Original Article

Comparison of the Effect Of 0.2% Chlorhexidine and Xylitol Plus 920 Ppm Fluoride Mouthwashes on Count of Salivary Streptococcus Mutants, a Pilot Study

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ABSTRACT

Statement of the Problem: Dental caries is a common chronic disease. Mouthwashes and other preventive approaches play an important role in caries prevention. Finding the most efficient mouthwash in the market is always a concern for dentists and patients.

Purpose: This study aimed to assess the effect of chlorhexidine (Behsa, Iran) and xylitol plus 920 ppm fluoride (FX) (Fuchs, Germany) mouthwash on salivary *Streptococcus mutans* (*S. mutans*), which is the main microorganism responsible for dental caries.

Materials and Method: This single-blind randomized clinical trial was conducted on 30 dental students, divided into two groups. The salivary count of *S. mutans* was measured at the beginning of the study. Group 1 students used chlorhexidine mouthwash while group 2 used FX mouthwash for two weeks. Saliva samples were collected again and salivary count of *S. mutans* was determined. Data were analyzed using Mann Whitney and Wilcoxon signed rank tests

Results: Salivary count of *S.mutans* significantly decreased in the two groups after using the mouthwashes ($p< 0.05$) and no significant difference was noted in the mean colony count between the two groups after the use of mouthwashes ($p> 0.05$).

Conclusion: Within the limitations of this study, the results showed that both mouthwashes could decrease *S. mutans* count.

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Introduction

Dental caries is an infectious disease, which is caused by complex interactions of cariogenic oral flora and degradable carbohydrates in diet. [1]

Cariogenic microorganisms such as *S. mutans* and *Lactobacillus acidophilus* (*L. acidophilus*) use carbohydrates for energy production, and organic acids are the byproducts of this reaction. These organic acids cause demineralization in the underlying surface. [2] Microbial flora, time, carbohydrates, and tooth structure are the four main factors playing a role in development of caries. Diet, oral hygiene practice, socioeconomic factors, age, and race are all involved in development of caries. [2-3]

According to the World Health Organization (WHO), 60-90% of students have dental caries. [4] Some studies [5-6] have reported the prevalence of caries to be 30% and 82%, respectively.

Evidence shows that a combination of mechanical and chemical methods is required to prevent caries. The commonly used chemical methods include mouthwashes, toothpastes, gels, and varnishes. [7]

Mouth rinses commonly used include chlorhexidine (CHX), Listerine, fluoride, and xylitol. Clinical studies have shown fluoride and CHX mouthwashes can effectively decrease the count of *S. mutans*. [8] Some of the side effects of CHX include tooth discoloration, impaired sense of taste, and mucosal desquamation. [9]

Xylitol is a five-carbon sugar, which is often used in chewing gums, mouth rinses, and toothpastes, which prevents the proliferation and metabolism of *S. mutans*. Thus, it can decrease its count in the oral cavity. [10]

Studies on the effect of xylitol-fluoride mouthwash in comparison with CHX on salivary *S. mutans* are limited. Thus, this study aimed to assess the effect of FX (Fuchs, Germany) mouthwash (combination of xylitol and 920 ppm fluoride) and 0.2% CHX (Behsa, Iran) on salivary S. mutans.

Materials and Method

This clinical trial (code number: IRCT2016073129133 N1) was conducted on 30 dental students (15 male and 15 female, 20-23 year-olds) selected by convenience sampling in Zanjan Dental School. They were thoroughly informed about the study and willingly signed informed consent forms. The participants had DMFT 5 to 7, had no history of antibiotic therapy in the past three months, and had no history of using mouthwashes or chewing gums containing xylitol. Individuals with any systematic disease, drug usage, mucosal lesions, pregnancy, or oral habits were excluded from the study.

Unstimulated saliva samples were collected using the spitting method. Saliva samples were collected between 9-10 a.m. The participants were requested to refrain from eating, drinking, or tooth brushing for one hour prior to saliva collection. Saliva samples were stored in an icebox and transferred to a laboratory to determine the *S. mutans* count.

The participants were then randomly divided into two groups. Group one received 0.2% CHX mouthwash while group 2 received FX mouthwash, which contains xylitol, Sodium monofluorophosphate, and Limonene and Hydrogenated castor oil. The patients were instructed to use the mouthwash twice a day (15 mL of the mouthwash was used each time for 30 seconds) for two weeks. They were requested not to change their routine oral hygiene practice. In addition, they were requested not to eat or drink or rinse their mouth for 30 minutes after using the mouthwash. At the end of two weeks, saliva samples were collected again. During the study, two patients were excluded because of using antibiotics. Samples were cultured, gram- stained, and evaluated under adequate lighting. In addition, the number of colonies was counted and marked by a marker. Number of counted colonies for each participant was multiplied by the dilution coefficient (which was 100 in this study). The data were reported as colony forming units per one milliliter (CFU/mL) of saliva.

Kolmogorov-Smirnov test was used to assess normal distribution of data. Since the data were not normally distributed, the Mann Whitney test was used to compare the groups before and after using the mouthwashes. The Wilcoxon signed rank test was used for within group comparisons at $p= 0.05$ level of significance.

