Original Article

Comparative Evaluation of Efficacy of Green Tea Mouth Rinse and Green Tea Gel on the Salivary *Streptococcus mutans* and *Lactobacillus* Colony Count in 12–18-year-old Teenagers: A Randomized Clinical Trial

Maryam Haji Ahmadi, Leila Sarrami[1], Afsaaneh Yegdaneh[2], Alireza Homayoni[3], Zohreh Bakhtiyari[4], Nasrin Danaeifar[5], Najmeh Akhlaghi

Department of Pediatric Dentistry, Dental Research Center, Isfahan University of Medical Sciences, [1]General Dentist, Isfahan University of Medical Sciences, Isfahan, Iran, [2]Department of Pharmacognosy, Faculty of Pharmacy, Pharmacology Research Center, Isfahan University of Medical Sciences, [3]Department of Pharmaceutics, Faculty of Pharmacy, Research Center for Advanced Pharmaceutical Systems, Isfahan University of Medical Sciences, [4]Department of Pharmacy, Pharmacognosy Laboratory, Faculty of Pharmacy, Isfahan University of Medical Sciences, Isfahan, Iran, [5]Pediatric Dentistry Resident, Louisiana State University, New Orleans, Louisiana, United States

Address for correspondence:
Najmeh Akhlaghi, DDS, MS, Hezar Jarib Ave, Tadbir Building, Isfahan University of Medical Sciences, Pediatric Dentistry Department, Isfahan, Iran.
Email: najme.a2008@yahoo.com

Abstract

Aims: Green tea is an antibacterial agent with no significant side effect. This feature makes green tea safe for children to use. The purpose of this study was to compare the effectiveness of green tea gel and mouth rinse on salivary level of *Streptococcus mutans* and *Lactobacillus* of teenagers aged 12–18 years. **Subjects and Methods:** In this randomized controlled clinical trial study, 30 children aged 12–18 years were included in the study according to the inclusion criteria and were randomly divided into two groups. Participants in mouth rinse group were asked to rinse their mouth with 0.5% green tea mouthwash twice a day for 2 weeks. In the gel group, participants were requested to brush their teeth with 0.5% green tea gel twice a day for 2 weeks. After 4 weeks of washout period, mouthwash group applied the gel and the gel group rinsed the mouthwash for 2 weeks and with the same instruction as mentioned. Colony count of *S. mutans* and *Lactobacillus* was determined before and after intervention and data were analyzed using *t*-test. **Results:** According to the independent *t*-test, there was no significant difference in the salivary levels of *S. mutans* before and after intervention regarding age and gender ($P = 0.33$). Results from paired *t*-test showed significant decrease in the mean count of *S. mutans* and *Lactobacillus* colonies in both groups before and after intervention ($P < 0.001$). **Conclusions:** Green tea gel and mouthwash contribute to a significant reduction of salivary levels of *S. mutans* and *Lactobacillus* colonies, with a greater effect of mouthwash than the green tea gel, which was not statistically significant.

Keywords: *Green tea, Lactobacillus, mouthwash, Streptococcus mutans*

Introduction

Dental caries is one of the most prevalent chronic diseases. It results from different factors including tooth anatomy, oral bacterial flora, nutrition, and diet. *Streptococcus mutans* and *Streptococcus sobrinus* are the main bacteria contributing to the development of caries. In addition, *Lactobacillus* and *Actinomyces* may promote the caries process.[1-3] It has been shown that salivary contamination with *S. mutans* is directly related to caries development. Hence, reducing the number of colonies of aforementioned bacteria will reduce the risk of dental caries.[4,5]

Plaque-induced dental caries is categorized as a local disease. Therefore, local application of antimicrobial agents would have higher efficiency than systemic application.[6,7] Green tea is one of the antimicrobial agents which has been highlighted because of its several clinical features.[8,9] It can decrease the rate of cardiovascular diseases,[10] heart attack, stroke,[11] obesity,[12] and cancer[13] development.

