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ORIGINAL ARTICLE

Effectiveness of 2% Black Tea (*Camellia sinensis*) Infusion in Increasing Salivary pH and Fluoride in Children

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ABSTRACT

Black tea is the most commonly consumed tea in Indonesia. It has antibacterial properties and can inhibit the attachment of *Streptococcus mutans* to tooth enamel. **Objective:** This study, conducted for 1 month, aimed to compare the effectiveness of a 2% black tea infusion with that of 0.2% fluoride solution mouth rinse, used as a positive control, in increasing salivary pH and fluoride levels in children aged 7–8 years. **Methods:** This was an experimental study with a double-blind randomized controlled trial design. The participants comprised 28 first-grade students of a state elementary school, who were selected using simple random sampling technique. Salivary pH was measured using a digital pH meter, and salivary fluoride levels were measured using TECAN Infinite M200 Pro® UV-VIS spectrophotometer. **Results:** An independent t-test revealed no significant differences between the groups of students who rinsed their mouths using 2% black tea infusion and those who rinsed using 0.2% fluoride solution mouth rinse ($p < 0.05$). **Conclusions:** Mouth rinsing with a 2% black tea infusion is equally effective as rinsing with a 0.2% fluoride solution in increasing salivary pH and fluoride levels.

Key words: black tea; fluoride; mouth rinse; pH

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INTRODUCTION

The number of dental and oral problems experienced by Indonesians, particularly the incidence of dental caries in children, remains high. The eruption of permanent teeth, particularly that of the incisors and molars, initiating at the age of 6 years, occurs in primary school-age children. At this age, the awareness and ability of children regarding dental hygiene and health maintenance are low, making their permanent teeth prone to caries. Previous studies have shown that the average prevalence of dental caries in children in Indonesia reaches 90%.[1,2] Therefore, prevention of caries are crucial at this age, and there is a need for further research to develop safe and effective oral hygiene aids that can help prevent caries in children.

To improve dental and oral health in children, the Indonesian government has launched the School Dental Health Program, which is a public health effort aimed at maintaining and improving the dental and oral health of students in target schools.[3] Some of the dental health programs that have been running include implementation of daily tooth brushing with a fluoride-containing toothpaste, guided by a teacher, at least for children in grades I–III.[4] This activity has been implemented by the government in target schools; however, the proportion of children with caries remains high.

An easy and cost-effective way to improve the oral health of children is to establish a routine in which they rinse their mouth with a fluoride-containing solution. Rinsing with 0.2% NaF solution every week has been shown to result in a decrease in caries incidence by 15–65.6% and rinsing with a 0.05% NaF solution every day has been shown to result in a decrease in caries incidence by >48%–67%.[5] The use of herbal tea, particularly black tea, is common in Indonesia but it is

not used as a mouthwash. Tea contains catechin, which is known to have antibacterial effects and inhibit the attachment of *Streptococcus mutans*.[6] The anti-caries effect of tea is a bactericidal effect against *S. mutans* and *S. sobrinus*. It prevents bacterial attachment to teeth by inhibiting glucosyltransferase and bacterial amylase formation.[7]

Black tea not only inhibits the growth of *S. mutans*, it also contains fluoride.[8] However, little is known about the effect of black tea on salivary fluoride levels and pH during and after use as a mouth rinse. This study aimed to determine whether rinsing with a 2% infusion of black tea was effective in preventing caries compared with controls using 0.2% fluoride solution, which was evaluated by changes in salivary pH and fluoride levels.

METHODS

This study was designed as a randomized, double-blind controlled trial with a pre-test-post-test control group. To determine the research location, a purposive sampling technique was used. Furthermore, simple random sampling was used to determine the samples of children in the classroom. Informed consent was provided by the children's parents. Ethical clearance was obtained from the Ethical Committee of Universitas YARSI. Informed consent was obtained from the subjects prior to the study.

The subjects of the study were first-grade students of SDN 012 Pagi Sumur Batu who met the following inclusion criteria: grade I students aged 7–8 years, no systemic disease, no orthodontic treatment or prosthodontics (fixed or removable), and no previous habit of rinsing with tea or other mouthwashes. The exclusion criteria were as follows: use of topical fluoride treatment, taking medication in the last 3 months, and not being cooperative at the time of the study.

A total of 31 children participated in this study; 28 of the children met the inclusion criteria, one child was excluded due to having autism syndrome, and two children were not cooperative during the study. Therefore, the final number of participants was 28 children, consisting of 12 boys and 16 girls.

