Clinical and Microbiological Effects of Subgingival Irrigation with Propolis Extract (Propolis Platinum™) and Chlorhexidine (Periogard®) as an Adjunct to Scaling and Root Planing in Patients Affected with Chronic Periodontitis – A Comparative Study

ABSTRACT

Background  Conventional periodontal therapy of plaque removal such as scaling and root planing is inefficient in complete removal of plaque and bacteria from deeper pockets. Subgingival irrigation has been shown to reduce dental plaque, gingivitis, bleeding, probing depth, and periodontal pathogens.

Aim  To assess the clinical and antimicrobial efficacy of Propolis (Propolis Platinum™) and Chlorhexidine (Periogard®) as a subgingival irrigant in the management of patients affected with chronic periodontitis.

Materials and Methods  A total of 45 patients of both sexes, ranging from 30 to 55 years with pocket depths ≥5 mm with chronic periodontitis were divided into three groups (Group A, B & C) of 15 patients each. Plaque Index (PI), Gingival Index (GI), Sulcus Bleeding Index (SBI), Pocket Probing Depth (PPD), Relative Attachment Level (RAL) and Microbial Colony Count (MCC) were recorded at baseline visit and 6 weeks interval. Complete scaling and root planing was performed in all patients at baseline followed by subgingival irrigation with Normal Saline in Group A, Propolis in Group B & Periogard Group C.

Statistical Analysis  The results were compiled and statistically analysed to get a meaningful conclusion using Wilcoxon Sign Rank test for intragroup comparison and Mann–Whitney U test for intergroup comparison.

Results  Group B & C showed highest reduction in microbial colony counts and improvement in all clinical parameters as compared to Group A.

Conclusion  Subgingival irrigation with propolis extract and periogard as an adjuvant to periodontal treatment was more effective than scaling and root planing as assessed by clinical and microbiological parameters.

KEYWORDS  chronic periodontitis, propolis, periogard, subgingival irrigation

INTRODUCTION

Periodontitis is one of the most common chronic inflammatory diseases of adults. Pathogenesis of periodontitis involves the presence of bacterial plaque that inhibits a local inflammatory response in host that leads to edema, leukocyte infiltration and release of inflammatory mediators. These inflammatory changes cause periodontal pocket formation, connective tissue detachment and alveolar bone resorption, ultimately leading to tooth loss. Subgingival irrigation attempts to directly reduce pocket microflora to prevent initiation of periodontal diseases.
Various agents are used for subgingival irrigation namely chlorhexidine, povidone iodine, stannous fluoride, metronidazole, sanguinarine, tetracycline and hydrogen peroxide. Various herbal extracts have been tested for their antimicrobial, analgesic, hemostatic, antibacterial, anti-inflammatory, antifungal and antiviral activity e.g. propolis, turmeric, neem, menthol etc. Chlorhexidine digluconate is a biguanide compound that was introduced in the United Kingdom in 1954 by Davies et al. as disinfectant and topical antisepitic. It is most effective and safest antiplaque agent. It has broad antimicrobial spectrum active against both gram positive and gram negative bacteria which acts bacteriostatically at low concentration and bactericidal at higher concentration. Periogard (Periogard Colgate Oral Pharmaceuticals, Subsidiary of Colgate-Palmolive Company, 300 Park Avenue, New York, NY 10022) oral rinse provides antimicrobial activity during oral rinsing. Microbiological sampling of plaque has shown a general reduction of counts of certain bacteria, both aerobic and anaerobic, ranging to $54\text{-}97\%$ through 6 months use.

Propolis (Propolis Platinum™, K-Link Healthcare India Pvt. Ltd. Chennai. Manufactured by Yi Wang Honey Garden (m) SDN BHD, Selangor Darul Ehsan, Malaysia) is a natural antibiotic with antibacterial, anti-inflammatory, antiseptic, antymycotic, antipsoriatic and bacteriostatic properties. Propolis reportedly acts on the bacterial cell walls and their specific components such as lipopolysaccharides reducing the protein content of oral biofilm.

