



Streptococcus Mutans Colonies on Plaque of Fixed Orthodontic Users after Gargling with Black Tea (*Camellia Sinensis*)

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ABSTRACT

Background and aim: Black tea contains catechin compounds that inhibit bacterial attachment to the enamel. One of the caries-causing pathogenic microbes found in the plaque of fixed orthodontic users is *Streptococcus mutans*. This study aims at determining the difference in the number of *Streptococcus Mutans* colonies in the plaque of fixed orthodontic users after being given black tea.

Material and methods: It is an experimental laboratory study with a pretest-posttest control group design. The sample consisted of 12 YARSI University students using fixed orthodontics, divided into two groups. The intervention group was given 2% black tea as a mouthwash, and the control group was only given distilled water for gargling. Samples in the form of plaque smears on several parts of the teeth were taken the day before treatment, on day 14, and on day 21 to see the number of bacterial colonies by direct counting method at YARSI University Microbiology Laboratory. Research samples were also calculated as plaque index before and after gargling.

Results: This study showed a significant decrease in treatment colonies. The p-value = 0.0001 was obtained. It shows a significant difference in 2% black tea on the growth of *Streptococcus mutans* bacteria. There was also a significant difference in score plaque index before and after gargling, $p = 0.004$ ($p < 0.05$).

Conclusions: Gargling with black tea effectively reduces the number of *Streptococcus Mutans* in fixed orthodontic users.

1. Introduction

Orthodontic treatment, especially for fixed orthodontic appliance users, can impact changes in the oral environment and the composition of the oral flora and increase the number of bacteria that can cause dental caries. It is caused by the difficulty of the patient's oral hygiene procedures. Increasingly complex oral hygiene procedures result in forming a layer that sticks tightly to the surface of the teeth containing bacteria, known as plaque. Dental plaque can be defined as a soft deposit that forms a biofilm and sticks to the surface of the teeth or other hard surfaces of the oral cavity. Dental plaque consists of various kinds of microorganisms.^[1] The presence of orthodontic appliance components attached to the tooth surface, such as brackets, archwires, and elastics, creates a new retention area that can increase the accumulation of *Streptococcus mutans* bacteria, the primary bacterial strain causing dental caries. *Streptococcus Mutans* Colonies are found in 40-85% of patients using fixed orthodontics. There is a close relationship between the number of colonies of *Streptococcus Mutans* Colonies bacteria and the prevalence of dental caries. One of the efforts to prevent caries is to reduce the number of cariogenic bacteria.^[2] There are many ways to reduce the number of bacterial colonies in the oral cavity. One of them is by using mouthwash. The use of

tea, especially black tea, is widespread in Indonesia. Tea contains polyphenols known as catechins, namely epicatechin (EC), epicatechin 3-gallate (ECG), and epigallocatechin gallate (EGCG). These compounds have antioxidant, anti-mutagenic, anti-carcinogenic, hypocholesterolemic, and antibacterial effects. Catechin compounds have antibacterial effects and can reduce inflammation by inhibiting bacterial growth and reducing the concentration of bacteria in dental plaque.^[3] Another study on the effect of gargling black tea solution on plaque accumulation also showed significant results, such as reducing the amount of dental plaque.^[4] This study aims to determine the difference in the number of *Streptococcus Mutans* Colonies in the plaque of fixed orthodontic users after being given black tea.

2. Material and methods

It is an analytical study with an experimental laboratory design. This study used a single blinding method. Therefore, the researcher did not know which treatment and control groups were. The study sample was taken using a simple random sampling method so that the number of samples obtained was 12 people, six for the black tea group and six for the aquadest group. The YARSI University ethics committee team has approved this study with

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registration number:368/KEP-UY/BIA/XII/2017. The data was collected by filling in informed consent to YARSI University students using fixed orthodontics who were willing to be respondents. After obtaining informed consent, the researchers conducted a baseline plaque index examination using the Loe and Silness method and initial sampling. A plaque index examination was conducted in the dental unit of YARSI University, and the measurement of number of colonies. Streptococcus Mutans was conducted at the YARSI University Microbiology Laboratory.

