Original Article

Assessment of benzyl isothiocyanate as an adjunct to conventional periodontal therapy

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Abstract:

Background: Conventional nonsurgical periodontal therapy eliminates the pathogenic microbes, yet residual deposits promote the recurrence of the disease. As antimicrobials may pose undesirable effects, alternate therapies are probed. Aim: The aim of the study is to evaluate the efficacy of locally delivered benzyl isothiocyanate (BITC) as an adjunct to scaling and root planing to treat patients with chronic periodontitis. Materials and Methods: The study included 30 patients diagnosed with chronic periodontitis. Test (scaling and root planing along with BITC intervention) and control (scaling and root planing) sites were randomly assigned to each patient. These sites were in the contralateral quadrants, having a probing depth of 4–6 mm. The plaque index (PI), gingival index (GI), pocket probing depth (PPD), clinical attachment level (CAL), and microbial load (colony forming unit [CFU]) were assessed at baseline, 1-week, and 6-week time interval. Data were analyzed by ANOVA/Friedman test, Mann–Whitney U-test, pairwise paired t-test, and Wilcoxon test, with P ≤ 0.05 set as statistically significant. Results: The scores of PI, GI, PPD, and CAL from baseline to 6-week follow-up within both the test and control sites were noted to be statistically significant (P < 0.0001). The CFU showed a significant reduction (P = 0.0229) within the test site at varying time intervals. The change in the mean PI score from baseline to 6-week time interval between the test and control site was noted to be statistically significant (P = 0.0039). Conclusion: The local application of BITC chips effectively reduced the PI, GI, PPD, and CFU, subsequently with the gain in CAL, and improved the tissue integrity and thereby oral hygiene.

Key words:

Benzyli isothiocyanate, chronic periodontitis, oral hygiene, root planing

INTRODUCTION

Periodontitis is caused by a specific or a group of microorganisms, resulting in the progressive destruction of the periodontal ligament and the alveolar bone with periodontal pocket formation, gingival recession, or both. It is precipitated during an interplay of bacterial challenge and host response. It is modified by environmental, acquired risk factors and genetic susceptibility. Anaerobes, facultative aerobes, microaerophiles, or capnophiles are the periodontal pathogens identified. Common bacterial species in the periodontal environment (Actinomyces, certain Streptococcus, and Staphylococcus spp.) can provoke opportunistic infections creating a disturbance in the microbial milieu. While localized periodontitis is strongly associated with the presence of Actinobacillus (Haemophilus) actinomycetemcomitans, Eikenella corrodens, and Capnocytophaga, a major part of periodontitis has no correlation with a definite composition of the bacterial flora.

Treating periodontitis is a challenge because the infection occurs due to a bacterial biofilm, which could be highly resistant to antimicrobials and host response. A thorough elimination of the bacteria from the periodontal cavity is a tedious task and the bacteria could remain in the oral cavity, due to which a chance of recurrence is a possibility. The severity of the disease is mainly dependent on the bacterial components present as well as on the host response.

Either surgical or nonsurgical treatment could be followed to achieve these goals, depending upon the severity of the disease. Scaling and root planing, considered a “gold standard” method, effectively reduces the microbial level in the periodontal pocket and improves the clinical parameters such as bleeding on probing and probing depths and clinical attachment level (CAL).

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Chemotherapeutic agents administered either systemically or incorporated locally at the site of inflammation could hasten the treatment process, especially in case of microbial infections. However, they may cause undesirable adverse effects such as hypersensitivity, gastrointestinal intolerance, and development of bacterial resistance. To overcome the side effects of these synthetic molecules, recent research has explored the therapeutic application of natural products such as herbs and plant extracts.

Miswak (Salvadora persica) root and bark have been traditionally used over 1000 years as a chewing stick or natural toothbrush to strengthen the gums, prevent caries, clean the mouth, whiten the teeth, and sweeten the breath and is widely used throughout India, Africa, and Arab nations. Miswak extracts possess various biological properties, containing significant antifungal, antibacterial, antiviral, analgesic effect, anti-inflammatory, and hypoglycemic effect.

The antibacterial property of S. persica is mainly attributed to one active component called benzyl isothiocyanate (BITC). It penetrates the outer bacterial membrane and interferes with the bacterial redox systems. BITC is reported to be active against Gram-negative periodontal pathogens such as Aggregatibacter actinomycetemcomitans and Porphyromonas gingivalis. It was reported to have moderate inhibition against four oral bacteria, namely P. asaccharolytica, P. gingivalis, Streptococcus sobrinus, and Streptococcus mutans.

