

Efficacy of two mouthwashes with cetylpyridinium chloride: a controlled randomized clinical trial

Cassiano Kuchenbecker RÖSING^(a)
Juliano CAVAGNI^(a)
Eduardo José GAIO^(a)
Francisco Wilker Mustafa Gomes
MUNIZ^(a)
Nicolle RANZAN^(a)
Harry Juan Rivera OBALLE^(a)
Stephanie Anagnostopoulos
FRIEDRICH^(a)
Raísa Maldonado SEVERO^(a)
Bernal STEWART^(b)
Yun Po ZHANG^(b)

^(a)Universidade Federal do Rio Grande do Sul – UFRS, Faculty of Dentistry, Department of Periodontology, Porto Alegre, RS, Brazil.

^(b)Colgate-Palmolive Technology Center, Piscataway, NJ USA.

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Corresponding Author:
Cassiano Kuchenbecker Rösing
Email: ckrosing@hotmail.com

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Abstract: This study aimed to evaluate the anti-plaque and anti-gingivitis effects of two mouthwashes containing cetylpyridinium chloride (CPC), in comparison to negative control mouthwash. One hundred and twenty subjects were randomly assigned to study groups: test (0.075% CPC and 0.28% zinc lactate), positive control (0.07% CPC) and negative control mouthwash without CPC. All volunteers were examined by a calibrated examiner for the Quigley-Hein Plaque Index (Turesky modification) and Löe-Silness Gingival Index (GI). Gingival severity was also measured by the percentage of sites with positive gingival bleeding. During six weeks, oral hygiene consisted of brushing twice daily with a toothbrush and toothpaste and rinsing with their assigned mouthwash. Plaque and gingival parameters were assessed at baseline, after four and six weeks of product use. Statistical analyses were performed separately for plaque and gingival indices, by ANOVA, paired t-test and ANCOVA ($\alpha < 0.05$). After 4 and 6 weeks, all mouthwashes groups presented statistically significant reductions in plaque and gingival parameters as compared to baseline. In comparison to the positive control, the test group presented additional reductions in dental plaque of 19.8% and 16.8%, after 4 and 6 weeks, respectively. For GI, the additional reductions in the test group were 9.7% and 14.3%, at 4 and 6 weeks, respectively. The test group showed additional reduction of 35.3% and 54.5% in the gingival severity, at week 4 and 6, respectively. It is concluded that the mouthwash containing CPC and zinc lactate presents significant anti-plaque and anti-gingivitis effects as compared to positive and negative control mouthwashes.

Keywords: Mouthwashes; Cetylpyridinium; Periodontal Index; Treatment Outcome.

Introduction

The importance of supragingival plaque control is unanimous in the literature with positive effects on caries, periodontal diseases and tooth loss demonstrated over long periods.¹ Mechanical plaque disruption is the most usual form of oral hygiene. However, self-performed plaque control has limitations including motivation and



dexterity.² Chemical solutions have been used to compensate such difficulties with interesting results, mainly by increasing the anti-plaque and anti-gingivitis effects in comparison to toothbrushing alone.^{3,4} Also, the use of adjunct rinsing solutions has increased worldwide.⁵

Among the largely used rinsing solutions, cetylpyridinium chloride (CPC) presents high potential both for its antibacterial effect as well as the absence of serious adverse effects.^{3,6} The clinical superiority in terms of plaque and gingivitis of a mouthwash with CPC and sodium fluoride (NaF), in comparison to a mouthwash containing only NaF, was previously showed in a 6-months study.⁷

A systematic review stated that CPC provides a small but significant anti-plaque and anti-gingivitis effect when used as adjuvant to mechanical oral hygiene.⁸ Additionally, the antibacterial effects of CPC are comparable to chlorhexidine in shorter periods, including as pre-procedural rinses.⁹ Zinc salts are safe and efficacious in oral environment, with potential in different oral conditions, including reduction of oral malodor.⁶ Additionally, one systematic review stated that zinc containing mouthwashes should be included in the management of halitosis due to its greater reduction in the organoleptic scores.¹⁰ The literature reported positive anti-plaque and anti-gingivitis outcomes in zinc containing mouthwashes. One study showed the combination of amine fluoride/stannous fluoride with zinc lactate presented a higher inhibitory activity on plaque regrowth in comparison to a alcohol-free essential oils containing mouthwash, using an *in vivo* plaque regrowth model of 3 days.¹¹

