ATIMICROBIAL EFFECT OF GREEN TEA POLYPHENOL ON
STREPTOCOCCUS MUTANS, INVITRO

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Abstract
Tea is a traditional drink which is beneficial for our health, one of it's benefits is capability to control the process of dental caries, because it has active substance polyphenol. Polyphenol has property as antibacterial agent and as antiseptic. Polyphenol works by denaturing the protein bacteria and inhibiting glucosiltransferase enzyme of mutans streptococci. Objectives: The aim of the study is investigating ant-microbial effect of green tea polyphenol on mutans streptococci, in vitro. Methods: The subjects of this study were 9 colonies of local strains of mutans streptococci that have been isolated from plaques and labeled as: S. mutans1, S.mutans2, S. mutans3, S.mutans4, S.mutans5, S.mutans6, S.mutans7, S.mutans8 and S.mutans9. A sensitivity test was done by using a serial dilution method of Schaub et al, (1958) and the test microorganism in this research was Streptococcus mutans. The concentration of green tea polyphenol used research in this research was 0.1/ml, 0.01/ml, 0.001/ml, 0.0001/ml and 0.00001/ml. Results: showed that green tea polyphenol has bactericidal effect on the local strains of Streptococcus mutans at Minimum Inhibition Concentration (MIC) of 0.001/ml. Conclusion: Therefore it could be concluded that green tea polyphenol has bactericidal effect on local strains of Streptococcus mutans.

Key words: Green tea polyphenol, Streptococcus mutans

INTRODUCTION

Streptococcus mutans are, the prime cause of dental caries. They cause the dental caries by forming dental plaque1,2,3,4,5. Efforts to control dental caries in the community have been done with various methods, i.e with tooth brushing, gargling with antiseptics and fluoridation in the toothpaste.

Tea leaves of which we all know are commonly available in the market, actually contain active substance flour. Beside that, tea leaves also contain polyphenol compound. From medical dictionary we know that polyphenol is one of disinfectants which have antibacterial effect.

We still need further research to determine antibacterial property of polyphenol in tea leaves in controlling of dental caries. It needs to be done so the public as the consumers can consume medically accountable food and drinks.

OBJECTIVE OF RESEARCH

The objective of this research is determining the sensitivity of polyphenol in tea leaves to inhibit the local strains of Streptococcus mutans, in vitro.
METHOD OF RESEARCH

Material Research

Polyphenol in this research is an extracted substance from green tea, and the bacteria used as analysis unit are cultivated *Streptococcus mutans* bacteria.

The objective of this research is to determine the sensitivity of *Streptococcus mutans* to polyphenol. In this case *Streptococcus mutans* is cultivated in BHI (Brain Heart Infusion Broth) culture medium. The specimen were incubated in an anaerobic jar at 37°Celsius degree for 3 X 24 hours.

Working Method

Making the bacteria Culture Medium

1. From the cultivated *Streptococcus mutans* bacteria in TYS20B5 solid culture medium, determine bacteria colony which will be cultivated again.
2. After determining bacteria colony which will be taken, we take data of bacteria i.e their diameter, color, surface and edge. After that, we take one loop of bacteria and cultivate them in liquid culture medium, BHI. Then close the tube with cotton, and incubate it in anaerobic jar at 37°Celsius degree for 48 hours.
3. After 2 days, compare the turbidity of bacteria medium culture in BHI broth with Brown III standard solution.
4. If after bacteria culture in BHI broth is more turbid, add sterile physiologic salt solution little by little until the turbidity is equal to Brown III standard solution.
5. If the turbidity of bacteria culture medium is equal to Brown III standard solution, the number of bacteria cell/millimeter in bacteria culture medium can be counted, their number is ±9 X 10⁸ bacteria/millimeter.

