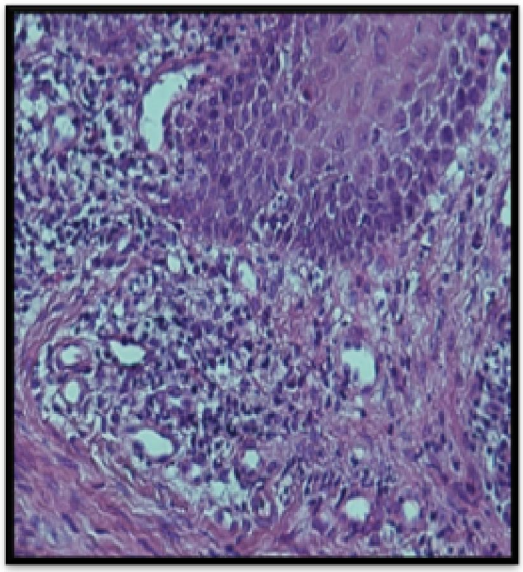

Figure 10: higher magnification of the previous fighter showing numerous blood capillaries distraction of collagen fibers, collection of edema fluid and heavy infiltration of chronic inflammatory cells (×400)

Test

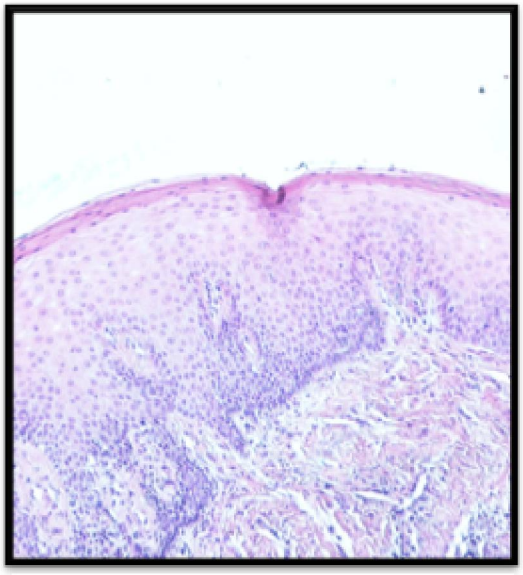

Figure 9: H and E stained soft tissue section from the interdental papilla of the test group displaying epithelial hyperplasia; note: absence of inflammatory cells and increased collagen formation (×160)

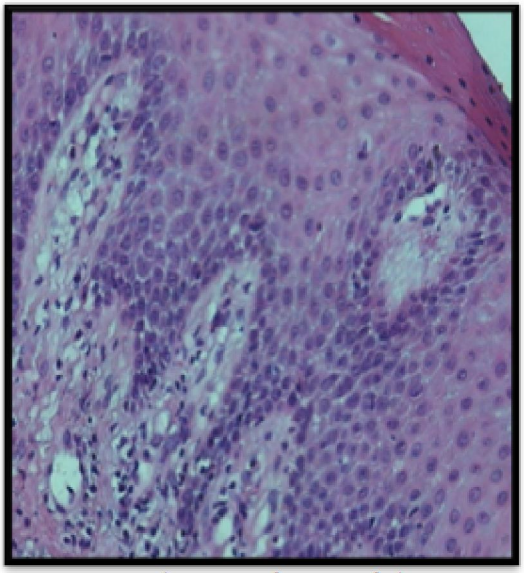

Figure 11: Higher magnification of the previous figure showing hyperplasia of the oral gingival epithelium; mild perivascular inflammatory reaction and increased collagen fibers (×400)

4. Discussion:

Dental plaque is a prerequisite for periodontal disease. Bacteria present in dental plaque have direct pathologic effect on the periodontal tissues. Bacterial lipopolysaccharides stimulate production of catabolic cytokines and inflammatory mediators including

arachidonic acid metabolites such as prostaglandin E2 (PGE2), Interleukin-1 (IL-1), Interleukin-6 (IL-6), TNF–α (Tumour Necrosis Factor–α). These cytokines and inflammatory mediators stimulate the release of tissue-derived enzymes, the matrix metalloproteinases, which cause destruction of the extracellular matrix and bone. [23]