A 2% black tea solution was prepared as reported in a previous study.[9] Briefly, 6 g of black tea was added to 300 ml of boiled water and heated for 15 min. The brewed tea was then filtered and stored in a sterilized plastic bottle. Furthermore, 0.2% fluoride solution was produced by grinding a 1-mg fluoride tablet, from which 0.4 g was weighed out and dissolved in 200 ml of distilled water. The resulting 0.2% fluoride solution obtained was used as a positive control.[10,11]

During the first visit, scaling was performed on all research subjects. The saliva sampling process was performed 2 days after scaling. Before collecting the saliva sample, the subjects were instructed to rinse their mouths with 10 ml of either the 2% black tea infusion or 0.2% fluoride solution for 1 min. The subjects were also instructed not to eat, drink, or rinse their mouth with anything except water for 1 h before and after rinsing. To collect the saliva sample into a tube, the subjects were asked to chew paraffin wax for 10–15 min until 5 ml of saliva was obtained. The measurement of salivary pH was performed using a digital pH meter (Royal Scientific-Haryana, India). The collected saliva samples were placed in a cooling box containing ice gel. Subsequently, the samples were taken to the YARSI University Herbal Laboratory. The salivary fluoride levels were measured using the TECAN Infinite M200 Pro® UV-VIS spectrophotometer. The collection of saliva and examination of pH and fluoride levels were performed three times, namely at baseline, in the first 2 weeks, and the second 2 weeks.

The data then were analyzed using the Shapiro-Wilk normality test, followed by the homogeneity independent t-test and two-way ANOVA.

RESULTS

Table 1 shows the results of independent t-test of salivary pH between the 2% black tea infusion group and 0.2% fluoride solution group. The significance of the difference in salivary pH in the first 2 weeks was $p = 0.342$ ($p > 0.05$), indicating no significant difference between the 2% black tea infusion group and the 0.2% fluoride solution group. In addition, the significance of the difference between salivary pH in the second 2 week and baseline was $p = 0.241$ ($p > 0.05$), indicating there was also no significant difference between the 2% black tea infusion group and 0.2% fluoride solution positive control group.

Table 2 shows the results of the independent t-test of salivary fluoride levels of the 2% black tea infusion group and 0.2% fluoride solution. The significance of the difference in salivary fluoride levels in the first 2 weeks and baseline was $p = 0.422$ ($p > 0.05$), indicating no significant difference between the 2% black tea infusion group and the 0.2% fluoride solution group. In addition, the significance of the difference between salivary fluoride levels in the second 2 weeks and baseline was $p = 0.507$ ($p > 0.05$), indicating no significant difference between the 2% black tea infusion group and the 0.2% fluoride solution group.

Table 3 shows the differences in the mean salivary pH measurements at baseline, in the first 2 weeks, and in the second 2 weeks. The results of two-way ANOVA showed a significant difference in salivary pH at these three measurement time-points for both the 2% black tea infusion group and the 0.2% fluoride solution group. The p-value was 0.001 ($p < 0.05$).

Table 1. Test results of independent t-test for salivary pH levels

	Checks	Group (mean ± SD)		P-value	95% CI
		Black tea	Fluor-2M1		
Salivary pH	ΔpH first two weeks-baseline	4.77 ± 0.06	4.75 ± 0.69	0.342	−0.02 to -0.07
	ΔpH second two weeks−baseline	4.94 ± 0.08	4.90 ± 0.06	0.241	−0.02 to - 0.09

Table 2. Test results of the independent t-test for salivary fluoride levels

	Checks	Group (mean ± SD)		P-value	95% CI
		Black tea	Fluor		
Salivary pH	Δ salivary fluoride level first two weeks-baseline	−31.11 ±8.27	−33.69 ± 8.45	0.422	−3.92 to -9.09
	Δ salivary fluoride level second two weeks−baseline	−21.68 ± 8.25	−23.78 ± 8.23	0.507	−4.32 to-8.52

Table 3. Results of two-way ANOVA for salivary pH

Salivary pH	Group			
	Black tea		Fluor	
	Mean ± SD	P-value	Mean ± SD	P-value
Baseline	7.27 ± 0.13	0.001	7.39 ± 0.07	0.001
First 2 weeks	7.56 ± 0.07		7.56 ± 0.07	
Second 2 weeks	7.72 ± 0.07		7.71 ± 0.05	

Table 4. Results of two-way ANOVA for salivary fluoride levels

Salivary fluoride	Group			
	Black tea		Fluor	
	Mean ± SD	P-value	Mean ± SD	P-value
Baseline	5.60 ± 0.75	0.001	5.83 ± 0.76	0.001
First 2 weeks	7.01 ± 0.80		7.95 ± 0.41	
Second 2 weeks	10.27 ± 0.93		10.81 ± 1.51	

Table 4 shows the difference in the average salivary fluoride levels at baseline, in the first 2 weeks, and in the second 2 weeks. The results of the two-way ANOVA in Table 4 show a significant difference in salivary fluoride levels at the three measurement time-points for the 2% black tea infusion group and the 0.2% fluoride solution group with a p-value of 0.001 ($p < 0.05$).

