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The anticariogenic effect of xylitol on seven *Streptococcus mutans* strains

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ABSTRACT

Introduction: Xylitol can affect caries-inducing bacteria; however, different *Streptococcus mutans* strains might respond differently. *Aim:* To investigate the effect of xylitol on biofilm formation and metabolic activity of seven *S. mutans* strains. *Methods:* Seven *S. mutans* strains (UA159, A32-2, NG8, 10449, UA130, LM7, and OMZ175) were inoculated into 96-well microtiter plates and were tested with various xylitol concentrations (0.0, 0.0016, 0.0031, 0.0063, 0.0125, 0.025, 0.05, 0.1, 0.2, 0.4 and 0.8 g/mL) for inhibition of biofilm formation and bacterial metabolic activity by recording absorbance values. Lactate dehydrogenase and extracellular polysaccharide assays were conducted at 0.0, 0.1, 0.2, 0.4, and 0.8 g xylitol/mL. Data were analyzed by one-way analysis of variance, Tukey's, paired t, and LSD tests at 0.05 significance level. *Results:* Xylitol produced a significant decrease in bacterial biofilm formation compared to controls at 0.4 g/mL, with almost complete lack of biofilm formation at 0.8 g/mL. This was consistent with metabolic activity which demonstrated a significant activity reduction occurring for all strains at 0.4 g/mL, and a complete lack of activity at 0.8 g/mL for all seven strains. There was a trend for lower LDH and EPS production with the increase in xylitol concentration especially with UA159, UA130, and NG8. *Conclusion:* Xylitol has a clear anticariogenic effect on *S. mutans* which was slightly different depending on the tested strain confirming that the benefit of xylitol might vary from one patient to another. The effect is more apparent at concentrations of 0.4 g/mL and higher.

Keywords: xylitol, *Streptococcus mutans*, biofilm, viability, caries

1. INTRODUCTION

Dental caries disease is a bacterial disease that affects the hard tissue structures in the oral cavity. It is considered to be a chronic global burden disease affecting more than two billion people worldwide of both developing and developed countries (World Health Organization, 2020). Evidence suggests that caries is a multifactorial process which makes its management very challenging since its discovery. The imbalance between protective and pathological factors would participate in the development and progress of this disease (Hurlbutt and Young, 2014; Nassar, 2017). Thus, the management and

treatment principles of this disease have remarkably changed throughout the years (Konig, 2004; Hurlbutt and Young, 2014). The use of natural products to prevent caries development and progression seems to be a modern growing principle to challenge and modulate the effect of this multifactorial disease (Brambilla et al., 2016; Oza et al., 2018; Padminee et al., 2018). Xylitol, a natural carbohydrate sugar alcohol sweetener that is used as a sugar substitute in many oral products was shown to positively improve the oral health environment by modulating the bacterial pathogenic properties of this disease (Riley et al., 2015; Alanzi et al., 2016; Janakiram et al., 2017; Nassar, 2017).

Xylitol is considered safe with no side-effects if used in concentrations up to 8-10 g/day (Forster et al., 1982). The major reported side-effect in such a high dose was a laxative effect (Dodds, 2012; Jang et al., 2012). Xylitol seems to accomplish its anticariogenic effect through several mechanisms including the inhibition of bacterial metabolic pathways, the interference with cariogenic bacteria adherence to teeth structures, decrease vertical transmission, and stimulation of salivary flow that will result in the reduction of bacterial biofilm and the promotion of teeth mineralization (Janakiram et al., 2017; Nassar, 2017).

Streptococcus mutans is known to be one of the primary cariogenic bacteria capable of causing dental caries (Loesche, 1986). Several *S. mutans* strains were identified (Kuramitsu, 1993) and the effect of xylitol on these strains still not fully elucidated. In addition, the response of each *S. mutans* strain to antibacterial agents is different (Li and Bowden, 1994; Nassar et al., 2012; Nassar and Gregory, 2017). Thus, the main aim of this study was to study the effect of xylitol on biofilm formation and metabolic activity on seven *S. mutans* strains.

2. METHODOLOGY

Bacterial strains and media

This study was conducted between January and March 2021. Seven *S. mutans* strains were used in this study: UA159 (American Type Culture Collection ATCC 700610), A32-2 (isolated in this lab), UA130 (ATCC 700611), NG8 (serotype *c*), 10449 (ATCC 25175), OMZ175 (serotype *f*) and LM7 (serotype *e*). Agar plates (Mitis Salivarius Sucrose Bacitracin) were used to culture the bacterial strains. Growth conditions were 5% CO_2level at 37°C unless otherwise stated. Tryptic soy broth with 1% sucrose (TSBS; Difco Laboratories, Detroit, MI) was used as the main culture media.

Xylitol concentrations and preparations

Several xylitol concentrations were tested by mixing xylitol into TSBS. A stock concentration of 0.8 g/mL was prepared and then serial dilution was used to obtain the other xylitol concentrations (0.0016, 0.0031, 0.0063, 0.0125, 0.0250, 0.05, 0.1, 0.2, and 0.4 g/mL) in TSBS. In addition, TSBS alone, and TSBS with bacteria were used as negative and positive controls, respectively.

