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Guava leaf extract - Phytochemical therapy for periodontitis patients

Research Article

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Abstract

Introduction: Periodontitis is one of the most prevailing diseases associated with the oral cavity. It is important to treat the disease effectively as it results in tooth loss and also affects the host extensively. Treatment of periodontitis includes various surgical and non-surgical modalities. Non-Surgical modalities include Scaling and Root Planing (SRP), Local Drug Delivery (LDD), laser and HMT therapy. In the present study, the effect of guava leaf extract gel as a local drug delivery agent for the treatment of chronic periodontitis is evaluated. Aim: The aim of this study is to analyse the efficacy of guava gel extract as a local drug delivery agent in patients with periodontitis following scaling and root planing (SRP). Materials and method: A randomised, controlled study was conducted in 21 systemically healthy patients suffering from moderate periodontitis with isolated pockets (pocket depths 45mm). Clinical parameters GI, PI and PPD were recorded at baseline and subsequently after 7th, 14th and 21st day. The study design consisted of three groups, each group having 7 subjects, allocated in these groups randomly. Group A - Guava leaf extract gel (7), Group B – Chlorhexidine group (7), Group C - Control group (7). Results: Both the groups, Group A and Group B showed similar reduction in Gingival Index, Plaque Index and Probing Pocket Depth values when compared at baseline and on 21st day but the differences failed to reach the level of significance. Conclusions: The results suggest that Guava leaf extract gel as a local drug delivery is highly effective and comparable to gold standard.

Keywords: Local drug delivery (LDD), Periodontitis, Guava leaf extract gel, Chlorhexidine, Scaling and root planing (SRP).

Introduction

Periodontitis is a result of polymicrobial biofilm that is adherent to the tooth structure which causes tissue destruction. The soft and hard tissue destruction is due to release of various cytokines, inflammatory mediators and as well as due to increased reactive oxygen species which causes direct tissue damage along with increasing the inflammation by increasing the release of pro inflammatory factors (1,2).

Untreated periodontitis is a major cause of tooth loss in adults as well as disability both of which leads to compromised function and aesthetics (3,4). Several studies have also found periodontitis is also associated with diabetes, cardiovascular diseases, and pregnancy complications like premature birth (3). Pooled prevalence estimates of periodontitis were assessed from various studies and it was found that overall prevalence of periodontal disease was 51% (CI: 41.9-60.1). Prevalence of mild to moderate periodontitis was 26.2% (CI: 21.1-31.6)(4). Severe periodontitis is the 11th most prevalent disease in the world according to Global Burden of Disease (GBD)(5,6). The prevalence of periodontal disease was recorded to range from 20% to 50% around the world(6). Being one of the most prevalent diseases it poses a huge economical burden on the country (7).

The goals of periodontal disease treatment are to prevent the disease occurrence which can be accomplished by oral health awareness and motivation amongst people, stop its progression by removal of causative factor which is fulfilled by scaling and root planing, non-surgical methods like host modulation therapy [HMT], local drug delivery system [LDD], adjunct systemic antibiotic therapy in the cases of mild to moderate periodontitis or by surgical methods like access flap surgeries, regenerative surgical procedures in severe periodontitis cases (8,9).

In 1979, Max Goodson developed the concept of controlled release local drug delivery with added benefits of higher drug concentration at target site (9). LDD ensures higher concentration of drug at the required site for a longer duration of time (10). Various pharmaceutical drugs are available for local drug delivery like chlorhexidine, tetracycline, simvastatin (11). Chlorhexidine is the most commonly used drug that is used in LDD which has a bactericidal or bacteriostatic effect which reduces microbial load in the inaccessible areas (9,10). These pharmaceutical drugs have several side effects like bacterial resistance, staining of teeth and tongue, xerostomia, hypogeusia

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and unpleasant taste(12). Recent advances in the field of alternative medicine introduced various products for the treatment of periodontitis. The utilisation of natural products has served as a major antidote for centuries. Research in phytosciences, an upcoming integrative science, has revealed the role of various plants in periodontal therapeutics. The metabolites from plants have important biological activities such as antimicrobial, antioxidant, anti-inflammatory, antiseptic, and anti-collagenase properties. Extract of various plants like aloe vera, curcumin, lemon grass, green tea in the form of gels and mouthwashes have proven to be effective in treatment of oral diseases.

