JOURNAL OF APPLIED ORAL SCIENCE
www.fob.usp.br/jaos or www.scielo.br/jaos
J Appl Oral Sci. *2009;17(5):404-7*

ANTIPLAQUE AND ANTIGINGIVITIS EFFECT OF *LIPPIA SIDOIDES*. A DOUBLE-BLIND CLINICAL STUDY IN HUMANS

Ítalo Sarto Carvalho RODRIGUES[1], Vinícius Nascimento TAVARES[1], Sérgio Luís da Silva PEREIRA[2], Flávio Nogueira da COSTA[3]

1- Graduate Student, University of Fortaleza, Fortaleza CE, Brazil.
2- PhD in Periodontics, University of Fortaleza, Fortaleza CE, Brazil.
3- Specialist in Organic Chemistry, University of Fortaleza, Fortaleza CE, Brazil.

Corresponding address: Sérgio Luís da Silva Pereira - Av. Engenheiro Leal Lima Verde, 2086 - Alagadiço Novo - 60833-520 - Fortaleza, CE - Phone: +55-85-34744108 - Fax: +55-85-34773059 - e-mail: luiss@unifor.br

Received: September 29, 2008 - Modification: May 20, 2009 - Accepted: May 22, 2009

ABSTRACT

Objectives: The antiplaque and antigingivitis effect of *Lippia Sidoides* (LS) was evaluated in this *in vivo* investigation. Material and Methods: Twenty-three subjects participated in a cross-over, double-blind clinical study, using 21-day partial-mouth experimental model of gingivitis. A toothshield was constructed for each volunteer, avoiding the brushing of the 4 experimental posterior teeth in the lower left quadrant. The subjects were randomly assigned initially to use either the placebo gel (control group) or the test gel, containing 10% LS (test group). Results: The clinical results showed statistically significant differences for plaque index (PLI) ($p<0.01$) between days 0 and 21 in both groups, however only the control group showed statistically significant difference ($p<0.01$) for the bleeding (IB) and gingival (GI) index within the experimental period of 21 days. On day 21, the test group presented significantly better results than the control group with regard to the GI ($p<0.05$). Conclusions: The test gel containing 10% LS was effective in the control of gingivitis.

Key words: Dental plaque. Gingivitis. Periodontology.

INTRODUCTION

Gingivitis is one of the most frequent periodontal diseases, affecting more than 90% of the population, regardless of age, sex or race[21]. Brazilian epidemiologic studies have shown a high prevalence of gingival inflammation, ranging from 74% to 100%, although media individual percent of gingival bleeding vary from 28% to 35%[16].

Dental plaque can be visible on dental surfaces after 1 or 2 days if oral hygiene procedures are unsatisfactory. The localization and rate of its formation are varied, depending of determinant factors such as diet and salivary flow[20]. After 10 to 21 days of supragingival dental plaque accumulation, clinical signs of gingivitis appear, such as redness, edema and a tendency to marginal bleeding on gentle probing[20].

Chemotherapeutic agents had been used as antiplaque agent, avoiding development of gingivitis[13]. Chlorhexidine is considered as the gold standard, showing positive results by inhibition or retarding the bacterial proliferation[15]. However, due to undesirable effects after prolonged use, such as pigmentation and taste disturbance[11], phytotherapic agents have been investigated as alternatives[4,6,14,16].

Lippia sidoides (*LS*) is a typical shrub commonly found in the Northeast of Brazil. Its camphorate foliage is indicated as topic antiseptic agent for skin and mucosa surface and also for throat infections[9]. Essential oil obtained from this phytotherapic is constituted mainly by thymol (56.7%), carvacrol (16.7%) and other substances, such as felandreno, cariofileno, p-cimeno and mirceno[3,9]. Previous studies indicated that these major components had showed potent antimicrobial activity against fungi and bacteria[3,7,9], including species of the genus *Streptococcus mutans*[3] and reduced the severity of gingivitis, bacterial plaque and histological inflammatory infiltrate in dogs[5]. In an only controlled short-term clinical study in humans, a LS essential oil mouthrinse reduced bacterial plaque and gingival inflammation[4].