Biofilm assay

Ninety six - well sterile polystyrene microtiter plates with flat bottom were used (Fisher Scientific, Pittsburgh, PA, USA). The TSBS with the previously mentioned prepared concentrations of xylitol were used in each well of the microtiter plate, and after that inoculation of the cultured seven strains of bacteria was done. Each of the seven strains was organized in the microtiter plate in a manner of which each strain had different concentrations of xylitol. Bacterial inoculum (10 μL) in TSBS (290 μL) were used as positive control. The plates were incubated for 24 hours in 5% CO_2 and 37°C. After the incubation, planktonic bacteria were removed through pipetting, and then saline was used to wash the wells twice gently. The bacterial biofilm was fixed by the addition of 100 μL of 10% formaldehyde solution and left at room temperature overnight. Formaldehyde was removed the following day and wells were washed twice by saline. Afterwards, 100 μL of 0.1% crystal violet staining solution was added to the wells and kept for one day at room temperature. The following day wells were washed and 250 μL of isopropanol was added and left for one hour at room temperature. Finally, a spectrophotometer (molecular appliances, Inc., Sunnyvale, CA, USA) was used to read each well absorbance values at 490 nm with isopropanol because the blank. Each set were done in triplicate wells and each assay was performed at least 3 times.

Bacterial metabolic activity assay

A sodium 3`-[1-[(phenylamino)-carbonyl]-3,4-tetrazolium]-bis(4-methoxy-6-nitro) benzene-sulfonic acid hydrate (XTT) assay was utilized (Huang et al., 2012) in order to research the impact of various xylitol concentrations on the metabolic activity. In brief, biofilms of all seven strains of S. mutans were developed in 96-well microtiter plates. This was achieved by the addition of 10 μL overnight cultures at 290 μL 1% TSBS; then the plates were incubated for 24 h in 5% CO_2 at 37°C. After that, nonadherent cells were removed by washing the biofilms 3 times with sterile 0.9% NaCl and three hundred μL of every xylitol solution or TSBS control

were added to designated wells (in triplicate) and incubated at 37°C. After 24 h of incubation, the handled biofilm was washed thrice, and the metabolic activity of the biofilm was determined by the XTT assay. After 2 h of dark incubation at room temperature, a spectrophotometer was used at 490 nm to measure the absorbance of the XTT solution after being transferred into a brand new microtiter plate.

Lactate dehydrogenase assay

LDH assay was used to determine cellular activity of the seven *S. mutans* strains. The seven strains were treated with the different xylitol concentrations (0, 0.1, 0.2, 0.4, and 0.8 g/mL) diluted in TSBS and have been incubated for twenty-four h at 37°C and 5% CO_2. The plates were washed with deionized water three times, 10 µL of lysis buffer (Pierce LDH cytotoxicity Assay, Thermo Scientific, Rockford, IL, USA) become added to every well and was incubated at 37°C, 5% CO_2 for 45 min. A fresh LDH reagent mix was prepared according to the manufacturer's instruction, 100 µL of the reagent mix was added to the wells containing the biofilm cells and lysis buffer and was incubated for 30 min. at room temp in the darkness. Finally, 50 µL of the stop solution was added to each well, the absorbance was measured at 490 and 680 nm using the spectrophotometer and the 680 nm absorbance values were subtracted from the 490 nm values for each well.

Extracellular polysaccharide assay

EPS assay measured the cellular ability of the *S. mutans* to produce extracellular polysaccharide when treated with xylitol (0, 0.1, 0.2, 0.4, and 0.8 g/mL). The biofilm of the seven strains of *S. mutans* were prepared the same way as the LDH assay. The plates were washed thrice times with deionized water, 150 µL of undiluted sulfuric acid was transferred to each well that contained biofilm cells and immediately followed by 30 µL of 5% phenol. The plates were incubated by floating the plates at 90°C for 5 min. in a bath of hot water followed by a 5 min. cool down at room temperature. Finally the absorbance of the EPS produced from the biofilm cells become measured at 490 nm using the spectrophotometer.

Statistical testing

Absorbance values from biofilm and metabolic activity assays were analyzed using one-way-analysis of variance (ANOVA) accompanied via Tukey's multiple comparison test. Comparisons were made between strains at each concentration as well as for all concentrations within a particular strain. Overall biofilm mass at the conclusion of the experiment was compared between the seven tested strains using one-way ANOVA.

LDH and EPS assays comparisons were performed the use of one-way ANOVA accompanied by Least Significant Difference (LSD) post hoc tests to investigate differences between strains. Paired t-test was used to compare mean absorbance values at 0.1, 0.2, 0.4, and 0.8 g/mL compared to the corresponding control. All statistical analyses were conducted using SPSS Statistical software Ver. 23 (IBM Corporation, Armonk, NY, USA) at a 0.05 importance level.

