



Effect of Chewing Xylitol Containing and Herbal Chewing Gums on Salivary *Mutans Streptococcus* Count among School Children

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ABSTRACT

Background: The present study aims to assess and compare the reduction in salivary *Mutans Streptococci* counts after chewing Xylitol, herbal and placebo gums among high school children.

Methods: The study was conducted among 72 school children (12–15 years) from 3 randomly selected schools (blocks). Xylitol, herbal and placebo gums were randomly allocated to 3 blocks. Subjects were instructed to chew one pellet four times a day for 21 days. The mean reduction in salivary *Streptococcus mutans* count was assessed.

Results: The 100% Xylitol sweetened chewing gum “Xylitol” has shown statistically significant reduction in salivary *Mutans Streptococci* colony forming units at the end of 21 days ($P < 0.01$). The reduction was not statistically significant in herbal and placebo chewing gum.

Conclusions: Hundred percentage Xylitol sweetened chewing gum was found to be more effective in reducing salivary *Mutans Streptococci* count when compared to herbal and placebo chewing gums.

Keywords: Gum base, herbal chewing gum, salivary *Mutans Streptococci*, xylitol


INTRODUCTION

Dental caries and periodontal disease are wide spread and pose a significant public health problem across the globe. In India, the prevalence of dental caries is as high as 60–80% among children.^[1] Despite the high prevalence

of dental caries, oral health has not received sufficient importance in India.^[2]

The etiology of dental caries is quite complex involving interaction between the agent, host, and environmental factors. Dental caries can be prevented by modifying the agent, host and environmental factors involved in its causation. *Mutans Streptococci* are important agent factor and major causative bacteria implicated in dental caries.^[3,4] Many studies have shown a direct relationship between the occurrence of carious lesions and salivary *Streptococcus Mutans* count.^[5,6]

Chewing gums that are used by a large proportion of populations have gained a lot of consideration in caries prevention as they stimulate saliva, one of the most

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significant important host factors that determine the occurrence of dental caries. Stimulated saliva has an increased concentration of bicarbonates that result in elevated plaque pH and enhanced acid buffering capacity.^[7] The stimulated saliva is also a state of mineral supersaturation that promotes enamel remineralization.^[8] Traditional chewing gums sweetened with sucrose may contribute to the cariogenicity of the diet, hence sugar substitutes such as, lactitol, maltitol, mannitol, sorbitol, and Xylitol are commonly used in foods to replace sugars. In the midst of a variety of chewing gums, sugar free chewing gums containing the sugar substitute have proved valuable against dental caries. Study results have shown that oral bacteria do not use these sugar substitutes to produce acids that demineralize enamel and dentin.^[7] Chewing gums containing Xylitol have received special attention, since along with the mechanical cleaning due to saliva stimulation it also own anti-bacterial effects.^[9] Among the various polyols Xylitol alone has a taste comparable to sucrose in terms of sweetness.^[10] Xylitol chewing gum is found to reduce salivary and plaque *Mutans Streptococci* count.^[9-14]

Certain herbal chewing gums are also marketed claiming to provide a beneficial effect against dental caries. "Orbit White" is one herbal chewing gum available in the Indian market for the last few years. It contains "pudina" that is an herbal mint and traditionally used in India as a flavoring agent in preparing dishes. It has a known anti-bacterial effect against *Proteus mirabilis*, *Staphylococcus aureus*, *Alcaligenes fecalis* and *Bacillus cereus*,^[13] herbal chewing gums containing pudina probably may have an influence on oral microorganisms but literature reveals no evidence in this regard.

A major criticism of the clinical trials on chewing gums relates to their designs. Xylitol chewing gums have been consistently evaluated against control groups wherein the subjects were advised not to chew the test chewing gum. However, the effect of chewing per se should not be ignored. It is, therefore, not possible to assess the effect of sweeteners and other additives in chewing gum without a control group that chews a control (placebo) gum. Exploration of available literature revealed no studies comparing the anti-bacterial effect of Xylitol containing chewing gums with herbal chewing gums against salivary *Mutans Streptococci*. The present study was conducted with an aim to assess and compare the relative effect of these two chewing gums and a placebo gum (gum base) on salivary *Mutans Streptococci* counts, among high school children in Davangere city.