Although these initial studies showed positive results, there are no sufficient data about the clinical effects of *LS* on gingivitis. Thus, a cross-over, double-blind clinical study in humans was conducted to evaluate the in vivo antiplaque and antigingivitis effect of a gel containing *LS*.

MATERIAL AND METHODS

Subjects

Twenty-six undergraduate dental students from the University of Fortaleza (13 female and 13 male, aged 19 to 25 years) were enrolled in this study. All subjects had at least 20 natural teeth, among which the 4 posterior teeth in the lower left quadrant (experimental teeth). All randomly screened students were informed about the nature of the study and signed an informed consent form in compliance with the guidelines of the Brazilian National Health Council. Participants with medical disorders and under antimicrobial therapy, as well as smokers, pregnant women and individuals presenting a probing depth $\geq$ 3 mm associated with any mandibular teeth were excluded from the trial. Subjects with retentive factors of dental plaque, such as carious cavity and restoration excess in the test area were not included in this clinical study as well. The protocol was approved by the University's Ethics Committee (Report Coética no. 205/ 2005, University of Fortaleza) and an ISRCTN register was assigned (no. 26530238).

Toothshield Fabrication

An alginate impression of the experimental teeth was taken and poured in die stone to obtain casts. On each stone cast, a 0.3-mm-thick thermoplastic mouthguard material space was made using a vacuum former. Upon the spacer, an individual toothshield was made of a 2-mm-thick thermoplastic mouthguard material, using the same vacuum former. The toothshield was trimmed 2 mm beyond the gingival margin to assure that gel would be in contact with the gingival margin of the experimental teeth during toothbrushing of the remaining teeth.

Control and Test Products

The control and test gels were formulated and packed into tubes in the Laboratory of Pharmaceutics at the University of Fortaleza. The tubes were previously coded to warrant that neither the examiner nor the participants knew their content, which was revealed by the pharmacist only after the study was completed. All students used both gels in alternate periods, according to a cross-over study.

Preparation of the Gels

The essential oil from *LS* was extracted by steam distillation in the Pharmaceutics' Laboratory at the University of Fortaleza. Initially 1 mL of essential oil was diluted in 9 ml of ethylic alcohol (1:9), preparing a 10% mixture. As much as 50 g of carboxymethylcellulose was added to the *LS* infusion (1000 mL) and the mixture was kept boiling until its complete dissolution to obtain the 10% gel concentration. A glycerin/ethanol mixture (50 mL: 50 mL) was added and the solution was vigorously stirred during 15 min until gel formation. A very small amount of menthol (flavoring) and conserving agent were then added. The control gel had the same formulation except for the *LS* extract.

Clinical Design

This study was a randomized, double-blind comparison of 2 crossover groups of dental students performed in 2 experimental phases of 21 days each with a 1-month washout interval between them. A partial mouth experimental model was used[18]. To standardize the groups, the participants were submitted to a meticulous evaluation (pre-experimental phase) to score the Plaque Index (PLI)[19], the Gingival Index (GI)[10] and the Bleeding Index (BI)[1] of each tooth. All teeth of each subject were polished and flossed by the examiner to eliminate dental plaque remnants. The importance of oral hygiene was strongly reinforced.

Thirty days after the initial phase, the volunteers were randomly assigned to 2 groups by random permutation of three and the experimental phase began. On day 0 of both experimental periods, PLI, GI and BI were recorded. A personal "kit" containing a toothshield, a tube with 90 g of control or test gel and a commercial dentifrice with no antiinflammatory properties was given to all students (Sorriso, Kolynos do Brazil Ltda., Osasco, SP, Brazil). During each 21-day experimental period, the participants were instructed to fill the toothshield with the gel prior to insertion in the mouth and seat it over the experimental teeth three times a day for at least 1 min. The students refrained from brushing the test quadrant, while the other teeth were normally brushed three times a day using the commercial dentifrice. In addition to verbal instructions, the students were given written recommendations to follow at home. On the last day of each period (21st day), the indexes were recorded and the teeth were polished with pumice.

