# Effects of xylitol on salivary mutans streptococcus, plaque level, and caries activity in a group of Saudi mother-child pairs

## *An 18-month clinical trial*

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## ABSTRACT

**الأهداف:** تقييم تأثير استخدام الزايلتول على مستوى البكتريا اللعابية العقدية المتحورة، واللويحة السنية ونشاط التسوس على مجموعة من الأطفال وأمهاتهم السعوديين.

**الطريقة:** شملت عينة الدراسة 60 زوج مكون من الأطفال وأمهاتهم ممن قاموا بمراجعة عيادات مستشفى جامعة الملك عبد العزيز، جدة، المملكة العربية السعودية وذلك خلال الفترة من فبراير 2009م إلى يوليو 2010م. لقد تم اختيار المشاركين على أساس وجود ارتفاع في مستوى البكتريا اللعابية العقدية المتحورة. قُسمت عينة الدراسة عشوائياً إلى مجموعة الدراسة ومجموعة الشاهد (30 زوج لكل منهما)، وقد وصل عدد العينة بعد 18 شهر إلى 21 أم وطفل في مجموعة الدراسة، و13 أم وطفل في مجموعة الشاهد. لقد أُعطيت مجموعة الدراسة الزايلتول كعلاج، في حين أعطيت المجموعة الشاهد ورنيش الفلورايد. وكان العلاج بالزايلتول يُعطي للأمهات في شكل علكة وللأطفال على شكل أقراص 3 مرات يومياً لمدة 3 أشهر. تلقت المجموعتان تعليمات نظافة الفم والمشورة الغذائية والعلاج التحفظي. وتم فحص جميع المشاركين في الدراسة لتقييم التسوس، واللويحة السنية ومستوى البكتريا اللعابية العقدية المتحورة بعد مرور 6، و12، و18 شهراً.

**النتائج:** أظهرت النتائج في مجموعة الدراسة الانخفاض الملحوظ لعدد الأطفال وأمهاتهم ذوي المستويات المرتفعة من البكتريا العقدية المتحورة بعد مرور 18 شهر مقارنة بنقطة البداية ($p$=0.001, $p$=0.000). ولقد لوحظ وجود فرق ملحوظ في اللويحة السنية لدى الأطفال في مجموعة الدراسة مقارنة بمجموعة الشاهد بعد مرور 6، و 12 شهر ($p$=0.000, $p$=0.006)، فيما أظهرت مجموعة الدراسة في حالة الأمهات نقص ذو دلالة إحصائية في اللويحة السنية بعد مرور 18 شهر مقارنةً بنقطة البداية. كما أظهر الأطفال وأمهاتهم في مجموعة الشاهد زيادة ذات دلالة إحصائية في نشاط التسوس طوال فترة الدراسة ($p$=0.040, $p$=0.040).

**خاتمة:** أثبتت الدراسة بأن استخدام الأمهات لعلكة الزايلتول وتناول الأطفال أقراص الزايلتول يؤدي إلى انخفاض كبير في عدد البكتريا العقدية المتحورة واللويحة السنية فضلاً عن نشاط التسوس.

**Objectives:** To assess the effect of xylitol on salivary mutans streptococcus (MS), plaque level, and caries activity in a group of Saudi mother-child pairs.

**Methods:** A clinical trial of 60 mother-child pairs with high MS levels attending at King Abdulaziz University clinics were randomly grouped into experimental (received xylitol) and control (received fluoride varnish) groups (30 pairs each). The study was conducted from February 2009 to July 2010 for 18 months period. At 18 months, the sample dropped to 21 (experimental) and 13 pairs (control). Xylitol gum were given to mother and chewable tablets were given to children 3 times a day for a period of 3 months. Both groups received oral hygiene instructions, dietary counseling, and restorative treatment and examined to assess caries, plaque and MS levels at 6, 12, and 18 months.

**Results:** The number of mother-child pairs with high MS level in the experimental group decreased significantly at 18 months compared to baseline ($p$=0.001, $p$=0.000). A statistically significant difference in plaque level was found between the experimental and control groups at 6 and 12 month in children ($p$=0.000, $p$=0.006), while in mother, a significant decrease was recorded in the experimental group only at 18 month compared to baseline. Control group showed statistical significant increase in caries throughout the study period ($p$=0.040, $p$=0.040).

**Conclusions:** The use of xylitol chewing gum by mother and chewable tablets by children, showed significant reduction of MS count, plaque score as well as caries experience.

