ORIGINAL ARTICLE

Effect of Green Tea Extract Mouthwash on Salivary *Streptococcus mutans* Counts in a Group of Preschool Children: An *In Vivo* Study

Mohamed Th Salama[1], Zeyad A Alsughier[2]

Abstract

Aim: This study was conducted to evaluate the effectiveness of green tea mouthwash on the salivary level of *Streptococcus mutans* in the preschool children.

Materials and methods: In this randomized controlled clinical trial, 40 cooperative children (4–5 years old) were divided into two groups. The study group included 20 children who did the routine tooth brushing 3 times/day, and then green tea extract mouthwash (8 mL/day) 2 times/day for 4 weeks. The control group included other 20 children who did the routine tooth brushing as the study group but did not use any green tea extract mouthwash. The quantitative microbiological laboratory cultivation method of *S. mutans* was carried out for each child at the baseline, after 2 weeks, and after 4 weeks of the study period.

Results: Statistically, the results showed that there was a statistically significant difference in the mean log *S. mutans* counts between the study and control groups in both follow-up periods after 2 weeks and after 4 weeks. Also, there were statistically significant mean percentage decreases in log *S. mutans* counts for the two groups.

Conclusion: The use of green tea mouthwash showed promising results in reducing the cariogenic salivary *S. mutans* counts.

Clinical significance: Green tea extract mouthwash is a nontoxic and safe, particularly for children. Catechins, the main bioactive ingredient of green tea, show an antibacterial action; thus, it has a promising effect in decreasing the count of salivary *S. mutans* and in the prevention of dental caries.

Keywords: Catechin, Green tea, Mouthwash, Preschool children, Randomized controlled clinical trial, *Streptococcus mutans*.

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Introduction

Dental caries is a multifactorial, chronic, preventable, and localized transmissible disease as a result of interaction among host, bacteria, diet, and time, causing cavitation of inorganic components of the enamel and dentin.[1,2] It can affect children's life quality by causing pain, malnutrition, and premature loss of teeth and alters the growth and development.[3] It is an infectious disease caused by the presence of oral bacteria mainly *Streptococcus mutans* for its initiation and lactobacillus for its progression. *Streptococcus mutans* is a Gram-positive, nonmotile, coccus-shaped, and anaerobic facultative bacterium that is found in the human oral cavity. It adheres to the tooth surface in the dental plaque biofilm and favors the initiation and progression of dental caries.[4,5] Pathogenicity of *S. mutans* occurs as a result of its acidogenicity in the presence of dietary sucrose and its accompanying acid tolerance, together support changes in the dental plaque ecology by choosing for a cariogenic flora, raising the enamel demineralization probability, and, eventually, formation of dental caries.[6]

Several modalities such as water fluoridation, fluoride dentifrices, mouth rinses, varnishes, and gels have played an important role in the decline of dental caries.[7]

It has been found that as an antimicrobial and antiplaque agent, mouthwashes are one of the effective and safe delivery systems. These mouthwashes are able to inhibit the adhesion of bacteria, colonization, and the metabolic activity which eventually affect the growth of bacteria.[8]

There is an increasing demand for the usage of medicinal plants with antibacterial property. Green tea is a nonfermented type of tea and is considered as one of the ancient and widespread therapeutic beverages consumed worldwide.[9] Green tea extract mouthwash is a nontoxic and safe mouth rinse, particularly for children.[10] Catechins, the main bioactive ingredient of green tea, own an antibacterial action and have shown utility in the treatment of oral and topical infection.[11] Some evidences showed that green tea has an indirect antibacterial effect by the stimulation of protective components such as immunoglobulins, lactoferrin, lysosome, histatin, and mucin.[12] At a concentration of 40 mg/mL, green tea brewed at 90°C at 5, 20, and 40 minutes was determined to be reasonably effective against *S. mutans*.[13]

[1]Department of Oral Public Health, Pediatric Dentistry and Orthodontics, Alrass College of Dentistry, Alrass, Qassim, Saudi Arabia

[2]Department of Orthodontics and Pediatric Dentistry, College of Dentistry, Qassim University, Almulida, Qassim, Saudi Arabia

Corresponding Author: Mohamed Th Salama, Department of Oral Public Health, Pediatric Dentistry and Orthodontics, Alrass College of Dentistry, Alrass, Qassim, Saudi Arabia, Phone: +966 501323572, e-mail: dr.mohamed.tharwat@qudent.org

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The aim of this study is to monitor and evaluate the microbiological effect of green tea extract mouthwash on the salivary *S. mutans* count in a group of preschool children.

