Subgingival Irrigation with a Solution of 20% Propolis Extract as an Adjunct to Non-Surgical Periodontal Treatment: A Preliminary Study

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Abstract

Natural products, including propolis, are now frequently used to treat periodontal disease, but there are a few clinical studies in this area. The aim of this randomized clinical trial was to evaluate the effect of subgingival irrigation of periodontal pockets with a hydroalcoholic solution of propolis extract 20% (w/v) as an adjunct to periodontal therapy. Sixteen individuals were divided into a test group (TG), comprised 65 teeth (scaling and root planing + irrigation with propolis solution), and a control group (CG), comprised 62 teeth (scaling and root planing + irrigation with saline solution). Clinical data such as probing depth, plaque index, gingival index and oral hygiene index were collected at baseline (T0) and after 45 (T1), 75 (T2) and 90 (T3) days. Both groups showed significant differences among the evaluated periods. The TG presented more reduction ($p < 0.05$) of probing depth than CG at T1 and T3. Within the limits of this short-term study, these data suggest that irrigation with a hydroalcoholic solution of propolis extract 20% (w/v) as an adjunct in periodontal treatment was more effective than the mechanical treatment with saline solution in terms of reducing probing depth for up to 90 days from the beginning of treatment.

Keywords: Propolis, irrigation, periodontal pocket, periodontal therapy, scaling and root planing

Introduction

Accumulation of bacterial biofilm on the dental surface and host susceptibility contribute to the etiology of periodontal disease (Teles *et al.*, 2013). Bacteria directly related to the disease include *Aggregatibacter actinomycetemcomitans*, *Prevotella intermedia*, *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, *Tanerella forsythus*, and *Campylobacter rectus* (Teles *et al.*, 2013). Because of the infectious nature of periodontal disease, control of dental biofilm is essential for any periodontal treatment plan: the basic strategy of treatment for most periodontal manifestations includes the suppression of periodontal pathogens in the biofilm (Loesche and Grossman, 2001; Teles *et al.*, 2013). Mechanical treatment should be seen as the gold standard of periodontal therapeutics for the periods between chemical/surgical treatments (Ishikawa and Baehni, 2004).

Chemical treatment involves antimicrobial therapy with anti-inflammation drugs, antibiotics (locally or systemically administered) and antiseptic buccal compressed tablets (Loesche and Grossman, 2001; Fritoli *et al.*, 2015; Smiley *et al.,* 2015). The indiscriminate use of antibiotics to treat periodontal disease, mainly systemic, has led to the development of bacterial resistance to medicines. This trend, in addition to the high cost of synthetic medicines and the consumers' preference for natural products, has led to a growing world market for phytotherapy (Grunwald, 1995; Yoshinaga *et al.,* 2014). Natural products, including propolis, are now frequently used to combat periodontal disease and decay.

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According to the technical regulations of the Agricultural Ministry, propolis is a hive product that bees manufacture from balsamic resins actively secreted by plants on leaf buds and barks. The bees increase salivary secretions, wax and pollen for final elaboration of the product (Koo *et al.*, 2000; Palombo, 2011). Propolis has been used for thousands of years, credited with biological activity that is antibacterial, antiviral, antitumoral, immunomodulatory and anti-inflammatory (Grunberger *et al.*, 1988; Dobrowolski *et al.*, 1991; Amoros *et al.*, 1992; Dimov *et al.*, 1992; Kujumgiev *et al.*, 1999; Borrelli *et al.*, 2002; Gebara *et al.*, 2002). The propolis comprises a chemical mixture of variable composition, depending on the area of origin and the season of collection (Ghisalberti, 1979; Grange and Davey, 1990).

Despite increased use of propolis in several areas of the world (Marcucci, 1995) there are few studies that use propolis as a therapeutic agent in the treatment of periodontal disease. Because of the relevance of propolis in the treatment of buccal diseases, the objective of the present study was to evaluate the effects of a hydroalcoholic solution of propolis extract 20% (p/v) as an adjunct in the treatment of chronic periodontal disease.