Samples in the form of smears on several parts of the teeth were taken the day before treatment, on day 14, and on day 21 to see the number of bacterial colonies by direct counting method in the Microbiology laboratory of YARSI University. Sampling used a relatively easy swab method by wiping a sterile cotton swab on the buccal and labial surfaces of the maxillary and mandibular anterior teeth and all of the first molars. Streptococcus mutans bacteria used in this study were cultured directly from the dental plaque source. TYS20B was used for culturing because it can separate Streptococcus mutans from other bacteria. Bacterial colonies will grow optimally in TYS20B after being put in an incubator for 2x24 hours at 37oC. After being cultured and gram staining was carried out, the number of colonies was calculated using the CFU/ml method with OpenCFU software. The gargling activity was carried out for 21 days in the morning and evening with the researcher's steeping 2% black tea solution. The subjects should gargle with

10 ml of liquid for 1 minute and were not allowed to eat or drink except water for 1 hour after gargling. The gargling activity was carried out for 21 days in the morning and evening with a 2% black tea steeping solution made by the researcher. Subjects had to rinse their mouth with 10 ml of liquid for 1 minute and were not allowed to eat or drink except water for 1 hour after gargling. Plaque index examination was carried out before and after gargling, while the number of Streptococcus mutans colonies was measured weekly. Data processing in this study used the SPSS program, with the Shapiro-Wilk normality test, Independent T-test, and Two Way ANOVA test.

3. Results

The research was conducted at YARSI University and YARSI University Microbiology Laboratory from December 6 to December 20, 2017. The subjects of this study were YARSI University students using fixed orthodontics. Those students had met the inclusion criteria totaling 12 people. Six people were in the black tea group, and six were in the aquadest group. The Independent T-test results showed no significant difference between the black tea group and the distilled water group in reducing the number of Streptococcus mutans colonies in the second week (p>0.05) (Table 1). However, there was a significant difference between the black tea and distilled water groups in reducing the number of Streptococcus mutans colonies in the third week (p<0.05).

Table 1. Independent T-test results of the number of colonies.

	Inspection	Group(Average±SD) (CFU/ml)		P-value	95% CI
		Black Tea	Aquadest		
Number of colonies	Colony number Δm2- baseline	-613±641	100±800	0.23	-1308-119
	Colony number Δm3- baseline	-1022±800	164±286	0.007	-1960-413

Table 2 shows the results of the plaque index significance test in week 2, p-value = 0.27 (p>0.05), meaning that there is no significant difference between the 2% black tea group and the distilled water group. The significance of the plaque index in week three was p-value = 0.07 (p>0.05),

meaning there was no significant difference between the 2% black tea group and the aquadest solution group. The number of colonies in each group was measured using the Two Way ANOVA parametric test.

Table 2. Results of independent T-test dental plaque index score.

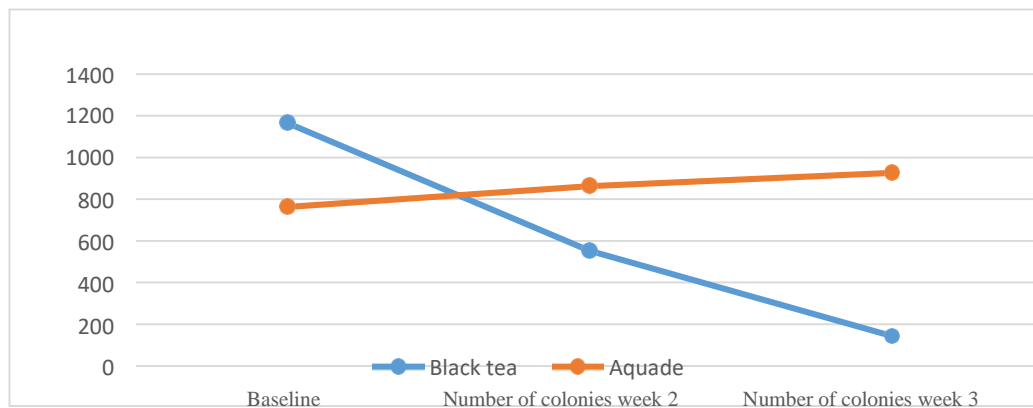
	Inspection	Group(Average±SD) (CFU/ml)		P-value	95% CI
		Black Tea	Aquadest		
Plaque Index	Δ Plaque after - before	-0.235±0.13	-0.2±0.06	0.004	-0,35-(-0,08)

The two-way ANOVA test results showed a significant difference in the number of colonies, p-value = 0.001 (p <0.05), at three measurement times in the 2% black tea group between baseline, week two week three

(Table 3). There was no significant difference in the number of colonies in the distilled water group, p-value = 0.373 (p>0.05), between baseline, week two, and week three (Fig. 1).