Materials and Methods

The present study was a randomized controlled clinical trial, conducted at Alexandria University, Egypt. Children were conveniently selected from the outpatient clinic of the Department of Pediatric Dentistry, Faculty of Dentistry; the green tea extract mouthwash was prepared at the Department of Pharmaceutics, Faculty of Pharmacy; microbiological examination of saliva samples was detected and evaluated at the High Institute of Public Health. This study was performed after receiving the approval from the Research Ethics Committee, Faculty of Dentistry; official consent was obtained from children's parents after explaining the type and the importance of this study; also the approval from the Department of Pharmaceutics was obtained for the preparation of green tea extract mouthwash.

Inclusion Criteria

- Forty healthy children 4–5 years of age have primary dentition after completing their restorative phase.
- No previous use of any green tea containing products.
- Children were classified as class I (healthy children) according to the American Society of Anesthesiologists (ASA) physical status (free of any systemic disease or syndrome).
- Did not receive any antibiotics 2 weeks before or during the study.
- Cooperative child (positive or definitely positive) according to Frankl's behavior rating scale.
- No interceptive orthodontic appliances and space maintainers.
- Absence of oral infections.

Materials

- Green tea (Tianjin Jianfeng Natural Product R&D Co., Ltd., Tianjin, China)
- Fluoride-containing toothpaste (Colgate fresh confidence)
- Toothbrushes for daily usage (Oral-B® Pro-Health Stages® 2 Disney Jr Jake and the Neverland Pirates Toothbrush)
- Mitis Salivarius Agar (Oxoid Microbiology Products)

Preparation of Green Tea Mouthwash[14]

Green tea extract mouthwash was prepared at the Department of Pharmaceutics; it was prepared by putting 1 g of the tea sample which was brewed in 100 mL boiling deionized water for 20 minutes, the infusions were then immediately poured on an ice container to cool down to 15°C, filtered through cellulose filters, and then transferred to brown glass vials to prevent oxidation of the prepared green tea extract.

Short Dental Hygiene Educational Message

All the children included in the study were given a short dental hygiene educational message by which each child was given a new toothbrush to be used during the study; children were instructed about the proper method of teeth brushing and the use of pea-sized amount of pediatric toothpaste by using a "horizontal scrub" tooth brushing technique. Each child was given a follow-up table to be signed under the supervision of his or her caregiver after each time of teeth brushing. The table includes the child's data regarding the name, age, group, and the serial number given to the child.

Grouping of the Patients

Selected patients were allocated randomly by a toss into two groups using blind concept (children in both groups did not know in which group they belong to) as follows:

Group I (Study Group)

Group I consisted of 20 children who did the routine tooth brushing with a commercially available pediatric toothpaste, 3 times/day (after breakfast, lunch and dinner), and were given green tea extract mouthwash (8 mL/day) 2 times/day (after breakfast and before bed) for a period of 4 weeks.

Group II (Control Group)

Group II consisted of 20 children who did the routine tooth brushing with the same pediatric toothpaste, 3 times/day (after breakfast, lunch, and dinner) for a period of 4 weeks, and did not use any green tea extract mouthwash.

Saliva Sampling[15]

Saliva samples were collected at the baseline during the implementation of the short dental hygiene educational message, after 2 weeks, and 4 weeks of the study period. On the day of saliva sampling, each child was refrained from tooth brushing in the morning and from eating or drinking (except water) at least 2 hours before the saliva sampling time. The child was asked to chew on a paraffin wax 1 minute until the piece of wax became soft and was allowed to swallow the saliva in his mouth. Then to chew similarly for another 2 minutes. Finally, the child was asked to keep the piece of wax in his mouth and spit the collected saliva in a sterile-labeled container containing transport media.

