

Original Article

Antimicrobial Effect of Toothpastes Containing Fluoride, Xylitol, or Xylitol-Probiotic on Salivary *Streptococcus mutans* and *Lactobacillus* in Children

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ABSTRACT

Aim: This *in vivo* study was designed to compare the antimicrobial effect of toothpastes containing fluoride, xylitol, or xylitol-probiotic on *Streptococcus mutans* and *Lactobacillus* in 13-15-year-old children. **Materials and Methods:** The study consisted of 60 pediatric patients who were randomly divided into three groups of 20 each. Group 1 received fluoride toothpaste (Colgate Max Fresh), group 2 used xylitol toothpaste (Xyliwhite), and group 3 used xylitol-probiotic toothpaste (PerioBiotic). Participants were asked to use the allocated dentifrice two times a day for 6 weeks. We performed tests on the saliva samples at the beginning of the study and after 6 weeks' duration following the use of toothpaste. The Statistical Package for the Social Sciences version 15 software and chi-square test were used for data analysis. **Results:** PerioBiotic toothpaste and Colgate Max Fresh toothpaste showed significant reduction in the number of participants who, prior to commencing the study, were diagnosed with a high number of cariogenic bacteria. On the contrary, no significant changes were observed with respect to the number of participants with a very high number of *S. mutans* and *Lactobacillus* on Xyliwhite toothpaste group. **Conclusion:** In the light of the data obtained in this *in-vivo* study, a new innovational aspect has been reached in the use of probiotics to ensure the balance between bacterial flora in the oral cavity. In daily routine, the administration of probiotics to children is difficult. The toothpaste can be used as a vehicle to transport probiotics to children's mouth.

KEYWORDS: *Lactobacillus*, *Streptococcus mutans*, toothpaste

Date of Acceptance:

12-Jan-2017

INTRODUCTION

There is an imbalance between the loss and gain of the minerals; therefore, together with the cariogenic bacteria, dental caries appear.^[1] Dental caries start and proceed with the influence of *Streptococcus mutans* and similar predisposing factors, which are among the salivary microflora.^[2] There are various microorganisms that have been identified to have the potential of being highly cariogenic; however, extensive studies have been conducted mostly on *S. mutans* and *Lactobacillus*.^[3]

The initiation of caries has a close relation with *S. mutans*. On the other hand, further progress of carious lesions has a relation with *Lactobacillus*.^[4] Throughout

history, brushing and flossing have been considered as the golden standard in controlling the plaque and preventing dental caries.^[5] According to recent developments, the oral microbial ecology is modulated for the purpose of being able to control the dental caries and dental diseases that appear due to microbial activity. Probiotics are used in toothpastes and mouth-rinses as mechanical methods and have significant effects in oral health as well as in preventing oral caries and diseases.

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How to cite this article: Maden EA, Altun C, Ozmen B, Basak F. Antimicrobial Effect of Toothpastes Containing Fluoride, Xylitol, or Xylitol-Probiotic on Salivary *Streptococcus mutans* and *Lactobacillus* in Children. Niger J Clin Pract 2018;21:134-8.

Access this article online	
Quick Response Code:	Website: www.njcponline.com
	DOI: 10.4103/njcp.njcp_320_16

If probiotics, which are in fact living organisms, are used in sufficient amount, they provide health benefits.^[6] Studies have been conducted *in vivo* thus far, and it has been claimed according to the conclusions that probiotic *Lactobacillus* and bifidobacteria strains decrease the caries, which are related with the bacteria in the saliva.^[7] In the light of these data, the innovative dentistry technology has presented a new product which is made from *Lactobacillus paracasei*, which is a probiotic microorganism strain; this new product is named PerioBiotic Probiotic Toothpaste. This newly-developed product, which is a therapeutic innovation, can be beneficial in the war against dental caries.^[8] The content of the PerioBiotic Probiotic toothpaste binds and eliminates salivary microbial counts (*S. mutans* and *Lactobacillus*) in the mouth.^[8]

The influence of the fluoride in preventing caries is a well-known fact. Among the anti-caries substances, there are F-dentifrices, which are also widely-known.^[9] In many developed countries, reduction in caries has been achieved with toothpastes containing fluoride.^[10] Dentifrices that contain fluoride are recommended for everybody from every age group.^[11] Fluoride is plentiful in the mouth and has considerable bioavailability because of its solubility in ionic (free) form (F⁻ of NaF) or in ionizable form (MFP). The anticaries activity is ensured by the bioavailable form of fluoride.^[12]

