Original Article

One-stage Full Mouth Disinfection Using 20% Propolis Hydroalcoholic Solution: A Clinico-microbiologic Study

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Abstract

Background: Propolis is a resinous substance produced by honeybees which has many therapeutic properties because of its unique composition. It has been widely used since many years for different medicinal purposes. **Aim:** The aim of this study was to investigate the effects of one-stage full mouth disinfection (OSFMD) using 20% propolis hydroalcoholic solution in chronic periodontitis patients. **Materials and Methods:** Thirty patients diagnosed with chronic periodontitis and presenting three or more nonadjacent teeth with deep pockets were selected for the study. Clinical parameters including gingival index, plaque index, bleeding on probing, probing pocket depth, and clinical attachment level were recorded at baseline in all the patients followed by subgingival plaque sampling. All the thirty patients were randomly allocated into two groups; 15 patients (control group) were subjected to scaling and root planning (SRP) alone, and in remaining 15 patients (test group), SRP was done followed by OSFMD using 20% propolis hydroalcoholic solution after 24 h. All the patients were kept at periodic recall, and clinical and microbiological parameters were again taken at 4 weeks and 12 weeks. **Results:** There was a significant improvement for all the clinical parameters, with higher probing depth reduction and attachment gain in the test group when compared to the control group. Furthermore, the microbiological counts of the periodontopathogens were found to decrease considerably more in the test group. **Conclusion:** SRP followed by OSFMD with propolis extract after 24 h was more effective than SRP alone in chronic periodontitis patients.

Keywords: *Microbiological count, one-stage full-mouth disinfection, propolis, scaling and root planning*

Introduction

Propolis, also known as bee glue, is a natural resinous material produced by honeybees (*Apis mellifera*) from substances collected from different parts of plants. It is a complex mixture of 50% resins, 30% waxes, 10% essential oils, 5% pollen, and 5% of various organic compounds. The word propolis is derived from the Greek word *pro* (meaning "in front of") and *polis* (meaning "community").[1-3]

Propolis has been extensively used by man since ancient times due to the outstanding therapeutic properties it has. Egyptians used bee glue to embalm their cadavers, Greek and Roman physicians used it as mouth disinfectant and as an antiseptic and healing product in wound treatment. It was also used by many Arab physicians. It was listed as an official drug in the London pharmacopoeias of the 17th century. Propolis became very popular in Europe between the 17th and 20th centuries due to its antibacterial activity. In the end of 19th century, propolis was widely used due to its healing properties.[1,3]

Currently, several propolis products are being used worldwide including capsules (either pure or combined with aloe gel or pollen), extracts (hydroalcoholic or glycolic), mouthwash solutions, throat lozenges, creams, powder, and also in more purified products from which the wax was removed.[1] The several therapeutic properties of propolis include antibacterial, anti-inflammatory, anesthetic, anticariogenic, antifungal, antiprotozoan, and antiviral.[2] The principal compounds responsible for biological activities of propolis are flavonoids, aromatic acids, diterpenic acids, and phenolic compounds.[1]

However, propolis cannot be used directly as raw material and a simple fractionation to obtain compounds is difficult due to its complex composition. Hence, to solve this problem, the usual procedure is the use of a solvent, which should remove the inert

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material and preserve the desired compound. Solvents used for propolis are water, methanol, ethanol, chloroform, dichloromethane, ether, and acetone, out of which ethanol is the most common solvent choice.[1,2] The active substances of propolis are easily soluble in ethanol.[4]

The concept of bacterial specificity in periodontal infections has been largely accepted. A susceptible host, the presence of periodontopathogens, and the absence of beneficial species are considered to be the three factors responsible for the establishment of an active periodontal infection. Periodontopathogens have been found to spread subgingivally, including at sites without clinical loss of periodontal attachment. Hence, in a normal periodontal treatment strategy, a reinfection of a disinfected area might well occur before the completion of the treatment. Thus, one-stage full mouth disinfection (OSFMD) is preferred as an adjunct to scaling and root planning (SRP) as it aims to eradicate or reduce the periodontopathogens in all the intraoral niches and it also reduces the probability of intraoral transmission of periodontopathogens from one niche to the other.[5]

This study aims to examine the result of OSFMD with 20% propolis hydroalcoholic solution, both clinically and microbiologically, after 24 h of SRP when compared to SRP alone in chronic periodontitis patients.

