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Original Article

Comparing *Streptococcus mutans* and *Lactobacillus* colony count changes following green tea mouth rinse or sodium fluoride mouth rinse use in children (Randomized double-blind controlled clinical trial)

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ABSTRACT

Background: Green tea contains phenolic compounds which could be considered as an anticariogenic agent. In addition, there has not been any significant side effect reported compared to sodium fluoride. So it seems that any comparison between the effects of green tea extract on the level of cariogenic bacteria with sodium fluoride is beneficial. The purpose of this study was to compare the effect of sodium fluoride and green tea mouth rinses on the level of salivary *Streptococcus mutans* and *Lactobacillus* of children.

Materials and Methods: In a double-blind randomized controlled parallel study, 60 children (8- to 12-year old) were selected according to inclusion criteria and were randomly divided into two groups. Subjects were instructed to rinse their mouth with 0.05% sodium fluoride mouth rinse or 0.5% green tea mouth rinse, twice a day for 2 weeks. Before intervention and after 2 weeks, salivary levels of bacteria were measured. Bacterial level changes were compared using *t*-test ($\alpha = 0.05$).

Results: Independent *t*-test showed no significant differences in the average number of bacterial colonies before and after intervention in both groups ($P>0.05$). According to the paired *t*-test there was a significant difference between the mean number of bacterial colonies, before and after intervention, in each group ($P<0.05$).

Conclusion: Green tea mouth rinse resulted in significant reduction of colony number of salivary *S. mutans* and *Lactobacillus* which is comparable with sodium fluoride mouth rinse. Due to fewer side effects, it seems that green tea can be used with less concern compared to sodium fluoride in children.

Key Words: *Camellia sinensis*, *Lactobacillus*, mouth wash, sodium fluoride, *Streptococcus mutans*

INTRODUCTION

Dental caries is one of the most common chronic diseases among children. The main bacterial agents in caries development are *Streptococcus mutans* and *Lactobacillus*. Reducing these microorganisms causes a significant decrease in dental caries.[1]

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Plaque-induced caries is a local disease; therefore, local use of antimicrobial agents is more efficient than their systemic use.[2] Fluoride is an established antimicrobial agent, and due to its anticariogenic and remineralization characteristics, it is extensively used in prevention of dental caries.[3] In addition, a high amount of systemic fluoride application is toxic.[4] Green tea is reported to be an efficient agent in the prevention of dental caries. Polyphenols, particularly catechin, account for the highest proportion of green tea phenolic components and are responsible for most of its useful characteristics.[5] It was shown that green tea can also prevent cardiovascular diseases,[6] stroke,[7] obesity,[8] and cancer.[9] No specific side

effect has been reported due to its use.[10-13] Several studies have indicated that bioactive components of green tea are able to influence the process of caries formation through several different mechanisms: they may inhibit proliferation of the *Streptococcal* agent, interfere with the process of bacterial adhesion to tooth enamel, and act as inhibitors of glucosyl transferase and amylase.[14-17] In a double–blind clinical trial, it has been indicated that green tea extract containing polymerized polyphenols leads to significant inhibition of dental plaque deposition.[18] Liu *et al*. found that green tea's tablets significantly inhibit dental plaque deposition.[19] Signoretto *et al*. showed that drinking tea and coffee causes significant inhibition of dental plaque deposition and also decreases *S. mutans* and *Lactobacillus* in dental plaque and saliva.[20] The findings of a cohort study conducted on 25078 volunteers indicated an association between green tea consumption and decreased rate of tooth loss.[21] Awadalla *et al*. showed statistically significant reduction of *S. mutans* count in saliva and dental plaque, and gingival bleeding index using green tea. This study supports the effectiveness of local application of green tea as antibacterial and anticariogenic material, so it could be cost-effective caries prevention measure especially in developing countries.[22]

Regarding mentioned findings and the fact that green tea seems safe and has fewer side effects compared to sodium fluoride, it seems that a comparison between the effects of green tea extract with sodium fluoride (as gold standard of caries prevention) on the level of cariogenic bacteria is beneficial and logical. The purpose of this study was to compare the effect of sodium fluoride mouth rinse and green tea mouth rinse on the level of salivary *S. mutans* and *Lactobacillus* of children.

