

## Effect of Xylitol on *Candida albicans* Resistance in Serum (In Vitro Study)

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### Abstract

Xylitol is reported to inhibit the growth of *C. albicans*. **Objectives:** Investigating serum factor role in inhibiting the growth of *C. albicans* and the effect of 1%, 5%, 10% xylitol on *C. albicans* resistance in serum in vitro. **Methods:** Identification of *C. albicans* (oral swab of candidiasis patient) was conducted using CHROMAgar, confirmed by germ tube test. The cultures were serially diluted, inoculated in Sabouroud Dextrose Broth (SDB) contained 0% (control), 1%, 5%, or 10% xylitol, and kept for 3 or 7 days. These inoculations were then exposed to either active or inactive serum (Fetal Bovine Serum heated in 65°C for 30 minutes) for 2 hours in 37°C. The colony forming unit (CFU) of *C. albicans* in Sabouroud Dextrose Agar (SDA) were counted after 2 days. *C. albicans* ATCC 10231 strain was used as a comparison. One-way ANOVA with a 0.05 was used. **Results:** After 3 days cultured in media with or without xylitol, the CFU of *C. albicans* exposed to active serum were significantly lower than those exposed to inactive serum ( $p=0.032$ ). Although not statistically significant ( $p=0.689$ ), increased concentration of xylitol lead to increased resistance of *C. albicans* in active serum. Only 7 day exposure of 10% xylitol resulted in significantly higher growth of *C. albicans* ( $p=0.034$ ). No significant difference of *C. albicans* CFU in active or inactive serum ( $p=0.404$ ). **Conclusion:** Serum factor has role in inhibiting *C. albicans* growth in vitro. Exposure of 1%, 5%, or 10% xylitol for 3 or 7 days has no significant effect on *C. albicans* resistance in serum.

**Key words:** *Candida albicans*, resistance, serum factor, xylitol

### Introduction

Candidiasis is a most common fungal infection on human, that mostly caused by *Candida albicans*.<sup>1</sup> In the last two decades, infection

cases due to *Candida* species has increased significantly.<sup>2</sup> *Candida* species are commensal flora commonly found in the oral cavity, gastrointestinal tract, and vagina. Its



transformation from commensal to pathogen is triggered by the presence of various predisposing factors, among others are long exposure to broad-spectrum antibiotics or reduced host defenses like in immunocompromised patients, that can lead to bloodstream invasion of *C. albicans*.<sup>1,3</sup>

High intake of dietary carbohydrate is one risk factor of oral candidiasis commonly occur in population with modern life style. A study reported that adhesion of *C. albicans* to human buccal epithelial cells was significantly promoted by incubation in medium containing a high concentration of fructose, galactose, glucose, maltose, sorbitol, or sucrose.<sup>4</sup> Substitution of sugar with xylitol had been reported to decrease oral candidiasis as well as to repress the growth of *Candida*.<sup>5</sup>

Xylitol is a sugar alcohol which does not contain effective carbohydrate. This natural sweetener can be found in the fibers of many fruits and vegetables, including various berries, corn husks, oats, and birch tree.<sup>5</sup> Instead of having six carbons like glucose or other sugar that commonly used, xylitol only consists of five carbons sugar. Such different chemical structure is believed to be the reason of the difficulty in

digesting xylitol by microbes.<sup>6</sup>

Many studies have reported a lot of xylitol benefit to reduce caries.<sup>5</sup> Xylitol is also reported to inhibit the growth of *C. albicans*.<sup>7,8</sup> Chewing xylitol gum is reported to reduce risk of oral candidiasis and angular cheilitis, influence oral candidiasis pathogenesis, and enhance the effectiveness of antifungal therapy.<sup>9,10</sup> However, the effective concentration of xylitol in inhibiting *C. albicans* growth as reported by previous studies were varied, 1%, 5%, and 10%.<sup>7,8,9</sup> Report from Makinen et al (1975) showed that *C. albicans* colonies start to decrease after 72 hours exposed to xylitol.<sup>8</sup>