Propolis extracts are effective in inhibiting growth and adherence of Streptococcus mutans, Porphyromonas gingivalis, Prevotella intermedia, Fusobacterium species, Capnocytophaga species and Eikenella species. So, the aim of this present study is to evaluate clinically and microbiologically, the efficacy of subgingival irrigation with propolis extract (Propolis Platinum™) and chlorhexidine (Periogard®) in patients affected with chronic periodontitis.

**MATERIALS AND METHODS**

**Selection of subjects**

A total of 45 patients of both sex of age group 30–55 years suffering from chronic periodontitis were selected. The research protocol was initially submitted to the Ethical Committee. After ethical approval, all subjects were verbally informed and written informed consent was taken for participation in the study.

**Inclusion criteria**

Chronic periodontitis patients with pocket probing depth $\geq 5$ mm; patients who had not taken any chemotherapeutic mouth rinse and oral irrigation during past three months; patients with no history of systemic antibiotic intake for the last three months; patients without any systemic disorder.

**Exclusion criteria**

Smokers/tobacco users, patients with drug/alcohol abuse, pregnant/lactating women; patients who had received any surgical/non surgical therapy three months prior to study; patients who were allergic to Propolis and chlorhexidine.

**METHODS (STUDY DESIGN)**

A randomized single blind design was used. After education and motivation, each patient was put on mechanical plaque control measures followed by scaling using hand scalers and curettes.

The study was divided into three groups of 15 patients each:

- **Group A**: Scaling and root planing followed by subgingival irrigation with Normal Saline (control group).
- **Group B**: Scaling and root planing followed by subgingival irrigation with Propolis Platinum™.
- **Group C**: Scaling and root planing followed by subgingival irrigation with PerioGard®.

**Preparation of propolis ethanolic solution from propolis extract**

Propolis ethanolic solution was prepared for subgingival irrigation, by mixing propolis extract with 99.9% ethanol in ratio of 1:2 (1 part propolis extract and 2 parts of ethanol) (Fig. 1).

![Fig. 1 Prepared ethanolic solution of Propolis.](image)
**Procedure of subgingival irrigation**

After recording the clinical and microbiological parameters at baseline, complete scaling and root planing were performed. After scaling and root planing, the area was irrigated subgingivally with 3 ml of Normal Saline in Group A (control group); 3 ml of propolis ethanolic solution in Group B and 0.12% chlorhexidine in Group C; delivered via a syringe, by inserting it into the deepest part of the pocket (Fig. 2). Subgingival irrigation was done twice a week for 2 weeks at an interval of 4 days. Each site received subgingival irrigation of 12 ml.

**Microbiological sampling**

Microbiological sampling was done at baseline and after 6 weeks. After clinical assessment, the site selected for sampling was dried and isolated. The site was sampled for subgingival microflora with a sterile curette. Each sample was taken by insertion of a sterile curette in the pocket depth and was left for about 30 seconds. The curette was then removed and inserted in a glass tube containing 5 ml of thioglycollate broth. (Fig. 3a and b) The specimen was immediately transported for microbiological analysis.

**Procedure for culturing of microbiological sample**

Inoculation using streak method

0.004 ml of the thioglycollate broth was inoculated on two petri dishes containing sheep blood agar using the streak method.

Preparation of blood agar. Blood agar base is specially devised to permit the maximum recovery of Streptococci, Pneumococci and other fastidious pathogenic organisms without interfering with their haemolytic reactions. Blood agar was prepared by adding 5–10% sterile sheep blood to sterile nutrient agar that was melted and cooled to 50°C.

Incubation

1) One of the inoculated blood agar medium was incubated anaerobically for 48 hours in an anaerobic jar and the anaerobic conditions were maintained in the jar by placing anaerobic gas pack (HiMEDI, HiMedia Anaerogas Pack 3.5 L, HiMedia Laboratories Pvt. Ltd. Mumbai-4000086, India.) and tightly screwing the lid.

2) The other plate was incubated aerobically in the bacteriological incubator at 37°C for 48 hours.

