

Original Research Article

Effect of dentifrices containing sorbitol, combination of xylitol and sorbitol on salivary *Streptococcus mutans* and *Lactobacillus* counts in 14-15 year old children: a randomized trial

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ABSTRACT

Background: Xylitol, a naturally occurring sugar alcohol which cannot be fermented by oral microorganisms has been shown to reduce *Streptococcus mutans* levels in plaque and saliva and to markedly reduce tooth decay. Studies have proved that combination of xylitol and sorbitol added in chewing gum is more effective in reducing tooth decay compared to xylitol alone. Purpose of this double blinded, parallel, randomized trial was to compare the relative effect of sorbitol and combination of xylitol and sorbitol containing dentifrices on salivary *S. mutans* and *Lactobacillus* in a group of high school children.

Methods: Sixty school children with age group of 14-15 years were randomly allocated into 2 study groups (S-sorbitol containing dentifrice and X+S- Xylitol and Sorbitol containing dentifrice), with 32 participants in each group. Subjects were asked to use the experimental dentifrices twice daily for 3 months period. Resting whole saliva was collected at baseline, at the end of 1 month, 2 months and 3 months interval after the use of assigned dentifrice. Saliva samples were used for microbial analysis. *S. mutans* was cultured on Mitis salivarius bacitracin agar and *Lactobacilli* on Rogosa SL agars. Results were expressed in frequencies. Nonparametric tests namely Wilcoxon's signed rank test and Mann Whitney U test were used for testing the statistical significance. The level of significance was set at $p=0.05$.

Results: There was no significant difference in salivary *S. mutans* count amongst the two groups at baseline ($p=0.271$). There was no significant difference in counts of *S. mutans* and lactobacilli among the subjects in the sorbitol containing dentifrices (S) group at different time intervals of the study whereas there was significant reduction in counts of *S. mutans* and *Lactobacillus* count among the subjects in the xylitol and sorbitol (X+S) containing dentifrices at all the time interval. At the end of 3rd month interval there was significantly low *S. mutans* and lactobacilli count for X+S group compared to S group with $p<0.001$ and $p=0.02$ respectively.

Conclusions: The use of dentifrices containing combination of xylitol and sorbitol, twice daily showed significant antimicrobial properties against salivary *S. mutans* and *lactobacillus* by the end of 3 month compared to sorbitol containing dentifrice.

Keywords: Sugar alcohols, Dentifrices, Caries prevention and control

INTRODUCTION

Dental caries is a localized, transmissible infectious process that ends up in the destruction of hard dental

tissues.¹ There is practically no geographic area in the world whose inhabitant does not exhibit some evidence of dental caries. It affects both the sexes, all races, all socioeconomic status and all age groups. It not only

causes pain and discomfort, but also in addition, places a financial burden on the patients. The prevention of dental caries has long been considered as an important task for the health profession.²

Streptococcus mutans is one of the main opportunistic pathogen of dental caries, which plays a central role in fermenting carbohydrates that result in acid production, leading to the corrosion of tooth enamel. In addition, other microflora like *Lactobacillus* species is also associated with active carious lesions.¹ Oral cavity provides an ideal environment for bacterial growth and poor oral hygiene is one of the reasons for accumulation of these microbes and their harmful activities.³ Hence effective oral hygiene plays an important role in reducing dental plaque accumulation and maintaining oral health.

A number of approaches through mechanical and chemical means for improving routine oral hygiene have been documented in the literature. One common approach is the routine use of dentifrices formulated with antimicrobial ingredients like chlorhexidine, Triclosan, essential oils, fluorides and polyols as an important adjunct to help control the dental plaque biofilm.³ A dentifrice is defined as a semi-aqueous material for removing naturally occurring deposits from teeth and is supposed to be used simultaneous with a toothbrush.⁴