Materials and methods

In this study, we included 18 individuals of both genders who were diagnosed with mild to moderate and moderate to severe chronic periodontal disease, in agreement with the American Academy of Periodontology (1999) criteria. This study was previously approved by the Committee of Ethics in Research of UNITAU (CEP/UNITAU n°252/06). The participants received detailed information regarding the study, and written informed consent was obtained from each patient.

Subjects were enrolled if they presented with at least ten teeth and two or more sites with periodontal pocket ≥ 5 mm deep in uniradicular teeth; age ≥ 30 years old; good general health, without any evident systemic alteration visible by clinical exam or detected in the anamnesis; no periodontal treatment in the last 6 months; and non-smoker. Exclusion criteria were antibiotic therapy in the last 3 months or during the study, diabetes, immunosuppressive drugs, pregnancy or lactation.

The periodontal clinical examination for establishment of the diagnosis was accomplished after anamnesis, by a single examiner blinded, trained and previously calibrated, using a manual William's periodontal probe. The evaluation of the intra-examiner error was performed by means of two measurements of probing depth of the test and control teeth with an interval of one week between each measurement. Both were submitted to linear regression analysis in order to calibrate the examiner and verify the degree of reliability of the measurements performed. The results of this analysis demonstrated a value of R2 = 0.714889 of the test group and R2 = 0.71986 for the control group. The values of p showed that both straight lines pass through the origin with a slope of 45°, for a confidence interval of 5%.

The probing depth (PD) was determined in all teeth, except third molars, at three points of the vestibular surface and three points of the palatal surface. Other clinical parameters included: oral hygiene index (OHI; O'Leary *et al.*, 1972), plaque index (PI; Silness and Löe, 1964) and gingival index (GI; Löe and Silness, 1963). Instructions for oral hygiene were explained to all the patients. PD, PI, GI and OHI evaluations were performed at baseline (T0), and after 45 days (T1), 75 days (T2) and 90 days (T3). Non-surgical periodontal treatment was quantified by the tactile sensibility criteria through verification of the planing of the root surface with exploring probe number 5. After scaling and root planing, the patients were divided randomly, using dice, into two groups: even numbers were the test group (TG), nine individuals (65 teeth) with pocket probing depth ≥ 5 mm who received irrigation with a hydroalcoholic solution of propolis extract 20% (p/v), and odd numbers were the control group (CG), nine individuals (62 teeth) with pocket probing depth ≥ 5 mm who received irrigation with saline solution. Both solutions were applied after scaling and root planing (SRP) of all teeth included in the study and 15 days after the first irrigation.

The sites included in the study were isolated with cotton rolls and irrigated with approximately 2 mL of each solution, deposited in the deepest area of the periodontal pocket, with light pressure and soft application movements. The subgingival irrigation was carried out using syringes with rhomb tips, inserted in the bottom of the periodontal pocket. Local suction with a disposable sucking sterile surgical vacuum was used to avoid overflow of the product.

Crude samples of *Apis mellifera* bee propolis were obtained at several places in the Vale do Paraíba – São Paulo, Brazil, in order to procure a mixture representative of the area. The entire manipulation process and preparation of the hydroalcoholic solution of propolis extract 20% (p/v) was done at the Laboratory of Quality Control of Apícolas Products at the Center for Apícolas Studies of the University of Taubaté - CEA/UNITAU, in Taubaté - SP.

The hydroalcoholic solution of propolis extract 20% (p/v) was analyzed by physiochemical analysis utilizing high performance liquid chromatography (HPLC) for evaluation of the presence of the following flavonoids: quercetin, artepellin-C, galangine,

kaempferol, ferulic acid, and caffeic acid. Ultraviolet spectrophotometry analysis of the propolis hydroalcoholic extract 20% (p/v) solution was carried out at the Laboratory for Quality Control of Apícolas Products at the Center for Apícolas Studies of the University of Taubaté - CEA/UNITAU, in Taubaté - SP.

