



# Randomized, double-blind, placebo-controlled clinical trial on the effects of propolis and chlorhexidine mouthrinses on gingivitis

Ensaio clínico duplo-cego randomizado e placebo-controlado dos efeitos de enxaguatórios a base de própolis e clorexidina na saúde gengival, para análise

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## ABSTRACT

**Objective:** The aim of this study was to compare the effects of typified propolis and chlorhexidine mouthrinses on gingival health in a randomized double-blind placebo-controlled clinical trial. **Material and Methods:** Sixty participants were randomized to 3 mouthrinse study groups: 1) 2% typified propolis (n = 20); 2) 0.12% chlorhexidine (n = 20), and 3) placebo (n = 20). Participants rinsed unsupervised twice a day for 28 days. The Papillary Bleeding Score (PBS) was measured on the mesio-buccal surfaces of all teeth at baseline and 28 days thereafter. Co-variance analysis was employed to compare PBS average values and the number of sites with PBS  $\geq 2$  among study groups. Sub-group analysis was further applied to participants who were  $< 40$  years-old. **Results:** The results show efficacy of propolis mouthrinse when comparing before and after treatment protocols significantly for a reduction of mean PBS scores. For younger participants propolis mouthrinse was superior to all groups in reducing mean PBS scores and significant when compared to 0.12% chlorhexidine mouthrinse. **Conclusion:** The efficacy of 2% typified propolis mouthrinse was demonstrated in reducing the levels of gingival inflammation. These results need to be duplicated by other investigators under similar study protocols.

## KEYWORDS

Propolis; Chlorhexidine; Gingivitis.

## RESUMO

**Objetivos:** O propósito deste estudo foi o de comparar os efeitos de enxaguatórios a base de própolis tipificada e clorexidina na saúde gengival, empregando-se um ensaio clínico duplo-cego, randomizado, e placebo-controlado. **Material e Métodos:** Sessenta participantes foram randomizados em 3 grupos de enxaguatórios, a saber: própolis tipificada 2% (n = 20), clorexidina 0,12% (n = 20) e placebo (n = 20). Os participantes bochecharam duas vezes ao dia por 28 dias os respectivos enxaguatórios. Medidas do índice de sangramento papilar (PBS) foram feitas na superfície méso-bucal de todos os dentes dos participantes no tempo basal e após 28 dias. Análise de co-variância foi empregada permitindo comparações entre os grupos das médias do PBS e do número de sítios  $\geq 2$ . Análise de sub-grupo foi efetuada em participantes com idade  $< 40$  anos. **Resultados:** Constataram-se os efeitos positivos do enxaguatório de própolis a 2% na redução da inflamação gengival após uso não supervisionado por 28 dias. Análise no sub-grupo de participantes com idade  $< 40$  anos constatou superioridade do enxaguatório de própolis quando comparado com o enxaguatório de clorexidina a 0,12%. **Conclusão:** Este ensaio clínico demonstrou a eficácia de enxaguatório de própolis tipificada a 2% na redução da inflamação gengival. Estes resultados necessitam ser duplicados por outros investigadores.

## PALAVRAS-CHAVE

Própolis; Clorexidina; Gengivite.

## INTRODUCTION

The use of oral rinses for preventing and controlling of gingivitis has been recommended in the repertoire of oral hygiene procedures. Chlorhexidine mouthrinses have been employed for promotion of gingival health over 45 years [1]. The efficacy of chlorhexidine mouthrinses in combating gingivitis is well documented as meta-analysis studies have clearly demonstrated [2].

Propolis is synonymous of national heritage (in Brazil) with nutritious and therapeutic attributes, where its therapeutic properties have been described universally. The therapeutic effects of propolis have been the subject of research over 100 years, and recent guidelines suggest its potential for use in the future [3]. One of the most studied applications of propolis worldwide has been in dentistry where scientific reports date back to 1952 [4]. However, there is not evidence of the therapeutic effects of propolis in dentistry, and rarely in medicine, with the use of randomized clinical trials employing rigorous methodology.

The aim of this study was to compare the effects of typified propolis and chlorhexidine mouthrinses on gingival health in a randomized double-blind placebo-controlled clinical trial.

## MATERIAL & METHODS

### Inclusion/Exclusion Criteria

One hundred-fifty patients were screened from a patient pool attending the Dental Clinics at University Bandeirante of São Paulo, São Paulo, Brazil. After signing informed consent approved by the Institutional Review Board (UNIBAN-Protocol N.0038/2007), patients were submitted to eligibility criteria. Inclusion/exclusion criteria included: the presence of at least 20 teeth, no clinical signs of periodontal disease, age range of 18 to 55 years-old, not being a current smoker, normal saliva secretion rate, not being pregnant, and not under any oral topical or systemic medication.

