

EFFECT OF XYLITOL WITH VARIOUS CONCENTRATION AND DURATION ON THE GROWTH OF *CANDIDA ALBICANS* (IN VITRO STUDY)

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Abstract

The growth of *C. albicans* is influenced by glucose intake. Xylitol is commonly used as sugar substitute. Reported effective concentrations of xylitol in reducing *C. albicans* growth *in vitro* were varied, 1%, 5%, and 10%. **Objectives:** Investigate the effect of different concentration and duration of xylitol exposure in inhibiting *C. albicans* growth *in vitro*. **Method:** Identification of *C. albicans* from oral swab of a male candidiasis patient was conducted using CHROMagar, confirmed by germ tube test. *C. albicans* suspension (10^8 cells/ μ l) were inoculated in SDB contained 1%, 5%, 10% xylitol, and without xylitol (as control), for 3 and 7 days, then incubated in 37°C on SDA and counted for their CFU after 48 hours. The *C. albicans* ATCC 10231 strain was used as a comparison. **Results:** After 3 days, increased concentration of xylitol (1%, 5%, 10%) lead to decrease growth of *C. albicans*, both the ATCC 10231 (125%; 51%; 14% respectively) and the clinical isolate (103%; 81%; 42%), $p = 0.044$. Significant lower growth of *C. albicans* compared to control were only seen in those exposed to 10% xylitol ($p = 0.024$). After 7 days, exposure of 1%, 5%, 10% xylitol did not significantly affect the growth of *C. albicans* ($p = 0.396$). **Conclusion:** The growth of *C. albicans* could be inhibited by 10% xylitol for 3 days.

Key words: *C. albicans*; xylitol

Introduction

Candidiasis is the most common fungal infection of human and mostly caused by *Candida albicans*.^{1,2,3} In the last two decades, infection cases due to *Candida* species has increased significantly.⁴ *C. albicans* is indigenous to the oral cavity, gastrointestinal tract, female genital tract and sometimes the

skin; hence infection is usually endogenous, although cross-infection may occur, e.g. from mother to infant, and among infant siblings.⁵ In oral cavity, the main oral site where it is most often found is the tongue, particularly in the posterior dorsum area in the region of the circumvallate papillae.⁶

As commensal organisms and part of an individual's normal microflora, *Candida species*

can be detected in approximately 50% of the population.⁷ However, if the balance of the normal flora is disrupted or the immune defenses are compromised, *Candida* species often become pathogenic.^{7,8}

Dietary intake of the host is one predisposing factor required for the development of *Candida* infection. Carbohydrate-rich diet and vitamin-deficiencies are well regarded as the vital predisposing factors of this infection.^{9,10} *In vitro* studies showed that glucose intake is a determinant promoter of *C. albicans* growth.^{10,11} In contrary to this finding, *in vivo* studies showed that chewing xylitol gum could decrease the adherence of *C. albicans* to buccal epithelial cells¹² and the risk of candidiasis and angular cheilitis.¹³

Xylitol, a natural sweetener, can be found in foods as berries, fruit, vegetables and mushrooms. It is even generated by the human body during metabolism. Xylitol is a polyol sweetener, a sugar alcohol. It differs from other sweeteners such as sorbitol, fructose and glucose because the xylitol molecule has five, instead of six carbon atoms.¹⁴ Xylitol has 40% less calories and 75% less carbohydrates than sugar and is slowly absorbed and metabolized.¹⁵ Different from intense artificial sweeteners, including aspartame, acesulfame-K, and sucralose, xylitol has no adverse effects or bitter aftertaste.¹⁶

Compared to other polyols, xylitol has been reported to be effective in inhibiting *C. albicans*, a serious systemic yeast problem, and other harmful intestine bacteria including *Helicobacter pylori*, implicated in periodontal disease, bad breath, gastric and duodenal ulcers, and even stomach cancer.¹⁵ The inhibiting effect of xylitol on the *Candida* growth is known as dose dependent. Previous different studies reported various effective concentrations of xylitol, 1%,¹⁷ 5%,¹¹ 10%¹⁸ in reducing the growth of *C. albicans in vitro*. Besides that, it was known that optimum duration for the growth of *C. albicans* is 3 days.¹⁹

Therefore, the objectives of this study were to investigate the inhibiting effect of those various concentrations (1%, 5%, 10%) and

durations (3 and 7 days) of xylitol exposure on *C. albicans* growth *in vitro*.

