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ORIGINAL RESEARCH

Effectiveness of Oral Antiseptics on Tooth Biofilm: A Study *in vivo*

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ABSTRACT

Aim: To evaluate the effectiveness of five different mouthwashes through measurement of the plaque index.

Materials and methods: Fifty subjects took part in this blind study, randomized into blocks of five groups according to the active ingredient of the mouthwash: CHX group (0.12% chlorhexidine gluconate), essential oils (EO) group, cetylpyridinium chloride (CPC) group, Tri group (triclosan) and *Hamamelis virginiana* (HV) group. All subjects were evaluated for a reduction in the bacterial plaque index at 7, 14 and 21 days.

Results: There was a significant reduction in the mean plaque index during the period of evaluation ($p < 0.01$), and the reduction during the period of evaluation was different between mouthwashes ($p < 0.01$). The reduction in the plaque index at the end of 21 days was, in decreasing order, CHX > EO > CPC > Tri > HV.

Conclusion: The reduction in the plaque index during the period of evaluation was different between the types of mouthwash. The mouthwash containing the active ingredient chlorhexidine was the most effective, followed by the essential oil, cetylpyridinium chloride, triclosan and *H. virginiana*.

Keywords: Antimicrobial activity, Mouthwash, Tooth biofilm.

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INTRODUCTION

Being the main etiological factor in gingivitis, the mechanical control of tooth biofilm is the most important strategy for the prevention and treatment of periodontal diseases; however, the majority of people fail to maintain adequate control, so other methods are used as complementary means to help control supragingival/subgingival plaque and gingivitis.[1-4] Of these methods, the use of mouthwashes with antimicrobial active ingredients helps to remove tooth biofilm and also prevent it from forming.[4-7]

Several active ingredients used in mouthwashes, including chlorhexidine gluconate (CHX), present substantiveness, i.e. they remain active within the oral cavity for approximately 12 hours, which is explained by their dicationic nature. Thus, one cationic end of the molecule attaches itself to the film, which has a negative charge and the other cationic end is free to interact with bacteria.[8] Moreover, CHX has a wide spectrum over both Gram-positive and Gram-negative bacteria, viruses and yeasts.[9]

Cetylpyridinium chloride (CPC) is a quaternary ammonium compound capable of killing Gram-positive pathogens and yeasts through interaction with the bacterial membrane, infiltrating the cytoplasm and deregulating equilibrium between cells.[10] Triclosan (Tri), on the other hand, is a nonionic, low-toxicity agent with both hydrophilic and hydrophobic properties. It has a wide bacterial spectrum, being effective against Gram-positives

and Gram-negatives as well as promotes the inhibition of inflammatory mediators.[11] There is evidence to suggest that Tri may reduce the formation of tooth biofilm.[12-13]

The essential oils (EO) are a mixture of eucalyptol, thymol, menthol and methyl salicylate. Its action mechanism consists of denaturing bacterial proteins, altering the permeability of the outer membrane of Gram-negative bacteria and the chelation of cations present in the bacterial cytoplasm, rendering the enzymes inactive.[14]

Hamamelis virginiana is an alternative product, antiseptic, astringent, hemostatic, coolant, sedative, tonic, and antioxidant. Among the main active ingredients found in HV are flavonoids and tannins.[15] Due to its antiphlogistic and astringent properties, HV extracts have been used to treat small wounds and local inflammation.[16,17] Antimutagenic and antioxidant properties have also been described in HV.[18-20]

At present, many mouthwashes with different compositions and active ingredients are available in the market. However, some formulations have still not been effectively tested for proving their clinical efficiency. Therefore, the objective of this study was to evaluate the effectiveness in reducing the plaque index of five mouthwashes with the following ingredients: 0.12% chlorhexidine gluconate (Periogard®), essential oils (Listerine®), cetylpyridinium chloride (Cepacol®), triclosan (Plax Fresh Mint®) and *H. virginiana* (Maravilha Curativa®).

