



Comparing the effects of green tea/ green coffee bean extracts and chlorhexidine gluconate on salivary *Streptococcus mutans* count in children with early childhood caries

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ABSTRACT

Dental caries is known as a major oral health problem induced by *Streptococcus* . and *Lactobacillus* as primary contributing microorganisms responsible for this condition. The aim of this study was to compare the effect of Green tea/ Green coffee Bean Extracts and Chlorhexidine Gluconate on *Streptococcus mutans* count. The study was conducted among 90 preschool children aged from 4 to 6 years. Statistical analysis was done using the Shapiro-Wilk test, Friedman test, Chi-square test, paired sample t-test, repeated measures analysis of variance (ANOVA) and Mann-Whitney U test at a significance level of 0.05. The results demonstrated a significant difference in the mean of salivary *S. mutans* count after administration of CHG (p-value=0.001) and GT (p-value=0.02) between three different times. The study results indicate the beneficial effects of Green Tea/ Green Coffee Bean extracts and Chlorhexidine Gluconate on salivary *Streptococcus mutans* count.



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INTRODUCTION

Dental caries is known as a major oral health problem induced by *Streptococcus mutans* (*S. mutans*), *Streptococcus sobrinus* (*S. sobrinus*), *Streptococcus salivarius* (*S. salivarius*), and *Lactobacillus* as primary contributing microorganisms responsible for this condition. Research studies in this area have further revealed that a reduction in microorganisms can lead to a major decrease in dental caries (Tanner et al., 2011). Currently, early childhood caries (ECC) has been recognized as one of the most important conditions in pediatric dentistry. It refers to the occurrence of one or more decayed, missing, or filled primary teeth in children at the age of 71 months or younger. ECC also initiates with white-

spot lesions in the upper primary incisors alongside the border of the gingiva (Dean, 2015). The etiology of the ECC is thus focused on the establishment of oral cariogenic bacteria such as *S. mutans* that produce acids via fermentation of nutritive sugars, which can demineralize the tooth surface. The presence of this microorganism in the mouth is observed before tooth eruption. Therefore, timely removal of *S. mutans* can decrease the risks of dental caries. Many efforts have been thus made to reduce oral bacteria in the mouth like tooth brushing and the use of mouthwashes and gels. Some advantages of mouthwashes have also been mentioned as elimination of bad breath and prevention of dental caries and infections. Moreover, daily use of mouthwashes has been characterized by significant antibacterial effects (Berkowitz, 2003).

In this regard, even though a few antibacterial agents are favorable, chlorhexidine gluconate (CHG) has been reported to be predominantly effective against cariogenic bacteria. CHG is a cationic biguanide whose profits are centered on its bactericidal and bacteriostatic properties. It is also known as a safe disinfectant with limited side effects whose long-term usage is related only to minimal alterations in oral microbial plaque as well as insignificant changes in susceptibilities. Discoloration of teeth, irritation of mucosa, changed taste impression, parotid inflammation, and accumulation of supragingival calculus are accordingly some of the side effects of CHG, which can confine its consumption as a therapeutic agent (Addy and Moran, 2008; Prayitno and Addy, 1979).

Recently, herbal substitutes have gained acceptance with no adverse properties. In this regard, green coffee (GC) bean extract with its antibacterial effects against gram-negative and gram-positive bacteria has been gaining greater consideration among other herbal extracts (Yadav et al., 2017; Toda et al., 1989). It is the main source of chromogenic acid (CGA) and 5-12 grams/100 grams of the polyphenolic compound are composed of CSAs with antibacterial and antifungal influence as their health benefits (Farah et al., 2008).

Another herbal substitute is green tea (GT) which is considered mildly refreshing and produces an overall feeling of happiness. Furthermore, GT has been revealed to reinforce capillaries, enable weight loss, and even stop implanted malignant cell progress (Menendez et al., 2005). The above-mentioned beneficial effects of GT are often ascribed to its antioxidant properties, which can remove free radicals. Antioxidants can also provide electrons to free radicals to prevent chemical instability. As

well, GT is comprised of a diverse grouping of antioxidants, vitamins, and minerals such as vitamin C and water-soluble vitamins known as the vitamin B-complex group. These chemical compounds are thus rapidly released and a cup of GT can deliver a small amount of manganese, potassium, and fluoride (Farah et al., 2008).

