

International Journal of Dental Science and Innovative Research (IJDSIR)

IJDSIR : Dental Publication Service

Available Online at: www.ijdsir.com

Volume – 4, Issue – 5, September - 2021, Page No. : 366 - 374

Psidium guajava: A fruity way to halt periodontal disease progression

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Citation of this Article: Dr. Apeksha S. Gajghate, Dr. Salman T. Ansari, Dr. Rajvir Malik, Dr. Namrata M. Khetal, Dr. Paiham B. Shaikh, "Psidium guajava: A fruity way to halt periodontal disease progression", IJDSIR- September - 2021, Vol. – 4, Issue - 5, P. No. 366 – 374.

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Conflicts of Interest: Nil

Abstract

Background: Periodontal diseases are infectious diseases caused by bacteria present in dental plaque which is the main etiologic and initiating factor for gingivitis. Regular methods for controlling it have been found to be ineffective, which have paved the way for the use of herbal products as an adjunct to Scaling and root planing (SRP). Psidium guajava (Guava) has an enormous wealth of medicinal value. It has been known for its anti-inflammatory, antimicrobial, antioxidant, antidiarrheal, antimutagenic properties.

Aim: The aim of this study is to evaluate and compare the anti-inflammatory effect of a gel containing Psidium guajava (Guava) extract with chlorhexidine gel in treatment of Chronic Gingivitis.

Materials and Methods: In this double-blind, splitmouth study 25 individuals were included. The Control group was SRP + Chlorhexidine gluconate gel and the Test group was SRP+ Gel containing Psidium guajava (Guava) extract. Clinical parameters- Plaque Index (PI), Gingival Index (GI) and Sulcus Bleeding Index (SBI) were evaluated at 0 and 21st day. **Results:** There was no significant difference when the efficacy of Psidium guajava (Guava) extract gel was compared with 1% chlorhexidine gluconate gel in treatment of patients with gingivitis. However, a statistically significant difference was observed in PI, GI and SBI when both groups were compared after 21 days. (P < 0.05)

Conclusions: Though Chlorhexidine has earned eponym of gold standard for the treatment of periodontal disease at the same time the test gel containing Guava extract when used as an adjunct to scaling and root planing was efficient in treating Gingivitis.

Keywords: Chlorhexidine; Dental Plaque; Gel; Gingivitis; Periodontal diseases; Psidium

Introduction

Gingivitis is an inflammatory condition of the gingiva and is the most common periodontal disease affecting more than 90% of the population, regardless of age, sex or race. The frequently seen types of gingivitis are plaque-induced, hormonal, acute ulcerative necrotizing, drug-induced, or spontaneously presenting hyperplastic gingivitis. Categorically, the most important form of gingivitis is plaque-induced [1]. It is caused by the dental plaque which is the main etiologic and initiating factor. Dental plaque biofilm consists of microorganisms which includes species of Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Fusobacterium, Veillonella, and Treponema. Bacteroides such as Capnocytophaga, and Eikenella are also potentially linked to the etiology of the disease. There are also local or systemic etiologic factors that exaggerate plaque because of the microbial attack [2].

The foremost step in prevention and management of gingivitis includes maintaining oral hygiene. Disruption of plaque is done by professional scaling and root planning (SRP) and also homecare mechanical methods

which is the use of regular tooth brushing with proper technique and interproximal hygiene aids such as dental floss or the use of interproximal brushes [3]. There are certain limitations of the mechanical debridement like unfavorable anatomy of the teeth which have paved the way for the use of chemotherapeutic agents [4]. Systemic and local antibiotics are proposed as an adjunct to SRP. However, side effects such as resistance, toxicity, sensitivity, and growth of opportunistic infection have made their use limited [5]. Chlorhexidine (CHX) is bisbiguanide and is considered as a gold standard because of its substantivity and broad-spectrum antibacterial activity [6-10]. CHX is known to have a number of side effects like brown discoloration of teeth, salty taste, oral mucosal erosions which limit its longterm use [11-13].

In view of the growing ineffectiveness of conventional oral biofilm eradication, the implementation of innovative treatments inspired by nature has gained increasing attention. Ayurvedic medicines are rich depot of effective herbal remedies. In the modern times, there is an enormous awareness and scientific trials on medicinal plants and its formulations [14]. Natural products such as *Curcuma longa*(turmeric), Aloe vera, *Ocimum tenuiflorum* (basil), *Punica granatum Linn* (pomegranate) etc. have been reported to have antibacterial and anti-inflammatory properties [15-16].