### Subject Population/Demographics

This was a randomized double-blind placebo-controlled clinical trial. Sixty patients met the entry criteria. These participants were 18-55 years old of both genders and in good general health. Table 1 depicts the demographic characteristics of study participants. Study groups were well balanced at baseline for all demographic variables.

**Table 1** - Demographics and clinical parameters of study participants at entry

Parameter/Group	Chlorhexidine	Propolis	Placebo	Sig.
<b>Age</b>	41.6 (13.4)*	39.4 (9.8)	39.0 (11.7)	ANOVA NS
<b>Gender</b>				
Male	7	8	9	Chi-square Test NS
Female	13	12	11	
<b>Race</b>				
White	15	13	14	Chi-square Test NS
Black	2	4	4	
Brown	3	3	2	

\*mean (standard deviation); NS = not significant.

### Treatment Products and Protocol

The medical history of participants was obtained and an oral soft tissue examination was rendered to all participants. Participants were randomized to 3 experimental groups: 1) alcohol-free, 2% typified propolis mouthrinse (n = 20). 2% Própolis rinse was manufactured at the laboratories of the Department of Pharmacology at Federal University of Santa Catarina, Florianópolis, SC, Brazil. The formulation included 2% typified propolis, mint flavor, polioxyethelers, sorbitol, blue color and water; 2) a commercially available 0.12% chlorhexidine mouthrinse (n = 20); 3) placebo mouthrinse that matched propolis mouthrinse

without the active ingredient ( $n = 20$ ). Patients rinsed 15 ml of mouthrinse, according their allocated groups. Rinsing was performed in the morning and before bedtime after ordinary oral hygiene procedures.

All adverse reactions were documented and patient accountability/continuance criteria were recorded at all visits.

### **Allocation Concealment**

For allocation of groups a computer-generated list of random numbers was used. Rinses were prepared in dark-bottles, which were consecutively numbered according to the randomization schedule. Participants were randomized to one of the three test color-matched rinses. Study coordinator, examiners, and participants were unaware of group allocation. The group identity was generated and kept in Florianopolis, SC, Brazil while the study was conducted in São Paulo, SP, Brazil.

### **Papillary Bleeding Score (PBS)**

PBS measurements were made on the mesial buccal surfaces of all teeth [6], excluding third molars. This index utilized a triangle-shaped toothpick (STIMUDENT, J&J, New Jersey, USA) made of soft, pliable wood to stimulate the interproximal papilla. The test was performed one quadrant at a time. The STIMUDENT is inserted horizontally between the teeth from the facial surface, depressing the interproximal papilla by up to 2 mm. The STIMUDENT was inserted one time and then the site was scored after 15 s. PBS values ranged from 0 (healthy gingiva) to 5 (severe inflammation, marked redness and edema; tendency to spontaneous bleeding). The PBS has been reported to be the most reproducible and reliable index (both intra- and inter-examiner) for measuring the gingival status of patients when compared to established indexes for gingivitis [7]. PBS measurements were performed by an experienced examiner (AAA). Intra-examiner reliability exercises revealed a Kappa test score of 0.85, indicating adequate reproducibility of PBS measurements.

### **Statistical Analysis**

Each participant provided mean values for PBS and the number of sites with a  $PBS \geq 2$  for the entire dentition at baseline and 28 days thereafter was considered. Covariance analysis was employed allowing for comparisons among groups of mean values for PBS and the number of sites with a  $PBS \geq 2$ . The baseline results for PBS were treated as a covariable in the analytic models. General linear models were employed adjusted for age, gender and mean PBS (or the number of sites with  $PBS \geq 2$ ) at baseline. Similar sub-group analysis was performed in participants < 40 years old ( $n = 10$  for each group).

## **RESULTS**

All groups reported adverse reactions. Propolis formulations presented with the least number of reports ( $n = 7$ , including breath alteration, burning sensation, yellow teeth, taste alteration, bitter taste). Chlorhexidine formulations had the highest number of adverse reactions ( $n = 23$ , with emphasis on burning sensation, taste alterations, yellow teeth, breath alteration, tongue burning, mucosal irritation, bitter taste). The placebo group reported the majority of reactions ( $n = 9$ ) related to taste alterations.

Table 2 shows the results by experimental group of the effects of rinsing on mean PBS values and on the number of sites with  $PBS \geq 2$ . At baseline groups were well balanced on both mean PBS values and on the number of sites with  $PBS \geq 2$ , where no statistically significant differences were detected among groups.

The same results were observed at the end of the study period (28 - day visit). However, paired analysis revealed that there was a reduction in mean PBS values between baseline and after 28 days for the propolis group only ( $p < 0.05$ ). Sub-group analysis in patients < 40 years old revealed that comparisons among experimental groups showed a significant difference between propolis and chlorhexidine groups on mean PBS values by pairwise tests (Table 3) at the 28-day visit.

