Original Research Paper

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Punicagranatum as a Local Drug Delivery System: A Clinico Microbiological Study

Abstract:

The use of herbal/ natural compounds can be comparatively safer alternative to those of synthetic compounds for periodontal therapy. The present study aims to investigate the effect of pomegranate extracts in form of chip for treating adult patients affected with periodontitis followed with scaling and root planing. The study was conducted in two parts: Invitro and Invivo. The study was a randomized controlled study and was addressed by clinical and microbiological parameters at base line and subsequent intervals.

Material and methods: In vitro release was performed by using Keshary-chien diffusion cell for randomly selected strip. In vivo30patients with adult periodontitis having initial pocket depth ≥4 mm were enrolled into this research. For each subject two experimental sites were chosen located in symmetrical quadrants. Sites were randomly assigned to control group or test group. Then subgingival application of medicated chips was done in both groups. The clinical parameters were recorded at baseline, 30 days and 90 days. Descriptive statistical analysis has been carried out in present study.

Results: The in vivo study confirmed significant improvements of plaque index ratings with significant reduction in gingival index, plaque index and relative attachment level scores at 90 days as compared to control group. While Porphyromonasgingivalis, Prevotellaintermedia, Fusobacteriumintermedia and Aggregatibacteractinomycetemcomitans showed a more significant reduction at 90 days as compared to control group. In vitro results showed complete drug release in – 72- 80 hours .Matrix degrades between 3 to 4 days. Pomegranate extracts in chip may provide additional advantages to scaling and root planing for improving periodontal status.

Key-words: Chip, gingiva, periodontal, pomegranate, punicagranatum

Introduction:

Diseases of the oral cavity continue to be one of the major health issues worldwide. The interlink between oral diseases and microbial species activities that form part of the macro biota of the oral cavity is well established.[1] Periodontal diseases which are subgingival and also associated with large number of anaerobic gram-negative bacteria such as (Porphyromonasgingivalis, Actinobacillus sp., Prevotella sp. and Fusobacteriumsp).[2] Although plaque control methods have the potential to maintain adequate levels of oral hygiene and clinical experience. Population-based studies have shown, such methods are not being employed as accurately as they should be. Therefore, several chemotherapeutic agents such as: triclosan, essential oils and chlorhexidine have been developed in order to control bacterial plaque, aiming to improve the efficacy of daily hygiene control measures.[3] Despite of various agents being commercially available, these chemicals can alter oral microbiota and also don't have side-effects such as antibiotic resistance ,vomiting, diarrhoea and tooth staining .

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Hence, alternative products searchstill continues and natural phytochemicals which are isolated from plants used in traditional medicine are considered to be as good alternatives to the synthetic chemicals.

Punicagranatum Linn. (Punicaceae) is a shrub or small tree native to Asia where its parts have been used as an astringent, haemostatic, for diabetes patients, as an anthelmintic and for treating diarrhoea and dysentery. Phenolic compounds like phenolic acids flavanoids, phenylpropanoids , tannins etc. are responsible for its functional properties.[4]

The aim of this study is to investigate the adjunctive benefits of Punicagranatum/ pomegranate as a local drug delivery system in the treatment of periodontal pockets. Also to gauge the consequences as well as benefits of herbal chip from extracts of pomegranate/ Punicagranatum as a subgingival adjunct to scaling and root planing with their effect on clinical parameters such as plaque scores, gingival scores, pocket probing depth, and relative gingival attachment level.

Material and method:

A total of 30 healthy patients affected by chronic periodontitis who reported to the outpatient department of periodontology at our institution were recruited. The study was conducted in two parts: *Invitro*and*Invivo*

Chip preparation:

Pericarp of the fruit was used as an active ingredient for preparation of chips. Hydroxypropyl cellulose was used as carrier for delivery of punica extracts.

The chips were prepared by transferring 20 mL of ethanol to a cleaned 100 mL beaker, which was placed on a magnetic stirrer. A magnetic bead was added and the stirrer was set to 500 RPM. A small quantity of hydroxyl propyl cellulose (HPC) was added and dissolved until the entire quantity (800 mg) was added. The stirrer was set to 1,000 RPM and the mixture was stirred for 45 minutes. The weighed quantities of polyvinyl pyrrolidone and polyethylene glycol were added and the mixture was stirred for 60 minutes. Once the polymer dispersion was homogeneous, 60 mg of Punicagranatum was added and the complete mixture was stirred for approximately 30 minutes, at which point it was transferred to a Petri dish precoated with glycerine. The mixture was allowed to dry, forming a patch, at which point it was removed from the Petri dish. Using a sharp sterilized blade, the patch was cut into chips, which were stored in aluminium foil until further use. (Figure.1)