GT polyphenols are also retained as they do not undertake fermentation. A benzene group is thus bonded together with a hydroxyl one to form a phenol and the term polyphenol is used when multiple phenols are attached together. Additionally, the polyphenols are attributed to antimicrobial properties (Koo and Cho, 2004).

A real-time polymerase chain reaction (real-time PCR), also recognized as quantitative PCR (qPCR), is a laboratory method of molecular biology centred in a polymerase chain reaction (PCR). The amplification of a targeted DNA molecule can also be observed during PCR. As well, qPCR suggests a number of gains in comparison with conventional PCR, such as high sensitivity, better accuracy, and assessment of data without any post-PCR exposure procedures. There are also several bacteria types in the oral cavity and qPCR has been introduced as one of the best methods to measure these bacteria (Chen et al., 2007).

The main objective of this study was to assess the effects of GT/GC bean extracts in comparison with CHG on salivary *S. mutans* count in preschool children with ECC using qPCR. Studies on the antibacterial and anti-cariogenic properties of herbal species were very limited, so it was decided to compare the effects of methanolic extract of GT/GC and CHG on salivary *S. mutans* count in preschool children with ECC through qPCR.

MATERIALS AND METHODS

Ethical Considerations

This study was approved by the Ethics Committee of Kerman University of Medical Sciences (project code of ethics no. IR.KMU.REC1395.553). At first, researchers visited preschool children and a short educational program was held in which oral dental health issues, notably oral public health management including prophylactic and therapeutic measures, were discussed using face-to-face communications. All the preschool children, authorities, teachers, and especially their parents also became aware of the objectives and procedures of this study, and those who were willing to participate and signed written informed consent forms were included in the study.

Study Subjects

A sample size of 90 subjects was selected, including female and male children aged from 4 to 6 years. The sample size was selected out of preschool children in kindergartens. Commercially available 0.12% CHG was then used as positive control and methanolic extract of 20% GT and 10% GC were also considered as intervention groups.

GT/ GC Preparation

CHG, GT, and GC gel were prepared by a pharmacist. First, the leaves of GT were grounded to a small size. Then, distilled water was added to the leaves to prepare the extract. Correspondingly, 20% GT was prepared by dissolving 20 grams of GT in 100 ml of distilled water (Papetti *et al.*, 2007). As well, 100 ml of sterile water was added to 10 grams of coffee to have 10% (Prayitno and Addy, 1979).

Randomization Description

Simple randomization or unrestricted method was used and an individual was considered as the randomization unit. Die rolling probability from simple randomization methods was thus used in this study. There were three groups in this study, so numbers 1 and 2 were assigned to the first group (CHG), numbers 3 and 4 were given to the second group (GC), and numbers 5 and 6 were allotted to the third one (GT).

To meet the allocation concealment criteria, a non-transparent, sealed, random-sequence envelope was also used. In this way, a statistical specialist provided 90 envelopes and each of the randomly generated links were recorded on a card and the cards were then arranged in the envelopes. At the time of the implementation of the study, according to the order of entry of eligible subjects, one of the envelopes was respectively and the group assigned to that subject was determined. In this study, a pediatric dental assistant examined the subjects in terms of inclusion and exclusion criteria. Randomization methods were also fulfilled by a statistical specialist and the study was performed by a trained dental student.

Inclusion Criteria

1. Children aged from 4 to 6 years with no history of systemic
2. Children whose parents signed the consent form
3. Children who had not taken any antimicrobials or medications over the past 4 weeks

Exclusion Criteria

1. Children with a history of medication or any mouthwash use
2. Children with no soft tissue lesion in their mouth as well as no active and severe periodontal disease

Blinding Description

A double-blinded method was employed in this study. All the materials were in the form of gels with the same consistency. The study subjects and the outcome evaluator who performed the qPCR were also unaware of the mentioned materials and groups.