One among several laboratory methods for identifying *C. albicans* is germ tube test in which the yeast culture were incubated in serum for 2 hours at 37°C. However, incubation of yeast cells in serum not only induces germ tube formation, but also inhibits its growth.<sup>11</sup> Serum factor, such as complement, has an antimicrobial effect.<sup>12</sup> Serum also contains transferrin and lactoferrin which can inhibit *C. albicans* growth due to its binding capacity with iron, which is an essential nutrient for the growth of *Candida*. Those serum component is also found in gingival crevicular fluid of the oral cavity, and known to



inhibit the growth of pathogenic microorganism, including *C. albicans*.<sup>13,14,15</sup>

*C. albicans* is also known to have several mechanisms to support its sustainability in serum.

*C. albicans* can bind the complement regulators so that they can avoid the complement-mediated phagocytosis.<sup>16</sup> *C. albicans* also has calcineurin, a protein phosphatase that is required to survive calcium stress in serum.<sup>17</sup>

Therefore the hypothesis in this study was that xylitol could inhibit the growth of *C. albicans* and suppress its resistance in serum *in-vitro*.

The objectives of this research were to investigate the effect of 1%, 5%, 10% xylitol for 3 and 7 days on *C. albicans* resistance in serum *in vitro*, and to investigate whether serum factor plays role in inhibiting the growth of *C. albicans*.

### Materials and Methods

*C. albicans* used in this research was a clinical isolate taken from a male oral candidiasis patient in RSUPNCM who was receiving chemotherapy for his nasopharyngeal cancer. *C. albicans* laboratoric strain ATCC (American Type Culture Cell) 10231 was used as a comparison (Microbiology Laboratory –

Faculty of Medicine Universitas Indonesia). The clinical yeast sample was taken from oral lesion

with swab technique, and inoculated in CHROMagar (Parasitology Laboratory –

Faculty of Medicine Universitas Indonesia) which was then incubated at 37°C for 2 days.

CHROMagar is a media conveniently used to identify different *Candida* species accurately through its pigmentation.<sup>18</sup> After 2 days, *C.*

*albicans* could be identified as pale green colonies. Confirmation of *C. albicans* was

conducted using germ tube test by incubation in serum for 2 hours at 37°C. Germ tube

formation was observed under microscope with 40 times magnification. Group of yeast cells

with filamentous extension without constriction indicated *C. albicans* species. The ATCC

10231 strain and the clinical *C. albicans* isolate then inoculated in Sabouraud Dextrose Agar

(SDA) for 2 days at 37°C.

The inoculation were serially diluted in Phosphate-Buffered Saline (PBS), then inoculated in SDA for 2 days at 37°C, and counted to get the initial Colony Forming Unit (CFU). The initial CFU was the CFU from cultures before exposed to xylitol (kindly provided by PT Lotte Indonesia) and serum.



To get the percentage of *C. albicans* growth, the initial CFU score was to be compared to the CFU score of those exposed to xylitol and serum.

Xylitol exposure to the *C. albicans* suspension was conducted after particular stage of serial dilution, from which 10 µl was taken and inoculated in 1 ml solution of Sabouraud Dextrose Broth (SDB) containing 0% (control), 1%, 5%, or 10% xylitol and kept for 3 or 7 days. SDB was used in this study because of its ability to maintain *C. albicans* viability.

After 3 days or 7 days, these inoculations were washed in PBS and purified by centrifugation at 10,000 rpm for 2 minutes twice. To test the *C. albicans* resistance in serum, Fetal Bovine Serum (Biowest, South America) were used. Following centrifugation, *C. albicans* then grouped as those exposed to active serum, inactive serum, or non serum (PBS) for 2 hours in 37°C. Inactive serum was prepared by heating the FBS in 65°C for 30 minutes. The CFU of *C. albicans* in SDA were counted after 2 days, and compared to the initial CFU. Data was analyzed using one-way ANOVA with  $\alpha$  0.05.