Failure of the active component to reach an adequate concentration at the site of action is due to its inability of being retained locally for an adequate time period. The incorporation of antimicrobial agents into controlled release delivery systems, facilitating their placement directly into the periodontal pocket, could be advantageous. Chitosan is considered excellent to be used as a drug delivery vehicle because of it is highly stable, biodegradable, nontoxic, permeation, improving factors and ease of availability.

Multiple in vitro-based studies in the literature endorse using BITC to treat periodontitis. A dearth of studies correlating these findings among clinical scenarios was observed. Thus, the present study proposed to evaluate the clinical and microbiological effectiveness of locally delivered BITC incorporated in miswak as an adjunct to scaling and root planing in treating patients with chronic periodontitis.

**MATERIALS AND METHODS**

A total of 30 participants, aged 35–55 years who were diagnosed with chronic periodontitis (as per the American Academy of Periodontology 1999 classification), were included in the study. The sample size was calculated as per the proportionality formula based on previous literature. Considering the effect size of 0.40, significance level of 0.05, and power of the study as 0.80, a sample size of 30 was obtained. This was conducted at a tertiary care center, during the year 2016–2018, after acquiring approval from the institutional ethics committee and informed consent from the patients participating in the study. This was a double-blinded study and one examiner was involved in recording the observations.

Patients with a pocket probing depth (PPD) of 4–6 mm in the contralateral quadrants, those without any systemic disorders, and those who had not undergone any form of nonsurgical or surgical periodontal therapy in the past 6 months were included in the study. The study excluded patients with a history of antibiotics and nonsteroidal anti-inflammatory drug use in the past 6 months, pregnant and lactating females, smokers, consuming tobacco in any form, those with <20 scorable teeth, and those with a history of allergy to Miswak.

Two sites in each patient were randomly selected (computer-based software) in the contralateral quadrants, with a probing depth of 4–6 mm. A split-mouth design was followed to reduce bias influenced by patient variation. The groups were delineated as follows: test sites – scaling and root planing was performed along with the placement of local drug delivery chips containing BITC; control site – scaling and root planing alone was performed [Figure 1a and b].

A pilot study was conducted to determine the minimum inhibitory concentration (MIC) of BITC (Sigma Aldrich, Germany) against S. mutans using the brain–heart infusion media. After 24 h incubation at 37°C, the MIC was defined as the lowest concentration of BITC which prevents visible growth of bacteria.

The BITC-chitosan chips were prepared by mixing 600 µg of chitosan powder (HiMedia Laboratories) in 20 ml of 1% acetic acid with 250 µg/ml BITC solution. This homogenous mixture was poured onto a Petri dish and left for drying overnight at room temperature. The BITC-chitosan chips measuring 4 mm × 5 mm were cut, sterilized by ultraviolet radiation, and stored at room temperature [Figure 2a and b].

Sites with 4–6 mm pocket depth were randomly chosen as test and control sites. Clinical parameters were assessed, and plaque samples were procured for microbiological analysis from both the sites. Supragingival plaque was removed before the plaque collection to avoid contamination or dilution of the subgingival sample.

The samples were then transferred to a microcentrifuge tube containing 0.5 ml of reduced transport fluid and the culture was prepared. Following which, a full-mouth thorough scaling and root planing was carried out. The test site was then isolated and tin foil of the size of the BITC-chitosan chip was inserted in the pocket to verify proper subgingival placement of the chip. The chip was then trimmed according to the tin foil measurement and inserted deep in the periodontal pocket such that the chip rested subgingivally at the base of the pocket and was not exposed. A periodontal dressing, COE-PAK, was placed over both the test and control sites [Figure 2c and d]. Patients were instructed not to brush over the pack and to follow modified bass technique for brushing the remaining teeth. Patients were also advised not to use any other plaque control methods. Patients were recalled after 1 week and 6 weeks for a follow-up evaluation.