METHODS

The present study is a randomized, clinical, follow-up study with a concurrent parallel design conducted among high school children in Davangere city. Davangere city consists of a total of 155 higher primary schools. Sample size determination was based on the data obtained from a pilot study. For α error fixed at $<5\%$ ($P < 0.05$) and β fixed at 20%, the sample size was determined as 72 (24/group). Ethical clearance was obtained from the ethical review board of Bapuji Dental College and Hospital, Davangere. The study included students having, at least one frank carious cavity and salivary *Mutans Streptococci* counts of equal to or $>10^5$ cfu/ml of saliva.

Method employed to obtain the desired sample

Out of a total of 155 schools, eight schools (two schools from each zone) were selected among which three schools were randomly selected by employing the lottery method. List of all the students belonging to age groups 12–15 years from the selected schools was obtained from the school records. Among these those who were, (1) had been taking anti-biotic therapy within the last 2 weeks, (2) had hypersensitivity to any product used in the study, (3) were unable to comply with the study appointment schedule, (4) were suffering from acute or chronic systemic disease with potential oral manifestations, (5) wore a removable prosthesis, (6) were undergoing orthodontic treatment, (7) were suffering from temporomandibular joint disorders, (8) had a history of gastro intestinal problem, or (9) were habitual gum chewers, were all excluded.

The remaining students were examined and those having at least one frank carious cavity were selected. The examination of the frank cavity was carried under natural light and the tooth suspected of having a carious lesion was further confirmed by drying the tooth with a chip blower, removing the overlying debris and feeling the base of the lesion using a cloud particle imager probe. These students were further subjected to salivary analysis to assess the salivary *S. mutans* counts.

Assessment of salivary *Streptococcus Mutans* count

One ml of unstimulated saliva from the selected students was collected. The children were instructed to let saliva collect without swallowing for at least 1 min, and then to expectorate into the sterile test tube and was repeated until a minimum of 1 ml of saliva was collected. It was diluted with saline (1:10). The diluted sample was streaked on mitis salivarius bacitracin agar plates and incubated for 48 h at 37°C in an atmosphere of 95% nitrogen and 5% carbon dioxide.

Children who had salivary *Mutans Streptococci* counts of $\geq 10^5$ were considered as possible participants and among these 24 children/school were selected randomly. 24 students from each school were considered as one sub group. This resulted in a total sample of 72 children (24×3) for the final study. A detailed schedule of visits to the selected schools in assigned time frame was prepared well in advance by discussing with the school authorities and based on the study design. Written informed consent was obtained from the parents of the children participating in the study (Since Sec 90 of IPC suggest that the age for giving valid consent of 12 yrs or above child for any procedures).

The chewing gums used in the study were: Herbal chewing gum-“Orbit white” (Xylitol, Lotte India Corporation Ltd., orbit white, Wrigley Company) – Group A, 100% Xylitol chewing gum-“Xylitol” (Lotte Company) – Group B, Placebo chewing gum-“gum base” – Group C.

Packing of chewing gum

The chewing gum pellets were removed from their respective wrappers and packed in identical boxes.

In the pretested questionnaire, all the required and relevant information of the participants' socio-demographic data, diet history, and oral hygiene habits interview method was recorded. The saliva was collected and analyzed for salivary *M. streptococcus* which formed the baseline data.

