Original Article

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Comparison between the Effects of Green and Black Tea, and Fluoride on Microhardness and Prevention of Demineralization of Deciduous Teeth Enamel

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ABSTRACT

BACKGROUND AND OBJECTIVE: Dental caries in preschool children impose serious problems on parents and children due to lack of cooperation. Since tea leaves contain fluoride and polyphenol, this study was conducted to compare the effects of green and black tea, and fluoride in preventing demineralization of deciduous teeth enamel.

METHODS: This experimental study was performed on 40 extracted human deciduous incisors. The teeth were divided into 4 groups of 10 and were then placed in distilled water, green tea, black tea and oral sodium fluoride (0.05%) for four minutes in seven consecutive days. Surface microhardness before and after tooth extraction was determined in these four solutions by Micro Vickers Hardness Testing Machine. To cause primary caries, each sample was kept for one week in demineralization solution and the concentrations of the released calcium, phosphorus and fluoride ions were analyzed by spectrophotometric and potentiometric laboratory methods.

FINDINGS: Primary microhardness showed no significant difference between the groups. Secondary microhardness compared to primary microhardness in the two groups of green tea (mean of 317 ± 65.47 to 386 ± 85.42, p = 0.003) and fluoride (mean of 319 ± 71.76 to 446 ± 117.15, p = 0.003) increased significantly. The concentration of calcium and phosphorus ions in the four study groups showed a significant difference after being placed in the demineralization solution (p = 0.001) (p = 0.003). However, this difference was not observed in fluoride concentrations.

CONCLUSION: The results of this study showed that drinking green tea can increase the microhardness and green and black tea increase the resistance to demineralization of the deciduous teeth enamel.

KEY WORDS: *Enamel, Microhardness, Green Tea, Black Tea, Demineralization.*

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Introduction

Dental caries is one of the causes of disability in society, which is associated with a decline in quality of life and direct and indirect costs, such as expensive treatments and disability in access to work and education (1). Caries initially begins by decalcifying the inorganic part of the tooth and then continues with the destruction of the organic matrix, leading to progressive demineralization in the tooth structure (2). Many studies have investigated the differences between erosion in deciduous teeth enamel and erosion in permanent teeth enamel, and their results showed that due to the lower mineralization of the deciduous teeth enamel in comparison with the permanent teeth and lower density of minerals at their outer layer, they are more prone to erosion and demineralization (3). Today, the use of tea and medicinal plants has grown steadily due to fewer side effects than chemical drugs in the treatment of many diseases (4). Tea is a product that is affordable and inexpensive and has a lot of home use, while its properties have been known since 4,000 years ago (4, 5). Based on the processing method in the factory, it is possible to divide the kinds of tea into three categories. In non-fermented tea (green tea), the oxidation process of polyphenols is not performed. In semi-fermented tea (oolong tea), the oxidation process of polyphenols is limited (6). In fermented tea (black and red tea), the oxidation process of polyphenols is completely carried out (6). Green tea is fermented for a short time and has plenty of polyphenols, while black tea is fermented for a longer time and more catechin is decomposed (5). Tea leaves are rich in fluoride and other components such as polyphenols (catechins) that play an important role in the resistance of teeth (7). With high levels of fluoride and organic compounds, tea prevents bacterial activity and results in enamel remineralization (8). Barbosa et al., and Denise et al. concluded in their study that consuming beverages that contain green tea extract could reduce teeth erosion (9, 10). Studies have shown that green tea can protect against various oral diseases such as dental caries, gingivitis, periodontitis, halitosis and oral malignancy, inflammation from smoking, and dentin erosion (11). Since the treatment of teeth in young children is difficult and costly, and many of the chemicals used to prevent caries are not prescribed in young children or should be used with caution, the use of substances that have less side effects, and are cheap and accessible can be useful. So far, there has been no research on the effect of tea extract on microhardness and the prevention of demineralization of deciduous teeth enamel. Therefore, this study was conducted to compare green and black tea and sodium fluoride mouthwash in preventing demineralization of enamel in dental caries.

Methods

This experimental study was approved by the Ethics Committee of Babol University of Medical Sciences (with the code of mubabol.rec.1395.226), and was conducted on 40 human anterior deciduous incisors (consistent with other studies) through convenience sampling method from healthy teeth without decay or cracks and the teeth that were extracted due to lack of space or being loose, while they were extracted not more than three months ago (12). Immediately after extraction, the teeth were placed in normal saline 0.09% (DarouPakhsh Co. Tehran, Iran) at room temperature and was replaced daily, and for the sake of disinfection, they were kept in a solution of Chloramine – T 1% (Sigma, Sweden) for one week. The teeth were cleaned and the crown was detached from the cementoenamel junction by disc (D & Z, Germany). Prior to hardness testing, the teeth were mounted in clear polyester casting resin, so that the lingual surface of the crown was immersed in the mold containing polyester content to the extent that only the labial surface of the crown was placed outside of the polyester. Because the intact surface enamel has a higher fluoride content and is more resistant to acids than the underlying layers (13), it was attempted to minimally polish using silicon carbide papers 800, 1000, 1500, so that we can see a nearly flat, scratch-free surface below the optical microscope. A 2 x 2 mm window was then placed on the enamel on the buccal side of the tooth, so that the rest of the surfaces were covered with a protective lacquer.