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Dental caries is a significant public health problem for a large proportion of the Saudi population especially in children.[1,2] Many preventive programs have been introduced to control dental caries mainly focusing on dietary modification, the use of fluoride and pit and fissure sealants to increase the host resistant. Mutans streptococci (MS), particularly streptococcus mutans is the major causative bacteria that are involved in dental caries.[3,4] Many clinical studies have been conducted to test the effect of suppression of MS level on primary dentition. The use of sugar substitute therapy replacing harmful dietary habits seems to have positive effect on caries levels. Xylitol is a polyol- a pentatol that occurs widely in nature is used originally to sweeten a number of sugar-free products and is most frequently used as chewing gum.[5] Several recent studies have been published documenting the inhibitory effect of xylitol against dental caries.[6-11] Xylitol sugar substitute exerts inhibitory caries effect by inhibiting the cariogenic bacteria to ferment it.[5] Other studies reported that xylitol reduces the ability of MS to adhere, makes it more easily removed from plaque.[12,13] The study reported by Holgerson et al[14] among school children suggested that chewing gum with xylitol can reduce the amount of dental plaque and acid production and interfere with the microbial composition. Bacteriological studies reported by a number of investigators[15,16] showed the transmission of MS in mother-child pair reveals a relationship between salivary MS levels in mothers and initial acquisition of MS by their infants. Children with early colonization by MS showed high caries experience compared to children with late colonization.[16-19] Xylitol containing chewing gums are being studied for caries preventive action among mothers and the reduction of MS acquisition in children during tooth eruption.[8,20,21] Reviews of the dental literature revealed an impressive clinical data in regard to xylitol, however, no research has dealt with its potential action as a practical caries preventive tool among Saudi population groups who are recognized as being high caries risk. Therefore, the present study was designed to assess the effect of xylitol consumption on salivary MS and plaque levels as well as caries activity in a group of Saudi mother-child pairs identified as high caries risk individuals.

**Methods.** This is a clinical study on caries experience, plaque, and MS level in mother and child with an initial high salivary MS level over a period of 18 months.

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***Subject selection.*** Sixty pairs of mothers aged 22-45 years and children aged 2-5 years (120 subjects) were selected in this study. The study was conducted from 28 February 2009 to 15 July 2010 for 18 months period. The sample was selected from patients attending at King Abdulaziz University Hospital (KAUH), Well Baby Clinics, and the Faculty of Dentistry Pediatric Clinics, Jeddah, Saudi Arabia. Selection was based on the following criteria: a) Both mother and children with a high count of salivary MS (≥105), b) The presence of one or more decayed or filled primary tooth in children and one or more decayed or filled permanent tooth in mothers. Children with systemic disorders such as diabetes, hyperglycemia or sleeping disorders or irregular medications were excluded from the study. Also, children wearing removable dental prosthesis or prone to temporomandibular joint complaints or attending the clinics without the mothers or reared by nanny were also excluded from the study.

The initial number of pairs included in the preliminary screening was approximately 124 pairs. After completion of the baseline clinical and microbial investigations, only 60 mother/child pair was eligible to enroll in the study. Screening process lasted 3 months. The high cost of the experimental material needed for the 18-month follow up study was the limiting factor to extend further screening. Candidates were listed and a serial number were assigned to them.

Informed written consent was obtained from mothers after they were provided with verbal and written information concerning the importance and the procedures of the study. Sealed envelopes containing the serial number of the pairs were used to randomly allocate the candidates to experimental and control groups in a consecutive order. The experimental group received xylitol treatment for a period of 3 months. Xylitol chewing gums were given to the mothers, while the children were given xylitol chewable tablets. The pairs were instructed to participate in 5 minutes chewing sessions after breakfast (8:00 am), after lunch (1:00 pm), and also after snacks (6:00 pm). Detailed instructions on the administration and monitoring the use of tablets were given to mothers. Chewing gums and tablets (Fennobon Oy, Yrittäjäntie, Karkkila, Finneland) contained xylitol as the only sweetener (100/ww). Each chewing gum pellet (1.8 g) contained 66% xylitol per weight providing a total dose of 3.64 g. A xylitol chewable tablet (1.2 g) contained 84% xylitol per weight making up a total daily dose of 3 g. The control group received the conventional treatment namely Fluoride varnish (Durafat 5% NaF [Ultradent Products, Inc, South Jordan, Utah, USA), and received fluoride vanish every 6 months throughout the study period, which was not applied to the experimental groups. Both the experimental and control groups

received oral hygiene instruction, dietary counseling, and restoration treatment including amalgam and composite restorations as well as fixed dental prosthesis if needed.

