Antiplaque and antigingivitis efficacy of cetylpyridinium chloride with zinc lactate compared with essential oil mouthrinses

Randomized clinical trial

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ABSTRACT

Background. The authors of this study aimed to evaluate the clinical antiplaque and antigingivitis effects of 3 oral hygiene regimens: toothbrushing with standard fluoride toothpaste and manual toothbrush and using a mouthrinse containing cetylpyridinium chloride, zinc lactate, and fluoride (CPC + Zn + F) in an alcohol-free base; toothbrushing with standard fluoride toothpaste and manual toothbrush and using a mouthrinse containing essential oils (EO) in an alcohol-free base; and toothbrushing with manual toothbrush and standard fluoride toothpaste and manual toothbrush (control).

Methods. The participants (N = 120) were randomly assigned to study groups and followed the assigned regimens twice daily for 6 weeks. The participants were examined by a calibrated examiner for the Quigley-Hein plaque index (Turesky modification) and Löe-Silness gingival index at baseline, week 4, and week 6. Statistical analyses were performed separately for plaque and gingival indexes by means of analysis of variance, paired t test, and analysis of covariance (α = 0.05).

Results. At week 4, the CPC + Zn + F group presented additional reductions in dental plaque compared with EO and control groups of (21.4% [P < .001] and 31.4% [P < .001], respectively). After 6 weeks, these values were 26.7% (P < .001) and 44.8% (P < .001), respectively. For Löe-Silness gingival index, additional reduction in the CPC + Zn + F group compared with EO were 10.6% (P < .001) and 13.7% (P < .001) at 4 and 6 weeks, respectively. Compared with control, these reductions were 13.6% (P < .001) and 17.8% (P < .001), respectively.

Conclusions. The regimen including a mouthrinse containing CPC + Zn + F presented higher antiplaque and antigingivitis effects than EO and control regimens.

Practical Implications. A mouthrinse containing CPC + Zn + F is an effective protocol for the control of dental plaque and gingivitis.

Key Words. Mouthrinses; cetylpyridinium; essential oils; gingivitis; dental plaque.

The importance of the removal of supragingival plaque has been well established in the literature regarding oral health care. Rates of caries, periodontal disease, and tooth loss have been shown to be lower in populations with good standards of plaque control. The most widely used method of mechanically disrupting supragingival biofilm is with toothbrushes, and, over time, this practice results in improved oral health. In addition to mechanical toothbrushing, mouthrinses have been used with varied formulations to control biofilm chemically in an effort to...
compensate for the limitations associated with toothbrushing. Literature reports that patients with motor or cognitive problems, those with a lack of motivation, those in postsurgical phases, and those with orthodontic devices may benefit from the use of mouthrinses containing antimicrobials. Studies have found potential antibacterial effects, with limited adverse events, of mouthrinses containing cetylpyridinium chloride (CPC), which is 1 of the types most commonly used. For example, additional oral health benefits result when CPC is added to sodium fluoride mouthrinses compared with mouthrinses containing only sodium fluoride. A systematic review of the literature has reported a small but significant antiplaque and antigingivitis effect of CPC as an adjuvant to routine mechanical oral health hygiene.

In a 2020 systematic review that evaluated the efficacy of adjunct use of chemical agents and mechanical plaque control in systemically healthy people, the authors reported that after 6 months, antiseptic agents were clinically efficacious in reducing gingival index (GI). No clear evidence is detected in the literature regarding who would benefit the most from these agents. However, as shown via analysis of the results from published studies in patients with higher accumulation of dental plaque, the use of these agents may provide benefits in the long term (> 6 months) regarding dental plaque and gingivitis reduction. However, adverse effects may be expected, mostly dental staining.