The main component of green tea is polyphenols particularly flavonoids as catechins which are responsible for preventing from streptococcal adhesion to tooth surface,[14] indirect antibacterial effect through increasing salivary secretion of immune system components,[6] and reduction of acid production through lactate dehydrogenase inhibition.[15,16]

Plaque control chemical agents have been used in mouth rinses, toothpastes, and gels.[17-19] Gel has been highlighted due to its ease of application and has improved acceptability by children due to similarity with toothpaste regarding shape and way of application.

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The aim of the present study was to compare the effectiveness of green tea mouth rinse with green tea gel on the level of salivary *S. mutans* and *Lactobacillus* in children aged 12–18 years.

Subjects and Methods

The study design was a randomized controlled clinical trial. The study protocol was approved by the Ethical Committee of Isfahan University of Medical Sciences (grant #396170). It was also registered on the Iranian Registry of Clinical Trials (IRCT, www.irct.ir) with the IRCT code of 36552.

In the present study, 30 teenagers (16 girls and 14 boys) aged 12–18 years were selected from patients who were referred to the pediatric clinic of Isfahan Dental School between January and September 2016. The sample size was calculated with the power of 80%, 0.75 difference in average number of bacteria between two groups, and α set at 0.05. Children and their parents were informed verbally and with written information about the study procedure, and informed consent form was signed by the parents.

The inclusion criteria were good oral hygiene (brushing twice a day), no untreated active carious lesion, no existing gingival or periodontal disease, no history of taking systemic antibiotic or fluoride therapy within the last 4 weeks, no regular habit of chewing xylitol-containing gum, drinking tea, coffee, or cocoa, no systemic disease, absence of orthodontic appliances, having no active abscess or drainage, with decayed-missed-filled primary teeth (dmft) of 2 or higher. Participants were asked not to take xylitol-chewing gum, systemic antibiotics, topical fluoride, tea, coffee, and cocoa during the study period and to report any changes in their general health status and medication.

Study design

Participants were randomly divided into two groups regarding the initial *Streptococcus* count; Following saliva sample collection, the *S. mutans* colonies were measured; the names of children with similar bacterial count were written on a piece of paper and mixed together and were randomly assigned into two groups. Information about each participant's health, medication, dental care, and habits was collected at the beginning of the study through a questionnaire and was performed again at the end of the study.

After microbiological assessment, 0.5% green tea mouthwash and 0.5% green tea gel were given to mouthwash and gel group, respectively. Mouthwash was presented in 350 mL bottles and the gel in 250 mL tubes. To make them more attractive for children, containers were labeled with animation figures and direction of use. Participants in the mouthwash group were requested to rinse their mouth after brushing at morning and at night with 20 mL of 0.5% green tea mouth wash, for 60 s, twice a day. After each application, they were asked to prevent from eating or drinking for 1 h. Participants in the gel group also were asked to brush their teeth with 0.5% green tea gel twice a day for 2 min. Saliva samples were gathered 24 h after participants stopped 2 weeks of application of green tea mouthwash and gel, and the level of *S. mutans* and *Lactobacillus* was determined. After 4 weeks of washout period, mouthwash group used the gel and gel group rinsed the mouthwash for the same amount of time and with the same instruction as mentioned. It is also necessary to mention that the participants were given the same tooth brush and fluoride tooth paste to brush their teeth twice a day during the study.

Decayed/missed/filled teeth

An experienced dentist examined subject's oral health according to the WHO criteria[20] and recorded number of dmft and decayed/missed/filled permanent teeth.

Preparation of green tea mouth rinse

1.8 g green tea extract containing 6% phenolic compound was diluted by double-distilled water to 20 mL to produce green tea mouthwash containing 0.5% phenolic compound. Then, 20 g sodium lauryl sulfate was added for transparency and 36 g methyl-paraben and 4 g propyl-paraben were added to stabilize the solution. Finally, edible mint color (0.4 g), mint flavor (20 mL), and aspartame sweetener (80 g) were added to the solution.[21]

Preparation of green tea gel

To produce 7 kg of green tea gel, 140 g of carbopol-934 was dissolved in 7 L of double-distilled water. After 24 h, 20 mL 3-ethanolamine was added to neutralize the acidity. To stabilize the gel, 6.12 g of methyl-paraben and 1.4 g of propyl-paraben were added. Then, 0.7 g of green tea extract was added to the gel. The green tea gel was finalized by adding 28 g aspartame as a sweetener, 140 mg of edible mint color, and 7 mL of mint flavor.