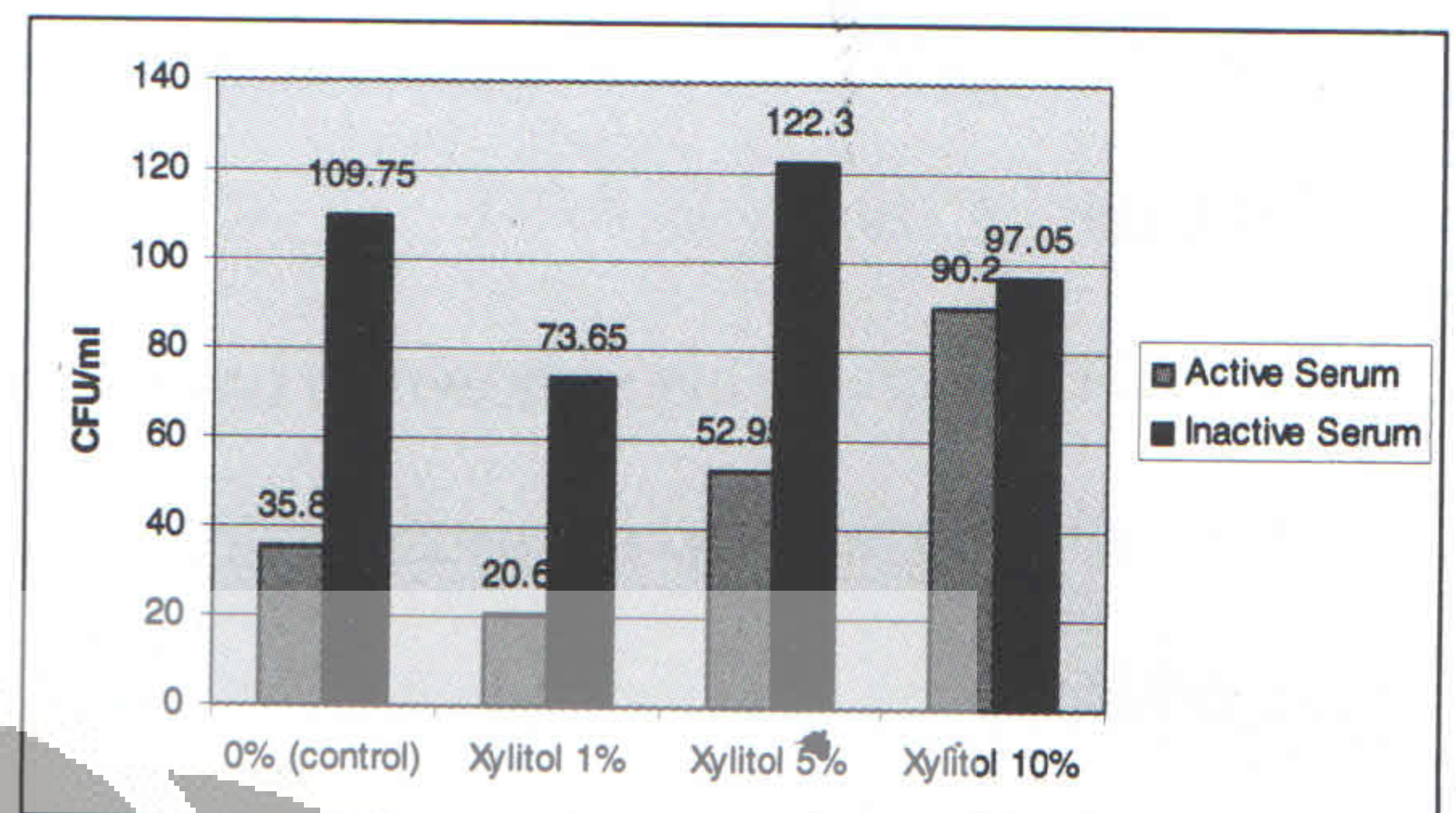


Fig 1. Clinical Isolate *C. albicans* Resistance in Serum after Xylitol Exposure for 3 Days

In active serum, clinical isolate *C. albicans* growth was significantly inhibited after 3 days both in those with or without xylitol exposure ( $p = 0.032$ ). Compared to those exposed to inactive serum, the CFU of *C. albicans* in active serum was always less. This result was in line with the hypothesis that serum contains several active components that can inhibit the growth of *C. albicans*. It could be assumed that after heated in 65°C, some serum factors became inactive, so that *C. albicans* was not inhibited in inactive serum.