MATERIALS AND METHODS

This study was approved by the research ethics committee at Ceuma University (opinion number 832.068/2014), and was conducted in the dental clinics of Ceuma University, São Luis, in the Brazilian state of Maranhão, during the period commencing August 6 and ending September 15, 2014.

Participants

A convenience sample of 50 participants consisting of 10 patients per group and a plaque index varying between 60 and 80%, according to the index proposed by O'Leary et al, were invited to take part in the study.[21] The sample size was determined by taking into consideration the statistical test known as repeated-measures between-factors analysis of variance (ANOVA), with an effect size of 0.5, power of 0.95 and α of 0.05, using five groups and four repetitions (G Power version 3.1.4, Heinrich-Heine Universität, Düsseldorf, Germany). The allocation of patients was performed in blocks, i.e. the participants were allocated in a predetermined group sequence, such that all the groups had the same number of participants by the end of the exercise.

Each participant had to have a minimum of 20 permanent teeth and not be undergoing orthodontic treatment. Teeth with extensive restorations, with the presence of a full crown and third molars, were not included in the tooth count. Patients who were pregnant or had a systemic disease, were smokers or patients that made use of antibiotics, anti-inflammatory or systemic medication affecting gum condition within the last 4 weeks, were excluded from the study. Similarly, patients with generalized caries or advanced periodontal disease were also excluded.

Intervention

The selected participants initially received oral hygiene instruction at the point where the same types of toothpaste and toothbrushes were handed out, and were instructed to brush their teeth twice a day for 1 minute immediately after meals, and to use 10 ml of one of the following mouthwashes for 30 seconds:

- *Group 1*: Periogard® (Colgate-Palmolive Company, New York City, USA)—0.12% chlorhexidine gluconate.
- *Group 2*: Listerine® (Johnson and Johnson, New Jersey, USA)—essential oils.
- *Group 3*: Cepacol® (Sanofi Aventis Farmacêutica Ltda., Rio de Janeiro, RJ, Brazil)—cetylpyridinium chloride.
- *Group 4*: Plax Fresh Mint® (Colgate-Palmolive Company, New York City, USA)—triclosan.
- *Group 5*: Maravilha Curativa® (Humphreys Pharmacal Inc., Rio de Janeiro, RJ, Brazil)—*Hamamelis virginiana.*

The participants underwent a plaque index evaluation 7, 14 and 21 days after the initial evaluation.

The plaque index evaluation was carried out individually by a single examiner (JCMJ), blind as to the type of mouthwash used by the participants, and previously trained and calibrated (Kappa = 0.85), using the index proposed by O'Leary et al.[21]

STATISTICAL ANALYSIS

The data were input to spreadsheets in Microsoft Excel 2010 for Windows (Microsoft Corporation®, USA) for the appropriate storage of information. The statistical analyses were carried out using the software application SPSS 21.0 (IBM, SPSS Statistic, USA).

A descriptive statistical analysis was carried out, highlighting the estimated means and their respective 95% confidence intervals, for each type of mouthwash and period of evaluation.

The data were submitted to statistical analysis by analysis of variance/general linear models (ANOVA/GLM) mixed design test (one between-groups variable and one within-subjects variable), in which the effect was

evaluated of the different mouthwashes on reducing the plaque index over different periods of evaluation. For the purposes of comparing the plaque index between the evaluated periods within just one mouthwash group, the ANOVA/GLM one-way repeated-measures test was applied. To compare the plaque index between mouthwashes within the same evaluation periods, the ANOVA one-way test was applied. A level of significance of 5% was employed for all the tests.