The Kolmogorov-Smirnov test showed that the data from both test groups displayed no normality, indicating the use of non-parametric tests for comparisons between the groups. Kruskal-Wallis tests for more than two independent samples were used for evaluation of the PD and OHI within groups. The PI and GI were subjected to the hypothesis test for differences among proportions, the binomial test for two proportions. For between-group evaluation, the Mann-Whitney test was used for two independent samples. To evaluate the statistical differences between groups and among group measures of PD (for TG and CG) at T0 - initial, T1 - 45 days, T2 - 75 days and T3 - 90 days, we used non-parametric tests. All the tests used possessed a level of significance of 95%.

Results

Two individuals from the CG were excluded because they did not attend all appointments, so 16 individuals (10 men and 6 women) concluded the study. Two individuals of the TG displayed lesions similar to ulcers that healed without recurrence in less than one week (*Table 1*). The sites included in the present study underwent relative isolation and subgingival irrigation with 2 mL of solution containing propolis or saline solution. Local suction of the solution prevented overflow of the antimicrobial solution and alleviated patient discomfort. In two individuals, the sites where the irrigation solution was applied showed lesions similar to ulcers or burns. Those lesions might have appeared due to the presence of caffeic acid in the solution.

Table 1. Characteristics (mean + SD) of the study population.

Parameter	Test group	Control group	*p* value
N	9	7	
Age (years)	50.22 ± 7.75	48.00 ± 9.16	0.4574
# of teeth	65	62	
# of sites	174	135	
Gender	3F/6M	5F/4M	
PD (mm)	5.75 ± 1.17	5.63 ± 0.84	0.3334
PI	1.50 ± 0.94	0.95 ± 0.80	<0.0001
GI	0.94 ± 0.84	1.10 ± 0.93	0.0183
IHO (%)	61.71 ± 23.97	54.85 ± 11.80	0.5011

Values are presented as mean ± standard deviation. PD, probing depth; PI, plaque index; GI, gingival index; IHO, oral hygiene index.

Table 2. Probing depth within-group evaluation (*p* value) for the test group (TG) and control group (CG) at baseline (T0), 45 days (T1), 75 days (T2), and 90 days (T3).

Comparison	TG	CG
T0 & T1	< 0.05	< 0.05
T0 & T2	< 0.05	< 0.05
T0 & T3	< 0.05	< 0.05
T1 & T2	ns	ns
T1 & T3	ns	ns
T2 &T3	ns	ns

$p = 0.001$, Kruskal-Wallis

For both groups, PD showed statistically significant clinical improvement when baseline values were compared to those obtained at later time points (*Table 2*). With respect to PI, both groups displayed clinical improvement in the pattern of plaque accumulation, considered as an increase in the number of dental faces without visible plaque (*Figure 1a, 1b*). There was a significant improvement in GI, considered as an increase in the number of dental faces without bleeding on probing (*Figure 1c, 1d*). Within-group assessment of OHI did not show a significant difference with time.

In the PD analysis there was a significant difference between TG and CG when the initial time was compared with the other evaluations. The hydroalcoholic solution of 20% (w/v) propolis extract was shown to be more effective than saline in reducing PD (*Table 3*).

Table 3. Probing depth between-group evaluation (*p* value) for the test group (TG) and control group (CG) at baseline (T0), 45 days (T1), 75 days (T2), and 90 days (T3).

Comparison	TG	CG	*p*-value
T0 – T1	1.42 ± 1.37	1.13 ± 1.29	0.0031*
T0 – T2	1.48 ± 1.39	1.23 ± 1.34	0.0108*
T0 – T3	1.50 ± 1.40	1.30 ± 1.41	0.0398*
T1 – T2	0.06 ± 0.72	0.09 ± 0.69	0.5851
T1 – T3	0.08 ± 0.78	0.16 ± 0.83	0.3451
T2 – T3	0.02 ± 0.55	0.07 ± 0.57	0.3943

*$p < 0.05$

There was no significant difference in the evaluation of PI and GI between TG and CG, when comparing the number of dental faces that presented with a score of 0, 1, 2 or 3 at 90 days.

