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Effect of Chlorhexidine, Fluoride and Green Tea Oral Gel on Pediatric Salivary Cariogenic Bacteria: A Clinical Trial Study

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Abstract

Background: The purpose of this study was to investigate the effects of chlorhexidine, fluoride and green tea gel on salivary *S. mutans* and Lactobacillus in children.

Materials and Methods: The present single-blinded clinical trial was conducted on 60 children aged 4- 6 years in pediatric dental clinic of Kerman University of Medical Sciences, Kerman, Iran, in 2019. Children randomly were divided into three groups of 20. In each group, one of green tea 5% gel, chlorhexidine 2% gel or fluoride 0.2% gel was applied to teeth of children for five minutes before spitting out. Unstimulated saliva samples were taken from children before intervention, 30 minutes and one week after gel applications. Real-time quantitative Polymerase Chain Reaction method was used for counting bacteria. Flavor acceptability of each gel was also recorded using the Visual Analogue Scale. Data were analyzed using SPSS software version 21.0.

Results: Participants were equally distributed in terms of gender and the mean DMFT score was not different between three groups. Green tea 5%, chlorhexidine 2% and fluoride 0.2% gel reduced the count of salivary *S. mutans* and Lactobacillus at 30 minutes and one week after intervention (P=0.000). Bacterial count increased one week after the application of chlorhexidine gel. However, a week after the application of green tea gel, the bacterial count was still less than what it was 30 minutes post application. The flavor acceptability of green tea was higher than chlorhexidine (P=0.007).

Conclusion: The gel form of green tea, fluoride and chlorhexidine reduced salivary *S. mutans* count. However, the durability and flavor acceptance of green tea was more than chlorhexidine and fluoride gel.

Key Words: Child, Chlorhexidine, Fluoride, Green tea, *Streptococcus mutans*, Real-time PCR.

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1- INTRODUCTION

Dental caries is a multifactorial disease in which the diet, microbial agents and host responses play an important role. The pain caused by untreated dental caries in children can affect school activity, nutrition, speaking and subsequent growth and development (1). The presence of bacteria is known to be a critical factor in the development of dental caries. Two bacteria that play a major role in the development and progression of caries are Streptococcus mutans and Lactobacillus (2). *S. mutans* is associated with the onset of caries whereas Lactobacillus plays a more important role in progression of carious lesions. Both of these bacteria have the ability to produce acids and survive in low PH environments. Unlike *S. mutans*, Lactobacillus adheres poorly to oral tissues and is mainly detected in sites of carious dentin rather than being found in a healthy oral environment (3). Antibacterial compounds can reduce the level of bacterial plaque and prevent dental caries by inhibiting colonization, growth and metabolism of bacteria and by impairing bacterial plaque maturation (4, 5).

Chlorhexidine and fluoride are among the compounds containing antibacterial agents. Chlorhexidine is a biguanide compound with effective cationic agents against *S. mutans* and Lactobacillus. A systematic review revealed a statistically significant reduction in Streptococcus mutans levels in saliva after the use of a chlorhexidine mouthwash. However there is still inadequate evidence regarding its ability to prevent dental caries (6, 7). Also due to its local side effects such as staining and altered taste sensation, it's long term use has been limited (8). The effectiveness of topical fluoride as a cariostatic agent has been well established, therefore it is commonly used as a professional treatment or by patients at home, in an attempt to arrest the progression of active caries (9). Studies indicate that fluoride has an inhibitory effect on colonization of bacteria thus it can prevent plaque maturation and also reduce the acid produced by bacteria, especially *S. mutans* (10). However, when used improperly, one of the major concerns of fluoride therapy is the potential of toxicity. Repeated ingestion of small amounts of fluoride frequently observed in young children who cannot fully control their swallowing reflex can result in manifestations of chronic fluoride toxicity, such as dental fluorosis (2). Considering the drawbacks of the chemical mouthwashes mentioned above, there is a growing tendency towards the use of herbal products, which provide a wide range of biological and medicinal activity, have higher safety margins and cost less. Amongst the herbal products, green tea is becoming very popular. Green tea is known to be a health promoting beverage with a vast spectrum of medicinal benefits, mainly attributed to the catechin content of it. Catechin is a polyphenol which brings about the anti-bacterial, anti-cariogenic and anti-oxidative effects of green tea (8).