Studies assessing the efficacy of mouthwashes containing CPC and zinc salts simultaneously have not been published. The aim of this study was to evaluate the anti-plaque and anti-gingivitis efficacy of a mouthwash containing 0.075% CPC, 0.28% zinc lactate and 0.05% sodium fluoride and compared to a positive control mouthwash with only CPC and sodium fluoride and negative control mouthwash with fluoride over a 6-week period. The null hypothesis of this study is that there are no significant differences, regarding plaque and gingivitis parameters, among the three mouthwashes.

Methodology

Study design

This study was designed as a randomized, single-center, examiner-blind, and parallel-group clinical trial.

Ethical aspects

The study was approved by the Institutional Review Board of the Federal University of Rio Grande do Sul under protocol 1.016.222, and all volunteers signed an informed consent form. The study was conducted according to good clinical practice (GCP).

Main outcome

The main outcome of the present study is gingival parameters measured by the Gingival Index.¹²

Sample size estimate

The sample size calculation utilized historical data from a previous study. It was determined based on a standard deviation for the response measure in gingival index of 0.58, an alpha level of 0.05, a 10% attrition rate, and 80% power. The sample size of 120 (40 per group) individuals was considered necessary to detect a minimal statistically significant difference between the study groups of 15% units.¹³

Subjects

One hundred and twenty (120) males and females, aged 21-70 years, were enrolled in this study. Recruitment consisted of a convenience sample, and the study was conducted between May and July 2015, at the Dental School of the Federal University of Rio Grande do Sul, 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 by Turesky modification of the Quigley-Hein Plaque Index,^{14,15} a mean Gingival Index score of at least 1.0 determined by the Löe-Silness Gingival Index,¹² and a minimum of 20 natural teeth, excluding third molars.

Subjects were excluded from the study if they had any of the following conditions: orthodontic bands; partial removable dentures; subjects who had tumor(s) or significant pathology in the soft or hard tissues of

the oral cavity; individuals presenting moderate or advanced periodontal disease (purulent exudates, tooth mobility, and/or extensive loss of periodontal attachment or alveolar bone); five or more carious lesions requiring immediate care; subjects who used antibiotics any time during the one month prior to entry into the study; subjects who participated in any other clinical study or test panel within 30 days prior to the start of the study; pregnant or breast feeding women; subjects who received a dental prophylaxis in the past two weeks prior to the baseline examination; subjects with history of allergies to oral/personal care consumer products or their ingredients; subjects on any prescription medicines that might interfere with the study outcome; subject with a medical condition which prohibits not eating/drinking or chewing gum for 4 hours prior to their scheduled visit; and subjects with a history of alcohol or drug abuse.

Experimental procedures

Qualifying subjects reported to the clinical study site after refraining from any oral hygiene procedures for twelve hours and from eating, drinking, or smoking for four hours.

Baseline examination comprised soft and hard tissue evaluation of the oral cavity and perioral region, followed by plaque and gingivitis assessments. Supragingival plaque examination was performed, using the modified Quigley and Hein Index,^{14,15} with a plaque disclosure tablet with 2% basic fuchsin (Eviplac, Biodinâmica, São Paulo, Brazil). Plaque was assessed in six sites per tooth and scored from 0 (no plaque) to 5 (plaque covering 2/3 or more of the side of the crown of the tooth). Subject wise scores were calculated by summing all scores for all sites and dividing by the total number of sites scored.

The degree of gingival inflammation was scored at 6 sites per tooth according to the criteria of the Gingival Index (GI) system.¹² GI scores vary from 0 (absence of inflammation) to 3 (severe inflammation) with tendency to spontaneous bleeding. Subject wise 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 site scored 2 or 3, which represents the sites that bleed on marginal probing.¹⁶

Following, subjects were randomized into three groups of 40 individuals each. Randomization list was computer generated. Allocation concealment was under responsibility of an external researcher and all products were covered with white over-wrapping paper in order to conceal product identity. The material was sequentially numbered and kept inside opaque plastic bags.