The results of the physiochemical analysis showed that the solution was appropriate for use, according to the norms of the Ministry of Agriculture, Livestock and Provisioning published in the Official Diary of the Union.

Evaluating the hydroalcoholic solution of propolis extract 20% (w/v) through HPLC revealed the presence of the following flavonoids: quercetin, 4.0 mg/100 g; artepellin-C, 50.0 mg/100 g; galangin, 1.5 mg/100 g; kaempferol, 1.5 mg/100 g; ferulic acid, 4.2 mg/100 g; and caffeic acid, 5.3 mg/100 g. Ultraviolet spectrophotometry analysis at 560 nm absorbance registered a reading strip of 0.144, which corresponds to the coloration of clear amber.

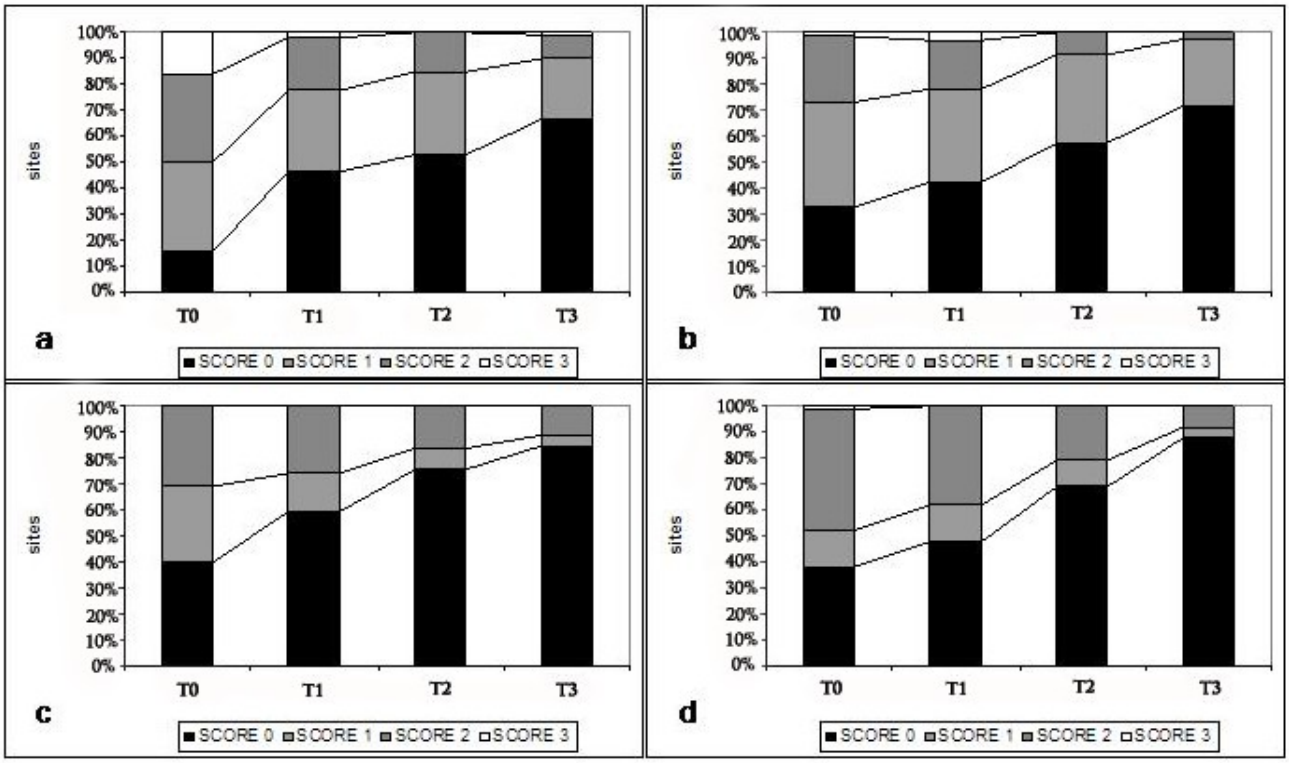

Figure 1. Graphical representation of the plaque index (PI) and the gingival index (GI) in the test (TG) and control groups (CG) studied over time. Plaque index evolution over time in the a) TG, and b) CG. Gingival index evolution over time in the c) TG, and d) CG. T0, baseline; T1, 45 days; T2, 75 days; T3, 90 days.