DISCUSSION

The results of this study showed that there were no significant differences between 2% black tea and 0.2% fluoride solution in their ability to increase salivary pH ($p > 0.05$). Therefore, rinsing with 2% black tea was likely to have the same effect as rinsing with 0.2% fluoride solution (as a positive control) in increasing salivary pH and preventing caries due to the existence of phenol in tea in the form of catechins.[12,13] Green tea consists of five major groups, namely epigallocatechin-gallate, epigallocatechin, epicatechin-gallate, epicatechin, and gallocatechin, which can maintain salivary pH in the normal range. It was stated that green tea can inhibit acid formation and thus the growth and the glucosyltransferase activity of *S. mutans* and bacteria in plaque. Previous studies have also shown that black tea can significantly reduce plaque, it helps to prevent the decrease in salivary pH following glucose consumption and increases the rate of salivary pH increase.[14,15]

The results of the present study were in accordance with a study by Shalal, in which there was an increase in salivary pH following drinking black tea, which may protect the oral cavity from caries-causing bacteria.[16] The present study is also in line with a study by Rosma and Aritonang, which reported an increase in salivary pH following rinsing with green tea among fifth-grade elementary school students. This occurred due to chemical and mechanical stimuli from rinsing with green tea.[17] According to a study by Amalia et al., there was a significant increase in salivary pH after rinsing with white tea as the catechin has bacteriostatic and bactericidal effects against *S. mutans*. Catechin functions by inhibiting the activity of glucosyltransferase enzymes, inhibiting acid formation in the oral environment.[18] Catechin can also damage bacterial cell walls and cytoplasmic membranes and cause protein denaturation. Reports on the effects of black tea in increasing salivary pH remain limited. The majority of previous studies [17,18] examined other types of tea, such as green tea and white tea, as their ingredients are the same. Srinidhi et al. showed that rinsing with black tea or green tea increased salivary pH, although rinsing with green tea resulted in a marginally higher pH.[19]

The acidity (pH) of unstimulated saliva is usually slightly acidic and hypotonicwith values between 5.7 and 6.2.[20] In the present study, the salivary pH at baseline was neutral, but there was a significant increase in the pH following rinsing with black tea. This may be influenced by the chemical stimulation of catechins in the black tea, the mechanical stimulation from rinsing, and the mechanical stimuli from chewing paraffin wax prior to the collection of saliva samples.[12] The salivary pH can be affected by the presence of a stimulus. Chemical stimuli increase salivary pH more than mechanical stimuli. The longer the duration of the stimulus, the more the rise in salivary pH.

The results of the independent t-test (Table 2) showed that there were also no significant differences between the 2% black tea infusion and 0.2% fluoride solution in their ability to increase salivary fluoride levels ($p > 0.05$). Saliva helps prevent caries by drawing fluoride ions, which enter the enamel and help protect it against acid.[16] Fluoride can increase the resistance of enamel by forming fluorapatite bonds in the enamel, resulting in remineralization.[21,22]

The results of the present study are in accordance with those of a study by Suyama et al. on the increase in salivary fluoride levels following chewing gum made from green tea. This led to remineralization and increased the resistance of enamel to acids.[23] Another study by Nasiri and Domang (2014) investigated the increasing levels of fluoride in saliva after drinking tea in 10 subjects aged 22–26 years. The results of this study revealed that there was an increase in fluoride levels in saliva for up to 10 min after black tea intake (0.04 ± 0.2 vs. 0.97 ± 0.32 mg/L, $p < 0.01$). Fluoride levels remained elevated compared with those at baseline at 5 min (0.04 ± 0.02 vs. 0.12 ± 0.08 mg/L, $p < 0.01$) and 10 min (0.04 ± 0.02 vs. 0.09 ± 0.07 mg/L, $p < 0.05$) but not at 20 min (0.04 ± 0.02 vs. 0.05 ± 0.03 mg/L, $p = 0.056$).[23] However, evidence on the effectiveness of rinsing with black tea infusion to increase salivary fluoride level remains limited. Previous investigations have used green tea as the ingredients contained are the same.[24]

In the present study, the salivary fluoride levels were high. Although the reason for this is debated, it may be that the method used for collecting samples affected the salivary fluoride levels.[18] Saliva samples were collected by chewing paraffin wax for 10–15 min until 5 ml was obtained. A minimum sample of 5 ml was required to measure salivary fluoride levels. Therefore, there were deposits in the collected samples that affected the salivary fluoride levels, it should use an unstimulated salivary flow rate between 0.2 and 0.7 mL / min to minimize errors due to differences in salivary flow rates.[25] The turbid or concentrated solution affected the absorption value by spectrophotometry. The absorption value was proportional to the fluoride concentration in the test substance measured using the UV-VIS spectrophotometer.[26] The advantage of this study was that it was performed for 1 month with children aged 7–8 years using black tea infusion. Therefore, the results are expected to be used in a caries prevention program.

CONCLUSION

Based on the results of the study, it was concluded that rinsing with 2% black tea infusion was likely to have the same effect as rinsing with 0.2% fluoride solution, the gold standard for increasing salivary pH and fluoride levels. Certain factors need to be considered when using the unstimulated saliva sample collection method in order to obtain a more accurate pH change value and to minimize the bias toward absorption (absorbance) by spectrophotometry. It is also suggested that the absorbance values of 2% black tea infusion and 0.2% fluoride solution are calculated several times to obtain significant data results. Additionally, further investigations can be performed on the effectiveness of 2% black tea rinsing on salivary pH and fluoride levels with unstimulated salivary samples, which can be performed in minutes with a study duration of at least 6 months, to observe changes in dental caries in the subjects.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest related to the research

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