Materials and methods

This laboratory experiment was conducted in the Oral Biology laboratory, Faculty of Dentistry, Universitas Indonesia. The laboratoric strain ATCC 10231 (American Type Culture Cell) which was taken from Microbiology Department, Faculty of Medical, Universitas Indonesia, was used as a comparison. The clinical isolate was taken from a male oral candidiasis patient who came to Oral Medicine Department, RSCM, Jakarta and received chemo-radiation therapy for his nasopharyngeal cancer.

The clinical yeast sample was taken with swab technique, and inoculated in CHROMagar. CHROMagar is a media conveniently used to identify different *Candida* species accurately through its pigmentation.²⁰ After 2 days, *C. albicans* could be identified as pale green colonies. Confirmation of *C. albicans* was conducted by examining the formation of germ tube after incubation in Fetal Bovine Serum (FBS) for 2 hours at 37°C. The ATCC 10231 strain and the clinical *C. albicans* isolate then were inoculated in Sabouraud Dextrose Agar (SDA) for 2 days.

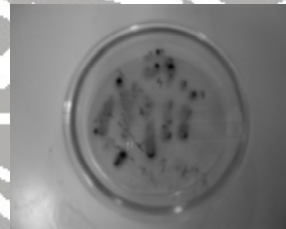


Figure 1. *C. albicans* identified as

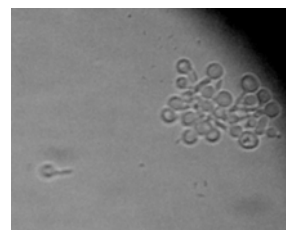


Figure 2. Germ tube formation pale green colonies in CHROMagar of *C. albicans*

The inoculation were serially diluted, then inoculated in SDA for 2 days at 37°C, and counted to get the initial Colony Forming Unit (CFU) which is the CFU from cultures before exposed to xylitol. This score was to be compared to the CFU of those exposed to xylitol after 3 or 7 days and thus the percentage of *C. albicans* growth could be analyzed.

Xylitol exposure to the *C. albicans* suspension was conducted after several stages of serial dilution, from which 10 µl was taken and inoculated in 990 µl solution of Sabouraud Dextrose Broth (SDB) containing 1%, 5%, or 10% xylitol, and without xylitol as control and each kept for 3 or 7 days.

After 3 days and 7 days, these inoculations were washed in Phosphate-Buffered Saline (PBS) and purified by centrifugation at 10,000 rpm for 2 minutes. The CFU of *C. albicans* in SDA were counted after 2 days. Data was analyzed using General Linear Model (GLM) Univariate with α 0.05.

Results

Before exposed to xylitol, the mean results of extrapolation that have been achieved

from *C. albicans* clinical isolate was 9×10^8 CFU/ml and from the ATCC 10231 strain was $1,5 \times 10^8$ CFU/ml. This score will be compared to the CFU of those exposed to xylitol with various concentrations (1%, 5%, 10%) and different duration (3 or 7 days) to get the percentage of *C. albicans* growth. To be able to compare the growth of different groups of *C. albicans* colonies (those exposed & not exposed to xylitol), the CFU difference between those two groups was considered as 100%.

For example, the control *C. albicans* colony after 3 days was 970×10^8 CFU/ml, while the initial CFU was 9×10^8 CFU/ml. Thus, the range between those two groups above is 970×10^8 CFU/ml - 9×10^8 CFU/ml = 961×10^8 CFU/ml. This score was considered as 100% (Table 1). For *C. albicans* colony exposed to 1% xylitol for 3 days, the score was 1000×10^8 CFU/ml. Thus the CFU range compared to colony control group for 3 days was 1000×10^8 CFU/ml - 970×10^8 CFU/ml = 30×10^8 CFU/ml. This increase was considered as $(30 \times 10^8$ CFU/ml : 970×10^8 CFU/ml) \times 100% = 3%. Thus, the percentage for *C. albicans* exposed to 1% xylitol for 3 days is 103% (Table 1).