***Saliva sample collection.*** Stimulated saliva samples were collected from each participant for salivary bacterial level assessment. Saliva sampling was performed before conducting the clinical examination between 9:00-11:00 am for a period of 15 minutes. Subjects were instructed not to eat or drink or chew a gum for 2 hours before sampling. On the morning of saliva sampling, mothers and children refrained from tooth brushing. Each subject was asked to chew a pellet of paraffin wax (1g) and to expectorate the stimulated saliva into a calibrated cylinder (15 ml). Chewing was carried out under close supervision of the examiner and children were asked to imitate their mother's action. If expectorations were found to be difficult with younger children, saliva was collected with the pipette supplied by the Calreticulin (CRT) kit (Vivadent, Ivocaire Line, Crt bacteria [for determining the streptococcus mutans and lactobacillus count in saliva] Ivoclar Vivadent AG, FL-9494 Schaan, Liechtenstein). Saliva was not taken if the child has received antibiotics within 1-month prior to examination. Saliva was collected at the end of xylitol treatment after 3, 6, 12, and 18 months to assess changes in salivary MS levels.

***Microbiology screening.*** Mutans streptococcus level in stimulated saliva sample was determined using the (CRT) bacteriological screening method at baseline, 3, 6, 12, and 18 months period. According to the manufacturer's instructions, agar surface was wetted with saliva, and then placed in the test vial, which were incubated at 37$^0$C for 48 hours. The density of the MS colonies was compared with the corresponding evaluation figure in the enclosed model chart. Based on the manufacturer's criteria, findings of $10^5$ CFU or more of MS indicated a high caries risk, whereas findings less than $10^5$ CFU were considered low caries risk.

***Clinical examination.*** A comprehensive dental examination for each subject was conducted by one calibrated examiner to collect the baseline data on caries and plaque levels. Examinations were performed in optimal light using mouth mirror and explorer. The diagnosis of dental caries was based on the World Health Organization (WHO)[22] criteria. In addition, bitewing radiographs for each subject were taken before clinical examination to detect proximal caries. Dental caries level was expressed using the decayed, missing filled, teeth (DMFT) index for permanent teeth and decayed, missing filled, teeth (dmft) for deciduous teeth. Plaque levels were assessed using the Green and Vermillion simplified Oral Hygiene Index (plaque component).[23] Clinical examination was repeated at 3, 6, 12, and 18 months period to assess dental caries and plaque levels.

***Calibration.*** To reach good intra-examiner reliability, calibration of the examiner was conducted prior to baseline registration. Ten children and their mothers were examined by one of the examiner to assess their caries, plaque, and salivary SM levels. They were re-examined the second day and the level of agreement between corresponding readings was assessed using the Kappa method.

***Statistical analysis.*** Statistical analysis was carried out using Statistical Package for the Social Sciences (SPSS) version 16. Measures of central tendency (mean and standard deviation) were used to describe quantitative variables, while frequencies and percentages were used to describe qualitative variables. Student t test and paired t test were used to evaluate differences between experimental and control groups and differences throughout the study period for DMFT and plaque scores. For difference between the groups in MS count, Pearson Chi-square test and Fisher's exact test of significance was used. McNemar test was used to detect differences in MS levels before and after intervention. Mann-Whitney and Wilcoxon signed rank test was performed to detect changes in plaque scores throughout the period of the study. Significant level was set at 0.05 level.

**Results.** Intra-examiner results using Kappa statistical for permanent and primary teeth were 0.8 and 1.00, which represent an excellent agreement. This study was conducted for a period of 18 months to compare the effect of xylitol in a group of mother-child pairs. **Figure 1** shows the flow of participants throughout the study period.

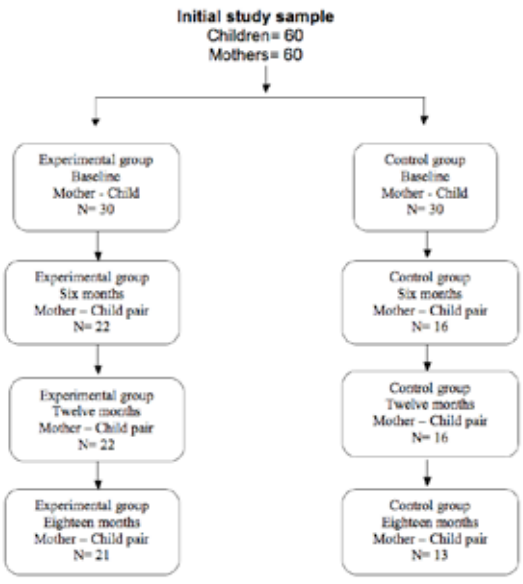


**Figure 1 -** Flow chart showing the initial screened patients at baseline, and the number of patients in the experimental and control groups throughout the study period.