Materials and Methods

Thirty systemically healthy patients, who came to the Outpatient Department of Periodontology of Rungta College of Dental Sciences and Research, Bhilai, were selected for the study. All the patients were in the age group of 25–55 years of either sex and they were diagnosed with chronic periodontitis having three or more nonadjacent teeth with pockets ≥5 mm. All patients were otherwise systemically healthy, nonsmokers, and nontobacco users. None of the patients had undergone subgingival instrumentation within 12 months before the baseline examination, had compromised medical conditions which required prophylactic antibiotic coverage, or had used antimicrobial agents 4 months before the study. Patients who were uncooperative and showed unacceptable oral hygiene were excluded from the study. Furthermore, patients who had ongoing drug therapy, which might affect the clinical symptoms of periodontitis, were not included in the study.

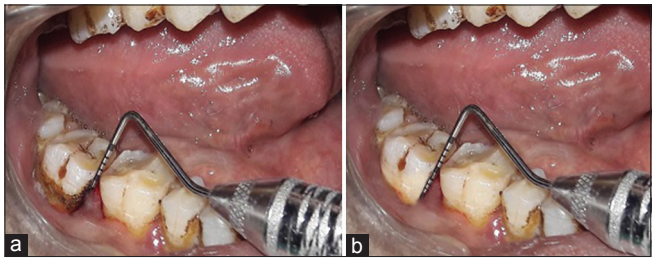


Figure 1: (a) Measurement of probing pocket depth at baseline. (b) Measurement of probing pocket depth after 12 weeks

The clinical parameters of the study included gingival index (GI),[6] plaque index (PI),[7] modified sulcus bleeding index,[8] probing pocket depth (PPD), and clinical attachment level (CAL). The microbiological parameters included the microbiological counts of *Aggregatibacter actinomycetemcomitans* (Aa), *Prevotella intermedia* (Pi), and *Porphyromonas gingivalis* (Pg).

All of the thirty patients were randomly allocated into two groups – the test group (15 patients) and the control group (15 patients). The control group was subjected to SRP alone whereas the test group was subjected to OSFMD, using 20% propolis hydroalcoholic solution, 24 h after SRP. The clinical and microbiological parameters were recorded at baseline, 4 weeks, and 12 weeks, respectively.

The propolis extract powder was commercially bought and stored at room temperature and then it was mixed with 99.8% (v/v) ethanol in hermetically sealed glass vessels at a ratio of 1 g of propolis powder to 3 ml of ethanol. Vessels were then incubated for 1 week at room temperature in darkness, with constant agitation. The resulting ethanol solutions were clarified by centrifugation at 7000 g for 60 s, and the supernatants were collected and filtered through Whatman #4 filter paper. Ethanol-soluble components were then collected by evaporation to dryness. The extracts were re-dissolved in pure ethanol to obtain 20% (w/v) solutions. The final solutions were stored in hermetically sealed brown-glass bottles at room temperature.[9]

At baseline, all the clinical parameters were recorded [Figure 1a]. The subgingival plaque samples were taken from the deepest pockets. All samples were taken from an undisturbed subgingival flora and after removal of the supragingival plaque. Before being sampled, the sites were isolated from saliva by the application of cotton rolls. Then, sterile paper points were inserted into the selected pockets and kept in place for at least 10 s [Figure 2a]. Following removal, the paper points were transferred into a screw-capped vial containing 1 ml of transport medium.[5] Then, the samples were cultured for Aa, Pg, and Pi as shown in Figure 3a-c. All the patients underwent SRP followed by recall of test group patients 1 day after SRP for OSFMD using the 20% propolis hydroalcoholic solution. OSFMD involves brushing the dorsum of the tongue for 60 s with

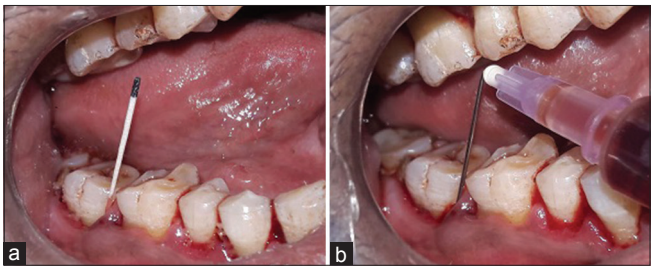


Figure 2: (a) Subgingival plaque samples being taken using paper points. (b) Irrigation tip being placed into the pocket for subgingival irrigation using 20% propolis hydroalcoholic solution

the solution and rinsing the mouth twice with the solution for 1 min followed by repeated subgingival irrigation of all the pockets by means of a syringe with a blunt needle [Figure 2b].[5] All the patients were recalled after 4 weeks and 12 weeks and again the clinical parameters were recorded [Figure 1b]. Also, the subgingival plaque samples were collected and cultured.