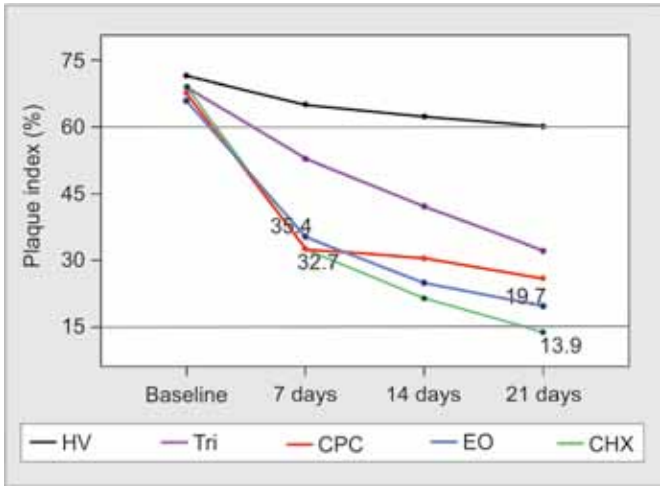
RESULTS

All 50 participants were included in the analysis. The average age of the participants was 33.9 ± 5.4 years. Table 1 shows the estimated plaque index means, with the respective 95% confidence intervals for the groups and periods evaluated.

The results of the ANOVA/GLM mixed design test showed that there was a significant reduction in the mean plaque index during the period of evaluation of at least one type of mouthwash ($p < 0.001$). For the measures *vs* mouthwash interaction also, the result was significant ($p < 0.001$), showing that the reduction in the plaque index over the period of evaluation depended on the type of mouthwash, i.e. the reduction in the plaque index occurred differently between mouthwashes. This finding may be observed by analyzing Graph 1, where it can be seen that the reduction in the plaque index took place in a different way between the mouthwashes.

Table 1: Estimated means for the different mouthwashes used in the different periods and evaluation with their respective confidence intervals of 95%

			95% confidence interval	
Groups	*Time*	*Mean (SD)*	*Lower bound*	*Upper bound*
CHX	Baseline	69.10 (± 7.29)	64.87	73.32
	7 days	32.70 (± 15.62)	23.28	42.11
	14 days	21.50 (± 9.36)	13.78	29.21
	21 days	13.90 (± 4.86)	7.98	19.81
EO	Baseline	66.90 (± 7.02)	61.77	70.22
	7 days	35.40 (± 17.09)	25.98	44.81
	14 days	25.00 (± 7.80)	17.28	32.71
	21 days	19.70 (± 6.12)	13.78	25.61
CPC	Baseline	67.70 (± 5.67)	63.47	71.92
	7 days	32.60 (± 17.84)	23.18	42.01
	14 days	30.50 (± 17.09)	22.78	38.21
	21 days	26.10 (± 12.27)	20.18	32.01
Tri	Baseline	69.00 (± 6.99)	64.77	73.22
	7 days	52.90 (± 9.20)	43.48	62.31
	14 days	42.10 (± 12.36)	34.38	49.81
	21 days	32.30 (± 10.06)	26.38	38.21
HV	Baseline	71.44 (± 6.05)	67.37	75.82
	7 days	64.89 (± 1310)	55.68	74.51
	14 days	60.78 (± 11.34)	54.68	70.11
	21 days	59.22 (± 11.06)	54.28	66.11



Graph 1: Estimated mean plaque index values of the evaluator mouthwashes over different periods of time (CHX: Chlorhexidine gluconate; CPC: Cetylpyridinium chloride; EO: Essential oils; Tri: Triclosan; HV: Hamamelis virginiana)

The comparison of plaque index between the mouthwashes within the same period of evaluation can be seen in Table 2, while the comparison of the plaque index between periods evaluated within the same mouthwash group can be seen in Table 3.

Comparing the mouthwashes by the Tukey test showed that there was a significant difference ($p < 0.001$). Table 3 shows the paired comparison between mouthwashes.

DISCUSSION

The evaluation of the effectiveness of different brands of mouthwash in the reduction of dental plaque is important because the formulations contain several active ingredients that may not produce the desired effect.

