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Research Article

EFFICACY OF MOUTH RINSE FORMULATION BASED ON CETYLPYRIDINIUM CHLORIDE 0.1% IN THE CONTROL OF DENTAL CALCULUS BUILDUP

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ABSTRACT

Objective: This study aimed at comparing the antiplaque, anticalculus, and antigingivitis potentials of a mouth rinse containing essential oil, alcohol, zinc, and fluoride with a mouth rinse containing cetylpyridinium chloride (CPC) 0.1% over 1-, 2-, and 3-month periods.

Methods: This study was a double-blind, parallel randomized clinical trial with a 3-day run-in phase. Respondents were asked to gargle twice daily with 15 ml of mouth rinse for 30 seconds after brushing teeth. Respondents were 80 females with a mean age of 21 years, and a single dental examiner was employed throughout the study to decrease the variance. Prophylaxis was performed for all respondents before the intervention. Three mouth rinses were tested: Group 1 with the mouth rinse containing CPC 0.1%, Group 2 as the negative control, and Group 3 as the positive control with a mouth rinse containing alcohol. Evaluations were conducted by plaque index, gingival index, calculus index, and CariScreen examinations.

Results: The clinical trial showed that the mouth rinse with alcohol and the mouth rinse containing CPC 0.1% were effective in inhibiting bacterial buildup (antiplaque) and have anticalculus properties, but with no statistically significant antigingivitis effect.

Conclusion: It was found that the mouth rinse containing alcohol has similar effectiveness with CPC 0.1% mouth rinse, but side effects, such as a burning sensation, were reported in the alcohol-containing mouth rinse.

Keywords: Mouth rinse, Cetylpyridinium chloride, Anticalculus.

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INTRODUCTION

Oral biofilms relate to the development and severity of gingivitis and periodontitis. Therefore, controlling for oral biofilm plays a key role in the prevention, treatment, and decrease of the recurrence of periodontal diseases [1]. Although this relationship has long been recognized, adequate self-performed biofilm control, such as brushing and rinsing with oral health products, still remains an area with the opportunity for improvement. As mentioned, noninvasive techniques, such as rinsing with adequate mouth rinse to prevent plaque buildup, which indicates early onset of calculus buildup, are low cost, feasible, and sustainable [2]. Therefore, research on the efficacy of mouth rinse is important.

There are several over-the-counter products to control plaque buildup, such as mouth rinses containing cetylpyridinium chloride (CPC), and there are various concentrations of CPC in mouth rinses. CPC-containing mouth rinses are indicated for regular daily use and are aimed at preventing and controlling biofilms and gingivitis [3]. Choices of CPC concentration may lead to different patterns of effectiveness [4]. CPC is a quaternary ammonium compound that has the ability to reduce plaque and gingivitis [5]. An additional study about CPC-containing mouth rinses supported the benefit of CPC in decreasing plaque accumulation. CPC may also decrease gingival inflammation provided by this agent when used in combination with either supervised or unsupervised oral hygiene [6]. The aim of this study was to test whether the CPC-containing mouth rinse has progressive cumulative clinical benefits. A group of Indonesians was selected to be the study population to test this new formula, because the literature has shown higher prevalence and severity of gingivitis in developing countries than in populations in developed countries.

The present study aimed to compare the efficacy and safety of three mouth rinse formulations. The study was designed for a 6-month observation period. However, due to the occurrence of side effects, including, a painful sensation, irritation, and ulcer of the mouth by one mouth rinse formulation during the study's execution, the length of the study was shortened. The presence of side effects would have affected compliance and violated ethical principles if we had continued carrying out the study for the 6-month period. However, reducing the length of the study from 6 to 3 months did not compromise the validity of the study, as the number of participants could be statistically analyzed. Nevertheless, the use of highly sensitive CarieScreen equipment to analyze dental plaque enabled us to detect the formation of dental plaque at an earlier time, hence allowing for adequate data despite the reduced observation period.

