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RESEARCH ARTICLE

Comparative Effectiveness of Mouthwash Containing Chlorhexidine 0.12% and Cetylpyridinium Chloride 0.1% with Chlorhexidine 0.2% against *Porphyromonas gingivalis*

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ABSTRACT

Background: *Periodontitis is an inflammatory disease that affects the supporting tissue of teeth and causes damage to the periodontal ligament and alveolar bone, generally caused by microorganisms. Chronic periodontitis is often caused by gram-negative anaerobic bacteria, especially Porphyromonas gingivalis. Mouthwash active ingredients that frequently used such as chlorhexidine (CHX) or cetylpyridinium chloride (CPC) have an antibacterial effect and prevent plaque formation.* ***Objective:*** *This research aims to compare the effectiveness of mouthwash containing CHX 0.12% and CPC 0.1% with CPC 0.2% on the bacterium Porphyromonas gingivalis ATCC 33277.* ***Materials and Methods:*** *This research is an experimental laboratory by giving treatment to Porphyromonas gingivalis with mouthwash CHX 0.12% and CPC 0.1%, CHX 0.2%, and distilled water as the negative control, then calculating the inhibition zone for each treatment and continued with One-Way ANOVA test.* ***Results:*** *The result showed the largest average of the inhibitory zone diameter was on CHX 0.2% which was 12,7 mm. Meanwhile, mouthwash containing CHX 0.12% and CPC 0.1% showed an average diameter of inhibitory zone resulted in 10,97 mm.* ***Conclusion:*** *In conclusion, CHX 0.2% has greater ability of inhibitory than mouthwash containing CHX 0.12% and CPC 0.1% against the growth of Porphyromonas gingivalis.*

Keywords: *Antibacterial Agents, Cetylpyridinium Chloride, Chlorhexidine, Mouthwash, Porphyromonas Gingivalis*

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INTRODUCTION

Oral health and hygiene are important things that need attention, because poor oral condition or inflammation can cause pain and discomfort.[1] Periodontal disease is one of the inflammatory condition that often found in oral cavity. The most common periodontal disease are gingivitis and periodontitis.[2] An inflammatory condition known as periodontitis affects the tissue that supports teeth and usually caused by certain bacteria, where there is damage to periodontal ligament and alveolar bone.[3] The spread of inflammation from the epithelium to the connective tissue causes damage to the collagen fibers, followed by loss of attachment which is a sign of change from gingivitis to periodontitis. Periodontitis can be broadly classified into chronic and aggressive periodontitis.[4] Chronic periodontitis is the most common type of periodontitis. The disease progression of chronic periodontitis is slow to moderate and related to the plaque accumulation and calculus. The development of periodontitis disease may be caused by multifactorial, such as systemic, local or environmental factors that disrupt the interaction of normal host-bacteria.[3] Although it can affect people of all ages, adults are more likely to be affected from chronic periodontitis. The level of local factors is correlated with the level of disease progression. Certain bacteria cause chronic periodontitis to develop more slowly.[4] Chronic periodontitis is often caused by gram-negative anaerobic bacteria, especially *Porphyromonas gingivalis*.[5]

Bacteria that often cause periodontal disease are *Porphyromonas gingivalis, Aggregibacter actinomycetemcomitans, Prevotella intermedia, Tannerella forsythia, Fusobacterium spp*.[6] *Porphyromonas gingivalis* is a gram-negative bacteria that often found in the subgingival area, and sometimes found in tongue and tonsils. The characteristics of *Porphyromonas gingivalis* are gram-negative, coccobacilli, non-motile, asaccharolytic and pleomorphic. *Porphyromonas gingivalis* grows anaerobically with dark pigmentation in media containing blood.[6,7] *Porphyromonas gingivalis* has several virulence factors, such as gingipains, lipopolysaccharide (LPS), fimbriae, lectins (erythrocytes), capsules, collagenase, and proteases which release harmful and toxic metabolites and crucial in the early stages of periodontitis development.[8]

Periodontal disease can be prevented by maintaining oral hygiene, generally through plaque control by mechanical and chemical methods. Examples of mechanical methods are brushing teeth, using dental floss or an interdental brush. Examples of chemical methods are using toothpaste and mouthwash.[9] The use of mouthwash can control supragingival plaque and gingivitis to clean the oral cavity mechanically.[10]