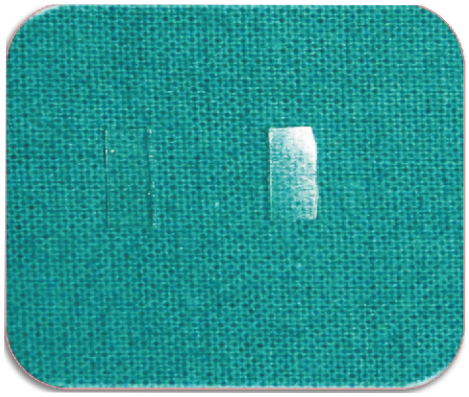


Figure.1 Punicagranatum chi

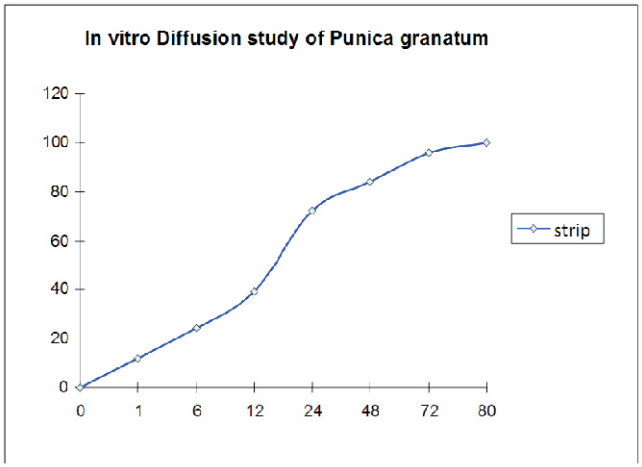
The overall ingredients of chip are given in the table below: (Table.1)

Sl. No.	Punicagranatum chip	
	Ingredients	Quantity (mg)
1	Punicagranatum extract	5%(60 mg)
2	Hydroxy Propyl Cellulose	800
3	Polyvinyl Pyrrolidone	100
4	Poly Ethylene Glycol	100
5	Ethanol+Water mixture	Q.S.

Note: Q.S. = Quantity Sufficient.

In vitro drug release studies:

In vitro release was performed by using Keshary-chien diffusion cell for randomly selected strip. Isotonic phosphate buffered saline (pH 6.8) was used as diffusion medium. One millilitre of the IPBS was withdrawn at different time intervals (for three days) and immediately replaced with 1 ml fresh IPBS. The content of the drug was estimated by measuring the absorbance after achieving suitable dilution. Further the percentage drug release was calculated and the result was subjected for kinetic treatment to know the order of release. (Graph.1) Describes *In vitro d*iffusion study for Punicagranatum.



Graph.1 Duration of complete drug release – 72- 80 hrs .Matrix degrades between 3 to 4 days

In vivo part:

30 systemically healthy subjects aged between 18-65 years diagnosed with chronic periodontitis with pocket depth between 5-8 mm were selected for the study. Patients who had periodontal therapy in the past six months, patients on medications such as antibiotics, anticoagulants, steroids or on hormonal therapy, pregnant women and lactating mothers were excluded.

The study was a randomized controlled study and was addressed by clinical and microbiological parameters at base line and subsequent intervals. For each subject two experimental sites were chosen located in symmetrical quadrants. Sites were randomly assigned to control group or test group

Control group: Scaling and root planing alone.

Test group: Scaling and root planing along with application of Punicagranatum chips.

Clinical procedure:

The subjects were briefed about the study and a written informed consent from each of the subject was taken. Plaque index (Turesky- Gilmore-Glickman modification of the Quigley and Hein plaque index)[5](Figure.2), Gingival index (Loe and Silness, 1963)[6](Figure.3), Relative attachment level (Figure.4)were recorded and microbiological samples were collected using paper points and transferred to transport media(Figure5). Full mouth scaling and root planing was subsequently performed at baseline & placement of punicagranatum chips was done in test sites (Figure 6). The clinical parameters were recorded at baseline, 30 days and 90 days.