The experimental groups were:

- a. A test mouthwash group containing 0.075% CPC and 0.28% zinc lactate with 0.05% sodium fluoride in an alcohol-free base (Colgate-Palmolive Co., Brazil);
- b. A positive control mouthwash group containing 0.07% CPC with 0.05% sodium fluoride in an alcohol-free base (Oral-B Pro-Saúde, Procter & Gamble Co., Brazil);
- c. A negative control mouthwash group without CPC (Colgate-Palmolive Co., Brazil).

Home use instructions consisted of brushing twice daily (morning and evening) for one minute with a commercially available fluoride toothpaste (Colgate Cavity Protection Toothpaste) and manual soft-bristled toothbrush and rinsing twice daily (morning and evening) for 30 seconds with 20 mL of their assigned mouthwash for a period of 6 weeks. The subjects were asked to follow this protocol and not to use any other mouthwash or interproximal cleaning devices, such as dental floss or interproximal brushing during the experimental period.

After the baseline examination, one researcher not involved in the clinical examination provided all the information to each subject. Additionally, the first rinse was performed at the clinic under supervision.

Subjects were instructed to return to the clinical facility after 4 and 6 weeks of product use. At each visit, subjects were questioned about any adverse event related to the use of the mouthwash. When any adverse event was reported, one of the researchers performed an oral examination in order to give a proper treatment or explanation to the volunteer. The allocation concealment was not broken at any moment during the study. Additionally, they were examined for dental plaque and the degree of gingival inflammation, using the same procedures employed at baseline. Oral hard and soft tissue examinations were also performed at

each visit. These evaluations were performed by the same trained and calibrated examiner (CKR), who remain blinded to product assignment during the course of the study. Intra-examiner reproducibility was assessed prior to the commencement of the study both for plaque and gingival inflammation, with good levels of reliability ($\kappa > .7$).

Statistical analysis

Comparison of the treatment groups with respect to gender was performed using a Chi-Square analysis and for age using an analysis of variance (ANOVA). Statistical analyses were performed separately for the gingivitis assessments and dental plaque assessments. Mean plaque index was analyzed for all surfaces, interproximal aspects of the buccal and palatal/lingual surfaces and severity from the average of all surfaces with scores of 3, 4 and 5.¹⁶ Mean gingival index was analyzed for all surfaces, interproximal surfaces and severity defined as sites with gingival scores of 2 and 3.¹⁶ Comparisons of the treatment groups with respect to baseline gingival index scores and plaque index scores were performed using ANOVA. Within-treatment

comparisons of the baseline versus follow-up gingival and plaque index scores were performed using paired t-tests. Comparisons of the treatment groups with respect to baseline-adjusted gingival and plaque scores at the follow-up examinations were performed using analyses of covariance (ANCOVA's). Post-ANCOVA, pair-wise comparisons of the study treatments were performed using the Tukey's test for multiple comparisons. All statistical tests of hypotheses were two sided, and employed a level of significance of $\alpha=0.05$, using the package SPSS (version 18.0, SPSS, Chicago, IL, USA). During these analyses, the statistician was also blinded to the product allocation of each subject.

Results

One hundred and twenty (120) subjects entered the clinical study and were randomized into one of the three treatment groups. One hundred twelve (112) subjects completed the 6-week clinical study and their data were analyzed. The flowchart of the study with reasons for exclusion are shown in Figure 1.