Neturi et al. reported green tea mouthrinse to be as effective as chlorhexidine (which is commonly considered to be the gold standard) in reducing *S. mutans* counts (8). Moreover studies revealed that the anti-cariogenic effect of green tea is due to inhibition of proliferation of streptococcal compounds, interference with bacterial adhesion to enamel and inhibition of glucosyltransferase (11, 12). A practical solution to control this silent epidemic disease can be achieved not only through the use of fluoride and preventive restorations (fissure sealants and preventive resin restorations) but also with the use of a variety of antimicrobial agents in the daily diet (1). Hence, an inexpensive and easily available product should be introduced so that people with different socioeconomic status can benefit from it. On the other hand, many people around the world have switched to herbal products for the treatment and prevention of many diseases. Therefore, this study was conducted to investigate the effect of chlorhexidine, fluoride and green tea on salivary *S. mutans* and Lactobacillus bacteria in children using Real-time quantitative Polymerase Chain Reaction (RT-qPCR). Also, the three compounds were compared regarding their taste, which is an important factor in children's acceptance of the compounds.

2- MATERIALS AND METHODS

2-1. Study design and population

This study was a single-blinded clinical trial. Convenience sampling method was used to recruit 71 children who referred to dental clinic of Kerman University of Medical Sciences, Kerman, Iran, in 2019. Among them, 11 were excluded from the study because they did not meet the inclusion criterion. Finally 60 children who had the inclusion criteria (30 girls and 30 boys) aged 4-6 years were enrolled in the study. The study was carried out in the pediatric clinic of Kerman Dental School.

2-2. Inclusion and exclusion criteria

Children who were in good physical health with no systemic diseases who had not used antibiotics at least 3 weeks before the study, were included. Other inclusion criteria were the absence of oral soft tissue lesions, any active or severe periodontal disease and no prosthetic or orthodontic appliances. Also, it was confirmed that participants had no history of food or drug allergies and had not used xylitol gums or any type of mouthwash or gel containing fluoride at least 3 weeks before the study (3, 6, 7).

2-3. Substances used

Substances used in this study were; methanolic extracts of green tea 5% gel, chlorhexidine 2% gel and fluoride 0.2% gel, all of which were prepared in a similar form as gel by the same pharmacologist.

2-3-1. Preparation of green tea gel

The Green tea used in this study was made in a company in Lahijan, North of Iran. It was grinded and every 100 grams was added to 500 ml of methanol and left for 48 hours. Then the solution was filtrated and poured into plates and kept in room temperate. The crystal powder formed in plates was then scraped and added to carbomer gel 934 base to make the green tea methanolic gel 5%.

2-4. Methods

A complete oral examination was performed for each child and their DMFT score was recorded by a trained dentist using the WHO guidelines (13). Parents of participants were educated to practice proper oral hygiene behaviors and supervise their children. They were then asked to monitor their children for effective oral hygiene behaviors (tooth brushing without tooth paste) for a period of one week as the washout period. They were also asked not to use xylitol chewing gum and any type of mouth wash or gel containing fluoride or chlorhexidine during the study period. Also, they were required to inform the researchers in case of a disease needing antibiotics. Children were then randomly divided into three groups of 20. On the day of the experiment, participants were asked to eat their breakfast and brush their teeth (without toothbrush) at the research place and not to eat anything else until the first unstimulated saliva sampling which was carried out an hour later. For the saliva sampling, they were asked to sit in a comfortable chair at the coachman position. Subsequently, they were required to spit out 0.25-0.5 ml of their saliva in a graded microtube which was then kept as the samples before intervention.

2-5. Intervention

A trained pediatric dentistry resident carried out the intervention procedure for all participants. In order to apply the three type of gels, each child's teeth were rinsed with water and dried with air suction. The teeth were then isolated with cotton roles and a suction was placed in their mouth. Each gel was applied to teeth of children belonging to the specific group using a cotton swab. After five minutes the child was told to spit out and rate the flavor felt in their mouth by showing a shape on a VAS chart. These shapes had numbers from 1 to 5. Larger numbers indicated a more unfavorable taste (**Figure.1**). After the application of gel, children were asked not to eat or drink anything for half an hour, after which the sampling was repeated as before and unstimulated saliva was collected and restored as the 30 minutes post intervention sample. Subsequently, parents were asked to supervise their children at home regarding proper oral hygiene behaviors according to instructions that they had received for another one week when the unstimulated saliva sampling was repeated as before and a week post intervention samples were restored. Therefore, three samples of unstimulated saliva were collected for each child (one before intervention, 30 minutes after intervention and a week after intervention). Based on laboratory guidelines, each time a sample was obtained, samples were stored in a container with ice cubes and transferred to the laboratory for analysis regarding the count of lactobacillus and *S. Mutans*.