Clinical Assessment

All the indexes were recorded by the same examiner on the mesiobuccal, buccal, distobuccal, mesiolingual, lingual and distolingual surfaces of the experimental teeth. The values of six sites of each tooth were recorded to obtain the PLI, GI and BI means. Then, the means for the four experimental teeth were calculated to determine index means of each volunteer. Intra-examiner agreement for all indexes was calculated by repeating the measurements in 10 patients, with at least 1 h of interval[2]. The Kappa coefficient was used to verify the agreement between the examinations. PLI, GI and BI means were 0.75, 0.83 and 0.86, respectively.

Statistical Analysis

The Mann-Whitney non-parametric test was used to estimate the difference between control and test group on days 0 and 21 ($p<0.05$). In each group, the mean scores of all indexes were compared between baseline and the end of the trial by the Wilcoxon test ($p<0.01$). However, for illustration, the results are presented as mean and standard deviation.

RESULTS

Twenty-two subjects completed the clinical trial. Four students were excluded from the study during the

experimental phase due to third molar extraction. The test gel had good acceptance and did not show adverse effects, such as abscess, ulcerations or allergic reactions.

On day 0 the control and test groups did not show statistically significant difference from each other with respect to PLI (p=0.9813), BI (p=0.4455) and GI (p=0.4455) means. These results indicated that both groups were well balanced at baseline (Tables 1, 2 and 3). At the 21st day, plaque (p=0.3242) and gingival bleeding (p=0.0707) were present in both groups, but the difference between them was not statistically significant (Tables 1 and 2). However, in this same period, the GI means differed statistically, favoring the test group (p=0.0299) (Table 3).

Comparing the means between day 0 and day 21 in each group, there was a statistically significant difference in the PLI index – control group (p=0.0003) and test group (p=0.0005) (Table 1). The control group showed statistically significant difference for the BI (p=0.0049) and GI (p=0.0017) indexes (Tables 2 and 3). However, for the test group, BI (p=0.0277) and GI (p=0.0277) means did not differ significantly between baseline and trial end periods (Tables 2 and 3).

TABLE 1- Plaque index (PLI) means and standard deviation on day 0 and day 21 for the control and test groups

	Control	Test
Day 0	1.61 + 0.45 A,a	1.71 + 0.37 A,a
Day 21	2.56 + 0.71 A,b	2.39 + 0.74 A,b

Means followed by the same uppercase letters on day 0 and day 21 do not differ statistically (p>0.05). Means followed by different lowercase letters in the same column differ statistically (p<0.01).

TABLE 2- Bleeding Index (BI) means and standard deviation on day 0 and day 21 for the control and test groups

	Control	Test
Day 0	0.02 + 0.05 A,a	0.01 + 0.04 A,a
Day 21	0.13 + 0.14 A,b	0.06 + 0.08 A,a

Means followed by the same uppercase letters on day 0 and day 21 do not differ statistically (p>0.05). Means followed by different lowercase letters in the same column differ statistically (p<0.01).

TABLE 3- Gingival Index (GI) means and standard deviation on day 0 and day 21 for the control and test groups

	Control	Test
Day 0	0.05 + 0.10 A,a	0.03 + 0.09 A,a
Day 21	0.31 + 0.35 A,b	0.13 + 0.16 B,a

Means followed by the same uppercase letters on day 0 and day 21 do not differ statistically (p>0.05). Means followed by different lowercase letters in the same column differ statistically (p<0.01).

DISCUSSION

The inability of adult population to perform adequate mechanical tooth cleaning has stimulated the search for chemotherapeutic agents in mouthrinses or added to dentifrices to improve plaque control and prevent gingivitis[13]. The gold standard in this scope is the use of chlorhexidine. Studies showed that two diary rinsing with 10 mL of a 0.12% chlorhexidine solution inhibited dental plaque, calculus and gingivitis in humans[8,12].