According to the World Health Organization expert committee the decline in dental caries prevalence observed in many countries can be attributed to the “widespread use of toothpastes that contain fluoride”. Although fluoride has had a profound effect on level of caries prevalence, it is far from complete cure. It is unlikely that there is any concentration of fluoride, which will eliminate caries totally. This need, has redirected dental research to develop novel preventive agents that can act as an adjunct to fluoride or independent of it. Attempts are on to develop a “non-toxic anti-cariogenic agent” that could be added to toothpaste, mouthwash and food in an approach to lower the caries experience.⁵

Sugar substitutes are one such agent that has been proposed to have anticariogenic properties. These include lactitol, maltitol, mannitol, sorbitol, isomalt, and xylitol and are commonly used in foods to replace sugars.⁶ Dentifrices in the current market usually contain sorbitol and very few contain xylitol as sweeteners.⁷

Sorbitol can be fermented at a slower rate by *S. mutans* and *Lactobacilli* and can serve as a substrate for them.⁶ Xylitol, a naturally occurring sugar alcohol was approved for use in food by the U.S. Food and Drug Administration (FDA) since 1963. Xylitol which cannot be fermented by oral microorganisms has been shown to reduce *S. mutans* levels in plaque and saliva and to markedly reduce tooth decays.⁶

As xylitol does not produce acid, it does not lower the pH of saliva. Xylitol lowers the temperature of the oral cavity

slightly when it dissolves, which most people find refreshing. In contrast to other sugar alcohol, xylitol facilitates salivary secretion, thus immediately recovering a decline in pH. All these factors increase the amount of soluble calcium in dental plaque, which in turn facilitates remineralization of the enamel.⁸ Today, more than 50% of chewing gums and candies are sweetened with xylitol. One major obstacle with the use of both gums and candies for xylitol administration is high frequency and the rather large number of pellets that are required to deliver the therapeutic amounts. In addition, the costs for a long-term use could be a barrier. Therefore, novel low-cost delivery system for xylitol is necessary which is targeted to various ages.⁹

Due to the relatively high cost of xylitol, sorbitol and xylitol are often combined, supposedly with a better clinical effect. In vitro studies suggest that a combination of xylitol and sorbitol would enhance anti- microbial effect compared with xylitol alone.^{10,11} Hence clinical trials are being conducted to use mixture of xylitol and sorbitol in various products. In the present study an attempt was made to compare the relative effect of sorbitol and combination of sorbitol and xylitol containing dentifrices on *S. mutans* and lactobacillus in a group of high school children.

METHODS

Trial design

This was a double- blinded; parallel arm randomized trial with an allocation ratio of 1:1. The study was approved by ethical review board of College of Dental Sciences, Davangere, Karnataka. No modification to the trial methods were performed after trial commencement. This randomized controlled trial is reported in accordance with the CONSORT (consolidated statement of reporting trial) statement.

Table 1: Predefined inclusion and exclusion criteria for the recruitment of subjects.

Exclusion criteria

- 1) Children with orthodontic appliance
- 2) Recent antibiotic therapy
- 3) The presence of any systemic illness
- 4) Using xylitol containing products.
- 5) History of hypersensitivity to any product used in the study.

After excluding the subjects who fulfilled the exclusion criteria, the remaining subjects were examined for the inclusion criteria.

Inclusion criteria

- 1) School children aged between 14-15 years
- 2) Minimum of 20 natural teeth must be present
- 3) Patients with parental consent.
- 4) Patient with DMFT values ranging 0 to 3.

Participants

Taralabalu Residential School, a residential school located in Davangere city with strength of 300 in which students aged 14-15 years, were selected for the study. Although various factors directly or indirectly affect the variables that are under study, diet appears to be one of the major factors that can have a profound influence on the variables. Hence a single residential school was selected for the study and for the same reason only students staying in the school hostel and not day-scholars were included as study samples. From this school 64 children fulfilling all the inclusion and exclusion criteria were selected. The eligibility criteria for the recruitment of the study cohort are listed in (Table 1).