After obtaining baseline saliva samples from each of the subjects in all the three subgroups, chewing gums were allocated randomly (lottery method) and by a person who was not aware about the study to each subgroup, so children in each school had the same type of chewing gum. The three chewing gums the investigator was blinded about the group allocation and the chewing gums used by the children. The chewing gum assignment was done as mentioned below: Block I (Group A) – “Orbit white”, block II (Group B) – “Xylitol” and block III (Group C) – “Gum base”. The chewing gums were distributed once every week (28 pellets each time). Subjects were instructed to chew one pellet each time for four times a day for 10 min after breakfast, lunch, evening snacks and dinner for 21 days. All subjects were advised to maintain their routine dietary habits, daily oral hygiene regimens and not to consume any other chewing gum except that which is provided to them during the study period. To keep a check on the compliance check list was provided to the students. The class teachers were also asked to remind the students to chew the chewing gums every day. The investigator visited the schools every alternate

day to check for the compliance. On every visit, the students were reminded to chew the chewing gums as according to the given instructions. On the 22nd day, unstimulated salivary sample was collected and assessed for salivary *Mutans Streptococci*. On the same day, all subjects were asked to complete a compliance questionnaire which included three questions related to the compliance and experience of the students during the study period.

Statistical analysis

Statistical analysis was done using SPSS (version 15) USA. Comparison of salivary *Mutans Streptococci* counts at baseline (pretest), and after the intervention (posttest) was done by paired *t*-test. Comparison of salivary *Mutans Streptococci* counts between the groups at baseline and after 21 days was done by one way ANOVA. Followed by Tukey's honestly significant difference test. The difference in salivary *Mutans Streptococci* counts before and after the intervention (pretest-posttest) was compared between the three sub groups using Kruskal Wallis test followed by Mann-Whitney U-test. Logistic regression, a multivariate analysis was performed to understand the effect of confounders over the outcome.

RESULTS

The study was conducted among 72 school children (12–15 years) from three randomly selected schools (blocks). Xylitol, herbal and placebo gums were randomly allocated to three blocks. Subjects were instructed to chew one pellet four times a day for 21 days. The mean reduction in salivary *S. mutans* count was assessed.

Table 1; shows the age and sex wise distribution of the subjects. The mean age was approximately 14 in all three groups ($P > 0.005$). Participants of both genders distributed equally in the sub groups. Distribution of the study subjects based on various oral hygiene and dietary factors is presented in Table 2, brushing frequency, material used to brush their teeth, habit of rinsing their mouth after every meal showed significant difference between the subgroups. Mean pre and posttest salivary

Table 1: Age and sex wise distribution of the subjects

	Group A (herbal)	Group B (Xylitol)	Group C (placebo)	Significance
Age				
Mean±SD	14.3±0.7	14.5±0.6	14.2±0.9	$P > 0.05$ (NS)
Sex number (%)				
Male	15 (63)	14 (58)	16 (67)	$P > 0.05$ (NS)
Female	9 (37)	10 (42)	8 (33)	

NS: Not significant, SD: Standard deviation

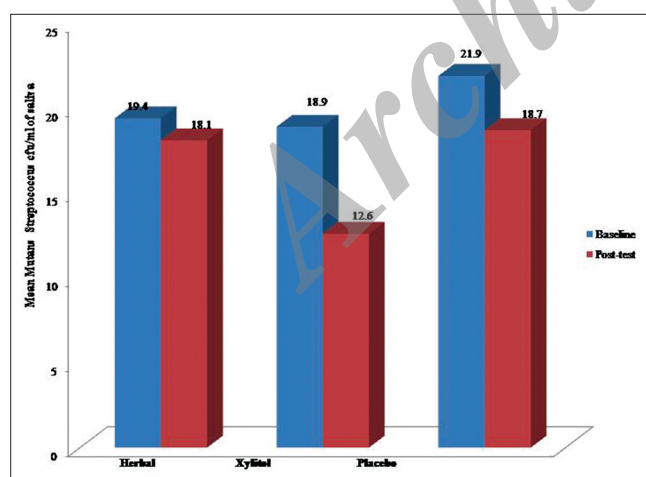
Table 2: Distribution of the study subjects according to their oral hygiene and dietary practices