The addition of zinc lactate in the composition of the mouthrinse was also seen to be safe in an oral environment, with beneficial potential for various oral conditions (for example, reduction of plaque and gingival inflammation) and a decrease in oral malodor. In addition, the antigingivitis effect of the combination of CPC and zinc lactate was shown to be significantly greater when the mouthrinse was used as an adjuvant with a toothbrush in a 6-week clinical trial than a formulation without the zinc lactate. In addition to the abovementioned benefits of zinc, the results of an in vitro study found that this substance has antimicrobial properties when tested against bacteria associated with halitosis. The benefits of zinc also were reported in a randomized clinical trial (RCT), which found that a zinc-based mouthrinse may have similar clinical benefits in halitosis reduction compared with a chlorhexidine mouthrinse. This formulation also was found to be more effective at reaching interproximal areas than comparison groups. The authors of another study observed that the effect of CPC is comparable with that seen in chlorhexidine as a preprocedural mouthrinse.

Essential oils (EO) also are used in combination with mechanical plaque removal. The results of a systematic review have shown their potential to remove significantly more plaque and reduce gingival inflammation more effectively than both a placebo and a CPC formulation without zinc lactate. To our knowledge, studies assessing the efficacy of mouthrinses containing CPC and zinc lactate simultaneously compared with mouthrinses containing EO have not been published. The concept of our study relies on a possible synergistic effect of zinc lactate and CPC. As such comparison between this combination and EO has not yet been undertaken, to our knowledge, the aim of our study was to evaluate the clinical efficacy of 3 oral hygiene multicomponent regimens in controlling established dental plaque and gingivitis over a 6-week period of use:

- a commercially available regular fluoride toothpaste, an adult soft-bristled manual toothbrush, and a mouthrinse containing 0.075% CPC and 0.28% zinc lactate and fluoride in an alcohol-free base;
- a commercially available regular fluoride toothpaste, an adult soft-bristled manual toothbrush, and a mouthrinse containing EO in an alcohol-free base;
- a commercially available regular fluoride toothpaste and a soft-bristled manual toothbrush.

**METHODS**

**Study design**

We designed this study as a phase III randomized, single-center, 3-cell, examiner-blind, and parallel-group clinical study. We followed the Consolidated Standards of Reporting Trials statement.

**Ethical considerations**

The protocol was approved by the Institutional Review Board of the Federal University of Rio Grande do Sul, Brazil, and all the participants signed an informed consent form.

**ABBREVIATION KEY**

| CPC | Cetylpyridinium chloride. |
| CPC + Zn + F | Cetylpyridinium chloride, zinc lactate, and fluoride. |
| EO | Essential oils. |
| GI | Gingival index. |
| RCT | Randomized clinical trial. |
Sample size estimate
We determined the sample size of 120 (40 per group including attrition) on the basis of the standard deviation for the response measures of 0.58, a significance level of \( \alpha \) at 0.05, and an 80% level of power. This study was powered to detect a mean difference between groups of 15% in dental plaque. The sample size calculation used historical data from a previous pilot study, and having 32 participants was considered necessary.

Sample population
The sample consisted of 121 men and women, aged 21 through 70 years, who were recruited by convenience, and we randomized 120 into 1 of the 3 treatment groups (Figure 1). We conducted the study from August through September 2018, at the School of Dentistry of the Federal University of Rio Grande do Sul, Porto Alegre, Brazil. Inclusion criteria comprised good general health, availability for the 6-week duration of the study, an initial mean plaque index score of at least 1.5 as determined via Turesky modification of the Quigley-Hein plaque index,\(^{17,18}\) a mean GI score of at least 1.0 as determined via the Löe-Silness GI,\(^{19}\) and a minimum of 20 natural teeth, excluding third molars.

Participants were not included in the study if any of the following applied: orthodontic bands; partial removable dentures; tumors or significant pathology in the soft or hard tissues of the oral cavity; moderate or advanced periodontal disease; 5 or more carious lesions requiring immediate care; antibiotics use at any time 1 month before entry into the study; pregnant or breast-feeding women; underwent a dental prophylaxis in the 2 weeks before the baseline examination; a history of allergies to oral or personal care consumer products or their ingredients; any prescription medicines that might interfere with the study outcome; any medical condition that required not eating, drinking, or chewing gum within 4 hours of their scheduled visit; or history of alcohol or drug abuse.