Setting and Procedure

Initially, a questionnaire containing demographic characteristics information (i.g. age, gender, history of medication use, history of systemic diseases, etc.) was completed. The next step was an explanation of oral hygiene and brushing to parents and asking them to brush their children's teeth without toothpaste for 2 weeks and not to allow them to use gums containing xylitol or any mouthwashes containing CHG and fluoride during the study. Upon the completion of the 2 weeks, considered as a washout period, the children were randomly divided into three groups of 30 subjects and asked to spit into a sterile container for 3 min in the upright position until 0.5-1 cc of unstimulated saliva was collected. After taking saliva samples, they were transferred to a laboratory for determining their *S. mutans* count. Then, their mouth was washed with water and air dried. At the subsequent step, the teeth were isolated by the cotton roll and 1 cc of one of the three substances was swabbed on all teeth. After 30 min, the children were requested once again to spit their saliva into a sterile container. Finally, parents were asked to monitor their children's brushing for one week. After one week, the unstimulated saliva from children was recollected as before.

Microbiological Method

Frozen saliva samples were thawed at 37° C and 300 μ l of each sample was used for DNA extraction using RIBO-prep extraction kit according to manufacturer's instructions. qPCR was also performed through the Applied Biosystems StepOnePlus Real-Time PCR. Each reaction contained 20 μ l of reaction mixture including 5X HOT FIREPol Eva Green qPCR Mix Plus (4 μ l), a specific primer (0.5 μ l), PCR grade water (5 μ l), and template DNA (10 μ l).

Amplification was then carried out as follows: initial denaturation for 15 min at 95° C, 35 cycles of denaturation for 20 s at 95° C, annealing for 15 s at

Table 1: Primers used for the real-time PCR

Primer	Sequences (5'-3')	Position	Product size (bp)
Sm F	AGC CAT GCG CAA TCA ACA GGT T	2007-2028	415
Sm R	CGC AAC GCG AAC ATC TTG ATC AG	2421-2399	

Table 2: Mean Salivary *S. mutans* count after applying three compounds in terms of time interval

Compounds	Before administration		Half an hour after administration		One week after administration	
	Mean	SD	Mean	SD	Mean	SD
CHG	42.10	10.58	14.01	12.62	34.01	12.44
GC	24.22	13.89	17.58	11.83	18.64	9.59
GT	18.76	9.35	17.58	9.05	11.31	3.51

63° C, and extension for 30 s at 72° C. Sequences of primers specific to the *gtfB* gene of *S. mutans* used in this study are shown in Table 1.

To determine the sensitivity and the detection range of the qPCR assay, a standard curve was generated for *S. mutans* as follows: the extracted DNA solution of *S. mutans* ATCC35668 was diluted to a concentration of 9 ng/ μ l. Starting with this concentration, ten-fold serial dilution was prepared.

Statistical Analysis

Data analysis was completed using the SPSS Statistics software (version 22). The statistical analysis was thus fulfilled using the Shapiro-Wilk test, Friedman test, Chi-square test, paired sample t-test, repeated measures analysis of variance (ANOVA) and Mann-Whitney U test at a significance level of 0.05.

RESULTS AND DISCUSSION

Table 2 shows the mean and the standard deviation (SD) of salivary *S. mutans* count after applying three compounds in terms of study time intervals. The results of the Shapiro-Wilk test also revealed that the data from CHG, GC, and GT had not followed a normal distribution at three different times. For this purpose, the mean difference within three different times was evaluated via the Friedman test. Additionally, the results demonstrated a significant difference in the mean of salivary *S. mutans* count after administration of CHG (p-value=0.001) and GT (p-value=0.02) between three different times. The paired comparison was also utilized to compare salivary *S. mutans* count after administration of CHG and GT at three different times (Table 3).

Moreover, the Mann-Whitney U test was employed to compare salivary *S. mutans* count after applying three different compounds as a paired comparison

at different times. The results indicated a significant difference between mean salivary *S. mutans* count after administration of the three compounds during one week at a 0.05 significance level (Table 4).