One such herbal alternative is guava leaf extract. Guava is considered to be a poor man's apple in tropical regions (15). ***Psidium guajava*** is a plant that belongs to the myrtaceae family. The plant has various properties like anti-inflammatory, antimicrobial, antioxidant, antibacterial, antimutagenic which are due to the presence of many bioactive compounds in it. The antioxidant action can be attributed to quercetin, carotenoids, vitamin C, polyphenols present in guava (14). Therefore guava plants are of extreme medicinal value. Leaves of guava are rich in flavonoids, guaijaverin and quercetin which makes it a potent antibacterial agent. Extract of guava leaves is effective against both gram negative as well as gram positive bacteria (13,16). Extract of guava acts as an antiplaque agent because it inhibits the growth of *Staphylococcus aureus* (*S. aureus*) and *S. mutans*. Guava is rich in vitamin C which is essential in maintaining the periodontium as it regulates the fibroblast differentiation and collagen structure. Therefore guava is also helpful in wound healing (13). Guava has the ability to inhibit prostaglandin (PG), kinin, and histamine due to which it has a good anti-inflammatory action (14). Guava leaf extracts have the ability to scavenge free hydrogen peroxide, superoxide anion radical and inhibit the formation of hydroxyl radical thereby reducing the chances of oxidative tissue damage (13,14). The present study was undertaken to evaluate the effect of 5% tender guava leaf extract on gingival health and its comparison with chlorhexidine PI, GI and PDD were considered as gingival health markers.

Guava leaf extract gel

Formation of guava leaf extract gel

Guava leaves approximately 250 gm were dried and ready for extraction using Soxhlet Apparatus. To increase the surface area, it must be crushed with a pestle and mortar. The plant material should be enough to completely fill the porous thimble (in our experiments we dry packaging with porcelain added to the cotton plug). Following that, the solvent (1 L of ethanol) is added to a round bottom flask that is attached to an isomantle with a Soxhlet extractor and condenser. The crushed plant material is placed inside the Soxhlet extractor in the thimble. Glass wool is used to lag the side arm.

The solvent is heated with the isomantle and begins to evaporate as it moves through the apparatus to the condenser. The condensate then drips into the reservoir, which holds the thimble. When the solvent level reaches the siphon, it returns to the flask, and the cycle begins again. This operation is repeated until complete extraction is achieved. The entire process should take 16 hours, this was ensured with a small yield of extracted plant material (about 2 to 3 ml) in the glass bottom flask. Following the completion of the process, the ethanol should be evaporated using a rotary evaporator(17). After the process is completed, the ethanol is separated by distillation, resulting in ethanol as well as a concentrated yield of guava extract (about 2 to 3 ml) in the glass bottom flask.

Determination of minimum inhibitory concentration (MIC)

The MIC estimation was carried out in the Department of Pharmacology, DIPSAR, Delhi, following the preparation of Guava Extract (Liquid form). A micro-dilution conduction 0.5 mL of BHI broth was added in tubes labeled from 2 to 10; 1 mL of the test material was taken in the first tube; 0.5 mL of the guava extract from the first tube was transferred to the second tube containing the BHI broth, mixed well, and 0.5 mL was serially transferred to the third tube, until the fifth tube; Thus, guava extract solutions were serially diluted to obtain concentrations of 32,16,8, 4, and 2 mg/mL for EGE and L/mL for AGE. After that, the tubes were inoculated with 0.1 mL of cultures. The MIC was defined as the lowest concentration of guava extract that completely inhibited the growth of the organisms.

Preparation of Guava Extract Mouth Gel

Carbopol-940 and sodium CMC were dispersed in 20 mL of distilled water by mechanical stirring. 5 mL of distilled water was mixed with the appropriate amount of sodium benzoate and then heated in a water bath to dissolve properly. After cooling the solution, polyethylene glycol-4000 was added and mixed with it. The required amount of Guava leaf extract was then added to the above mixture, and the volume was adjusted using the remaining distilled water. Finally, the full mixture of ingredients was added to the Carbopol-940 gel in a proper manner with continuous stirring, and triethanolamine was added drop by drop to the formulation to adjust the pH and obtain the desired consistency of the gel.