3) Two nutrient agar plates inoculated with *Pseudomonas aeruginosa* were used as control. These nutrient agar plates were kept along with the test specimen in anaerobic jar and bacteriological incubator.

**Colony counting.** Colony count was done from both plates using the colony counters and the microbial colony count was calculated to obtain the total bacterial count.

**Statistical analysis**

The recorded data were compiled and analysed using SPSS version 19.0. Intergroup comparison of Group A & B, Group B & C, Group A & C was done using Mann–Whitney U test. The evaluation of the intra-group differences of PI, GI, SBI, PPD, RAL and MCC at baseline and 6 weeks was done using Wilcoxon Sign Rank test.

Level of significance for the present study was 5% (0.05).

**RESULTS**

Measurement of Pocket Probing Depth (PPD), Relative Attachment Level (RAL) readings of Group A (Fig. 4a and b); Group B (Fig. 5a and b) and Group C (Fig. 6a, and b) was done at baseline and 6 weeks.

Microbial colony count (MCC) of Group A (Fig. 7a and b); Group B (Fig. 8a and b) and Group C (Fig. 9a and b) was done at baseline and 6 weeks.
Fig. 4 (a) Measurement of pocket probing depth at baseline, (b) Measurement of pocket probing depth at 6 weeks. Group B - Scaling and Root Planing + Propolis.

Fig. 5 (a) Measurement of pocket probing depth at baseline, (b) Measurement of pocket probing depth at 6 weeks. Group C - Scaling and Root Planing + Periogard.

Fig. 6 (a) Measurement of pocket probing depth at baseline, (b) Measurement of pocket probing depth at 6 weeks. Group A - Scaling and Root Planing + Normal Saline.
Adjunct to scaling and root planing in patients affected with chronic periodontitis

Fig. 7  (a) Bacterial growth on blood agar plate before treatment, (b) Bacterial growth on blood agar plate after treatment. Group B - Scaling and Root Planing + Propolis.

Fig. 8  (a) Bacterial growth on blood agar plate before treatment, (b) Bacterial growth on blood agar plate after treatment. Group C - Scaling and Root Planing + Periogard.

Fig. 9  (a) Bacterial growth on blood agar plate before treatment, (b) Bacterial growth on blood agar plate after treatment.
When the intragroup comparison was made between baseline and 6 weeks interval for three groups – Group A, Group B, Group C, intra-group difference between two intervals was found to be statistically significant for all three groups for all parameters (Tables 1–6).

When the intergroup comparison was made between groups for mean plaque reduction scores & mean gingival index scores, difference was found to be statistically non-significant between Group A and Group B, Group B and Group C, Group A and Group C (Tables 7 and 8).

When the intergroup comparison was made between groups for mean reduction in SBI & RAL scores, difference was found to be statistically significant between Group A and Group B, Group A and Group C. However, intergroup comparison between Group B and Group C revealed statistically insignificant difference between groups (Tables 9 and 10).

When the intergroup comparison was made between the Group A and Group B for reduction in PPD scores, a statistically significant difference was observed. The intergroup comparison of Group B and Group A with the Group C also revealed significant difference in PPD reduction scores between the groups (Table 10).

When the intergroup comparison was made between Group A and Group B for mean reduction (MR) in colony count, significantly higher percentage reduction (%R) was observed in Group B (MR: 6.34 ± 4.04; %R: 93.11%) as compared to Group A (MR: 0.28 ± 0.16; %R: 8.50%) (P = 0.001). The similar significant difference for reduction in colony count between two intervals was observed between Group A (MR: 0.28 ± 0.16; %R: 8.50%) and Group C (MR: 4.82 ± 2.22; %R: 92.22%). When the intergroup comparison was made between Group B (MR: 4.82 ± 2.22; %R: 92.22%) and Group C (MR: 4.82 ± 2.22; %R: 92.22%), there was no significant difference in mean and percentage reduction of colony count between two groups (P = 0.345) (Table 12).