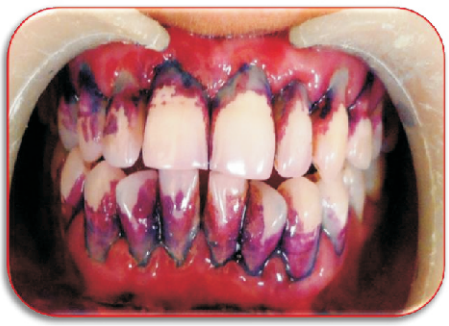


Figure.2 Plaque index

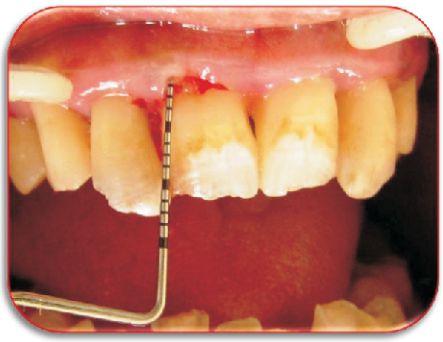


Figure.3 Gingival index

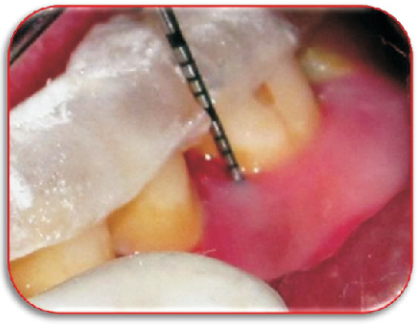


Figure.4 Relative attachment level

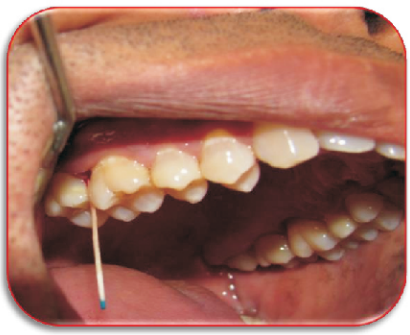


Figure.5 Collection of samples using paper points

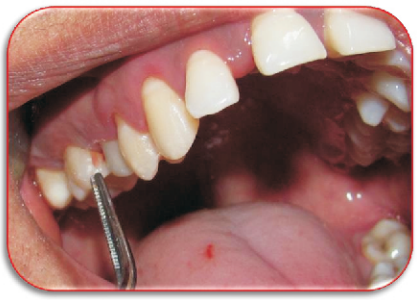


Figure.6 Placement of chips

Statistical analysis:

Descriptive form of statistical analysis has been carried out in this present study. Results are presented on Mean ± SD (standard deviation).

Student paired T test has been used to find the significance of study parameters on continuous scale within each group. In this, comparison of baseline with other 2 intervals i.e. 30 days & 90 days in each group is done.

Results:

In vivo results:-

Duration of complete drug release – 72- 80 hours .Matrix degrades between 3 to 4 days.

In vitro clinical results:

According to the plaque index, gingival index and relative attachment levels, the punicagranatum group showed more statistically significant reduction in gingival index, plaque index and relative attachment level (RAL)scores at 90 days as compared to control group.(Table 2 , Table 3, Table 4)

Table 2 :- Paired t test for intra-group comparison between the groups for Plaque index.

Paired Samples Test										
GROUP			Paired Differences					t	df	Sig. (2-tailed)
			Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
						Lower	Upper			
Control Group	Pair 1	P.i baseline - Pi30 Days	1.70667	.29633	.07651	1.54257	1.87077	22.306	14	<.001**
	Pair 2	P.i baseline - Pi90 Days	1.25333	.32921	.08500	1.07102	1.43565	14.745	14	<.001**
	Pair 3	P.i 30 Days - Pi90 Days	-.45333	.22318	.05762	-.57693	-.32974	-7.867	14	<.001**
Test Group	Pair 1	P.i baseline - Pi30 Days	2.26667	.27689	.07149	2.11333	2.42000	31.705	14	<.001**
	Pair 2	P.i baseline - Pi90 Days	1.96000	.24727	.06385	1.82307	2.09693	30.699	14	<.001**
	Pair 3	Pi30 Days - Pi90 Days	-.30667	.12799	.03305	-.37754	-.23579	-9.280	14	<.001**

Note: P.i= Plaque Index, t= test result, df= Degree of freedom=14

Table.3 Paired t - test for intra- group comparison between the groups for GI.