Bacteria Dilution

If equalization process has been done, culture of Streptococcus mutans bacteria shall be diluted as follows:
1. Prepare 7 tubes containing physiologic salt solution, each contains 9 ml. Also prepare 1 tube containing 5 ml of physiologic salt solution.
2. Take 1 ml using pipette from main culture.
3. Put the bacteria into the tubes, respectively 1 ml and shake them thoroughly so the bacteria will spread out in physiologist salt solution.
4. Do the same thing through the seventh tube.
5. Take 5 ml of bacteria from the seventh tube and put them into the eight tube and shake them thoroughly so the bacteria will spread out in physiologist salt solution.
6. After the number of bacteria is estimated ±50 cells/ml each tube shall be labeled.
The Sensitivity test of Bacteria to Polyphenol.

1. Prepare 9 sets of test tubes, with each set consists of 5 test tubes. Each tube is filled with 9 ml of BHI and is labeled 1 - 5 respectively.
2. Put 1 ml Polyphenol extract as much with 1 : 1 concentration into first tube then stir it well.
3. From the first tube, take 1 ml of polyphenol extract and put it into the second tube, do the same thing through the fifth tube.
4. After the first set is completely done, do the same thing with the second set through the ninth set.
5. After dilution is finished, label each set of test tubes pursuant to type of bacteria cultivated in it.
6. Put 1 ml of diluted Streptococcus mutans into those five test tubes.
7. After all those processes have been completed, put them into anaerobic jar and incubate them for 72 hours.
8. After 72 hours, macroscopically we can see in which tube, the bacteria cannot grow. Record the result to determine the minimum inhibiting concentration.

RESULTS OF RESEARCH

The results of sensitivity test of Streptococcus mutans to polyphenol dilution Series, shows that specimens in several tubes IV begins to shows turbidity and all specimens in tubes V are fully turbid. But there is no turbidity at all in tubes number I.II and III

Table: Results of sensitivity test of Streptococcus mutans to polyphenol extract in green tea leaves

<table>
<thead>
<tr>
<th>TYPE OF BACTERIA</th>
<th>THE CONCENTRATION OF POLYPHENOL (/ml)</th>
<th>TOTAL MINUS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0,1 (I)</td>
<td>0,01 (II)</td>
</tr>
<tr>
<td>S.mutans1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>S.mutans2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>S.mutans3</td>
<td>-</td>
<td>-</td>
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<tr>
<td>S.mutans4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>S.mutans5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>S.mutans6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>S.mutans7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>S.mutans8</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>S.mutans9</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>TOTAL MINUS</td>
<td>9</td>
<td>9</td>
</tr>
</tbody>
</table>

+:Growing  - : Not Growing
From the table above shows that:

* All *Streptococcus mutans* strains are sensitive to Polyphenol with concentration 10^{-3}/ml - 10^{-4}/ml.
* *Streptococcus mutans1, Streptococcus mutans5, and Streptococcus mutans7*, are sensitive to Polyphenol with concentration 10^{-4}/ml.
* All *Streptococcus mutans* strains are not sensitive to Polyphenol with concentration 10^{-5}/ml.
* *Streptococcus mutans1* is sensitive to Polyphenol with concentration 10^{-1}/ml, 10^{-2}/ml, 10^{-3}/ml and 10^{-4}/ml is not sensitive to Polyphenol with concentration 10^{-5}/ml.
* *Streptococcus mutans2* is sensitive to Polyphenol with concentration 10^{-1}/ml, 10^{-2}/ml, 10^{-3}/ml and is not sensitive to Polyphenol with concentration 10^{-4}/ml and 10^{-5}/ml.
* *Streptococcus mutans3* is sensitive to Polyphenol with concentration 10^{-1}/ml, 10^{-2}/ml, 10^{-3}/ml and is not sensitive to Polyphenol with concentration 10^{-4}/ml and 10^{-5}/ml.
* *Streptococcus mutans4* is sensitive to Polyphenol with concentration 10^{-1}/ml, 10^{-2}/ml, 10^{-3}/ml and is not sensitive to Polyphenol with concentration 10^{-4}/ml and 10^{-5}/ml.
* *Streptococcus mutans5* is sensitive to Polyphenol with concentration 10^{-1}/ml, 10^{-2}/ml, 10^{-3}/ml, 10^{-4}/ml and is not sensitive to Polyphenol with concentration 10^{-5}/ml.
* *Streptococcus mutans6* is sensitive to Polyphenol with concentration 10^{-1}/ml, 10^{-2}/ml, 10^{-3}/ml and is not sensitive to Polyphenol with concentration 10^{-4}/ml and 10^{-5}/ml.
* *Streptococcus mutans7* is sensitive to Polyphenol with concentration 10^{-1}/ml, 10^{-2}/ml, 10^{-3}/ml, 10^{-4}/ml and is not sensitive to Polyphenol with concentration 10^{-5}/ml.
* *Streptococcus mutans8* is sensitive to Polyphenol with concentration 10^{-1}/ml, 10^{-2}/ml, 10^{-3}/ml and is not sensitive to Polyphenol with concentration 10^{-4}/ml and 10^{-5}/ml.
* *Streptococcus mutans9* is sensitive to Polyphenol with concentration 10^{-1}/ml, 10^{-2}/ml, 10^{-3}/ml and is not sensitive to Polyphenol with concentration 10^{-4}/ml and 10^{-5}/ml.