Table 1: Ingredients

Ingredients	Quantity Taken
1 Carbopol-940 (g)	1.5
2 Sodium CMC (g)	1
Sodium Saccharin (g)	0.5
SLS (g)	2
Polyethylene Glycol- 4000 (g)	2
Sodium Benzoate (0.05%) (g)	0.5
Triethanolamine (ml))	q.s
Distilled Water(ml)	q.s.
Guava Extract (ml)	5

During the formulation trial phase, various issues such as homogeneity, spread-ability, and viscosity arise and must be resolved by manipulating Carbopol-940 and sodium CMC concentrations either increased or decreased. As a result, other batches are removed at the start, leaving only one batch in the end. The table shows the chemical and plant extract composition.

Materials and Methods

This is an experimental study conducted in the Outpatient Department of Department of

Periodontics, SGT University, Gurugram, Haryana, India. 30 patients from the Outpatient Department of Periodontics were selected. Detailed examination of the patients was done and case history recorded.

Inclusion criteria

- Patients in the age group 20-50 years
- Presence of at least 20 functional teeth
- Presence of pocket depth of 5mm or more

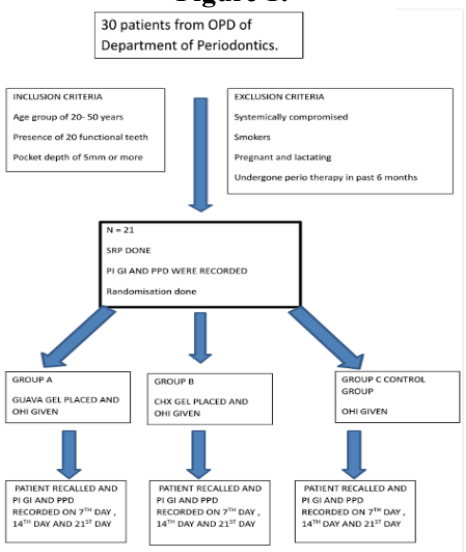
Exclusion criteria

- Systemically compromised patients
- Patients with smoking habit
- Pregnant or lactating female patients
- Patients who have undergone periodontal therapy in past 6 months

Based on the inclusion and exclusion criteria, 21 patients out of 30 patients were selected and the patients were allocated into groups randomly.

Oral Hygiene Instructions were given to all the patients and administration of gel was done at 2 sites in the oral cavity where the periodontal pocket depth was found to be 5mm or more. Patients in Group A were administered guava gel as LDD. In Group B, chlorhexidine was administered. Finally, Group C patients were labeled as a control group. Patients were recalled on 7th, 14th and 21st days. Further based on the clinical parameters, PI, GI and PPD were recorded.

Figure 1:



Results

Statistical analysis was performed using the Wilcoxon signed rank test for intragroup comparison and Mann Whitney U Test for intergroup comparisons. Mean value and standard deviation were calculated for baseline and 21st day readings. The mean GI, PI and PPD values of both the groups were analysed using SPSS 21 software.

Inter group comparison table

Table 1: GI Comparison

S.No	Day	LDD Used	Mean	SD	P value
1	Baseline	Guava	1.514	0.1069	0.318, ns
		CHX	1.443	0.1512	
2	21st DAY	Guava	0.886	0.2545	0.097, ns
		CHX	1.071	0.1704	
ns: non significant, sig:significant, SD: Standard Deviation					

The GI scores at baseline for guava and CHX were compared using Mann Whitney U test. The difference failed to reach the level of significance.