METHODS

This study was cleared for ethics by the Faculty of Dentistry, Universitas Indonesia Institutional Review Board, and each participant gave informed consent. This study was a before-and-after parallel, randomized, double-blind clinical study. Females, aged 18 years and over, were recruited. The participants were given a prophylaxis before the run-in phase and then a silica fluoride toothpaste to use for 3 days as the run-in phase. Oral hygiene scores included the plaque index (PI), gingival index (GI), and calculus index (CI). Further, the bacterial load was also measured and was taken and analyzed [7]. Each respondent was randomly allocated to the test product. The respondents used the study products as part of their normal oral hygiene regimen. Oral soft tissues were assessed at each examination for detecting any adverse event, such as an allergic reaction. Adverse events were monitored

with the ethical standards throughout the study. Any concomitant medication, including food supplements and prophylactic treatments, were recorded. A single examiner performed the examinations to minimize potential biases. An intrarater examiner analyzed 10% of samples to optimize the reliability of examinations.

At baseline and after 1, 2, and 3 months of using the mouth rinse, the participants underwent an oral examination. Following randomization, the participants received prophylaxis and began brushing two times daily with fluoride toothpaste, which was provided by the researchers. This procedure was followed by rinsing twice daily with 15 ml of the assigned mouth rinse for 30 seconds. Purposive sampling was employed, concordant with the stated inclusion and exclusion criteria. Participants were monitored for compliance by keeping a good contact with them by regular texting. Mouth rinses were bottled for a weekly proportion, so participants needed to refill with the new mouth rinse bottles when meeting the research administrator. Motivational interviews were conducted during meetings, and potential adverse events were well monitored. Remunerations were conducted periodically.

The inclusion criteria were females, aged 18-30 years, willing to participate, and who sign the informed consent. Moreover, the participants should be able to comply with the study procedures, no medical conditions that prevent a person from brushing their teeth, a minimum of 20 natural healthy teeth with no indication of extraction, a Turesky modification of the Quigley and Hein Index more than 1.5, a Loe and Silness index of more than 1, Class 3 calculus in lower anterior lingual teeth, and being nonsmokers [8]. Exclusion criteria were the occurrence of an adverse event, withdrawal, and sickness that could bias the results. Moreover, the participants were excluded from the study if they do not comply with procedures of the study that could bias the research results, such as using xylitol gum, casein phosphopeptide–amorphous calcium phosphate, gargling solution, and other medications.

Participants were monitored for the primary outcomes of the GI and PI at baseline and after 1, 2, and 3 months. Participants were randomly assigned to mouth rinse groups to do unsupervised rinsing twice daily with the CPC or positive or negative control rinse parallel with normal brushing. The coding of the mouth rinses was as follows: 1 = mouth rinse containing CPC 0.1%; 2 = negative control; and 3 = positive control, a mouth rinse containing zinc chloride, alcohol, and essential oil. Participants were allowed to discontinue the clinical study at any time. Participants participated in this study of their own free will, full consciousness, and without any coercion. In addition, the principal investigator had the right to withdraw participants for any reason that was stated in the exclusion criteria and also for other reasons that were in the best interests of the respondent. Clinical assessment of the mouth rinse's efficacy was assessed before and after 1, 2, and 3 months of mouth rinse usage using a CariScreen meter and intraoral health indexes.

The CariScreen measurement

Swab samples were taken of the plaque from the participants' teeth, which when combined with special bioluminescence reagents within the swab, created a reaction that then was measured with the CariScreen meter. The CariScreen meter gave a score between 0 and 9999. A score under 1500 is considered relatively healthy, while above that shows a relatively high bacterial load. A previous study using oral clinical specimens from pediatric patients demonstrated that adenosine triphosphate (ATP)-driven bioluminescence can be used in direct determination of bacterial numbers and can serve as a general assessment indicator for oral hygiene [9]. ATP-driven bioluminescence may potentially serve as a component of tartar-buildup risk assessment. The CariScreen measurement was based on subject analysis.

The PI measurement

The PI score was analyzed using the disclosing solution from GC Corp, Tokyo, Japan. The description of the PI was as follows: 0 = no plaque, 1 = dot plaque on gingival margin, 2 = line plaque reaching 1 mm from gingival margin, 3 = plaque more than 1 mm to 1/3rd of the tooth's surface, 4 = plaque from 1/3rd–2/3rd of the tooth's surface, and 5 = plaque more than 2/3rd of the tooth's surface. The disclosing gel was applied with a micro brush to all participants' teeth, and afterward, they were asked to gargle with water to see the 3-dimensional examinations of GC Tri Plaque ID Gel, a dental plaque-disclosing gel, of every tooth surface. The measurement results could be representative of the surface- and subject-level analyses. Surface-level analyses were conducted by a categorical yes-or-no plaque appearance on each surface. Subject-level analyses were calculated by summing the total number of the PI scores for each tooth divided by the number of teeth examined multiplied by 100%.