GROUP			Paired Differences					t	df	Sig. (2-tailed)
			Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
						Lower	Upper			
Control Group	Pair 1	Gi baseline - Gi30 Days	.6867	.3907	.1009	.4703	.9030	6.806	14	<.001**
	Pair 2	Gi baseline - Gi90 Days	.10000	.52780	.13628	-.19229	.39229	.734	14	.475
	Pair 3	Gi 30 Days - Gi90 Days	-.58667	.42906	.11078	-.82427	-.34906	-5.296	14	<.001**
Test Group	Pair 1	Gi baseline - Gi30 Days	1.1200	.3745	.0967	.9126	1.3274	11.581	14	<.001**
	Pair 2	Gi baseline - Gi90 Days	.71333	.38334	.09898	.50104	.92562	7.207	14	<.001**
	Pair 3	Gi 30 Days - Gi90 Days	-.40667	.21536	.05561	-.52593	-.28740	-7.313	14	<.001**

Note:Gi= Gingival index, t= test , df= Degree of freedom=14

Table 4:- Paired t test for intra- group comparison between the groups for RAL

Paired Samples Test										
GROUP			Paired Differences					t	df	Sig. (2-tailed)
			Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
						Lower	Upper			
Control group	Pair 1	Ral baseline - Ral30 Days	1.00000	1.06904	.27603	.40798	1.59202	3.623	14	.003**
	Pair 2	Ral baseline - Ral90 Days	.73333	1.57963	.40786	-.14144	1.60810	1.798	14	.094
	Pair 3	Ral 30 Days - Ral90 Days	-.26667	1.09978	.28396	-.87571	.34237	-.939	14	.364
Test group	Pair 1	Ral baseline - Ral30 Days	3.20000	1.26491	.32660	2.49952	3.90048	9.798	14	<.001**
	Pair 2	Ral baseline - Ral90 Days	2.93333	1.09978	.28396	2.32429	3.54237	10.330	14	<.001**
	Pair 3	Ral 30 Days - Ral90 Days	-.26667	.88372	.22817	-.75605	.22272	-1.169	14	.262

Note: Ral= relative attachment level, t= test ,df=Degree of freedom=14

Porphyromonasgingivalis , Prevotellaintermedia , and Aggregatibacteractinomycetemcomitans,Fusobacteriuminte rmediashowed a more significant reduction at 90 days as compared to control group.(Table 5 , Table 6 , Table 7 , Table 8)

Table 5:- Paired Samples Test forP.g

Group			Paired Differences					t	df	Sig. (2-tailed)
			Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
						Lower	Upper			
Control group	Pair 1	P.g Baseline - 30 Days	227.133	101.582	26.228	170.879	283.388	8.660	14	<.001**
	Pair 2	P.g Baseline - 90 Days	144.800	75.392	19.466	103.049	186.551	7.439	14	<.001**
	Pair 3	30 Days - 90 Days	-82.333	60.610	15.649	-115.898	-48.769	-5.261	14	<.001**
Test group	Pair 1	P.g Baseline - 30 Days	356.733	98.279	25.376	302.308	411.158	14.058	14	<.001**
	Pair 2	P.g Baseline - 90 Days	281.133	100.246	25.883	225.619	336.648	10.862	14	<.001**
	Pair 3	30 Days - 90 Days	-75.600	51.586	13.319	-104.167	-47.033	-5.676	14	<.001**

Note: P.g= Porphyromonasgingivalis, t= test, df= Degree of freedom=14

Table 6 :- Paired Samples Test for P.i

Group			Paired Differences					t	df	Sig. (2-tailed)
			Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
						Lower	Upper			
Control group	Pair 1	P.i Baseline - 30days	259.067	74.920	19.344	217.577	300.556	13.392	14	<.001**
	Pair 2	P.i Baseline - 90 Days	169.333	82.066	21.189	123.887	214.780	7.991	14	<.001**
	Pair 3	30 days - 90 days	-89.733	44.356	11.453	-114.297	-65.170	-7.835	14	<.001**
Test group	Pair 1	P.i Baseline - 30days	276.467	83.473	21.553	230.241	322.692	12.828	14	<.001**
	Pair 2	P.i Baseline - 90 Days	245.600	80.898	20.888	200.800	290.400	11.758	14	<.001**
	Pair 3	30days - 90 Days	-30.867	20.273	5.234	-42.093	-19.640	-5.897	14	<.001**

Note: P.i=Prevotellaintermedia, t=test ,df=Degree of freedom=14

Table 7:- Paired Samples Test for A.a

Group			Paired Differences					t	df	Sig. (2-tailed)
			Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
						Lower	Upper			
Control group	Pair 1	A.a Baseline - 30days	17.267	8.371	2.161	12.631	21.902	7.989	14	<.001**
	Pair 2	A.a Baseline - 90 Days	10.933	7.620	1.968	6.713	15.153	5.557	14	<.001**
	Pair 3	30 days - 90 days	-6.333	4.152	1.072	-8.633	-4.034	-5.908	14	<.001**
Test group	Pair 1	A.a Baseline – 30 days	22.467	12.665	3.270	15.453	29.480	6.870	14	<.001**
	Pair 2	A.a Baseline - 90 days	18.200	11.995	3.097	11.557	24.843	5.876	14	<.001**
	Pair 3	30 days - 90 days	-4.267	2.576	.665	-5.693	-2.840	-6.414	14	<.001**