The initial study sample of 30 mother/child at baseline has been subjected to decrease. Twenty-two experimental and 16 control subjects were present at 6 and 12 months period, however, at 18 months the number of subjects dropped to 21 and 13 respectively. Data was only obtained from subjects who completed the study period and attended the final examination. Also those who reported irregular use of xylitol were excluded. Result of the 3 months compared to baseline was accepted for publication in the Journal of Clinical Pediatric Dentistry 2011 and not included in this article.

**Tables 1 and 2** shows the MS counts in experimental and control groups in mothers and children at different study phases. **Table 1** shows significant difference between the experimental and control group of children at 6 and 18 months period ($p$=0.049, $p$=0.024) using Pearson Chi-square test.

Statistically significant difference was obtained in experimental group between baseline and 18 months period ($p$=0.000), using McNemar test.

**Table 2** shows no statistically significant difference between mothers with high MS of the experimental and control group at 6, 12, and 18 months period using Pearson Chi-square. However, statistically significant differences was obtained between baseline and 18 months in the experimental group ($p$=0.001) using McNemar test.

**Tables 3 and 4** shows results of plaque scores in experimental and control groups in mothers and children pairs. **Table 3** showed statistically significant lower plaque score at 6 and 12 months period of the experimental group compared with the control group using Mann-Whitney test. However, a reduction of plaque score at 18 months was observed, but not of significant value. Also, no significant difference was observed between baseline and 18 months in the experimental and control group using Wilcoxon signed rank test.

**Table 4** shows no statistically significant difference in plaque scores between experimental and control groups in mothers at 6, 12, and 18 months period using Mann-

**Table 1 -** Frequency distribution and percentage of children with high mutans streptococci level (≥$10^5$) at different study phases.

| Study phases | Study groups with high level | | *P* value† |
|---|---|---|---|
| | Experimental n (%) | Control n (%) | |
| Base line | 30/30 (100.0) | 30/30 (100.0) | |
| 6 months | 11/22 (50.0) | 13/16 (81.2) | 0.049* |
| 12 months | 13/22 (59.1) | 14/16 (87.5) | 0.078 |
| 18 months | 9/21 (42.9) | 10/12 (83.3) | 0.024* |
| Baseline /18 months P value‡ | 0.000* | 0.500 | |

*significant difference at <0.05. †Chi-square, ‡McNemar test

**Table 2 -** Frequency distribution and percentage of mother with high mutans streptococci level (≥$10^5$) at different study phases.

| Study phases | Study groups with high level | | *P* value† |
|---|---|---|---|
| | Experimental n (%) | Control n (%) | |
| Base line | 30/30 (100.0) | 30/30 (100.0) | |
| 6 months | 13/22 (59.1) | 10/16 (62.5) | 0.832* |
| 12 months | 12/22 (54.5) | 11/16 (68.8) | 0.376 |
| 18 months | 10/21 (47.6) | 9/12 (75.0) | 0.126* |
| Baseline/18 months P value‡ | 0.001* | 0.250 | |

*significant difference at <0.05, †Chi-square, ‡McNemar test

**Table 3 -** Plaque scores of children in the experimental and control groups at different study phases.

| Study phases | Study groups | | Experimental and control groups | | |
|---|---|---|---|---|---|
| | Experimental Mean±SD | Control Mean±SD | t test P value | Mann-whitney Z | Mann-whitney *P*-value |
| Baseline | 1.07 ± 0.25 n=30 | 1.13 ± 0.57 n=30 | 0.561 | -1.172 | 0.241 |
| 6 months | 0.59 ± 0.59 n=22 | 1.56 ± 0.73 n=16 | 0.000 | -3.670 | 0.000 |
| 12 months | 0.68 ± 0.59 n=22 | 1.44 ± 0.81 n=16 | 0.002 | -2.938 | 0.006 |
| 18 months | 0.76 ± 0.77 n=21 | 1.23 ± 0.73 n=13 | 0.087 | -1.928 | 0.076 |
| Baseline /18 months | P value | | | | |
| Paired t test | 0.137 | 0.639 | | | |
| Wilcoxon signed rank | | | | | |
| z | -1.459 | -1.000 | | | |
| P value | 0.145 | 0.317 | | | |