Interventions

Formulation of the dentifrices

Formulation of the dentifrices was done at Department of Pharmacognocny, Bapuji College of Pharmacy, Davangere. The formulations differed only with respect to the presence of the active ingredient that is sorbitol or combination of xylitol and sorbitol. The sorbitol dentifrices(S) contained 70% sorbitol as the only humectants, dentifrice containing both Xylitol and Sorbitol (X+S) contained 10% xylitol and 70% sorbitol (in 1:4 ratio).¹ Xylitol and sorbitol concentrations were based on minimum inhibitory concentration and glycerol concentration determined in previous study.⁷ Preparation of toothpastes was carried out in planetary mixer rotating at 320 rpm. Viscosity was maintained between 70,000-100,000 centipoises (cP) and pH of all the dentifrices was maintained at 7.¹²⁻¹⁵ The prepared dentifrices were then transferred to laminate collapsible plastic tubes. All the dentifrices contained same amount of fluoride.

Subjects were randomly allocated by lottery method into 2 study groups (S and X+S). Subjects in the S group were provided with sorbitol dentifrices and subjects in the X+S group were provided with dentifrice containing both Xylitol and Sorbitol.

18 subjects who fulfilled the inclusion criteria were included in pilot study in order to check the feasibility and validity of the study and also to assess the acceptability of the prepared formulations. Subjects who participated in the pilot study were not included in the main study.

Subjects were provided with the test dentifrices and Colgate[®] toothbrushes (soft bristled) and were asked to use the experimental dentifrices twice daily before breakfast and bedtime throughout a three months period.

Sample size

Sample size determination was based on the expected minimum reduction in colonies of microorganisms in

treated group, as observed in a previous study.⁷ The desired statistical power was set at 80% ($1-\beta=0.8$) at a significance level of 5% ($\alpha<0.05$). Twenty five subjects per group were required but additional numbers of samples were considered in order to eliminate drop out effect on the study and thirty subjects in each group were included for final study.

Randomization and blinding

The random allocation sequence was generated manually in the department of public health dentistry, college of dental sciences, Davangere, Karnataka. A block randomization was carried out (block size=4; allocation ratio=1:1). The randomly allocated sequence was implemented and concealed in sequentially numbered sealed envelopes. They were opened only prior to intervention after patient enrolment and receiving signed written consent form from parents of subjects. Single investigator, who was not involved in the participant's enrolment process, generated the random allocation sequence. Consecutive subjects who fulfilled the inclusion criteria were enrolled in the trial. An investigator recruited the participants for the study to two groups and he was blinded to the participant's group allotment.

Outcome measures

The primary outcome measure determining the effectiveness of the test dentifrices was to evaluate the change in counts of *S. mutans* and Lactobacillus count in saliva. Unstimulated saliva was collected between 8:00 am – 9:00 am and children were instructed not to eat or drink anything for at least one hour before the collection of saliva sample. The collected samples were processed on the same day; *S. Mutans* was cultured on Mitis salivarius bacitracin agar and Lactobacilli on Rogosa SL agar. After 48 hours of incubation colonies were identified and counted using an electronic colony counter. Samples were collected at baseline, at the end of 1 month, 2 months and 3 months interval after the use of assigned dentifrice. There were no changes to the outcome measures after trial commencement.

Study protocol

The oral pathology faculty and the author who was an primary investigator for the present study after having detailed discussion of the methods involved in assessing the *S. mutans* and *Lactobacillus* count underwent a calibration session in the department of oral pathology, College of Dental Sciences. The aim of this session was to train the investigator in counting and recording the *S. mutans* counts as colony forming units. The data recorded by both the examiners were subjected for kappa statistical analysis in order to find the degree of consistency or variation in judgments between the examiners. The unweighted kappa coefficient value for interexaminer reliability with respect to *S. mutans* count was 0.86 and

Lactobacillus count was 0.81. After initial review and examination, the patients were given detailed written information about the trial. Baseline clinical examinations, including complete oral health check-up, caries status were performed for all participants. A complete full mouth oral prophylaxis was performed and oral hygiene instructions were given prior to the start of the study.