The data were expressed as mean ± standard deviation The values of PI, GI, BOP, PPD, and CAL at baseline, after 4 weeks, and 12 weeks were compared and analyzed using student's paired *t*-test and Student's unpaired *t*-test [Table 1]. Similarly, the microbiological counts were also compared and analyzed [Table 2]. Statistical significance was set at $P < 0.05$.

Results

All of the thirty patients (15 in each group) completed the 3-month follow-up period. At baseline, there were no differences between the two groups in clinical or microbiological parameters. After 12 weeks, statistically significant difference was found in PI, GI, BOP, PPD, and CAL between the two groups, as shown in Table 1. When the patients were recalled after 12 weeks, the PI and GI mean value of the test group was 1.28 ± 0.1 and 1.3 ± 0.2, respectively, and that of the control group was 1.56 ± 0.4 and 1.59 ± 0.43, respectively, *P* value being 0.013 and 0.026. Furthermore, the BOP and PPD mean value after 12 weeks in the test group was 1.12 ± 0.26 and 3.87 ± 0.92, respectively, and in the control group was 1.47 ± 0.31 and 4.53 ± 0.52, respectively, *P* value being 0.0025 and 0.02. Figure 1a and b shows the PPD at baseline (8 mm) and after 12 weeks (5 mm). Similarly, the CAL mean value after 12 weeks in the test group was 1.47 ± 1.51 and in the control group was 2.53 ± 0.52, *P* value being 0.015.

When the microbiological data were compared, the *P* value was found to be significantly less after 12 weeks for all the three microorganisms Aa, Pg, and Pi. Furthermore, the reduction in the number of microorganisms was significantly greater in the test group when compared to the control group for Aa, Pg, and Pi, as shown in Table 2. The microbial colonies of Pi, Aa, and Pg at baseline and after 12 weeks are as shown in Figure 4a-c.

Discussion

Propolis has been widely used for its medicinal properties all around the world. Due to its strong, anti-infective activity, propolis has often been called a "natural antibiotic." However, only a few studies have examined the antimicrobial properties of propolis against periodontopathogens. Some studies have shown the advantages of using full mouth disinfection as an adjunct to SRP.[10-13] To our knowledge, there is no study in which full mouth disinfection has been done using propolis. Hence, this study was done to find out the effects of full mouth disinfection of propolis solution using both clinical and microbiological parameters.

In our study, we found that the PI and GI values were similar at baseline in both the groups; however, in the test group, the values were similar or reduced after 12 weeks as compared to those after 4 weeks, unlike in the control group where the values increased after 12 weeks as compared to 4 weeks. Furthermore, the number of sites

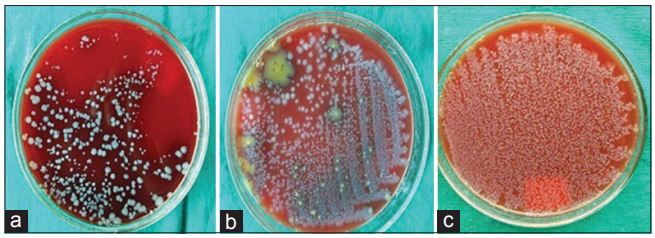


Figure 3: (a) Microbial colonies of *Prevotella intermedia* at baseline of test group. (b) Microbial colonies of *Aggregatibacter actinomycetemcomitans* at baseline of test group. (c) Microbial colonies of *Porphyromonas gingivalis* at baseline of test group

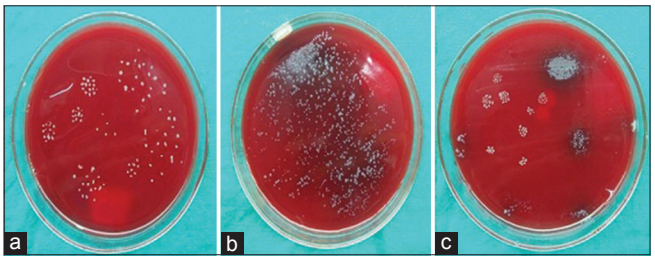


Figure 4: Microbial colonies of *Prevotella intermedia* at 12 weeks of test group. (b) Microbial colonies of *Aggregatibacter actinomycetemcomitans* at 12 weeks of test group. (c) Microbial colonies of *Porphyromonas gingivalis* at 12 weeks of test group