*significant difference at <0.05

**Table 4 -** Mean plaque scores of mothers in the experimental and controls groups at different study phases.

| Study phases | Study groups | | Experimental and control groups | | |
|---|---|---|---|---|---|
| | Experimental Mean±SD | Control Mean±SD | *P* value† | Mann-whitney | |
| | | | | Z | *P*-value |
| Baseline | 1.13 ± 0.86 n=30 | 17.17 ± 0.95 n=30 | 0.887 | -0.040 | 0.968 |
| 6 months | 0.59 ± 0.91 n=22 | 0.94 ± 0.85 n=16 | 0.242 | -1.466 | 0.181 |
| 12 months | 0.36 ± 0.49 n=22 | 0.38 ± 0.72 n=16 | 0.954 | -0.436 | 0.737 |
| 18 months | 0.19 ± 0.40 n=21 | 0.54 ± 0.97 n=13 | 0.152 | -0.958 | 0.484 |
| Baseline /18 months P-value | 0.000*,‡ | 0.054‡ | | | |
| Wilcoxon sign rank test z | -3.466 | -1.299 | | | |
| *P*-value | 0.001 | 0.194 | | | |

*significant difference at <0.05, †t-test, ‡paired t test

**Table 5 -** Comparison of dmft score of children in the experimental and controls groups at different study phases.

| Study phases | Study groups | | *P*-value† |
|---|---|---|---|
| | Experimental | Control | |
| ***Baseline*** | n=30 | n=30 | 0.191 |
| Mean±SD | 8.37 ± 5.73 | 10.27 ± 5.39 | |
| Confidence interval | 6.23-10.51 | 8.25-12.28 | |
| ***6 months*** | n=22 | n=16 | 0.024* |
| Mean±SD | 8.95 ± 5.59 | 13.00 ± 4.68 | |
| Confidence interval | 6.12-11.12 | 10.51-15.49 | |
| ***12 months*** | n=22 | n=16 | 0.041* |
| Mean±SD | 9.64 ± 4.66 | 13.12 ± 5.44 | |
| Confidence interval | 7.57-11.70 | 10.23-16.02 | |
| ***18 months*** | n=21 | n=13 | 0.001* |
| Mean±SD | 9.19 ± 4.06 | 14.69 ± 4.42 | |
| Confidence interval | 7.34-11.04 | 12.02-17.37 | |
| Baseline /18 months *P* value | 0.574‡ | 0.040*,‡ | |

*significant difference at <0.05, †t-test, ‡paired t-test.
dmft - decayed, missing filled, teeth for deciduous teeth.

**Table 6 -** Comparison of DMFT score of mothers in the experimental and controls groups at different study phases.

| Study phases | Study groups | | *P*-value† |
|---|---|---|---|
| | Experimental | Control | |
| ***Baseline*** | n=30 | n=30 | 0.070 |
| Mean±SD | 13.30 ± 6.44 | 16.63 ± 7.50 | |
| Confidence interval | 10.89-15.71 | 13.83-19.44 | |
| ***6 months*** | n=22 | n=16 | 0.033* |
| Mean±SD | 14.48 ± 6.72 | 19.50 ± 6.91 | |
| Confidence interval | 11.42-17.54 | 15.82-23.18 | |
| ***12 months*** | n=22 | n=16 | 0.012* |
| Mean±SD | 15.59 ± 6.83 | 20.94 ± 5.13 | |
| Confidence interval | 12.56-18.62 | 18.20-23.67 | |
| ***18 months*** | n=21 | n=13 | 0.001* |
| Mean±SD | 13.10 ± 7.60 | 21.54 ± 5.38 | |
| Confidence interval | 9.64-16.55 | 18.29-24.79 | |
| Baseline /18 months *P* value | 0.918‡ | 0.040*,‡ | |

*significant difference at <0.05, †t-test, ‡paired t-test.
DMFT - decayed, missing filled, teeth index for permanent teeth

Whitney. However, statistically significant decrease was observed in the experimental group between the baseline and 18 months, while the control group showed no statistical significance using Wilcoxon signed rank test.

**Tables 5 and 6** demonstrates results of DMFT and dmft in experimental and control groups in mother-child pairs. **Table 5** shows statistically significant increase in dmft in the control compared to experimental group in children at 6, 12, and 18 months periods using t test. No significant difference was observed between baseline and 18 months of the experimental group. However, in the control group comparison between baseline and 18 months period showed statistically significant increase in the dmft ($p$=0.040) using Paired t test.

