

Comparative Evaluation of Bromelain-Quercetin Gel with Chlorhexidine Gel as Subgingival Local Drug Delivery Following Scaling and Root Planning in Stage I /II and Grade B Periodontitis - Randomized Control Clinical Trail

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Abstract

Background: Periodontitis is a dysbiotic disease affecting the tooth and supporting structures. Different treatment options have been tried in form of nonsurgical and surgical therapy. Antimicrobials agents (plant extracts) have been used as an adjunct to nonsurgical therapy. Pineapple (bromelain) and guava (quercetin) are one such agents. As there is less literature in this combination, we intend to study combination of bromelain and quercetin for treatment in periodontitis as gel form. This study will be first of its kind to check the efficacy of 2% bromelain-quercetin gel and to compare its effects with 0.2% Chlorhexidine in stage I/II and Grade B periodontitis.

Material and Methods: Patients with Stage I/II and Grade B periodontitis with periodontal pocket depth measuring ≤ 5 mm and radiographic evidence of horizontal bone loss were included and treated with local drug delivery bromelain-quercetin gel and chlorhexidine gel following Scaling and Root Planning (SRP) for 3 weeks. Scaling and Root Planning is an integral part of periodontal therapy. Clinical parameters included are Plaque Index (PI), Gingival Index (GI), Modified Sulcular Bleeding Index (mSBI), Probing Pocket Depth (PPD) and Clinical Attachment Level (CAL) were assessed before intervention and at 21st day. Parameters recorded were statistically analysis will be $p < 0.005$.

Results: Intragroup comparisons of PPD, CAL, GI, PI and MSBI at 3 weeks does not shows statistically significant results. Intergroup comparison of parameters (PPD, GI, PI, CAL, mSBI) shows statistically significant results between the groups. Intergroup comparison of bromelain-quercetin gel and chlorhexidine gel of clinical parameters shows statistically significant results at baseline and at 3weeks.

Conclusion: The present study favors the use of combination therapy over SRP alone in order to achieve greater pocket depth reduction and greater attachment level gain.

Keywords: Scaling and Root Planning; Dysbiotic Disease; Periodontitis; Antimicrobials Agents

Introduction

Periodontitis a dysbiotic inflammatory disease that induces an irreversible immune state that leads to destruction of the supporting structure of the teeth. Management of periodontal disease through thorough removal of biofilm which is pivotal for having better oral health. Scaling and Root Planning (SRP) forms fundamental treatment for periodontitis [1]. Years of documented research have established that Chlorhexidine di-gluconate (CHX), as gold standard of chemical plaque control

agent safe, stable and effective in preventing and controlling plaque formation. A various medicinal herb have been used in periodontal therapy [2]. Bromelain is one such agent derived from *Ananas comosus* (popularly known as pineapple), contains proteinases that exhibits anti-inflammatory properties, antibacterial effect against periodontopathogens [1]. Bromelain gel has shown to have chemomechanical property for caries removal [3]. The bromelain ora-base gel evaluated in periodontitis showed a positive response for clinical parameters tooth mobility, gingival bleeding index and probing pocket depth, myeloperoxidase, malonaldehyde and alveolar bone loss of the oral tissues, preventing alveolar bone resorption caused by the disease, highlighting the potential as an adjuvant treatment of periodontitis, in addition to reducing the systemic effects on the liver tissue [4]. Quercetin, another phytochemical present in guava have excellent antibacterial actions against periodontal pathogens, *Aggregatibacter+ actinomycetemcomitans* (Aa), *Porphyromonas gingivalis* (Pg), *Prevotella intermedia* (Pi) and *Fusobacterium nucleatum* (Fn) [5]. 2% Quercetin has shown to be advantageous for subgingival application after scaling and rootplaning [5].

Recently, bromelain-quercetin, in quadruple therapy as adjunct effect in treatment of COVID-19 patients, has shown to have a potent anti-inflammatory action [6]. As there is lack of literature evaluating combination of bromelain-quercetin for treatment in oral cavity, we have used bromelain-quercetin intervention in periodontal therapy. This study is first of its kind to check the efficacy of 2% bromelain-quercetin gel and to compare its effects with 0.2% Chlorhexidine in Stage I/II and Grade B periodontitis.

