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EVALUATION OF ANTIBACTERIAL POTENTIAL OF LICORICE AND XYLITOL CONTAINING SUCROSE FREE HARD CANDIES IN HIGH CARIES RISK PATIENTS

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ABSTRACT

Objectives: To evaluate the efficacy of sucrose-free hard candies containing xylitol and licorice for preventing dental caries in high-risk individuals.

Materials and Methods: Total 80 participants were enrolled based on inclusion criteria. Participants were divided at random into four groups of twenty. Group A consumed stevia-sweetened hard candies including licorice extract, Group B consumed stevia-sweetened hard candies containing xylitol extract, Group C consumed stevia-sweetened hard candies, and Group D consumed sucrose-sweetened hard candies. For the first ten days, participants were asked to eat 2 candies each day; saliva samples were collected at Day 0, after fourteen days following the baseline saliva collection, and after six months after the intervention. Each participant was asked to spit into a sterile plastic container until a sufficient quantity (5ml) of un-stimulated saliva was collected. It took place between 9 and 10 a.m. For at least an hour before the saliva sample was taken, the individual was requested to abstain from all forms of oral care. The statistical analysis was done by SPSS version 20.

Results: Eighty participants finish the experiment and follow the instructions. CFU and pH data for streptococcus mutans in saliva were done as experimental analysis. The mean percentage change in CFU of Streptococcus mutans (%) varied significantly across hard candies over the course of each time period (baseline to fourteen days, $P < 0.001$; fourteen days to six months, $P < 0.001$; and baseline to six months, $P < 0.001$). There was a statistically significant difference in mean salivary pH across various assessment periods within Base line to fourteen days ($P < 0.001$) and Fourteen days to six months ($P = 0.019$) and Base line to six months ($P < 0.001$). The connection between bacterial count and salivary pH was found to be -0.142 ($P = 0.212$) using Pearson's correlation coefficient.

Conclusion: Licorice or xylitol extract are used to make sugar-free hard candies that are anti-bacterial, although the effect wears off with time. Hard candies without sugar have been proven to decrease cariogenic bacteria, while having an essentially neutral effect on pH.

Key words: Dental caries, Licorice extract, Xylitol extract, S. mutans, Oral, Risk Patients, Sucrose Free

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INTRODUCTION

Sugary flavors evoke feelings of warmth and appreciation which lead to the dental carries. Numerous epidemiological studies from all over the globe

contribute to the idea that a rise in dental caries may be traced back to changes in people's eating habits¹. According to survey, there is 56.62% Pakistani population is affected from dental carries². Diet has a significant role in the carious process, as proven by the Vipeholm, Hopewood home, and Turku sugar studies³. Plaque's pH may be lowered to levels that may cause some demineralization of enamel due to the use of sucrose, glucose, fructose, and other simple carbohydrates by several oral bacteria to create organic acids (lactic, acetic, and propionic). Although many things might contribute to an illness, germs are generally accepted as the primary cause⁴. There are between five hundred and thousand different kinds of bacteria, fungus, protozoa, and viruses living in the oral cavity. Eliminating all bacteria from a given area is quite unlikely, but reducing the overall bacterial concentration may slow the cariogenic process⁵.

Dental caries is often caused by a diet heavy in sucrose. Sugar tops the cariogenic scale. Sucrose helps Streptococcus mutans colonies new places and become digested by oral bacteria. Sucrose helps dental biofilms synthesise extracellular polysaccharides (EPS)⁶. The stability and physical integrity of biofilm matrix are maintained by extracellular polysaccharides (EPSs), which may be a pathogenic component of biofilm growth and cariogenic bacteria. The oral cavity's pH may need to be raised to prevent cariogenic bacteria from producing acid and promote remineralization. Passive diffusion leaches tooth minerals, causing demineralization. Remineralization requires adjusting the mouth cavity's pH to prevent demineralization and mineral diffusion^{7,8}.