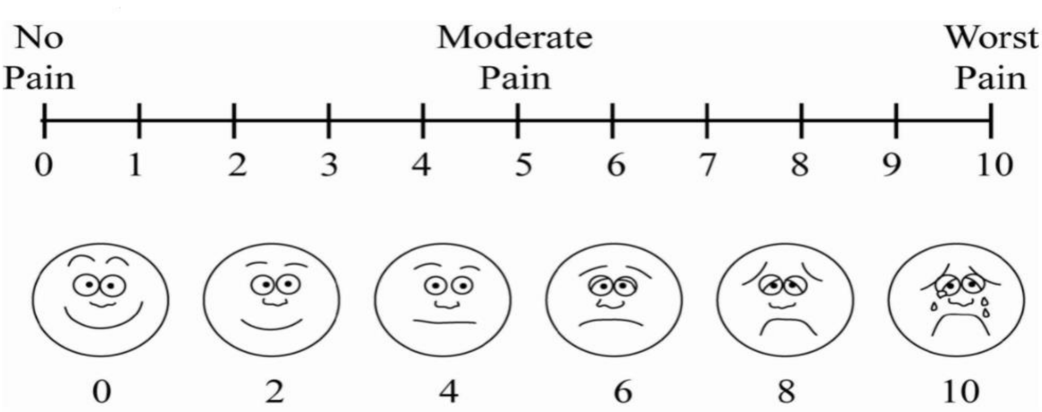

Fig.1: VAS shapes used for indicating the taste acceptability of different gels.

VAS: Visual Analogue Scale.

2-6. Measuring tools

Real-time PCR was used to determine the number of Lactobacillus and *S. mutans*. Visual Analogue Scale (VAS) was used to determine taste preferences (14).

2-7. Ethical consideration

Ethics Committee of Deputy of Research and Technology at Kerman University of Medical Sciences, Iran approved this study. Ethics code of k/92/472 was allocated to the study. RCT code. The study objectives and methodology were explained to parents of the children participating in the study, and written informed consent was obtained from them.

2-8. Data Analyses

For data analysis mixed model ANOVA and Tukey test was carried out using SPSS software version 21. Significance level was set as 0.05.

3- RESULTS

The present study sample consisted of 71 children aged 4-6 years of which 11 were excluded from the study due to lack of parental cooperation and failure to refer for follow-up sessions. Therefore, 60 children were enrolled with equal sex distribution (50% female and 50% male). They were randomly assigned to 3 groups of 20. The mean dmft in the three groups had no statistically significant difference and had normal distribution (P>0.05). **Table 1** compares the three gel types regarding mean count of *S.mutans* after application. The results showed that the mean count of *S. mutans* one week after the intervention was significantly lower in the green tea group than in the chlorhexidine and fluoride groups (P <0.05). Compared with the time before intervention, the mean count of salivary *S. mutans* decreased significantly 30 minutes and one week after application of all three gel types (P<0.05) (**Table.2**). Although not statistically significant, a week after the application of fluoride and chlorhexidine gel, the mean count of *S.mutans* increased compared to 30 minutes post application of the gels. For chlorhexidine, this increase was borderline (P=0.054). In contrast, the mean count of *S.mutans* decreased a week after application compared to 30 minutes post application of the gel, but this decrease was not statistically significant (P>0.05) (**Table.2**). The results of comparing salivary Lactobacillus count among the gel groups at three different times showed that the mean bacterial count in the chlorhexidine group at 30 minutes after the intervention was lower than that of the green tea group, which was statistically border line (P=0.052) (**Table.3**).

Table 1- Comparison of the effect of three gels on the count of salivary *S. mutans* at three different times.

Time period	Gel type		Mean difference	*P-value
Before intervention	Chlorhexidine	Fluoride	1.795	0.300
	Chlorhexidine	Green tea	2.211	0.132
	Fluoride	Green tea	0.416	1.000
Thirty minutes after intervention	Chlorhexidine	Fluoride	0.898	1.000
	Chlorhexidine	Green tea	1.704	0.518
	Fluoride	Green tea	0.807	1.000
A week after intervention	Chlorhexidine	Fluoride	1.649	0.385
	Chlorhexidine	Green tea	4.675	0.000
	Fluoride	Green tea	3.026	0.019

*Statistically significant at 0.05 level (mixed model ANOVA test and post hoc Tukey test).