In order to calculate the microhardness of samples, Vickers Hardness Testers (MH1, Coopa, Iran) were used in this study. The initial surface microhardness of the samples was calculated at three points so that the force applies by the tip of the indentor was 50 g and the time of applied force was 10 seconds (14). After applying the force by rotating the microscope with magnification of 10, the instrument read the effect of the diameter and was entered into the control unit to calculate the microhardness. The mean of the three obtained figures was recorded as the initial hardness of each sample in terms of VHN (Vickers hardness number). The samples were then randomly divided into 4 groups of 10. Two groups were selected for testing the

solutions; the first group was spring green tea and the second group was spring black tea obtained from the Refah Company in Lahijan, Iran. Each tea solution was prepared by boiling 2 grams of leaves dried and crushed in 100 milliliters of non-ionized water for 30 seconds. Two other remaining groups included positive control solution containing sodium fluoride (NaF = 0.05%) (Behsa, iran) and negative control group (non-ionized water). The teeth in each group were immersed in 20 ml of solution for 4 minutes and then washed in a deionized water of 37 °C and held in deionized water and incubator (LTE SCIENTIFIC LTD, UK) until the next day. This process was repeated for 7 days with fresh solutions. The microhardness of each sample was repeatedly measured and recorded in the same manner after 7 days by the Vickers device. In order to produce primary carious lesions, each sample was kept in 25 ml demineralizing solution with pH = 4.4 at 37 °C for one week. The demineralizing solution contained 2.2 mM CaCl2, 2.2 mM KH2PO4, 50 mM acetic acid and 1 M KOH (15). The concentration of calcium and phosphorus ions released in the demineralizing solution was evaluated by spectrophotometry in a soil lab. The concentration of fluoride ion was also measured using the potentiometric method (ZAG CHEMIE CO., IRAN) in the chemistry laboratory of the Faculty of Chemistry, Mazandaran University, Babolsar. The results were statistically analyzed by ANOVA, paired T Test, Mann-Whitney Test and Kruskal-Wallis, Post Hoc Tukey Tests, while $p < 0.05$ was considered significant.

Results

Primary microhardness was close between all groups and did not have a significant difference. After performing the interventions, secondary microhardness increased significantly in both groups of green tea (p=0.003) and sodium fluoride (p=0.003), while in the other two groups (distilled water and black tea), the primary and secondary microhardness of the teeth were not significantly different (Table 1).

There was a significant difference in the secondary microhardness between groups (p=0.11). The secondary microhardness of the sodium fluoride group was significantly higher than that of distilled water and black tea. After calculating Diff and comparing the four groups, there was a significant difference between the groups (p=0.005). There was a significant difference between the two groups of green tea and sodium fluoride compared to black tea and distilled water. The concentration of calcium and phosphorus ions and fluoride released in the demineralizing solution is given in Table 2.

Based on ANOVA, the concentration of calcium and phosphorus ions in the studied groups showed a significant difference. However, this difference was not observed in fluoride concentration (Table 2). In the Tukey analysis between groups, the concentration of calcium after one week of being placed in the demineralizing solution in the distilled water group was significantly higher than the rest of the groups (p <0.001), while there was no significant difference in calcium content between other groups.

The concentration of phosphorus released in the demineralizing solution in the distilled water group was significantly higher than that of the sodium fluoride group (p=0.001), while this difference was not observed among the rest of the groups. The concentration of fluoride ion in the studied groups did not show any significant difference.

Table 1: Mean, standard deviation and Diff of primary and secondary microhardness of teeth

Variable	Primary microhardness (kg / mm^2) Mean ± SD	Secondary microhardness (kg / mm^2) Mean ± SD	Diff	P- value$^{\zeta}$
Distilled water	315.70 ± 10.40	320.82 ± 90.70 a	5.80±8.34 a(11)	0.505
Black tea	318.49 ± 40.61	333.51 ± 00.83 a	14.60±6.67 a(7)	0.057
Green tea	317.65 ± 20.47	386.85 ± 90.42 ab	67.70±17.56 b(71.50)	0.003
Sodium fluoride	319.71 ± 80.76	446.117 ± 10.15 b	126.30±31.43 b(118.50)	0.003
P-value†	0.999	0.011	⍰ 0.005	--

* The same letters in each column indicate no significant difference between the groups. The numbers in the table are standard deviation ± mean.