Various kinds of mouthwash ingredients containing antimicrobials such as chlorhexidine and cetylpyridinium chloride, have shown efficacy in decreasing plaque and preserving oral hygiene.[11] Chlorhexidine (CHX) is one of mouthwash with bisbiguanide ingredients that can kill microorganism by damaging their membrane cell, which damages the cytoplasm. Based on experimental studies, CHX is the gold standard for evaluating how effectively other mouthwashes work. Long-term use of CHX needs to be considered because it has the potential to cause staining on teeth and changes in taste.[12] Side effects that are often complained by the patients are stains on the teeth, mouth and buccal mucosa. There is also irritation of the oral mucosa, burning sensation and changes in taste perception.[13] Side effects of CHX use are usually proportional to the duration of treatment.[14]

Chlorhexidine (CHX) is available in concentrations of 0.12% and 0.2% which affect plaque inhibition, the plaque inhibitory properties diminishing at lower concentrations.[13] CHX 0.2% is bactericidal and CHX 0.12% is bacteriostatic. The decrease in CHX concentration is to reduce side effects while maintaining the effectiveness

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of the ingredient.[15] CHX 0.2% is effective for preventing plaque and gingivitis.[16]

Cetylpyridinium chloride (CPC) is one of the mouthwash's active ingredients, that is made up of quaternary ammonium compounds, which are known to inhibit the growth of bacteria. CPC can also be used as a treatment for halitosis. CPC with a concentration of 0.05%-0.1% effectively acts as an antimicrobial. A further approach for preventing periodontal disease is to use CPC as an antibacterial ingredient in mouthwash since it is considered to be safe, effective and has no serious adverse effects.[8] CPC can cause extrinsic staining effects but only slightly compared to CHX mouthwash, because CPC is available in preparation alcohol-free, so the side effects that occur are less than CHX and more beneficial for all individuals.[16]

There is a combination of CHX and CPC mouthwash to eliminate the side effects of CHX and be more effective in inhibiting the growth of *Porphyromonas gingivalis* bacteria than using CHX mouth wash alone. This research aims to compare the effectiveness of CHX 0.12% and CPC 0.1% mouthwash with CHX 0.2% against *Porphyromonas gingivalis.*

MATERIALS AND METHODS

This field-experimental research examined the effects of different treatments on research subjects. The Randomized Controlled Clinical Trials with a pretest-posttest control group design was used.

This research took place in Tresna Werda Budi Mulia 1 Nursing Home, Jakarta in March 2022. Elderly aged 45-90 years were the population of this research, from which 30 samples were selected. This research is preliminary research done to obtain initial data regarding the effect of black tea candy in increasing saliva volume. Since this research was carried out during the COVID-19 pandemic, the researchers were restricted to including more samples and had to strictly apply safe saliva draw technique.

The inclusion criteria included the people aged 45 - 90 years, did not use drugs and therapies that affect saliva, did not suffer from diabetes, rheumatoid arthritis (RA), HIV, or Sjogren's syndrome, and were willing to sign a letter of consent as research subjects. Meanwhile, the exclusion criteria included subjects who did not consent and were not cooperative during the sampling. A simple random sampling technique was performed to select 30 samples. The independent variables of this study were the SXI score and treatments with 0.2% black tea jelly candy, jelly candy without black tea, and control without treatment, while the dependent variable was saliva volume. This research has received ethical approval from the ethics committee of YARSI University with registration number: 017/KEP-UY/BIA/I/2022.

The ingredients of black tea jelly candy were black tea, water, sucrose, distilled water, tamarind salt, and beef gelatin. These ingredients are mixed, then heated and then left at room temperature so that candy with the consistency of jelly will be formed. The procedure for collecting saliva at the baseline for the control group was to collect unstimulated saliva in a measuring tube. After 5 minutes, subjects gargled in distilled water before their saliva was collected again. As for the black tea treatment group, before consuming the jelly candy without tea or in the black tea jelly candy, subjects were instructed to slightly bow their heads during saliva collection. Saliva collection was carried out for 5 minutes with the interval of spitting into the saliva container once every 1 minute. The saliva volume in the containers was measured. Then the treatment group was instructed to chew jelly candy for approximately 5 minutes. During the saliva collection in the post-intervention examination after consuming black tea and non-black tea jelly candies, subjects were instructed to slightly bow their heads slightly. Saliva collection was carried out for 5 minutes with the interval of spitting into the saliva container once every 1 minute. The volume of saliva in the container was measured

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according to the number printed on the container.

