Propolis Extract as an Adjuvant to Periodontal Treatment

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Purpose: The aim of this study was to evaluate the effect of subgingival irrigation with propolis extract by clinical and microbiological parameters.

Materials and Methods: Twenty patients diagnosed with chronic periodontitis presenting three non-adjacent teeth with deep pockets were selected. After scaling and root planing, the selected periodontal sites were submitted to one of the following treatments: irrigation with a hydro alcoholic solution of propolis extract twice/week for two weeks (group A); irrigation with a placebo twice/week for two weeks (group B); or no additional treatment (C). Subgingival plaque sampling and scaling and root planing were performed two weeks after clinical data recording. Two weeks later irrigation procedures were started (Baseline). Microbiological and clinical data were collected at baseline, and after 4, 6 and 24 weeks.

Results: A decrease in total viable counts of anaerobic bacteria (p=0.007), an increase in the proportion of sites with low levels ($\leq 10^3$ cfu/mL) of *Porphyromonas gingivalis* (p=0.005), and a decrease in the number of sites with detectable presence of yeasts (p=0.000) were observed in group A sites when compared to group B and C sites. Propolis treatment did not lead to an increase in organisms such as coagulase positive Staphylococci and *Pseudomonas* spp. 24 weeks after treatment there was an increased proportion of sites showing probing depth (PD) $\leq$ 3 mm in Group A sites.

Conclusion: Subgingival irrigation with propolis extract as an adjuvant to periodontal treatment was more effective than conventional treatment both by clinical and microbiological parameters.

Key words: propolis, bacteria, periodontitis, irrigation, periodontal pocket

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Human periodontal disease has been associated with a complex microbiota. Several studies have shown that the presence of bacteria such as *Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis* and *Prevotella intermedia* are related to active periodontal disease (Slots et al, 1986; Bragd et al, 1987; Dzink et al, 1988; Slots and Listgarten 1988; Dahlén et al, 1989). The infectious nature of periodontal disease and inherent limitations of scaling and root planing lead sometimes to the use of antimicrobial agents in order to reduce periodontal pathogens (Committee on Research, Science and Therapy, 1996). Locally delivered antimicrobials are an alternative to systemic antibiotics and may help to arrest periodontal disease progression (Greenstein, 1987; Rams and Slots, 1996).

Propolis is a natural balm that shows anti-inflammatory and antibacterial properties (Lindenfelser, 1967; Grange and Davey, 1990; Dobrowolski et al, 1991; Focht et al, 1993; Bankova et al, 1995; Gebara et al, 1996; Steinberg et al, 1996; Park et al, 1998; Nieva et al, 1999; Sforcin et al, 2000; Gebara

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Table 1 Study groups

A	B	C
Scaling and root planing + Irrigation with hydro alcoholic propolis solution (20µg/ml) 2 times a week for 2 weeks	Scaling and root planing + Irrigation with placebo (14% ethanol) 2 times a week for 2 weeks	Scaling and root planing (S.R.P.)

