Antimicrobial Activity of Dentifrice Containing Xylitol on Mutans Streptococci

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Abstract
In several human trials, xylitol products have been shown significantly reduce the incidence of caries. *Streptococcus mutans* is the main cause of dental caries by triggering dental plaque formation, effort to control dental caries in the community has been done in many ways. Recent study showed that the amount of Colony Forming Units (CFU) of salivary Mutans Streptococci after tooth brushing with dentifrice containing xylitol is lower compared with non-xylitol dentifrice. **Objective:** The aim of the study was to determine the sensitivity of dentifrice containing xylitol on mutans streptococci.

**Methods:** Dentifrice containing xylitol was examined in vitro to inhibit the bacterial growth by determining the inhibition zone (agar diffusion method), minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC). The microorganisms tested were *Streptococcus mutans* Ing-Britt, *Streptococcus mutans* KPSK2, *Streptococcus mutans* LM7, *Streptococcus mutans* JC2, *Streptococcus sobrinus* B13. Data obtained was done in a descriptive method. **Results:** showed that Xylitol in dentifrice had effect on all of mutans of *Streptococcus mutans* Ing-Britt (inhibition zone 2.00mm; MIC 10^{-3}/ml, MBC 10^{-1}/ml); *Streptococcus mutans* KPSK2 (inhibition zone 1.80mm; MIC 10^{-3}/ml, MBC 10^{-1}/ml); *Streptococcus mutans* LM7 (inhibition zone 2.10mm; MIC 10^{-3}/ml, MBC 10^{-1}/ml); *Streptococcus mutans* JC2 (inhibition zone 1.50mm; MIC 10^{-3}/ml, MBC 10^{-1}/ml); *Streptococcus sobrinus* B13 (inhibition zone 2.20mm; MIC 10^{-3}/ml, MBC 10^{-1}/ml), and their MBC value was three times bigger than MIC.

**Conclusion:** We conclude that dentifrice containing Xylitol has antimicrobial activity against *Streptococcus mutans* Ing-Britt, *Streptococcus mutans* LM7, *Streptococcus mutans* KPSK2, *Streptococcus mutans* JC2 and *Streptococcus sobrinus* B13. It can be used to anticipate caries risk in the future.

Key words: Dentifrices, Xylitol, Mutans Streptococci.

Introduction

Dental caries remains as a significant health threat in everywhere and also as a prominent target of many dental health care in Indonesia, where most of the population suffers from dental caries and periodontal diseases.

Dental caries is a main dental health problem throughout the world although its prevalence in several industrial countries has declined. On the other hand in Indonesia caries prevalence tends to increase.

The evidence is overwhelming that *Streptococcus mutans* is the primary etiological agent in the dental caries by triggering dental plaque formation.

For this reason early prevention is needed by controlling dental caries in the community has been done in many ways, such as by tooth brushing regularly using dentifrice, mouth rinsing with antiseptics, and adding fluoride to toothpaste and drinking water in certain community. But actually dental caries still showed a high figure. This
condition is a challenge for dentistry profession to prevent the dental caries and periodontal disease.

In this study is still needed further research to determine antibacterial property of dentifrice containing xylitol in controlling of dental caries.

The objective of this research is determining the sensitivity of toothpaste containing xylitol by measuring the inhibitory zone, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) on mutans streptococci, in vitro.

The result will provide information about tooth paste containing xylitol and its effectiveness in preventing dental caries for dentistry in particular and for researcher in general. This will hopefully lead another research that eventually will produce a new, save and inexpensive in dental treatment.

Materials and Methods

The material used in this study is tooth paste containing xylitol was examined in vitro. The microorganisms tested were laboratory strains of mutans of Streptococcus mutans Ing-Britt, Streptococcus mutans LM7, Streptococcus mutans KPSK2, Streptococcus mutans JC2 and Streptococcus sobrinus B13.

In this case, those mutans streptococci are cultivated in TYS20B\(^5\), BHI (Brain Heart Infusion Broth) and diagnostic sensitivity test (DST) was performed. Those specimen are incubated in anaerobic jar at 37\(^\circ\) Celsius for 3 X 24 hours.

Working method

Sensitivity test to antibiotic can be done in two ways\(^6\):

I. Drug serial dilution method:

a. Making the bacteria culture medium

a.1. From the cultivated mutans streptococci in TYS20B, take one loop of bacteria and cultivate them in liquid culture medium BHI, then incubated it in anaerobic jar at 37 Celsius degree for 2 X 24 hours.

a.2. After 2 days, compare the turbidity of bacteria media culture of BHI with Brown III standard solution.

a.3. If it is found that bacteria culture in BHI media is more turbid, add sterile saline solution, little by little until the turbidity is equal to Brown III standard solution.

a.4. If the turbidity of bacteria culture medium is equal to Brown III standard solution, the number of bacteria cell/ml in bacteria culture medium can be counted, their number is \(\pm 9 \times 10^8\) bacteria/ml.

b. Bacteria dilution

If equalization process has been done, culture of mutans streptococci bacteria shall be diluted as follows:

b.1. Prepare 7 tubes of each 9 ml containing physiologic saline solution, and also prepare 1 tube containing 5 ml physiologic saline solution.