At baseline the mean GI for Group A (Guava) and Group B ((CHX) was similar, showing no significant difference, i.e., in both the groups the patients had almost similar gingival health. When the Baseline mean was compared to 21st day, for group A and group B, both the groups showed significant improvement in gingival health, but in intergroup comparison, the difference was not significant ,ie, guava leaf extract gel is as effective as CHX in improving gingival health. (Table 1, Graph 1)

Table 2: PI Comparison

S.No	Day	LDD Used	Mean	SD	P value
1	Baseline	Guava	1.4214	0.04880	0.073, ns
		CHX	1.5214	0.14100	
2	21st DAY	Guava	0.957	0.2760	0.710, ns
		CHX	0.986	0.1773	
ns:non significant, sig:significant, SD: Standard Deviation					
Mann whitney U test, level of significance set at $p < 0.05$					

The PI scores at baseline for guava and CHX were compared using Mann Whitney U test. The difference failed to reach the level of significance.

The PI scores on 21st day for guava and CHX were compared using Mann Whitney U test. The difference failed to reach the level of significance.

When baseline mean was compared to the 21st day, it was observed that the mean PI in Group A(guava) was comparable to Group B(CHX). But the difference was not statistically significant (p=0.710), i.e. both CHX and guava extract gel improve the plaque index score of the patients. (Table 2, Graph 2)

Table 3: PPD Comparison

S.No	Day	LDD Used	Mean	SD	P value
1	Baseline	Guava	5.429	0.5345	0.383, ns
		CHX	5.714	0.4880	
2	21st DAY	Guava	3.714	0.4880	0.165, ns
		CHX	4.857	0.6901	
Mann whitney U test, level of significance set at $p < 0.05$					
Ns:non significant, sig:significant					

The PPD scores at baseline for guava and CHX were compared using Mann Whitney U test.

The difference failed to reach the level of significance.

The PPD scores at 21st day for guava and CHX were compared using Mann Whitney U test.

The difference failed to reach the level of significance.

Mean PPD of both the groups are shown in Table 3. On evaluation, the p value of both the groups were greater than 0.05 on baseline (p=0.383). On the 21st day PPD of both the groups showed reduction (p=0.165). However, the result was not statistically significant. (Table 3, Graph3)

Discussion

Dysbiosis of the oral microbiome triggers the most common biofilm-mediated oral diseases such as caries and gingivitis. While gingivitis is characterized by bleeding and swelling due to inflammation of gums, eventually leading to the destruction of tooth-supported tissues, this is termed as periodontitis. Both diseases culminate in loss of tooth, impaired mastication, phonetics, respiration, swallowing, and the quality of life to a great extent(18).

Mild and moderate forms of periodontitis, is highly prevalent in adult-aged populations all over the world, with prevalence rates around 50% , while its severe form increases mostly during the third and fourth decades of life(19). And shockingly, lack of awareness is the largest contributing factor.

Several researches have revealed the important role of plants in periodontal therapeutics. Plant metabolites possess bioactive compounds which contribute to anti-microbial, antioxidant, anti-inflammatory, antiseptic and anti-collagenase properties to name a few.

Although the difference in values is not statistically significant, Guava Leaf Extract gel proved to be a worthy competitor of the gold standard, Chlorhexidine gel, commonly used in dentistry.

The guava plant has exceptional perks, its leaves are rich in guaijaverin and quercetin, making it a strong antibacterial agent(20), the fruit itself has excellent antioxidant properties due to presence of carotenoids, vitamin C, polyphenols etc. Moreover, it is effective against both gram-positive and gram-negative bacteria and also inhibits the formation of hydroxyl radicals, eventually reducing the chances of oxidative tissue damage, thereby reducing the potential damage on oral tissues, this property of guava leaves is ascribed to the presence of flavonoids and polyphenols. Vitamin C regulates fibroblast differentiation and collagen structure.

In the present study, there was significant improvement in Gingival health, Plaque index and Pocket Probing Depth in the test group where 5% of guava leaf extract gel was used as a local drug delivery agent. Guava leaf extract acts as an antiplaque agent as it inhibits the growth of S. aureus and S. mutans.

Conclusion

The phytochemical Guava leaf extract was equally efficient in reducing the GI, PI and PPD as Chlorhexidine gel which is a gold standard in periodontal therapy.

Guava leaf extract is a wonderful alternative to Chlorhexidine gel in periodontitis patients.

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