Pomegranate is considered" A pharmacy unto itself". It means that it has many potential effects including bactericidal, anti-fungal, antiviral, immune modulation, anti-inflammatory effects. [24]

The major ingredient of pomegranate fatty acids, punicic acid, is an excellent anti‐inflammatory compound with a property to suppress prostaglandin production. [25] Cold pressed pomegranate seed oil has inhibited both cyclooxygenase and lipoxygenase enzymes in vitro. Both these are key enzymes in production of various inflammatory mediators. [24] Pomegranate fruit extract has a broad inhibitory effect on matrix metalloproteinaises (MMPs) expression and IL–1β induced tissue destruction. [24] Apart from the above mechanisms, anti‐inflammatory effect of pomegranate could be due its immunoregulatory action on macrophages and T and B lymphocytes, so Pomegranate could be beneficial in treating periodontitis as it possesses excellent anti‐inflammatory effect. [26]

Pomegranate could produce an anti-gingivitis effect as the flavonoids possess direct antioxidant properties [27] and indirect effects by enhancing the free radical scavenging activity of hepatic enzymes catalase, super oxide dismutase and peroxidase. [24]

Several studies have been used pomegranate components in multiple forms such as mouthwashes, gels, periodontal chips, irrigants for treating periodontal problems including gingivitis and periodontitis.

Sastravaha et al. in a preliminary study reported that, local delivery of Centella asiatica and Punica granatum periodontal chips following scaling and root planing showed significant improvements in pocket depth and attachment level compared to placebo. [28] In a siccessive follow‐up study, they observed that there were significant improvement in the periodontal parameters and a decrease in the IL–1β and IL-6 compared to baseline. [29] Sastravaha and his coworkers concluded that, Punica granatum extract may provide a synergistic action in collagen stabilization as tannins have the affinity for proteins, thus, forming bonds with collagen fibers. [29]

Pomegranate components could promote oral health, including reducing the risk of gingivitis. The daily mouth rinsing with pomegranate extract dissolved in water increased the levels of antioxidant activity and decreased activities of aspartate aminotransferase as reported by DiSilvestro RA et al. [30] Aspartate aminotransferase is considered an effective indicator of cell injury and is elevated among periodontitis patients. [31]

In contrast to previous studies, another study conducted by Salgado AD et al. found that A 10% Punica granatum extract gel was not efficient in preventing dental plaque formation and gingivitis. [11] Whereas, a pomegranate gel (5g of carboxymethyl cellulose in 100mL of pomegranate juice) showed excellent anti-gingivitis effect and significant reduction in plaque scores when used as an adjunct to mechanical debridement. [17]

Pomegranate extract is also known for its wound healing properties. It is known to induce increased fibroblast migration and proliferation, formation of collagen and angiogenesis. [32-34]5%, 10% and 15% methanol peel extract ointment of pomegranate resulted in a complete and faster wound healing as applied by Singer AJ et al. and Yan H on rats and Hayouni EA et al. on pigs. [32, 35] The wound healing properties can be attributed to the presence of tannins and polyphenols. [36]

Hence in the present study, pomegranate gel was found to be effective in removing local factors that cause periodontitis when performed along with scaling and root planing. The experimental material was well accepted by the patient. Neither complications nor allergic reactions were found.

Due to the lack of clinical trials with a similar design investigating the effect of pomegranate gel on periodontitis, future trials are needed with adjustments of the methodology, including increasing the sample size for better extrapolation of the results. Extending the duration of the follow-up period may also enable detection of further changes over time.

Conclusion:

The clinical, microbiological and histopathological results of current study support the anti-inflammatory and antimicrobial role of Punica granatum gel, and support its use as adjunctive to scaling and root planing for patients with chronic periodontitis.

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