Saliva samples

Saliva sampling was carried out before and after each intervention. As the microbial count in saliva fluctuates during the day,[22] sampling was performed at the same time (between 7.30 and 8.30), 1 h after breakfast, and before brushing. To obtain saliva sample, sterile cotton stick was soaked at the mouth floor for 5 min and transferred to 10 ml sterile tubes immediately. One calibrated operator performed all saliva samplings.

Microbial evaluation

The microbial analysis was performed within 45 min after sample collection. To count *S. mutans*, 20 µl of saliva sample was spread on mitis salivarius agar (Difco) containing 0.2 U/ml bacitracin and sucrose (15% w/v). In addition, to measure the number of lactobacilli colonies, 20 µl of saliva sample was spread on Rogosa agar (Unipath,

Basingstoke, UK). Both groups of plates were incubated anaerobically (85% N_2, 5% CO_2, and 10% H_2) at 37°C for 3 days. The colony-forming units (CFUs) were determined by morphology, size, and color and were counted using a stereomicroscope.

Statistical methods

Appropriate descriptive statistics (mean and standard deviation) were determined for each participant at different evaluations. To analyze the difference between baseline and follow-up values in each group, paired sample *t*-test was used. To test between-group differences, independent sample *t*-test was used. Data analysis was performed using SPSS 11.5 (SPSS Inc., Chicago, IL, USA), with the significance level set at 0.05.

Results

Thirty participants (46.7% boys and 53.3% girls) performed and completed the study. The mean age of participants was 14 ± 1.84 years. Nineteen (63.3%) children were under 15 years and 11 (36.7%) were 15 years or older. The mean age of girls and boys was 13.79 ± 1.85 and 14.19 ± 1.9 years, respectively. According to the independent sample *t*-test, no significant differences was found in the mean age of boys and girls ($P = 0.56$).

The average colony count (CFUs) of the salivary *S. mutans* was reduced in both groups after 2 weeks of intervention, which was statistically significant ($P < 0.001$) based on paired sample *t*-test [Table 1]. Mean reduction in the *S. mutans* colony count in mouth rinse and gel group was 52% ± 25% and 30% ± 14%, respectively.

Repeated measures analysis of variances showed similar changes in the number of *S. mutans* colonies in two groups ($P = 0.33$). In fact, both mouth rinse and gel were effective in decreasing in colony count.

Salivary *S. mutans* colony count decreased by 37% ± 20% in boys and 47% ± 29% in girls [Table 2]. According to the independent sample *t*-test, no significant difference between two genders was found in colony decrease ($P = 0.33$). In addition, no significant difference was shown between two genders within each group.

Table 3 shows the mean change in the number of *S. mutans* colonies in both mouth rinse and gel groups, separated by their age. It must be noted that according to repeated measures analysis of variances, age and gender variables did not have any confounding effect on the changes in colony count.

Two participants had positive *Lactobacillus* test which turned to negative after intervention. The *Lactobacillus* colony count was 8 in one case and 5 in the other participants at the baseline. There was no significant difference in the mean numbers of colonies between two groups ($P = 0.17$).

Table 1: Changes in *Streptococcus mutans* colony count (mean±standard deviation) in each group before and after intervention

	Intervention group		*P*
	Mouthwash	Gel	
Before intervention (CFU/ml)	154.3±86.9	159±86.9	0.33
After intervention (CFU/ml)	75.9±56	108.2±59.4	
P	<0.001	<0.001	

CFU: Colony forming units

Table 2: Changes in *Streptococcus mutans* colony count (mean±standard deviation) in each gender

Gender	Intervention group		*P*
	Mouthwash	Gel	
Male (CFU/ml) %	−0.38±0.25	−0.65±0.28	0.06
Female (CFU/ml) %	−0.37±0.06	−0.31±0.15	0.57