**DISCUSSION**

In this research, we use 9 strains of *Streptococcus mutans* (classified pursuant to their morphology) cultivated in BHI. The result shows:

1. That all strains of *Streptococcus mutans* are sensitive to concentration 10^{-3}/ml-10^{-4}/ml.
2. That *Streptococcus mutans1, Streptococcus mutans5 and Streptococcus mutans7* are sensitive to concentration 10^{-4}/ml.
3. That all strains of *Streptococcus mutans* are not sensitive to concentration 10^{-5}/ml.
Polyphenol in tea has anti-microbial effect that can inhibit the growth of *Streptococcus mutans*. The result above shows that only some strains of *Streptococcus mutans* are sensitive to polyphenol with concentration $10^{-4}$/ ml because of difference sensitivity of *Streptococcus mutans* to *Streptococcus mutans*. Polyphenol inhibits *Streptococcus mutans* by denaturing and inhibiting glucosiltransferase enzyme of *Streptococcus mutans*.

Tea with a Latin name of *Camellia sinensis* belongs to the genus *Camellia*. Most of them grow in the tropical region with an altitude of 200 to 2000 meters above sea level with atmospheric temperature 14 - 25 degree Celsius. Tea harvesting can be done after 5 years with a productive period of 40 years and after that it shall be replanted.

Tea leaf contains several chemical substances classified as: phenol substances, non-phenol substance, aromatic compound and enzyme. Two phenol substances in tea leaves according to Stahl and Bukuchava are tannin i.e. catekin with its gallat and non-tannin which consists of flavonols, protein, xanthon pigment, caroten pigment, vitamin, fat sterol. Wax cellulose and tigmin.

Aromatic compound that makes the taste of tea is aldehyde and unsaturated keton. Enzyme found in tea leaf are polyphenol oxidase, peroxidase protease, amylase and peptidase.

In dentistry, one of the most important effects of tea is that it can reduce caries incidence of drinkers and it can also help bringing back mineral onto enamel again, it is because of its flour contents.

Tea also has antibacterial effect on *mutans streptococci*, because of its polyphenol peroxidase enzyme or GTF contents, that inhibit formation of glucan from sucrose. It is this glucan that makes the bacteria stick to plaque.

Chinese Green Tea could be inhibited the growth of *mutans streptococci* after contacted with concentration of 0.1% CGT for 5 minutes.

The crude tea polyphenolic compounds from the leaf of *Camellia sinensis* were found to effectively inhibit the attachment of *Streptococcus mutans* strain JC-2 (serotype c) to saliva-coated hydroxyapatite.

**CONCLUSION AND SUGGESTION**

From the research we know that Polyphenol extract of Green tea has bactericide effect on *Streptococcus mutans* with Minimum Inhibiting Concentration (MIC) 0.001/ml.

Tea is a traditional drink from ancient time, but some people also use tea as traditional medicine to cure certain diseases. So generally tea is used as daily drink and for preventing some diseases.

Because tea contains Polyphenol antiseptic, so it also can be used as mouthwash. Inhibiting effect of Polyphenol can reduce the population of bacteria in oral cavity, so early infection can be anticipated. It's better to gargle the tea before swallowed.
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