The absence of adverse effects using test gel showed that it was well tolerated, supporting safety for the clinical use. These results were already expected once biocompatibility of *LS* was reported previously[5], although mild and transient burning after using mouthrinse containing this natural agent had been related in the literature[4].

An in vitro study showed that *LS* was effective in inhibiting the growth of oral pathogens[3], which allow us to deduce that this phytotherapic could be used as antiplaque agent. However, this antibacterial effect was obtained in vitro conditions, which does not mimic completely the oral environment[22]. This fact was confirmed in this study in which both groups did not avoided plaque accumulation, although the test group had presented at day 21 less plaque, but not significantly, as compared control group.

Since the composition of the gels differed only for the presence of *LS*, it can be inferred that this phytotherapic agent had no effect on the bacterial growing in vivo. However, Botelho, et al.[4] (2007) showed an antiplaque effect of a 1% LS-based mouthrinse similar to chlorhexidine, using a 7-day treatment regimen. Based on a previous study[16], the present work tested a gel containing 10% *LS*. Perhaps, mouthrinsing per se presented a mechanical effect on dental plaque and the bioavailability of the phytotherapic might be higher than in the gel. The test gel was placed on the toothshield in a non-diluted way and it is possible that a solubilization by saliva or toothbrush's mechanical action in order to have an antibacterial effect would be necessary[13]. The experimental model used in this present study discarded other factors that could jeopardize the clinical results, such as mechanical plaque control, which could hide the phytotherapic agent's actual effect. However, the efficiency of other dentifrices has been shown using this clinical design[13].

In this study, the Turesky, et al.[19] (1970) index was used to assess plaque due its sensitivity to detect small deposits of plaque. However, the cut-off between the scores can be difficult to assess and could interfere in the results, so calibration of examiners was performed to solve this problem

assuring the confidence of the results.

Despite the lack of interference of *LS* in plaque accumulation, the phytotherapic had a positive effect on gingivitis, agreeing with another works[4,5]. In spite of insufficient data in the literature about the antiinflammatory mechanisms of *LS*, this property has been highlighted previously[4,5]. Another explanation for this fact is that the test gel exerted its antigingivitis effect indirectly through the dental plaque pathogenicity. However, this possibility was not confirmed in the literature yet due to complex mechanisms involved in the etiology of gingivitis.

There is no "gold standard" index for assessing the severity of gingivitis. Since the GI has been the most widely used index in studies investigating oral hygiene products, it was employed in the present study to allow for comparison of the present findings to those of other investigations. This index uses a scale in which color changes in the gingival tissues precedes bleeding on probing; however this parameter is not necessarily an accurate indicator of gingivitis[17]. For this reason, gingival inflammation was evaluated by BI as well.

Finally, more controlled trials using the same concentrations of *LS* extract are necessary to investigate its action on the treatment of gingivitis, comparing to chlorhexidine. Further studies should be developed to identify the real benefits of *LS* as a therapeutic and preventive agent for gingivitis, in addition to its common use in popular medicine.

CONCLUSION

The 10% *LS* gel was not a good antiplaque agent, but it was effective in the control of gingivitis.

ACKNOWLEDGMENTS

The authors would like to thank the undergraduate dental students of the University of Fortaleza, who participated in the study.

REFERENCES

1- Ainamo J, Bay I. Problems and proposals for recording gingivitis and plaque. Int Dent J. 1975;25(4):229-35.

2- Blieden TM, Caton JG, Proskin HM, Stein SH, Wegener CJ. Examiner reliability for an invasive gingival bleeding index. J Clin Periodontol. 1992;19(4):262-7.

3- Botelho MA, Nogueira NAP, Bastos GM, Fonseca SGC, Lemos TLG, Matos FJA, et al. Antimicrobial activity of the essential oil from Lippia sidoides, carvacrol and thymol against oral pathogens. Braz J Med Biol Res. 2007;40(3):349-56.

4- Botelho MA, Bezerra JG Filho, Correa LL, Fonseca SGC, Montenegro D, Gapski R, et al. Effect of a novel essential oil mouthrinse without alcohol on gingivitis: a double-blinded randomized controlled trial. J Appl Oral Sci. 2007;15(3):175-80.