Table-2: Comparison of salivary *S. mutans* counts in the three gel groups according to intervention periods.

Gel type	Time period		Mean difference	P-value
Chlorhexidine	Before intervention	30 minutes after intervention	4.669	0.000
	Before intervention	A week after intervention	3.061	0.000
	30 minutes after intervention	A week after intervention	-1.608	0.054
Fluoride	Before intervention	30 minutes after intervention	3.771	0.000
	Before intervention	A week after intervention	2.915	0.000
	30 minutes after intervention	A week after intervention	-0.857	0.599
Green tea	Before intervention	30 minutes after intervention	4.162	0.000
	Before intervention	A week after intervention	5.524	0.000
	30 minutes after intervention	A week after intervention	1.363	0.131

*Statistically significant at 0.05 level (mixed model ANOVA test and post hoc Tukey test).

Table-3: Comparison of the effect of the three compounds on the count of salivary Lactobacillus at three different times.

Time period	Gel type		Mean difference	P- value
Before intervention	Chlorhexidine	Fluoride	0.834	1.000
	Chlorhexidine	Green tea	-0.722	1.000
	Fluoride	Green tea	-1.556	0.443
Thirty minutes after intervention	Chlorhexidine	Fluoride	-1.862	0.679
	Chlorhexidine	Green tea	-3.687	*0.052
	Fluoride	Green tea	-1.825	0.649
A week after intervention	Chlorhexidine	Fluoride	1.177	1.000
	Chlorhexidine	Green tea	-1.283	0.936
	Fluoride	Green tea	-2.460	0.147

*Statistically significant at 0.05 level (mixed model ANOVA test and post hoc Tukey test).

The results also indicated that the mean Lactobacillus count decreased 30 minutes and one week after intervention compared to the time before intervention in all three gel groups, which was statistically significant (P<0.05) (**Table.4**). In addition, a week after the application of chlorhexidine gel, the mean count of Lactobacillus increased compared to 30 minutes post application; although this increase was not statistically significant (P>0.05). On the other hand, a week after the application of green tea and fluoride gel, the Lactobacillus count was lower than 30 minutes post application. This decrease was also not statistically significant (P>0.05). Regarding the flavor acceptability of the gels, results revealed that the flavor acceptance of green tea was significantly higher than chlorhexidine (P=0.007) (**Table.5**).

Table-4: Comparison of salivary Lactobacillus counts in the three groups according to intervention periods.

Gel type	Time period		Mean difference	*P-value
Chlorhexidine	Before intervention	30 minutes after intervention	5.643	0.000
	Before intervention	A week after intervention	3.637	0.000
	30 minutes after intervention	A week after intervention	-2.007	0.234
Fluoride	Before intervention	30 minutes after intervention	2.948	0.001
	Before intervention	A week after intervention	3.980	0.000
	30 minutes after intervention	A week after intervention	1.032	0.998
Green tea	Before intervention	30 minutes after intervention	2.679	0.001
	Before intervention	A week after intervention	3.076	0.001
	30 minutes after intervention	A week after intervention	0.39	1.000

*Statistically significant at 0.05 level (mixed model ANOVA test and post hoc Tukey test).

Table 5: Comparison of flavor acceptability in the three compounds studied.

Gel types		*P-value
Chlorhexidine (2%)	Fluoride (0.2%)	0.581
	Green tea (5%)	0.007
Fluoride (0.2%)	Chlorhexidine (2%)	0.581
	Green tea (5%)	0.080

*Statistically significant at 0.05 level (ANOVA and post hoc Tukey test).

4- DISCUSSION

This study showed that all three gel types (chlorhexidine2%, green tea 5% and fluoride 0.2%) were effective in reducing cariogenic bacteria. Green tea revealed a more durable effect and was mostly accepted regarding taste. Dental caries is a common dental disease and *S.mutans* and Lactobacillous are among the main cariogenic microorganisms (15). *S.mutans* is known as the main cariogenic bacteria. It tolerates an acidic environment, produces organic acid and creates a biofilm (16). *S,mutans* is also related to the chronicity of the disease (17). The decrease of *S.mutans* is associated to lower dental caries. Lactobacillus are among other bacteria involved in dental caries specially dentinal cavities and early childhood caries (18). The count of *S.mutans* in saliva is known to be useful in predicting caries and the transmission risk of the disease (19).

