Efficacy of Gargling the Green tea Solution to the Level of pH Saliva on Teenager Students Population in Deli Serdang District of Indonesia

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Abstract
Objectives: Green tea contains have antimicrobial, anticariogenic and therapeutic effects on several diseases. The goal of this research to determine the effect of gargling the green tea solution on salivary pH on teenage students population. This research is a Quasy experiment with a pre-post test randomized single blind control trial design.
Methods: Interventions include gargling green tea solution and measuring the pH saliva before and after intervention. The sample consisted of 60 subjects, divided into 3 groups consist of 20 respondents. Saliva was measured by a Hanna digital pH meter. Statistical analysis using Kruskal Wallis and Wilcoxon test. Significance is indicated by the value of p <0.05 with a confidence level of 95%.
Results: It showed that the data were not normally distributed (p <0.05). There was a significant difference between salivary pH before and after gargling green tea solution (p <0.05). Based on the Kruskal Wallis test, there was a significant difference between the control group and gargling with green tea solution group.
Conclusion: It was concluded that Gargling with 20 ml green tea solution more effective than 30 ml group. The suggestion was gargling with green tea solution was helpful to neutralize the saliva pH to inhibits the process of dental caries.
Keywords: Green Tea, Saliva pH, Gargling.

Introduction
Saliva is a biological liquid in the oral cavity which is a mixture of major and minor salivary gland secretions with the composition of organic, inorganic and macromolecular materials (Fabian et al, 2007) Saliva is an important factor to maintain mineral balance and oral health (Almeida et al, 2008; Wu et al, 2008)). The condition of saliva affects the formation of plaques and tartar, especially against supragingival tartar (Jin and Yip, 2002).

Over time, studies about green tea has the potential to prevent dental and oral diseases. Green tea contains catechins (polyphenol compounds) which have antimicrobial, anticariogenic and therapeutic effects on some diseases (Taylor, 2005). Green tea is also proven effective against periodontal disease, oral cancer, halitosis and preventing dental caries. Various benefits of green tea can not be separated from the presence of beneficial compounds such as polyphenol, theophylline, tannin, catechins, and...
a number of minerals such as Zn, Se, Mo, fluoride, minerals that can prevent inflammation of the gums and cavities. Polyphenols may reduce plaque and acid production by oral bacteria that cause caries and gum disease. Green tea is also proven effective against periodontal disease, oral cancer, halitosis and preventing dental caries (Arab et al., 2011). Every milliliter of saliva 10-200 million bacteria are found, one of which is Streptococcus Mutans (Tarigan, 2013). Streptococcus mutans bacteria that multiply will cause the formation of plaque in the enamel layer and will cause the pH of the oral cavity to decrease, become acidic, while reducing the Streptococcus mutans bacteria in the oral cavity causes to become alkaline and even neutral. The lower of the pH value of saliva, the more acid in the solution. Conversely, the increase in salivary pH value based on the background of green tea containing catechin compounds, where this substance plays a role in inhibiting the growth of streptococcus mutans.

The aim of this study was to determine the effect of gargling with green tea solution on salivary pH in teenage students population in deli serdang district of Indonesia and to find out the difference in average salivary pH difference before and after gargling 20 ml and 30 ml green tea solution.

Methods
1.1 Research Design
This research is a Quasy experiment with a pre-post test randomized single blind control trial design (Arikuntoro 2006). In this study the intervention was gargling the green tea solution and measured saliva pH before and after intervention. In this study the sample consisted of 60 subjects, which divided by simple random sampling using random number tables, and divided into 3 groups consist of 20 people.

1. Group I: The untreated (control) group who was given drinking water by blind coloring according to the color of green tea to disguise the respondent.

2. Group II: The treatment group gargling with 20 ml green tea solution.

3. Group II: The treatment group gargling with 30 ml green tea solution.

1.2 Research Locations
This research was carried out in several locations. Data was collect by oral cavity examination and taking the saliva while salivary pH measurements were carried out in the Laboratory.

1.3 Research Population and Samples
The population is all teenage students with total 400 peoples. Sampling was done by consecutive sampling method according to inclusion and exclusion criteria. The inclusion criteria were willing to fill out informed consent, was not in sick condition, cooperatively, the composition of the teeth were not crowded or the teeth were lightly packed and carious teeth had a maximum of 2 teeth, while the exclusion criteria were not in orthodontic treatment or prosthetic use.