Psidium guajava (Guava) is one such plant that has been used to manage many systemic conditions and improve oral hygiene [17-18]. The important elements of guava are vitamins, tannins, phenolic compounds, flavonoids, essential oils, sesquiterpene alcohols and triterpenoid acids [19]. The leaves of guava contain two important flavonoids quercetin known for its spasmolytic, antioxidant, antimicrobial, anti-inflammatory actions [20]and guajaverin known for its antibacterial action [21]. The Pulp contains ascorbic acid, carotenoids (lycopenes, β -carotene) possessing antioxidant properties whereas the seeds contain glycosides, carotenoids, phenolic compounds which have antimicrobial actions [19].

Quercetin present in guava has shown excellent antibacterial actions against periodontal pathogens such as A.actinomycemtemcomitans (*Aa*), *P.gingivalis* (*Pg*), *P*.

intermedia (Pi), Fusobacterium nucleatum (Fn) [22]. The anti-inflammatory action of guava is in its ability to inhibit prostaglandin, kinin and histamine [23]. Aa is a chief periodontal pathogen which is associated with aggressive periodontitis. Guava fruit extract completely neutralizes the cytolytic, proinflammatory response of human leukocytes induced by Aa leukotoxin and prevents the release and activation of Interleukin-1ß (IL- 1β) [24]. The antioxidant action can be credited to quercetin, carotenoids, vitamin C, and polyphenols present in guava [25]. Owning to this approach that the numerous properties guava has, the present study was undertaken to evaluate and compare the antiinflammatory effect of a gel containing Psidium guajava (Guava) extract with chlorhexidine gel in treatment of Chronic Gingivitis.

Materials and methods

Study design: In this double-blind, randomized controlled trial, the participants were selected from outpatient department of Department of periodontology, Swargiya Dadasaheb Kalmegh Smruti Dental college and Hospital, Nagpur in February 2020. The procedure was conducted according to the 2010 CONSORT (Consolidated Standards of Reporting Trials) guidelines, and an ethical approval was obtained from the Institutional Ethic Committee of Swargiya Dadasaheb Kalmegh Smruti Dental college and Hospital.

The minimum required sample size was calculated as 22 patients, to compensate the dropouts, it was planned that 25 patients would be recruited. Patients having gingivitis with more than 20 teeth with plaque and calculus and clinical signs of gingival inflammation were screened for the study. Total 105 patients were screen and finally, a total of 25 patients with a split-mouth design, in the age group of 20-65 years, were selected in the study. Informed written consent was obtained from the patients. simple randomization approach [26] using Α computer-generated random numbers was employed to assign patients in a split-mouth design (the sequence was concealed until interventions were assigned) to one of the following treatment modalities:

Group I-SRP + Chlorhexidine gluconate gel

Group II-SRP+ Gel containing Psidium guajava (Guava) extract.

The patients were instructed apply the gel on the gingiva twice daily for two weeks, without disclosing its nature for blinding the procedure. The patients were instructed to continue with their routine oral hygiene measures and to keep a gap of 30 minutes before rinsing. The Plaque index (PI), Gingival index (GI) and Sulcus Bleeding Index (SBI) were evaluated at 0 and 21st day by two different trained examiners to avoid bias. The examiners were calibrated before the study to reduce the inter examiner variability. The primary efficacy outcome was changes in the PI, and the secondary outcome was changes in the GI and SBI. Furthermore, patients were asked to report any side effects and were checked for any intraoral effects at each appointment.

Preparation of Guava gel: The fruits were thin sliced using a sterile knife, dried at room temperature for 5 days and then manual grinding was done to obtain powder form. An infusion was prepared with powdered material at a ratio of 25 g powder to 250 mL distilled water, cooled at room temperature and then filtered with the help of filter paper. Thereafter, 50 g of carboxymethylcellulose was added to the infusion (250 mL) and the mixture was kept boiling until complete dissolution to obtain the 10% gel concentration. Glycerine (50 mL:50 mL) was added as a humectant and the solution was vigorously stirred for 15 minutes until gel formation occurred. Lastly, 2 mg methyl paraben was added as a preservative agent (Fig. 1).

The preparation of this guava gel is registered under Extracts from the register of copyrights, Copyright office, Government of India (Registration number-L-104596/2021).

Statistical analysis

Statistical analysis was done by using descriptive and inferential statistics using student's paired and unpaired t test for the intergroup comparison was applied using the mean difference values of the PI, GI and SBI at day 0 and day 21 and software used in the analysis was SPSS 27.0 version and P<0.05 is considered as level of significance.

Results

Study population: A total of 25 patients (15 males and 10 females) selected from a single centre, irrespective of their medical problems, socioeconomic status, and oral habits, completed the study after randomization with no dropouts. CHX was served as the positive control (Control group). PI was considered the primary and GI and SBI the secondary end points. The inter- and intra-examiner variability was calculated using Cohen's kappa statistics.