Discussion

The main results verified in this study were that the groups presented significant differences between the evaluated periods. The propolis group showed a greater reduction ($p < 0.05$) in the depth of probing than CG at 45 and 90 days. The literature shows a few clinical studies in which subgingival irrigation with solutions containing propolis was used as an adjuvant in the periodontal treatment. de Freitas *et al.* (2016) compared the treatment of periodontal disease with scaling and root planing associated with irrigation with 0.9% saline solution, chlorhexidine 0.1 and 0.5%, sodium hypochlorite and propolis extract 11% in rats. The results showed no differences between groups. Clinical studies have also been done with mouthwash containing propolis. Murray *et al.* (1997) showed that propolis did not significantly inhibit the formation of bacterial plaque. However, Koo *et al.* (2002) demonstrated that in a group rinsing with propolis solution, the plaque index after three days was significantly less than in the placebo group. Additionally, Morawiec *et al.* (2015) demonstrated the antimicrobial effect of the extract of Brazilian green propolis used for hygiene maintenance following minor oral surgeries, and Tanasiewicz *et al.* (2012) showed that hygienic preparations with a 3% content of ethanol propolis extract efficiently support removal of dental plaque and improve the state of marginal periodontium.

There are few clinical studies examining subgingival irrigation with propolis-containing solution as adjuvant to periodontal treatment (Coutinho, 2012; Sanghani *et al.*, 2014; Gebara *et al.*, 2003). Although the sample size was small, the results obtained in this study were interesting. The mechanical treatment was effective in both groups, independent of irrigation solution, and PD improved in both groups. The propolis increased the benefits of conventional mechanical periodontal treatment when baseline values were compared to those of later time points. In patients with PD of at least 5 mm, adjuvant treatment with propolis effectively reduced PD from the onset of treatment to the 45-day time point.

Other clinical parameters such as PI, GI and OHI were also evaluated. Both PI and GI significantly improved with adjuvant therapy from the initiation of treatment to the 90-day time point; Between-group differences were not significant. Similarly, when Gebara *et al.* (2003) and Coutinho (2012) evaluated clinical and microbiological parameters among patients, they found that irrigation with a hydroalcoholic solution of propolis extract 20% (w/v) as adjuvant to periodontal treatment was more effective than conventional mechanical treatment alone. These studies and our research utilized the same concentration of propolis, but they used four irrigation washes as compared to our two procedures.

In addition, Sanghani *et al.* (2014) also demonstrated subgingival delivery of propolis showed promising results as an adjunct to scaling and root planing in patients with chronic periodontitis when assessed by clinical and microbiological parameters; however, this study used propolis at a concentration of 5 mg.

One study showed that the use of oral propolis (400 mg of propolis once daily) for 6 months reduced the levels of HbA1c, fasting plasma glucose (FPG), serum N (carboxymethyl) lysine (CML), and improved periodontal therapy outcome in people with DMt2 and CP. The propolis group showed significantly greater probing depth reduction and clinical attachment level gain than the control group after 3 and 6 months (El-Sharkawy *et al.,* 2016). Recently a group presented a study with dental paste in patients with orthodontic appliances in which they analyzed plaque index and gingival index, and found that there was a greater reduction of the indices in the group that used propolis (Machorowska-Pienidhek *et al.,* 2016). The efficacy of a gel containing 3% of ethanolic extract of Brazilian green propolis (EEP-B) was tested for maintenance of oral hygiene in patients with wounds in the postoperative buccal mucosa of mandibular fracture. Hygiene was evaluated by means of plaque index, bleeding index and oral hygiene index. A better efficacy was observed in the group using EEP-B-containing gel (Niedzielska *et al.,* 2016).