Chewing-gums and candy contain xylitol, a natural sugar alcohol which is used as a sweetener.^[13] To prevent caries, the use of xylitol is approved by the American Academy of Pediatric Dentistry (AAPD).^[14] Gummy bears, syrup, and gum have been used in tests conducted in young children as several xylitol delivery modalities.^[15] The fact that xylitol products have bacteriostatic effect on *S. mutans* has been documented in clinical trials.^[16] Only one study, which was conducted *in vitro*, claims that xylitol toothpastes have a potential in preventing tooth decays.^[17] The clinical evidence at this stage is conflicting.^[18] In the literature, there are no studies evaluating the effects of toothpastes with fluoride, xylitol, or xylitol-probiotics on *S. mutans* in the saliva of young children. The purpose of this study is to compare the effects of fluoride, xylitol, or xylitol-probiotics on levels of *S. mutans* and *Lactobacillus* in the saliva of children who were between the ages of 13 and 15.

MATERIAL AND METHODS

This research study was conducted on a sample of 60 patients, children 13–15 years of age, whose parents volunteered after receiving verbal and written information over a 6-week period. Sample size ($n = 20$) was calculated using power analysis and data from a previous

publication. None of the participants had received any antibiotic or antimicrobial agent or professional fluoride therapy within the last month. Other exclusion criteria included individuals with a habitual use of dairy probiotics or xylitol chewing gums. The participants had good oral health with no active and untreated carious lesions or signs of periodontal disease. The study protocol was approved by Ethics Committee (B.10.4.ISM.4.06.68.49). All volunteers were asked to read the informed consent form, and to sign it if they agreed to its contents. The participants were randomly divided into the groups (each comprising 20 children): Group 1-fluoride toothpaste (Colgate Max Fresh, Colgate-Palmolive, New York, USA), Group 2-xylitol toothpaste (Xyliwhite, Now Foods, Bloomingdale, IL), and Group 3-xylitol-probiotic toothpaste (PerioBiotic, Designs for Health, Arlee, MT). Patients were provided with toothbrushes of medium hardness and were instructed to brush their teeth thoroughly two times a day for 2 minutes using their allocated toothpastes and a modified Bass brushing technique. During the study duration, participants were restrained from using any other toothpaste or oral product from another supplier and were instructed not to change their oral hygiene habits. The levels of *S. mutans* and *Lactobacillus* were evaluated by CRT bacteria (Vivadent, Schaan, Liechtenstein) at the baseline and after 6 weeks of intervention. The participants were instructed not to eat or drink for 2 hours before collection saliva and to brush their teeth only once in the morning on the day of the sample collection. Two millilitre of stimulated saliva was collected into a sterile plastic cup for 5 min during paraffin-wax chewing. The saliva was transferred to a selective culture medium in glass tubes and then was incubated at 37°C for 48 h. After incubation, the colony forming units score of *S. mutans* and *Lactobacillus* were obtained by comparing the test strip with an evaluation chart provided by the manufacturer [low *S. mutans* and *Lactobacillus* count ($<10^5$) or high *S. mutans* and *Lactobacillus* count ($\geq 10^5$)].

The Statistical Package for the Social Sciences version 15 program was used to evaluate the collected data. The data were gathered by using forms that were prepared for this specific purpose. The evaluation of the differences in the beginning and after the 6-week duration among the groups were made by using the chi-square test.

RESULTS

All the study participants stated that they brushed their teeth well twice a day by using the toothpastes given to them for 6 weeks. Tables 1 and 2 present the pretreatment and posttreatment levels of *S. mutans* and *Lactobacillus* in the saliva. Majority of the participants

had high counts of salivary *S. mutans* and *Lactobacillus* at baseline. When the number of cariogenic bacteria (*S. mutans* and *Lactobacillus*) at the start of the PerioBiotic Probiotic toothpaste usage were compared with the results seen after 6 weeks, an important decrease was observed in the *S. mutans* and *Lactobacillus* bacteria according to the CRT tests. After the 6-week use of Probiotic toothpaste, the *S. mutans* ($\geq 10^5$) decreased to 20% from 75% in the group with *S. mutans* [Figure 1]. In addition, there was a decrease in the number of participants with *Lactobacillus* ($\geq 10^5$) from 60% to 30% after 6 weeks of application [Figure 2]. In addition, an important decrease was recorded in the participants with the *S. mutans* percentage (80–45%) and *Lactobacillus* (70–55%) after using the Colgate Max Fresh toothpaste for 6 weeks. On the other hand, it was determined using the chi-square test that there were no important changes between the Xyliwhite toothpaste group and the other

groups regarding *S. mutans* (80–75%) and *Lactobacillus* (75–65%). It was also determined after the comparison of the groups that the decrease in the *S. mutans* was higher than the group using PerioBiotic Probiotic toothpaste at a significant level when compared with the Colgate Max Fresh toothpaste and Xyliwhite toothpaste. *S. mutans* were more sensitive to the antimicrobial activity of the PerioBiotic Probiotic toothpaste and to the Colgate Max Fresh toothpaste when compared with the *Lactobacillus*.