As an infectious disease, caries can be avoided by antimicrobials targeting cavity-causing bacteria. Effective oral antimicrobials reduce tooth decay. According to many researches, sugar-free products may prevent caries and enhance oral health. This approach can help reduce oral cavity-causing Streptococcus mutans¹⁰. Glycyrrhiza glabra, or licorice, is therapeutic. Egyptians, Greeks, and Romans used this medicine for many ailments¹¹. Several studies have found anticancer, antiviral, anti-inflammatory, and antibacterial properties in licorice compounds, particularly against streptococcus mutans¹². Antibacterial actions against Streptococcus mutans, sobrinus, gingivalis, and prevotella intermedia can minimise plaque generation¹³. Companies utilise xylitol to substitute sugar and sweeten naturally. Dental cavities

are reduced by xylitol products. Saliva boosts saliva production and enamel remineralization. Xylitol also reduces cariogenic bacteria CFU in dental plaque and saliva¹⁴.

After realizing the beneficial effects of sugar replacing products and very less data found in Pakistan, we designed this study to replacing the regular consumption of sucrose-containing hard candies with a more beneficial product that may reduce your risk of contracting streptococcus mutans without adversely affecting any other bacteria in the mouth. Therefore, the objective of this research was to evaluate the efficacy of sucrose-free hard candies containing xylitol and licorice for preventing dental caries in high-risk individuals.

MATERIALS AND METHODS

After taking approval from IRB of hospital, this cross-sectional study was conducted at Oral Pathology department of Frontier Medical and Dental College, Abbottabad from August 2022 to January 2023. Those who fit as study participants were recruited. A total of eighty participants were selected via a random process to take part in the research. Twenty participants were grouped in Group A to D.

Sample size calculation was done by using WHO calculator (www.openepi.com) with a 95% confidence interval and a 5% significance level, assuming 80% of the study's power based on the prevalence of 57% of dental carries in Pakistani population.² Total 80 sample size was calculated. We divided equal number of patients in four groups.

Participants with the age group between 16-22 years old and free of any known or suspected systemic illnesses were included. Participants who consume candy on a regular basis as a sugary snack despite practicing average oral hygiene (brushing their teeth twice a day with fluoride toothpaste) are considered high caries risk candidates according to CAMBRA (caries management by risk assessment). In addition, according to the radiographic categorization of dental caries, they need to have at least two teeth in the back of their mouth that exhibit first proximal lesions E1 or E2. Subjects who wore orthodontic appliances or removable prostheses, those with any medical or systemic ailment, and those allergic to any of the hard candy components were not eligible to participate.

Subjects were divided at random into four sets

of twenty. Group A consumed stevia-sweetened hard candies including licorice extract, Group B consumed stevia-sweetened hard candies containing xylitol extract, Group C consumed stevia-sweetened hard candies, and Group D consumed sucrose-sweetened hard candies. For the first ten days, participants were asked to eat 2 candies each day; saliva samples were collected on Day 0, after fourteen days following the baseline saliva collection, and after six months after the intervention.

Each participant was asked to spit into a sterile plastic container until a sufficient quantity (5ml) of unstimulated saliva was collected. It took place between 9 and 10 a.m. For at least an hour before the saliva sample was taken, the individual was requested to abstain from all forms of oral care. Before eating any of the hard candies, a baseline sample was collected. After waiting 14 days, a second sample was collected. Six months after the third sample was collected. Before taking a saliva reading, the pH meter was calibrated using the supplied 4 (acetic acid) and 7 (phosphate buffered saline) pH buffer solutions. After collecting saliva, an electrode was placed in it to measure its pH. The electrode was cleaned with running water after each measurement. The data were noted manually.

100 μ L of diluted saliva samples were dispersed equally on the surface of Mitis Salivaris Agar with Bacitracin (MSBA) media plates. The plates were then placed at 37 $^{\circ}$ C in anaerobic incubator for 24 hrs without the presence of oxygen. Next day, the microscopic examination of each plate was done and *S. mutans* were seen like granular, frosted-glass

seeming, raised, convex, opaque colonies of a dark blue hue with rough borders. *S. mutans* showed up as chains of Gram-positive cocci.

Data analysis was performed in SPSS 20. Continuous data were represented by mean and standard deviation and we used an analysis of variance (ANOVA). Statistical significance was determined using a 95% confidence interval and a P value less than 0.05. Wherever possible, we used a test based on the Pearson correlation coefficient.