**DISCUSSION**

To attain and maintain periodontal health, subgingival plaque must be removed. Oliver et al. (1973)\(^8\) and Mashimo et al. (1980)\(^9\) both concluded that rinses did not permeate subgingivally. Pitcher et al. (1980)\(^10\) also
demonstrated that rinses or mechanical irrigation at the gingival margin did not reach >3 mm intrasulcularly.

The results of the present study showed that subgingival irrigation with Propolis and 0.12% chlorhexidine (PerioGard®) has shown significant improvement in relation to all clinical and microbiological parameters. Several authors reported the use of antimicrobial substances as irrigant after mechanical therapy in periodontal pockets. Antimicrobial activity of propolis in vitro against periodontopathogenic organisms reported by Gebara et al. (2002) led to evaluate its use in vivo, by assessing clinical and microbiological data. The primary function

| Table 7 | Intergroup comparison of change in plaque index scores between the two intervals – baseline and 6 weeks. |
|---|---|---|---|---|---|
| **Groups** | **Baseline (Mean ± SD)** | **6 week interval (Mean ± SD)** | **Mean change b/w intervals** | **% change b/w the intervals** | **Mann Whitney test for significance of change** |
| **Group A and Group B** | | | | | |
| Group A | 2.26 ± 0.25 | 1.18 ± 0.63 | 1.01 ± 0.51 | 48.09 ± 21.58 | 0.072 (NS) |
| Group B | 2.31 ± 0.38 | 1.01 ± 0.44 | 1.36 ± 0.32 | 59.30 ± 11.88 | |
| **Group B and Group C** | | | | | |
| Group B | 2.31 ± 0.38 | 1.01 ± 0.44 | 1.36 ± 0.32 | 59.30 ± 11.88 | 0.143 (NS) |
| Group C | 2.15 ± 0.28 | 0.92 ± 0.28 | 1.19 ± 0.25 | 56.53 ± 9.93 | |
| **Group A and Group C** | | | | | |
| Group A | 2.26 ± 0.25 | 1.18 ± 0.63 | 1.01 ± 0.51 | 48.09 ± 21.58 | 0.443 (NS) |
| Group C | 2.15 ± 0.28 | 0.92 ± 0.28 | 1.19 ± 0.25 | 56.53 ± 9.93 | |

NS: Non-significant.

| Table 8 | Intergroup comparison of change in gingival index scores between the two intervals – baseline and 6 weeks. |
|---|---|---|---|---|---|
| **Groups** | **Baseline (Mean ± SD)** | **6 week interval (Mean ± SD)** | **Mean change b/w intervals** | **% change b/w the intervals** | **Mann Whitney test for significance of change** |
| **Group A and Group B** | | | | | |
| Group A | 2.21 ± 0.44 | 0.91 ± 0.48 | 1.30 ± 0.72 | 56.73 ± 25.73 | 0.191 (NS) |
| Group B | 2.30 ± 0.31 | 0.73 ± 0.22 | 1.56 ± 0.30 | 68.08 ± 9.52 | |
| **Group B and Group C** | | | | | |
| Group B | 2.30 ± 0.31 | 0.73 ± 0.22 | 1.56 ± 0.30 | 68.08 ± 9.52 | 0.665 (NS) |
| Group C | 2.18 ± 0.34 | 0.53 ± 0.12 | 1.65 ± 0.33 | 75.27 ± 6.32 | |
| **Group A and Group C** | | | | | |
| Group A | 2.21 ± 0.44 | 0.91 ± 0.48 | 1.30 ± 0.72 | 56.73 ± 25.73 | 0.082 (NS) |
| Group C | 2.18 ± 0.34 | 0.53 ± 0.12 | 1.65 ± 0.33 | 75.27 ± 6.32 | |