1.4 Research Instrument
Instruments used were pH meters, collecting pot saliva, diagnostic set (windshield, tweezers, near becken), check sheets, stopwatches, ice boxes, water thermometers, green tea solution, masks, gloves, aquades, and ice gels.

1.5 Measurement
The primary data is the salivary pH data that has been taken by the direct-mouth examination technique of the students while the secondary data is the data needed as a complement in the research obtained from the school, namely data on the number of students.

1.6 Implementation Research
Preparation stage: Make a green tea solution with a volume of 20ml and 30ml. Green tea solution was made using green tea dipping as much as 8gr (4 bags) with 320 ml of distilled water so that a 2.5% green tea solution obtained by brewing process carried out at the optimum temperature of 70 - 80°C so that the levels of polyphenols in green tea are not reduced (Nubatonic et al., 2016). Every 20 ml of green tea solution is made from mixing green tea with a concentration of 2.5% plus 17.5 ml of distilled water, while every 30 ml
of green tea solution is made from mixing green tea with a concentration of 2.5% and added 27.5 mL of distilled water. The control solution was made from mixing 30 mL of distilled water mixed with green food coloring until the color was similar to the color of the green tea solution.

Phase:
1. Early Saliva is carried out 1 hour after consumed food snacks as usual. After 30 minutes of resting hours the patient is not allowed to eat and drink. Respondents were instructed to sit in an upright position with their heads slightly bent forward, to help collect saliva in 5 minutes. Respondents were asked to spit out saliva into the collecting pot saliva for 5 minutes until 1-2 mL of saliva was obtained.
2. The collected saliva is stored in a closed pot container which is labeled (with sample code for each different group), stored in ice box filled with ice gel, and immediately taken to the laboratory.
3. Gargling green tea solution (20 ml, 30 ml, control) is done for 30 seconds strongly by sucking fluid between the teeth, around the mouth with movement of the muscles of the lips, tongue, and cheeks closed.
4. Measuring stage: Saliva pH is measured using a pH meter until all pH meter electrodes are submerged in saliva. Then the salivary pH is recorded on the data sheet provided.

1.7 Data analysis
If the data is normally distributed (Saphiro-Wilk test) so statistical analysis is used a one-way ANOVA test to compare each parameter between the three intervention groups, while the dependent t test to compare before and after intervention in each group. Significance is indicated by the value of p <0.05 with a confidence level of 95%.

Result
The data were tested for normality by the Shaphiro-Wilk test, shows that the data is not normally distributed, as presented in table 1 below.

Table 1. Normality test of Saliva pH

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre (Before)</td>
<td>I</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>20</td>
</tr>
<tr>
<td>Post (After)</td>
<td>I</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>20</td>
</tr>
</tbody>
</table>

*=Homogeneous data (p>0.05)

Table 1 shows that data is not normally distributed (p <0.05) so the data was tested using nonparametric test, the Wilcoxon test, and the Kruskal Wallis test.

Table 2. Baseline

<table>
<thead>
<tr>
<th>Variable</th>
<th>I (20ml)</th>
<th>II (30ml)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>Mean± SD</td>
<td>Mean± SD</td>
<td>Mean± SD</td>
</tr>
<tr>
<td></td>
<td>6.9 ± 0.48</td>
<td>6.76 ± 0.38</td>
<td>6.9 ± 0.32</td>
</tr>
</tbody>
</table>

The results of statistical tests in table 2 show that the initial conditions of salivary pH before intervention in each group did not different (p >0.05). It means that the initial conditions of all respondents are the same (homogeneous). Based on the table above the initial average value of saliva pH of the three groups lest than 7 which shows the acidic conditions.

Table 3. Average Saliva for each group (based on the Wilcoxon test)

<table>
<thead>
<tr>
<th>Saliva pH</th>
<th>n</th>
<th>Mean± SD</th>
<th>Δ</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group Before</td>
<td>20</td>
<td>6.9 ± 0.48</td>
<td>0.35</td>
<td>0.072</td>
</tr>
<tr>
<td>After</td>
<td>20</td>
<td>6.95 ± 0.51</td>
<td></td>
<td></td>
</tr>
<tr>
<td>II (20 ml) Before</td>
<td>20</td>
<td>6.76 ± 0.38</td>
<td>0.68</td>
<td>0.000*</td>
</tr>
<tr>
<td>After</td>
<td>20</td>
<td>7.32 ± 0.37</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III (30 ml) Sebelum</td>
<td>20</td>
<td>6.90 ± 0.32</td>
<td>0.61</td>
<td>0.000*</td>
</tr>
<tr>
<td>Sesudah</td>
<td>20</td>
<td>7.63 ± 0.30</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*=Significance value : p<0.05

In Table 3 show that there is a significant difference between salivary pH before and after gargling green tea solution (p <0.05) in groups II and III, but salivary pH after gargling green tea in the control group did not significant difference. The average difference between before and after the intervention in the three groups most occurred in group II. It means that group II has the biggest
change in salivary pH increases compared to groups III and I.