Every subject was required to answer the SXI questionnaire which consisted of 5 questions that had been validated to determine the prevalence of xerostomia. Each question is answered in a 3-point Likert scale expressing never = 1; sometimes = 2; and often = 3. All the answers were summarized with a total score ranging between 5-15. The total score was then categorized into categories of normal or no xerostomia complaints (5-7); mild (8-10); moderate (11-13); and severe complaints (greater than 13).[11] The data were then statistically analyzed in Shapiro Wilk normality test, dependent T-Test, Wilcoxon and Oneway ANOVA using SPSS program.

RESULTS

The results of measuring the diameter of the inhibitory zone for mouthwash containing CHX 0.12% and CPC 0.1%, CHX 0.2%, and distilled water against *Porphyromonas gingivalis* can be seen in table 1.

Table 1. Results of Measurement of Inhibitory Zone Diameter

Inhibitory Zone Diameter	Treatment		
	CHX 0.12% dan CPC 0.1% (mm)	CHX 0.2% (mm)	Negative control
1	11.57	14.55	0.00
2	10.80	12.1	0.00
3	11.05	13.42	0.00
4	11.35	12.82	0.00
5	11.15	12.65	0.00
6	11.52	12.02	0.00
7	9.7	12.05	0.00
8	10.85	13.6	0.00
9	10.72	11.05	0.00
Mean (mm)	10.97	12.7	0

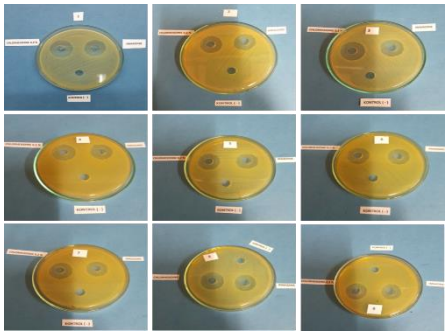

Figure 1. The results of observation of the inhibitory zone diameter of CHX 0.12% and CPC 0.1%, CHX 0.2%, and control negative against *P. gingivalis*

Based on research, the largest diameter of the inhibition zone was in the CHX 0.2% mouthwash treatment, which was 12.7 mm. The next diameter of inhibition zone is in the mouthwash treatment containing CHX 0.12% and CPC 0.1%, which was 10.97 mm. While in the negative control treatment, no inhibition zone was developed or the inhibition zone was zero. Based on the classification of inhibition, the result of measurement of the inhibitory zone diameter for CHX 0.12% and CPC 0.1% and CHX 0.2% included in the strong category (Table 2).

Table 2. Classification of inhibition zones of CHX 0.12% and CPC 0.1% and CHX 0.2% against *P. gingivalis*

Treatment	Diameter mean (mm)	Classification of Inhibition Zone
CHX 0.12% dan CPC 0.1%	10.97	Strong
CHX 0.2%	12.7	Strong

The normality test was done using *Shapiro-Wilk* because the samples were less than 50. The normality test results for CHX 0.12% and CPC 0.1% showed a significance value of 0.138 and for CHX 0.2% showed significance value of 0.905. The data results show a p-value>0.05, it can be conclude that the data is normally distributed, then it can be continued with analysis statistic using *One Way ANOVA* test. After normality test, continued with homogeneity test using the *Levene* test to find out whether the data is homogeneous or not. The homogeneity test results show a significance value of 0.002 (p-value <0.05) so it can be assumed that the data is not homogeneous.