MATERIALS AND METHODS

This prospective study had a randomized, double-blind, controlled clinical trial with two parallel groups. The study was approved by the school of Dentistry Ethics committee at the Isfahan University of Medical Sciences, Iran (granted 390013). To conduct the study, 60 children aged 8-12 year old (22 girls and 38 boys) who referred from different schools of Isfahan city to Oral and Dental Health Center for oral health examination were selected according to inclusion criteria. Children and their parents were given verbal and written information, and they signed informed consent. The inclusion criteria were included: 1-A non compromised oral health (brush the teeth twice a day), 2-No untreated active carious lesions, 3-No signs of either gingivitis or periodontal disease, 4-No history of systemic antibiotic use or topical fluoride treatment within 4 weeks prior to baseline, 5-No regular xylitol chewing gum, tea, coffee, or cocoa habit, 6-No systemic diseases, 7-Absence of orthodontic appliances. According to initial salivary *Streptococcus* count, participants were randomly allocated into two groups which were through the following process: the salivary samples were collected and the levels of salivary *S. mutans* were measured. The names of children having similar bacterial count were written on small pieces of paper by a man who did not involve in this study, and mixed together and were randomly allocated into two groups *(n*=30). The information about each subject's demographic characteristic, health, medication, habits and dental care was recorded through a questionnaire at baseline and was repeated at the end of the study. Both kinds of mouth rinses were bottled and coded in similar containers (250 ml), so that the subjects, investigator, and the staff of microbiologic laboratory were blinded about the kind of mouth rinse throughout the study. The children were asked to rinse their mouth after brushing at morning and night, with 20 ml of the mouth rinse containing 0.05% sodium fluoride or 0.5% green tea, for 60 s, twice a day, for 2 weeks. After each application, they were requested not to eat or drink for 1 h. Children's proper application of mouth rinse was supervised by their parents. After 2 weeks of regular application, the participants were instructed to stop using mouth rinses and salivary samples were collected 24 h afterward and the levels of salivary *S. mutans* and *Lactobacillus* were determined. It must be noted that the participants were given the same tooth brush and fluoride tooth paste to brush their teeth twice a day during the study. The subjects were requested not to use xylitol-containing products, tea, coffee, cocoa, systemic antibiotics, and topical fluoride treatment for 4 weeks before and during the study. They were also asked to report any change in health status or medicine being used. Any participant violating the rules would be excluded.

Preparation of mouth rinses

Green tea (Golestan Company, Iran) was purchased and grounded to desirable size using an electrical mill. The green tea was then extracted by percolation method, using ethanol 70% (Yasan Company, Iran) as

solvent. To prepare green tea mouth rinse containing 0.5% phenolic compound, green tea extract containing 6% phenolic compound was diluted by double-distilled water. In order to prepare fluoride mouth rinse containing 0.05% sodium fluoride, 10 g of sodium fluoride (Merk Company, Germany) dissolved in double-distilled water. Finally, by addition of strawberry color (20 g/l, Authorized additive Company, Iran), strawberry flavor (5.4 ml/l, Oils and color Abyaz chemical Company, Iran) and aspartame sweetener (4 g/l, Condrel Company, Germany) to green tea and sodium fluoride solutions and mixing well, both mouth rinses were prepared.

Fluoride analysis of mouth rinses

Fluoride content of prepared green tea and sodium fluoride mouth rinses, and also fluoride content of 0.05% sodium fluoride mouth rinse (Oral-B, UK) available in the market was determined. After adding 0.1 ml TISABIII to 1 ml of solution in three samples by the fluoride ion specific electrode (Ion selective combination fluoride electrode, Orion, USA), fluoride ion was examined. The results were observable on monitor screen in part per million (ppm) (Ion meter 97^{A+}, Orion, USA). The results indicated that prepared green tea and sodium fluoride mouth rinses contain 1.4 and 221 p.p.m fluoride, respectively. The fluoride content of prepared sodium fluoride mouth rinse was similar to that of 0.05% sodium fluoride (Oral-B) available in the market.