Participants were then instructed to use the test products. They were asked to abstain from dentifrices other than those provided for the study, and to maintain their normal dietary habits throughout the study. They were also instructed to refrain from visiting to the dentist, and use of any xylitol containing product other than assigned dentifrices. Specific instruction on brushing was given to the participant that is twice daily for 2 minutes and to use approximately 1 gram of toothpaste every time. All the subjects were regularly supervised by the examiner in the morning by visiting the schools every day for first one week later on alternate week. Guardians for the residential school were instructed to supervise the brushing at night times. Recall examination were performed at end of 1(T1), 2 (T2) and 3 (T3) months interval after intervention. The trial concluded after all the participants were examined at T3, as originally intended in the protocol

Statistical analysis

Data collected by experiments were computerized and analyzed using the Statistical Package for Social Sciences (SPSS) version 17.0. Results were expressed in frequencies. Nonparametric tests namely Wilcoxon-signed rank test and Mann Whitney U test were used for testing the statistical significance.

Mann-Whitney U test for group wise comparison of salivary microbial counts at various time interval and Wilcoxon-signed rank test for intra group comparison. For all tests a p value of 0.05 or less was considered statistically significant.

RESULTS

A total of 69 participants were assessed for eligibility, studying in a residential school in Davangere city. Five of them were excluded and finally 64 participants (S group n=32 and X+S group n=32) were randomized and enrolled in the study to receive intended intervention. After trial commencement three participants (S group n=1 and X+S group n=2) were excluded from the study because they were lost to follow up. Hence a total of 61 participants (S group n=31 and X+S group n=30) were included in the final analysis for the primary outcome measures. The CONSORT flow diagram of the phases in the participant recruitment, randomization, follow up and analysis is presented (Figure 1).

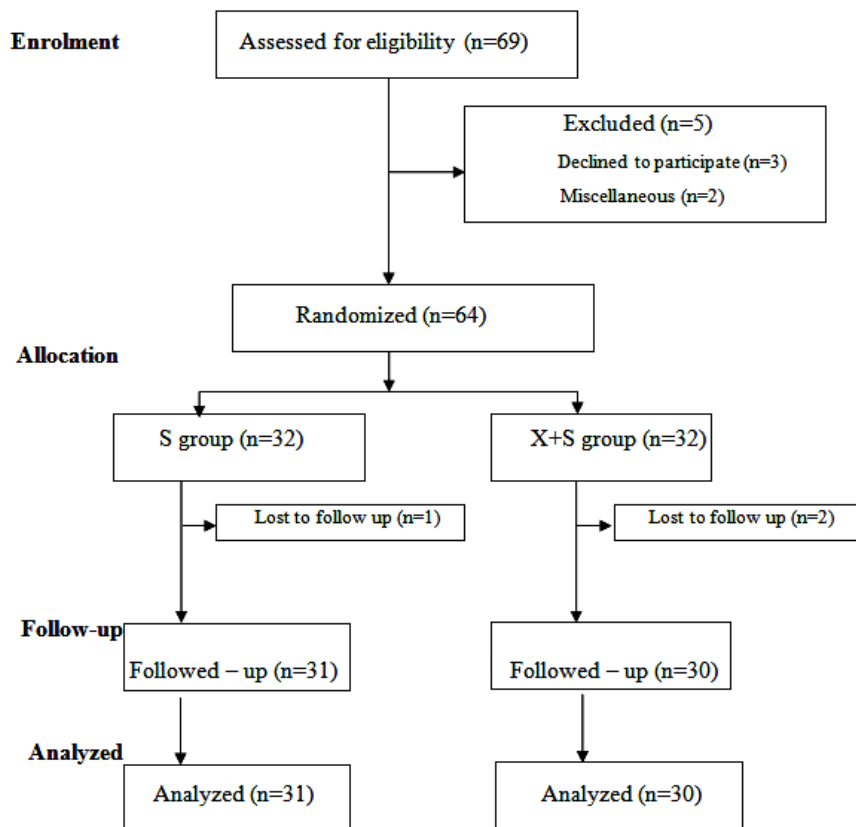


Figure 1: Flow chart of the phases of the two study groups in the trial (n, number of patients).