Materials and Methods

Various adjuncts have been explored for their efficacy in restoring periodontal health. Both natural and synthetic material have been used natural variants. This prospective randomized controlled clinical trial has been done in Krishnadevaraya College of Dental Science and Hospital, Hunasamaranahalli, Bengaluru 562127. The present clinical trial has been done according CONSORT 2013 pro extended.

Ethical Statement

The study has been approved from Institutional Review Board and Ethical Committee (IRB) (Ref: KCDSHEC/IP/2023/V1/P4a). The study has been registered under clinical.gov with registration Identity number is NCT06775392.

The present study included preparation of Bromelain-Quercetin gel and Chlorhexidine gel at Department of Biotechnology, Sir M. Visvesvaraya Institute of Technology, Prepared gel was stored at room temperature before clinical analysis. Cytotoxicity of the prepared gel on human gingival fibroblast was done at Stroma biotechnology lab; Bangalore and the results show no cytotoxicity was observed on human gingival fibroblasts. Patients enrolled for the study were from Out-Patient Department of Periodontology, Krishnadevaraya College of Dental science and Hospital. The gel was used as local drug delivery, as part of non-surgical therapy in patient presenting with Periodontitis. Patients with Stage I/II and Grade B periodontitis were enrolled for the study based on the inclusion and exclusion criteria. The eligible subjects were informed in detail about the procedure and a written signed consent was obtained from each patient (Table 1).

Inclusion Criteria	Exclusion Criteria
1. Systemically healthy patients	1. Patients who have received any surgical or nonsurgical therapy during past 6 months
2. Patient having ≥ 20 teeth.	2. Pregnant or lactating females.
3. Patients with localized pockets probing depth of ≤ 5 mm	3. Use of systemic antibiotics in the past 6 months.
4. Patient who have stage I/II and grade B periodontitis in the age group between 30-50 years.	4. Patient who are not willing to give a written informed consent
5. Patient with radiographic evidence of horizontal bone loss in atleast two teeth	
6. Patients who are cooperative and able to attend the hospital for regular follow-up	

Table 1: Study criteria.

Study Design

A total of 30 probing sites in stage I/II and Grade B periodontitis patients with Pocket Probing Depth (PPD) ≤ 5 mm and radiographic evidence of horizontal bone loss were included. The sites selected were divided into groups

- Group I (Control) -10 Probing sites with probing depth of ≤ 5 mm were treated with SRP alone
- Group II -10 Probing sites with probing depth of ≤ 5 mm were treated with SRP followed by placement of bromelain-quercetin gel
- Group III -10 Probing sites with probing depth of ≤ 5 mm were treated with SRP followed by placement of chlorhexidine gel

Clinical parameters were recorded at baseline and at 3 weeks

- Plaque Index (PI) (silness and loe 1964) [7]
- Gingival Index (GI) (loe and silness 1963) [8]
- Modified Sulcular Bleeding index (MSB) [9]
- Probing Pocket Depth (PPD) of ≤ 5 mm recorded with UNC-15 probe
- Clinical Attachment Level (CAL) recorded with Williams graduated non-colour coded Periodontal probe

Clinical Procedure

All the patients presenting with stage-I/II and Grade B Periodontitis satisfying inclusion and exclusion criteria were subjected to clinical examination and scheduled for periodontal therapy. Clinical parameters were recorded and considered as baseline values. All patients underwent Non-Surgical Periodontal Therapy (NSPT) under oral hygiene phase. Scaling and root planing (SRP) with oral hygiene instructions were executed for all the included patients. A total of 30 probing sites were included for the study [10]. Probing sites were followed up for evaluation for 3 weeks. Remaining 20 probing sites underwent adjunctive therapy along with SRP. Twenty probing sites received Chlorhexidine gel and Bromelain-Quercetin gel as adjunct, each adjunct comprising of 10 sites. Each probing sites were evaluated at 3 weeks.

Local Drug Delivery

The medicament (Chlorhexidine and Bromelain-Quercetin gel) was placed at the depth of the pocket using tuberculin syringe with applicator tip. This was followed by placement of periodontal dressing (COE-PACK)TM. Patients were instructed following site-specific delivery to maintain oral health.

Post Operative Instructions

Patient were asked to refrain for 60 minutes with any oral activity. They were instructed not to brush the treated area till the removal of the periodontal dressing. Patient were recalled after 12 days for the removal of the periodontal dressing. All Patients was instructed to maintain oral hygiene at regular intervals and were evaluated at 21st day for re-recording the parameters and maintains hygiene by cleaning with dampened clean cloth.