Table 2: Comparison of the plaque index between mouthwashes for the different periods of evaluation

	CHX	*EO*	*CPC*	*Tri*	*HV*
Baseline	a	a	a	a	a
7 days	a	ab	a	bc	c
14 days	a	a	ab	b	c
21 days	a	ab	bc	c	d

Different letters on the horizontal line: significant difference $p < 0.05$ (ANOVA one-way Tukey test)

Table 3: Comparison of plaque index between periods of evaluation for the different mouthwashes

	Baseline vs 7 days	*7 days vs 14 days*	*14 days vs 21 days*
HV	0.07	0.02*	0.38
Tri	0.01*	0.01*	0.01*
CPC	0.01*	0.18	0.06
EO	0.01*	0.01*	0.01*
CHX	0.01*	0.01*	0.01*

*Statistically significant $p < 0.05$ (ANOVA one-way repeated measures)

Accordingly, the objective of this study was to investigate the reduction in supragingival plaque after using five brands of mouthwash with different formulae, evaluated at periods of 7, 14 and 21 days. According to Loe et al, these periods are sufficient to evaluate the effectiveness of mouthwashes.

So that groups would all have a similar plaque index at the beginning of the intervention (baseline), only participants with an index in the 60 to 80% range were recruited. The data were analyzed, confirming that there was no significant difference between the groups at the initial evaluation ($p = 0.511$) demonstrating that the baseline had been adjusted to fit.

A reduction in the plaque index occurred between the groups during the periods evaluated. It is possible that the cause of the reduction was in part due to the initial instruction on oral hygiene; however, it is possible to conclude that the use of mouthwashes was influential to the extent that the reduction was different across the groups. This assertion can be evidenced when we see that, while groups CHX, CPC and EO underwent a sharp reduction in the first 7 days (rates below 40%), those using HV and Tri were only moderately reduced. Moreover, the groups with CHX, CPC and EO maintained a similar reduction pattern between days 7 and 21, unlike the HV and Tri groups. The CHX group achieved a plaque index lower than 15% at the end of the 21 days (13.9%), while the HV group did not manage a reduction of less than 60% in the same period of evaluation.

It was also found that the groups with EO, Tri and CHX always obtained significantly greater effects when compared to earlier evaluations (Table 3). The same was not the case with the group that used CPC, which after a sharp reduction in the first 7 days, flattened out and did not show a significant difference in the remaining evaluation periods in comparison with the earlier periods (at 14 and 21 days). This result agrees to that of Haps et al, who carrying out a systematic review, concluded that CPC provided a small but significant additional benefit in the reduction of plaque buildup and gum inflammation.[4] Moreover, Osso and Kanani found that the majority of studies showed that mouthwashes with CHX or EO afford significant antigingivitis and antiplaque benefits, and that the CPC provided limited benefits when compared with the control group.[22]

The present study showed that the oral antiseptic with the active ingredient CHX stood out as an efficient antimicrobial agent in the control of bacterial plaque through to 21 days. It is known that CHX has a high level of antibacterial, antiviral and antifungal action as well as high substantiveness.[23] However, despite it being generally safe to use, it is not free from adverse side effects. For example, usage over long periods is limited due to the tendency to stain the teeth, not only this but also it has an unpleasant taste.[6]

In the present study, the reduction found in the group using HV was not sufficiently clear to be able to conclude that the active ingredient was responsible for this reduction. Moreover, the evaluation at 21 days showed that the use of HV was lower than in the other groups.

Taking into account just the final evaluation (at 21 days), it was found that reduction in the plaque index observed the following decreasing order: CHX > EO > CPC > Tri > HV. This outcome agrees with that of Gunsolley, who showed that mouthwashes containing CHX had a highly effective antiplaque and anti-gingivitis action, followed by EO and CPC.[24] Similarly, Van Leeuwen et al, carrying out a systematic review, demonstrated the effectiveness of CHX and EO.[25]

The outcome of this study demonstrates that chemical control has an influence on the reduction of dental plaque, thus becoming an important adjunct in its reduction; therefore, it should be incorporated as complementary to mechanical controls, and its daily use should be encouraged. Mechanical methods for removing tooth biofilm should not be replaced but should serve as an adjunct.

The reduction in the plaque index over the course of the evaluation was influenced by the type of mouthwash used. The mouthwash containing chlorhexidine was the most effective in controlling bacterial plaque, followed by essential oils, cetylpyridinium chloride, triclosan and *H. virginiana*.

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