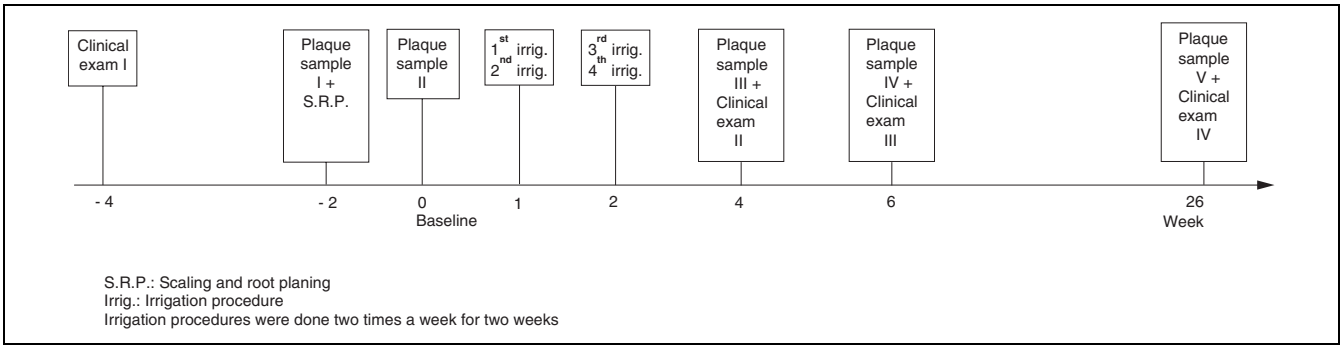

Fig 1 Project Schedule

et al, 2002). Its safety for human usage has already been shown (Magro-Filho et al, 1990; Arvouet-Grand et al, 1993; Magro-Filho et al, 1994). Its antibacterial spectrum includes inhibition of several periodontopathogenic bacteria (Gebara et al, 2002).

This study aimed to evaluate the additional effect of subgingival irrigation with propolis extract after mechanical therapy on deep periodontal pockets by clinical and microbiological parameters.

MATERIALS AND METHODS

Twenty patients (14 females and 6 males, aged 25 to 57 years) diagnosed with chronic periodontitis, exhibiting at least 3 single-rooted teeth with periodontal pocket depths ≥ 5 mm, and who had not taken systemic antibiotics for the previous 6 months were selected for this study. The Ethical Committee of the School of Dentistry, University of São Paulo, approved all procedures and a written informed consent was obtained from each patient. All patients were clinically examined in order to register plaque index (Silness and Löe, 1964), gingival index (Löe and Silness, 1963), pocket probing depth, bleeding upon probing and clinical attachment level. All patients received oral hygiene instructions and were requested to report any eventual usage of systemic antibiotics or antimicrobial rinses during the experimental period.

Three non-adjacent single-rooted teeth exhibiting bleeding upon probing and pocket probing depth of at least 5 mm were selected from each patient. These periodontal pockets were treated as group A, B or C (Table 1), in such a way that each patient had one tooth allocated in each group. Two weeks after the first exam, subgingival plaque was sampled (Sample I) and scaling and root planing of all teeth included in the study was performed with curettes. All other teeth were treated with an ultrasonic device. Teeth from group A received irrigation with 3ml of a propolis hydro alcoholic solution (20% propolis extract) twice a week, for two weeks. Teeth from group B received irrigation with 3ml of a placebo solution (containing 14% ethanol-propolis extract vehicle) twice a week, for two weeks. Teeth from group C (control group) did not receive any additional treatment. Clinical and microbiological parameters were evaluated before any treatment (I), 2 weeks after scaling and root planing (II); 2, 4 and 24 weeks after irrigation procedures (III, IV and V respectively – Fig 1). All patients were engaged in a supportive periodontal treatment program for the following 6 months.

Table 2 Proportion of sites with ≥ 10^5 cfu of total viable counts/sample

Schedule	n	A		B	C
Plaque sample I	20	85%		90%	95%
Plaque sample II	20	85%	Q=4.89	80%	80%
Plaque sample III	20	60%		70%	80%
Plaque sample IV	20	40%	Q=6.03	70%	70%
Plaque sample V	14	50%		100%	79%