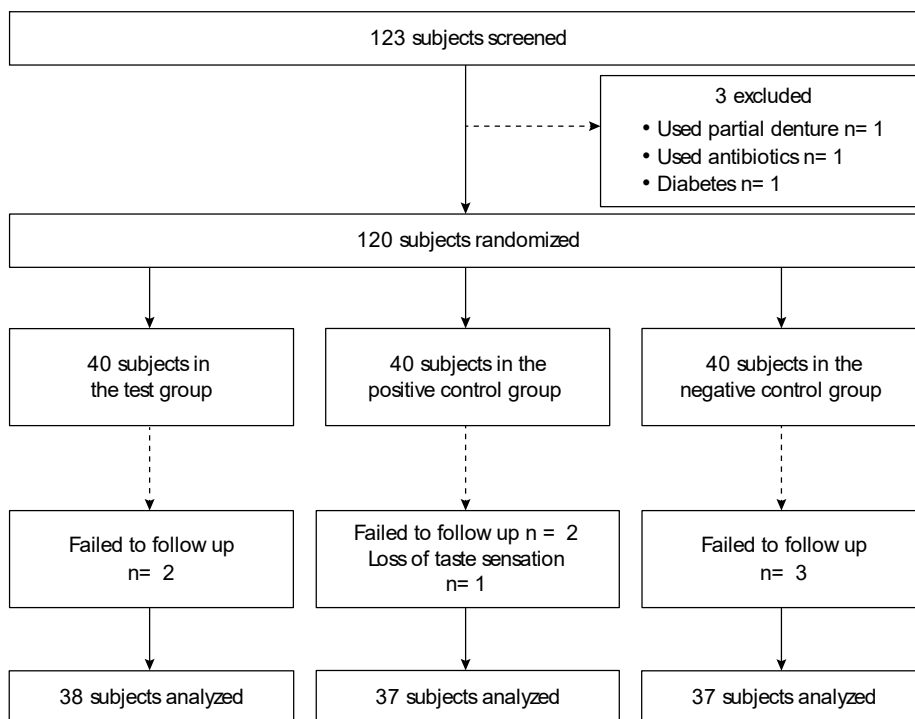


Figure 1. Flowchart of the study.

Table. Demographical and baseline characteristics of the study subjects.

Variables	Test group (n = 37)	Positive control group (n = 37)	Negative control group (n = 38)	p-value
Male/Female	15/22	14/23	14/24	0.994*
Mean age ± SD	31.86 ± 9.93	30.73 ± 10.03	31.26 ± 10.35	0.890**
Range	(22–59)	(21–63)	(22–60)	
Mean plaque index (whole-mouth)	3.44 ± 0.43	3.43 ± 0.48	3.42 ± 0.44	0.978**
Mean plaque index (severity)	0.86 ± 0.15	0.88 ± 0.14	0.84 ± 0.14	0.606**
Mean plaque index (interproximal)	3.54 ± 0.43	3.52 ± 0.47	3.53 ± 0.43	0.988**
Mean gingival index (whole-mouth)	1.63 ± 0.17	1.57 ± 0.18	1.62 ± 0.18	0.387**
Mean gingival index (severity)	0.63 ± 0.17	0.58 ± 0.18	0.62 ± 0.17	0.385**
Mean gingival index (interproximal)	1.71 ± 0.17	1.67 ± 0.20	1.72 ± 0.18	0.492**

*chi-square test; **one-way ANOVA test.

A summary of gender, age and the baseline characteristics of the study population are presented in Table. No statistically significant differences were found among the treatment groups with respect to gender ($p = 0.944$) and age ($p = 0.890$). Furthermore, at baseline, no statistically significant difference was encountered in Plaque, Plaque Severity and Plaque Interproximal, Gingival, Gingival Severity (Gingival Bleeding) and Gingival Interproximal indices.

All mouthwashes groups, which is test, positive, and negative control, showed statistically significant reductions ($p < 0.05$) in dental plaque and gingivitis after 4 and 6 weeks of product use when compared to baseline. Figure 2 shows mean (SD) Plaque scores at the three experimental time points. After 4 weeks, test, positive control and negative control groups exhibited the following reductions from baseline: 59.8%, 49.9% and 37.0%, respectively. The test group showed a statistically significant higher Plaque reduction when compared to positive control and negative control groups ($p = 0.002$ and $p < 0.001$, respectively). Additionally, when compared to the negative control group, the positive control group exhibited a statistically significant ($p < 0.001$) higher reduction in this index.