There was no difference in mean for colony counts of salivary *S. mutans* and *Lactobacillus*, at baseline, in

sorbitol containing dentifrices (S) group and dentifrice containing both sorbitol and xylitol (p=0.242 and p=0.894) respectively (Table 2).

Table 2: Shows comparison of salivary *S. mutans* and *Lactobacillus* counts at baseline amongst different groups, mean *S. mutans* and *Lactobacillus* counts in saliva expressed as 10³ CFU/ml.

Groups		<i>S. mutans</i>	<i>Lactobacillus</i>
Sorbitol group		21.13±2.3	9.82±3.007
Xylitol+Sorbitol group		23.87±4.208	9.73±3.648
Mann Whitney U test	Mann Whitney U value	371.0	441.0
	P value	0.242	0.894

Table 3: Presents comparison of sorbitol (S) and combination of xylitol and sorbitol (X+S) group with respect to *S. mutans* and *Lactobacillus* counts in saliva at various intervals. Mean *S. mutans* and *Lactobacillus* counts in saliva expressed as 10³ CFU/ml.

Groups		Baseline		T1		T2		T3	
		SM	L	SM	L	SM	L	SM	L
Sorbitol group		21.1±2.3	9.8±3.0	22.6±4.1	9.6±2.6	21.7±4.1	9.5±2.0	21.5±4.1	8.9±1.8
Xylitol +Sorbitol group		23.8±4.2	9.7±3.6	22.9±3.7	8.3±3.2	19.1±2.5	8.8±3.5	16.6±2.2	6.3±2.8
Mann Whitney U test	Mann Whitney U value	371.0	441.0	388.5	325.5	305.0	383.5	131.5	245.5
	P value	0.242	0.894	0.361	0.064	0.031 (S)	0.322	<0.001 (HS)	0.002 (S)

Table 4: Presents comparison of salivary *S. mutans* counts within the groups at different time intervals, expressed as 10³CFU/ml of saliva.

	Sorbitol group	Xylitol +sorbitol group
Baseline	21.13±2.3	23.87±4.208
T1	22.60±4.132	22.97±3.718
T2	21.77±4.183	19.13 ± 2.543
T3	21.53±4.150	16.67± 2.202
Wilcoxon signed rank test		1>2>3>4
	3>4(p=0.028)	1>2(p<0.001)
		2>3(p<0.001)
		3>4(p<0.001)

Table 5: Presents comparison of salivary *Lactobacillus* counts within the groups at different time intervals, expressed as 10³CFU/ml of saliva.

	Sorbitol group	Xylitol +sorbitol group
Baseline	9.82±3.007	9.73±3.648
T1	9.63±2.606	8.30±3.239
T2	9.53±2.080	8.80±3.508
T3	8.93±1.874	6.30 ±2.867
Wilcoxon signed rank test	3>4 (p=0.005)	2>4 (p=0.002)

Table 3 presents comparison of sorbitol (S) and combination of xylitol and sorbitol (X+S) group with respect to *S. mutans* and *Lactobacillus* counts in saliva at various intervals respectively. At the end of T1 there was no difference seen in mean colony counts of *S. mutans* and *Lactobacillus* between sorbitol (S) and combination of xylitol and sorbitol (X+S) group with a p value of 0.361 and 0.064 respectively.

At the end of T2 the subjects in the combination of xylitol and sorbitol containing dentifrices (X+S) group showed significantly low *S. mutans* count compared to subjects in the sorbitol containing dentifrices (S) group (p=0.031). There was no difference seen with respect to mean colony counts of *Lactobacillus* between two groups (p=0.322). Results at the end of T3 showed significantly low *S. mutans* count for X+S group compared to S group

($p < 0.001$), there was also significantly low *Lactobacillus* count for X+S group compared to S group with a p value of 0.002.

Table 4 and 5 presents comparison of salivary *S. mutans* and *Lactobacillus* counts within the groups at different time intervals.