Microbiological Examination

Microbial examination was done on several steps; it began with the preparation of the fresh mitis salivarius bacitracin (MSB) agar media. The MSB agar was prepared according to the instruction from the manufacturer by suspending 9 g of MSB agar powder in 100 mL distilled water, the medium was then sterilized by autoclaving at 121°C for 15–20 minutes. Bacitracin-impregnated discs were used; bacitracin solution was prepared by dissolving 4 bacitracin discs, each containing 10 IU of bacitracin antimicrobial in 2 mL of distilled water followed by vigorous shaking for 2 minutes and sterilized by filtration; after autoclaving the medium, the flask was allowed to cool to 50°C. Bacitracin solution and 0.1 mL of 1% potassium tellurite solution were added to the medium; 20 mL of the medium were poured into each of the sterilized Petri dishes in the lab in a sterile area away from air currents, no more than 10 cm away from a torch/flame. The plates were left to solidify at room temperature for 20–25 minutes. Before cultivation, the plates were placed inverted and uncovered to dry in an incubator for 30 minutes. Then, the plates were ready for bacterial cultivation.

Dilution of the Collected Saliva Samples

Serial dilution of samples allowed easy and possible accurate bacterial count to obtain a countable plate, which contains 15 to 150 CFU/mL. To obtain the dilution of 1:10, 1:100, and 1:1000, serial dilution methods were done as follows:

- Each saliva sample was vigorously shaken before dilution.
- To prepare a dilution of 1:10, of saliva samples, 1 mL of each sample was added to a test tube containing 9 mL of transport medium using an automatic micropipette. The test tube was vigorously shaken for homogenous distribution.

- To prepare a dilution of 1:100, 1 mL of 1:10 dilution was added to a test tube containing 9 mL of transport medium. The test tube was vigorously shaken for homogenous distribution.
- To prepare a dilution of 1:1000, 1 mL of 1:100 dilution was added to a test tube containing 9 mL of transport medium. The test tube was vigorously shaken for homogenous distribution.

Cultivation of the Collected Saliva Samples

- Using an automatic micropipette, 0.1 mL of each dilution was taken from the test tube and placed on the center of labeled MSB plates.
- A sterile glass rod was used to spread the sample on the agar surface. This provided a smooth surface to avoid scratching or indenting the agar surface.
- The inoculated plates were incubated anaerobically in a gas pack jar and the incubator was adjusted to 37°C for 48 hours.

Colony Identification and Bacterial Counting

After 48 hours, the plates were removed from the incubator; the most suitable plates for counting were selected. The *S. mutans* colonies were identified by their characteristic morphology. The colonies were identified being 0.5–1 mm in diameter, raised, convex, opaque of dark blue color with rough margins, and granular frosted appearance (Fig. 1).

Colonies Counting

The number of colonies or colony forming units (CFU) was counted. The number of colonies per milliliter saliva was calculated by the following equation: number of colonies/mL (CFU/mL) = number of colonies counted × the dilution × the cultured volume.

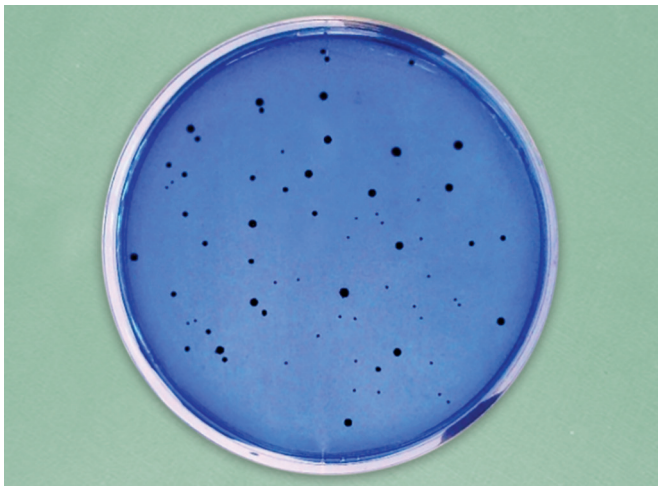

Fig. 1: Appearance of colonies

Statistical Analysis of the Data

Data were fed to the computer and analyzed using the IBM SPSS software package version 20.0. Quantitative data were described using range (minimum and maximum), mean, and standard deviation. A bar chart was used to graphically describe the data. The distributions of the quantitative variables were tested for normality using the Kolmogorov–Smirnov test. It revealed that the data were normally distributed, and the parametric test was applied. A comparison between two independent populations was done using an independent *t* test; also, the paired *t* test is used to analyze two paired data. Bacterial counts were transformed to $\log_{10}$ (CFU/mL). The paired *t* test was used to compare between the two groups in log *S. mutans* counts and to study the changes by time in each group. The level of statistical significance was set at $p \leq 0.05$.