The CI measurement

The measurement results could be representative of the surface- and subject-level analyses. Surface-level analyses were conducted by a categorical yes-or-no calculus appearance on each tooth's surface. Subject-level analyses were calculated by summing the total number of CI for each tooth divided by the number of teeth examined multiplied by 100%. The criteria for classifying calculus were as follows: 0 = no calculus present, 1 = supragingival calculus covering not more than 1/3rd of the exposed tooth surface, 2 = supragingival calculus covering more than 1/3rd but not more than 2/3rd of the exposed tooth surface or the presence of individual flecks of subgingival calculus around the cervical portion of the tooth or both, and 3 = supragingival calculus covering more than 2/3rd of the exposed tooth surface or a continuous heavy band of subgingival calculus around the cervical portion of the tooth or both.

The GI measurement

Clinical appearance (color, texture, shape, size, and absence of ulceration) of all gingival surfaces was observed. Probing was performed on all the four surfaces of the gingival sulcus. Occurrence of bleeding after 10 seconds was observed and noted. The index was scored according to the Loe and Silness GI to describe the location and gingival inflammation clinical severity. The scores were described as follows: 0 = normal; 1 = mild inflammation with slight color change, mild swelling, and slight texture change; 2 = moderate inflammation with redness, hypertrophy, swelling, shiny color, and presence of bleeding if pressured or stimulated with probe; and 3 = severe inflammation with clear redness, hypertrophy, swelling, ulceration, and presence of spontaneous bleeding. All distal, mesial, lingual, and buccal surfaces of the teeth were examined. The GI for a particular tooth is the result of the average of the four tooth surface measurements. The GI for a particular tooth type (molars, premolars, incisive) is the average of the measurements of the same tooth type on both jaws. The GI for each individual is the average score of all the teeth examined.

Bleeding may occur on probing and refers to a hemorrhage caused by a soft touch to the gingival soft tissue on the gingival sulcus using a device called a probe. Capillary dilation and increased blood flow happen in inflamed tissues. Erythema appears in early gingivitis lesions due to capillary proliferation and decline in collagen production caused by changes in cytotoxic fibroblasts. At this stage, bleeding on probing could be detected. Probing on the four surfaces of the dental gingival sulcus was performed. The Sensor Probe Type C pocket probe by Pro-Dentec was used. Probing pressure was assured not to be too high or too low with the help of the pocket probe's sensor, with the amount of pressure at 2.5 cm/g. When the given pressure was too high, the head of the probe would curve, whereas when the given pressure was too low, there would be noticeable gaps on the head of the probe.

RESULTS

Side effects occurred, and respondents complained of the strong flavor of the mouth rinse containing alcohol. The data were analyzed for a normal distribution using Shapiro–Wilk or Kolmogorov–Smirnov test for normality using the computer software SPSS Statistics version 20.

Table 1: Mean of the CariScreen measurement result and the p values

Mouth rinse	Before	After 1 month	After 2 months	After 3 months	p-values (Friedman)
1	3,451	4,036	3,108	6,560	0.384
2	5,888	5,345	6,801	7,711	0.302
3	6,384	4,292	4,213	2,541	0.001*
p-values (Kruskal–Wallis test)	0.229	0.480	0.021*	0.000*	0.229

*p<0.05

Parametric or nonparametric tests were used as appropriate. All the statistical tests were two tailed, and significance level was set at 0.05. This means that any p-value <0.05 showed a statistically significant difference. The coding was 1 = mouth rinse containing CPC 0.1%, 2 = negative control, and 3 = positive control mouth rinse with alcohol.