Data Analysis

Statistical Package for Social Sciences [SPSS] for Windows Version 22.0 Released 2013. Armonk, NY: IBM Corp., will be used to perform statistical analyses.

Descriptive Statistics

Descriptive analysis of all the explanatory and outcome parameters will be done using mean and standard deviation for quantitative variables, frequency and proportions for categorical variables.

Inferential Statistics

One-way ANOVA test followed by Tukey's post hoc analysis / Kruskal Wallis Test followed by Dunn's post hoc test [based on data distribution] will be used to compare the mean clinical parameters between 3 groups. Repeated Measures of ANOVA Test / Friedman's Test followed by Wilcoxon Signed Rank Post Test was used to compare the mean clinical parameters between different time intervals in each group. The level of significance [P-Value] will be set at $P < 0.05$ and any other relevant test, if found appropriate during the time of data analysis will be dealt accordingly.

Results

The present study included preparation of the bromelain-quercetin gel and chlorhexidine gel at Department of Biotechnology, Sir M. Visvesvaraya Institute of Technology campus, Krishnadevaraya College of Dental science and Hospital, Bangalore-562157. Bromelain has shown to exhibit various fibrinolytic, antioedematous, antithrombotic, and anti-inflammatory activities both *in-vitro* and *in-vivo*. Ever since bromelain was known chemically, it has been used as a phyto medical agent [1]. Quercetin is a group of flavanols found in vegetables, fruits and nuts such as shallots, cranberries, blueberries, tomatoes, broccoli, and apples. It also provides a variety of health benefits and resistance to diseases including anti-carcinogenic, anti-inflammatory, antiviral, antioxidant, psychostimulant activity and also ability to inhibiting lipid peroxidation, platelet aggregation, capillary permeability and stimulation of mitochondrial biogenesis. Quercetin has been reported as anti-inflammatory substance that last long and have strong anti-inflammatory capacities. It has demonstrated antimicrobial activity that potentially decrease the inflammatory marker, cholesterol reduction and inhibit bone loss [14]. The following clinical parameters were recorded at baseline and at 21 days. Plaque Index (PI), Gingival Index (GI). Modified Sulcular bleeding index (MSB), Probing Pocket Depth (PPD) of ≤ 5 mm recorded with UNC-15 probe and Clinical Attachment Level (CAL) recorded at the selected probing site.

Means scores regarding PPD and GI at baseline shows statistically significant results between the groups whereas mean scores regarding PI, CAL and mSBI between three groups at baseline does not show statistically significant results (Table 2). Mean scores of PPD, CAL, GI, PI and mSBI at 3 weeks does not shows statistically significant results (Table 3). Intergroup comparison of PPD, GI, PI, CAL, MSBI shows statistically significant results between the groups (Table 4). Intragroup comparison of bromelain-quercetin gel and chlorhexidine gel of clinical parameters shows statistically significant results at baseline and 3 weeks (Table 5).

Variables		Range	Minimum	Maximum	Mean	SD	P-value
PPD	Group I	1.00	2.66	3.66	3.1130	.39460	0.03
	Group II	1.06	2.10	3.16	2.6870	.37175	
	Group III	.66	2.50	3.16	2.8470	.22647	
CAL	Group I	2.67	6.33	9.00	7.5620	.99653	0.08
	Group II	4.66	4.00	8.66	6.8160	1.43089	
	Group III	2.00	7.00	9.00	7.9400	.81677	
PI	Group I	.75	.25	1.00	.5250	.24861	0.36
	Group II	.75	.25	1.00	.5500	.25820	
	Group III	.75	.25	1.00	.6750	.23717	
GI	Group I	.50	.25	.75	.5750	.16874	<0.001
	Group II	.75	.00	.75	.4750	.21890	
	Group III	.75	.50	1.25	.9400	.27264	
MsBI	Group I	1.00	1.00	2.00	1.6000	.51640	0.69
	Group II	1.00	1.00	2.00	1.4000	.51640	
	Group III	1.00	1.00	2.00	1.5000	.52705	

Table 2: Comparison of mean score of different study variable at base line between three group.