Saliva samples

Due to more constant salivary microbial count than plaque, saliva samples were collected in preference to plaque sample.[23-26] Saliva sampling from each subject was carried out before and after intervention. Possible fluctuations in saliva microbial counts, which usually occur throughout the day,[27] were controlled by sampling saliva at the same hour (between 7.30 and 8.30), 1 h after breakfast before tooth brushing. Saliva samples were collected using sterile cotton stick that was soaked at the bottom of the mouth for 5 min and cotton was transferred to 10 ml sterile tubes immediately. Saliva sampling steps for all participants were carried out by one practitioner. An experienced dentist examined subject's oral health according to WHO criteria[28] and recorded number of decayed/missed/filled primary and permanent teeth (DMFT).

Microbial evaluation

The microbial analysis process was commenced within 45 min after sample collection. For microbiological analysis, 20 µl of saliva sample were spread on mitis salivarius agar (Difco)[29] supplemented with 0.2 U/ml bacitracin and sucrose (15% w/v) for *S. mutans* count. Also 20 µl of saliva samples were spread on Rogosa agar (Unipath, Basingstoke, UK) for the count of total *Lactobacilli*. Both groups of plates were incubated anaerobically (85% N_2, 5% CO_2, and 10% H_2) into chambers at 30°C for 3 days. The colony forming units (CFU) were identified by morphology, size and color, and were counted using a stereomicroscope.

Statistical methods

The analyses were processed by SPSS (11.5 Chicago, IL, USA). A comparison of bacterial counts within groups from baseline to follow up was performed with a paired *t*-test. An independent *t*-test was used to compare changes in the bacterial levels during the intervention between groups. Critical *P* values of significance were set at 0.05.

RESULTS

Totally 60 subjects (36.7% girls and 66.3% boys) completed the trial. The mean age of subjects was 9.6±1.31 years. At baseline, before any intervention, 88.3% of all subjects exhibited levels of ≤6000 CFU of salivary *S. mutans* and 87.1% of all subjects showed levels of ≤ 500 CFU of salivary *Lactobacillus*. After 2-week intervention, 99.8% of subjects exhibited decreased levels of the *S. mutans* count in sodium fluoride mouth rinse group and 98.7% in green tea group. There was also 83.3% decrease in *Lactobacillus* count for both groups. An independent *t*-test showed no significant differences in the average number of bacterial colonies before and after intervention in both groups [Table 1]. According to the paired *t*-test, there were significant differences between the mean number of bacterial colonies, before and after intervention, in each group [Table 2]. No side or adverse effects were reported during the course of the study. Two mouth rinses were well accepted by the participants and no one in the study could identify the kind of the mouth rinse. The compliance was monitored and was excellent in the subjects that remained to the end of the study.

DISCUSSION

The purpose of this study was to evaluate the effect of sodium fluoride and green tea mouth rinses on the level of salivary *S. mutans* and *Lactobacillus* of children. Sodium fluoride is regarded as a gold

Table 1: Comparison of bacterial level changes between the two groups

Intervention group	Before intervention			After intervention		
	Green tea	Sodium fluoride	*P* value	Green tea	Sodium fluoride	*P* value
Bacterial type						
Streptococcus mutans (CFU/ml) Mean±SD	103.3±459.7	33.3±182.6	0.443	4706.7±3854.7	5225±6764.8	0.717
Lactobacillus (CFU/ml) Mean±SD	150±116.7	255±367.9	0.145	536.7±483.8	511.7±495.1	0.844

CFU: Colony forming units

Table 2: Comparison of bacterial level changes within two groups

Intervention group	Sodium fluoride			Green tea		
	Before intervention	After intervention	*P* value	Before intervention	After intervention	*P* value
Bacterial type						
Streptococcus mutans (CFU/ml) Mean±SD	5225±6764.8	33.3±182.6	<0.001	4706.7±3854.7	103.3±459.7	<0.001
Lactobacillus (CFU/ml) Mean±SD	511.7±495.1	255±367.9	0.015	536.7±483.8	150±116.7	<0.001

CFU: Colony forming units

standard of caries prevention. It is extensively used to prevent and treat dental caries due to its anticariogenic and remineralization properties.[3] In addition, the studies showed that bioactive components of green tea are able to influence the process of caries formation by several different ways.[14-17] The results of this research indicated that before any intervention, there were no significant differences in the average number of salivary *S. mutans* and *Lactobacillus* between two groups. So it was possible to make a comparison between the effectiveness of two different mouth rinses on the mean number of cariogenic bacteria.