Table 3. Two-way ANOVA test results number of colonies.

Number of Colony	Group			
	Black tea		Aquadest	
	Mean±SD	P-value	Mean±SD	P-value
Colony baseline	1165.8±912.1	0.0001	762.8±328.7	0.373
Colony week 2	552±389.2		863.1±365.5	
Colony week 3	143.1±132.2		927±543.2	

**Fig. 1. Compare the number of colonies at baseline, week two, and week three.**

The number of colonies between the 2% black tea group and the aquadest solution can be seen in the figure, which shows that the number of colonies tends to decrease in the black tea group in weeks two and three. Then to find out the difference between the number of colonies in each group, the Post Hoc

Test was carried out. The Post Hoc test results showed a significant difference, $p\text{-value} = 0.0001$ ($p < 0.05$), in the 2% black tea group between the baseline compared to week two, the baseline compared to week three, and week two compared to week three (Table 4).

Table 4. Significance of the post-hoc two-way ANOVA test results on the number of colonies in the 2% black tea group.

	Week 2	Week 3
Baseline	0.0001	0.0001
Week 2	-----	0.0001

4. Discussion

The Independent T-test results showed no significant difference between the black tea group and the distilled water group in reducing the number of Streptococcus mutans colonies in week two ($p > 0.05$). In comparison, there was a significant difference between the black tea 2% with a solution distilled water group in reducing the number of Streptococcus mutans colonies in week three ($p < 0.05$). It is due to the chemical content of the phenol group in tea in the form of catechins. Catechins consist of four major groups, epigallocatechin-3-gallate (EGCG), epigallocatechin (EGC), epicatechin-3-gallate (ECG), and epicatechin (EC). Catechins also have many benefits.^[5] Previous studies stated that green tea catechins keep the oral cavity's pH normal, thus creating unfavorable conditions for Streptococcus Mutans to colonize and multiply.^[6] This study's results align with previous studies that there was a decrease in the number of Streptococcus mutans after drinking black tea. Therefore the oral cavity could be protected from caries-causing bacteria.^[6-8] This study is also in line with research on the decrease in

Streptococcus mutans colonies on the plaque after gargling with 2% green tea because the catechins contents function as bacteriostatic and bactericidal against Streptococcus Mutans.^[9, 10] Catechins work by inhibiting the activity of the glucosyltransferase enzyme so that acid formation is inhibited. Catechins can damage bacterial cell walls and cytoplasmic membranes and cause protein denaturation.^[11] The results of this study contradict the study conducted by Ramadhan et al., 2019 which stated that black tea did not significantly affect cariogenic bacteria. However, in this study, the measurement of bacteria was done one hour after the subjects were asked to drink black tea, not gargle.^[12] The method used in this study to calculate the number of colonies has a drawback. The drawback was the density of the number of colonies that were too piled up; thus, the results could not be determined when the researchers observed. It then caused the researcher to use openCFU software. The Independent T-test results showed a significant difference between 2% black tea and aquadest solution in reducing plaque index ($p < 0.05$). It is in line with a previous study that stated a decrease in

plaque accumulation after gargling with black tea. The polyphenols in black tea caused a decrease in plaque. It inhibited glucosyltransferases and prevented the formation of the matrix material used by a plaque to stick to the surface of the tooth enamel.^[13] This study aligns with previous studies regarding decreasing dental plaque index after gargling with black tea. It is due to the antibacterial effect produced by polyphenols in black tea.⁴ This study is also in line with the conclusions from the meta-analysis stating that there is a decrease in plaque index after gargling with green tea solution.^[14]

5. Conclusion

The study results showed a significant difference in the number of *Streptococcus mutans* colonies p -value=0.001 ($p<0.05$) in the 2% black tea group for three weeks. In addition, there was no significant difference in plaque index before and after gargling black tea at 2% for three weeks. Gargling with black tea effectively reduced the number of *Streptococcus mutans* colonies in the plaque of fixed orthodontic users. Our suggestion for future study is needed on the effectiveness of gargling black tea on reducing *Streptococcus mutans* colonies by considering specific time and concentrations.

Conflict of Interest

The authors declared that there is no conflict of interest.

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