Experimental procedures
Qualifying participants attended the clinical study site after refraining from any oral hygiene procedures for 12 hours and from eating, drinking, or smoking for 4 hours. The baseline examination consisted of soft- and hard-tissue evaluation of the oral cavity and perioral region, followed by plaque and gingivitis assessments.

Supragingival plaque examination was then performed with plaque disclosure rinsing with 10 milliliters of 0.04% basic fuchsine solution (Replasul, Iodontosul). The supragingival plaque examination was performed with the modified Quigley and Hein plaque index.\(^{17,18}\) Plaque was assessed in 6 sites per tooth and scored from 0 (no plaque) through 5 (plaque covering two-thirds or
more of the side of the crown of the tooth). Individual scores were calculated by means of summing all scores for all sites and dividing by the total number of sites scored.

Gingival inflammation was scored at 6 sites per tooth according to the criteria of the GI system.\(^{19}\) Individual scores were calculated by summing all scores for all sites and dividing by the total number of sites scored. Furthermore, gingival severity was calculated by the percentage of sites scored 2 or 3, which represents sites that bleed on marginal probing.\(^{20}\)

Participants were randomized into 1 of 3 groups with 40 members each. The randomization list was computer generated. Allocation concealment was the responsibility of an external researcher, and products were covered with white paper to conceal product identity. The material was numbered sequentially and kept inside opaque plastic bags.

The experimental group regimens were
- a test group given mouthrinse containing 0.075% CPC and 0.28% zinc lactate with 0.05% sodium fluoride in an alcohol-free base (Colgate-Palmolive Company) together with an adult soft-bristled manual toothbrush and standard fluoride toothpaste (CPC + Zn + F group);
- a positive control group given mouthrinse containing essential oils (EO) in an alcohol-free base (Johnson & Johnson) together with an adult soft-bristled manual toothbrush and standard fluoride toothpaste (EO group);
- a negative control group given an adult soft-bristled manual toothbrush and standard fluoride toothpaste (control group).

Participants were instructed to brush their teeth twice daily with the standard commercially available sodium fluoride toothpaste and the adult soft-bristled manual toothbrush for 2 minutes (morning and evening). In addition, the CPC + Zn + F and EO groups were instructed to rinse for 30 seconds twice per day with 20 mL of their assigned mouthrinse for 6 weeks. The participants were instructed to refrain from using any other type of oral hygiene product or method, such as mouthrinse or interproximal cleaning devices (dental floss or interdental brushes) for the duration of the study period. These instructions were given to participants after the baseline examination by a researcher uninvolved in the clinical examination. The first rinse was performed at this time at the clinic and under supervision.

At weeks 4 and 6, participants were instructed to return to the clinical study site with the products, and any adverse events were recorded. When an adverse event was reported, an oral examination was performed to provide proper treatment or explanation to the participant. The allocation concealment was kept unbroken during the entire study. In addition, participants were examined for dental plaque and degree of gingival inflammation, using the same procedures used at baseline. These evaluations were performed by the same trained and calibrated examiner (C.K.R.), who remained blind to product assignment during the course of the study. Intraxaminer reproducibility was assessed before the study for both plaque and gingival inflammation, with appropriate levels of reliability ($\kappa > .7$).

### Statistical analysis

We performed statistical analyses separately for dental plaque and gingivitis assessments. We also calculated and separately analyzed whole-mouth mean values and interproximal mean values. We performed comparisons of the treatment groups with respect to baseline GI scores and plaque index scores using an analysis of variance in a per-protocol approach. We performed within-treatment comparisons of the baseline versus follow-up gingival and plaque index scores using paired t tests. We performed comparisons of the treatment groups with respect to baseline-adjusted gingival and

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<td>Cetylpyridinium Chloride, Zinc Lactate, and Fluoride</td>
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plaque scores at the follow-up examinations using analyses of covariance. All statistical tests of hypotheses were 2-sided and used a level of significance of \( P \) below .05.