3. RESULTS

Biofilm assay

For the biofilm absorbance values, at baseline, there was a significantly different ($p< 0.05$) between values for strains LM7 and OMZ175 compared to 10449 (Table 1). Xylitol at a 0.0016 g/mL concentration produced a slight decrease in biofilm formation compared to controls (Figure 1). A sustained gradual reduction was observed over all concentrations until 0.2 g/mL was reached in all strains; after which a significant decrease ($p< 0.05$) occurred at 0.4 g/mL with almost complete lack of biofilm formation produced at 0.8 g/mL. Comparing the different strains, UA159, LM7, UA130, and OMZ175 produced more biofilm mass compared to the other three strains between 0.0031 and 0.1 g/mL (Figure 1). The 10449 *S. mutans* strain produced the smallest quantity of biofilm formation compared to other strains over all concentrations below 0.1 g/mL. At 0.4 g/mL, only strains UA130 and OMZ175 produced some biofilm amounts compared to other strains.

Regarding cumulative biofilm mass at the conclusion of the research for all concentrations, UA159, LM7, UA130, and OMZ175 produced significantly more biofilm mass ($p< 0.05$) compared to A32-2, NG8 and 10449; with the latter producing the least amounts of biofilm overall (Figure 2 & table 2).

Table 1 Mean absorbance values of biofilm formation of seven strains of Streptococcus mutansat different xylitol concentrations.

S. mutans strain	Xylitol concentration (g/mL)										
	Control	0.0016	0.0031	0.0063	0.0125	0.025	0.05	0.1	0.2	0.4	0.8
UA159	0.51 ± 0.1 $^{A,B/c}$	0.42 ±0.1 $^{A,D/a,c}$	0.44 ± 0.1 $^{A/c}$	0.42 ± 0.2 $^{A/a,c}$	0.43 ± 0.1 $^{A/c}$	0.43 ± 0.1$^{A/c}$	0.41 ± 0.1$^{A,B/a,c}$	0.39 ± 0.0 $^{A/a,c}$	0.31 ± 0.1 $^{A/a}$	0.01 ± 0.0 $^{A,C/b}$	0.01 ± 0.1 $^{A/b}$
A32-2	0.43 ± 0.1 $^{A,B/a}$	0.29 ± 0.1$^{A,B,D/a}$	0.36 ± 0.1 $^{A,B/a}$	0.35 ± 0.1$^{A,B/a}$	0.34 ± 0.1$^{A,B/a}$	0.34 ± 0.1$^{A,B/a}$	0.37 ± 0.2 $^{A,B/a}$	0.34 ± 0.2$^{A/a}$	0.26 ± 0.2 $^{A/a}$	0.00 ± 0.0 $^{C/b}$	0.00 ± 0.0 $^{A/b}$
NG8	0.45 ± 0.1 $^{A,B/c}$	0.38 ±0.1$^{A,B,C,D/a,c}$	0.35 ± 0.1 $^{A,B/a,c}$	0.36 ± 0.1 $^{A,B/a,c}$	0.36 ± 0.1 $^{A,B/a,c}$	0.36 ± 0.1$^{A,B/a,c}$	0.33 ± 0.1 $^{A,B/a,c}$	0.34 ± 0.1 $^{A/a,c}$	0.30 ± 0.1 $^{A/a}$	0.01 ± 0.0 $^{C,D/b}$	0.00 ± 0.0 $^{A/b}$
10449	0.39 ± 0.1 $^{A/c}$	0.26 ±0.1 $^{B/a,c}$	0.29 ± 0.1 $^{B/a,c}$	0.25 ± 0.1 $^{B/a}$	0.27 ± 0.1 $^{B/a,c}$	0.28 ± 0.1$^{B/a,c}$	0.28 ± 0.1 $^{A/a,c}$	0.29 ± 0.1 $^{A/a,c}$	0.31 ± 0.1$^{A/a,c}$	0.03 ± 0.0$^{A,D,E/b}$	0.00 ± 0.0 $^{A/b}$
UA130	0.48 ± 0.1 $^{A,B/a}$	0.40 ±0.1$^{A,B,C,D/a}$	0.38 ± 0.1 $^{A,B/a}$	0.40 ± 0.1$^{A,B/a}$	0.46 ± 0.1 $^{A/a}$	0.41 ± 0.1$^{A,B/a}$	0.42 ± 0.1 $^{A,B/a}$	0.43 ± 0.1 $^{A/a}$	0.43 ± 0.2 $^{A/a}$	0.15 ± 0.0 $^{B/b}$	0.00 ± 0.0 $^{A/b}$
LM7	0.51 ± 0.1 $^{B/c}$	0.48 ±0.1$^{A,C,D/c}$	0.44 ± 0.1 $^{A/a,c}$	0.43 ± 0.1 $^{A/a,c}$	0.45 ± 0.1 $^{A/a,c}$	0.43 ± 0.1$^{A/a,c}$	0.44 ± 0.1 $^{B/a,c}$	0.43 ± 0.1 $^{A/a,c}$	0.31 ± 0.1$^{A/a}$	0.04 ± 0.0 $^{E,F/b}$	0.01 ± 0.0 $^{A/b}$
OMZ175	0.54 ± 0.1 $^{B/c}$	0.42 ±0.2 $^{D/a,c}$	0.47 ± 0.1 $^{A/a,c}$	0.44 ± 0.1$^{A/a,c}$	0.43 ± 0.1 $^{A/a,c}$	0.42 ± 0.1$^{A,B/a,c}$	0.44 ± 0.1 $^{B/a,c}$	0.39 ± 0.1 $^{A/a}$	0.35 ± 0.1 $^{A/a}$	0.08 ± 0.0 $^{G/b}$	0.00 ± 0.0 $^{A/b}$