**Table 2** - Effects of mouthrinses on mean PBS values and on the number of sites with PBS  $\geq 2$ 

Group	Baseline Mean (SD) PBS	28 days Mean (SD) PBS	Baseline Mean (SD) Sites PBS $\geq 2$	28 dias Mean (SD) Sites PBS $\geq 2$
Propolis 2% (n = 20)	1.0 (0.5) <sup>a</sup>	0.5 (0.5) <sup>a</sup>	4.7 (2.9)	3.3 (3.9)
Chlorhexidine 0.12% (n = 20)	1.1 (0.5)	0.9 (0.6)	5.7 (4.7)	4.4 (5.2)
Placebo (n = 20)	0.9 (0.4)	0.7 (0.4)	4.7 (3.8)	3.8 (2.9)
<b>ANOVA</b>	<b>0.456</b>	<b>0.141</b>	<b>0.603</b>	<b>0.172</b>

a- Numbers with same superscripts are significantly different between baseline and 28-day visit comparisons ( $p < 0.05$ ).  
SD - Standard deviation.

**Table 3** - Effects of mouthrinses on mean PBS values and on the number of sites with PBS  $\geq 2$  in participants < 40 years old after 28 days

Group	28 days Mean (SD) PBS	28 dias Mean (SD) Sites PBS $\geq 2$
Propolis 2% (n=10)	0.4 (0.5) <sup>a</sup>	2.8 (3.3)
Chlorhexidine 0.12% (n=10)	1.0 (0.8) <sup>a</sup>	6.2 (6.6)
Placebo (n=10)	0.7 (0.3)	2.7 (3.2)
<b>ANOVA</b>	<b>0.08</b>	<b>0.179</b>

a - Numbers with same superscripts are significantly different by Fischer pairwise test ( $p < 0.05$ ).  
SD- Standard deviation

## DISCUSSION

Initial studies utilizing propolis gel and rinses in the 1980's were conducted in Eastern Europe and Cuba [8-10] to determine the efficacy of these propolis vehicles in combating gingivitis. These publications however are difficult to access and therefore do not permit an adequate evaluation of these studies. Consequently, it is problematic to compare and to extrapolate results from those studies.

Recent studies (Phase II studies) were performed in Brasil evaluating an alcohol-free 5% propolis mouthrinse and its acceptability [11]. This same propolis prototype was evaluated regarding its efficacy in 18 subjects with promising results in reducing gingivitis [12].

The present clinical study does not permit comparisons with the study mentioned previously with particular emphasis on the prevention of gingival inflammation. Randomized clinical

trials allow for an assessment of efficacy controlling for bias eliminating therefore systematic error in the interpretation of results [13]. We employed 3 study groups comparing propolis, chlorhexidine and placebo mouthrinses in a randomized double-blind placebo-controlled trial. The current literature lacks studies of propolis in the treatment of gingivitis employing the methodology mentioned above rendering comparisons difficult. Furthermore, it was not possible to conduct sample size and power analysis for our trial before study commencement because of lack of randomized clinical trials of propolis on gingivitis.

The results of the present study clearly demonstrated that typified propolis rinse was effective in reducing gingival inflammation with unsupervised rinsing twice a day for 28 days. (by comparing pre- and post-rinse gingival parameters) (Table 2). These attributes were not observed for the chlorhexidine and placebo

groups. However, comparisons among the 3 experimental groups did not reach statistical significance. We next performed sub-group analysis in participants < 40 years old (Table 3). The underlying premise for this analysis is based on the fact that the older group (> 40 years old) possesses a higher number of subgingival restorations. Subgingival restorations comprise a risk factor for increased dental plaque accumulation (influencing respectively gingival and periodontal health) [14] and, consequently, would potentially influence the results of this study. The results of the sub-group analysis in the age group < 40 years old showed superiority of the propolis mouthrinse when compared to the chlorhexidine mouthrinse in the control of gingival inflammation after 28 days (Table 3).

Whereas mechanistic studies are necessary to elucidate the effects of propolis on the gingival health that were evident in our study results, it is conceivably possible to propose that these effects resulted from anti-inflammatory and antimicrobial properties on the gingival tissues. The anti-inflammatory properties of propolis are well documented [15]. With the advent of new technology for analysis of the oral microbiome [16] and its functional capability (metagenomic analysis) in disease and in health, it will be possible to explain in greater detail the clinical results presently observed.

Lastly, it is important to point out that the results from this study need to be duplicated by other investigators in order to confirm or not these results.

## CONCLUSION

Typified propolis rinses may be of value in the prevention of gingivitis when compared to existing and placebo rinses.

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