ISSN 2664-4177 (Print) & ISSN 2664-7931 (Online)
South Asian Research Journal of Oral and Dental Sciences
Abbreviated Key Title: South Asian Res J Oral Dent Sci

| Volume-7 | Issue-2 | May-Jun- 2025 **DOI: https://doi.org/10.36346/sarjods.2025.v07i02.007**

Original Research Article

The Effect of Xylitol on the Antimicrobial Activity of Chlorhexidine Against Mutans Streptococci and Lactobacilli

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Article History
Received: 04.04.2025
Accepted: 10.05.2025
Published: 03.06.2025

Abstract: ***Background***: Dental caries continues to be a major public health problem in many countries. The etiology of dental caries is multifactorial, and it is widely accepted that Mutans streptococci and Lactobacilli play a major role in the formation of dental caries through adhering to the tooth surface and producing acid from dietary sucrose. Chlorhexidine is active against both gram positive and negative bacteria and Xylitol is a naturally occurring non-cariogenic sugar substitute used as an artificial sweetener in foods, ***Objective***: The purpose of this study was to determine the impact of xylitol on the antibacterial activity of chlorhexidine against salivary Mutans streptococci and lactobacilli. ***Results***: To evaluate the effects of these substances salivary mutans streptococci and lactobacilli were isolated and identified, Agar diffusion technique was used to study the antimicrobial effects of both xylitol and chlorhexidine. The minimum inhibitory concentration was determined for the xylitol and chlorhexidine alone and in combination using tube dilutions method, the combination effects of xylitol and chlorhexidine were evaluated using fractional inhibitory concentration (FIC) indices. ***Conclusion***: ½ MIC of chlorhexidine with ¼ MIC of xylitol was efficient and superior to single treatments in controlling suppressing *mutans streptococci and lactobacilli in-vitro* and xylitol may be used as sweetener and flavoring chlorhexidine.

Keywords: Xylitol, chlorhexidine, mutans streptococci, lactobacilli.

INTRODUCTION

The most widespread and consequential oral disorders are dental caries and periodontal disease, and oral hygiene routines continue to be the primary prevention measures against them. Dentists have long used various equipment and chemicals to keep the oral health of their patients in prime condition. Scientists have been obliged to hunt for novel antibacterial compounds from other sources, such as medicinal plants [1, 2]. But an effective anti-plaque agent till date is chlorhexidine [3, 4]. Chlorhexidine has a substantive positive charge. It binds to various surfaces including enamel pellicle, hydroxyapatite and mucosa membranes. It binds to surface and disrupts bacterial cytoplasmic membranes, including leakage of low molecular weight components and the precipitation of cell content. Chlorhexidine inhibits key metabolic enzymes such glucosyltransferase, phosphoenolpyruvate and phosphotransferase of mutans streptococci [5-7]. Also, chlorhexidine has Synergistic effect with ethyl alcohol against streptococcus mutans in dental plaque [8].

Many of the currently available mouth rinses including chlorhexidine do have drawbacks, such as alteration in taste sensation and staining of teeth. In order to overcome such side effects, the WHO has advised to investigate the possible use of natural products (herb and plant extracts). [1, 2, 9, 11]. Because chlorhexidine has an extremely bitter taste, it is often necessary to flavor and sweeten mouth rinses and gel product. Al-mizraqchi *et al*. (2008) showed that aspartame may be used as sweetener and flavoring in concentrations up to 16% with chlorhexidine rinse [12]. Xylitol is anaturally occurring non-cariogenic sugar substitute used as an artificial sweetener in foods, cannot be metabolized by oral bacteria thereby contributing to caries prevention, Xylitol present in chewing gum inhibits bacterial growth through two

mechanisms: Direct inhibition of the glycolytic route resulting from the xylitol 5-phosphate derivative and/or indirect inhibition resulting from the competition for the HPr-P (phosphorylated phosphor carrier protein) carrier between glucose and xylitol [13, 14].

Oral hygiene is essential for reducing the accumulation of dental plaque, a film of germs and food that form on teeth. Mechanical aids such as toothbrushes, floss, and interdental cleaners include oral hygiene procedures. Chemical aids such as mouthwashes, dentifrices, and chewing gum assist preserve oral health, providing a risk-free and effective technique to reduce or remove plaque accumulation [15, 16].