Mean PI was 1.30 ± 0.27 and 1.10 ± 0.23 , mean GI was 1.24 ± 0.21 and 1.00 ± 0.21 , mean SBI was 1.23 ± 0.29 and 1.08 ± 0.15 at 0 and 21 days respectively for the test group and for the control group, mean PI was 1.31 ± 0.46 and 1.04 ± 0.38 , mean GI was 1.14 ± 0.38 and 0.92 ± 0.30 ,

mean SBI was 1.31±0.39 and 1.05±0.29 at 0 and 21 days respectively (Table 1 and Table 2).

The intragroup analysis conducted (Fig. 2 and Fig. 3) using a student paired t-test showed statistically significant results for the PI, GI and SBI values of both group (P=0.0001). This signifies that there was a statistically and clinically significant changes in the clinical parameters after 21 days in the test and control groups (Table 3 and 4). There were no side effects reported by patients.

Discussion

Herbal therapies have been pursued to attain antimicrobial, antioxidant, antiseptic, anti-inflammatory, and anti-collagenase effects. The beginning of periodontal diseases is a multifactorial process. It is well-known that oxidative stress is an important cause of cell damage associated with the initiation and progression of many chronic diseases [27]. Oxidative stress is the disturbance in the pro-oxidant and antioxidant balance, resulting in potential tissue damage. Plaque biofilm lets the expansion of anaerobic bacteria, which eventually results in the activation of neutrophils. This further results in the up regulation of proinflammatory cytokines and also ends up in the discharge of neutrophilic enzymes and ROS (Reactive Oxygen Species) [28]. To combat the oxidative stress, all the cells within the body are armed with an intrinsic store of molecules called "antioxidants." Antioxidants are "those substances which when present at low concentrations, compared to those of an oxidizable substrate, will delay, or inhibit oxidation of that substrate" [29]. Many studies have revealed that the total antioxidant capacity in periodontitis patients is significantly lower when compared to healthy controls or in subjects who have received periodontal therapy [30]. These findings have caused the use of exogenous

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supplements for the treatment of periodontal disease. Thereafter, Herbal antioxidant remedies are the attention of research in recent times.

The current study evaluated the efficacy of guava gel application along with SRP, in comparison with chlorhexidine gluconate gel with SRP alone, on the clinical parameters in treatment of gingivitis. This was a comparative study, with split-mouth design carried out over a period of 21 days. The split-mouth design was adopted to avoid the inter-patient variability. The primary objective was to evaluate the PI and GI, SBI were the secondary objectives. All the parameters were assessed at baseline and on 21st day. CHX is often used as a standard control as they are accepted as effective, the most effective, or the "gold standard" [31].

Guava being rich in vitamin C is considered an excellent remedy for periodontitis. It works as an anti-plaque agent and helps remove plaque accumulated on the teeth and gingiva. Its anti-inflammatory and analgesic properties help reduce swelling and pain on the gingiva [21]. In a previous study, Site-specific assessment of indices was carried out to assess the effects of 3% P. guajava gel on the gingival health. In, test sites, there was a significant decrease in the scores of plaque index (1.63 ± 0.018) , gingival index (1.5 ± 0.032) , and bleeding index (56.4 \pm 3.79). The control sites also showed a significant decrease in the scores of plaque index (1.53 \pm 0.002), gingival index (1.4 \pm 0.020), and bleeding index (47.9 \pm 2.32). Among the sites, the results were significant at three months, with test sites showing a greater reduction in all the indices (P < P)0.001). Also, the antimicrobial efficacy and Minimal inhibitory concentration (MIC) of *P. guajava* was analysed by using the agar well diffusion method, 3% P. guajava gel showed a greater zone of inhibition, indicating better antimicrobial activity [32].

In our current study, PI, GI and SBI showed a statistically significant difference in both groups after 21 days (p<0.005) which signifies both the gels are antiplaque and antigingivitis agents. Apparently, there have been no studies performed to evaluate the effects *P*. *guajava* gel as a gel in the treatment of Gingivitis which makes this study one of its kind. Hence, we are comparing our study with other herbal products.

Subgingival use of *E. officinalis* (Amla) sustainedrelease gel adjunctive to SRP in chronic periodontitis was evaluated by Grover et al, the mean PPD reduction was 2.484 ± 0.432 mm and 3.096 ± 0.491 mm and the mean CAL gain was 2.423 ± 0.681 mm and $3.088 \pm$ 0.782 mm at two and three months, respectively [33]. Boyapati et al. assessed local application of Achyranthes aspera gel in the management of chronic periodontitis. The mean PPD reduction was 2.24 ± 0.85 mm and mean CAL gain was 0.73 ± 0.40 mm at three months [34]. The results in the current study are similar to the earlier mentioned studies where herbal agents are used and clinical effects are evaluated at one and three months except our study had a very short follow-up period.