The CariScreen results

Research results summarized in Table 1 show the mean of CariScreen measurement results before and after 1, 2, and 3 months of mouth rinse usage. CariScreen gives a score between 0 and 9999. The higher the score is, the higher is the bacterial load. Mouth rinse 3 was further analyzed with *post hoc* Wilcoxon test. Comparison between, before, and after the 2-month results showed a statistical significance of 0.032. Comparison between, before, and after the 3-month results showed a statistical significance of 0.007. Mouth rinse 3 showed the highest reduction in bacterial load, followed by mouth rinse 1, then 2. This means that mouth rinse 3 has a good capability to inhibit bacterial growth *in vivo*, followed by mouth rinses 1 and 2 (Tables 1 and 2).

In addition, CariScreen results were statistically analyzed. The analysis was conducted with person as the primary sample unit and with the CariScreen results after 2 months' use of the mouth rinse. Table 3 summarizes the comparison between the three mouth rinses. Then, the analyses were decomposed to the CPC- and alcohol-containing mouth rinses (Table 4). Tables 3 and 4 summarize the summary of the CariScreen analysis. It can be concluded that mouth rinses 1 and 3 both have antibacterial effects, which fight bacteria, but the CPC-containing mouth rinse was more likely to be more effective than the alcohol-containing mouth rinse.

The PI results

Further results were regarding the PI. Tables 5 and 6 show the description of the PI. Mouth rinse 3 showed the lowest growth of plaque, followed by mouth rinse 1, then 2. This means that mouth rinse 3 has a good capability to control for plaque *in vivo*.

The CI results

Table 7 summarizes the surface-level analyses regarding the calculus buildup comparing the three mouth rinses. The analyses were in categorical values. After 1 month of mouth rinse use, there were no significant differences between all groups, but after the 2nd and 3rd months, there were statistically significant differences between the mouth rinses' effectiveness. The negative control (mouth rinse 2) showed a low capability to inhibit calculus buildup. On the other hand, the tested mouth rinse (No. 1) showed better calculus-inhibiting capability, better than mouth rinse 3 (Table 8).

The GI results

Further results were regarding the GI. Table 9 shows the description of the GI. The research results showed the mean of the GI measurement results before and after mouth rinse usage. The GI showed no difference in any groups. This might be caused by the short period of the use of the mouth rinse, which might not affect the gingiva or the periodontal tissue. The bacterial growth might not be enough to evoke inflammation. The results in Table 10 were presented in two statistical aspects: (1) Using the GI in a numerical analysis with the person as a primary sample unit and (2) using the bleeding measurement in a categorical analysis with the tooth surface as a primary sample unit.

Table 2: *Post hoc* analysis (p-value) of the CariScreen results of Table 1 by Mann–Whitney test

Mouth rinse	p-values after 2 months	p-values after 3 months
1 versus 2	0.017*	0.660
1 versus 3	0.635	0.030*
2 versus 3	0.018*	0.000*

*p-value<0.05

Table 3: Comparison between mouth rinses according to increasing or decreasing bacterial load

Mouth rinse	Before<after (increasing bacterial load)	Before>after (decreasing bacterial load)
1	8	16
2	15	9
3	10	14

Table 4: Comparison between mouth rinses according to percentage of increasing or decreasing bacterial load

Mouth rinse	Before<after (increasing bacterial load)	Before>after (decreasing bacterial load)
1	8 (45%)	16 (53%)
3	10 (55%)	14 (46%)
Total	18 (100%)	30 (100%)

Table 5: Number of tooth surfaces (n=3777) with plaque measurement results with categorical statistical approach, which were yes-or-no plaque presence (surface-level analysis)

Mouth rinse	After 1 month	After 2 months	After 3 months
1	382	382	385
2	512	453	478
3	381	343	359
p-values (Pearson's Chi-square test)	0.001*	0.001*	0.001*

*p-value<0.05

Table 6: *Post hoc* analysis of Table 5 in P values from the continuity correction results

Mouth rinse	After 1 month	After 2 months	After 3 months
1 versus 2	0.000*	0.012*	0.001*
1 versus 3	0.178	0.003*	0.016*
2 versus 3	0.000*	0.000*	0.000*

*p-value<0.05

DISCUSSION

Calculus formation is the result of petrification of dental plaque biofilm, with mineral ions provided by bathing saliva or crevicular

Table 7: Number of tooth surfaces (n=3291) with calculus measurement results, with categorical statistical approach, which were yes-or-no calculus buildup (surface-level analysis)