All clinical parameters were recorded preoperatively at baseline and postoperatively 1 week and 6 weeks posttreatment. The plaque index (PI) was selected for this study as it was easy to assess, reliable, and more objective. The clinical scoring...
procedure used to assess supragingival plaque formation in the gingival third was a modification of the Quigley-Hein (Turesky Modification) plaque scoring index. Quantitative assessment of the disclosed plaque was done to evaluate the extent of plaque over the tooth area without disturbing the plaque using a dental probe as done in other plaque indices.[20] The gingival index (GI) was used in the study since the sensitivity and reproducibility of this index is known to be good. The scoring system for the gingiva was based as per Löe and Silness.[21] Conventional procedure was followed, and the scores were obtained. Pressure-sensitive probe (Hawe Click probe, Kerr company, Switzerland) was used to measure the PPD (the distance from the gingival margin to the sulcus depth) and CAL (measures the position of the soft tissue in relation to cementoenamel junction [CEJ] – in this case, distance from CEJ to the sulcus depth was measured).[22]

Plaque samples collected at varying time intervals from both test and control sites were cultured using lawn streak method on blood agar plates for 18–24 h (work carried out at Dr. Prabhakar Kore Basic Science Research Centre, Belagavi). The colonies formed were identified based on morphological/staining (gram/lactophenol cotton blue) characteristics. Colonies were counted manually to determine the colony forming units (CFUs).[22]

Data were analyzed using R v386 3.6.0 software CRAN package. Continuous data were represented in the form of mean ± standard deviation, and the categorical variables were represented as frequency (%). Within-group comparison for the clinical and microbiological parameters was done using repeated-measure ANOVA/Friedman test. Pairwise paired t-test and pairwise paired Wilcoxon test with Bonferroni correction were considered as post hoc test for repeated-measure ANOVA and Friedman test, respectively. Between-group comparison was done using t-test/Mann–Whitney U-test. The change in the values from baseline to subsequent follow-ups was compared separately for each site using pairwise Wilcoxon sign-rank test. Level of significance was set at \( P \leq 0.05 \).

RESULTS

The study included 30 participants, aged 35–55 years, diagnosed with chronic periodontitis. The CONSolidated Standards of Reporting Trials flow diagram for the study is represented in Figure 3. The MIC obtained against \textit{S. mutans} in this study was 1.56 \( \mu \)g/ml. BITC was incorporated within chitosan which was used as a vehicle to release the drug in a sustained mode for 8–10 days.

On comparing the parameters within the test and control sites, a decrease in the scoring was noted from the baseline to 6-week time interval. Regarding the parameters in the test site, the mean PI score \( (P < 0.0001) \), mean GI score \( (P < 0.0001) \), mean PPD \( (P < 0.0001) \), mean CAL \( (P < 0.0001) \), and mean CFU \( (P = 0.0229) \) reduced significantly. Similar significant reduction in the scores regarding the parameters in the control sites with the mean PI \( (P < 0.0001) \), mean GI \( (P < 0.0001) \), mean PPD \( (P < 0.0001) \), mean CAL \( (P < 0.0001) \) from baseline to 6-week time interval was noted. Significant difference in the PI \( (P = 0.0087) \) score and CFU \( (P = 0.0152) \) count between the test and control site was noted during the 6th week follow-up [Table 1 and Figure 4].

![Figure 1](image1.png)

**Figure 1**: (a) Chronic periodontitis and (b) postscaling and root planing

| Parameter | Parameter time | Test site | Control site | \( P^a \) |
|-----------|----------------|-----------|--------------|---|
| PI (score) | Baseline | 4.03±0.65 | 3.90±0.76 | 0.4848\( ^* \) |
|           | 1 week     | 1.07±0.64 | 1.20±0.61 | 0.4165\( ^* \) |
|           | 6 weeks    | 1.53±0.68 | 1.97±0.61 | 0.0087\( ^* \) |
| GI (score) | Baseline | 2.47±0.63 | 2.60±0.50 | 0.4746\( ^* \) |
|           | 1 week     | 0.83±0.65 | 0.90±0.66 | 0.6963\( ^* \) |
|           | 6 weeks    | 1.02±0.61 | 1.33±0.61 | 0.0620\( ^* \) |
| PPD (score) | Baseline | 5.1±0.76 | 5.0±0.64 | 0.5598\( ^* \) |
|           | 1 week     | 4.17±0.83 | 4.20±0.85 | 0.8489\( ^* \) |
|           | 6 weeks    | 4.20±0.85 | 4.33±0.99 | 0.4108\( ^* \) |
| CAL (score) | Baseline | 5.4±1.13 | 5.13±1.07 | 0.3517\( ^* \) |
|           | 1 week     | 4.60±1.16 | 4.43±1.10 | 0.6459\( ^* \) |
|           | 6 weeks    | 4.6±1.16 | 4.57±1.28 | 0.9022\( ^* \) |
| CFU (counts) | Baseline | 148.23±72.67 | 170.70±83.70 | 0.2715\( ^* \) |
|           | 1 week     | 130.77±76.43 | 156.30±88.01 | 0.2351\( ^* \) |
|           | 6 weeks    | 107.17±69.31 | 154.03±75.64 | 0.0152\( ^* \) |