A: p=0.007
Statistically significant results: p ≤ 0.050 and Q ≥ 3.858

After removal of supragingival plaque with a sterile curette, subgingival plaque was obtained by inserting two paper points into the deepest portion of the pocket for 10 seconds. The paper points were immediately transferred to VMGA III transport media (Möller, 1966). Samples were processed within 24 hours after collection. The flasks were maintained at 37ºC for 10 minutes, vortexed for 30 seconds and diluted in phosphate buffer saline (PBS). Aliquots of 10 µL of each dilution were inoculated in triplicate in plates with Brucella Agar (Difco, Detroit, USA) enriched with blood (2%), hemin (0.1%-Sigma, St. Louis, USA) and menadione (0.01%, Sigma, St. Louis, USA), Saboraud Agar (Difco, Detroit, USA), Mac Conkey Agar (Difco, Detroit, USA), S110 Agar (Difco, Detroit, USA) and TSBV (Triptone Soy Agar – Difco, Detroit, USA – enriched with 0.1% yeast extract, 10% horse serum and added with 75 µg/mL of bacitracin and 5 µg/mL of vancomycin, Sigma St. Louis, USA). The plates containing Brucella Agar and the ones with TSBV were incubated under anaerobic condition (Gas Pak – BBL, Sparks, USA) at 37º C for 5 and 4 days respectively. Mac Conkey Agar and Agar S110 plates were incubated under aerobic conditions at 37ºC for 48 hours. Saboraud Agar plates were incubated at room temperature for 4 days.

Total viable counts were evaluated on Brucella Agar plates. *P. melaninogenica* group, *P. intermedia* group, *P. gingivalis,* and *A. actinomycetemcomitans* were identified following standard protocols (Slots, 1986; Slots, 1987; Alcoforado et al, 1987). Colonial morphological analysis and methylene blue staining were used to identify yeast colonies on Saboraud Agar. *Pseudomonas* was identified on Mac Conkey Agar after colony morphological analysis and oxidase test. The colonies on agar S110 were evaluated through morphological analysis and coagulase test (Coagu-plasma, Inlab, Brazil).

Results for the clinical and microbiological parameters were compared among the 3 groups, at different intervals. Statistical analysis was performed using proportion comparison and chi-square test.

RESULTS

Fourteen of twenty selected patients were followed to the end of the study. The reasons for drop out were the use of systemic antibiotics (5), and non-attendance at the scheduled appointment (1).

At baseline, no differences in clinical or microbiological parameters could be detected among the three groups. A statistically significant correlation was observed between the presence of yeasts and the levels of *P. gingivalis* (≥ 10^3 cfu/sample).

The effect of treatment with propolis in sites from group A was compared with clinical and microbiological data obtained from sites of group B and C. A decrease in total viable counts of anaerobic bacteria (Table 2), and an increase in the proportion of sites with low levels of *P. gingivalis* (≤ 10^3 cfu/mL) (Table 3) were observed in sites from group A, when compared to the other groups. There were no differences in the prevalence or amount of coagulase positive Staphylococci and *Pseudomonas* spp among the groups. Table 4 shows that a decrease in the number of sites with detectable yeasts was observed in group A sites within time

Table 3 Proportion of sites with ≤ 10^3 cfu of Porphyromonas gingivalis/sample

Schedule	n	A		B	C
Plaque sample I	20	45%		35%	35%
Plaque sample II	20	45%	Q=8.74	55%	65%
Plaque sample III	20	45%		40%	60%
Plaque sample IV	20	90%	Q=5.45	55%	60%
Plaque sample V	14	79%		43%	57%

A: p=0.005
Statistically significant results: p ≤ 0.050 and Q ≥ 3.858

Table 4 Proportion of sites positive to yeasts

Schedule	n	A*		B**		C***	
Plaque sample I	20	65%	Q=4.89	45%	Q=5.37	50%	Q=4.00
Plaque sample II	20	80%		75%		65%	
Plaque sample III	20	35%	Q=8.36	90%		85%	
Plaque sample IV	20	15%		75%	Q=6.70	85%	Q=4.76
Plaque sample V	14	93%		100%		100%	

A: p=0.000* ; B: p=0.002** ; C: p=0.005***
Statistically significant results: p ≤ 0.050 and Q ≥ 3.858

(p=0.000). An increase in the number of positive sites to yeasts was observed in group B (p=0.002) and group C (p=0.005) within time after treatment. The antimicrobial activity of the propolis extract used against *P. intermedia* group, *P. melaninogenica* group and *A. actinomycetemcomitans* could not be evaluated due to the low prevalence of these bacteria at the sampled sites at baseline.

