

ASSESSMENT OF ANTI-MICROBIAL EFFECTS OF XYLITOL ON STREPTOCOCCUS MUTAN

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ABSTRACT

Objective: The purpose of this study was to evaluate the effects of xylitol on salivary s.mutans level in children. **Subject and Methods:** This Clinical trial was conducted at Government primary school Dar-ul-Uloom Hirabad for the period of 2 months. The trial begins by using performa which was consisting of two parts. The first part consisted of general information, and second part was consisted of clinical oral examination, it was carried out to find out active lesions by the help of using (WHO) criteria with the use of plane mouth mirror, and WHO probe. Collection of saliva was scheduled after the clinical examination. This sampling of Saliva is done at the day 1st and also at the end of the study trial. Xylitol products given to students, for twice a day (500mg xylitol/day) for at least 20 minutes. The colonies of bacterial growth were measured in (cfu/ml) colony forming unit per milliliter of saliva sample by anaerobic jars.

Results: Mean age was $8.5 \pm (SD= 2.32)$ range from 4 years to 13 years, the compliance was excellent, and no side effects were reported. The growth of S.Mutans at baseline were observed $6.16 \pm (SD=1.37)$ and after chewing xylitol the growth of S.Mutan was reduced $3.52 \pm (SD=1.15)$. All subjects had detectable levels of S. Mutans at baseline, and after the 4-week intervention period, significantly reduce levels ($P < 0.05$).

Key Words: Dental Caries, S.mutan, Xylitol, Children, Prevention

How to cite this article: Panhwar M¹, Rajpar SP², Talpur N³, Pirohit O⁴, MemonP⁵, Shams S⁶. **ASSESSMENT OF ANTI-MICROBIAL EFFECTS OF XYLITOL ON STREPTOCOCCUS MUTAN.** JPUMHS; 2021;11:01,19-23.

<http://doi.org/10.46536/jpumhs/2021/11.01.284>

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Received On Wed, Dec 9, 2020, Accepted On 15 March 2021, Published On 31 March 2021

INTRODUCTION

Dental caries are alarming for every men kind regardless of gender, age, or their socioeconomic conditions and its one of the most prevalent infectious diseases effecting up to 60% to 90% proportion of population the burden of the disease is considerable within all age groups.¹ According to “National Call to Action for Promoting Oral Health care,” Dental caries is the most common chronic childhood disease.² It impacts on quality of life with pain and subsequently poorer general health Hence classified as one of the main burdening worldwide public health problems by the WHO.³ Human oral cavity is consists so many type of different bacteria. From all of Streptococcus mutans are to be considered as an important humanoid cariogene. Thus, an increase in the growth of these bacteria (streptococcus mutans) in the oral cavity is reflected as a “Major risk factor for the incidence of dental caries” . In addition, according to literature there is an uncertainty about the connection of streptococcus mutans and the caries experience in mankind, Giacaman et al. investigate if presence of streptococcal mutans colonies were

high along with the presence of biofilm can we predict caries susceptibility in particular person .Their results showed us that “The cariogenic potential of streptococcus mutans can be based presence of individual’s own microorganism pathology ,also with including biofilm formation” he also Indicating further more studies are essential

Caries control approaches were always aimed to limiting exposure to sugar contains products and these have been used from many generations. These strategies for restriction were always been flop. This is all because people always found them very tough. In dentistry caries-control strategy and prevention has become an important issue especially regarding the development of new different methods which involves replacement of ingestion of fermentable sugar mainly (sucrose)—with non-fermentable sugar alternate. By joining this principle with a socially acceptable habit such as gum chewing can be help us to establish caries-control policy.^{4,5}

As worldwide incidence of dental caries decreasing in developed countries like United States, and simultaneously increasing in

developing nations. Reason for decrease in the west is due to increased use of fluoride⁶, but increase in developing countries is due to consumption of sugar (sucrose).⁷ After this information we are substituting sucrose (sugar) with xylitol (its natural sugar alcohol helps in prevention of caries). In this study we will find out: can the daily use of chewing gum containing xylitol effects on carcinogenic bacteria of children 6 to 12 years old. Mostly collective all of polyols presences in sugar-free chewing gum are known as xylitol, mannitol and sorbitol. Evidence also proved that if any people take sugar free chewing gums just instantly after every meal will have caries-reducing effects.^{8,9} It is known that chewing gum can help in stimulation of salivary flow rate.¹⁰ This is a useful supplement to standard oral hygiene maintains.¹¹ It is also effective in increasing plaque Ph. Chewing sugarless gums after meals and snacks can help in promote remineralization of the enamel and reduce S. mutans rate.¹² Streptococcus mutans are primary species associated with the development of dental caries in humans and animals.^{13,14} Nutrition is one of the chief etiological cause for Dental caries occurrence.^{15,16}

Xylitol is one of important natural sweetener which is derived from the fibrous part of plants. Xylitol can limiting the growth of bacteria that is streptococcus mutans (main cariogenic bacteria) as these bacteria do not use xylitol for their growth and multiplication.¹⁷ Over time, the quality of the bacteria in the mouth changes with the use of xylitol survive on the tooth surface. Lower plaque forms and amount of acids that attack the tooth surface can be lowered to maintain a neutral pH in the mouth.¹⁸ Research has shown that the use of xylitol also helps to repair tooth enamel damage.¹⁹