Table 3: Mean difference in salivary *S. mutans* count after administration of three compounds at three different times

Mean difference test	Statistics	P-value
CHG	26.54	<0.001 *
GC	4.08	0.12
GT	7.97	0.02 *

*P<0.05

Dental caries is still the most common oral health problem. This condition develops as soon as the tooth erupts in the oral cavity (Seow, 1998). The results of the present study indicated that the administration of three compounds of 2% CHG and 5% methanolic extracts of GT and GC had resulted in a reduction in the mean salivary *S. mutans* count in 30 min and one week after administration. However, this reduction in bacteria count for CHG was higher than the rest of the agents. The results also showed that salivary *S. mutans* count had exhibited much more reduction during one week after administration of three compounds compared with the time before administration and 30 min after it (Table 5).

Clarke et al. first introduced *S. mutans* as the main cause of dental caries. In dental plaque, CHG has also been proposed as a useful agent in reducing *S. mutans* (Clarke, 1924). Axelsson and Lindhe additionally described CHG mouthwash as very effective in inhibiting plaque and gingivitis (Axelsson and Lindhe, 1987). Despite the numerous benefits of CHG, it has also been characterized by some disadvantages. The most common side effects of CHG

Table 4: Comparison of salivary *S. mutans* count after administration of three compounds at three different times

Compounds	Comparison of administration time (p-value)		
	Before administration Half an hour after administration	Before administration One week after administration	Half an hour after administration One week after administration
CHX	<0.001 *	0.10	0.008 *
GT	0.93	0.04 *	0.06

*P<0.05

Table 5: Comparison of salivary *S. mutans* count after administration of three compounds in terms of different compounds

Time intervals	Comparison of mean difference	p-value
Half an hour after administration	CHX - GC	0.12
	CHX - GT	0.16
	RC - GT	0.57
A week after administration	CHX - GC	<0.001*
	CHX - GT	<0.001*
	RC - GT	0.04*

*P<0.05

in this respect are tooth discoloration, increased plaque formation, and taste alteration (Gales et al., 2008).

Herbal mouthwashes are also well-considered because they are not chemically manufactured and have been used in medications since ancient times. GT has been similarly introduced as a healthy drink from the past and has been used as a healthy product. GT leaves also contain three important ingredients that make people healthy, including essential oils, theophylline, and polyphenolic compounds.

As stated by Zhang et al., the reaction between polyphenols and dental matrix prevents tooth demineralization. This reaction contains hydrogen as well as covalent and ionic bonds that change the organic enamel matrix (Zhang et al., 2009; Kashket et al., 1985). This modified enamel matrix also reduces the loss of mineral ions. As well, polyphenols prevent glycosyltransferase formation via cariogenic bacteria. According to Antonio et al., polyphenols with caffeic acid and five caffeoylquines display some activities against *S. mutans*.

Early Chinese practitioners have also introduced GT to increase longevity, to improve headaches and body pain, and to boost energy and detoxification (Cabrera et al., 2006). In the study by Awadalla et al., CHG mouthwash moderates *S. mutans* only slightly more than GT. Due to more cost-

effectiveness of GT and its ease of use in home care, it can be an alternative of CHG (Awadalla et al., 2011).

Coffee is also a good drink that has many benefits. Its components include caffeic acid, caffeine, chlorogenic acid, trigonelline, and melanoidine, which have antibacterial effects. In the investigations by Daglia et al. and Papetti et al., *S. mutans* suppression by trigonelline had been also observed (Daglia et al., 2007; Papetti et al., 2007).

As well, polyphenols can have antioxidant properties. Antimicrobial effects are similarly mediated by chlorogenic acid. In the study by Yadav et al., the influence of GC on *S. mutans* had been investigated. Accordingly, GC had been introduced as a good alternative for CHG reducing *S. mutans*. In the present study, GC could also lower *S. mutans* half an hour and one week after administration (Yadav et al., 2017).

In this study, a very meticulous statistical analysis was performed. Still, the limitations of the present study included parents' lack of consent with their children's participation in the study, absence of some children on the days of saliva collection, and difficulty in collecting saliva from children. However, the researchers made attempts to encourage children to continue participating in the study by giving gifts.

CONCLUSIONS

According to the results of our study, it can be concluded that GT and GC could be reasonable cost effective choices to CHG. However, more studies will be needed to assess any possible adverse effects with the long-term use of these extracts.

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Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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