\( ^* \)Significant \( (P \leq 0.05) \); \( P^a \) within group analysis; \( P^b \) between group analysis; \( P^c \) Mann–Whitney U-test; \( P^d \) t-test; \( P^e \) Repeated ANOVA; PI – Plaque index; GI – Gingival index; PPD – Pocket probing depth; CAL – Clinical attachment level; CFU – Colony forming unit; SD – Standard deviation; \( P \) – Probability value

![Figure 2](image2.png)

**Figure 2**: (a) Chitosan incorporated with benzyl isothiocyanate; (b) benzyl isothiocyanate-chitosan chips 4 mm x 5 mm size; (c) placement of benzyl isothiocyanate chip at test site and; (d) COE-PAK placement at test site
The distribution of PI score was significantly different for at least 2 time points in both the test and control sites and hence was inferred that the PI distribution significantly changed over time points in both the sites ($P < 0.05$). The distribution of the GI score at baseline was significantly different from the 1st week ($P < 0.0001$) and 6th week ($P < 0.0001$) in both the sites. The median of GI score was noted to be significantly different between the 1st week and 6th week in the control site ($P = 0.0027$) but not in the test site ($P = 0.2$). The distribution of PPD and CAL score at baseline was significantly different from the 1st week ($P < 0.0001$) and 6th week ($P < 0.0001$) in both the sites, but both PPD and CAL distribution was not significantly different between 1st week and 6th week in the test ($P = 0.545$) and control ($P > 0.99$). The mean CFU count at 6th week was significantly different from baseline in the test site ($P = 0.016$), whereas the mean CFU count at 1st week was not significantly different from mean of CFU count at baseline ($P = 0.763$) and 6th week ($P = 0.371$) in the test site.

The change in the scores of PI, GI, PPD, CAL, and CFU from baseline to 1st week, baseline to 6th week, and 1st–6th week between the test and control sites is represented in Table 2. On comparing the scores, only the change of the mean PI score from baseline to 6-week time interval between the test (61.98%) and control (49.57%) site was noted to be statistically significant ($P = 0.0039$).
The bacteria on blood agar plates were identified as *Streptococcus* spp., *Actinomyces* spp., *Diphtheroids*, and Gram-negative bacilli [Figure 5].

**DISCUSSION**

Chronic periodontitis is a multifactorial inflammatory disease that affects the supporting structures of the teeth. The use of local drug delivery with antibiotics within the periodontal pocket has shown promising results in case of mild and moderate chronic periodontitis. In the arena of local drug delivery, the literature is replete with the application of herbs such as curcumin, green tea, pomegranate, aloe vera, and neem.[9] Owing to the paucity of studies with BITC, this study focused on evaluating the therapeutic benefits of locally delivered BITC incorporated in chitosan, as an adjunct to scaling and root planing in patients with periodontitis. Clinical and microbiological parameters such as PI, GI, PPD, CAL, and CFU at varying time intervals (baseline, 1 week, and 6 weeks) were evaluated at the test and control sites.

The pilot study conducted determined the MIC of BITC against *S. mutans* as 1.56 µg/ml. A comparable result was observed in a study by Dufour et al., with the MIC ranging between 1.25 and 5 µg/ml.[10] Following this, BITC was incorporated within chitosan that was used as a vehicle allowing the drug to be released in a sustained mode for 8–10 days based on a study reported by Ahmed et al.[13] The concentration of BITC used in this study was approximately 150 times higher than its MIC. It was chosen so because, the earlier conducted biofilm experiments indicated that the necessary MIC of antimicrobial agents should be at least 50 times or even up to 210,000 times higher than for bacteria growing under planktonic conditions.[24]