Results

The results of this study were derived from 40 children of age ranging from 4 to 5 years old. They were conveniently selected according to the inclusion criteria. Selected patients were divided into two groups (study group and control group).

Table 1 shows the distribution of the studied sample groups regarding their gender and age. The number of males and females in the study group was 11 and 9 with a percentage of 55 and 45, respectively. The number of males and females in the control group was 12 and 8 with a percentage of 60 and 40, respectively. There was no statistically significant difference between the mean gender in the two groups with a *p* value of 0.749. The mean ± SD of age in the study group and the control group was 4.53 ± 0.31 and 4.55 ± 0.33, respectively. There was no statistically significant difference between the mean age in the two groups with a *p* value of 0.902.

Table 2 shows a comparison between the log bacterial count at the baseline, 2 weeks, and 4 weeks in the study and control groups. It was shown that the mean ± SD of the log bacterial count at the baseline in the study group and the control group was 93.20 ± 18.86 and 92.45 ± 19.54, respectively. The mean ± SD of the log bacterial count after 2 weeks in the study group was 71.55 ± 25.44 and in the control group was 88.25 ± 22.35. The mean ± SD of the log bacterial count after 4 weeks in the study group was 55.35 ± 28.18 and in the control group was 83.25 ± 22.61. On the one hand, there was no statistically significant difference between the two groups at the baseline ($t = 0.123$, $p = 0.902$), on the other hand, there was a statistically significant difference between the two groups after

Table 1: Comparison between the two studied groups according to gender and age

	Study group (n = 20)		*Control group (n = 20)*			
	No.	*%*	*No.*	*%*	*Test of sig.*	*p*
Gender						
Male	11	55.0	12	60.0	$\chi^2 = 0.102$	0.749
Female	9	45.0	8	40.0		
Age (years)						
Min.–max.	4.0–5.0		4.0–5.0		$t = 0.124$	0.902
Mean ± SD	4.53 ± 0.31		4.55 ± 0.33			

χ^2, Chi-square test
t, Student *t* test

Table 2: Comparison between the bacterial log count at the base line, 2 weeks, and 4 weeks in the study group and the control group

Result	*Study (n = 20)*	*Control (n = 20)*	*t*	*p*
Baseline × 10^4				
Min.–max.	67.0–132.0	65.0–135.0	0.123	0.902
Mean ± SD	93.20 ± 18.86	92.45 ± 19.54		
2 weeks × 10^4				
Min.–max.	32.0–119.0	54.0–140.0	2.205*	0.034*
Mean ± SD	71.55 ± 25.44	88.25 ± 22.35		
4 weeks × 10^4				
Min.–max.	19.0–108.0	43.0–129.0	3.454*	0.001*
Mean ± SD	55.35 ± 28.18	83.25 ± 22.61		

t, Student *t* test
*Statistically significant at $p \leq 0.05$

Table 3: Comparison between the studied periods according to log bacterial count in the control group

	Baseline	*2 weeks*	*4 weeks*
Result × 10^4			
Min.–max.	65.0–135.0	54.0–140.0	43.0–129.0
Mean ± SD	92.45 ± 19.54	88.25 ± 22.35	83.25 ± 22.61
% of change		↓4.5	↓10.0
p_1		0.035*	0.002*
p_2		0.016*	

p_1, *p* value for the paired *t* test for comparing between the baseline and each other periods
p_2, *p* value for the paired *t* test for comparing between 2 weeks and 4 weeks
*Statistically significant at $p \leq 0.05$

Table 4: Comparison between the studied periods according to log bacterial count in the study group