ζ: Paired t-test results; ⍰: Kruskal-Wallis statistical test; † : ANOVA test.

Table 2: The concentration of calcium and phosphorus ions and fluoride released in different groups after placement in the demineralizing solution

Variable	Fluoride (ppm) Mean ± SD	Phosphorus (ppm) Mean ± SD	Calcium (ppm) Mean ± SD
Distilled water	0.0 ± 04.004	9770.223 ± 40.01 a	271.23 ± 30.77 a
Black tea	0.0 ± 064.046	9586.195 ± 00.55 ab	193.16 ± 60.73 b
Green tea	0.0 ± 0.65.029	9606.199 ± 00.27 ab	191.48 ± 90.81 b
Sodium fluoride	0.0 ± 0.61.029	9416.147 ± 50.64 b	197.41 ± 10.57 b
P-value	0.634	0.003	< 0.001

* The same letters in each column indicate no significant difference between the groups. The numbers in the table are standard deviation ± mean.

Discussion

In the present study, secondary microhardness increased significantly in both groups of green tea and sodium fluoride compared to primary microhardness; while in the other two groups, (distilled water and black tea), the primary and secondary microhardness of the teeth were not significantly different. These results can be justified by the presence of fluoride in the sodium fluoride group and higher percentage of compounds such as fluoride and polyphenols, including catechins in green tea compared to black tea (12).

A study by Jose et al. showed that green tea and white tea extract increased the microhardness of dentin (16). In a study by Barbosa et al. on forty permanent human anterior teeth, it was concluded that consuming beverages containing green tea extract could reduce teeth erosion (9). Khamverdi et al., in their study of 100 premolar teeth, found that green tea varnish significantly reduced the tooth decay around orthodontic brackets (17). Denise et al. in their study showed that green tea extract reduces the erosion and roughness caused by erosion of permanent teeth (10). Tahani et al. in their study showed that green tea can have a positive effect on oral health by reducing the incidence of caries and periodontal disease (4).

In our study, contrary to the study of Rezaei et al., which was done on 50 permanent premolar teeth, there was no significant difference in the level of secondary microhardness of green tea and sodium fluoride mouthwash, while according to Rezaei et al., The effect of green tea on decay rate was significantly less than fluoride and chlorhexidine mouthwashes and did not have a significant difference with normal saline (18). This difference can be attributed to the fact that our study used green tea leaves, while in their study, the green tea polyphenol extract was used, indicating the presence of other substances in the structure of green tea except polyphenol, which requires further study in future studies. Second, in that study, the teeth were placed one day in a solution of the polyphenol extract, while in our study teeth were kept daily in boiled tea. Third, this study was conducted on deciduous teeth, contrary to the study of Rezaei et al.

Our study showed that the concentration of calcium ion in green and black tea groups and sodium fluoride mouthwash was significant compared to the distilled water group, while the difference in phosphorus ion concentration was observed only between sodium fluoride and distilled water, and there was no significant difference in concentration of fluoride ion between the four groups. In a study by Yu et al., it was concluded that, in addition to fluoride, organic tea components increase acid resistance in teeth enamel, and the fluoride in tea prevents calcium from being released in acidic solutions (19).

Rukhosh et al. in their study showed that green tea had the best effect on remineralization of teeth, while black tea, mentha spicata tea and ocimum basilicum tea were respectively the most effective on the remineralization of early caries of enamel. The chemical analysis also showed that in the two types of mentha spicata tea and ocimum basilicum tea, the amount of released calcium and phosphorus ions was higher than green tea and black tea, and green tea had the lowest levels of fluoride and phosphorus compared to two the groups of mentha spicata tea and ocimum basilicum tea significantly (8).

In our study, the amount of phosphorus released after being placed in the demineralizing solution in the green and black and sodium fluoride group was lower than that of distilled water, but the difference was significant only between sodium fluoride and distilled water. It is recommended that further studies be conducted to clarify the cause of this difference. The

amount of fluoride released in demineralizing solution in different groups was not statistically significant compared to distilled water. This may be caused by the presence of fluoride in the composition of the materials used in the case groups, which lead to the release of absorbed fluoride after being placed in the acidic medium, otherwise the enamel would be degraded more. Green tea consumption can increase the microhardness of the enamel of children's teeth, and green tea and black tea can reduce their demineralization. Therefore, green tea and black tea can be used as a drink and a proper mouthwash solution for children to prevent tooth decay. However, further studies are recommended to confirm this finding.

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