RESULTS
Of the initial 120 randomized participants, 110 completed the 6-week clinical study. The flowchart of the study, with reasons for exclusion, is shown in Figure 1. The table contains a summary of sex and age of the study participants. Throughout the study, 8 adverse effects on the oral hard or soft tissues of the participants were observed by the examiner or reported by the participants. No adverse events were reported in the CPC\(_{+}\)Zn\(_{+}\)F group, 3 were reported in the EO group, and 5 were reported in the control group. The reported adverse events comprised dentin hypersensitivity (1 in the EO, 1 in control group), gingival bleeding (1 in the EO, 1 in control group), palate and tongue burning (1 in the EO group), headache (2 in the control group), and swelling and gingival bleeding (1 in control group).

Clinical results
We present clinical results separately for plaque and gingivitis. Figure 2 shows the mean plaque index score (full mouth) at different study periods. At baseline, we observed no statistically significant difference among groups for plaque index (\( P = .115 \)). CPC + Zn + F, EO, and control groups presented a mean (standard deviation [SD]) plaque index score of 4.15 (0.44), 4.32 (0.41), and 4.08 (0.63), respectively. At weeks 4 and 6, we observed a higher reduction in plaque for the CPC + Zn + F group, followed by EO and control groups. At week 4, the additional reductions in dental plaque for the CPC + Zn + F group compared with EO and control groups were 21.4% (\( P < .001 \)) and 31.4% (\( P < .001 \)), respectively. After 6 weeks, these values were 26.7% (\( P < .001 \)) and 44.8% (\( P < .001 \)), respectively. When we analyzed the mean interproximal plaque index score, we obtained the same results. The CPC + Zn + F group had the highest reduction in interproximal
plaque, followed by the EO and control groups. We observed statistically significant differences among all groups (Figure 3).

Figure 4 shows mean full-mouth GI scores for experimental groups at different study periods. The mean GI did not show any statistically significant difference at baseline; CPC + Zn + F, EO, and control groups had a mean (SD) GI of 1.69 (0.15), 1.68 (0.15), and 1.67 (0.18), respectively. These values were reduced in all groups throughout the study, and, at both weeks 4 and 6, higher reductions were observed for the CPC + Zn + F group than the EO and control groups. EO performed better in GI reduction than control. For GI, the additional reductions at week 4 in the CPC + Zn + F group compared with EO and control groups were 10.6% (P < .001) and 13.6% (P < .001), respectively. At week 6, these values were 13.7% (P < .001) and 17.8% (P < .001), respectively.

When the GI was dichotomized and bleeding sites were analyzed, we obtained baseline values of 69%, 68%, and 67% for the CPC + Zn + F, EO, and control groups, respectively. The CPC + Zn + F group had these values reduced to 28% and 20% at weeks 4 and 6, respectively. The EO group reduced bleeding sites to 43% and 39%, and the control group reduced them to 47% and 46% at weeks 4 and 6, respectively. The analysis of these results revealed the same result as the mean GI score, with better performance for the CPC + Zn + F group. Results related to GI score for interproximal surfaces are shown in Figure 5. We observed no statistically significant difference at baseline. At weeks 4 and 6, the highest interproximal GI reduction was achieved with the use of the CPC + Zn + F regimen compared with EO and control regimens. EO had a higher reduction in interproximal GI score than the control.