Parameters	Number of subjects (%)			Significance level
	Group A (herbal)	Group B (Xylitol)	Group C (placebo)	
Brushing frequency				
Once a day	24 (100)	16 (66.7)	23 (95.8)	$P < 0.01$
Twice a day	0 (0)	8 (33.3)	1 (4.2)	
Aids used for brushing teeth				
Toothbrush	24 (100)	24 (100)	23 (95.8)	$P > 0.05$
Finger	0 (0)	0 (0)	1 (4.2)	
Material used to brush their teeth				
Toothpaste	24 (100)	19 (79)	23 (96)	$P < 0.05$
Toothpowder	0 (0)	5 (21)	0 (0)	
Charcoal	0 (0)	0 (0)	1 (4)	
Habit of rinsing their mouth after every meal				
Yes	3 (13)	13 (54)	11 (46)	$P < 0.01$
No	21 (87)	11 (46)	13 (54)	
Frequency of sweets consumption				
Once/twice a week	18 (75)	21 (88)	22 (92)	$P > 0.05$
More than twice a week	6 (25)	3 (12)	2 (8)	

Table 3: Pairwise intergroup comparison of mean *Mutan Streptococci* counts at baseline, posttest, and mean reduction count in the study groups

Groups	Baseline	Posttest	Mean reduction (baseline-posttest)
A-B	NS	0.05 (S)	$P < 0.05$ (S)
A-C	NS	0.96 (NS)	$P > 0.05$ (NS)
B-C	NS	0.03 (S)	$P > 0.05$ (NS)

S: Significant, NS: Not significant



Graph 1: Mean pretest and posttest salivary *Mutans Streptococci* counts among the study groups

Mutans Streptococci counts among the study groups are presented in Table 3 and Graph 1. The reduction in salivary *Mutans Streptococci* values at posttest in Group A (herbal), mentioned B (Xylitol) and C (placebo)

was statistically significant [Graph 2]. The difference between mean pretest and posttest salivary *Mutan Streptococci* count (mean reduction) was statistically significant only in Group B (Xylitol).

Table 4 shows the pair wise intergroup comparison of mean salivary *Mutans Streptococci* count at baseline, posttest, and the mean reduction in counts in the study and control group. It revealed statistically significant difference in mean *Mutans Streptococci* counts between Group A and Group B ($P < 0.05$) and group B and C ($P < 0.05$) at the end of 21 days. When mean reduction between groups was compared, the difference was statistically significant only between Group A and Group B ($P < 0.05$).

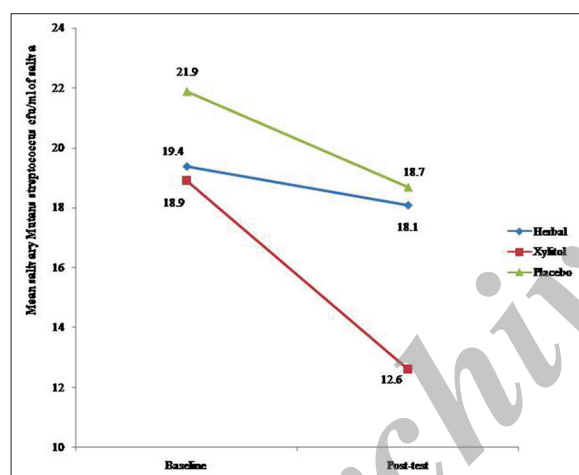
Table 4 shows the results of multivariate analysis. Frequency of brushing, material used to clean the teeth and rinsing after every meal among subjects varied between groups, and this difference was statistically significant. Logistic regression was performed using these variables along with chewing gum under trail as independent variable and salivary *Mutans Streptococci* as dependent variable. The effect of independent variables; frequency of brushing, material used to clean the teeth and rinsing after meal on dependent variable was statistically not significant, whereas the effect of chewing gums on salivary *Mutans Streptococci* (dependent variable) was statistically significant ($P < 0.2$).