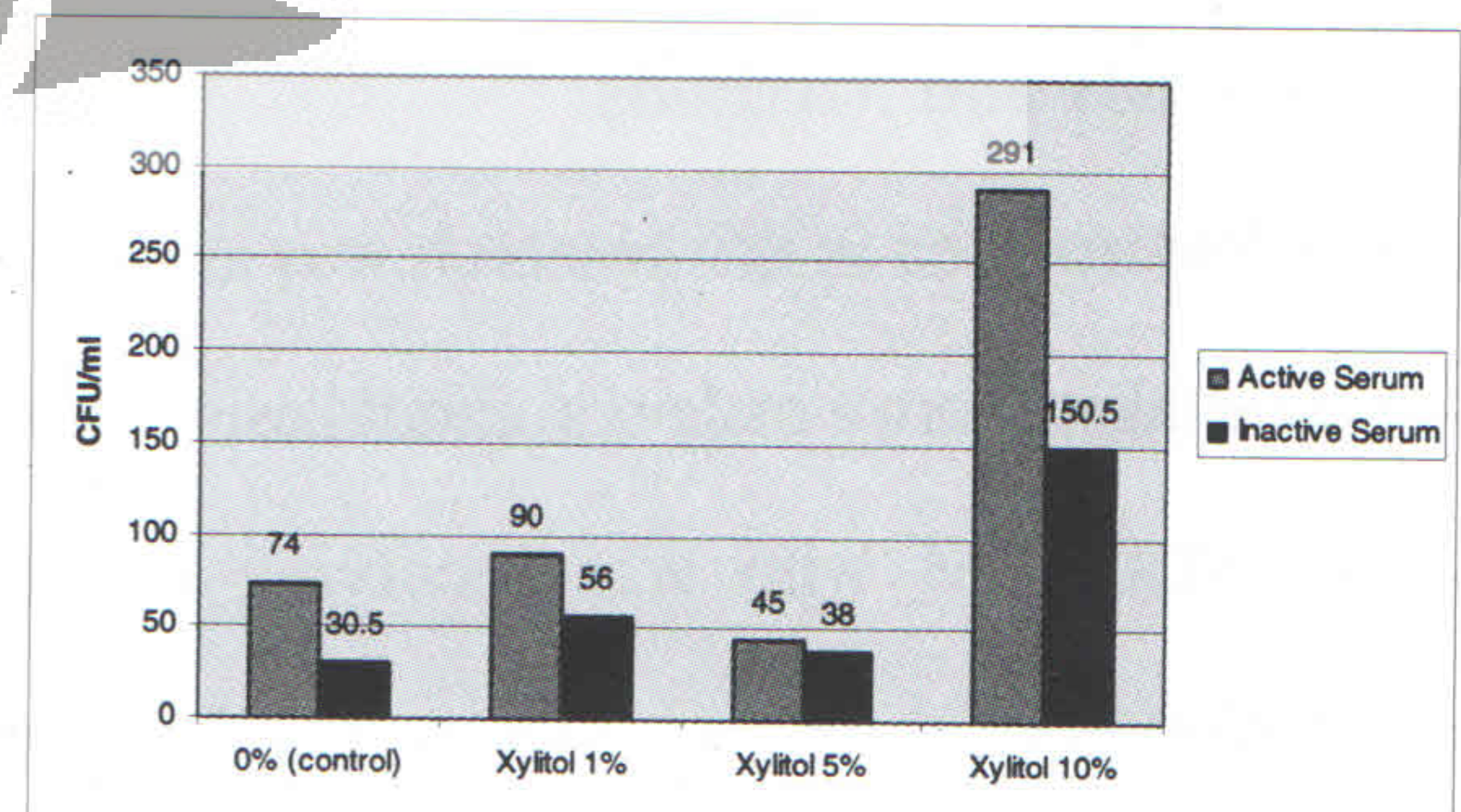


Fig 2. Clinical Isolate *C. albicans* Resistance in Serum after Xylitol Exposure for 7 Days



Conversely, after 7 days clinical isolate *C. albicans* CFU in active serum was always higher than the CFU in inactive serum. However this result was not statistically significant ( $p = 0.404$ ). From this result can be concluded that serum factor does not effective anymore to inhibit *C. albicans* growth after 7 days.