The results obtained in our study has shown that, if the PerioBiotic Probiotic toothpaste and Colgate Max Fresh toothpaste are used twice a day, a decrease in the number of the bacteria in the saliva was observed after 6 weeks. Brushing with xylitol toothpaste is no more efficacious in reducing *S. mutans* and *Lactobacillus* levels than a fluoride or xylitol-probiotic toothpaste in 13-15-year-old children.

Table 1: Chi-square test for *S. mutans* before and 6 weeks after using toothpaste

Groups		Streptococcus mutans count		p
		< 10 ⁵	≥ 10 ⁵	
Colgate Max Fresh	Initial visit (prior to using toothpaste)	4	16	0,016
	6 weeks after using toothpaste	11	9	
Xyliwhite	Initial visit (prior to using toothpaste)	4	16	1,000
	6 weeks after using toothpaste	5	15	
PerioBiotic	Initial visit (prior to using toothpaste)	5	15	0,001
	6 weeks after using toothpaste	16	4	

Table 2: Chi-square test for *Lactobacillus* count before and 6 weeks after using toothpaste

Groups		Lactobacillus count		p
		< 10 ⁵	≥ 10 ⁵	
Colgate Max Fresh	Initial visit (prior to using toothpaste)	6	14	0,250
	6 weeks after using toothpaste	9	11	
Xyliwhite	Initial visit (prior to using toothpaste)	5	15	0,688
	6 weeks after using toothpaste	7	13	
PerioBiotic	Initial visit (prior to using toothpaste)	8	12	0,031
	6 weeks after using toothpaste	14	6	

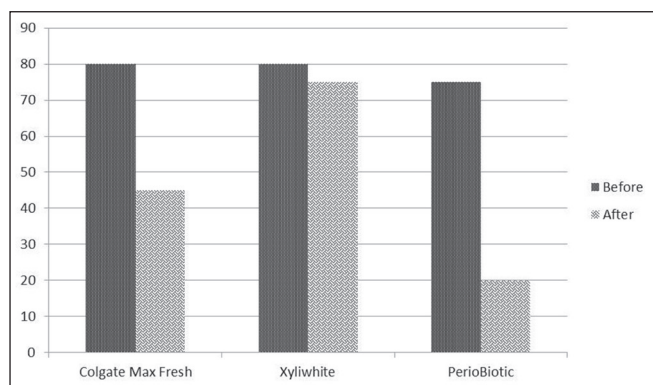


Figure 1: *S. mutans* counts at first (prior to using tothpaste) and 6th check-up

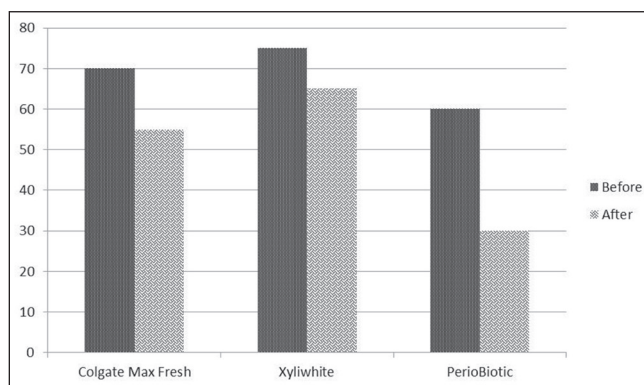


Figure 2: *Lactobacillus* counts at first (prior to using tothpaste) and 6th check-up

DISCUSSION

This study has been performed to determine whether the three potential anticaries agents, i.e., fluoride, xylitol, and probiotic bacteria would improve the suppressive effect on bacteria related with caries in the saliva. It is believed that *S. mutans* and *Lactobacillus* bacteria, which produce acids, have significant roles in the onset and development of dental caries.^[7] According to the definitions of the World Health Organization (WHO) and the American Food and Agricultural Organization (FAO), probiotic bacteria are living organisms that are useful for health when taken in sufficient amounts.^[19] There are many microorganisms that are called probiotics; *Lactobacillus* and *Bifidobacterium* are common probiotics. With the bacterial resistance against antibiotics being observed commonly, the idea of using probiotic treatment for oral health has emerged. Dental cavities, periodontal diseases, *Candida albicans* infections, and halitosis are among the target oral diseases that can be focused on.^[19,20]