Different uppercase letters indicate significant differences ($p < 0.05$) among strains at a given xylitol concentration using multiple comparison (column comparison). Different lowercase letters suggest significant differences ($p < 0.05$) at different xylitol concentrations within a particular strain using multiple comparisons (row comparison). Standard deviations are shown in parentheses.

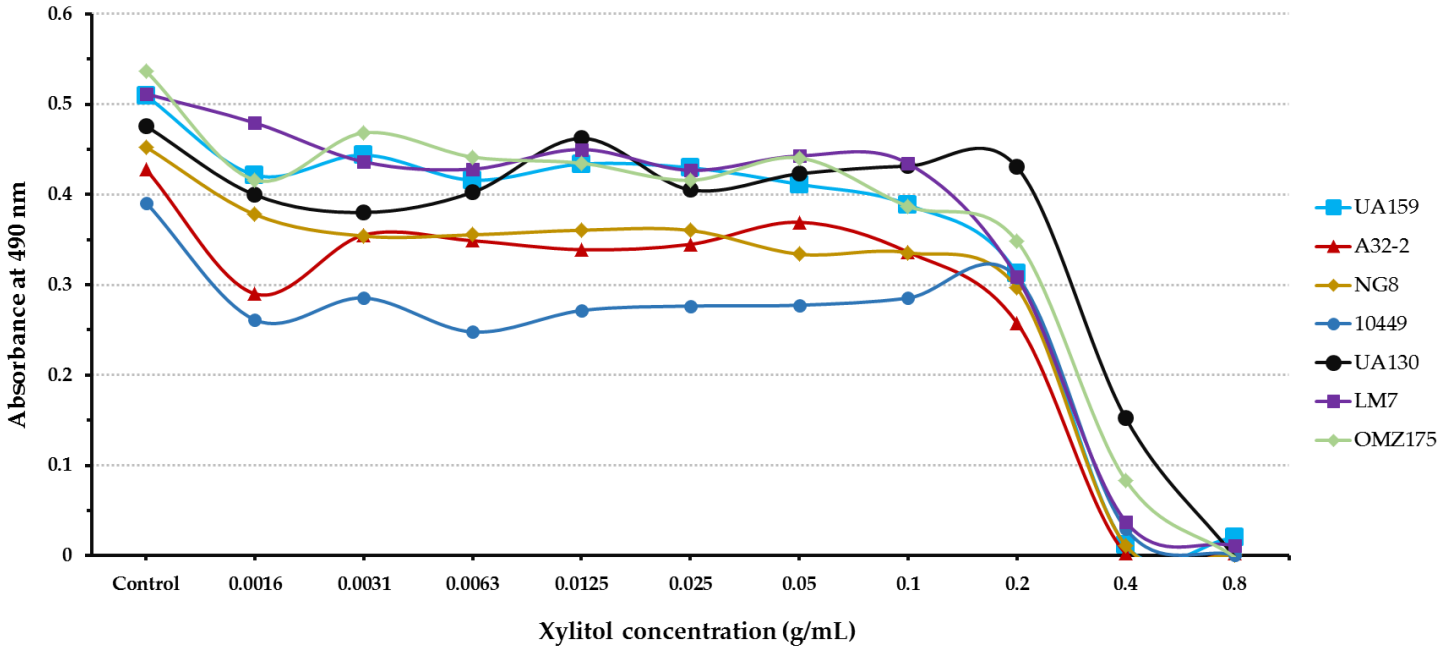

Figure 1 A line graph showing biofilm absorbance values for seven strains of Streptococcus mutans at different xylitol concentrations. Each data point is the average of three triplicates from three independent experiments.

