

COMPARATIVE EVALUATION OF THE EFFECT OF BLACK AND GREEN TEA ON ARTIFICIALLY DEMINERALIZED PERMANENT ENAMEL: IN VITRO STUDY

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ABSTRACT

Purpose: To evaluate and compare the effect of black and green tea on initial enamel caries like lesions of extracted human permanent teeth through microhardness analysis and polarized light microscopic evaluation.

Materials and methods: Fifty extracted young premolars with a standardized window on enamel were immersed in a demineralizing solution for 48 hours to produce subsurface enamel lesions. They were divided into two groups according to the type of treatment (n= 25), group I: teeth were treated by black tea infusion; group II: teeth were treated by green tea infusion. The enamel surface microhardness was measured at baseline, after the incipient enamel lesion, and after treatment. Additional twenty young premolars were selected and prepared for evaluation of the changes in enamel birefringence using the polarized light microscope.

Results: Both groups showed a statistically significant increase in enamel surface microhardness after treatment as compared to the demineralization phase. By comparing the two groups, there was a statistically significant difference in the percentage of surface microhardness recovery after treatment in favor of the black tea group (P <0.0001). The polarized light microscope showed an increase in the negative enamel birefringence in both groups with less degree in the green tea group.

Conclusion: Both black and green tea have a remineralizing effect on the initial enamel caries lesions with better effect of the black tea.

KEYWORDS: Initial enamel lesions, Black tea, Green tea, Microhardness, Polarized light microscope.

INTRODUCTION

Dental caries is a multifactorial disease which involves the gradual loss of mineral compounds

from dental hard tissues ⁽¹⁾. White spot lesions are the earliest macroscopic evidence of enamel caries; it remains a significant challenge in oral health

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care⁽²⁾. Following demineralization, a decrease in the enamel microhardness was noted, due to enamel softening through a chemical dissolution of enamel rods and voids creation⁽³⁾. Many approaches were introduced for caries eradication throughout the years⁽⁴⁾. Recently, the combat of caries took place through prevention rather than treatment⁽⁵⁾. Prevention of caries involves enhancing the remineralization of demineralized enamel⁽⁴⁾.

The World Health Organization and the Food and Agriculture Organization highlighted the importance of effective caries prevention through diet and administration of fluoride as part of public health programs^(6,7). Fluoride has been shown to promote the formation of apatite and enhance the remineralization of teeth even in very low concentration of less than 0.5 ppm in saliva⁽⁸⁾.

The advances in understanding the relationship between nutrition and health resulted in the development of the concept of a functional food⁽⁹⁾. The Institute of Medicine of the National Academy of Sciences has defined functional food as “any food or food ingredient that may provide a health benefit beyond the traditional nutrients it contains”⁽¹⁰⁾. Certain food and beverages such as herbs, spices, grape and tea exert beneficial effects on human health. Tea is the second most popular beverage consumed worldwide after water, therefore a great interest has been raised concerning tea health-promoting potential⁽¹¹⁾. Tea beverage is an infusion of dried leaves of *Camellia sinensis* (*C. sinensis*)⁽¹²⁾. There are three main varieties of *C. sinensis* tea; green, black and oolong⁽¹³⁾. Green and black tea are the most researchable upon among different varieties⁽¹⁴⁾. Earlier reports suggested that tea consumption reduces dental caries and dental plaque due to its high fluoride concentration and organic constituents⁽¹⁵⁾.

Fluoride accumulates in leaves of the tea plant, and some of this fluoride is released into the infusion which is drunk as a tea, therefore it provides an effective vehicle for fluoride delivery

to the oral cavity and may become associated with the oral cavity and their surfaces⁽¹⁶⁾. Green tea was found to have numerous oral health benefits due to its anti-inflammatory, antibacterial, antiviral actions and its remineralizing potential which help in caries prevention⁽⁴⁾. Moreover, it has been shown that black tea and its polyphenols inhibit growth, acid production, metabolism and glycosyltransferase enzyme action of *Streptococcus mutans* (*S. mutans*) and dental plaque bacteria⁽¹⁷⁾. To the authors' knowledge, no study tested the microhardness of the demineralized permanent enamel after treatment using green and black tea. Therefore, the present study aimed to evaluate and compare the effect of black and green tea on initial enamel caries like lesions of extracted human permanent teeth through microhardness analysis and polarized light microscopic evaluation. The null hypothesis for this study proposed that there will be no difference between the effect of black and green tea on enamel microhardness or the histological picture at the polarized light microscope level.