Variables		Range	Minimum	Maximum	Mean	SD	P-value
PPD	Group I	1.17	1.33	2.50	1.9570	.44907	0.28
	Group II	1.20	1.10	2.30	1.8930	.35030	
	Group III	.83	1.33	2.16	1.7080	.24462	
CAL	Group I	2.67	4.66	7.33	5.8340	.99349	0.41
	Group II	4.66	3.00	7.66	5.8820	1.44700	
	Group III	2.03	5.30	7.33	6.4190	.61363	
PI	Group I	.25	.00	.25	.1000	.12910	0.23
	Group II	.75	.00	.75	.2000	.22973	
	Group III	.25	.00	.25	.0750	.12076	

GI	Group I	.25	.00	.25	.1750	.12076	0.27
	Group II	.25	.00	.25	.1500	.12910	
	Group III	.50	.00	.50	.2500	.16667	
MsBI	Group I	1.00	.00	1.00	.6000	.51640	0.27
	Group II	1.00	.00	1.00	.3000	.48305	
	Group III	2.00	.00	2.00	.7000	.67495	

Table 3: Comparison of mean score of different study variable at 3 weeks between three group.

Variables		Time	Mean	SD	SE	P-value
PPD	Group I	Baseline	3.113	0.3946	0.12478	<0.001
		3 weeks	1.957	0.44907	0.14201	
	Group II	Baseline	7.562	0.99653	0.31513	<0.001
		3 weeks	5.834	0.99349	0.31417	
	Group III	Baseline	0.525	0.24861	0.07862	<0.001
		3 weeks	0.1	0.1291	0.04082	
CAL	Group I	Baseline	0.575	0.16874	0.05336	<0.001
		3 weeks	0.175	0.12076	0.03819	
	Group II	Baseline	1.6000 ^a	0.5164	0.1633	<0.001
		3 weeks	.6000 ^a	0.5164	0.1633	
	Group III	Baseline	2.687	0.37175	0.11756	<0.001
		3 weeks	1.893	0.3503	0.11078	
PI	Group I	Baseline	6.816	1.43089	0.45249	<0.001
		3 weeks	5.882	1.447	0.45758	
	Group II	Baseline	0.55	0.2582	0.08165	0.001
		3 weeks	0.2	0.22973	0.07265	
	Group III	Baseline	0.475	0.2189	0.06922	<0.001
		3 weeks	0.15	0.1291	0.04082	
GI	Group I	Baseline	1.4	0.5164	0.1633	<0.001
		3 weeks	0.3	0.48305	0.15275	
	Group II	Baseline	2.847	0.22647	0.07162	<0.001
		3 weeks	1.708	0.24462	0.07736	

Table 4: Comparison of mean score of different study variable at baseline and 3 weeks within each study group.

Variables		Time	Mean	SD	SE	P-value
PPD	Group II	Baseline	2.6870	0.37175	0.11756	<0.0001
		3 weeks	1.8930	0.35030	0.11078	
	Group III	Baseline	2.8470	0.22647	0.07162	<0.0001
		3 weeks	1.7080	0.24462	0.07736	
CAL	Group II	Baseline	6.8160	1.43089	0.45249	0.001
		3 weeks	5.8820	1.44700	0.45758	
	Group III	Baseline	7.9400	0.81677	0.25828	<0.0001
		3 weeks	6.4190	0.61363	0.19405	
PI	Group II	Baseline	0.5500	0.25820	0.08165	<0.0001
		3 weeks	0.2000	0.22973	0.07265	
	Group III	Baseline	0.6750	0.23717	0.07500	<0.0001
		3 weeks	0.0750	0.12076	0.03819	

GI	Group II	Baseline	0.4750	0.21890	0.06922	<0.0001
		3 weeks	0.1500	0.12910	0.04082	
	Group III	Baseline	0.9400	0.27264	0.08622	<0.0001
		3 weeks	0.2500	0.16667	0.05270	
SBI	Group II	Baseline	1.4000	0.51640	0.16330	<0.0001
		3 weeks	0.3000	0.48305	0.15275	
	Group III	Baseline	1.5000	0.52705	0.16667	0.003
		3 weeks	0.7000	0.67495	0.21344	

Table 5: Comparison of mean score of different study variable at baseline and 3 weeks within each study group.