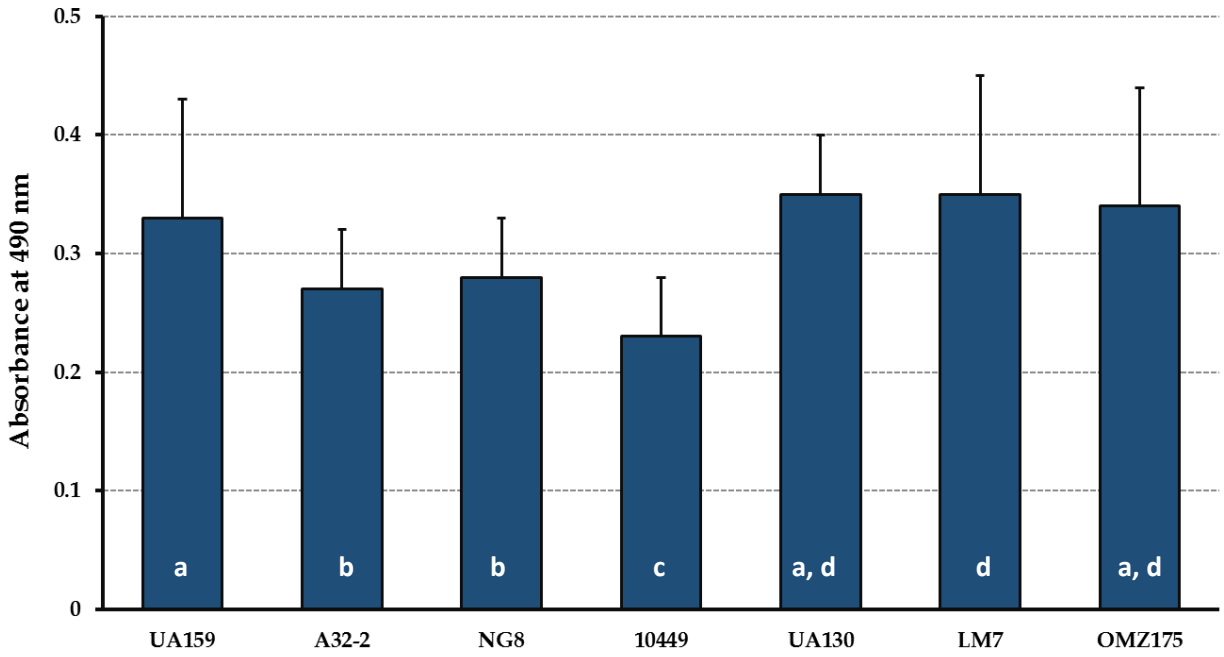

Figure 2 A bar graph indicating mean cumulative biofilm mass for each *Streptococcus mutans* strain at the conclusion of the experiment for all concentrations. Error bars represent standard deviations. The groups with completely different letters were statistically different ($p < 0.05$).

Table 2 The average absorption of metabolic activity of 7 strains of Streptococcus mutans at different xylitol concentrations.

S. mutans strain	Xylitol concentration (g/mL)										
	Control	0.0016	0.0031	0.0063	0.0125	0.025	0.05	0.1	0.2	0.4	0.8
UA159	0.44 ± 0.5 $^{A/a,b}$	0.49 ± 0.3 $^{A/a}$	0.52 ± 0.4 $^{A/a}$	0.61 ± 0.4 $^{A/a}$	0.53 ± 0.4 $^{A/a}$	0.61 ± 0.3 $^{A/a}$	0.34 ± 0.4 $^{A/a,b}$	0.24 ± 0.4 $^{A/a,b}$	0.36 ± 0.1 $^{A/a,b}$	0.01 ± 0.0 $^{A/b}$	0.01 ± 0.0 $^{A/b}$
A32-2	0.85 ± 0.2 $^{A/a}$	0.61 ± 0.2 $^{A,B/a}$	0.71 ± 0.3 $^{A/a}$	0.76 ± 0.3 $^{A/a}$	0.71 ± 0.3 $^{A/a}$	0.75 ± 0.3 $^{A/a}$	0.75 ± 0.3 $^{A/a}$	0.69 ± 0.4 $^{B,C/a}$	0.51 ± 0.1 $^{A/a}$	0.00 ± 0.0 $^{A/b}$	0.01 ± 0.0 $^{A/b}$
NG8	0.78 ± 0.1 $^{A/c}$	0.69 ± 0.2 $^{A,B/a,c}$	0.69 ± 0.2 $^{A/a,c}$	0.73 ± 0.2 $^{A/a,c}$	0.74 ± 0.2 $^{A/c}$	0.76 ± 0.2 $^{A/a,c}$	0.71 ± 0.2 $^{A/a,c}$	0.72 ± 0.2 $^{C/a,c}$	0.47 ± 0.2 $^{A/a}$	0.02 ± 0.1 $^{A/b}$	0.01 ± 0.0 $^{A/b}$
10449	0.89 ± 0.4 $^{A/a}$	0.59 ± 0.2 $^{A,B/a}$	0.68 ± 0.3 $^{A/a}$	0.55 ± 0.2 $^{A/a}$	0.62 ± 0.2 $^{A/a}$	0.62 ± 0.3 $^{A/a}$	0.54 ± 0.3 $^{A/a}$	0.58 ± 0.3 $^{A,B,C/a}$	0.52 ± 0.2 $^{A/a}$	0.07 ± 0.0 $^{A/b}$	0.00 ± 0.0 $^{A/b}$
UA130	0.57 ± 0.4 $^{A/a}$	0.49 ± 0.4 $^{A/a}$	0.45 ± 0.3 $^{A/a}$	0.47 ± 0.3 $^{A/a}$	0.56 ± 0.3 $^{A/a}$	0.41 ± 0.2 $^{A/a}$	0.50 ± 0.2 $^{A/a}$	0.52 ± 0.3 $^{A,B,C/a}$	0.47 ± 0.0 $^{A/a}$	0.04 ± 0.0 $^{A/b}$	0.00 ± 0.0 $^{A/b}$
LM7	0.85 ± 0.3 $^{A/a,c}$	0.90 ± 0.3 $^{B/c}$	0.82 ± 0.1 $^{A/a,c}$	0.76 ± 0.2 $^{A/a,c}$	0.81 ± 0.3 $^{A/a,c}$	0.58 ± 0.3 $^{A/a,c}$	0.58 ± 0.3 $^{A/a,c}$	0.59 ± 0.3 $^{A,B,C/a,c}$	0.46 ± 0.3 $^{A/a}$	0.06 ± 0.1 $^{A/b}$	0.00 ± 0.0 $^{A/b}$
OMZ175	0.69 ± 0.5 $^{A/a}$	0.43 ± 0.3 $^{A/a,b}$	0.42 ± 0.4 $^{A/a,b}$	0.42 ± 0.4 $^{A/a,b}$	0.46 ± 0.5 $^{A/a,b}$	0.40 ± 0.4 $^{A/a,b}$	0.41 ± 0.4 $^{A/a,b}$	0.28 ± 0.3 $^{A,B/a,b}$	0.29 ± 0.2 $^{A/a,b}$	0.05 ± 0.0 $^{A/b}$	0.01 ± 0.0 $^{A/b}$

