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ORIGINAL RESEARCH

Antifungal Activity of Xylitol against *Candida albicans*: An *in vitro* Study

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ABSTRACT

Aim: The most common fungal infection among human population is candidiasis, the etiology of which is mostly *Candida albicans*. As a result of a disrupted balance of the normal flora or a compromised immune system, *Candida* species can become pathogenic. Various *in vitro* surveys have shown that glucose intake is a promoter of *C. albicans* growth, whereas *in vivo* studies have found that xylitol can decrease the risk of candidiasis and angular cheilitis. Hence, we aimed to evaluate for the first time the inhibitory effects of xylitol on *C. albicans* by assessing its minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC).

Materials and methods: The standard strain of *C. albicans* was acquired. The fungi were incubated in supplemented brain–heart infusion broth for 48 hours at 37°C. The MIC was measured according to the Clinical and Laboratory Standards Institute (CLSI) M100-S24 standard. Microdilution method was applied using 360-µL sterilized polystyrene flat-bottomed 96-well plates. The antimicrobial effects were examined by the microbroth dilution method according to the CLSI M100-S24 standard.

Results: The MIC of xylitol for *C. albicans* was found to be 20×10^4 µg/mL. Furthermore, the concentration of 40×10^4 µg/mL with a decrease of 99.95% in the colony-forming units (CFUs) of the microorganism was found to be the MFC of xylitol for *C. albicans*.

Conclusion: According to the results of this survey, xylitol has considerable antimicrobial effects. Hence, this substance can be used as an effective element in gums, toothpastes, and antimicrobial mouthwashes, especially in patients with candidiasis.

Clinical significance: By knowing the MIC and MFC of xylitol, this substance can be effectively used in products aimed to cure this fungal infection.

Keywords: *Candida albicans*, Minimum fungicidal concentration, Minimum inhibitory concentration, Xylitol.

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INTRODUCTION

The most common fungal infection among human population is candidiasis, the etiology of which is mostly *Candida albicans*.[1,2] A significant increase in the prevalence of infections caused by *Candida* species has been observed in the past two decades.[1] *Candida albicans* can be found in the oral cavity, most frequently in the posterior dorsum area of the circumvallate papillae, female genitourinary tract, gastrointestinal tract, and sometimes the skin as the normal microflora in approximately 50% of the population.[3,4] As a result of a disrupted balance of the normal flora or a compromised immune system, *Candida* species can become pathogenic.[2-4] Most *Candida* infections only affect the mucosal lining, but the rare systemic manifestations may have a fatal course.[4] In the oral cavity, *C. albicans* can be seen as, various forms, such as pseudomembranous candidiasis, chronic plaque-type and nodular candidiasis, erythematous candidiasis, denture stomatitis, angular cheilitis, median rhomboid glossitis, and oral candidiasis associated with human immunodeficiency virus infection.[5-7] Before starting antifungal medication, it is necessary to eliminate any predisposing factor. Local

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factors are often easy to identify, but sometimes not possible to reduce or eradicate.[7,8] Antifungal drugs have a primary role in such cases. The most commonly used antifungal drugs belong to the groups of polyenes and azoles. Polyenes, such as nystatin and amphotericin B are the mostly used alternatives in the treatment of oral candidiasis.[8]

A diet enriched with carbohydrates and deficient in vitamins is an important predisposing factor for the development of *Candida* infection.[9,10] Various surveys have shown that glucose intake is a promoter of *C. albicans* growth,[9-11] whereas xylitol can decrease the risk of candidiasis and angular cheilitis and the adherence of *C. albicans* to buccal epithelial cells.[9,12,13] Furthermore, xylitol was found to reduce the production of acetaldehyde, a highly toxic and mutagenic product of alcohol, by *Candida*.[14]

Xylitol, categorized as a polyalcohol or sugar alcohol, is used as a natural sweetener. It can be found in fruits, vegetables, and mushrooms, and is also produced during metabolic reactions in the human body. Unlike other sweeteners with six carbon atoms, such as glucose, fructose, and sorbitol, the xylitol molecule is composed of five carbon atoms.[15,16] Compared with these carbohydrates, xylitol contains 40% less calories and its absorption and metabolism are slower.[16] On the contrary to artificial sweeteners including aspartame, sucralose, and acesulfame-K, no adverse effects have been reported for xylitol and it does not have a bitter aftertaste.[15-17]

Xylitol is found to be an effective inhibitor of *C. albicans* with a dose-dependent relation. Different concentrations of xylitol including 5,[18] and 10%[19] were reported to be effective in growth inhibition of *C. albicans in vitro*. Hence, we aimed to evaluate the inhibitory effects of xylitol on *C. albicans* by assessing its MIC and MFC. To the best of our knowledge, this is the first report on MIC and MFC for this sweetener.