After 6 weeks, the reductions in Plaque scores were 59.5%, 51.3% and 32.9%, respectively, compared to baseline. Similarly to the 4 week appointment, subjects in the test group showed statistically significantly greater reduction when compared to subjects in the positive and negative control groups ($p = 0.003$ and $p < 0.001$, respectively). Subjects in the positive control group also

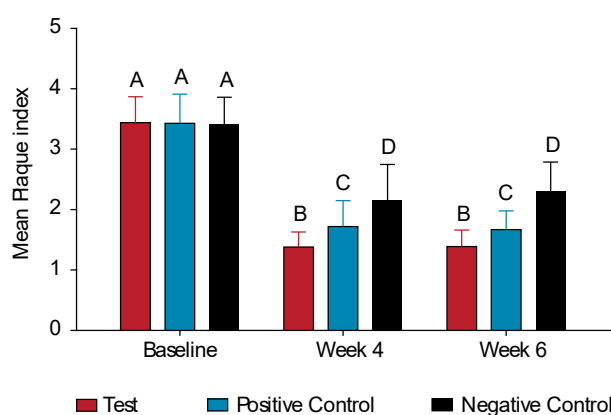


Figure 2. Mean (SD) Plaque index according to the group and the experimental timepoint. The comparisons were performed between the groups and within the groups related to the baseline examination. Equal letters represent no statistically significant difference. Different letters show statistically significant difference.

exhibited statistically significantly ($p < 0.001$) greater reduction when compared to negative control group.

Figure 3 shows the mean (SD) Gingival index of all groups at baseline, 4 and 6 weeks. From baseline, the test, positive control and negative control groups demonstrated a percent reduction of 28.8%, 16.8% and 9.3%, respectively, in this index. In comparison to the positive control and negative control groups, the test group exhibited higher statistically significant ($p = 0.006$ and $p < 0.001$, respectively) reductions. In relation to the negative control group, positive control group exhibited a higher statistically significant ($p = 0.007$) reduction in this parameter.

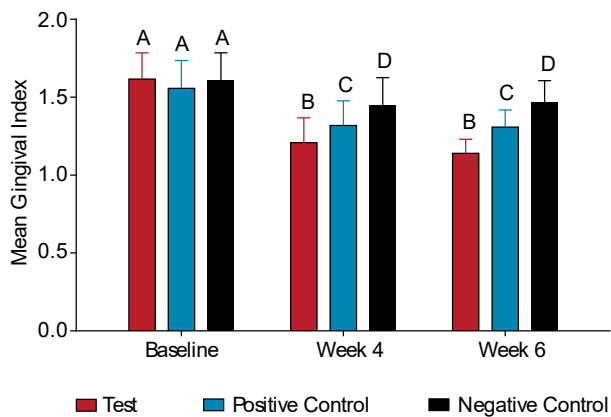


Figure 3. Mean (SD) Gingival index according to the group and the experimental timepoint. The comparisons were performed between the groups and within the groups related to the baseline examination. Equal letters represent no statistically significant difference. Different letters show statistically significant difference.

After 6 weeks, the reductions from baseline were 29.2%, 17.4% and 8.7%. It was demonstrated that the test group exhibited a higher statistically significant reduction as compared to the positive control and negative control groups ($p < 0.001$ in both analyses). Additionally, the positive control group demonstrated a statistically significant reduction in comparison to the negative control group ($p < 0.001$).

The mean (SD) Plaque Severity and Gingival Severity scores are shown in Figures 4 and 5, respectively. In the Plaque Severity, at both 4 and 6 weeks, no statistically significant differences were found between test and positive control group. On the other hand, both groups demonstrated higher statistically significant reduction in comparison to the negative control group at both examinations. Regarding the Gingival Severity scores, the test group showed a greater statistically significant reduction in this parameter when compared to both positive control and negative control groups at weeks 4 and 6. Furthermore, the positive control group showed a greater statistically significant reduction in comparison to the negative control group.