Table 1: Inter group comparison of clinical parameters

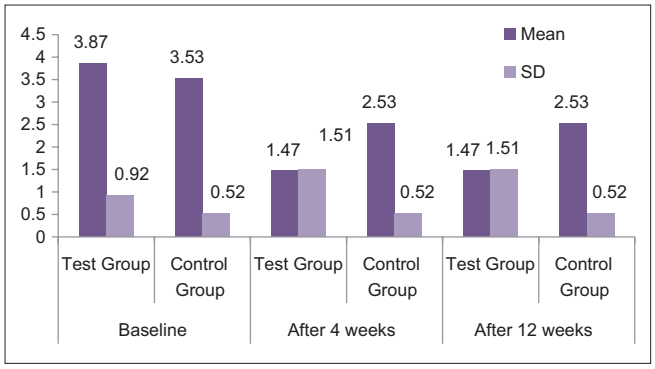
Parameters	Mean±SD								
	Baseline			4 weeks			12 weeks		
	Test group	Control group	*P*	Test group	Control group	*P*	Test group	Control group	*P*
PI	1.91±0.29	1.74±0.5	0.25	1.32±0.09	1.57±0.46	0.04	1.28±0.1	1.56±0.4	0.013
GI	1.85±0.31	1.78±0.51	0.67	1.34±0.21	1.59±0.4	0.042	1.3±0.2	1.59±0.43	0.026
BOP	1.77±0.32	1.62±0.44	0.28	1.12±0.26	1.21±0.34	0.40	1.12±0.26	1.47±0.31	0.0025
PPD	5.87±0.92	5.53±0.52	0.23	3.87±0.92	4.53±0.52	0.02	3.87±0.92	4.53±0.52	0.02
CAL	3.87±0.92	3.53±0.52	0.23	1.47±1.51	2.53±0.52	0.015	1.47±1.51	2.53±0.52	0.015

GI: Gingival index; PI: Plaque index; BOP: Bleeding on probing; PPD: Probing pocket depth; CAL: Clinical attachment level; SD: Standard deviation

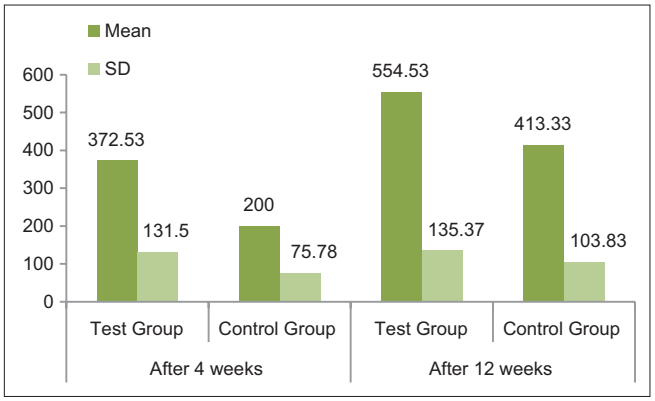
Table 2: Inter group comparison of reduction in microbial count

Parameters	Reduction in microbial count (mean±SD)					
	After 4 weeks			After 12 weeks		
	Test group	Control group	*P*	Test group	Control group	*P*
Aa	372.53±131.5	200±75.78	0.00014	554.53±135.37	413.33±103.83	0.003
Pg	269.47±131.88	196.4±61.16	0.04	470.8±87.21	383.07±114.6	0.025
Pi	332.73±149.21	178.13±61.73	0.0009	512.73±183.34	347.47±105.94	0.005

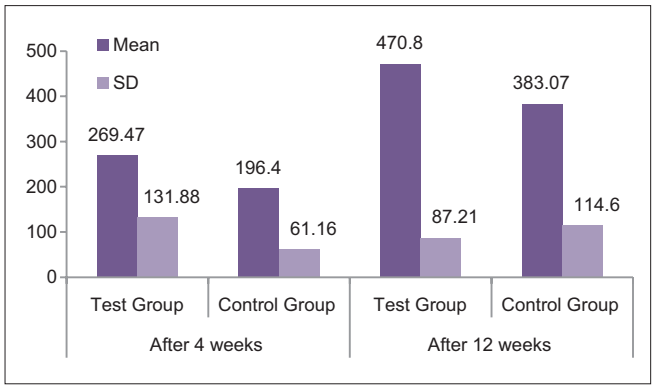
Aa: *Aggregatibacter actinomycetemcomitans*; Pi: *Prevotella intermedia*; Pg: *Porphyromonas gingivalis*; SD: Standard deviation