Different uppercase letters indicate significant differences ($p < 0.05$) among strains at a given xylitol concentration using multiple comparison (column comparison). Different lowercase letters suggest significant differences ($p < 0.05$) at different xylitol concentrations within a particular strain using multiple comparisons (row comparison). Standard deviations are shown in parentheses

Lactate dehydrogenase assay

Absorbance values for the LDH assay are presented in Figure 3. There was a clear trend of lower absorbance values with the increase of xylitol concentration from 0.1 to 0.8 g/mL. There was a significant decrease in LDH production at 0.4 g/mL xylitol with UA159, UA130, and LM7 and at 0.8 g/mL with UA159, UA130, LM7, and A32-2. Further, there were no significant differences ($p \geq 0.05$) among strains at control, 0.4, and 0.8 g/mL xylitol levels.

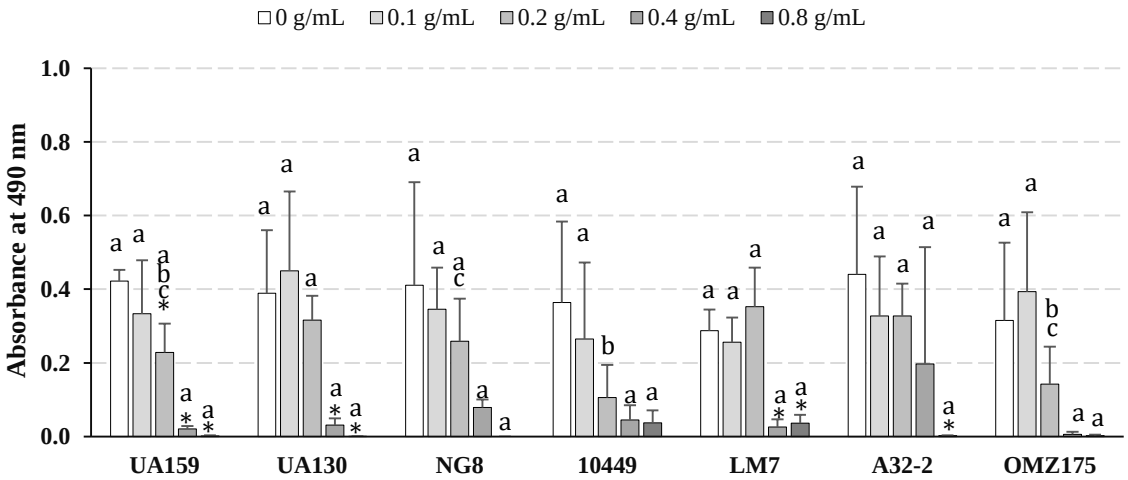

Figure 3 A bar graph showing lactate dehydrogenase assay (LDH) assay absorbance values for seven strains of *Streptococcus mutans* at different xylitol concentrations. Each data point is the average of three triplicates from three independent experiments. Error bars represent standard error. Asterisks indicate significant difference ($p < 0.05$) compared to control for that particular strain. Different letters indicate significant difference ($p < 0.05$) between strains at a specific concentration.

Extracellular polysaccharide assay

Absorbance values for the EPS assay are presented in Figure 4. At 0.4 and 0.8 g/mL xylitol levels, all strains had lower EPS production compared to control except UA130 and 10449. Still, there was a clear trend of lower absorbance values with the increase in xylitol concentration from 0.1 to 0.8 g/mL. Regarding interstrain comparisons, absorbance values were significantly different for control ($p< 0.001$) and at 0.1 g/mL and higher ($p< 0.001$). UA159, UA130, and NG8 had significantly lower EPS production compared to the other tested strains ($p< 0.001$).