NS: Non-Significant.
Table 9  Comparison of change in sulcular bleeding index scores between the two intervals – baseline and 6 weeks.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Baseline (Mean ± SD)</th>
<th>6 week interval (Mean ± SD)</th>
<th>Mean change b/w intervals</th>
<th>% change b/w the intervals</th>
<th>Mann Whitney test for significance of change</th>
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<tbody>
<tr>
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</tr>
<tr>
<td>Group A</td>
<td>2.21 ± 0.47</td>
<td>1.28 ± 0.43</td>
<td>0.93 ± 0.55</td>
<td>40.80 ± 19.29</td>
<td>0.004 (S)</td>
</tr>
<tr>
<td>Group B</td>
<td>2.15 ± 0.22</td>
<td>0.73 ± 0.19</td>
<td>1.41 ± 0.27</td>
<td>65.71 ± 9.59</td>
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<td>40.80 ± 19.29</td>
<td>0.004 (S)</td>
</tr>
<tr>
<td>Group B</td>
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<td>0.73 ± 0.19</td>
<td>1.41 ± 0.27</td>
<td>65.71 ± 9.59</td>
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</tr>
<tr>
<td>Group C</td>
<td>2.21 ± 0.33</td>
<td>0.81 ± 0.31</td>
<td>1.40 ± 0.43</td>
<td>62.68 ± 13.66</td>
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</tbody>
</table>

S: Significant; NS: Non-significant.

Table 10  Intergroup comparison of change in probing depth between the two intervals – baseline and 6 weeks.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Baseline (Mean ± SD)</th>
<th>6 week interval (Mean ± SD)</th>
<th>Mean change b/w intervals</th>
<th>% change b/w the intervals</th>
<th>Mann Whitney test for significance of change</th>
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</tr>
<tr>
<td>Group A</td>
<td>5.86 ± 0.63</td>
<td>5.53 ± 0.63</td>
<td>0.33 ± 0.18</td>
<td>5.39 ± 2.14</td>
<td>0.001 (S)</td>
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<tr>
<td>Group B</td>
<td>6.33 ± 1.11</td>
<td>3.00 ± 0.41</td>
<td>3.33 ± 0.83</td>
<td>52.76 ± 5.58</td>
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<tr>
<td>Group B</td>
<td>6.33 ± 1.11</td>
<td>3.00 ± 0.41</td>
<td>3.33 ± 0.83</td>
<td>52.76 ± 5.58</td>
<td>0.01 (S)</td>
</tr>
<tr>
<td>Group C</td>
<td>6.06 ± 0.25</td>
<td>3.26 ± 0.45</td>
<td>2.80 ± 0.41</td>
<td>46.18 ± 6.90</td>
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</tr>
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<td>Group A</td>
<td>5.86 ± 0.63</td>
<td>5.53 ± 0.63</td>
<td>0.33 ± 0.18</td>
<td>5.39 ± 2.14</td>
<td>0.001 (S)</td>
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<td>Group C</td>
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<td>3.26 ± 0.45</td>
<td>2.80 ± 0.41</td>
<td>46.18 ± 6.90</td>
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</tbody>
</table>

S: Significant.

of propolis in the hive is to act as a biocide, being active against invasive bacteria, fungi and even invading larvae. Antimicrobial activity of propolis ethanolic extract has been studied by several authors, however, few studies have investigated its activity towards oral pathogens.

Plaque index (PI) (Silness and Loe 1964)

In the intragroup comparison, significant improvements were observed for PI scores in all three groups (P-value < 0.05) when compared at 6 weeks time interval.
Table 11  Intergroup comparison of change in relative attachment level between the two intervals – baseline and 6 weeks.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Baseline (Mean ± SD)</th>
<th>6 week interval (Mean ± SD)</th>
<th>Mean Change b/w intervals</th>
<th>% change b/w the intervals</th>
<th>Mann Whitney test for significance of change</th>
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<tbody>
<tr>
<td></td>
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<td>P value</td>
</tr>
<tr>
<td>Group A</td>
<td>9.13 ± 0.83</td>
<td>8.80 ± 0.77</td>
<td>0.33 ± 0.48</td>
<td>3.49 ± 2.15</td>
<td>0.001 (S)</td>
</tr>
<tr>
<td>Group B</td>
<td>9.73 ± 1.27</td>
<td>6.33 ± 1.15</td>
<td>3.40 ± 0.73</td>
<td>35.12 ± 7.85</td>
<td></td>
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</tbody>
</table>