Figures 6 and 7 show, respectively, the mean (SD) Plaque Interproximal and Gingival Interproximal scores throughout the study in all groups. In both analyses, the test group showed a higher statistically significant reduction in comparison to the other groups at both 4

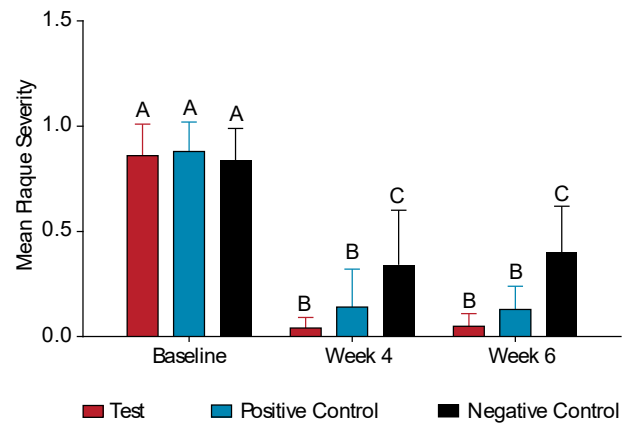


Figure 4. Mean (SD) Plaque Severity index according to the group and the experimental timepoint. The comparisons were performed between the groups and within the groups related to the baseline examination. Equal letters represent no statistically significant difference. Different letters show statistically significant difference.

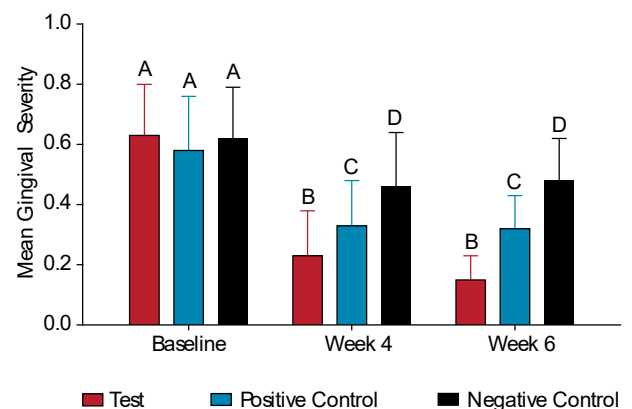


Figure 5. Mean (SD) Gingival severity index according to the group and the experimental timepoint. The comparisons were performed between the groups and within the groups related to the baseline examination. Equal letters represent no statistically significant difference. Different letters show statistically significant difference.

and 6 weeks. Additionally, also in both analyses and in both experimental periods, the positive control group showed a greater statistically significant reduction in comparison to the negative control group.

Throughout the study, 22 adverse events on the oral hard or soft tissues were reported by the subjects when questioned. From these, 20 completed the study. A larger number of adverse events (15 events) were reported in the positive control group. From these, tongue numbness

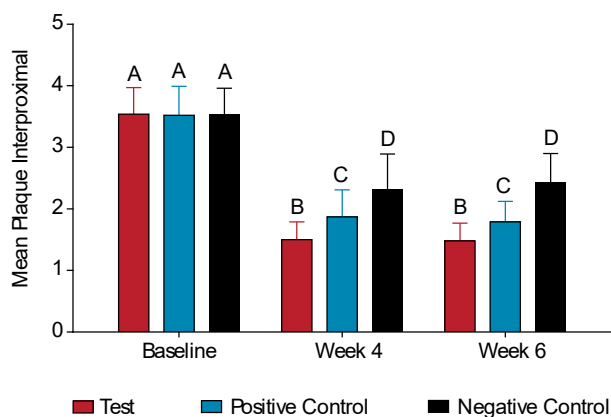


Figure 6. Mean (SD) Plaque Interproximal index according to the group and the experimental timepoint. The comparisons were performed between the groups and within the groups related to the baseline examination. Equal letters represent no statistically significant difference. Different letters show statistically significant difference.

(1 subject), decrease/loss of taste sensation (3 subjects), gingival bleeding (1 subject), sublingual swelling (1 subject), and tooth stain (9 subjects) were reported. In the test group, 3 adverse events were reported, bitter taste (1 subject) and dentin hypersensitivity (2 subjects). In the negative control group, tooth sensitivity (2 subjects), dentin hypersensitivity (1 subject) and nausea (1 subject) were reported. In both test and negative control groups no tooth staining was observed throughout the study.