**Table 6** shows statistically significant increase in mean DMFT score in the control group compared with the experimental group at 6, 12, and 18 months follow up visits ($p$=0.033, $p$=0.012 and $p$=0.001). Comparison of mean DMFT of mother between baseline and 18 months period in the experimental group using the paired t test showed no statistical significance ($p$=0.918). While in the control group a statistically significant increase in DMFT was observed between baseline and 18 months period ($p$=0.040).

**Discussion.** This is the third report of a randomized clinical study investigating the potential effect of xylitol consumed by Saudi mother-child pairs. Subject's selection was based on having high salivary MS level and presence of caries. Our study showed a highly significant reduction throughout the study in the number of children with high salivary MS level in the experimental group compared to the control. The results are consistent with data from a prospective trial carried out in Finland by Milgrom and Ly[24] in which

children and mothers treated with xylitol showed lower streptococcus mutans than children and mothers receiving chlorhexidine or fluoride varnish.

Significant reduction in salivary MS level was observed only among experimental mothers at the 18-month follow-up compared to the baseline period. This is consistent with other clinical findings reported among adult groups.[25-28] The distinct effect of xylitol seen in children compared to their mothers at different period is supported by the findings of Rekola,[26] who reported significantly high reduction of MS level in children when compared to their mothers. Furthermore, the reported superior results of chewable tablet over chewable gum, and related superior results to the texture, chemical composition, and its appealing flavor attributed. The statistically significant reduction in plaque observed in experimental children at different period suggests a positive impact of xylitol on the plaque accumulation. The result is similar to the study of Soderling et al.[12] Unlike children, mothers in the experimental and control groups showed no statistically significant difference at different study periods. This demonstrates the impact of the preventive program in which mothers of the control groups were exposed. Dentist should emphasize that xylitol is a reinforcement rather than a substitute to oral hygiene instructions (OHI), diet counseling, fluoride vanishes and regular dental checkups. The present result shows the positive impact of xylitol on salivary MS and plaque is consistent with data from other studies[24,27] that showed a dose response and a linear reduction in MS level with the use of xylitol chewing gum. Caries levels of children showed a statistically significant difference between the experimental and control groups at 6, 12, and 18 month periods. Unlike the experimental group, the controls showed higher values that were statistically significant at the 18 month period when compared to baseline. Similar results were seen in the DMFT scores of mothers. The intergroup differences provide evidence to support the potential anti-cariogenic effect reported by Kohler and Andreen,[28] Isokangas et al,[8] and Twetman,[29] who showed a positive effect of xylitol on caries level. Although, our study did not show significant caries reduction from baseline to the end of study period as presented by Thaweboon.[30] A significant increase in the control group from baseline to 18 month signifies the suppressive effect of xylitol on dental caries in experimental group similar to the finding of Hildebrandt and Sparks.[25] From the results, it is evident that xylitol can be used as a safe and convenient preventive tool in the Saudi population. A high level of compliance to the applied preventive program as reported by other studies,[10,31] could be achieved by committed and continuous educational effort. Accordingly, xylitol could be recommended as a cost-effective alternative method to fluoride varnishes, especially for young children living in remote areas where access to dental visit is very difficult. Future clinical trials are still needed to investigate treatment variations such as repeated xylitol consumption and different doses as well as the appropriate time to start and stop treatment in children.

In conclusion, use of xylitol chewable gum by mothers and xylitol chewable tablets by children 3 times/day for 3 months reduced mutans streptococcus (MS) level. Data showed a significant reduction of plaque level in mothers using xylitol throughout the 18 months period. In children, reduction of plaque was seen at 6 and 12 months period between the experimental and control groups. Also, reduction at 18 month was observed but did not reached a significant value. The 3 months consumption of xylitol 3 times/day by the mother-child pairs resulted in a significant difference in caries experience in comparison to the controls. Children using chewable tablets showed better results compared to their mothers using chewable gums. Xylitol containing products can be recommended as an adjunct treatment procedure to topical fluoride, and oral hygiene practices in caries control, reducing salivary MS and plaque accumulation.

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## Ethical Consent

All manuscripts reporting the results of experimental investigations involving human subjects should include a statement confirming that informed consent was obtained from each subject or subject's guardian, after receiving approval of the experimental protocol by a local human ethics committee, or institutional review board. When reporting experiments on animals, authors should indicate whether the institutional and national guide for the care and use of laboratory animals was followed.