These results showed that duration of xylitol exposure influenced *C. albicans* resistance to serum factor. Statistic test showed significant different between *C. albicans* CFU after 3 days and after 7 days, both clinical isolate ( $p = 0.012$ ) and ATCC 10231 strain ( $p = 0.000$ ).

Different with clinical isolate, serum factor did not have any significant effect to the growth of ATCC 10231 strain of *C. albicans*, either in 3 days ( $p = 0.397$ ) or 7 days culture ( $p = 0.821$ ). This different response might be due to different virulence between those 2 strains.

In 3 days clinical isolate *C. albicans* cultures, there was a tendency of increased *C. albicans* resistance in serum along with increased xylitol concentration. This pattern was in the contrary with the hypothesis of this research, that increased concentration of xylitol would lead to decreased *C. albicans* resistance in serum. Even though this result was insignificant statistically

( $p = 0.689$ ), but some previous research might clarify this tendency. Human serum contains several factors (i.e., transferrin, lactoferrin, lysozyme, and complement) that can inhibit or kill pathogenic microorganisms. According to Giles and Czuprynski (2003), the inhibitory effect of serum on most pathogenic fungi is dependent on the iron binding activity of transferrin, which caused lack of iron in the medium that inhibit *C. albicans* growth. Iron is an essential nutrient for *C. albicans* growth.<sup>15</sup>

Hamalainen and Makinen research on rat (1983) stated that 200 grams of xylitol added to 1 kg of the rat's diet, increase iron level in serum. Beside that, xylitol is one of chelating agents in plasma that affect iron discharge from transferrin to ferritin molecules.<sup>19</sup> Based on those knowledge, the effect of xylitol in increasing the *C. albicans* resistance in serum as showed in this study might be elucidated as the capacity of xylitol in increasing the iron level in serum and in releasing iron from transferrin, so that *C. albicans* can use the iron to support their metabolism and able to sustain the harmful component of serum.

Different from those seen in cultures exposed to xylitol for 3 days, in *C. albicans* exposed to



xylitol for 7 days no significant effect of the xylitol to *C. albicans* resistance could be seen ( $p = 0.058$ ). Only exposure of 10% xylitol for 7 days resulted in significant increase of *C. albicans* growth in serum ( $p = 0.034$ ). It could be assumed that addition of 10% xylitol for 7 days might strengthen the resistance of clinical isolate *C. albicans*, and even give benefit to support the growth of *C. albicans*.