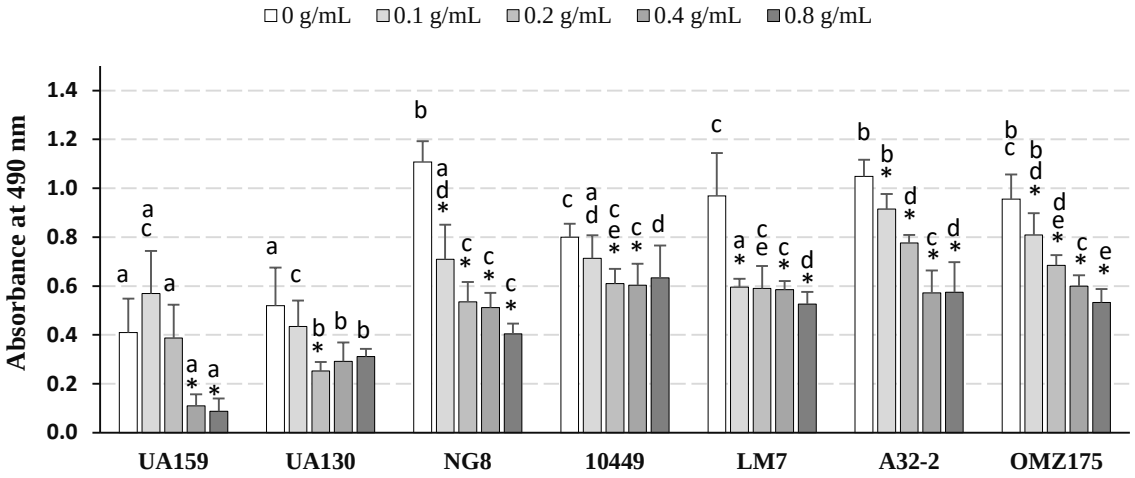

Figure 4 A bar graph showing extracellular polysaccharide assay (EPS) assay absorbance values for seven strains of *Streptococcus mutans* at different xylitol concentrations. Each data point represents the mean of triplicates from three independent experiments. Error bars represent standard error. Asterisks indicate significant difference ($p< 0.05$) compared to control for that particular strain. Different letters indicate significant difference ($p< 0.05$) between strains at a specific concentration.

4. DISCUSSION

The multifactorial nature of teeth decay implicates the need for a multidisciplinary approach to challenge the management and treatment of this disease. Prevention is one important and cost-effective parameter to control this disease, because surgical intervention was shown to not drastically change the conditions that led to the disease in the first place (Konig, 2004; Fontana et al., 2010; Marsh, 2010). Water fluoridation was one of the first preventive measures that were adopted since 1942 by Trendley Dean and his co-investigators (Dean et al., 1942). It was found to decrease the number and size of caries lesions (Young et al., 2007; Young et al., 2007). This is due to the mechanism of fluoride via reducing bacterial metabolism, and by enhancing mineral uptake and decreasing demineralization in enamel (Hamilton, 1990; Jenkins, 1999; Van Loveren, 2001). In addition, the use of fluoridated toothpastes with the proper brushing technique is another important factor to prevent dental caries (Bratthall et al., 1996).

Similarly, the use of naturally anticariogenic products such probiotic yogurt, sorbitol, propolis, maltitol and xylitol chewing gums have been demonstrated to have a positive effect on preventing and decreasing the incidence of caries (Haghgoo et al., 2015; Oza et al., 2018; Sivamaruthi et al., 2020). Since *S. mutans* is known to be an important cariogenic bacterium in the pathogenesis of dental caries (Loesche, 1986), it makes sense to focus our research to target and understand the effect of preventive products on the behavior of this bacterium, in order to decrease their role in the development and progression of tooth decay. The identification of several *S. mutans* strains that process different virulence factors and pathogenicity levels makes that even more challenging as certain strains may have different responses and behaviors to treatment approaches and environmental factors (Kuramitsu, 1993; Nassar and Gregory, 2017). Thus, the main aim of this research was to analyze the effect of xylitol on biofilm formation and metabolic activity on seven *S. mutans* strains.

Our data revealed that xylitol inhibited biofilm formation of *S. mutans* strains (UA 259, A32-2, NG8, 10449, and LM7) at 0.4 g/mL and all strains including UA130 and OMZ175 at 0.8 g/mL (Table 1 and Figure 1). This was consistent with bacterial metabolic activities as xylitol abolished the cellular activities of most strains starting at 0.4 g/mL and all strains at 0.8 g/mL (Table 2). These findings are further complemented by results from LDH and EPS assays, showing clear trends of reduced LDH and EPS production

(Figure 3 and Figure 4). Our outcomes are in consistence with others where xylitol demonstrated an inhibitory effect on certain *S. mutans* strains such as DSM 20523 (ATCC 25175) but not the clinical isolate *S. mutans* strain 117 (Salli et al. 2016). This indeed confirms the presence of different variabilities and sensitivities of *S. mutans* strains to xylitol concentrations. Moreover, the decrease in biofilm formation of these same seven *S. mutans* strains in our study was also similar to what was demonstrated by Nassar and Gregory (2017) when using fluoride as an antibacterial agent. Thus, all strains were significantly unable to produce biofilm at fluoride concentration greater than 225 ppm sodium fluoride, which is the concentrations of fluoride rinse products available over the counter (Nassar and Gregory, 2017).