The scores of PI, GI, PPD, and CAL from baseline to 6-week follow-up time within both the test and control sites were noted be significant (*P* < 0.0001). Similar observations of the clinical and microbial parameters either due to herbal drugs or laser therapy used to treat chronic periodontitis have been reported by Rathod *et al.* (*P* < 0.01) and Saglam *et al.* (*P* < 0.001) after 3 months of follow-up.[25,26] The reduction of the PI scores in the test sites can be attributed to the effective sustained release of BITC from chitosan chip which helped in eradicating biofilm bacteria from areas inaccessible by conventional therapy. This bacterial reduction was in accordance to the earlier studies, where the influence of sustained release of drugs from chitosan was proved to be useful in periodontal therapy.[17,27] The reduction of GI scores could be attributed to the anti-inflammatory property of BITC.[28] The reduction in the PPD was due to both gain in CAL and reduction in the swelling of the marginal gingiva as the inflammation reduced.[29] The difference in the PI, GI, PPD, and CAL scores between the test and control sites at varying time intervals was noted to be insignificant (*P* > 0.05), except during the 6th week follow-up with respect to PI score (*P* = 0.0087). Statistically insignificant observations between the test and control sites have been reported earlier by Saglam *et al.* (*P* > 0.05) for PI, GI, PPD, and CAL.[28] While considering the change in scores at varying time intervals, only the PI score was noted to be significant.

| Parameter | Change from baseline to 1st week | Change from baseline to 6th week | Change from 1st to 6th week |
|-----------|---------------------------------|---------------------------------|---------------------------|
| PI (score) | Test site: -2.97±0.72 (−73.55) | Control site: -2.70±0.53 (−69.23) | 0.1385† |
| GI (score) | Test site: -1.63±0.67 (−66.21) | Control site: -1.70±0.70 (−65.38) | 0.2754† |
| PPD (score) | Test site: -0.93±0.45 (−18.30) | Control site: -0.80±0.55 (−16.00) | 0.0653† |
| CAL (score) | Test site: -0.8±0.48 (−14.81) | Control site: -0.7±0.47 (−13.64) | 0.0775† |
| CFU (counts) | Test site: -17.47±82.26 (−11.78) | Control site: -14.40±96.24 (−8.44) | 0.8949† |

*†Significant (P≤0.05); *Mann-Whitney U-test; †T-test. PI – Plaque index; GI – Gingival index; PPD – Pocket probing depth; CAL – Clinical attachment level; CFU – Colony forming unit; sd – Standard deviation; *P* – Probability value

**Figure 5:** Microbial colonies acquired from test and control sites at varying time intervals
from baseline to 6-week follow-up ($P = 0.0039$), comparable to the findings of Hattarki et al. ($P = 0.000$), where the mean reduction in PI scores was significant from baseline to 6-week follow-up.$^{[20]}$

While performing the CFU count, the bacteria identified belonged to Streptococcus spp., Actinomycetes spp., Diphtheroids, and Gram-negative bacilli. The analysis of the microbial CFU showed a significant reduction ($P = 0.0229$) within the test site at varying time intervals but not in the control site ($P = 0.4905$). This shift in the microbiota at different time intervals was comparable to findings reported by Sağlam et al., where significance was noted within the test ($P = 0.001$) and control ($P = 0.01$) sites.$^{[28]}$ The difference in the CFU count between the two sites was significant ($P = 0.0152$) during the 6th week follow-up. The reduction of the CFU in test group is due to the antibacterial action of BITC and also that BITC might penetrate through the outer bacterial membrane and possibly interfere with the bacterial redox systems, thus hampering with the ability of the bacteria to maintain its membrane potential.$^{[10,11]}$

This pioneer study has validated the positive influence of BITC-incorporated chitosan chips as an adjunct to scaling and root planing and thus has added new insights to the existing literature. This method was proven to be safe and effective within 6 weeks of follow-up mainly attributed to the medicinal properties of BITC. Further, the reduction in the CFU that was observed endorsed the absence of recurrent infections.

Limitations of the study were smaller sample size and the patient’s follow-up was not carried out during the maintenance period. Future studies could include larger cohorts and vary the concentration of BITC, to arrive at the precise therapeutic concentration. The application of BITC-incorporated chitosan chip could be further evaluated against anaerobic microflora along with a long-term follow-up.

CONCLUSION

The use of local drug delivery system containing BITC incorporated in chitosan had a synergistic effect to scaling and root planing for the treatment of patients with chronic periodontitis, due to its antibacterial, anti-inflammatory, and wound-healing properties. The sustained release action of the drug allowed the eradication of bacteria from inaccessible areas in the subgingival environment. It resulted in the reduction of PI, GI, PPD, and microbial CFUs. A subsequent gain in the CAL was noted, resulting in improved oral hygiene status. This inferred that BITC chip is a valuable adjunct to treat patients with chronic periodontitis.

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Conflicts of interest
There are no conflicts of interest.

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