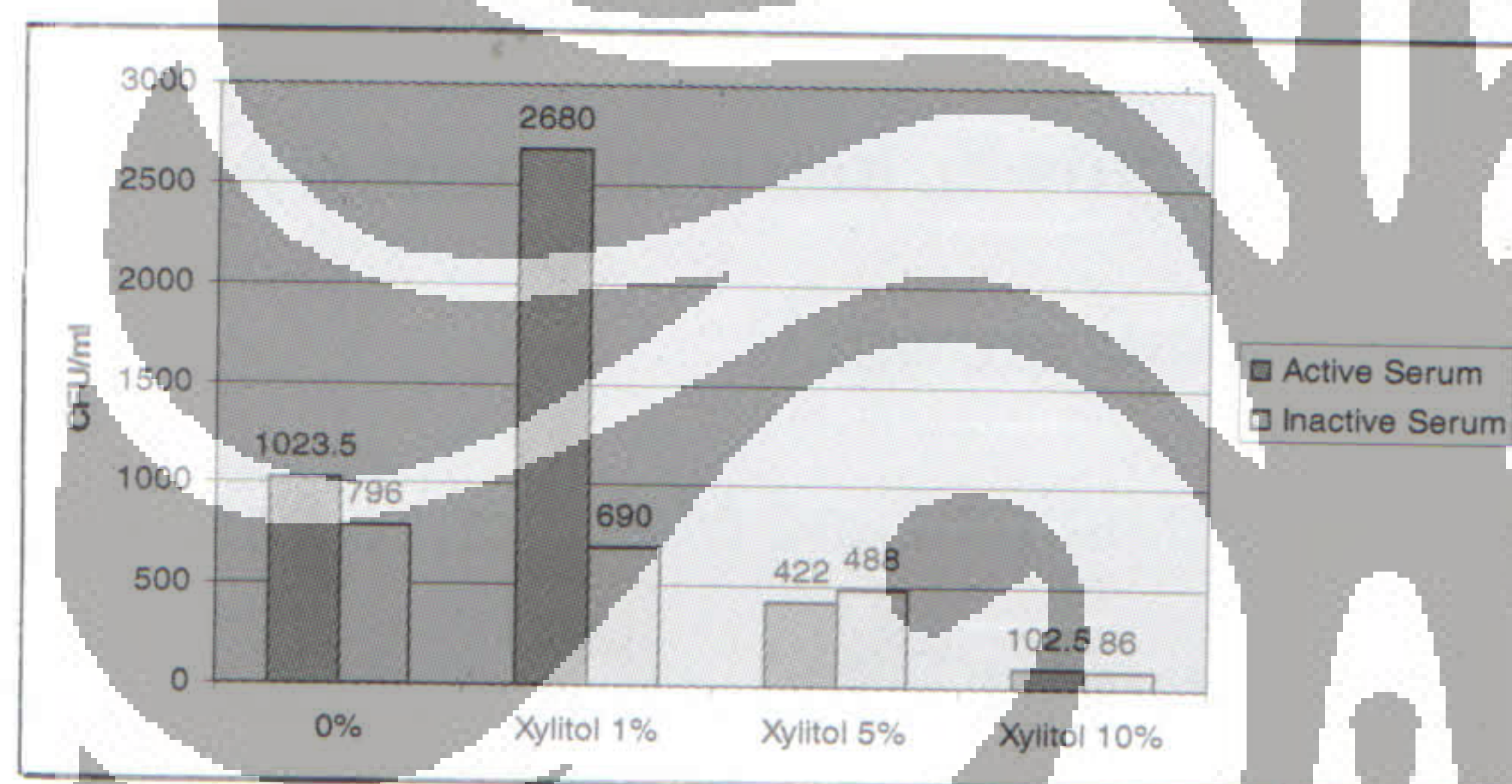


Fig 3. ATCC 10231 *C. albicans* Resistance in Serum after Xylitol Exposure for 3 Days

However, the tendency of increased *C. albicans* resistance in serum after xylitol exposure for 3 days was not seen in ATCC 10231 strain. There was no significant correlation between xylitol concentration exposed for 3 days and the growth of *C. albicans* ( $p = 0.294$ ). The effect of xylitol in increasing *C. albicans* resistance in serum might be influenced by the virulence of different strain type of *C. albicans*. It is likely that the more virulent clinical *C. albicans* is more responsive to xylitol or serum exposure, compared to the less virulent ATCC

10231 strain of *C. albicans*. Further research is required to investigate these different biologic and physiologic characters between different strains of *C. albicans*. Also no significant correlation was found on 7 days result of ATCC 10231 strain of *C. albicans* resistance in serum after exposed with xylitol ( $p = 0.118$ ). But statistically, there was a significant decrease of *C. albicans* CFU with exposure of 5% xylitol ( $p = 0.034$ ). This result was in line with clinical isolate result after 7 days, that *C. albicans* resistance was lowest with exposure of 5% xylitol. However, these similar trends might caused by different causal factor.