In Group A and B, 79% of the subjects had good and 21% had fair compliance level. In Group C, 46% of the subjects had good, 25% fair and 29% showed poor

Table 4: Multivariate analysis including frequency of brushing/day, material used to clean the teeth, rinsing after every meal and the chewing gums on trial as independent variables and salivary *Mutans streptococci* counts as a dependent variable in Xylitol group

Effect	Likelihood ratio tests			
	Model fitting criteria	Likelihood ratio tests		
		-2 log likelihood of reduced model	Chi-square	df
Intercept	28.154	0.0	0	
Frequency of brushing	29.376	1.222	1	0.269
Material used	29.694	1.54	2	0.463
Rinsing	29.237	1.083	1	0.298
Chewing gum	31.403	3.25	2	0.0197

The Chi-square statistic is the difference in -2 log-likelihoods between the final model and a reduced model. The reduced model is formed by omitting an effect from the final model. df=Degree of freedom



Graph 2: Reduction in salivary *Mutans Streptococcus* count in the Xylitol, Herbal and Placebo chewing gum groups at the end of twenty-one days

compliance. These differences between the groups were statistically significant ($P < 0.002$).

DISCUSSION

Dental caries gingivitis and periodontitis are the most common oral diseases throughout the world.^[16] Dental caries is a widespread, multifactorial, infectious disease the prevention of which is based on multifaceted approaches.^[17] In recent years, chewing gums have gained considerable attention in preventive dentistry. Chewing results in salivary stimulation that can neutralize or elevate the pH of plaque especially at the interface between the tooth and the plaque.

Stimulated saliva can also enhance the clearance of fermentable carbohydrates purely through its mechanical action.

The current study employed block randomization method for the following reasons; (1) the three test materials used in the current study were different in their color, consistency and taste. Applying block randomization was the only way to blind the participants with respect to three different test materials used in the study (2) to avoid trading and exchange of gums since a statistically significant difference was observed in health behavior of different blocks/subgroups which could have modified the results, multivariate analysis was performed [Table 4] which clearly suggested that the results observed were mainly due to chewing gum.

Salivary *Mutans Streptococci* counts were obtained using unstimulated saliva instead of stimulated saliva for the following reasons because salivary tests are generally more usable than tests based on plaque, which are often more technically sensitive.^[18] Additionally the *Mutans Streptococci* count obtained from plaque do not represent the whole mouth.^[19] By use of saliva samples also mechanical dislodging of *Mutans Streptococci* from plaque can be controlled thus avoiding falsely or inconsistently increasing their level.

Previous studies revealed that 5–10 g/day of Xylitol was required for its anti-bacterial effect.^[20-22] The product used in the current study contained 1.28 g of Xylitol/pellet, hence four pellets were advised/day so as to deliver around 5.14 g of Xylitol/day. Sugar free chewing gums when used after meals are suggested to have a beneficial effect for this reason in the present study subjects were instructed to chew the gum after every meal and evening snacks.^[23] The control group was maintained in order to balance the anti-bacterial effect caused due to salivary stimulation by chewing gums, so that any difference in the posttest salivary *Mutans Streptococci* count between the groups can be attributed to only the active ingredients present in the chewing gums. Due to it the compliance was poorer in placebo group but the difference in compliance between the three groups was not significant.

In the present study, although numerically a marginal reduction in salivary *Mutans Streptococci* count was observed in the herbal chewing gum group at the end of the follow-up period, the difference was statistically not significant. This is perhaps the first study to assess the anti-bacterial effect of herbal chewing gum. As there are no reports available in the literature, the scope for valid comparisons is severely constrained. Xylitol chewing gum has shown to possess a significant anti-microbial effect against salivary *Mutans Streptococci*. This is in

conformity with the results obtained in several previous studies.^[15,17,24-36] In a study conducted by Holerson *et al.* (2007) and in a study by Isotupa (1995) on orthodontic patients, the participants chewed Xylitol chewing gums 2 pellets three times/day and two pellets six times/day respectively for 4 weeks.^[14,36] Results of both studies have shown significant reduction in salivary *Mutans Streptococci* counts which are in agreement with the results obtained in the present study. In the present study, the subjects chewed the assigned chewing gum only 4 times a day. Based on the observation it can be speculated that chewing gums 4 times or 6 times a day has a similar effect on salivary *Mutans Streptococci*.