Mouth rinse	After 1 month	After 2 months	After 3 months
1	26	50	83
2	36	81	116
3	28	60	86
p-values (Pearson's Chi-square test)	0.341	0.011*	0.014*

*p-value<0.05

Table 8: *Post hoc* analysis of Table 7 in p values from the continuity correction

Mouth rinse	After 2 months	After 3 months
1 versus 2	0.005*	0.012*
1 versus 3	0.371	0.858
2 versus 3	0.066	0.024*

*p-value<0.05

Table 9: Gingival measurement results

Mouth rinse	Before	After 1 month	After 2 months	After 3 months	p-values (RM-ANOVA)
1	0	0.04	0.17	0.31	0.000
2	0	0.08	0.17	0.33	0.000
3	0	0.17	0.17	0.31	0.000
p-values	0.132 (Kruskal–Wallis test)	0.437 (Kruskal–Wallis test)	0.469 (Kruskal–Wallis test)	0.934 (1-way ANOVA)	

fluids [10]. Supragingival calculus formation can be controlled by chemical mineralization inhibitors, applied in toothpastes or mouth rinses [11]. These formulas act to delay plaque calcification, keeping deposits in an amorphous nonhardened state to facilitate removal with regular hygiene [12]. Clinical efficacy for these agents typically has been assessed as the reduction in tartar area coverage on the teeth between dental cleanings [13]. Research shows that topically applied mineralization inhibitors also can influence adhesion and hardness of calculus deposits on the tooth surface, facilitating removal [14,15].

Rapid ATP-driven bioluminescence assays have long been used in the quantitative determination of bacterial numbers and, most recently, in dental plaque assessment studies. Biofilm cell survival can be measured using the ATP-driven bioluminescence [10]. Using the luciferin substrate and luciferase enzyme, bacterial ATP can be quantified by measuring the release of visible light. CariScreen enables measuring intra-oral bacterial load by employing ATP-driven bioluminescence to identify oral bacterial load. CariScreen testing swabs were used in conjunction with the CariScreen testing meter for a painless and simple 1-minute chairside bacterial test to assess biofilm cell survival on patients, particularly in measuring the ATP-driven bioluminescence. The CariScreen testing meter was proven to be effective and accurate in detecting the level of the bacterial load *in vivo* [10].

Previous studies have described the antiplaque activity of some antimicrobial agents, such as chlorhexidine, CPC, and n-tetradecylamine. The results suggested that the antiplaque effect does not necessarily depend on high bactericidal activity [16]. Some of the authors concluded that a lower concentration of chlorhexidine and CPC in the mouth rinse demonstrated efficacy in reducing plaque and gingivitis levels, as well as decreasing the microbial load in saliva and oral gingival sulcus [6]. This study is valuable to help reduce the incidence of gingivitis and periodontitis among the Indonesian population. Currently, scant oral health studies analyze the efficacy of over-the-counter mouth rinse in Indonesia. This study showed that the tested mouth rinse containing CPC has a higher efficacy in preventing dental calculus than the control mouth rinse.

Findings from this research demonstrate that, when tested, the experimental CPC rinse had comparable anticalculus, antiplaque, and antigingivitis activities to the alcohol-containing rinse. Results were consistent with a previous research showing that a CPC rinse provides a comparable benefit to an alcohol-containing mouth rinse when used as an adjunct to tooth brushing [4]. Therapeutic rinses have become a common adjunct to oral hygiene regimens. The selection of an oral rinse is influenced by many factors, including product formulation and patient preference for esthetics. The majority of therapeutic rinses contain alcohol, which may not be desired for certain patient populations, including children, patients of certain religious faiths, patients with xerostomia, recovering alcoholics, and others [14]. High levels of alcohol also can produce a burning sensation during use and is a product attribute that may be unpleasant for some patients. This occurred in this study. Right after the use of mouth rinse 3, 10 patients reported a burning sensation during use and 1 reported the occurrence of an ulcer on the 1st day of use. Oral health treatment has shifted from treating the existing disease to preventing future disease. The CPC-containing mouth rinse, designed for the broad population, may be particularly appealing to those patient groups seeking an anticalculus, antiplaque, and antigingivitis rinse without alcohol or the burn of alcohol [14].