The antimicrobial used for subgingival irrigation - a hydroalcoholic solution of propolis extract 20% (w/v) - was submitted to the following physiochemical analyzes: high performance liquid chromatography (HPLC) and ultraviolet spectrophotometry. The antibacterial activity of propolis could thus be observed and the propolis solution checked for the presence of flavonoids, aromatic acids and esters (which differ among flora). Marcucci *et al.* (2001) report that a great diversity of propolis exists within Brazilian borders due to the vegetation present. Their physiochemical analysis (through HPLC) showed the presence of the following flavonoids: quercetin, artepellin-C, galangine, kaempferol, ferulic acid and caffeic acid.

Salomão *et al.* (2008) verified the bactericidal properties of caffeic acid and ferulic acid, while Marcucci *et al.* (1995) did so for galangine. Mirzoeva and Calder (1996) attributed anti-inflammatory properties to the presence of caffeic acid, quercetin, naringenin, the caffeic acid phenethyl ester (CAPE), apigenin, ferulic acid and galangine. While many authors attribute the antioxidant activity of the propolis to CAPE and kaempferol, Kimoto *et al.* (1998) studied the antitumoral action of the flavonoids and obtained quite promising results with artepillin-C; Jin *et al.* (2005) found similar results with CAPE. Sforcin (2007) verified the antitumoral activity of propolis; although the underlying mechanism remains unclear, it is known that propolis can exert antimicrobial effects (Santos *et al.*, 2002; Feres *et al.*, 2005; Koru *et al.*, 2007; Coutinho, 2012) either directly on the microorganism or indirectly, through stimulation of the immune system.

We found propolis extract to contain flavonoids with antimicrobial, anti-inflammatory, antioxidant and antitumoral effects. Marcucci and Bankova (1999) classified propolis samples collected throughout Brazil (except in the northern area) and determined their biological properties. They observed that the biological properties depend on the type of propolis tested. For example, the propolis that possesses antimicrobial activity against *Streptococcus aureus* (Group 12 - southeastern area) does not act in the same way on *Streptococcus mutans* (Group 3 - southern area). Various propolis samples differ in color as well. The propolis of the southeastern area has a greenish brown coloration; UV spectrophotometry performed on the propolis extract used in this study registered a value of 0.144, with a coloration similar to that of clear amber. According to Koo and Park (1997), the quantitative analysis of total flavonoids is not sufficient to rank various propolis samples because the types of flavonoid present control compound efficacy. Santos *et al.* (2002) suggested that the antimicrobial action of the propolis is probably caused by synergistic action among the different components.

Numerous studies have evaluated the antimicrobial efficacy of propolis. Siqueira *et al.* (2015) demonstrated antifungal activity of red propolis alcoholic extract against different *Candida* species isolated from chronic periodontitis cases. Lu *et al.* (2005), in microbiological studies *in vitro*, verified antimicrobial activity on some strains of cariogenic bacteria such as *Streptococcus aureus*. Gebara *et al.* (2002), Santos *et al.* (2002), Feres *et al.* (2005), Coutinho (2012) and Sanghani *et al.* (2014) observed similar activity against species related to periodontal disease, such as *Aggregatibacter actinomycetemcomitans*, *Fusobacterium nucleatum, Porphyromonas gingivalis* and *Prevotella intermedia*.

The results of this clinical study corroborate with the results of other studies and demonstrate that irrigation with a hydroalcoholic solution of propolis extract as an adjunct in periodontal treatment was more effective than irrigation with saline solution. Because the present study has limitations in both clinical design and sample size, further randomized clinical trials using propolis must be done to evaluate its possible clinical benefits in periodontal therapy, including trials using varying concentrations and frequencies of irrigation.

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