MATERIALS AND METHODS

This study was a randomized comparative in vitro study. It was approved by the Scientific Research Ethical Committee, Faculty of Dentistry, Alexandria University, Alexandria, Egypt (IORG000839). A total sample of 50 teeth was calculated based on the difference in microhardness between two groups means derived from previous studies^(1,18). Nineteen specimens per group, increased to 25 to make up for processing errors, were required considering a mean difference in microhardness values of 22.24 with pooled SD of 23.32 after 7 days of immersion in tea solutions, 80% power and 5% alpha error. The sample size was calculated using MedCalc comparisons of means calculator⁽¹⁹⁾. Additional twenty premolars were collected for qualitative evaluation using Olympus polarized light microscope CX31 (America Inc). Human maxillary premolars, extracted for orthodontic reasons,

were collected from the Oral Surgery Department, Faculty of Dentistry, Alexandria University to be included in the study. Teeth were rinsed with water and cleaned using fluoride-free pumice. Then they were examined using a magnifying lens for any defects. Only teeth free of caries, cracks or any developmental defects were included in the study. All teeth were stored in normal saline at room temperature until required for use to prevent their dehydration.

MATERIALS

- **Two different types of commercially available tea brands;** Black tea bags and green tea bags (Lipton tea imported and packed by fine tea company S.A.E New Borg El-Arab City, First Industrial Zone, Plot 5, Block 11/A, Alexandria).
- **Demineralization solution** composed of 2.2 mM calcium chloride, 2.2 mM potassium dihydrogen phosphate, 0.05 M acetic acid, and 1 M potassium hydroxide (KOH) to maintain a pH of 4.4⁽²⁰⁾.
- **Artificial saliva** was prepared by mixing 500 ml distilled water, 20 g potassium chloride, 0.843 g sodium chloride, 0.051 g magnesium chloride, carboxymethyl cellulose, 20 ml tricalcium phosphate, and 0.05 M sodium hydroxide to maintain a pH of 6.8⁽²¹⁾.
- **Distilled water.**

METHODS

Teeth preparation for microhardness evaluation (Quantitative evaluation)

Teeth included in the study were embedded in acrylic resin with their buccal surface facing upwards after removing their roots, with a water-cooled diamond saw, at the cement-enamel junction. The buccal enamel of teeth was ground using silicon carbide papers (grades 600 to 1200) under water irrigation and polished to produce a flat surface, as

the high fluoride content of the sound enamel renders the surface acid-resistant more than the underlying layer⁽²²⁾. Acid-proof nail varnish was used to coat the teeth surfaces exposing only a small standardized window in the cervical third of the buccal surface of enamel (4×4 mm) which were subjected to the demineralizing solution to produce caries-like lesion. Baseline microhardness assessment of all teeth was measured by applying a 50 g load to the buccal enamel surface for 10 seconds before being immersed in the demineralizing solution. The enamel surface microhardness (SMH) was assessed using digital display Vickers microhardness tester with a Vickers diamond indenter and a 20X objective lens. Five indentations were equally placed over a circle of 1 mm diameter at the cervical third of teeth. A built-in scaled microscope was used to measure the diagonal length of the indentations and Vickers values were converted into microhardness values. The following equation: $HV = 1.854 P/d^2$ [HV is Vickers hardness in Kef/ mm², P is the load in Kef and d is the length of the diagonals in mm] was used to obtain the surface microhardness values⁽²³⁾.