It's generally accepted that saliva flow and its composition are important for oral health, especially the prophylaxis of dental caries. Evidence shows that severe reduction of salivary secretion or alteration of salivary composition (may be caused by diseases of salivary glands, radiotherapy, use of certain drugs, etc.) could increase the risk of many oral diseases including dental caries, oral candidal infections, and mucosal ulceration.²⁰

The concepts of dental treatments were recently changed from traditional treatments towards new prevention for dental caries, along with the use of various types of different novel techniques. That is why a special emphasis has been paid to developing new preventive methods to treat dental caries. Apart from conservative treatment to other distinctive procedures in dentistry have vastly enhanced the quality of care to the general population also will be beneficial in reducing patients and operator fatigue.

The objective of this study is, consequently, to assess the impact of fluoride varnish application in children as an additional measure to a preventive program comprising of school-based oral health education. As seen in literature as simple preventive measure like application of

fluoride varnish can be helpful in prevention of dental caries in underserved community which can be very beneficial in developing country like Pakistan and its rural areas like Jamshoro, Sindh.

MATERIALS AND METHODS

This clinical trial was done for assessing the effects of xylitol containing products on dental caries prevention in primary school students. Participants of our study were students of Government primary Boys/Girls school Dar-ul-Uloom jail road Hirabad Hyderabad Sindh. A total of 20 healthy volunteers aged 6-12 years were included in this study as a study group and 20 healthy volunteers were taken as control group. The study and intervention involve was explained in detail to the school authorities, children and parents. A written informed consent was taken from parents or guardians of children. All participants who had moderate oral hygiene and had not received any antibiotic or antimicrobial agent or professional fluoride therapy within the last month were set as inclusion criteria. Subjects with chewing gums xylitol habit or who were undergoing orthodontic treatment were excluded from present study

The study was carried out by using proforma which was consisting of two parts. The first part consisted of general information from school students which was regarding age and gender and the second part was consisted of clinical oral examination of students. This oral examination of school children was carried out to find out active lesions by the help of using world health organization (WHO) criteria with the use of plane mouth mirror, and WHO probe. All the instruments were sterilized by autoclaving. The same examiner examined all children.

Collection of saliva was scheduled after the clinical examination, to minimize circadian rhythm effects, saliva sampling was done in the morning 10.00AM (which will more than 2 hour after the morning breakfast) the subjects will order not to eat or drink for a before the saliva sampling. Students were made to sit relax on the class chair. Children were instructed to swallow already existing saliva, to get rid their mouth of any debris in the mouth. Thereafter, approximately 2ml of unstimulated saliva samples from all children were collected in a sterile condition container, and then we put them in an ice box and transferred immediately to the laboratory of Liaquat University of Medical and Health Sciences. The saliva samples of all the participants were identified by a code number during the period of sampling collection and processing.

Exactly after 35 days, samples of saliva were withdrawn second time with same procedure as done in the beginning of the study. Then these saliva samples were also stored in an ice box than transferred to lab for analysis.

Xylitol products (chewing gums) were given to students by their class teachers and chief investigator. The suggested dose of gum for children was twice a day (500mg xylitol/day) for

at least 20 minutes only, which should be after breakfast and lunch break which is during school time was further checked by the main investigator. The school students were also guided to follow the same timing during the holidays at home. For this reason a bag containing 12 pieces of chewing gums was given a day before the weekend, so that they can take it in their home as well. Students were strongly being encouraged to reduce their carbohydrate diet and brush their teeth twice a day with any fluoride toothpaste.

The counts of streptococci mutans species were evaluated by culturing method. A thin rod shaped tool (loop) that is mainly used by lab technologist to separate an inoculum from a bacterial culture. The loop is used in the growth of germs on plates by shifting the inoculums for zipping, A loop which 10µl (0.01 ml of sample) was inoculated on the Blood AGAR plate and a zigzag line drawn on saliva sample.

The Streptococcus mutans used in the present study were grown on blood agar media. For anaerobic growth condition, the Agar dishes were kept in incubator on an anaerobic jars of (BBL, Cockeysville, Md.) which along with gas-generating kit (GasPak no. 70304, BBL) and we also an anaerobic indicator strip (GasPak no. 70404, BBL) for 37°C for up to (48 hours) were used. Then the appearances of colonies were

observed and the number of colonies of saliva sample was measured using a colony counter.

For different cultured colonies in size and shape, several biochemical tests, such as Gram staining, Catalase test, and Bacitracin discs (Padtan teb, Iran), Hydrolysis of Esculin (Himedia, India) and fermentation of the manitol and sorbitol (Sigma-Aldrich, USA) will be done. Based on Bergy's manual, the colonies similar to isolated MS will count same procedure also done in another study on salivary S .mutans (21)

The colonies of bacterial growth were measured in (cfu/ml) colony forming unit per milliliter of saliva sample by anaerobic jars.