There was no significant difference in counts of *S. mutans* and *Lactobacillus* among the subjects in the sorbitol containing dentifrices (S) group at different time intervals ($p = 0.567$ and $p = 0.306$). There was statistically significant difference in counts of *S. mutans* and *Lactobacillus* among the subjects in the dentifrices group containing both sorbitol and xylitol (X+S) at all the time interval ($p < 0.001$).

DISCUSSION

The present randomized clinical trial investigated the effect of two dentifrices containing sorbitol and combination of Xylitol and sorbitol on counts of salivary *S. mutans* and *Lactobacillus*. The result showed significant reduction of *S. mutans* and *Lactobacillus* in saliva of the participants using combination of Xylitol and sorbitol dentifrices compared to sorbitol dentifrices.

The results of present study are attributed to the fact that cariogenic microorganisms can 'learn' to metabolize sorbitol when their sugar supply is restricted; this form of adaptation to sorbitol has been demonstrated in animals. Firestone and Navia suggested that this adaptation could have occurred owing to selection by sorbitol-fermenting bacteria or induction by sorbitol-specific metabolizing enzymes.¹⁸ Combination of xylitol and sorbitol are used in various products. Study carried out by the Hujoell, Makinen on acid production in the oral cavity from sorbitol in the presence of xylitol in chewing gum containing sorbitol reduces acid production from sorbitol.¹³ This may be explained by the following effects of xylitol as: Reduction of plaque and the number of microorganisms on the teeth which are a consequence of the toxic effect of xylitol-5-phosphate and inhibition of acid production from sorbitol due to competitive blocking of the phosphotransferase system due to structural similarities between xylitol and sorbitol.¹⁷ Hence combination of xylitol and sorbitol can be effective.

70% sorbitol when used in the dentifrice was found to be ineffective in reducing the salivary *S. mutans* and *Lactobacillus* counts to a statistically significant level which was similar to the results obtained in studies done by Svanberg et al, Makinen et al.^{7,19} However in the above studies the concentration of sorbitol used was 20% whereas in the current study the concentration of sorbitol used was 70% (based on results of minimal inhibitory concentration determined in previous study.⁷ which was much higher compared to sorbitol concentration used in

other studies, still it failed to demonstrate the anticariogenic effect. Study done by Petersson et al investigated the influence of two concentrations 6% and 9% sorbitol plus 0.03% sodium fluoride and 0.8% MFP on both salivary *S. mutans* and *Lactobacillus* counts and showed that there was no significant reduction in the counts was seen which is consistent with our result although concentration of sodium fluoride used was in the concentration of 0.1%.²⁰

The results of present study using 10% xylitol and 70% sorbitol together in the dentifrice could not be compared with other studies as no studies have been reported in literature which has tried to assess the effect of combination of xylitol and sorbitol on *S. Mutans* and *Lactobacillus*. However study done by Petersson in which 6% sorbitol and 3% xylitol was used in a dentifrice along with 0.8% sodium mono-fluorophosphate (MFP) showed no difference in the salivary levels of *S. mutans* and *Lactobacillus*.²⁰

The results are similar to those obtained by the clinical studies following the use of a xylitol and sorbitol mixture containing chewing gum.^{12,13}

Present study unique as hitherto no similar studies have been reported in literature. Results of this study showed combination of sorbitol and xylitol dentifrices was most effective against *S. mutans* and *Lactobacillus*, hence the use of it may be promoted further in the dentifrices which can make the dentifrice cost effective. Further research is required to elucidate and understand the action of combination of sorbitol and xylitol on oral microbiota.

Although the methodology followed in this study protocol is robust, this study could be considered limited by its short term follow up. Despite a carefully planned calibration meeting, differences might have occurred between the investigators performing the protocol or in the features of the enrolled participants and therefore in the results obtained. However these differences did not preclude the significant differences between two groups. Due to our stringent patient selection criteria and small sample size, the generalizability of the results may be limited. Our study highlights the preventive effect of the use of dentifrice containing combination of xylitol and sorbitol, but further trials are necessary to confirm this effect.

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