Demineralization Phase: Artificial caries lesion has been produced by immersing the teeth in a demineralizing solution (10 mL for each specimen) for 48 hours. Then, they were rinsed with distilled water and stored in artificial saliva to simulate the oral cavity conditions⁽²⁴⁾. Every 12 hours, the demineralizing solution was renewed to prevent depletion of solution⁽²³⁾. Post-lesion microhardness test was conducted with the same static load and time applied for obtaining the post-lesion measurements (Second assessment).

Preparation of green and black tea:

One tea bag was added to 250 ml of freshly boiled water and stirred for 5 minutes then the bag was removed as this is the usual practice of tea preparation which produce the best flavor with little extraction of tannin. A volume of 250 ml was used as the average volume of a typical tea mug.

The solution was allowed to cool until it reached 37°C before testing⁽²⁵⁾.

Treatment phase: the 50 teeth were divided randomly into two groups according to the type of tea used:

Group I (n= 25): teeth were treated with black tea infusion for 5 minutes at 37°C, 3 times a day for 7 days; washed with distilled water; stored in artificial saliva

Group II (n=25): teeth were treated with green tea infusion for 5 minutes at 37°C, 3 times a day for 7 days; washed with distilled water; stored in artificial saliva.

The artificial saliva was changed frequently to avoid the risk of its saturation hence interfering with the treatment process⁽²⁶⁾.

The post-treatment microhardness test was conducted with the same static load and time applied for baseline and post-lesion measurements (Final assessment).

Teeth preparation for polarized light microscopic evaluation (Qualitative evaluation)

A uniform surface area of exposed enamel has been produced in the additional selected twenty premolars as has been mentioned before. After being immersed in the demineralizing solution, each tooth was sectioned using a diamond disc (911 pf-220-0.25 by Diatech Swiss Dental Instruments), through the lesion longitudinally into two halves (mesial half and distal half). One-half of each of the twenty teeth was treated with tea (black or green) and the other half remained untreated and served as control. Each half was considered as a specimen. All specimens were stored in artificial saliva till required for use. They were randomly distributed into four groups according to the type of treatment (ten for each group) Group A: were treated with black tea infusion Group B: were Left untreated

and serve as a control for group A, Group C: were treated with green tea infusion and Group D: were Left untreated and serve as a control for group C. Each specimen in the control group was the other half of the same tooth in the test group. Specimens were treated as mentioned before and after 7 days of immersion they were prepared for qualitative evaluation, using the polarized light microscope, of the changes in enamel birefringence through manual grinding of each specimen on a wet glass plate with aluminum oxide (AL₂O₃) powder with different granulation to obtain a section of 25 μm thickness. Each ground section was then washed under running water, then passed in ascending grades of alcohol (50, 70, 90 and 100%). Xylitol was used for clearance. Canada balsam was the mounting medium used to hold the specimen in place between the slipcover and the glass slide. Furthermore, a ground longitudinal section of normal enamel was prepared as previously described (reference section) to be compared histologically with those sections of the study. Photomicrographs were taken with a digital camera to achieve a comparison between the test and control groups. The histological features of the caries-like lesions of the four groups (treated with tea or left untreated) were examined at a magnification of x40 and were compared with the reference section⁽²⁷⁾.

Statistical Analysis

Differences between groups in hardness readings were assessed using independent t-test and within-group differences were compared using Two-Way Repeated Measures ANOVA followed by post hoc test. Percentage of surface microhardness recovery (SMHR) was calculated after remineralization as [(SMH final - SMH lesion) / (SMH baseline - SMH lesion)] × 100 and compared using independent t-test. Significance level was set at 0.05. Data were analyzed using IBM SPSS statistical software (version 25).

RESULTS

Results of microhardness (Quantitative evaluation)

Following demineralization, the microhardness values of both groups decreased significantly as compared to their baseline values (P=0.0001). While after treatment using black or green tea, the microhardness values of the two groups increased significantly as compared to the demineralization phase (P=0.0001). Although, it was still decreased significantly as compared to their baseline values (P=0.0001) (Table 1). By comparing the two groups there was no statistically significant difference in the microhardness values at baseline, after demineralization and after treatment between the two groups (P=0.883, 0.785, 0.091 respectively) (Table 1). Results of two way repeated measures ANOVA showed a significant effect of treatment within groups (P<0.0001). Between groups, comparison did not show a significant effect (P=0.541), while the interaction was significant (P<0.0001) (Table 2). Therefore, the percentage of surface microhardness recovery after treatment showed a statistically significant difference between the two groups with 80.40% of surface microhardness recovery for black tea and 50.54% for green tea (P <0.0001) (Table 3).