DISCUSSION
Our randomized and examiner-blind clinical study provides an investigative comparison of the efficacy, with respect to dental plaque and gingivitis reduction over a 6-week period, of an oral
hygiene multicomponent regimen encompassing a commercially available regular fluoride toothpaste, an adult soft-bristled manual toothbrush, and a mouthrinse containing 0.075% CPC and 0.28% zinc lactate with fluoride in an alcohol-free base compared with a positive control (similar regimen, with a mouthrinse containing EO in an alcohol-free base) and a negative control (only brushing with commercially available regular fluoride toothpaste and a soft-bristled manual toothbrush). The results generally show that the CPC + Zn + F group had higher reductions in plaque and gingivitis (that is, a higher antiplaque and antigingivitis effect) over the study period than both comparison groups.

The research design was a phase III RCT. RCT studies are considered 1 of the best ways of showing the efficacy of oral hygiene regimens and are used as primary data for systematic reviews, the highest level of evidence for clinical decision making.4 In our study, we made comparisons with EO and control groups, which provides perspective on the real efficacy of the regimen compared with 1 proven efficacious agent or the absence of a source of benefit. In addition, a blind examiner, allocation concealment, and randomization were taken into consideration, further proving the validity of the results. Regarding compliance, all participants were asked about their product use and reported they had used the mouthrinses. They returned with the unused liquids, which were discarded according to legal regulations.

Oral hygiene regimens traditionally include solely the use of toothbrushing with dentifrice and, in some cases, flossing. However, consumer consumption of dental floss, despite an increase over time, is still low.21 In an attempt to compensate for the lack of use (or lack of correct use) of dental floss, the use of mouthrinses has been studied.14 Also, different toothbrush designs have proven to increase the efficacy of toothbrushing.22

CPC has been shown to be efficacious since the 1970 study by Gjermo and colleagues.7 However, its clinical efficacy was limited owning to the lack of substantivity, which is the ability of an antimicrobial agent to retain its effect in the mouth for an extended period. Meta-analyses of

Figure 4. Gingival index at baseline, week 4, and week 6 examinations. Brackets represent statistically significant difference for comparisons between groups. All groups showed statistically significant improvements within each group (baseline versus week 4 and baseline versus week 6).
Clinical studies have confirmed a high heterogeneity in the benefits of the use of CPC. The addition of zinc lactate has been proposed to increase the capacity of CPC mouthrinses in both plaque and gingivitis reduction. An RCT found that this association substantially increased CPC’s antiplaque and antigingivitis effects compared with a mouthrinse with CPC only. In the formulation in our study, the presence of fluoride probably has no effect on plaque and gingivitis; however, it is interesting in terms of fluoride availability for caries prevention.

The EO group included in our study was given a solution of EO that has been found to be effective against plaque and gingivitis. We used an alcohol-free solution of EO. The presence of alcohol has been questioned in the literature, although no proven adverse effects, such as increased risk of developing oral cancer, have been reported. However, the use of alcohol is being limited in clinical settings owing to its strong taste and associated burning sensation. Studies comparing chlorhexidine solutions with or without alcohol have found that patients prefer the taste of nonalcoholic solutions. In a comparative study with other products, the taste of CPC was appreciated better. To increase patient compliance with the use of any rinsing solution, especially on a daily basis, taste is of utmost importance.

The response rate in our study was high, with more than 85% of participants completing the 6-week trial. Taking into consideration that the required number of participants was achieved in all groups, the internal validity of the study is warranted, with more than 80% of power. Adverse events occurred both in the EO and in the control groups but not in the CPC + Zn + F group. All adverse events were of low magnitude and promptly resolved. The loss of participants for follow-up was due to reasons not related to the protocol. These findings must be compared with the high occurrence of adverse effects when chlorhexidine, a reference standard agent for plaque control, is used.

The novel element of our study is the direct comparison of an oral hygiene regimen with mechanical toothbrushing together with a CPC + Zn + F mouthrinse with the same regimen including an alcohol-free EO solution. This, to the best of our knowledge, has not been performed.