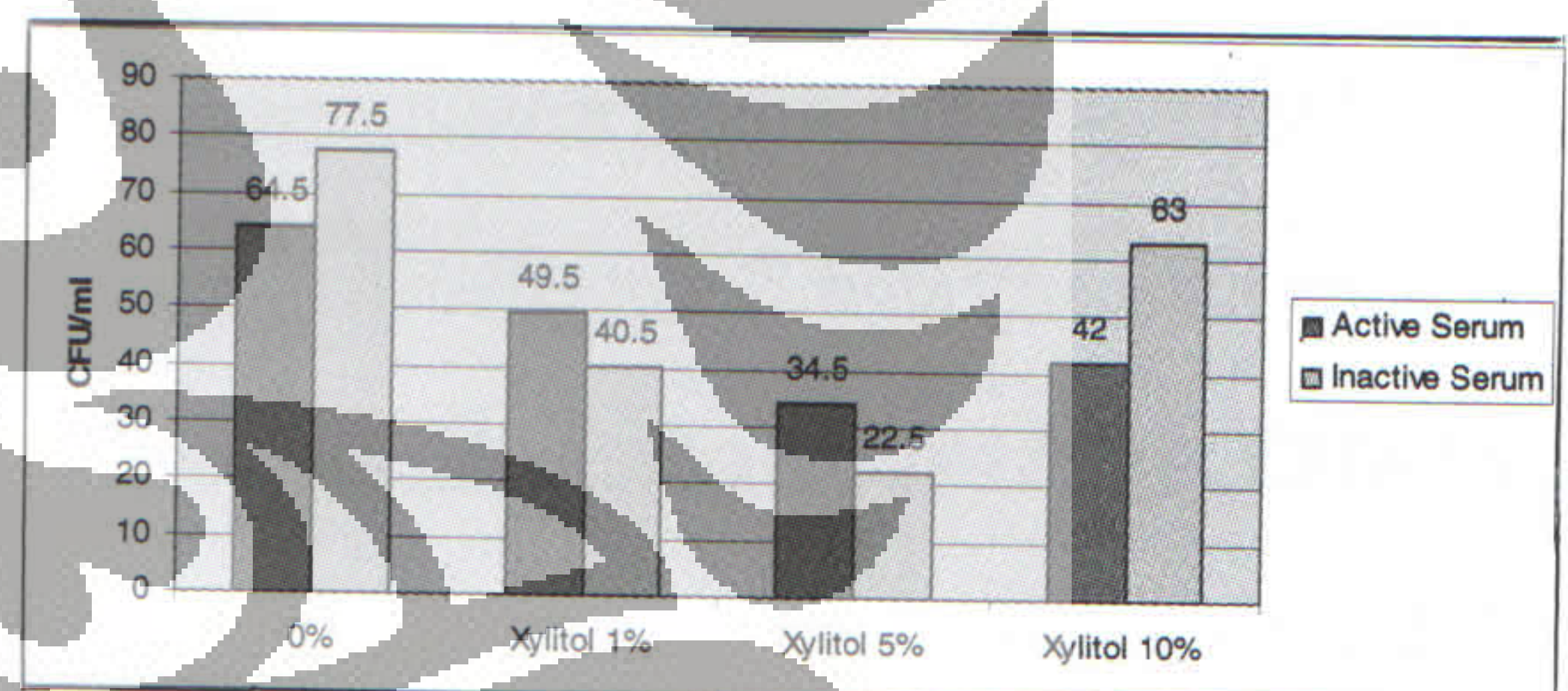


Fig 4. ATCC 10231 *C. albicans* Resistance in Serum after Xylitol Exposure for 7 Days

Different results of the effect of xylitol on the growth and resistance of *C. albicans* between those exposed for 3 days and those exposed for 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 duration effect to SDB properties, xylitol, and *C. albicans* is needed to clarify this phenomenon.



## Conclusions

The inhibition effect of serum factor to *C. albicans* growth *in-vitro* was significant after 3 days, but not effective after 7 days. Xylitol exposure for 3 and 7 days has no significant effect on *C. albicans* resistance in serum. However, there was a tendency of increased *C. albicans* resistance in serum along with increased concentration of xylitol after 3 days.

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