Group B and Group C

<table>
<thead>
<tr>
<th>Groups</th>
<th>Baseline (Mean ± SD)</th>
<th>6 week interval (Mean ± SD)</th>
<th>Mean Change b/w intervals</th>
<th>% change b/w the intervals</th>
<th>Mann Whitney test for significance of change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
<td>P value</td>
</tr>
<tr>
<td>Group B</td>
<td>9.73 ± 1.27</td>
<td>6.33 ± 1.15</td>
<td>3.40 ± 0.73</td>
<td>35.12 ± 7.85</td>
<td>0.780 (NS)</td>
</tr>
<tr>
<td>Group C</td>
<td>10.06 ± 1.22</td>
<td>6.66 ± 1.35</td>
<td>3.40 ± 0.51</td>
<td>34.13 ± 5.86</td>
<td></td>
</tr>
</tbody>
</table>

Group A and Group C

<table>
<thead>
<tr>
<th>Groups</th>
<th>Baseline (Mean ± SD)</th>
<th>6 week interval (Mean ± SD)</th>
<th>Mean Change b/w intervals</th>
<th>% change b/w the intervals</th>
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<tr>
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<td></td>
<td>P value</td>
</tr>
<tr>
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<td>8.80 ± 0.77</td>
<td>0.33 ± 0.48</td>
<td>3.49 ± 2.15</td>
<td>0.001 (S)</td>
</tr>
<tr>
<td>Group C</td>
<td>10.06 ± 1.22</td>
<td>6.66 ± 1.35</td>
<td>3.40 ± 0.51</td>
<td>34.13 ± 5.86</td>
<td></td>
</tr>
</tbody>
</table>

S: Significant; NS: Non-significant.

Table 12  Intergroup comparison of change in microbial colony count between the two intervals – baseline and 6 weeks.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Baseline (Mean ± SD)</th>
<th>6 week interval (Mean ± SD)</th>
<th>Mean change b/w intervals</th>
<th>% change b/w the intervals</th>
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<tr>
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<td>P value</td>
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<tr>
<td>Group A</td>
<td>3.60 ± 1.28</td>
<td>3.32 ± 1.26</td>
<td>0.28 ± 0.16</td>
<td>8.50 ± 4.99</td>
<td>0.001 (S)</td>
</tr>
<tr>
<td>Group B</td>
<td>6.91 ± 4.23</td>
<td>0.56 ± 0.31</td>
<td>6.34 ± 4.04</td>
<td>93.11 ± 5.90</td>
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Group B and Group C

<table>
<thead>
<tr>
<th>Groups</th>
<th>Baseline (Mean ± SD)</th>
<th>6 week interval (Mean ± SD)</th>
<th>Mean change b/w intervals</th>
<th>% change b/w the intervals</th>
<th>Mann Whitney test for significance of change</th>
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<tr>
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<td>P value</td>
</tr>
<tr>
<td>Group B</td>
<td>6.91 ± 4.23</td>
<td>0.56 ± 0.31</td>
<td>6.34 ± 4.04</td>
<td>93.11 ± 5.90</td>
<td>0.345 (NS)</td>
</tr>
<tr>
<td>Group C</td>
<td>5.14 ± 2.30</td>
<td>0.31 ± 0.13</td>
<td>4.82 ± 2.22</td>
<td>92.92 ± 4.07</td>
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</table>

Group A and Group C

<table>
<thead>
<tr>
<th>Groups</th>
<th>Baseline (Mean ± SD)</th>
<th>6 week interval (Mean ± SD)</th>
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<td>92.92 ± 4.07</td>
<td></td>
</tr>
</tbody>
</table>

S: Significant; NS: Non-significant.