CFU: Colony forming units

Table 3: Changes in *Streptococcus mutans* colony count (mean±standard deviation) separated by age

Age	Intervention group		*P*
	Mouthwash	Gel	
Under 15 years old (CFU/ml) %	−0.75±0.26	−0.38±0.15	0.37
15 years old and above (CFU/ml) %	−0.39±0.38	−0.27±0.16	0.25

CFU: Colony forming units

Discussion

The purpose of this study was to evaluate the effect of green tea mouth rinse and gel on the level of salivary *S. mutans* and *Lactobacillus*. As a result, the null hypothesis pertaining to similar efficacy of mouth rinse in comparison to gel was approved.

Studies have shown that bioactive components of green tea prevent dental caries through different mechanisms as interference in adhesion to enamel, prevention of streptococcal agent, and proliferation and inhibition of bacterial glucosyltransferase and amylase.[6,14]

In the present study, green tea mouth rinse and gel caused a significant reduction in the number of salivary *S. mutans* colonies. Nandan *et al*.[23] found that there is no significant difference between green tea and chlorhexidine mouth rinse on the level of *S. mutans* and mouth rinsing with green tea can be used as daily preventive measure in children. In another study by Thomas *et al*.,[24] it was reported that green tea had higher efficacy in reducing salivary levels of *S. mutans* when compared to chlorhexidine rinse. However, green tea was less effective on the salivary level of *Lactobacillus*. Tehrani *et al*.[25] compared the effectiveness of green tea and sodium fluoride mouth rinse on salivary *S. mutans* and *Lactobacillus* in their study; green tea mouth

rinse significantly decreased both bacteria. However, there was no significant difference between two mouth rinses. Tao *et al*.[26] found that the chewing gum enriched with green tea polyphenols inhibits dental caries. Behfarnia *et al*.[27] showed in their study in 2016 that green tea chewing gum improves plaque indices and decreases serum interleukin β1.

Green tea has polyphenols that inhibit growth of *S. mutans*, *S. sobrinus*, and *Lactobacillus*.[28] The polyphenolic compounds prevent bacterial adhesion to tooth surface through changes in the fibrils and fimbriae.[14] On the other hand, catechins inhibit glucosyltransferase and contribute to a significant reduction in plaque index.[6] In Das *et al*.'s study,[29] green tea mouthwash showed an antibacterial effect on two primary colonizing bacteria and also antiplaque effect comparable to 0.2% chlorhexidine.

The results of the present study indicated that there is no significant difference between effectiveness of green tea mouth rinse and gel on the salivary *S. mutans* level. However, mouth rinse was more effective in terms of reducing *S. mutans* count. Both mouth rinse and gel have common components including solvent, flavor, and color; the only difference was the form of the agent. Therefore, the type of ingredients was eliminated as a confounding variable in this study. It should be noted that the gel form was less acceptable to participants due to foam formation during application and stronger flavor because of its consistency.

Results of a study on children aged 6–16 years indicated that drinking catechin green tea enriched with catechin had no adverse effect on children. On the other hand, it decreased the obesity and risk of developing cardiovascular diseases in obese children.[30] Other studies also evaluated catechin safety, and no side effect was also reported.[30-32]

Studies have shown that early colonization of *S. mutans* in children's mouth increases the risk of developing caries in 4 years of age. In fact, the earlier the transmission of *S. mutans* to oral cavity, the higher the risk of dental caries at older ages.[33,34] Hence, prescribing a safe agent to inhibit cariogenic bacteria in children and teenagers would prevent caries development at older ages. Considering the present findings, it seems that green tea can be used as a safe preventive measure in children and teenagers.

Participants were chosen from children aged 12–18 years; this age range was selected due to the level of participant cooperation needed according to the crossover design of our study. Further studies are recommended to confirm the efficacy of green tea application in children younger than 12 years.

Conclusions

The results of the present study showed that green tea mouth rinse and gel resulted in a significant reduction in a number of salivary *S. mutans* colonies. Green tea mouth rinse was more effective than gel. However, the difference was not statistically significant.

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Conflicts of interest

There are no conflicts of interest.

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