Probiotics and their effect on oral health have been a focus of numerous trials in recent times. No documented trials have been reported with its focus on probiotic toothpaste use, especially in the pediatric population. Many clinical studies have demonstrated that the regular consumption of milk and cheese, which contain probiotics, leads to the decrease in the number of the cariogenic *Streptococci* in the saliva and dental plaque.^[21,22] Nikawa *et al.*^[22] reported that the consumption of yogurt containing *Lactobacillus reuteri* for more than 2 weeks led to a decrease of more than 80% in the *S. mutans* concentration in the saliva. Similar results have been reported in studies conducted on chewing gums and lozenges that contained probiotics.^[23,24] Majstorovic *et al.*^[24] conducted a study that dealt with probiotic pastes in 2013, and reported that the probiotic toothpaste that contained *Lactobacillus paracasei* probiotic strains led to a reduction of *S. mutans* in the saliva, which presented a new concept for oral hygiene.

The *Lactobacillus paracasei* has an advantage in terms of requiring 10 seconds in the oral cavity to become active.^[22] It has been reported in various studies conducted that there had been nearly 50% decrease in the *S. mutans* bacteria after the use of *Lactobacillus paracasei* mouthrinse.^[24] After the participants used the *Lactobacillus paracasei* candies, it was reported that there was a decrease in the number of the *S. mutans* bacteria in the oral cavity.^[24] In the light of this study, it has been proven that regular use of probiotic toothpaste influences the number of the plaque as well as the accumulated *S. mutans* and the *Lactobacillus* bacteria. These bacteria have been proven to have cariogenic effects and always to be active in the saliva. The probiotic activity of the

good bacteria, which are always present in the saliva, has been used as a proposed mechanism.

In a study conducted by Chi *et al.*,^[25] in 2014, it was reported that the traditional method of brushing the teeth with a soft xylitol/fluoride toothpaste is accepted as being no more influential in decreasing the caries of the level of the *S. mutans* when compared with the use of fluoride-only toothpaste in children who are at the highest risk. It has been demonstrated in various *in-vitro* studies and the studies that were conducted on animals that fluoride is effective on the xylitol/fluoride toothpaste against *S. mutans*.^[25] On the other hand, Randall *et al.*^[26] stated that fluoridated toothpastes and other components of toothpastes such as triclosan and sodium lauryl sulphate exhibited antibacterial activity against *S. mutans*.

A Cochrane Review was conducted on the fluoride toothpastes and it was reported that 14% increase was detected in the prevented fraction in teeth when the teeth were brushed twice a day when compared with the once-a-day usage of the fluoride toothpaste.^[27] These data show the significance of brushing the teeth twice a day and its importance in future studies. Regarding the type of the fluoride, the common toothpaste used in our study contained sodium fluoride. According to previously conducted studies, sodium fluoride is more effective in preventing caries in children when compared with the sodium monofluorophosphate.^[28]

Xylitol is used in food as a sugar substitute and does not lead to decrease in *S. mutans* salivary level.^[17] It must be used in full contact with the surface of the teeth in a frequent and direct manner to obtain the maximum effect.^[17,29] A study has been conducted among children. Children were made to brush their teeth twice a day with xylitol toothpaste (0.2 g xylitol/day). According to the results of the study, the children had low levels of salivary and plaque *S. mutans* after 6 months.^[18] There is triclosan in the toothpastes that contain xylitol, and therefore, confusion has appeared. It has been inferred from other similar studies, in which xylitol lozenge and gum were used that, to reduce *S. mutans* levels, higher dosages of xylitol were needed in the fluoride-xylitol toothpastes.^[30,31] In the study conducted by Chi *et al.*^[25] in 2014, no therapeutic benefits were observed in preventing the caries or in *S. mutans* reduction by brushing the teeth with toothpaste containing xylitol when compared with brushing the teeth with toothpaste which was sold in the malls. The conclusions in our study show similarities with this study in terms of the number of the participants and the amount of *S. mutans* and *Lactobacillus* on Xylitol toothpastes. It has also been concluded that the surfactant, which is an active ingredient in the xylitol toothpaste has

caused the toothpaste to become less active in terms of chemotherapeutical use.

CONCLUSION

This study was aimed to reveal the influence of fluoride, xylitol, or xylitol-probiotic on salivary *S. mutans* and *Lactobacillus* levels of children. Brushing with xylitol toothpaste is no more efficacious in reducing *S. mutans* and *Lactobacillus* levels than a fluoride or xylitol-probiotic toothpaste in 13–15-year-old children. According to the results, PerioBiotic Probiotic toothpaste that was tested is efficient at an important level. This situation may be related with the probiotic substance, which was synthesized and included in the toothpaste. It could be recommended as a therapeutic toothpaste, especially in children who are at high risk for caries.

Financial support and sponsorship

Nil.

Conflicts of Interest

There are no conflicts of interest.

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