These findings were in accordance with studies conducted by Hardy et al. (1982) and Coutinho (2012) who found propolis to be effective in reducing PI scores when used as a subgingival irrigant. Reynolds et al. (1992) concluded that chlorhexidine, when used as a subgingival irrigant, reduces PI scores to a great extent. In the intergroup comparison, change in PI scores between baseline and 6 weeks is non-significant.
(P-value > 0.05). This shows that subgingival irrigation with saline, propolis and chlorhexidine, after scaling and root planing has a bactericidal activity against the bacteria in plaque biofilm and in addition it inhibited the accumulation of experimental dental plaque in vitro. Similar results were reported by other authors.21–29.

**Gingival Index (GI) (Loe and Sillness, 1963)**

In the intragroup comparison, significant improvements were observed for GI scores in all the groups when compared at 6 weeks time interval (P-value < 0.05). Similar results were obtained by other authors.30

In the intergroup comparison, a change in GI scores between baseline and 6 weeks in all the three groups is non-significant (P-value > 0.05). This shows that subgingival irrigation with saline, propolis and chlorhexidine has a role in improvement in gingival health. Similar results were reported by various other authors.23–25,31,40,41 A greater reduction in GI scores is seen in Group B. This is probably justified by the antibacterial and anti-inflammatory effects of propolis.32

A reduction in the gingival inflammation after subgingival irrigation may be due to the dilution of plaque toxicity, interference with subgingival plaque maturation or possibly washing away unattached plaque as postulated by Derdivians et al. (1978),33 Fine et al. (1985),34 Flemming et al. (1990).35 All three irrigation groups improved significantly at 6 weeks with respect to their GI. This would seem to indicate that flushing of fluid into the periodontal pocket may have had a positive clinical effect, regardless of the irrigant. This is in agreement with studies evaluating subgingival irrigation, conducted by Ciancio et al. (1989).36

**Sulcus Bleeding Index (Muhlemann and Son 1971)**

In the intragroup comparison significant improvements were observed in all the groups when compared at 6 weeks interval (P-value < 0.05). Similar findings were obtained in studies conducted by Soh (1982),38 Haskan et al. (1986) and various other studies.23,25,31,40,41

In the intergroup comparison of three groups, highly significant results are found in Group B & C (P-value < 0.05). These results are identical to those of studies by Weider SG et al. (1983),39 Krucke et al. (2012).40 When Groups A & B, A & C are compared, the results are significant (P-value < 0.05). When Group B & C are compared, the results are non-significant (P-value > 0.5). This shows that subgingival irrigation with Propolis and PerioGard reduced bleeding upon probing when compared at 0–6 weeks. But, subgingival irrigation with saline does not reduce bleeding upon probing to a large extent. Other authors also reported that the flushing action of the subgingival irrigant had little effect on gingival bleeding.44–47 SBI scores reduce to a large extent in Group B and Group C, this is in accordance to study conducted by Wieder et al. (1983) who documented that initial mechanical debridement and irrigation of pockets with chlorhexidine seemed to be most important factors in subgingival plaque control. Scaling and root planing were effective in increasing the proportion of sites negative to bleeding on probing with time, as shown by Oosterwaal et al. (1987),48 Socransky et al. (1988)49, and Newman et al. (1994).50

**Pocket probing depth (PPD)**

PPD reduction is beneficial since it produces an environment less favorable for the establishment of periodontopathic microorganisms. In the present study, Group B & C showed significant reduction in PPD at 6 weeks as compared to baseline (P-value < 0.01). These results are similar to other studies.44,45,47

In intergroup comparisons the differences between Group A and B was significant (P-value < 0.05); between Group B and C was significant (P-value < 0.05); between Group A and C was significant (P-value < 0.05). The results are identical to studies by Vignarajah et al. (1989),52 and other authors.51,52,53,31,23 They also observed higher percentage of PPD reduction using Propolis and 0.12% chlorhexidine as compared to saline (placebo).

**Relative attachment level (RAL)**

Intragroup comparison in all the three groups between 0 and 6 weeks showed a significant improvement in RAL (P-value < 0.05). Intergroup comparisons between Groups A & C, A & B showed significant results (P-value < 0.05); whereas between Group B & C showed non-significant results (P-value < 0.05). These results resemble with Rosling et al. (1982),49 Westling et al. (1984),45 and studies by other authors who reported in their studies that mechanical effect of flushing action of irrigation alone had little effect on the attachment level.