The results of the current study are very much in agreement with the studies of Simon's *et al.*^[29] and Autio^[24] conducted among elderly adults and preschool children respectively, although the age groups and frequency of chewing gums/day were slightly different when compared to the present study. This suggests that the effect of Xylitol chewing gum is not restricted to a specific age group.

Longer period of Xylitol consumption does not appear to significantly decrease the number of MS in the saliva compared with a control group of nonconsumers^[37] in contrast to the results obtained with short periods of use.^[38,39] It is said that the reduction in salivary *Mutans Streptococci* persists for a specific period after instituting the chewing of Xylitol containing gums. After which the organisms develop resistance to Xylitol followed by the rise in the number of salivary *Mutans Streptococci*, these organisms were found to be less adherent to the tooth surface hence less pathogenic (cariogenic) thus providing long term effect anti-caries effect.

The effect obtained by Xylitol chewing gum on *Mutans Streptococci* counts may be due to the fact that Xylitol is not fermented by cariogenic plaque bacteria and thus, does not lower the pH of plaque.^[40] As the plaque pH does not decrease, enamel demineralization is prevented, and plaque bacteria do not proliferate. Xylitol, however, is absorbed and accumulates intracellularly in *Mutans Streptococci*. Xylitol competes with sucrose for its cell-wall transporter and its intracellular metabolic processes. Unlike the metabolism of sucrose, which produces energy and promotes bacterial growth, *Mutans Streptococci* only expend energy to break down the accumulated Xylitol without yielding energy in return.

In the present study Placebo chewing gum also showed a reduction in salivary *Mutans Streptococci* counts compared to the baseline even if the difference was statistically not significant. Similar results were obtained in the study done by Soderling and Trahan^[28] among

dental students where in unsweetened chewing gum base was chewed 3–5 times a day for a period of 2 weeks.

Xylitol chewing gum was found to be superior in its anti-bacterial effect on salivary *Mutans Streptococci* when compared to the placebo gum, but the difference was statistically not significant. The appreciable effect on salivary *Mutans Streptococci* counts by placebo chewing gum may be due to the texture of the gum base being harder to chew thus increasing salivation.

The compliance levels found in Xylitol, and herbal chewing gum groups was good compliance. In comparison, the compliance was poorer in the placebo group with only 46% showing good compliance. The reasons attributed to poor compliance by majority of the students were that, they had forgot to chew the gums, and few said that they had been out of the station and had forgot to carry the gums. The compliance level was not significantly different among the study groups (xylitol and herbal chewing gum) Majority of the subjects in the placebo group complained that the gum was harder in consistency and bland in taste. The subjects tolerated chewing gums well without reporting any side effects. This finding is in agreement with the once obtained in other studies.^[24,41,42]

The data obtained showed statistically significant difference between the sub groups with respect to the brushing frequency, material used to clean the teeth and rinsing after meal that in turn could have confounded the results. To account for this, logistic regression was performed to investigate their interaction effect. This analysis revealed that among all the independent variables, chewing of gum was the only variable which showed statistically significant ($P < 0.02$) association with reduction in salivary *S. mutans* counts (dependent variable) in the Xylitol groups.

CONCLUSIONS

From the present study it can be concluded that, 100% Xylitol sweetened chewing gum when chewed four times a day for 10 min each time for a period 21 days can significantly reduce the salivary *S. mutans* counts which may be beneficial in controlling dental caries among risk patients.

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