The mechanism of xylitol depends on the inhibition of metabolic pathways of oral microorganisms via affecting the phosphoryl transferase enzyme pathway which inhibits the transportation of sugars into the bacterial cell (Makinen, 2000). This would decrease the growth of *S. mutans* as well as hinder their adherence potential by affecting the production of extracellular polysaccharides (Loesche et al., 1984; Trahan, 1995). However, based on current data, this interference is strain-specific since UA159, UA130, and NG8 seem to be more affected compared to other tested strains. Production of LDH and EPS for UA159 strain was reduced clearly indicating a strain-specific sensitivity to xylitol concentrations of 0.4 g/mL and higher (Figure 3 and Figure 4). Significant LDH enzyme changes could require additional studies with larger sample sizes in order to be identified. Xylitol can be used in an additive manner with fluoride since their modes of action are different (Peldyak and Makinen, 2002).

Although our study was performed *in vitro*, it is still in agreement with others where xylitol in chewing gum exhibited reductions in salivary *S. mutans* counts compared to other products such as herbal and sorbitol gums (Chavan et al., 2015; Haghgoo et al., 2015; Oza et al., 2018). On the other hand, if xylitol chewing gum was selected to be used as an adjunct anticariogenic agent with other preventive key parameters, the dentist must be aware of the therapeutic and recommended dose of xylitol in chewing gums and also know the reliable companies that provide such a product. To achieve the anticariogenic effect of xylitol, the recommended dose of xylitol is about 6 g/day divided into 3-4 doses (Twetman, 2010; Alanzi et al., 2016); which can be translated into approximately 0.4 to 0.5 g/mL for each dose if a 1:3 dilution ratio occurs in the oral cavity due to the presence of saliva. These values fall in the range in which a significant reduction in both biofilm production and metabolic activity occurred in all seven *S. mutans* strains tested in the current investigation.

Unfortunately, there are numerous xylitol chewing gum products in the market with xylitol which are below the recommended therapeutic dose. In a study by Bouges *et al.*, (2017) the authors reported that only two out of ten brands of sugar free gums can deliver the recommended dose of 6 g/day using a reasonable number of gum sticks. According to previous research, Alanzi *et al.*, (2016) investigated xylitol content in sugar-free gums available in the countries within the Gulf Cooperation Council (GCC). They found that the majority of the companies did not provide the xylitol concentration shown on their product labeling, and they did not provide the customers with the advocated each day dose of xylitol to prevent dental caries. Moreover, as discussed earlier, because of the multifactorial nature of this disease, dentists must have the knowledge to manage dental caries from several aspects including educating and raising patient awareness about the disease and how to maintain a healthy oral hygiene. In addition, being aware of applying preventive protocols early enough before the development of the disease, emphasizing maintenance of a balanced diet to ensure a favorable oral environment as well as following evidence-based caries risk assessment protocols such as the Caries Management by Risk Assessment (CAMBRA), will aid in the proper prevention and management of dental caries (Konig, 2004; Tellez et al., 2013; Hurlbutt and Young, 2014; Nassar, 2020); hence, providing better standards of care to patients and the community.

Dental caries have been one of the most chronic and challenging diseases for decades. Applying preventive regimens is paramount to decreasing the incidence of the disease. This would include the use of anticariogenic products such as xylitol, which has been proven to have a promising effect against several *S. mutans* strains. In order to address the limitations of the current investigation, further studies are required to study the efficacy of using xylitol as an adjunct product in conjunction with other preventive measures in clinical settings. Still, xylitol was found to be efficient in decreasing biofilm production, metabolic activity, and production of EPS starting at 0.4 g/mL.

5. CONCLUSION

Xylitol has a clear anticariogenic effect on *S. mutans* which was slightly different depending on the tested strain. The difference in response between the tested strains can confirm that the benefit of xylitol might vary from one patient to the other. This goes with the current concepts of individualized caries preventive and management approaches and can guide clinicians in establishing effective treatment protocols. The effect of xylitol is more apparent at concentrations of 0.4 g/mL and higher in the current investigation.

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Ethical approval

The project was exempt from ethical approval process since the methodology did not involve or contain, in any part or form, human subjects or test animals or any tissue derived from them.

Authors' contributions

This project was carried in collaboration among all authors.

Declaration of competing interest

The authors have no conflict of interest to declare.

Data and materials availability

All data associated with this study are present in the paper.

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