Table 1. Percentage of *C. albicans* (clinical isolate and ATCC 10231) growth after exposed to xylitol (1%, 5%, and 10%) for 3 days and 7 days

Medium	<i>C. albicans</i> strain ATCC		<i>C. albicans</i> clinical	
	3 days	7 days	3 days	7 days
SDB (as control)	100%	100%	100%	100%
SDB + xylitol 1%	125%	86%	103%	46%
SDB + xylitol 5%	51%	94%	81%	157%
SDB + xylitol 10%	14%	73%	42%	94%

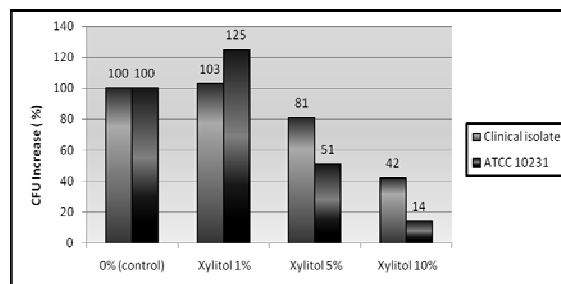


Figure 3. *C. albicans* Growth after Exposed to Xylitol for 3 Days

From figure 3, it can be seen that after 3 days, the increased concentration of xylitol lead to significant decline of *C. albicans* growth ($p = 0.044$). Significant lower growth of *C. albicans* compared to control ($p = 0.024$) was showed by those exposed to 10% xylitol.

Different from the results of xylitol exposure for 3 days, this study showed that the inhibitor effect of xylitol to *C. albicans* growth was not effective anymore after 7 days ($p = 0.396$).

Discussion

After 72 hours, 1% xylitol lead to increased growth of *C. albicans*, both in clinical isolate and ATCC 10231, compared to control (Figure 3). However, this difference was not statistically significant. This result differs from Lee's (2004) previous study.¹⁷ No statement about the duration of xylitol exposure to *C. albicans* in Lee's study. This study was designed to analyze the effect of 3 day xylitol exposure because it is known that optimal growth of *C. albicans in-vitro* is 72 hours.¹⁹

Three day exposure of 5% xylitol lead to decreased growth of *C. albicans*. Although such decrease of *C. albicans* growth was not statistically significant but the trend is in line with previous *in vitro* study from Makinen (1975). Makinen was comparing the growth of *C. albicans* exposed by xylitol 5%, xylitol 5% + glucose 0,2%, and glucose 0,2%. The result of his study was that 5% xylitol could inhibit the growth of *C. albicans* after 72 hours.¹¹

In this study, 3 day exposure of 10% xylitol lead to significant declined of *C. albicans* growth. This result is in line with previous study by Munita et.al (2002).¹⁸

Although in this study the growth of *C. albicans* could only be significantly inhibited by 10% xylitol for 3 days but the result showed that increased concentration of xylitol lead to significant decreased growth of *C. albicans*. Xylitol has 5 carbon atoms which is shorter than other sugars (glucose and fructose).²¹ Such different structure might be the reason for the inability of pathogenic bacteria to digest xylitol²² and the slower absorption of this sugar through human intestine wall.²³ Higher concentration of xylitol in the media of *C. albicans* as conducted in this study, might lead to increased difficulty in absorbing sufficient nutrient required to maintain the viability of the yeast.

Conversely, after 7 days, exposure of 1%, 5%, 10% xylitol did not significantly affect the growth of *C. albicans*. Different results of the effect of xylitol on the growth of *C. albicans* between those exposed for 3 days and those exposed to 7 days as shown in this study, might be due to the age of SDB media that influenced the medium acidity (pH). Further research about the effect of duration to SDB properties, xylitol, and *C. albicans* are needed to clarify this phenomenon.

The results of this study also showed that the effect of xylitol on the growth of *C. albicans* is influenced by the strain of the yeast. Different phenotype between strains might contribute in those different effects of xylitol. It is likely that the more virulent clinical *C. albicans* is more responsive to xylitol or serum exposure, compared to the less virulent²⁴ ATCC 10231 *C. albicans*. Further research is required to investigate the role of different biologic and physiologic characters between different strains of *C. albicans* on their response to xylitol.

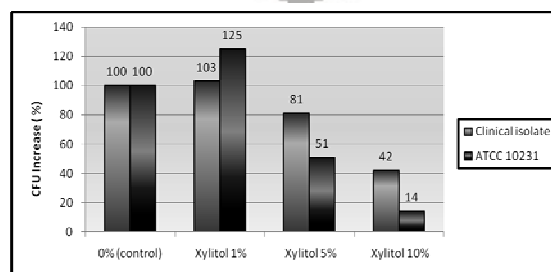


Figure 4. *C. albicans* Growth after Exposed to Xylitol for 7 Days

Conclusion

Increased concentration of xylitol after 3 days significantly inhibits *C. albicans* growth *in vitro*. The effective concentration of xylitol to inhibit the growth of *C. albicans* is 10% for 3 days.

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