WHO defines dental caries as a localized, post eruptive pathological process of extreme origin involving softening of the hard tooth tissue and proceeding to the formation of cavity. The process involves bacterial interactions in plaque accumulated on the surface of the teeth. Mutans Streptococci (MS) are the principle a etiological agents of dental caries [5]. Cariogenic features of these bacteria include synthesis of water–insoluble glucans, lactic acid production, ability to survive at allow pH, intracellular polysaccharide synthesis and the production of dextrin hydrolyzing enzyme (endodextranase) [17]. Mutans streptococci were found to be the predominant bacteria in caries process. *Streptococcus mutans* in plaque is the most commonly isolated organism among all other cariogens. It ferments sucrose and the resulting acid causes demineralization of tooth enamel. [18]. It is widely accepted that Mutans streptococci (MS) and Lactobacilli (LB) play important roles in the formation of dental caries through adhering to the tooth surface and producing acid from dietary sucrose. [17]. Lactobacilli may play a significant role only during the initiation of a low percentage of coronal caries lesions but may be more important in their progression. [5, 17]. Mutans streptococci are mainly responsible for the initial phase of the caries lesion especially in the enamel (initiation), whereas *Lactobacillus* is more involved with the progression of caries. [19].

The purpose of this investigation was to determine the impact of sugar alcohols sweeteners (xylitol) on the antibacterial activity of chlorhexidine against salivary Mutans streptococci and lactobacilli

Materials and Methods

The study was conducted according to the Iraqi national standard operative procedures (SOP) for microbiology. An ethical approval for the study in accordance with the Declaration of Helsinki was obtained from the Faculty of Dentistry Local Ethics Committee, Baghdad University. Following the ethical approval, verbal consent was obtained from each participant.

Twenty isolates of MS and LB were obtained under standardized conditions from Ten volunteers with no medical history aged 19-23 years (7male and 3 female) with at least one tooth with dental caries. Participants were asked to refrain from eating and smoking for at least one hr and were given a piece of sugar-free natural gum (0.4 - 0.5g) to chew for five min, then saliva was collected in a sterilized screw capped bottles [20]. The samples were homogenized and diluted with phosphate buffer saline. A 0.1 ml of two dilutions were cultured in duplicates on glucose-yeast extract-acetic acid agar (Rogosa agar, Oxoid, UK) to isolate the LB [21], and on Mitis Salivarius Bacitracin Agar (MSB) (Hi Media, India) to isolate MS [22]. Colonies were examined directly and under dissecting microscope (magnification x15). Gram staining, catalase production and carbohydrate (mannitol) fermentation tests were conducted on MS by using Cystine Trypticase-Mannitol Agar (Hi Media, India) to test the ability of MS to ferment the mannitol, in addition the spore forming and motility tests for LB were assessed to confirm bacterial identification. [5, 23]. Final identification had been done by using Vitek 2 system (bioMerieus). MS and LB were purified, one colony from each type of bacteria was transferred to 10 ml of sterilized Brain heart infusion broth (Oxoid, UK) and incubated for 24 hrs aerobically to prepare the stock culture.

Agar diffusion technique was applied to study the antimicrobial effects of both xylitol (Final concentration of xylitol 1-30 %) and chlorhexidine (final concentration 1-35 µg/ml) against the isolates spread on Brain Heart Infusion Agar: wells of 6mm were prepared in the agar using Cork borer. Each well was filled with a different concentration prepared from the stocks of the xylitol/ or chlorhexidine. Plates left for 15 minutes at room temperature and then incubated aerobically for 24 hrs at 37°C. Inhibition zones diameters were measured using a scientific ruler; resistance of the isolates to the tested agents was indicated when there were no zones of inhibition [24].

The minimum inhibitory concentration (MIC) was determined which as the lowest concentration of xylitol and chlorhexidine alone and in combination inhibits the growth of bacterial isolates using tube dilutions method in brain heart infusion broth media.