Figure 5. Interproximal gingival index at baseline, 4 week, and 6 week examinations. Brackets represent statistically significant difference for comparisons between groups. All groups showed statistically significant improvements within each group (baseline versus week 4 and baseline versus week 6).
The results comprise the study of reductions in plaque and gingivitis in 2 experiment periods, at 4 and 6 weeks. Such periods are used frequently in oral hygiene regimen studies. In relation to supragingival biofilm accumulation, all 3 regimens led to a statistically significant decrease in dental plaque at weeks 4 and 6 compared with baseline. Baseline plaque values did not differ among groups. This situation allows for better comparability of the encountered effects. The observed decrease may be related to both the Hawthorne effect28 (for example, as observed in the control group) and the antiplaque potential of the included substances. It should be emphasized that the CPC + Zn + F group exhibited the best performance in relation to plaque. The literature has reported this effect of CPC, although systematic reviews have led to the conclusion that this effect would be of low magnitude. However, the presence of zinc lactate likely increased the antiplaque capacity of CPC, as shown in a comparison with a mouthrinse without the zinc salt. In this respect, the reported effect substantiates the claim that the combination regimen of CPC + Zn + F was superior to EO in an alcohol-free base as well as the control regimen.

The analysis of gingival inflammatory signs is also of utmost importance, because it is even more a reflection of oral hygiene standards than plaque removal. The mean GI values observed in our study show that, similarly to plaque analysis, the 3 regimens were able to decrease gingival inflammation, with the CPC + Zn + F group showing the best performance. Mean GI frequently correlates in research studies with mean plaque index, which has been evident since the 1965 study by Löe and colleagues. In our study, the mean GI was reduced further in the CPC + Zn + F group, with an additional 10% through 14% reduction compared with the EO group and 13% through 17% compared with control. This reduction is of clinical interest. Therefore, the observed low-magnitude results in the systematic reviews of the use of CPC have been shown, both in our study and prior research, to enhance the quality of the performance.

Also of great clinical importance is the analysis of the effect of the 3 regimens on the presence of gingival bleeding. The GI for this study was dichotomized into bleeding and nonbleeding sites, as proposed by Ainamo and Bay. This dichotomization allows for a more clinically relevant interpretation of the results. Our study also found a better performance in the CPC + Zn + F group, indicating the superiority of such a regimen in decreasing gingival bleeding, a classic sign of gingival inflammation that has been a target of the dental profession for decades.

The participants in our study refrained from flossing during the study period. Even without performing mechanical interdental plaque removal, both plaque and gingival inflammatory signs were reduced clinically at both experimental periods. The additional use of mouthrinses showed an effect on interdental surfaces in both the CPC + Zn + F and EO groups. However, this effect was higher in the CPC + Zn + F group. In this respect, both the Hawthorne effect and the chemical effect of the rinsing solutions might be responsible for the encountered results. The superiority of the CPC + Zn + F group was shown, and the comparison with the control group mitigates the potential influence of the Hawthorne effect. Taking into consideration the need for interproximal plaque control and the higher prevalence of disease in these sites, our study has shown a clinically relevant finding.

The limitations of our study are related to the fact that convenience samples in an RCT have limited external validity and that the duration of the study was limited to 6 weeks. However, the comparisons among the 3 groups were performed under the same environmental and experimental conditions, which allows for clear conclusions. In addition, the clinical results reported in our study have an especially interesting characteristic, which is the consistency of the findings in both evaluation periods (after 4 and after 6 weeks). Independently of the parameter, the CPC + Zn + F group consistently showed the best performance, with statistically significant differences from the EO group. The EO group showed the second-best performance and, in turn, presented statistically significant differences from the control group. This shows that CPC + Zn + F had better clinical performance than either EO or the control for both supragingival plaque and gingival inflammatory signs. Such a finding, encountered in an RCT and performed with contemporary research paradigm tools, leads to the clinical indication of the regimen in a decision-making process.

CONCLUSIONS
The regimen including a mouthrinse containing CPC + Zn + F presented significantly higher antiplaque and antigingivitis effects than EO and the control, both in whole-mouth as well as in interproximal surfaces.
Costa Rica.
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