RESULT

Eighty participants finish the experiment and follow the instructions. CFU and pH data for streptococcus mutans in saliva were done as experimental analysis. The mean percentage change in colony forming unit of *Streptococcus mutans* (%) varied significantly across hard candies over the course of each time period (baseline to fourteen days, $P < 0.001$; fourteen days to six months, $P < 0.001$; and baseline to six months, $P < 0.001$). Groups A and B had a substantially larger mean percentage change from baseline to 14 days than groups B and D. When comparing the three groups throughout the span of 14 days to 6 months, candies in Group B exhibited the biggest mean percentage change. Group D showed the least amount of change statistically (Table I). Group C had the lowest percentage change from baseline to 6 months, whereas the other 3 groups showed no discernible difference

In table II, there was a statistically significant difference in mean salivary pH across various assessment periods within Base line to fourteen

Table 1: Bacterial percentage of *S. mutans* count based on (CFU) at each interval after consuming various hard candies.

Intervals		Mean	Std. Deviation	Minimum	Maximum	P value
Base line to fourteen days	Group A	42.43	1.48	41.00	45.30	<0.001
	Group B	51.93	0.31	51.70	52.60	
	Group C	18.79	0.33	18.30	19.40	
	Group D	-16.46	0.27	-16.80	-16.10	
Fourteen days to six months	Group A	-27.90	0.94	-29.10	-26.29	<0.001
	Group B	-52.37	1.10	-54.40	-51.40	
	Group C	-17.45	0.23	-17.87	-17.20	
	Group D	-4.29	0.15	-4.60	-4.10	
Base line to six months	Group A	28.50	0.41	27.60	28.90	<0.001
	Group B	28.91	0.62	27.80	29.60	
	Group C	4.35	0.14	4.10	4.54	
	Group D	-22.15	0.48	-22.60	-21.40	

days ($P < 0.001$) and Fourteen days to six months ($P = 0.019$) and Base line to six months ($P < 0.001$). The connection between bacterial count and salivary pH was found to be -0.142 ($P = 0.212$) using Pearson's correlation coefficient (Table III).

DISCUSSION

About 90% of children and 62% of adults throughout the world suffer from dental caries, making it one of the most widespread public health issues¹⁵. Practices have emerged in response to the widespread occurrence of dental caries like avoiding hygiene, careless for cleaning teeth, eating candies etc¹⁶. The allure of sucrose-free hard candies is that they provide a sweet fix without the negative health effects of sugar. This research was carried out to assess the impact of stevia-sweetened with licorice extract hard candies, stevia-sweetened with xylitol extract hard candies, on salivary streptococcus mutans count and salivary pH. Patients with high caries risk and at least two early enamel lesions were included in the trial because they represent the group in highest need of an intervention with an anticariogenic effect¹⁷. Previous studies indicated that most adults eat one sugar-sweetened snack every day or at least many times weekly, therefore the age range was standardized between 16 and 20 year old. It was followed by the previous research that high caries risk may be predicted by measuring the number of bacteria and the pH of the patient's saliva¹⁸. The acidic pH of the saliva/biofilm is caused by Streptococcus mutans' metabolism of the easily fermentable carbohydrates, which in turn promotes the proliferation of aciduric and acidogenic organ-

isms and demineralizes the tooth structure. Given the importance of saliva in controlling the remineralization/demineralization process, efforts should be made to maintain the saliva's pH around neutral¹⁹.

Hard candies containing xylitol extract have been shown to inhibit the growth of Streptococcus mutans, which may be due to the xylitol being phosphorylated and transported through the cell wall of S. mutans by a constitutive fructose-PTS (phosphotransferase) system, leading to an accumulation of xylitol-5-phosphate in intracellular substrate^{20,21}. Xylitol has a direct inhibitory effect on Streptococcus mutans and lowers the development of dental plaque and the adhesion of bacteria (making it antibacterial). Using a continuous-culture biofilm model, researchers discovered that sucrose greatly promotes bacterial colonization and proliferation, whereas xylitol has the opposite effect. S. mutans' ability to cling to hydroxyapatite is affected by xylitol, according to the findings²². It's possible that the quantity of alkaloids, tannins, and essential oils in licorice root, which limit the adhesion of bacteria to the tooth surfaces, the synthesis of glucan, and the activity of amylases, are responsible for the reason why the average percentage change in streptococcus mutans count with respect to of CFU (baseline to fourteen days) interval was higher in candies containing licorice extract and

Table 3: Pearson's Correlation between bacterial count and salivary pH.