The combination effects of xylitol and chlorhexidine were evaluated using fractional inhibitory concentration (FIC) indices, using the MIC concentration against most bacterial isolates. The following formula was used to calculate ∑FIC indices = (conc. A in combination /MIC A alone) + (conc. B in combination / MIC B alone)

The FIC indexes ≤ 0.5, 0.5<FIC≤1, 1<FIC≤2, and >2 will define as synergistic, additive, indifferent, and antagonism, respectively. (25).

RESULT

Statistically, the normality test of the diameter of inhibition zone against test microorganisms among concentrations of all experimental agents was performed using the Shapiro-Wilk test. The samples were normally distributed as the P value was not significant when > 0.05.

Mutans streptococci isolates were sensitive to different concentrations of chlorhexidine and xylitol was found to increase as the concentrations of the agents increased. The statistical analysis showed that there was a significant difference between concentrations for each type of agents against MS isolates (P<0.05), as shown in Table (1), Also, there was a significant difference between both agents' concentrations (P<0.05).

Table 1: **Descriptive and statistical test of diameter of inhibition zone(mm) against mutans streptococci (20 isolates) between the concentrations of chlorhexidine and xylitol**

Agents	Concentration µg/ml	minimum	maximum	mean	SD	F	P-value
chlorhexidine	1	10.5	13	11.5	0.536	303.881	0.000
	5	15.5	17	16.35	0.322		
	10	17	19	17.97	0.453		
	15	18	20	18.82	0.414		
	20	18	20.5	19.27	0.365		
	25	18	22	19.85	0.523		
	30	19	22.5	19.97	0.334		
	35	19	23.5	20.77	0.232		
Xylitol	Concentration %						
	1	8	9.5	8.5	0.445	10.141	0.002
	5	11	12	11.33	0.566		
	10	11.5	13.5	12.77	0.656		
	15	12	14	13.35	0.443		
	20	13.5	15	13.75	0.323		
	30	13.5	16	15.82	0.541		

The pattern against lactobacilli was slightly different, and both chlorhexidine and xylitol were highly active against mutans streptococci in comparison with lactobacilli, but still the lactobacilli isolates were sensitive to different concentrations of chlorhexidine and xylitol. The statistical analysis showed that there was a significant difference between concentrations for each agent against lactobacilli isolates (P<0.05), as shown in Table (2), Also, there was a significant difference between both agent concentrations (P<0.05).

Table 2: **Descriptive and statistical test of diameter of inhibition zone(mm) against lactobacilli (20 isolates) between the concentrations of chlorhexidine and xylitol**

Agents	Concentration /ml	minimum	maximum	mean	SD	F	P-value
chlorhexidine	1	8	8	8	0.222	118.220	0.000
	5	8	9	8.5	1.120		
	10	8	10	8.77	0.731		
	15	8.5	10.5	9.31	1.121		
	20	9	13	11.27	0.676		
	25	9	13	11.97	0.568		
	30	10	14	12.22	0.875		
	35	10	15.5	13.77	0. 631		
Xylitol	Concentration %						
	1	7	7	7	0.434	11.722	0.002
	5	7	7	7	0.879		
	10	8	8.5	8.22	0.878		
	15	9	10	9.23	1.342		
	20	11	14	12.75	0.812		
	30	11	16	13.97	0.655		

The MIC of both chlorhexidine and xylitol against lactobacilli was higher than that against mutans streptococci, and most isolates of mutans streptococci were inhibited by 10 µg/ml and 20%, while the MIC against most lactobacilli isolates were 15 µg/ml and 30% of chlorhexidine and xylitol respectively as shown in Tables 3 and 4.

Table 3: Minimum inhibitory concentration of the chlorhexidine and xylitol against mutan streptococci

Type of bacteria	No. of isolates	Type of agent	NO. of isolates inhibit within MIC of Chlorhexidine / xylitol						
MS	20	chlorhexidine µg/ml	**Concentrations**						
			1	5	10	15	20	25	30
			1		17	2			
		Xylitol %	1	5	10	15	20	25	30
					3	6	11		

Table 4: Minimum inhibitory concentration of the chlorhexidine and xylitol against lactobacilli

Type of bacteria	No. of isolates	Type of agent	NO. of isolates inhibit within MIC of Chlorhexidine / xylitol						
LB	20	chlorhexidine µg/ml	Concentrations						
			1	5	10	15	20	25	30
					6	12	2		
		Xylitol %	1	5	10	15	20	25	30
					1	1	3	5	10

The current study showed that the combination of ½ MIC of chlorhexidine with ¼ MIC of xylitol have an additive effect against mutans streptococci and lactobacilli and the ∑FIC against mutans streptococci and lactobacilli was 0.75. However, the antibacterial activity of chlorhexidine in MIC concentration was significantly reduced when the concentrations of xylitol were increased up to 30% against both mutans streptococci and lactobacilli.