b.2. Take 1 ml bacteria from item a.4, the put into the first tube, shake them thoroughly from the first tube take 1ml bacteria culture and put into the second tube, and do the same thing through the seventh tube.
b.3 Take 5 ml of bacteria from the seventh tube and put them into the eight tube and shake it thoroughly.

b.4. The number of bacteria is estimated ± 50 cells/ml each tube.

c. Sensitivity test of bacteria to xylitol in xylitol toothpaste.

c.1. Prepare 5 sets of test tubes, each tube is filled with 9 ml BHI and is labeled 1 – 5 respectively.

c.2. Put 1 gram of enzyme’s tooth paste as much with 1 : 1 concentration into first tube then stir it well.

c.3. From the first tube, take 1 ml of solution and put into the second tube, do the same thing through the fifth tube.

c.4. After dilution finished, then put 1 ml of diluted Streptococcus mutans from item b.4 in those five test tube. All test tubes put in anaerobic jar at 37 Celsius degree for 2 X 24 hours.

C.5. After 72 hours, macroscopically we can see in which tube, the bacteria can not growth. Record the result to determine the Minimum Inhibition Concentration (MIC)

II. Method using disk with drug in solid media

II.1 Diluted 1 ml of mutans streptococci in the agar DST petri disc, the bacteria suspension wet the DST agar thoroughly.

II.2. Then put dilution of xylitol on a disk and put it on the surface of DST agar.

II.3. Those petri discs are incubated in anaerobic jar at 37 Celsius degree for 3 X 24 hours.

II.4. Inhibition zone will show around the disc and measuring the diameter of the isolated zone around the samples.

Data obtained is analyzed in a descriptive.

Resultas of the research

Table 1 Showed that The Minimum Inhibitory Concentration MIC is 10^{-3}/ml and MBC 10^{-1}/ml for Streptococcus mutans Ing-Britt, Streptococcus mutans LM7, Streptococcus mutans KPSK2, Streptococcus mutans JC2 and Streptococcus sobrinus B13

Table1. Results of serial dilution test of Streptococcus mutans to xylitol in xylitol toothpaste

<table>
<thead>
<tr>
<th>Type of bacteria</th>
<th>The concentration of Enzyme toothpaste (/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>01 (I)</td>
</tr>
<tr>
<td>S. mutans Ing-Britt</td>
<td>-</td>
</tr>
<tr>
<td>S. mutans LM7</td>
<td>-</td>
</tr>
<tr>
<td>S. mutans KPSK2</td>
<td>-</td>
</tr>
<tr>
<td>S. mutan JC2</td>
<td>-</td>
</tr>
<tr>
<td>S sobrinus B13</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>+ growing</td>
</tr>
</tbody>
</table>

C(+) Positive control without Xylitol toothpaste)
C(-) Negative control (with Xylitol toothpaste)
Table 2 The measurement of inhibitory zone which is carried out from the border of disc to zone with bacterial growth showed that Inhibitory zone of *Streptococcus mutans Ing-Britt, Streptococcus mutans LM7, Streptococcus mutans KPSK2, Streptococcus mutans JC2* and *Streptococcus sobrinus B13*

Table 2. The result on inhibitory zone measurement in bacterial growth of mutans streptococci on DST agar media.

<table>
<thead>
<tr>
<th>Type of Bacteria</th>
<th>Inhibitory zone in mm</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Streptococcus.mutansIng-Britt</em></td>
<td>2.00</td>
</tr>
<tr>
<td><em>Streptococcus.mutansLM7</em></td>
<td>2.10</td>
</tr>
<tr>
<td><em>Streptococcus. mutansKPSK2</em></td>
<td>1.60</td>
</tr>
<tr>
<td><em>Streptococcus.mutansJC2</em></td>
<td>1.50</td>
</tr>
<tr>
<td><em>Streptococcus.sobrinusB13</em></td>
<td>2.20</td>
</tr>
</tbody>
</table>

The average of inhibition of mutans streptococci :1.80

**Discussion**

From the results showed that laboratory strains of *Streptococcus mutans Ing-Britt, Streptococcus mutans LM7, Streptococcus mutans KPSK2, Streptococcus mutans JC2* and *Streptococcus sobrinus B13* are sensitive to concentration $10^{-1}$/ml xylitol of Xylitol toothpaste.

Inhibitory zone of *Streptococcus mutansIng-Britt* is 2.00 mm; 2.10 mm for *Streptococcus mutansLM7*; 1.60 mm for *Streptococcus mutansKPSK2*; 1.50-mm for *Streptococcus mutansJC2*; 2.20 mm for *Streptococcus.sobrinusB13*.

In the past study it had been proven that Xylitol in Xylitol toothpaste can inhibit the growth of the population on salivary mutans streptococci. 8,9

**Conclusion**

The result showed that Xylitol in Xylitol toothpaste has bacterial activity against laboratory strains of mutans of *Streptococcus mutans Ing-Britt, Streptococcus mutans LM7, Streptococcus mutans KPSK2, Streptococcus mutans JC2* and *Streptococcus sobrinus B13*. It is expected that it can be used in preventing caries risk in the future.

**References**

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