MATERIALS AND METHODS

Microorganism

The standard strain of *C. albicans* (ATCC cultures 10231) was acquired from Rayen Biotechnology Ltd. The fungi were incubated in supplemented brain–heart infusion broth (produced by the Merck Co., Germany) for 48 hours at 37°C. Fresh fungal cultures were provided in the same conditions for the experiment. The cell numbers in the fungal suspensions were measured in a spectrophotometric wavelength of 600 nm in 1 mL cuvettes. Cultures were grown to reach the optical density of OD 600 ~0.08 to OD 600 ~0.1, which is indicative of a concentration of 1.5×10^8 CFUs/mL for *C. albicans*. To prepare the suspension, the optical density of this solution was compared with 0.5 McFarland solution through spectrophotometer. The standard 0.5 McFarland solution is a mixture of 0.05 mL $BaCl_2.2H_2O$ 1.175% and 9.95 mL H_2SO_4.

Minimum Inhibitory Concentration

Minimum inhibitory concentration was considered as the minimum concentration of the substance that can inhibit fungal growth completely. It was measured according to the CLSI M100-S24 standard. Microdilution method was applied using 360 µL sterilized polystyrene flat-bottomed 96-well plates (Fig. 1).

To prepare the standard concentration of xylitol solution, 400 mg xylitol is dissolved in 0.5 mL of serum and the volume is increased to reach 1 mL. The final concentration of the solution is equal to 400 mg/mL or 40×10^4 µg/mL. The solution was diluted to acquire another solution with a concentration of 10×10^4 µg/mL for comparison.

In the microdilution method, 96-well microplates were used; 3 rows were needed, and in every row, 12 wells existed. About 100 µL of the latter solution was transferred into the first and second wells of the first row of the plates. About 50 µL was taken from the second well and transferred to the third, and this was continued to the 12th well. Mueller–Hinton broth was added to the two first wells for their volumes to reach 100 µL. Serial two-fold dilutions were prepared in the next wells, correspondingly. About 100 µL of the fresh fungal culture was added to each well so that the final concentration of the fungi was 5×10^5 CFU/mL. In the second row of wells, similar concentrations of xylitol were mixed with Mueller–Hinton broth as a control group to evaluate external contaminations. A similar process was followed for the third row of wells except that the main solution used was the solution with the concentration of 40×10^4 µg/mL and continued like the first row to the 12th well of the third row.

Minimum inhibitory concentration of xylitol was evaluated after 48 hours of incubation of the microplates

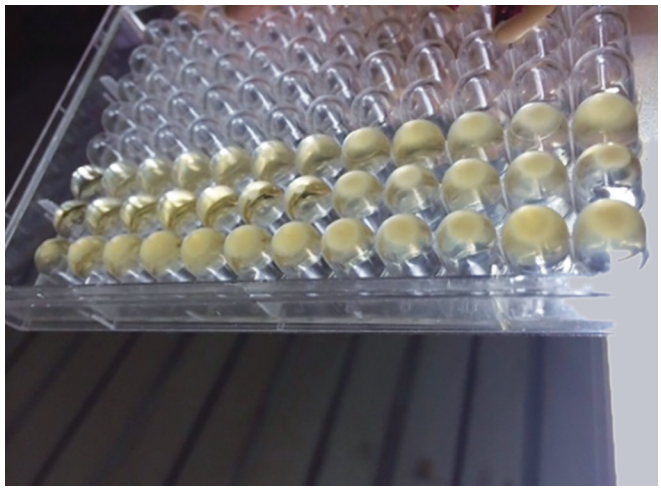

Fig. 1: Polystyrene flat-bottomed 96-well plates were used for the microdilution method

at 37°C. The experiment was repeated three times. Since the solutions were turbid themselves, aggregation of micro-organisms at the bottom of the wells was regarded to evaluate the MIC.