Discussion

Ramification of periodontal disease fueled by dysbiosis results in adverse clinical consequences graded as Periodontitis. Management of periodontal disease has been focused fundamentally toward the disruption of plaque micro-flora which involves the mechanical therapy and use of antimicrobial agents. Wide range of antibiotics have been tried and tested against periodontal pathogens. However, due the side effects of various antimicrobial drugs and development of various antibacterial resistant strains of microorganism, its use has been a challenge [1]. Hence, to overcome the shortcomings of antimicrobials and to increase patient acceptance, a wide array of herbal products and plant extracts have been tried and tested. As there is growing evidence on use of herbal agents as adjunct to Non-Surgical Periodontal Therapy (NSPT), the present study utilized the advantages of Bromelain-Quercetin combination gel and have prepared and clinically compared with chlorhexidine gel. Scaling and Root Planing (SRP) alone was used as control group [14].

Pineapple extract (bromelain) is one such product. Pineapple is the universal name of *Ananas comosus*. It belongs to the member of the family bromeliaceae, which is grown in a number of subtropical and tropical countries including, India. The medicinal property of pineapple is due to bromelain which is a rough aqueous extract from stem and fruit of pineapple. It has been used in dentistry as an chemo-mechanical, anti-bacterial anti-inflammatory and analgesic drug [1,3,4]. Another potent plant extract is 'Quercetin', a natural plant-derived from dietary polyphenol obtained from Guava possesses high safety profile and extensive beneficial properties including potent antioxidant, anti-inflammatory, anti-cancer, antiviral, anti-hypertensive and anti-aging effects [10-13]. Quercetin has been used to improve the disease outcomes in several disorders including rheumatoid arthritis, neuroinflammation and gastrointestinal disorders. Similarly, emerging evidence also indicates the oral-protective properties of Quercetin [14]. We have used combination of bromelain-quercetin gel as LDD in our study for its beneficial effects, as adjunct to SRP in disease specific site as test sites compared with 0.2 % chlorhexidine gel application as control group. According to Praveen NC, et al., in their study on *Streptococcus. mutans* showed sensitivity at the lowest concentration of 2 mg/ml as compared to *E. faecalis* (31.25 mg/ml) while *P. gingivalis* showed sensitivity at the lowest concentration of 4.15 mg/ml as compared to Aa (16.6 mg/ml) with bromelain. Therefore, Bromelain exerts an antibacterial effect against potent periodontal pathogens; hence, it may be used as an antibacterial agent [1]. in our study we have considered application bromelain-quercetin (2%) and chlorhexidine gel (0.2%) following SRP in probing dept ≤ 5 mm as LDD. Our study demonstrated statistically significant improvement in all the parameters.

Hossein, et al., in their study on One-week treatment with Anaheal (bromelain) at a dose of 1 g/d after pocket elimination surgery resulted in significantly lower BOP compared to placebo [15,16]. In our study, we have used Bromelain-Quercetin gel as local drug delivery agent for its antibacterial action. According to Sibarani Mega, et al., evaluated the effect of 2% quercetin gel subgingival application after scaling and root planing on IL-6 concentration of chronic periodontitis patients. The study concluded that quercetin gel application reduced IL-6 concentration in gingival crevicular fluid of chronic periodontitis [3]. In our study, we have used Bromelain-Quercetin gel as LDD for its antibacterial action results in PPD reduction and CAL gain. Mooney, et al., study on Quercetin supplement instigates a balanced periodontal tissue homeostasis through limiting inflammation and fostering an oral cavity microenvironment conducive of symbiotic microbiota associated with health [14]. Our study on Bromelain-Quercetin gel on sub gingival application had significant gain in attachment. Amr K Ahmed, et al., study on Quadruple therapy consisting of Zinc, Quercetin, Bromelain and Vitamin C showed promising results in improving clinical outcome among COVID-19 patients. Present study shows that the treatment of periodontal pockets with chlorhexidine and bromelain-quercetin gel in the maintenance phase provides a significantly greater improvement in clinical parameter when

compared with the improvement obtained with SRP alone. There was significant reductions observed in plaque and gingival scores following periodontal intervention. Lower plaque and gingival index scores observed in the test group may also be the result of the antiplaque and antibacterial effect.

Conclusion

The results in the present study thus favor the use of combination therapy over SRP in order to achieve greater pocket depth reduction and greater attachment level gain. The local drug delivery is effective in improving the clinical parameters even after 3 weeks following SRP, when the pockets are stabilized.

Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

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Data Availability Statement

Not applicable.

Ethical Statement

The project did not meet the definition of human subject research under the purview of the IRB according to federal regulations, and therefore, was exempt.

Informed Consent Statement

Informed consent was taken for this study.

Authors' Contributions

All authors contributed equally to this paper.

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