When clinical parameters were evaluated, the reduction in the proportion of sites positive to bleeding upon probing was significantly higher for group A (A: Q= 6.60) and group B (B: Q=4.57) when compared with group C (Table 5). Twenty-four weeks after the irrigation procedures a significant decrease in clinical probing depth was observed in group A when compared to groups B and C (Q=3.633), as shown in Table 6. No statistically significant differences were observed among the three groups in regard to plaque index, gingival index and attachment level through the study. A statistically significant correlation was observed between the total viable counts ≤ 10^5 cfu/sample and the absence of bleeding upon probing (p=0.015), when all 60 sites were evaluated at the end of the study (Table 7).

DISCUSSION

Several authors reported the use of antimicrobial substances as irrigant after mechanical therapy in periodontal pockets (Greenstein, 1987; Goodson, 1994; Rams and Slots, 1996). Antimicrobial activity of propolis *in vitro* against periodontopathogenic organisms (Gebara et al, 2002) led us to evaluate its use *in vivo*, by assessing microbiological and clinical data.

The effect of propolis irrigation was compared with the groups receiving placebo (B) or no irrigation (C) in the same patient in order to avoid differences in response to the treatment used in different individuals. In this study, the reduction of the total viable counts was detected 2 weeks after the irrigation procedures for group A, and was maintained for 24 weeks after the irrigation procedures. This reduc-

Table 5 Proportion of sites negative to bleeding upon probing

Schedule	n	A		B		C	
Initial exam	20	0	Q=12.23	0	Q=8.69	0	Q=10.49
Clinical exam II	20	50%		30%		40%	
Clinical exam III	20	85%	Q=6.60	60%	Q=4.57	75%	
Clinical exam IV	14	57%		43%		14%	

A: p=0.000, B: p=0.007 and C: p=0.001
Statistically significant results: $p \leq 0.050$ and $Q \geq 3.633$

Table 6 Proportion of sites with pocket depth of ≤ 3mm

Schedule	n	A		B		C	
Initial exam	20	0	Q=16.73	0	Q=13.10	0	Q=17.75
Clinical exam II	20	75%		55%		80%	
Clinical exam III	20	85%	Q=3.633	60%		75%	
Clinical exam IV	14	93%		64%		79%	

A: p=0.000 ; B: p=0.000 ; C: p=0.000
Statistically significant results: $p \leq 0.050$ and $Q \geq 3.633$

Table 7 Total viable counts X Bleeding on probing

	Bleeding on probing (+)	Bleeding on probing (-)
Total viable counts ≥ 105 cfu/mL	29	21
Total viable counts < 105 cfu/mL	1	9

Statistically significant correlation between total viable counts $\geq 10^5$ cfu/mL and bleeding on probing (p=0.015).

tion in total viable bacterial counts may be of clinical interest, since Wolff et al, (1994) reported that total viable counts as low as $\geq 10^3$ and $\leq 10^5$ cfu/sample are related to low numbers of periodontopathogenic organisms. In addition we have found a statistically significant correlation between the total viable counts $\leq 10^5$ cfu/sample and the absence of bleeding upon probing at the end of the study.

At the beginning of the experiment, as well as after scaling and root planing procedures, the majority of the evaluated sites exhibited high levels of *P. gingivalis* ($\geq 10^5$ cfu/sample). The use of the propolis extract in group A sites increased the proportion of sites with low levels ($\leq 10^3$ cfu/sample) of *P. gingivalis* from 45% to 79%. According to Wolff et al, (1994) the presence of levels of *P. gingivalis* below 10^3 cfu per sample of subgingival plaque, can be considered as a negative value for the presence of this bacteria.