The limitations of the current study were a short followup period of 21 days, no microbial analysis was carried out and a split-mouth design which could have resulted in cross-contamination of the microorganisms assessed at the test and control sites. Hence, further longitudinal studies are required to validate the results obtained in the current study.

Table 1: Comparison of PI, GI and SBI in two groups at Day 0.

Parameter	Test Group	Control	<i>P</i> -value
PI (%)	1.30 ± 0.27 .	1.31±0.46	0.94 ^{a)}
GI (%)	1.24±0.21	1.14±0.38	0.28 ^{a)}
SBI (%)	1.23±0.29	1.31±0.39	0.44 ^{a)}

Values are presented as mean±standard deviation. PI: plaque index, GI: gingival index, SBI: sulcus Bleeding index. Test group: patients were advised to apply guava gel on gingiva twice daily for two weeks, Control group: patients were advised to apply Chlorhexidine gel on gingiva twice daily for two weeks.

^{a)} Statistically non-significant difference compared to the baseline.

Table 2: Comparison of PI, GI and SBI in two groups at Day 21.

Parameter	Test Group	Control	<i>P</i> -value
PI (%)	1.10 ± 0.23	1.04±0.38	0.50 ^{a)}
GI (%)	1.00±0.21	0.92±0.30	0.27 ^{a)}
SBI (%)	1.08±0.15	1.05±0.29	0.63 ^{a)}

Values are presented as mean±standard deviation.

PI: plaque index, GI: gingival index, SBI: sulcus Bleeding index. Test group: patients were advised to apply guava gel on gingiva twice daily for two weeks, Control group: patients were advised to apply Chlorhexidine gluconate gel on gingiva twice daily for two weeks.

^{a)} Statistically non-significant difference compared to the baseline.

Table 3: Comparison of PI, GI and SBI in test group at Day 0 and Day 21.

Para	neter	Day 0	Day 21	<i>P</i> -value
PI	(%)	1.30 ±		0.0001 ^{a)}
25		0.27	1.10±0.23	
GI	(%)			0.0001 ^{a)}
25		1.24 ± 0.21	1.00 ± 0.21	
SBI	(%)			0.0001 ^{a)}

Values are presented as mean±standard deviation.

PI: plaque index, GI: gingival index, SBI: sulcus Bleeding index. Test group: patients were advised to apply guava gel on gingiva twice daily for two weeks. ^{a)}P<0.05 was considered significant.

Table 4: Comparison of PI, GI and SBI in control group at Day 0 and Day 21.

Parar	neter	Ι	Day 0	Day 21	<i>P</i> -value
PI	(%)	1	1.31 ±		0.0001 ^{a)}
25		0.46		1.04 ± 0.38	
GI	(%)	1	1.14±		0.0001^{a}
25		0.38		0.92±0.30	0.0001
SBI	(%)				0.0001 ^{a)}

Values are presented as mean±standard deviation.

PI: plaque index, GI: gingival index, SBI: Sulcus Bleeding index. Control group: patients were advised to apply Chlorhexidine gel on gingiva twice daily for two weeks.

^{a)}P<0.05 was considered significant.



Figure 1: Prepared Guava Gel

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Figure 2: Bar Graph showing comparison of PI, GI and SBI in two groups at Day 0.



Bar diagram showing comparison of mean plaque index (PI), gingival index (GI) and sulcus bleeding index (SBI) values for the two groups at 0 day. Test group: patients were advised to apply guava gel on gingiva twice daily for two weeks, Control group: patients were advised to apply Chlorhexidine Gluconate gel on gingiva twice daily for two weeks.

Figure 3: Bar Graph showing comparison of PI, GI and SBI in two groups at Day 21.



Bar diagram showing comparison of mean plaque index (PI), gingival index (GI) and sulcus bleeding index (SBI) values for the two groups at 21st day. Test group: patients were advised to apply guava gel on gingiva twice daily for two weeks, Control group: patients were advised to apply Chlorhexidine gluconate gel on gingiva twice daily for two weeks.

Conclusion

This randomized clinical trial climaxes that Guava (P.guajava) gel is as efficient as 1% Chlorhexidine gluconate gel in reducing plaque accumulation and gingival inflammation, thereby preventing periodontal disease in the studied patients. It is also well tolerated with no reported side effects.

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