Antimicrobial Effects

The antimicrobial effects were examined by the microbroth dilution method according to the CLSI, M100-S24 standard. The experiment was performed again with two dilutions higher and two dilutions lower regarding the results of MIC. Samples of fungal suspensions and specific concentrations of xylitol were transferred to the Sabouraud dextrose broth through a sterilized wire loop. After 24 hours of incubation at 37°C, the CFUs were counted. The concentration of xylitol that caused a 99.9% decrease in the number of microorganisms was regarded as the MFC. The decrease in the number of microorganisms was evaluated by the comparison with solutions composed of fungal suspensions and culture medium.

RESULTS

Fungi grew in all the wells of the first row. No growth was observed in the second row of wells, which were our control group. In the third row, starting with the concentration of 20×10^4 µg/mL, in the first well, no growth was seen. However, from the second well to the last, fungal aggregation was evident at the bottom of the wells. Therefore, the MIC of xylitol for *C. albicans* is 20×10^4 µg/mL. The three reruns of the experiment yielded similar results.

According to the MIC of xylitol, five concentrations of this substance (5×10^4 to 80×10^4 µg/mL) were evaluated to determine its MFC. Table 1 demonstrates the count of CFUs of *C. albicans* for each concentration in the three experiments performed (Table 1).

The decrease in the count of CFUs of microorganism was calculated through the following formula:

$$\text{Percentage of reduction} = 100 - \frac{\frac{\text{CFU}}{\text{mL}} \text{ in the specific concentration of xylitol}}{\frac{\text{CFU}}{\text{mL}} \text{ in the control group}} \times 100$$

Accordingly, the decreased percentage of CFUs for concentrations of 5×10^4, 10×10^4, 20×10^4, 40×10^4, and 80×10^4 µg/mL were 40.6, 74.6, 99.1, 99.95, and 99.99% respectively. Since a decrease by 99.9% was considered as the MFC of the substance, the concentration of 40×10^4 µg/mL with a decrease of 99.95% was found to be the MFC of xylitol for *C. albicans*.

DISCUSSION

In this study, we evaluated the effects of xylitol on the growth of *C. albicans*. According to the results, a concentration of 20×10^4 µg/mL was found to be the MIC of xylitol and a concentration of 40×10^4 µg/mL was calculated to be the MFC of xylitol for *C. albicans*. The result of this study showed that the effect of xylitol on the growth of *C. albicans* is an inhibitory effect.

Early reports on the effect of xylitol on *C. albicans* show diverse results. Samarayanake et al[20] evaluated the susceptibility of *C. albicans* to lysozyme regarding the sugar added to their culture medium. They evaluated the effect of dietary carbohydrates on the adherence of *C. albicans* to the epithelial monolayer and buccal epithelial cells in laboratory *C. albicans* and isolated *C. albicans*. They found that the fungi grown in the laboratory, i.e., *C. albicans* cultures supplemented by glucose, maltose, xylitol, galactose, and sucrose were more susceptible to lysozyme and the isolated cultures supplemented by glucose, sucrose, and maltose demonstrated a greater overall enhancement in adhesion. In this study, xylitol could not establish resistance against lysozyme; therefore, it could not cause inhibitory effect on the growth of *C. albicans*. Contrary to the Samarayanke study, in this study, we proved the inhibitory effect of xylitol on *C. albicans*. The different conditions of these two studies and lysozyme effect utilization in the Samarayanke study are the explanations for this difference in results.

In 1993, Vargas et al[11] compared the CFUs of *C. albicans* in neutropenic mice fed by glucose with the ones fed by xylitol. According to their study, glucose intake was associated with a significant increase in the growth of this fungus in the mucosa of the mice, and the differences between the two groups of mice were also found to be statistically significant. However, the CFU in mice fed by xylitol was very similar to their control group. Some years later, a study by Pizzo et al[9] found that xylitol in comparison with other carbohydrates, which enhance the adherence of *Candida* species to epithelial surfaces