The results of *One Way ANOVA* test showed a significance value of 0.000 where p-value<0.05, means there were significant differences in the three treatments given. The non-homogeneous data was continued with *Post Hoc* test using the *T-test* to determine which treatment was the most significant. *Post Hoc* test results showed p-value <0.05. It can be

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concluded that CHX 0.12% and CPC 0.12%, CHX 0.2%, and distilled water were significantly difference in inhibiting *Porphyromonas gingivalis.*

DISCUSSION

This research used well diffusion method to see the difference in effectiveness between mouthwash containing CHX 0.12% and CPC 0.1% with CHX 0.2%. The larger the clear zone formed around the hole, the higher the inhibition. From the results of the inhibition zone measurements, the largest average of inhibitory zone's diameter is on mouthwash containing CHX 0.2%. This shows that CHX 0.2% is the most effective mouthwash in inhibiting *Porphyromonas gingivalis* compared to mouthwash containing CHX 0.12% and CPC 0.1%.

Based on the research by Betadion R, *et al*., chlorhexidine has the strongest antibacterial effect against *Porphyromonas gingivalis*, so CHX is used as the gold standard and often as a positive control for antibacterial examination of other materials.[18] CHX is considered as gold standard for antimicrobial mouthwash due to its proven on long-term of effectiveness. However, due to the side effects of CHX such as staining/discoloration of teeth and oral mucosa, unpleasant taste, and alcohol content, certain individuals cannot use CHX and its can only be used in the short term.

The mechanism action of CHX as an antibacterial is described with the damage to bacterial cell membrane and leakage of cytoplasmic components. The positive charge (cations) in CHX attach the negative charge of bacterial molecules (anions). This will cause changes in the cell membrane which will disrupt the permeability of bacterial cell wall, then release the intercellular fluid or leakage of components resulting in cell death. The higher the concentration of CHX, the greater the damage to the bacterial cell membrane that occurs. At low and high concentrations, CHX is bacteriostatic (inhibits bacterial growth) and bactericidal (kills bacteria).[18]

CPC plays role in antimicrobial activity on supragingival plaque bacteria.[19] Several studies examining the combination efficacy of CPC and CHX and showed a decrease result in plaque levels and numbers of bacteria, as well as bleeding on probing (BOP) scores. The mouthwash combination of CPC and CHX may be effective for long-term use, due to the lower concentrations of CHX. It is hoped that this combination of mouthwash can maintain its effectiveness by minimizing the side effects that will occur.

The mechanism action of CPC as an antibacterial by increasing interaction with bacterial cells and causing damage to the cell membrane resulting in leakage of cytoplasmic components, metabolic disorders and eventually cause death cell of bacteria. At low concentrations, CPC affects cells by disrupting osmoregulation and homeostasis. At high concentrations, CPC cause membrane damage and leakage of cytoplasmic components. CPC can also inhibit the synthesis of glucans that it can inhibit the formation of biofilms.[20]

Based on research by Stela Lima F, *et al*., there are limitations to the use of CPC, where the *Porphyromonas gingivalis* only decreases with the use of CHX. Levels of periodontopathogens in CPC-treated biofilms were statistically similar to untreated biofilms. CPC only reduced 50% levels of *Porphyromonas gingivalis*.[21] In addition, the concentration of CHX in CHX 0.2% was higher compared to the combination of CHX 0.12% and CPC 0.1%. These things allow the use of CHX 0.2% to be better than CHX 0.12% and CPC 0.1% against *Porphyromonas gingivalis.*

There are various other factors that affect the results of the differences in the inhibition zones formed. Factors that can affect the inhibition zone include the sensitivity of the organism, incubation temperature, incubation time, turbidity of the bacterial suspension, and thickness of the agar medium. The incubation

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temperature must be done at 37°C and for 24 hours, temperatures less than 37°C can cause a larger inhibition zone diameter. In bacterial suspension turbidity, the diameter of inhibition zone will be larger if the suspension is not more turbid than the turbidity of standard McFarland 0.5, and will be smaller if the suspension is more turbid. Less thickness of agar media can cause the diffusion process to be faster and vice versa.[22]

CONCLUSION

Based on the results of the research, it can be concluded that CHX 0.2% mouthwash has greater inhibitory zone than CHX 0.12% and CPC 0.1% mouthwash against *Porphyromonas gingivalis*. There is a significant difference between the effectiveness of mouthwash containing CHX 0.12% and CPC 0.1% with CHX 0.2% mouthwash in inhibiting the growth of *Porphyromonas gingivalis*.

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