DISCUSSION

One of the most effective antimicrobial agents for oral use is chlorhexidine, which can be successfully formulated into a mouth rinse. This bisbiguanide has a broad spectrum of activity against yeasts, fungi, and a wide range of Gram-positive and Gram-negative bacteria. Chlorhexidine can reduce plaque, caries, and gingivitis in humans [26]. At high concentrations, chlorhexidine is bactericidal and damages the cell membrane. Chlorhexidine is substantive and is bound to oral surfaces from where it is released gradually into saliva over many hours at bacteriostatic concentrations. Chlorhexidine inhibits the PTS sugar transport system and thereby markedly inhibits acid production in streptococci and inhibits amino acid uptake and catabolism in some streptococci; chlorhexidine also affects various membrane functions, including the adenosine triphosphate (ATP)-synthase [5].

In the current study, Mutans Streptococci and lactobacilli isolates were sensitive to different concentrations of chlorhexidine. The antibacterial activity of the chlorhexidine (diameter of inhibition zone) was found to increase as the concentrations of the chlorhexidine increased. Mutans streptococci are particularly more sensitive to chlorhexidine than lactobacilli, this may be due to the differences in genetic information, composition of cell wall and cell-cell communication between bacterial cells. These cell–cell signaling strategies enable cells to sense and adapt to various environmental stresses and regulate (and coordinate) the expression of genes that influence the ability of pathogens to cause disease [5, 17, 23].

Xylitol inhibits bacterial growth through two mechanisms: direct inhibition of the glycolytic route resulting from the xylitol 5-phosphate derivative and/or indirect inhibition resulting from the competition for the HPr-P carrier between glucose and xylitol [13, 14]. The current study showed that the antimicrobial activity of chlorhexidine was greatest than xylitol in concentration up to 30%. However, the combination of ½ MIC of chlorhexidine with ¼ MICof xylitol have an additive effect against mutans streptococci and lactobacilli and the ∑FIC against mutans streptococci and lactobacilli was 0.75. Xylitol has been claimed to be superior to other sugar alcohols because of its effect on bacterial metabolism. Xylitol is transported into cells of mutans streptococci by the fructose-PTS where it enters a futile cycle of phosphorylation, dephosphorylation and eventual expulsion. This futile cycle reduces the rate of growth and acid production (from exogenous sugars such as glucose) of cells and leads to reduced levels of both mutans streptococci and caries in habitual users of xylitol-containing confectionery [5]. Nevertheless, the frequent use of xylitol chewing gum should be controlled to prevent the occurrence of microbial resistance. This finding may be related to factors such as the formation of acids resulting from glucose and/or polysaccharides resulting from sucrose [27, 28]. In addition xylitol as an alternative sweetener, could help to prevent dental caries by reducing the count of *S. mutans* in the saliva [29, 30].

In conclusion, based on the results found, we can conclude that combining of ½ MIC of chlorhexidine with ¼ MIC of xylitol was efficient and superior to single treatments in controlling suppressing *mutans streptococci and lactobacilli in-vitro* and xylitol may be used as sweetener and flavoring chlorhexidine. Further investigation should be carried out to confirm the results and develop strategies for using such a combination to prevent dental caries.

Acknowledgement

The authors are grateful to all volunteers who contributed to this study. Prof. Abbas Almizraqchi is thanked for his contribution.

Conflict of Interest: Authors of this work declare no conflict of interest.

Funding: This work was supported by the Ministry of Higher Education and Scientific Research/Iraq

Ethics: The scientific committee of the Pedodontics and Preventive Dentistry Department and the central ethical committee of the College of Dentistry at the University of Baghdad, Iraq gave approval for this study (Project No. 950324, date: October 14, 2024,

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