The present study utilized the real-time quantitative PCR (RT-qPCR) method to calculate the count of salivary *S. mutans* and Lactobacillus after application of fluoride, chlorhexidine and green tea extract gel. RT-qPCR is a simple and highly accurate technique for counting bacteria. Many studies have used RT-qPCR to find the association between different species of bacteria and dental caries (20-22). Because of high accuracy along with simplicity, this technique can be an alternative to previous methods; including bacterial culture. Another reason for the superiority of this method is that it does not need live bacteria to give an accurate count of the bacteria. In addition 50% of oral bacteria cannot be cultured in vitro and are only detectable by PCR methods (19). In this study, all gel types were effective in reducing both S.*mutans* and Lactobacillus counts 30 minutes and a week after application. The antibacterial characteristics of chlorhexidine and fluoride has been reported extensively in literature (23-25). Similar to this study, Tehrani et al. concluded that the use of fluoride and green tea as mouthwashes was effective in reducing salivary levels of both *S.mutans* and Lactobacillus after two weeks (9). Another recent study carried out on an adult population used qPCR method to reveal that the regular use of a brewed or commercially available green tea mouth wash resulted in a lower *S.mutans* count (26). The oral health beneficial characteristics of green tea are attributable to tea catechins that contribute to a less acidic plaque formation. Also, it serves a bactericidal function which inhibits bacterial aggregation. Chatechins are also known to suppress the genes responsible for bacterial virulence (27).

The two by two comparison of the three gels showed that they did not differ regarding the count of *S.mutans* 30 minutes post application. In contrast, after one week the group that had received green tea extract gel had significantly lower *S. mutans* count compared to those who had received fluoride or chlorhexidine gel. In contrast, after the administration of green tea and sodium fluoride mouthwashes twice a day for two weeks, Tehrani found no significant difference between the two mouthwashes regarding the mean count of *S. mutans* and Lactobacillus (9). The reason for this difference in results can be attributed to the difference in bacterial counting methods. In this study real-time qPCR method was used to quantify bacteria and the latter study was carried out using culture methods. The other cause can be the difference in the type of intervention. We used a gel form of antibacterial compounds and Tehrani used these compounds as mouthwashes. Additionally, there was a difference in the concentration of the compounds used. Thomas et al. also compared the antibacterial effect of green tea with chlorhexidine on *S. mutans* and Lactobacillus and reported that both compounds caused a significant reduction in *S. mutans* and Lactobacillus, but green tea had a greater effect on *S. mutans* (12). Similar to the studies mentioned above (9, 12), in this study, fluoride, green tea and chlorhexidine also reduced salivary lactobacillus and they did not present significant differences in reduction. In this study, the count of *S.mutans* a week post application of green tea extract gel was significantly lower than that of chlorhexidine and fluoride gel. Similarly, In a study by Lobo et al, after application of chlorhexidine 1% gel, the pediatric salivary *S. mutans* count started to increase on the fifteenth day after intervention (28).

This shows that green tea may have a more durable effect than chlorhexidine and fluoride. According to the findings of this study, green tea had the highest flavor acceptability, and chlorhexidine had the most unfavorable flavor. Thomas et al. similarly found that green tea mouthwash had a much higher flavor acceptance than chlorhexidine (12). On the other hand, side effects such as taste alterations and tooth staining are also reported for this compound (29). Findings revealed the effect of green tea on reducing the count of *S. mutans* and Lactobacillus bacteria with higher durability than other prevalent compounds. In addition, green tea had a higher flavor acceptability in children compared to chlorhexidine and fluoride, which is an important factor in obtaining a child's cooperation. Considering the lower cost and availability of this compound for everyone, the green tea can be suggested as an effective antibacterial compound, especially in the children. However, studies with a larger sample size and a longer follow-up period are suggested to achieve conclusions that are more definite.

4-1. Limitations of the study

Participants were recruited among those who referred to the pediatric dental clinic of Kerman University of Medical Sciences and patients attending other dental clinics and private practice offices were not included. Also this was a single blind clinical trial study and the researcher was aware of the type of gel applied to teeth and this might have contributed to a bias.

5- CONCLUSION

Green tea extract gel was effective on reducing cariogenic bacteria for a longer time compared to fluoride and chlorhexidine gel. The taste of green tea gel was also more acceptable. Therefore it might be considered for replacing routine preventive procedures in children.

6- ABBREVIATIONS

RT-qPCR: Real-Time quantitative Polymerase Chain Reaction,

VAS: Visual Analogue Scale.

7- ACKNOWLEDGMENTS

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8- CONFLICT OF INTEREST: None.

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