

In-vitro study investigating influence of toothpaste containing green tea extract on the microhardness of demineralized human enamel

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Abstract. The aim of this study was to analyze the influence of toothpaste containing green tea extract on the microhardness of demineralized enamel. Human tooth, which was demineralised in citric acid solution, had a toothpaste containing green tea extract of concentration of 5, 10 or 15% application. Microhardness measurement was carried out on each enamel surface of the teeth for initial, after the demineralization and after application of the tooth pastes. It showed that there was significant decrease between enamel microhardness of the teeth at the initial condition and after demineralization. After application of the toothpaste containing green tea extract of each concentration the microhardness increased significantly. However, there the microhardness was insignificant between the applications of each green tea concentration.

1. Introduction

Caries are the most prevalent disease in the world [1]. Caries prevalence in developing countries has increased up to 90% [2]. Based on the Basic Health Research of the Health Department of Indonesia (2007), 72.1% of Indonesians suffered from caries and 46.5% of these could have been prevented [3] by toothpaste containing fluoride [4].

Toothpaste containing fluoride can prevent caries by increasing the enamel microhardness that make the enamel more resistant to the demineralization process. The demineralization process decreases the minerals contained in the enamel, thereby reducing its microhardness [5-7]. Enamel microhardness can be increased by using fluoride, which is one of the minerals found in green tea.

Based on research by Zerabruk *et al* (2010), 100–430 mg/kg of fluoride can be found in tea leaves [8]. Green tea in toothpaste can be used to prevent caries by increasing enamel microhardness. Unlike in Taiwan and China that have toothpastes containing green tea, to date, Indonesia has not produced toothpaste containing green tea.

Most teas in Indonesia grow in the highlands in West Java, such as Puncak, Sukabumi, Pangalengan, Ciwidey, and Subang. Per capita tea consumption in Indonesia escalated from 1997 to



2003 [9]. The escalation of tea production shows that the amount of tea consumed by Indonesians is growing.

According to previous work, we assumed a one-time brushing for in vitro research took 15 seconds [13]. Therefore, in one day, it took 30 seconds to brush teeth. In a month, it took 900 seconds (15 minutes) of brushing, and in 6 months it took 5,400 seconds (90 minutes) of brushing. A 6-month application was based on the literature, which stated that 6 months were needed to have a fluoridation effect against caries [14]. This showed that the use of green tea in toothpaste may be a good option for preventing caries. The aim of this study was to determine the influence of toothpaste containing green tea on enamel microhardness.

2. Materials and Methods

Extraction of green tea was carried out by grinding dried green tea leaves and immersing them in aquadest 70 °C in 15 minutes. After a colorless solution was obtained, the green tea extract was dried to remove any remaining water. A toothpaste was prepared by mixing 90% calcium carbonate, 5% glycerin, and *sapo medicatus*, and toothpaste containing green tea was made by adding 0, 5, 10, or 15% of green tea extract into the toothpaste prepared. Toothpaste solutions with and without the green tea extract were obtained by dissolving the paste using aquabidestilate with a ratio of 1:2 (g:g). The first stirring was done manually. Subsequent stirrings were carried out using a magnetic stirrer to form a homogenous paste solution.

After approved by the Ethics Commission of the Faculty of Dentistry, University of Indonesia, a total of 16 extracted human premolar teeth with no caries and not cracked at the buccal surfaces were used as specimens. They were washed with water and immersed in a 0.9% NaCl solution. The teeth were then cut into two parts using a special tool for cutting teeth (Struers Accutom-2[®], Denmark). The crown part was planted in a decorated resin. The enamel was sanded, and polished using a sanding and polishing tool (Struers LaboPol-21[®], Denmark) to obtain a flat surface. The enamel was tested using a microhardness tester (Shimadzu Micro microhardness Tester HMV-2 series, Japan), with a load of 50 grams for 5 seconds [10]. After an initial enamel microhardness level was obtained, each specimen was immersed in a 1% citric acid solution (pH = 4) for 150 seconds to produce demineralized enamel [11-12] and followed by microhardness measurement. Each groups of 4 was applied with the toothpaste solution and the toothpaste solution containing green tea extract of 5%, 10% or 15%, as group A, B, C, and D, respectively.

Application of the toothpaste was performed using an orbital shaker for 90 minutes to prevent precipitation of the toothpaste. After the toothpaste with and without green tea extract was applied to the teeth, the enamel microhardness was tested by using the same tool to calculate enamel microhardness with a load of 50 grams for 5 seconds.

3. Results and Discussion

3.1 Results

The results of this research show the changes in enamel microhardness values involved decreasing and increasing the enamel microhardness as was shown in Table 1.

Table 1. Human enamel microhardness at the initial, after demineralization and after the application of the tooth pastes.