**Table 4.** Post intervention

<table>
<thead>
<tr>
<th>Variable</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saliva pH</td>
<td>6.95 ± 0.51</td>
<td>7.32 ± 0.37</td>
<td>7.63 ± 0.30</td>
<td>0.000*</td>
</tr>
<tr>
<td>Δ</td>
<td>0.35</td>
<td>0.68</td>
<td>0.61</td>
<td>0.000*</td>
</tr>
</tbody>
</table>

Based on table 4 above shows there is significant differences in the average salivary pH after intervention (p<0.05). Based on the Kruskal Wallis test also found the average difference in salivary pH of the three groups was also significantly different. Salivary pH values of the three groups included baseline criteria (greater than 7) but among the three groups, groups II and III were more basic than the control group.

**Discussion**

Based on the Wilcoxon test showed there were significant differences between salivary pH data before and after gargling green tea solution (p <0.05), so it can be said that gargling with a green tea solution has an effect on changes in salivary pH. Based on the Kruskal Wallis test, the treatment group produced a better increase in salivary pH than the control group. In group II the average difference in increase in salivary pH was 0.68, higher than the average difference in group III (0.61); whereas in the average control group the difference in increase in salivary pH was smaller than group II and III. It means that 20 ml of green tea has more influence on salivary pH than the 30 ml green tea solution group. Based on the Kruskal Wallis test it was found that there were significant differences in the mean differences between the three groups (p <0.05). An enhancement salivary pH happen because of the salivary secretion. This causes an increase in bicarbonate ions so that the salivary pH will increase. Increased salivary secretion can occur due to mechanical and chemical stimulation of the salivary gland and from stimulating the steeping content of green tea, which is bitter. This is in accordance with Permatasari’s study (2011), which showed an increase in salivary secretion in the gargling group of green tea due to mechanical stimulation of the salivary glands (gargling). As a result, salivary pH in the treatment group increased significantly compared to the control group.

Green tea has a polyphenol compound which consists mostly of catechins. Catechins from green tea work to inhibit the activity of glycotransferase enzymes, thus inhibiting the attachment of bacteria to the pellicle and the process of plaque formation is also hampered (Tehrani et al., 2011). Polyphenols also work to inhibit the growth of microorganisms because they have the ability to denaturate cell proteins and damage cell membranes of microorganisms on dental plaque (Anggayanti et al., 2013). Research conducted by Wijaya and Samad (2004) found that green tea solution has the ability to inhibit the development of Streptococcus mutans with a minimum concentration (MIC) is 2.5%. Research conducted by Awadala (2011) states that gargling with a solution of unsweetened green tea can inhibit the growth of Streptoccocusmutans bacteria on plaque and saliva which are the main bacteria that cause dental caries. Another study of green tea conducted by Fajriani (2014) states that gargling with 2.5% of green tea solution has the same effectiveness as 0.2% chlorhexidine in reducing Streptococcus mutans colonies in saliva in vitro.
Salivary pH is influenced by buffer capacity, average salivary rate, and oral microorganisms. Salivary pH at 6.5-7.5 is the optimal for bacterial growth and oral pH at 4.5-5.5 can facilitate the growth of acidogenic bacteria such as Streptococcus mutans and Lactobacillus. Green tea contains catechins (polyphenol compounds) which are proven to have antimicrobial, anticariogenic and therapeutic effects on several diseases. Green tea is also proven effective against periodontal disease, oral cancer, halitosis and preventing dental caries.

**Conclusion**
The average difference in salivary pH of the three groups was significantly different. Gargling with 20 ml green tea solution is more effective than 30 ml green tea solution to neutralize salivary pH.

**Competing Interests Statement**
The author declare that there are no competing or potential conflict of interest regarding the publication of the paper.

**Acknowledgment**
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**Conflict of Interest**
The authors involved in this research declare no conflict of interest.

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