Note: A.a= Aggregatibacteractinomycetemcomitans,t= test ,df= Degree of freedom=1

Table 8:- Paired Samples Test for F.n

Group			Paired Differences					t	df	Sig. (2-tailed)
			Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
						Lower	Upper			
Control group	Pair 1	F.nBaseline - 30 Days	15.00000	10.00000	5.00000	-.91223	30.91223	3.000	3	.058
	Pair 2	F.nBaseline – 90 Days	.00000	10.00000	3.33333	-7.68668	7.68668	.000	8	1.000
	Pair 3	F.n 30-90 Days	-20.00000	11.54701	5.77350	-38.37386	-1.62614	-3.464	3	.282
Test group	Pair 1	F.nBaseline – 30 Days	19.00000	12.72792	9.00000	-95.35584	133.35584	2.111	1	.041*
	Pair 2	F.nBaseline – 90 Days	19.75000	11.26573	5.63286	1.82371	37.67629	3.506	3	.039*
	Pair 3	F.n 30 -90 Days	.50000	.70711	.50000	-5.85310	6.85310	1.000	1	.500

Note: Fn=Fusobacteriumintermedia, t= test ,df=Degree of freedom

Discussion:

Punicagranatum (pomegranate) is considered as one of the oldest edible fruits. It has been widely used in traditional medicine in America, Asia, Africa and Europe for the treatment of different types of diseases [7]. Pomegranate has many potential effects including: bacteriocidal, antifungal, antiviral, immune modulation, astringent. Stomachic, styptic, laxative, diuretic and anthelmintic.[8]

In the present study the base material to deliver the herbal extract was hydroxyl propyl cellulose. HPC is a resorbable base material made up of water, methanol & ethanol. HPC has been shown to be stable, excellent plasticity, high viscosity & no reported irritation and toxicity. Studies by Noguchi et al, 1988 have used HPC as a material for slow release of tetracycline & Chlorhexidine and found it to be an effective delivery system.[9] A study conducted by Manoj et al in 2010 also used HPC as a base material for metronidazole & found it to an effective & sustained release delivery system.[10]

For the present study, a 60 mg/ mL concentration of Punicagranatum was emulsified in HPC. In vitro release was performed by using Keshary-chien diffusion cell for randomly selected strip. Duration of complete drug release was – 72- 80 hrs. The matrix degraded in 3 to 4 days.

For both the groups gingival index (GI), plaque index (PI), relative attachment level (RAL) & microbiological assessment was done at baseline, 30 days & 90 days.

The 30 days interval was selected to assess the response of periodontium to mechanical non-surgical therapy & local drug delivery, since shorter intervals than 1 month is not advised in order to allow for soft tissue healing & maturation [11]. The 90 days interval was selected for revaluation. The patient receiving periodontal therapy requires constant follow up hence in general 3 – 4 months has been frequently selected as a recall interval of the patient.[12]

Throughout the 90 days of the study,punicagranatum group showed reduction in plaque index, gingival index and relative attachment level. Reduction in clinical parameters can be attributed to anti-inflammatory properties of Punicagranatum. Studies done by Minakshi et al, 2008 have found extracts of Pomegranate fruit to have an anti-inflammatory effect by inhibiting the inflammation, cytokine induced production of PGE2 & IL-1β induced production of nitric oxide in-vivo.[13]

When an inter-comparison was done between test and control group at duration of 90 days for Pg , AA, Pi and Fn , significant decrease was seen in Punica granatum group. This finding attributed to anti-microbial properties of pomegranate fruit peel compound punigllacins which was confirmed by the study done by SaadSabar et al 2010 who confirm that the peel extract of Punica has highest anti-microbial activity as compared to other extracts.[14]

It seems from the above data that the chip distinctly of high value in enhancing the relative attachment. Limitations of the present research study were a shorter length of follow-up, and a smaller population size.

Conclusion:

It can be concluded that local drug delivery of Punica granatum improved periodontal status and reduced pathologic bacterial counts of Pg, Aa , Pi and Fn.Materials used in the study were accepted biologically with no oral side effects. Punica granatum chips improved clinical and microbiological parameters thus indicating that these chips can be used for local drug delivery. A larger sample size and a longer duration of study are needed to confirm these results.

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