	Baseline	*2 weeks*	*4 weeks*
Result × 10^4			
Min.–max.	67.0–132.0	32.0–119.0	19.0–108.0
Mean ± SD	93.20 ± 18.86	71.55 ± 25.44	55.35 ± 28.18
% of change		↓23.2	↓40.6
p_1		<0.001*	<0.001*
p_2		<0.001*	

p_1, *p* value for the paired *t* test for comparing between the baseline and each other periods
p_2, *p* value for the paired *t* test for comparing between 2 weeks and 4 weeks
*Statistically significant at $p \leq 0.05$

Table 5: Comparison between the two studied groups according to % of change in log bacterial count

% of change	*Study (n = 20)*	*Control (n = 20)*	*t*	*p*
Baseline–2 weeks				
Min.–max.	−15.38 to 52.94	−16.46 to 19.40	4.501*	<0.001*
Mean ± SD	24.68 ± 17.01	5.02 ± 9.61		
Baseline–4 weeks				
Min.–max.	−5.88 to 71.64	−12.99 to 35.82	5.040*	<0.001*
Mean ± SD	42.34 ± 24.43	10.37 ± 14.42		
2 weeks–4 weeks				
Min.–max.	−12.50 to 51.02	−38.10 to 20.37	3.948*	<0.001*
Mean ± SD	25.62 ± 18.89	5.69 ± 12.37		

t, Student *t* test
*Statistically significant at $p \leq 0.05$

2 weeks ($t = 2.205$, $p = 0.034$*); after 4 weeks, there was a statistically significant difference between the two groups ($t = 3.454$, $p = 0.001$*).

In Table 3 on comparing the mean of log bacterial count in the control group at the baseline and after 2 weeks, it was found that there was a statistically significant difference, $p = 0.035$*. In addition, comparing the mean of log bacterial count in the control group at the baseline and, after 4 weeks, it shows a statistically significant difference, $p = 0.002$*. There was a statistically significant difference, $p = 0.016$* between week 2 and after 4 weeks in the control group.

In Table 4 on comparing the mean of log bacterial count in the study group at the baseline and after 2 weeks, it was found that there was a statistically significant difference, $p = <0.001$*. In addition, comparing the mean of log bacterial count in the study group at the baseline and after 4 weeks, it shows a statistically significant difference, $p = <0.001$*. There was a statistically significant difference, $p = <0.001$* between week 2 and after 4 weeks in the study group.

Table 5 shows a comparison between the changes by time in log number of the bacterial count in each group. The mean ± SD of change by time in the study group and the control group at baseline and 2 weeks was 24.68 ± 17.01 and 5.02 ± 9.61, respectively. There was a statistically significant decrease in mean log *S. mutans* counts in the two groups with a *p* value of <0.001*. The mean ± SD of change by time in the study group and the control group at baseline and 4 weeks was 42.34 ± 24.43 and 10.37 ± 14.42, respectively. There was a statistically significant decrease in mean log *S. mutans* counts in the two groups with a *p* value of <0.001*. The mean ± SD of change by time in the study group and the control group at 2 weeks and 4 weeks was 25.62 ± 18.89 and 5.69 ± 12.37, respectively. There was a statistically significant decrease in mean log *S. mutans* counts in the two groups with a *p* value of <0.001*. The study group showed a higher decrease in log *S. mutans* counts than the control group.

Discussion

The present study was conducted to evaluate the effect of green tea mouthwash on the *S. mutans* salivary level in the preschool children. The study consisted of 40 healthy children of age ranging from 4–5 years old. This age represents the preschoolers who are considered at high risk to develop dental caries as most of the children stay outside homes (at nurseries) for a long period of time (above 6–8 hours) with frequent intake of sweets and candies. Moreover, practicing teeth brushing and other plaque control measures are unavailable while they are outside homes.

There were some limitations during the study; the age range of the patients was too young to teach them how to use the green tea mouthwash. In addition, the use of paraffin wax caused gag reflex to some of the children. Also patient/parent cooperation during follow-up periods was not regular, so a larger sample size was selected to match the sample size reported in the study.

Both variables (gender and age) at the baseline were compared in the two groups. The nonsignificant difference between mean gender and age in the two groups with *p* values of 0.749 and 0.902 ensures the similarity of the variables between the groups at the baseline as given in Table 1.