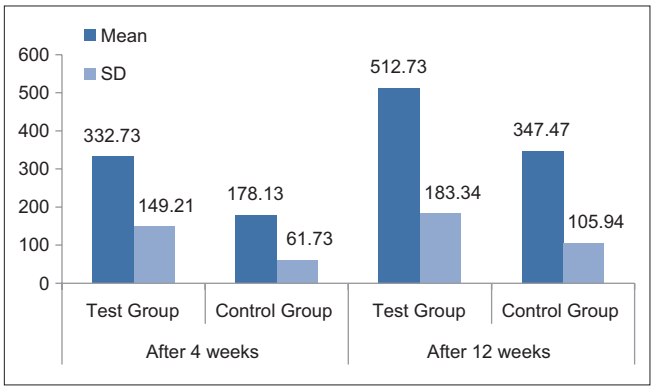
Graph 1: Comparison of clinical attachment level



Graph 2: Comparison of reduction in microorganism *Aggregatibacter actinomycetemcomitans*



Graph 3: Comparison of reduction in microorganism *Porphyromonas gingivalis*



Graph 4: Comparison of reduction in microorganism *Prevotella intermedia*

with bleeding on probing greatly reduced in the test group. This indicates the antimicrobial and anti-inflammatory effects provided by propolis extract even after 12 weeks of commencement of treatment. Furthermore, there was a greater reduction of PPD in the test group when compared to the control group. Similarly, there was a greater gain in the CAL in the test group [Graph 1]. This might be due to the tissue regeneration properties of propolis including healing which are possibly due to the antioxidant activity of propolis.[2] Microbiologically, the reduction in the number of colony-forming units (CFUs) per microliter was consistent in the test group after 12 weeks, unlike the control group where the number of CFU per microliter seemed to increase after 4 weeks [Graphs 2-4]. This might be due to the long-lasting effect of propolis leading to a change in the repopulation process occurring in the periodontal pocket.

Quirynen *et al*. in 1995 in his study examined both clinically and microbiologically, whether full-mouth disinfection within 24 h significantly improved the outcome of periodontal treatment. He divided ten patients into a test group (full mouth disinfection with chlorhexidine [CHX] after SRP) and a control group (only SRP). He concluded that the OSFMDD with CHX showed significant clinical (pocket reduction) and microbiological (shift toward a more beneficial flora) advantages on a short-term basis.[5]

Some of the propolis studies had similar results to this study, for example, a study of Dodwad and Kukreja in 2011[14] compared propolis-containing mouth rinse with 0.2% CHX (positive control) and with saline (negative control). They found out that CHX mouthwash was better than propolis and saline in inhibiting plaque formation and propolis was found to be only marginally better than CHX in improving gingival scores. They suggested that propolis might be used as a natural mouthwash instead of chemical mouthwashes such as CHX. Coutinho in

2012[9] compared the effect of propolis irrigation with that of irrigation with placebo and no irrigation in the same patient. She found that there were better results when propolis was irrigated into the pockets and concluded that subgingival irrigation with propolis extract as an adjuvant to periodontal treatment was more effective than SRP. Akca *et al*. in 2016[15] in his *in vitro* study compared the antimicrobial effectiveness of ethanolic extract of propolis (EEP) to CHX gluconate on different types of microorganisms and the results revealed that propolis was more effective in inhibiting Gram-positive bacteria than the Gram-negative bacteria in their planktonic state and it was suggested that EEP could be as effective as CHX on oral microorganisms in their biofilm state. The main limitations of our study were a small sample size that has been taken for the study and the short-term follow-up period. Furthermore, freshly prepared solutions should be used for each patient before OSFMD.

Conclusion

Based on our result, it can be concluded that both clinically and microbiologically, OSFMD using propolis solution as an adjunct to SRP was found to be better when compared to SRP alone in the treatment of chronic periodontitis patients. The increasing interest toward natural therapies, effective and healthy pharmacological compounds is a stimulus for further research on propolis. Further long-term randomized clinical trials are needed to establish the efficacy of propolis as a full mouth disinfection agent.

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Conflicts of interest

There are no conflicts of interest.

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