Discussion

The present study assessed the efficacy of a mouthwash containing 0.075% CPC, 0.28% zinc lactate and 0.05 ppm sodium fluoride compared both to a positive control mouthwash with CPC and fluoride and a negative control mouthwash with fluoride on plaque and gingival parameters over a period of 6 weeks.

In clinical dentistry, mouthwash solutions may be indicated in many clinical situations, such as patients with temporary or continuous impaired motor function, lack of dexterity to use toothbrushing, as adjuvant to mechanical biofilm control or halitosis treatment, and during a postoperative oral procedure.^{17,18}

The novelty of this study was the inclusion in the mouthwash of a zinc salt in order to increase efficacy of CPC alone. The study was performed under contemporary research paradigms, including blindness

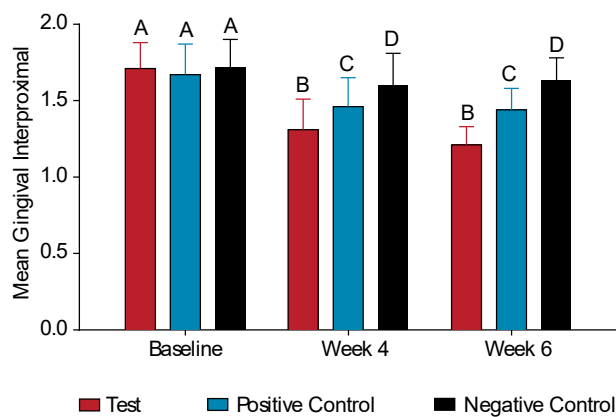


Figure 7. Mean (SD) Gingival Interproximal index according to the group and the experimental timepoint. The comparisons were performed between the groups and within the groups related to the baseline examination. Equal letters represent no statistically significant difference. Different letters show statistically significant difference.

of both the examiner and the patients, reproducibility of the examiner, comparison with positive and a negative controls, sample size estimate and was performed under good clinical practice standards.

The choice of the control groups was based on the primary objective of the study, which was to assess efficacy of a new formulation. Therefore, the positive control group was chosen from the market, as one solution that has virtually the same composition of the test mouthwash (0.07% CPC and 0.05 ppm sodium fluoride) without the zinc salt. The negative control group was developed without any active ingredient. All solutions were packed with opaque flasks, in order to warrant allocation concealment.

A 6-month follow-up clinical trial compared anti-plaque and anti-gingivitis efficacy of three different mouthwashes salutation, one containing essential oil, zinc, and fluoride, another with 0.05% CPC only, and a 5% hydroalcohol solution as a negative control.¹⁹ In this study, the volunteers received a complete dental prophylaxis after baseline examination, and interproximal cleaning was allowed during the study. Similarly to the present study, the mouthwash containing zinc showed significant superiority in plaque and gingivitis control. However, the differences in follow-up, active ingredient used in association with zinc salt, and the interproximal oral hygiene procedures does not allow a direct comparison between these studies.

The study was designed with analyses at 4 and 6 weeks. Inclusion and exclusion criteria used in the study are the ones recommended for studies on plaque and gingivitis, allowing reliable results.²⁰ The individuals were randomized in the three groups and no significant differences were observed among demographic or clinical parameters at baseline. Therefore, the groups were comparable at the start of the study. In the present study, compliance was considered as the panelists showing up for all visits and reporting continuous use of the mouthwash. A very high degree of compliance was obtained from the participants of the study, with more than 90% in all groups. No notable differences were observed in compliance rates among groups.

The main outcome of the present study is reduction in gingival parameters. However, we also assessed plaque parameters, since the reduction in gingivitis correlates with reductions in plaque.²¹ It should be emphasized that plaque indices reflect a momentary situation, whereas gingival inflammation is related to a habit.^{21,22,23} In general, all treatments reduced both plaque and gingival parameters. The observed effect, is probably due to the combination of the effect of the mouthwash with Hawthorne effect, in which the participation in a study motivates extra efforts in the self-performed measures.²⁴ However, significant differences were observed among groups in both plaque and gingival parameters, which are the portions that may be attributed to the effect of the rinsing solutions.