RESULTS

Mean age was 8.5 ±(SD= 2.32) range from 4 years to 13 years, the compliance was excellent, and no side effects were reported Table 1.

There were 20 students which included 11 male students and 9 female who participated in the study shows in Table 2. All of them completed the trial, the compliance was excellent, and no side effects were reported.

All subjects had detectable levels of S. mutans at baseline, and after the 4-week intervention period, significantly reduce levels (P < 0.05) of S. mutans compared to baseline shown in Table 3

TABLE 1 SHOWING STATISTICS OF AGE OF STUDENTS	
Mean	8.50
Std. Deviation	2.328
Range	9
Minimum	4
Maximum	13

TABLE 2 SHOWING GENDER STATISTICS					
Gender	N	Mean	Std. Deviation	Std. Error Mean	P value
Boys	11	5.7109	1.27299	0.38382	0.108
Girls	9	6.7111	1.36331	0.45444	

TABLE 3 SHOWING STREPTOCOCCUS MUTANS COLONY COUNTS IN AT DIFFERENT TIME INTERVALS	
Growth of S Mutans at base line	Growth of S Mutans after chewing Xylitol
6.1610	3.5285
1.37699	1.15860

DISCUSSION:

Our study focused on both important things which results in damaging the tooth surfaces, one is the presence of bacteria which causing dental caries and also clinically presence of different active untreated carious lesions on the teeth of children. After six months of our trial, we did again assessment of same school, during this specific period of time no other type of community-level caries prevention approaches were done at this school area. At the 1st month of study follow-up, we observed remarkable differences in caries overall in all school students. We reviewed several other studies which also showed if in short-period exposure of different polyols on MS results having reduce effects on (1, 22, 23) The inclusion criterion for our study there should be presence of carious lesions on tooth of study participates, a growth-reducing effect of xylitol on salivary MS has been described, indicating a long-term outcome of the polyol. (4, 24) In our present study confirmed that xylitol products consumption did not affect the salivary microbiota besides it decrease in salivary MS. While numerical reduction has been showed in students with having a high salivary MS concentration. (table no 1)

We used study design and xylitol doses which were previously shown to demonstrate xylitol-induced changes in plaque and saliva MS counts. We also used the same chewing gum as in this study and a 4-week trial period. (4).

According to gender comparison, we found that the reductions in the number of males were higher on both cariogenic bacteria and the number of females examined. One of limitation that may be considered was that the study was conducted on a small sample size.

In this trial xylitol has given us beneficial for prevention of oral disease as well as oral health education to school students and school staff. Overall, our approach showed an effective result towards the prevention and further development of new caries lesion. Our these results were also contrast to an pervious clinical trial, done by [Alanen et al., and Honkala et al.,] in which they suggested xylitol products (candies and lonzenges) were well effective towards caries prevention.

It has been proven that xylitol is completely safe and also promising sweetener it has several advantages as it is calorie-free and contains less laxative than any other poly(18, 25), another important property of xylitol is that this is non-glycemic and also having anti-oxidant properties. Due to this it has having lowest solubility which results in long term retention, Because of all its advantages it might play an important character as a preventive measure. Studies also revealed that human cariogenic bacteria (mutans) and other bacteria in our oral cavity do not consume xylitol for their multiplication.

Through by this study all school students, teachers and other staff members gets support on oral health maintenance as well as oral health

education at own door step (school) and also all were participated in proper clinical checkups, and therefore their performance towards oral health has been improved. The school teachers were actively participates in distribution of gums that might pay response towards oral hygiene and also prevention of disease.

Gummy bear in a form of a xylitol was given to school children in this study. Chewing gum has been reported by [Edgar,] that it is having effects of stimulating salivary flow this will leads to caries prevention.

In our study, we use chewing gummy bears that may have a saliva – stimulus effects, which results to the minimization of further caries progression (26).

Compliance of our study was very nice, as teachers and head teachers were helping in distribution and monitoring all the procedures including lab reports. The annual field visits by the investigators at the participating school also enhanced and gives motivation to the prevention of their tooth delays.

In this study we diagnosed caries by using ICDAS method by [Ismail et al.]. This is one of recently been acknowledged as a globally caries detection system by [EGOHID II.]. This method also having high validity as well, this has been shown in many of vitro studies [Ekstrand et al. 2007]

The baseline data of our study had showed the high consistency of the carious bacteria and which reduced after the intervention period.

CONCLUSION:

Dental caries is a multifactorial, diet-associated infectious disease. According to the results of this study, xylitol in chewing gums form when chewed two times a day for a about 15 mints were more effective than other sugar(sucrose)-containing products, This also help on individuals with having poor oral hygiene, having less salivary flow and patients high risk for dental caries. These chewing gums can also recommend an individual's of mental and/or physical disabilities.

ETHICS APPROVAL: The ERC gave ethical review approval

CONSENT TO PARTICIPATE: consent was taken from subjects and next of kin

FUNDING: The work was not financially supported

ACKNOWLEDGEMENTS: We would like to thank the all contributors

AUTHORS' CONTRIBUTIONS: All authors read and approved the final manuscript.

CONFLICT OF INTEREST: No competing interest declared.

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