Green Tea Groups	Green Tea Concentrations	Enamel microhardness Value \pm SD (KHN)		
		Initial	After demineralization	After application with toothpaste containing green tea
A	0%	425.5 \pm 4.43	345.75 \pm 16.58	404.75 \pm 9.21
B	5%	418.5 \pm 9.11	338.75 \pm 3.5	352.00 \pm 5.71
C	10%	432.25 \pm 25.96	337.75 \pm 7.67	364.00 \pm 7.25
D	15%	438.75 \pm 8.05	332.00 \pm 7.61	359.75 \pm 4.99

The enamel microhardness of all specimens after demineralization decreased and then increased following the application of the tooth pastes.

Group A had a decrease in enamel microhardness of 18.7% after being treated with demineralization using a citric acid and increased by 17.06% after being treated with a remineralization using a toothpaste without a green tea extract. In group B, the decrease after demineralization was 19.05% in the enamel microhardness after demineralization an increase by 3.76% occurred with remineralization using toothpaste containing a 5% green tea extract. In group C, the decrease after demineralization was 21.8% and the increase was 7.77% after a remineralization using toothpaste containing a 10% green tea extract. In group D, a decreased by 24.3% revealed in enamel microhardness after demineralization an increased by 8.36% after remineralization using toothpaste containing a 15% green tea extract was seen.

One-way ANOVA indicated statistically significant differences ($p < 0.05$) among the enamel microhardness of all specimens. The Tukey post-hoc test found the significant differences ($p < 0.05$) were found between each microhardness values, with the exception of the difference between the enamel microhardness of those demineralised and remineralized by the toothpaste containing a 5% green tea extract, between the enamel microhardness of those demineralised and remineralized by the toothpaste containing a 10% green tea extract, and between the enamel microhardness of those demineralised and remineralized by the toothpaste containing a 15% green tea extract ($p < 0.05$).

3.2 Discussion

Alterations in enamel microhardness were observed in this study included both the decreases and increases in enamel microhardness. This result is in agreement with a previous study showing that immersion in citric acid liquid (in vitro) with a pH of 4 for 140 seconds was similar to a 40-minute exposure of the enamel to orange juice (in situ). After this treatment, enamel microhardness was significantly decreased after the citric acid application [6]. Citric acid is widely contained in soft drinks and has a high potential for producing demineralization [11]. Attin *et al* (2005) and Lussi *et al* (1995) reported that citric acid levels can be assumed to be potentially high through daily drinks, resulting in demineralization.

The decrease in enamel microhardness in the current study was presumed to be because the citric acid dissolved the hydroxyapatite in the enamel; thereby minerals within the enamel were decreased. The more minerals that are dissolved the lower the enamel microhardness. Based on the results, the decreased amount of minerals in the enamel was presumed to cause a decrease in enamel microhardness following immersion of the specimens in a 1% (pH = 4) citric acid solution for 150 seconds. The demineralization process affects minerals in the enamel, especially calcium and phosphate. Citric acid binds to calcium on the enamel surface, thereby causing hydroxyapatite to dissolve [11-12].

The increase in enamel microhardness in the current research occurred with the application of each toothpaste with and without green tea extract. This was probably due to calcium carbonate content in the toothpaste. A study by J.A. Cury (2005) [16] reported that increased enamel microhardness was shown by toothpaste containing calcium carbonate and sodium monofluorophosphate increased remineralization. The use of calcium carbonate in toothpaste was as a buffer, so it could neutralize the acidic pH caused by carbohydrate fermentation. Under normal pH conditions, remineralization occur. The calcium and phosphate ions that are dissolved in a demineralization process are then replaced in the remineralization process. When calcium and phosphate ions are rebound, hydroxyapatite crystals are formed to cover the demineralization area and this help to increase enamel microhardness.

The increase in enamel microhardness occurred with the application of each toothpaste containing green tea extract of 5%, 10%, or 15% was presumed to be due to fluoride content in the green tea extracts used in this study. Samuel Zerabruk *et al* showed that 100–430 ppm fluoride was found in tea leaves [8]. Based on findings by Jie Wei *et al.*, fluoride has been proven to increase enamel microhardness by inducing remineralization through its chemical binding with hydroxyapatite, which forms fluoroapatite [15]. Fluoroapatite was formed by fluoride ions reacted with free Ca^{2+} and HPO_4^{2-} ions. Furthermore, fluoroapatite was more difficult to dissolve below a pH of 4.5 compared to hydroxyapatite.

No significant difference among the enamel microhardness applied with 5, 10 or 15% green extract showed that low concentration of fluoride in the green extract was enough to a formation of fluorapatite in the enamel. This provides information for dental practitioners about the benefits of toothpaste containing green tea for dental health, especially its effect on enamel microhardness following demineralization in tooth caries. Further study, however, is needed to explore a possibility of using lower green tea extract concentration below 5% in a toothpaste.

4. Conclusion

In conclusion, toothpaste containing a up to 15% green tea extract significantly promoted remineralization of a demineralized enamel and showed significantly higher microhardness value. However, there was no significant difference between the effects of each green tea extract with concentration of 5, 10 and 15% on enamel microhardness.

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