Results

This pilot study was conducted on 30 dental students; out of which, two were excluded since they used antibiotics during the course of study. The Mann Whitney test showed that the mean bacterial count was not significantly different between the two groups at the beginning of the study. ($p= 0.51$)

Both FX and 0.2% CHX were equally effective on salivary *S. mutans* count and both caused a significant reduction in *S. mutans* count in the saliva.

The difference in bacterial count after using the mouthwashes was not significant between the two groups either ($p= 0.6$). The Wilcoxon test showed that the mean colony count significantly decreased after the use of FX ($p= 0.001$) and CHX ($p= 0.001$) mouthwashes. (Table 1)

Table 1: Mean colony count at baseline and after the intervention in the two groups (CFUs/mL of saliva × 1000)

Mouthwash	Baseline Mean±Standard deviation	After the intervention Mean±standard deviation	*p* Value
FX	15.9±37.3	2.85±2.71	0.001
CHX	1.22±4.1	0.358±0.379	0.001
p Value	0.51	0.65	

Discussion

Currently, mouthwashes are increasingly used to prevent dental caries. A suitable mouthwash should not cause discoloration of teeth or mucosa; hence, it must be non-toxic and have an acceptable taste. However, no mouthwash is available in the market meeting all the required criteria. Therefore, studies are ongoing to find an efficient mouthwash.

Xylitol has been recently incorporated into chewing gums and mouthwashes. [9] This study evaluated the effect of 0.2% CHX and FX mouthwash on salivary *S. mutans*, which is the main microorganism responsible for dental caries. Before testing the efficacy of a material to decrease microbial count, bacterial tests must be performed to determine microbiological variables. Saliva samples can be tested to determine the count of salivary *S. mutans*. [10]

No previous study has evaluated the effect of xylitol and FX mouthwash on *S. mutans* count. Therefore, the results of this study were compared with those of studies on antimicrobial defects of xylitol and fluoride on microorganisms. The results of this study showed that both CHX and FX caused a reduction in *S. mutans* count and no significant difference was noted in antimicrobial efficacy of the two mouthwashes.

Some articles showed that CHX caused a reduction in *S. mutans* count, which was compatible with our findings [3-4]. This effect is because CHX can increase the permeability of cell membrane. In addition, CHX deactivates glycosyltransferase by changing the position of calcium and therefore, prevents the adhesion of bacteria to surfaces. In this regard, it has both bacteriostatic and bactericidal effects. [11]

In this study, the *S. mutans* colony count decreased after the use of mouthwashes. FX is a mouthwash containing xylitol and monofluorophosphate, which has been recently introduced to the Iranian market. Studies on the efficacy of this mouthwash in comparison with other mouth rinses are limited. Sadat Sajadi *et al.* [12] showed that the use of fluoride mouthwash caused a reduction in *S. mutans* count, which was in agreement with our study. It should be noted that the results of Jothika *et al.* [13] and Poureslami *et al.* [14] were different from ours, which may be due to difference in dose of fluoride used, since they used 400-ppm fluoride in their study, which was less effective than CHX. Another reason may be the frequency of using fluoride mouthwash. In our study, FX mouthwash containing 920-ppm fluoride and xylitol was used twice a day for two weeks.

The mechanism of action of fluoride is in such a way that in low concentrations, it inhibits the release of glycosyltransferase. This enzyme increases the adhesion of bacteria to surfaces, since it enables the use of glucose for synthesis of extracellular polysaccharides. In addition, presence of fluoride ion causes deposition of fluorapatite. In addition, it should be noted that xylitol is a five-carbon sugar that prevents the growth and proliferation of *S. mutans*. Moreover, *S.mutans* cannot metabolize xylitol and produce energy, thus, xylitol is accumulated within the cells, and it competes with sucrose to pass through the cell wall. In contrast to sucrose, which provides energy for *S. mutans*, xylitol causes energy loss and results in eventual death of microorganisms and thus, *S. mutans* count decreases in the oral cavity.

Hanno *et al.* [15] and Mojabi *et al.* [2] indicated that xylitol chewing gum and xylitol tablets decrease the salivary count of *S. mutans* and our results are similar to their reported results. It should be noted that in the aforementioned studies, aside from the effect of xylitol, chewing gum stimulates the secretion of saliva, which also plays an effective role in reduction of microorganisms in the oral cavity. However, we only used mouthwashes in our study. Zajkani *et al.* [16] evaluated the effect of Oral-B mouthwash and FX on *S. mutans* and *L. acidophilus in vitro* and showed that both mouth rinses caused a significant reduction in *S. mutans* count, which was in accordance with our findings.

Unfavorable taste of CHX and tooth discoloration following the use of CHX was among the limitations of our study, which could affect the frequency of using this mouthwash by the participants. However, since our study was conducted on dental students, our results are reliable regarding regular use of mouthwashes. In addition, this study was the first to assess the effect of FX mouthwash clinically, which was the strength of our study.

Considering the significant role of *S. mutans* in development of dental caries and also the side effects of CHX, our results showed that FX could be used as an adjunct to other preventive methods to decrease the

prevalence of caries. Future clinical studies with larger sample sizes and different frequency of use and dosage of FX mouthwash are required to obtain results that are more accurate.

Conclusion

Within the limitations of this study, the results showed that both mouthwashes could reduce *S. mutans* level. Thus, Xylitol mouthwash can also be considered as an effective oral hygiene regimen.

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Conflict of Interest

None declared.

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