5- Girão VCC, Nunes-Pinheiro DCS, Morais SM, Sequeira JL, Gioso MA. A clinical trial of the effect of a mouth-rinse prepared with Lippia sidoides Cham essential oil in dogs with mild gingival disease. Prev Vet Med. 2003;59(1):95-102.

6- Hebbar SS, Harsha VH, Shripathi V, Hegde GR. Ethnomedicine of Dharwad district in Karnataka, India-plants used in oral health care. J Ethnopharmacol. 2004;94(2):261-6.

7- Lacoste E, Chaumont JP, Mandin D, Plumel MM, Matos FJ. Antisseptic properties of essential oil of Lippia sidoides Cham: application to the cutaneous microflora. Ann Pharm Fr. 1996;54(3):228-30.

8- Lang NP, Hotz P, Graf H. Effects of supervised chlorhexidine mouthrinses in children: a longitudinal clinical trial. J Periodontal Res. 1982;17(2):101-4.

9- Lemos TL, Craveiro AA, Alencar JW, Matos FJ, Clarck AM, Mac-Chesney JD. Antimicrobial activity of essential oil of Brazilian plants. Phytother Res. 1990;4(3):82-4.

10- Löe H, Silness J. Periodontal disease in pregnancy: prevalence and severity. Acta Odontol Scand. 1963;21(3):533-51.

11- Löe H. Does chlorhexidine have a place in the prophylaxis of dental disease? J Periodontal Res Suppl. 1973;12:93-9.

12- Mikkelsen L, Jensen SB, Schiott CR, Löe H. Classification and prevalences of plaque Streptococci after two years oral use of chlorhexidine. J Periodontal Res. 1981;16(6):646-58.

13- Nogueira GR Filho, Toledo S, Cury JA. Effect of 3 dentifrices containing triclosan and various additives: an experimental gingivitis study. J Clin Periodontol. 2000;27(7):494-8.

14- Pai MR, Acharya LD, Udupa N. Evaluation of antiplaque activity of Azadirachta indica leaf extract gel: a 6-week clinical study. J Ethnopharmacol. 2004;90(1):99-103.

15- Quirynen M, Soers C, Desnyder M, Dekeyser C, Pauwels M, van Steenberghe D. A 0.05% cetylpyridinium chloride/0.05% chlorhexidine mouth rinse during maintenance phase after initial periodontal therapy. J Clin Periodontol. 2005;32(4):390-400.

16- Salgado ADY, Maia JL, Pereira SLS, Lemos TLG, Mota OML Antiplaque and antigingivitis effects of a gel containing Punica Granatum Linn extract: a double-blind clinical study in humans. J Appl Oral Sci. 2006;14(3):162-6.

17- Soukoulis S, Hirsch R. The effects of a tea tree oil-containing gel on plaque and chronic gingivitis. Aust Dent J. 2004;49(3):78-83.

18- Theilade E. The experimental gingivitis studies: the microbiological perspective. J Dent Res. 1996;68(sp. issue):1617-24.

19- Turesky S, Gilmore ND, Glickman I. Reduced plaque formation by the chloromethyl analogue of victamine C. J Periodontol. 1970;41(1):41-3.

20- Van der Weijden GA, Timmerman MF, Danser MM, Nijboer A, Saxton CA, Van Der Elden U. Effect of pre-experimental maintenance care duration on the development of gingivitis in a partial mouth experimental gingivitis model. J Periodontal Res. 1994;29(3):168-73.

21- Villalobos OJ, Salazar CR, Sánchez GR. Efecto de un enjuague bucal compuesto de aloe vera en la placa bacteriana e inflamación gingival. Acta Odontol Venez. 2001;39(2):16-24.

22- Wu CD, Savitt ED. Evaluation of the safety and efficacy of over-the-counter oral hygiene products for the reduction and control of plaque and gingivits. Periodontol 2000. 2002;28(1):91-105.