CONCLUSION

This study showed that the mouth rinse containing CPC 0.1% had a similar effectiveness compared to the alcohol-containing mouth rinse in inhibiting bacterial buildup and has anticalculus properties. Nonetheless, complaints of side effects in the alcohol-containing mouth rinse were reported.

REFERENCES

1. Darby I. Non-surgical management of periodontal disease. Aust Dent J 2009;54 Suppl 1:S86-95.
2. Bowen DM. Lasers and nonsurgical periodontal therapy. J Dent Hyg 2015;89(4):206-9.
3. Cortelli SC, Cortelli JR, Shang H, Costa R, Charles CA. Gingival health benefits of essential-oil and cetylpyridinium chloride mouthrinses: A 6-month randomized clinical study. Am J Dent 2014;27(3)119-26.

4. Costa X, Laguna E, Herrera D, Serrano J, Alonso B, Sanz M. Efficacy of a new mouth rinse formulation based on 0.07% cetylpyridinium chloride in the control of plaque and gingivitis: A 6-month randomized clinical trial. J Clin Periodontol 2013;40(11):1007-15.
5. García V, Rioboo M, Serrano J, O'Connor A, Herrera D, Sanz M. Plaque inhibitory effect of a 0.05% cetyl-pyridinium chloride mouth-rinse in a 4-day non-brushing model. Int J Dent Hyg 2011;9(4):266-73.
6. Lamont T. Lower concentration of chlorhexidine and cetyl-pyridinium chloride mouthwash demonstrates some efficacy. Evid Based Dent 2012;13(2):52-3.
7. Deinzer R, Jahns S, Harnacke D. Establishment of a new marginal plaque index with high sensitivity for changes in oral hygiene. J Periodontol 2014;85(12):1730-8.
8. Gupta D, Jain A. Effect of cinnamon extract and chlorhexidine gluconate (0.2%) on the clinical level of dental plaque and gingival health: A 4-week, triple-blind randomized controlled trial. J Int Acad Periodontol 2015;17(3):91-8.
9. Fazilat S, Sauerwein R, McLeod J, Finlayson T, Adam E, Engle J, *et al.* Application of adenosine triphosphate-driven bioluminescence for quantification of plaque bacteria and assessment of oral hygiene in children. Pediatr Dent 2010;32(3):195-204.
10. Asahi Y, Noiri Y, Miura J, Maezono H, Yamaguchi M, Yamamoto R, *et al.* Effects of the tea catechin epigallocatechin gallate on *Porphyromonas gingivalis* biofilms. J Appl Microbiol 2014;116(5):1164-71.
11. Cortelli SC, Cortelli JR, Wu MM, Simmons K, Charles CA. Comparative antiplaque and antigingivitis efficacy of a multipurpose essential oil-containing mouthrinse and a cetylpyridinium chloride-containing mouthrinse: A 6-month randomized clinical trial. Quintessence Int 2012;43(7):e82-94.
12. Noiri Y, Okami Y, Narimatsu M, Takahashi Y, Kawahara T, Ebisu S. Effects of chlorhexidine, minocycline, and metronidazole on *Porphyromonas gingivalis* strain 381 in biofilms. J Periodontol 2003;74(11):1647-51.
13. Rawlinson A, Pollington S, Walsh TF, Lamb DJ, Marlow I, Haywood J, *et al.* Efficacy of two alcohol-free cetylpyridinium chloride mouthwashes-a randomized double-blind crossover study. J Clin Periodontol 2008;35(3):230-5.
14. Sreenivasan PK, Haraszthy VI, Zambon JJ. Antimicrobial efficacy of 0·05% cetylpyridinium chloride mouthrinses. Lett Appl Microbiol 2013;56(1):14-20.
15. Latimer J, Munday JL, Buzza KM, Forbes S, Sreenivasan PK, McBain AJ. Antibacterial and anti-biofilm activity of mouthrinses containing cetylpyridinium chloride and sodium fluoride. BMC Microbiol 2015;15:169.
16. Murata Y, Miyamoto E, Ueda M. Antiplaque activity of some antimicrobial agents using a simple *in vitro* method. Caries Res 1990;24(2):113-6.