The present study investigated the effects of rinsing with green tea mouthwash for 4 weeks 2 times daily concerning salivary *S. mutans* count. The results in Table 2 revealed that there was a significant difference among study cases and controls after rinsing with green tea extract mouthwash after 2 weeks and 4 weeks. The results are in agreement with Wu-Yuan et al.[16] who stated that there was a significant difference among cases and controls concerning *S. mutans* salivary count before and after green tea application. Also, a study was done by Tehrani et al.[17] which showed that green tea mouthwash had a significant reduction in the number of salivary *S. mutans* and Lactobacillus colonies, which is comparable with the sodium fluoride mouthwash. In addition, another study was conducted by Neturi et al.[18] who compared green tea, chlorhexidine (CHX), and plain water on plaque and reported that green tea and CHX were similarly effective against *S. mutans*.

The results of the control group in Table 3 showed a statistically significant reduction in the *S. mutans* count after 4 weeks with a *p* value of 0.002 after oral health education to parents. These results are in agreement with Curnow et al.[19] who explained that practicing oral hygiene measures especially tooth brushing at least twice a day had a significant reducing effect on the plaque level with the result of reducing the count of cariogenic microflora.

Generally, the results of the study groups in Table 4 are in agreement with Ferrazzano et al.[20] who stated that 1 week of mouthwash with green tea (1.6 g of pulverized green tea in 40 mL double distilled water (DDW), 3 times a day) was able to significantly reduce the salivary levels of the virulent cariogenic pathogens *S. mutans* and lactobacilli. Hashimoto et al.[21] and Yang et al.[22] demonstrated *in vitro* microbial studies that that tea had high caries resistances properties. This is due to their high contents of fluoride and polyphenolic catechin components. Also, in agreement with a study done by Awadalla et al.[23] which showed a statistically significant difference between subjects pre- and postrinsing with 2% green tea for 5 minutes about *S. mutans* count in saliva and plaque, the pH values, and GBI. It supports the efficiency of local application of green tea as an antimicrobial and anticarcinogenic agent as it decreases the acidity of the saliva and plaque. In agreement with the present study, Matsumoto et al.[24] stated that active ingredients present in green tea showed a marked inhibitory effect against *S. mutans's* growth and activity with reduction of plaque accumulation around teeth.

Thomas et al.[25] showed that the effect of green tea mouthwash against salivary *S. mutans* was significantly better than CHX mouthwash. Goyal et al.[26] found that green tea mouthwash has better action against *S. mutans* in plaque when compared to saliva. Abdelmegid et al.[27] stated that there was a statistically significant decrease in the count of *S. mutans* at the baseline and postintervention in the children who were allocated to the green tea and honey groups.

Another study failed to demonstrate the antibacterial effect of green tea catechins on *S. mutans*. The reason for the conflicting evidence might be that green tea has indirect antibacterial activity through mediation of protective saliva components such as secretory immunoglobulins, lysozyme, lactoferrin, oral peroxidases, histatins, mucins, or others.[28]

This study supports the effectiveness of local application of green tea as an antibacterial and anticariogenic material and proved that its local application strongly inhibits salivary *S. mutans* count which is the main causative bacteria in the carious process.

Conclusion

The results of the present study concluded that:

- That local application (oral rinsing) with green tea mouthwash strongly inhibits salivary *S. mutans* count which is the main causative bacteria in carious processes.
- By increasing the duration (4 weeks) of using green tea mouthwash two times per day after breakfast and before bed time, there was a significant reduction in the *S. mutans* count.
- Tooth brushing even with pediatric toothpaste three times per day (after breakfast, lunch, and dinner) was an effective method in reducing the *S. mutans* count (control group).
- Green tea extract mouthwash with tooth brushing was very effective in both genders but it is not completely clear whether green tea potency is due to its active phenolic ingredients or other nutritional components.

Clinical Significance

Green tea extract mouthwash is a nontoxic and safe, particularly for children. Catechins, the main bioactive ingredient of green tea, show an antibacterial action; thus, it has a promising effect in decreasing the count of salivary *S. mutans* and prevention of dental caries.

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