When plaque is considered, higher statistically significant reductions were observed when the test solution is used. This effect was observed both in Plaque and Plaque Interproximal indices. The effects of CPC on plaque have been well documented.^{7,25,26} Therefore, the additional benefits observed in the test mouthwash in relation to the positive control group might be attributed to the presence of zinc lactate, since metal salts have been demonstrated to affect plaque.²⁷ One important finding of the present study is the effect on interproximal surfaces.

The results observed in the present study on gingival inflammation were of great interest, since the test mouthwash presented significantly greater reduction in mean whole mouth gingival index,

percent of bleeding sites (severity index) and in interproximal surfaces as compared to positive and negative controls. This reflects that after 4 and 6 weeks, a sufficient time for gingivitis to be evaluated, the use of a mouthwash with CPC, zinc lactate and sodium fluoride is effective reducing gingival parameters such as gingival inflammation and gingival bleeding. No statistically significant difference was observed after 4 weeks of study, demonstrating that in 4 weeks the effect especially on gingivitis was already demonstrated. Zinc salts have been previously studied and have demonstrated promising results.^{11,28} The present study suggests that the addition of zinc lactate enhanced the anti-gingivitis effect of a mouthwash containing CPC.

It should be emphasized that one of the most important results in the present study is the effect on interproximal surfaces. Those surfaces are the ones with highest prevalence of both caries and periodontal diseases²⁹ and really benefit from this effect, since the use of dental floss is not widespread.³⁰ Therefore, we can suggest that the greater efficacy in all surfaces as well as in interproximal surfaces in terms of gingival inflammatory signs are one of the important effects of the combination of CPC, zinc lactate and sodium fluoride.

The effect in terms of severity of gingival index should be also taken into consideration as an important and clinically relevant effect. The presence of gingival bleeding is the most important clinical finding in terms of gingival inflammatory signs. In the present study, the test mouthwash performed better in terms of clinically detectable gingival inflammation, supporting the clinical relevance of the encountered results.

It should be remembered that the concentration of CPC in the test and in the positive control solutions is slightly different (0.075% vs. 0.07%, respectively). It is very unlikely that the very small difference in concentration of CPC could explain the results in terms of plaque and gingivitis. It is most likely that the addition of zinc lactate is responsible for the clinically relevant effects demonstrated in the present study. Also, it should be remembered that zinc salts have additional benefits in oral health, including effect on halitosis³¹, that should be further studied. On the other hand, it must be highlighted that both

test and positive control mouthwashes were produced by different companies. Therefore, it is possible that some intrinsic differences in the formulation might account for the different results found in this study.

In randomized controlled trials, one of the important findings is the occurrence of adverse events. They were self-reported, therefore independent of the examiner. Some of them might have relationship with the substances under study. In the present study, adverse events were observed in all groups. However, a marked different pattern was observed in the positive control group, with 9 individuals reporting extrinsic staining. This effect was not reported by participants neither from the test nor from the negative control groups. When the volunteer reported any side effect, the researchers performed an oral examination. In the clinical analysis of the stains, it was detected a heavy staining on tooth surfaces of the 9 volunteers in the positive control group. Studies with CPC do not report such an

amount of staining.^{32,33,34} In seeking explanations for that, other compounds of the formula, such as preservers, coloring agents, flavors could account for the effect. The interpretation of such adverse effect needs to be further studied.

Conclusion

It is concluded that the mouthwash containing 0.075% CPC and 0.28% zinc lactate with 0.05% sodium fluoride in an alcohol-free base provided significantly greater reductions in Plaque, Plaque Interproximal, Gingival, Gingival Severity, and Gingival interproximal index after 4 and 6 weeks of product use as compared to a negative control mouthwash and a 0.07% CPC and 0.05% NaF mouthwash.

Acknowledgments

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