In the present study, sodium fluoride mouth rinse caused a significant reduction of salivary *S. mutans* and *Lactobacillus*. This finding is in agreement with several previous reports on fluoride application. Fluoride prevents dental caries through different processes: It inhibits adhesion of *S. mutans* to the tooth structure and therefore inhibits insoluble dextran production by this bacteria, it inhibits tooth demineralization and also remineralizes incipient carious lesions.[30] In this study, green tea mouth rinse also led to statistically significant reduction of salivary *S. mutans* and *Lactobacillus*. This result is consistent with previous studies on effectiveness of green tea on cariogenic bacteria. It was shown that green tea polyphenols inhibit growth of *S. mutans*, *Streptococcus sobrinus*, and *Lactobacillus*, significantly.[17] The polyphenolic compounds prevented the attachment of *S. mutans* to tooth structure through modification of its phenotype.[31] It also has been found that catechin present in green tea inhibits glucosyl transferase and leads in significant reduction of the plaque index.[14]

Comparing the results of this study, there were no significant differences between the effect of green tea and sodium fluoride mouth rinses on the number of salivary *S. mutans* and *Lactobacillus*. The base of both mouth rinses was similar in solvent, flavor, color, etc and the difference was just only between their effective substance (sodium fluoride and green tea polyphenols). So the probability of base effect on bacterial count was eliminated. In addition, fluoride analysis of green tea showed that its fluoride content was negligible, and therefore, its effect on bacterial count may not be attributed to its fluoride content. An *in vitro* study showed that green tea polyphenols are not effective on enamel remineralization. This finding supports the proposition that green tea polyphenols exert an anti-caries effect via an anti-microbial mode-of-action.[32] As the most important benefit of fluoride is remineralization of incipient carious lesions, it is the treatment of choice in the case of tooth remineralization. More studies are required on the effect of green tea polyphenol on enamel remineralization. Furthermore, an *in situ* study showed that there is no significant difference between the effect of 0.6% green tea solution and fluoride on the prevention of dentin erosion and abrasion. In fact, green tea polyphenols, especially catechin, inhibit matrix metalloproteinase which is localized in saliva and dentin and could be responsible for the matrix degradation in dentinal carious lesions.[33] So green tea polyphenols may decrease the rate of dentinal decay. A further study is required in this field.

The findings of a study on 6-16 year old children showed that regular daily consumption of green tea rich in catechin (576 mg per can) had no side effect on children's health. However, it can decrease obesity and cardiovascular risk factors in fat children.[11] There have been other studies on catechin safety and no side effects have been found.[10,12,13] Whereas high amount of fluoride ingestion may lead to acute poisoning and its low repeated ingestion causes fluorosis especially in children. So sodium fluoride mouth rinse is not recommended for children younger than 6 years who may swallow it,[4] so this study is conducted on 8-12 year old children.

It has been shown that earlier the colonization of *S. mutans* in the mouths of children, the higher the caries prevalence at 4 years of age. In fact, the earlier transmission of *S. mutans* occurs, the higher the caries risk at older ages.[34,35] Therefore, prescribing a safe and harmless antibacterial factor in children has an important role in prevention of dental caries at older ages. Regarding the results of this study and fewer side effects of green tea in comparison to sodium fluoride, it seems that green tea can be used with less concern compared to sodium fluoride mouth rinse in children. Further studies are recommended in this field and also for green tea application in children younger than 6 year old.

CONCLUSION

The results of the present study showed that green tea mouth rinse resulted in significant reduction of colony number of salivary *Streptococcus mutans* and *Lactobacillus* which is comparable with sodium fluoride mouth rinse.

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