Repopulation by periodontopathogenic organisms usually occurs 4–8 weeks after scaling (Magnusson et al, 1984), and may influence the suc-

Table 8 Propolis extract chromatography

Compound	µg/mL
B	More than 4500
C	153.30
D	4288.95
E*	137.43
G1*	570.21
L1*	80.24
L2*	144.99
Kaempferol	13.19
Kercetin	221.90

B – 3-Prenil 4-hidroxicinamic Acid
D – 3,5-direnil-4-hidroxicinamic Acid
* – unknown compounds

cess of the therapy (Shiloah and Patters, 1996). The increase in the proportion of sites with low levels of *P. gingivalis* for group A sites, which received irrigation with propolis, was observed 2 weeks after the last irrigation procedure and was maintained for 24 weeks after baseline. This data suggested that the effect of propolis irrigation might be long lasting, leading to a change in the repopulation process occurring in the periodontal pocket.

The propolis extract sites (group A) exhibited a decrease in the proportion of sites positive for yeasts from 65% to 35% at 2 weeks, and to 15% at 4 weeks after the irrigation procedures. However, 24 weeks after the irrigation procedures, an increase in the proportion of sites positive to yeasts was observed. Groups B and C showed an increase in the proportion of sites positive to yeasts throughout the study. The inhibitory activity of propolis against yeasts has been recognized (Gebara et al, 2002). However, the clinical relevance of these data should be confirmed since the significance of yeasts in the destructive process of periodontal disease remains to be elucidated.

The use of propolis extract did not result in selection of coagulase positive *Staphylococci* and *Pseudomonas* spp. in any of the observation periods of the experiment suggesting its safety for *in vivo* usage (Council on Scientific Affairs, 1998).

Scaling and root planing were effective in increasing the proportion of sites negative to bleeding upon probing within time, as shown by others (Oosterwaal, 1987; Socransky et al, 1988). A greater improvement in this clinical parameter was observed 24 weeks after the irrigation procedures for group A and B, but not for group C. This may indicate that the mechanical removal of bacteria by irrigation of the periodontal pocket (in an SPT program) was able to reduce the number of sites bleeding upon probing independent of the substance used which is in agreement with observations by Newman et al, (1994).

The scaling and root planing procedures were efficient in reducing the pocket probing depth to levels ≤ 3 mm, for all studied sites. There was a tendency to a decrease in clinical probing depth, 24 weeks after the irrigation procedures (Q=3.633) in group A, when compared to the other two groups, indicating that the irrigation with propolis extract was able to bring clinical benefits even 24 weeks after its usage. According to Ludovico et al, (1990) and in agreement with Lembariti et al, (1998) one session of scaling and root planing is not enough to maintain the subgingival microbiota compatible with health when patients with pocket probing depth ≥ 5 mm are considered. The benefits provided by propolis extract irrigation even after 24 weeks of the irrigation procedures, indicate that its use should be considered as adjuvant to scaling and root planing.

The propolis extract used exhibited antimicrobial activity against oral bacteria and *Candida*. The chromatogram of the propolis extract (Table 8) indicated that it was rich in flavonoides, which are considered the active compounds against microorganisms. Since we have shown that the irrigation with propolis extract exhibited additional effects to the mechanical treatment, the purification of the active compounds in propolis extract with antimicrobial and anti-inflammatory characteristics would be of interest. The use of these purified antimicrobial agents would avoid differences in antimicrobial activity among extracts from different places (Bankova et al, 1995), and provide the periodontist with another tool to control subgingival organisms.

The irrigation with propolis extract as adjuvant to periodontal treatment was able to bring more benefits than the utilization of a placebo irrigant solution or scaling and root planing alone. Additional studies are needed to show whether an increase in the concentration of the propolis extract used, increase in the frequency of application, incorporation of the propolis extract to slow release devices, or use of the purified active compounds present in propolis extract alone, could bring even better results.

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