Table 1: Count of CFUs for each concentration of xylitol

		Count of CFUs for each concentration (µg/mL)				
Experiment	*Control group*	*5×10^4*	*10×10^4*	*20×10^4*	*40×10^4*	*80×10^4*
1	250×10^5	145×10^5	65×10^5	22×10^4	10×10^3	2×10^3
2	260×10^5	160×10^5	70×10^5	25×10^4	14×10^3	3×10^3
3	255×10^5	150×10^5	60×10^5	22×10^4	12×10^3	2×10^3
Mean	255×10^5	151.6×10^5	65×10^5	23×10^4	12×10^3	2.3×10^3

reduces this adhesion, therefore, resulting in an inhibitory effect. Similar results were yielded from the survey conducted by Abu-Elteen[21] in 2005. They also reported that xylitol decreases the adherence of *Candida* species to buccal epithelial cells. In this study, the adherence of four *Candida* species to human buccal epithelial cells following treatment with the most commonly consumed dietary carbohydrates was investigated *in vitro*. Adhesion of these four *Candida* species was significantly promoted by incubation in minimal medium containing a high concentration of fructose, maltose, galactose, sorbitol, sucrose, and glucose. However, xylitol significantly reduced adherence of *Candida* species to buccal epithelial cells. Similar to Pizzo's study, this study stated the inhibitory effect of xylitol on the growth of *C. albicans*, but Pizzo explained the mechanism of inhibition of xylitol.

In 2008, a study revealed that benzethonium chloride had greater fungicidal effect when combined with sugars, such as xylitol than applied alone.[12]

The effect of various concentrations of xylitol on *C. albicans* growth was evaluated for the first time in 2009. Leepel et al found that, in a 3-day period, the growth of *Candida* colonies was adversely related to the concentration of xylitol; increased concentration of xylitol from 1 to 5% and 10% lead to a decrease in the growth of this fungus. However, a significant decrease in the growth of *C. albicans* compared with the control colonies was only seen in those exposed to 10% xylitol. On the contrary, in a 7-day period, no concentration of xylitol was able to decrease the growth of *Candida*, and no significant difference was observed comparing their growth with the control group. This laboratory experiment was accomplished on clinical isolates of *C. albicans* that were taken from a male patient with candidiasis. Moreover, *C. albicans* ACTT 10231 strain was used as a comparison.[19] This finding revealed the importance of the concentrations of xylitol on the growth of *Candida* compared with previous studies that somewhat reported minimum or no effect for xylitol in this matter.[9,11]

In 2011, Amaral et al[22] evaluated the antimicrobial effects of xylitol with MIC, and they found significant antimicrobial effects of this substance against *Staphylococcus aureus*, *Escherichia coli*, *C. albicans*, *Pseudomonas aeruginosa*, and *Aspergillus niger*. This study calculated the MIC for *S. aureus*, *E. coli*, *C. albicans* to be about 1.0 to 1.25%, and against *P. aeruginosa* and *A. niger* to be about 1 to 1.5%. According to this evaluation, the antimicrobial effect of xylitol was reliable, and it can be used as a microbial growth inhibitor. The same result in this study and Amaral's study emphasized the inhibitory effect of xylitol on the growth of *C. albicans*, but Amaral estimated the MIC at around 10×10^4 to 15×10^4 µg/mL. In this study, the MIC was about 20×10^4 µg/mL.

Xylitol was found to reduce the production of carcinogenic acetaldehyde from ethanol by *Candida* below the mutagenic level of 40 to 100 µM by Uittamo et al[14] Acetaldehyde is a highly toxic and mutagenic product of alcohol fermentation and metabolism, which has been classified as a class I carcinogen for humans. Many *Candida* species representing oral microbiota have been shown to be capable of marked acetaldehyde production.

In recent years, a study found that xylitol did not augment lysozyme- and peroxidase-related candidacidal activities.[23] However, other researchers found that xylitol combined with zinc has a more inhibitory effect on candidal growth than compared with xylitol alone.[24]

All the studies regarding this matter point to the fact that xylitol has considerable antimicrobial effects.

CONCLUSION

According to the results of this survey, xylitol has considerable antimicrobial effects. It can decrease the adherence of *C. albicans* to buccal epithelial cells and weaken this microorganism against our immune system by making it more susceptible to lysozyme. The growth of this fungus is also inhibited in the presence of xylitol.

Clinical Significance

Hence, this substance can be used as an effective element in gums and toothpastes and antimicrobial mouthwashes, especially in patients with candidiasis. Moreover, by knowing the MIC and MFC of xylitol, this substance can be effectively used in products aimed to cure this fungal infection.

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