**Microbial colony count (MCC)**

Intragroup comparison in all the groups at interval of 6 weeks showed significant reduction in microbial colony count (P-value < 0.05). Similar findings were observed in other studies.53,54,44 The intergroup comparisons between Group A & B; Group A & C showed significant results (P-value < 0.05). This is in accordance with Schmid et al. (1985),55 Boyd et al. (1985)56 who concluded that when pockets are irrigated with saline, no reduction in mean bacterial counts or shifts in proportions of microbial morphotypes were evident, suggesting that irrigation procedure alone had little impact on the pocket flora. The reduction in MCC after irrigation with Propolis is in conjunction with results obtained by Kujumgiev et al. (1999)57 and other authors.5,4,26,44,41,50,54–56 The reduction in MCC after irrigation with 0.12% chlorhexidine in Group C is in
agreement with Wennstrom et al. (1987)\textsuperscript{25} and other authors\textsuperscript{17, 43, 58, 59}.

According to Ludovic et al. (1990)\textsuperscript{68} and Lembariti et al. (1998)\textsuperscript{61} one session of scaling and root planing is not enough to maintain subgingival microbiota compatible with health when patients with pocket depth ≥ 5 mm are considered. The benefits produced by propolis and 0.12% chlorhexidine as subgingival irrigants after scaling and root planing, indicate that they should be considered as an adjunct to scaling and root planing.

In the present study, the maximum reduction in MCC is observed at 6 weeks in Group B, followed by Group C. This high improvement in propolis group is attributed to its mechanism of action, some of its constituents cause significant inhibition of bacterial motility, besides ion permeability alteration on the inner bacterial membrane\textsuperscript{62}. Takaisi-Kikuni NB and Schilcher H (1994)\textsuperscript{63} proposed that ethanolic extract of propolis interferes with division of bacteria, promoting cytoplasm disorganisation and protein synthesis inhibition.

The present study suggests that both Propolis and PerioGard possess high antimicrobial activity against the periodontal pathogens and when used as subgingival irrigant, these both can reduce the microbial colony counts to a large extent.

Antimicrobial agents (Propolis & 0.12% chlorhexidine) used as subgingival irrigants are significantly more effective than control irrigant (saline) in reducing supra and subgingival plaque. In the present study, we have used 0.12% chlorhexidine for subgingival irrigation in Group C. A study conducted by Segreto VA et al. (1986)\textsuperscript{17} showed that a 0.12% chlorhexidine mouthrinse provided the same clinical benefits as a 0.2% chlorhexidine mouthrinse when used under a twice daily regimen.

When a comparison was made between all groups, Propolis group showed slightly better but non-significant improvement as compared to the PerioGard group and both groups showed significant improvement as compared to normal saline group indicating that Propolis and PerioGard are very effective as an adjunct to scaling and root planing in treatment of chronic periodontitis.

The reduction seen even in the control group (normal saline) is due to the fact that SRP alone reduces the number of pathogenic microorganisms in periodontal pocket to a level below that required for inducing the disease\textsuperscript{19}.

Hence, further long-term studies with larger sample size are required to adequately assess these compounds as subgingival irrigants.

**SUMMARY AND CONCLUSIONS**

1. Subgingivally delivered Propolis and PerioGard as an adjunct to scaling and root planing in the treatment of chronic periodontitis have shown promising results. They both possess antibacterial activity against gram positive and gram negative bacteria.

2. Propolis is as effective as PerioGard (Chlorhexidine gluconate 0.12%) as antiplaque and antigingivitis agents, providing substantial reductions in gingivitis occurrence, severity and bleeding.

3. Mechanical therapy i.e., SRP by itself leads to a reduction in the clinical parameters (PI, GI & SBI) and it reduces number of pathogenic microorganisms in periodontal pocket to a level below that required for inducing the disease.

4. Subgingival irrigation is a potent method to control periodontopathogens when used as an adjunct to SRP in chronic periodontitis patients.

**REFERENCES**