Variables	Pearson Correlation	P value
Bacterial Count x Salivary pH	-0.142	0.212

Table 2: Salivary pH at each interval after consuming various hard candies.

Intervals		Mean	Std. Deviation	Minimum	Maximum	P value
Base line to fourteen days	Group A	7.0870	.02677	7.00	7.12	<0.001
	Group B	7.2410	.03024	7.20	7.29	
	Group C	7.3660	.01501	7.34	7.39	
	Group D	7.2380	.01508	7.21	7.26	
Fourteen days to six months	Group A	7.5185	.09132	7.46	7.78	0.019
	Group B	7.5245	.02762	7.48	7.59	
	Group C	7.2160	.92618	7.12	7.58	
	Group D	7.1560	.02998	7.11	7.19	
Base line to six months	Group A	7.4300	.01947	7.41	7.46	<0.001
	Group B	7.3255	.04261	7.24	7.39	
	Group C	7.4380	.04595	7.40	7.56	
	Group D	7.1360	.02644	7.10	7.18	

xylitol extract. This is consistent with the numerous studies that have shown glabridine, a major component of licorice root, to have a direct impact against both gram-positive and gram-negative types of bacteria. The antiadhesive and antibacterial activities of licorice root extract are enhanced by the presence of glycyrrhizin, glycyrrhetic acid, asparagine, and chalcones. Furthermore, flavonoids extracted from licorice root extract, including 1-methoxyficifolinol, licorisoflavan A, and diprenylgenistein, were demonstrated to totally block the development of biofilm, and are therefore suggested for prevention of dental caries²¹.

This rise in streptococcus mutans count was most pronounced over the time period spanning 14 days to 6 months when hard candies containing xylitol extract were consumed. Long-term use of xylitol alters the distribution of *S. mutans* between saliva and dental plaque, which contributes to the development of xylitol-resistant strains of streptococcus mutans in people. Some research has shown that xylitol-resistant *S. mutans* may be less cariogenic and less virulent than xylitol-sensitive *S. mutans*. However, some research suggests that acid and polysaccharide synthesis by xylitol sensitive and xylitol resistant streptococcus mutans are statistically indistinguishable. Hard candies containing sucrose showed the least noticeable percentage change over a span of 14 days to six months. Despite the fact that sucrose is the most cariogenic carbon source, cariogenic bacteria had the greatest cell division rate and the strongest acid production when sucrose was present in the environment, the cariogenic effect of sucrose-sweetened hard candies may have been mitigated in subjects who followed moderate oral hygiene measures, such as brushing their teeth twice a day with fluoridated tooth paste^{23,24}.

Unlike sucrose, stevia does not kill cariogenic bacteria, but it does not promote streptococcus mutans cell proliferation, either, which may explain why stevia-sweetened candies showed the smallest percentage change between the baseline and 6-month interval. Polysaccharide synthesis also seemed to be generally declining in stevia plants. The correlation between MS and plaque pH yielded some intriguing results. After 10 days of eating licorice extract hard candies, the patients' salivary pH rose considerably from the starting point. Licorice extract was shown to be effective in lowering acid production in an

experimental setting. It has been shown that the alkaline food licorice may prevent the development of peptic ulcers and gastroesophageal reflux disease. In addition, licorice's pleasant flavor might promote saliva production, which boosts buffering capacity due to the high concentration of bicarbonate ions in saliva^{11,25}. Similarly, after consuming sucrose, xylitol increases saliva flow in the oral cavity without raising acid content in dental plaque, regulating saliva pH and buffering capacity. Additionally, xylitol is a non-fermentable sugar produced by Streptococcus bacteria, most notably *S. mutans*, and it functions in the process of remineralization by inhibiting the development of cariogenic bacteria and therefore avoiding a decrease in oral pH. In this setting, xylitol's usage for mouthwash boosts saliva production, which in turn raises saliva pH to neutralize acids created by fermentable sugars²⁵.

The study of licorice and xylitol extracts as an antimicrobial activity is the strength of this study. However, due to the limited number of participants in the study, it is impossible to draw any definitive conclusions regarding the different age groups. In future, we need to do this on large scale population.

CONCLUSION

Sugar-free hard candies made with licorice or xylitol extract are antimicrobial, but their effect goes after interval of time. Hard candies without sucrose have been shown to reduce cariogenic bacteria, although their impact on pH is practically neutral.

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