TABLE (1) Microhardness values after immersion in the green and black tea.

	Black tea (n=25)	Green tea (n=25)	P-value
	Mean (SD)		
Baseline	195.36 (11.96) ^a	195.88 (12.89) ^a	0.883
After demineralization	174.76 (14.64) ^b	173.56 (16.22) ^b	0.785
After treatment	191.44 (12.16) ^c	185.04 (14.03) ^c	0.091
P-value	0.0001*	0.0001*	

*Statistically Significant difference at P-value ≤0.05

^{a,b,c} Different letters denote statistically significant difference between time points within each group.

TABLE (2) Two-Way Repeated Measures ANOVA model for the effect of treatment and change in microhardness.

	F test	η _p ²	P-value
Within groups effect	866.146	0.947	<0.0001*
Between groups effect	0.378	0.008	0.541
Interaction (effect of treatment xchange in microhardness)	23.645	0.330	<0.0001*

*Statistically Significant difference at P-value ≤0.05

η_p²: Partial Eta Squared

TABLE (3) Percentage of surface microhardness recovery (SMHR) after treatment with black and green tea.

	Black tea (n=25)	Green tea (n=25)	P-value
Mean (SD)	80.40 (7.51)	50.54 (10.23)	<0.0001*

*Statistically Significant difference at P-value ≤0.05

Histological results of polarized light microscope (Qualitative evaluation)

For polarized light microscope evaluation, normal enamel showed the normal course of enamel rods with alternative Hunter-Schreger Bands (HSBs) reflecting the normal mineralization and birefringence of enamel (Fig 1). After demineralization, the untreated specimens showed a relatively high degree of positive birefringence with loss of the typical enamel structure within the lesion (Fig 2 and 4). After seven days of treatment with black tea infusion, the body of the lesion showed a dramatic decrease in the extent of the body of the lesion and noticeable negative birefringence indicating an increased degree of remineralization (Fig 3). Specimens treated with green tea infusion showed a noticeable decrease in the extent of the body of the lesion and noticeable negative birefringence compared with the control group. However, the degree of negative birefringence was less than that of the black tea group which denotes less remineralizing effect (Fig 5).

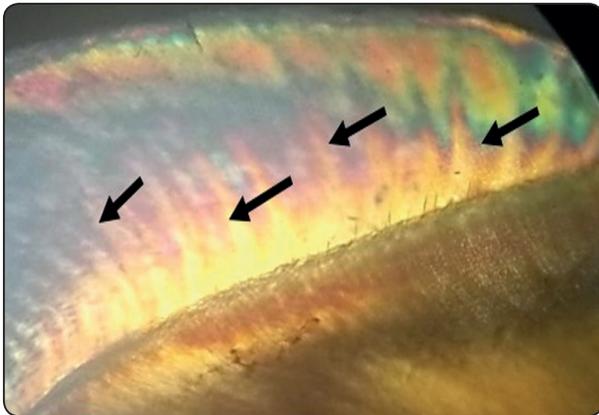


Fig. (1) Polarized light photomicrograph of a longitudinal ground section showing the normal course of the enamel rods with alternative Hunter-Schreger Bands (arrows) reflecting the normal mineralization and birefringence of enamel.



Fig. (2): Polarized light photomicrograph of a longitudinal ground section of a specimen with demineralized zone showing a high degree of positive birefringence with loss of the typical enamel structure within the lesion (circle), black tea group, original magnification x40.



Fig. (3) Polarized light photomicrograph of a longitudinal ground section of a specimen treated with black tea infusion showing a dramatic decrease in the extent of the body of the lesion and noticeable negative birefringence (circle). original magnification x40.

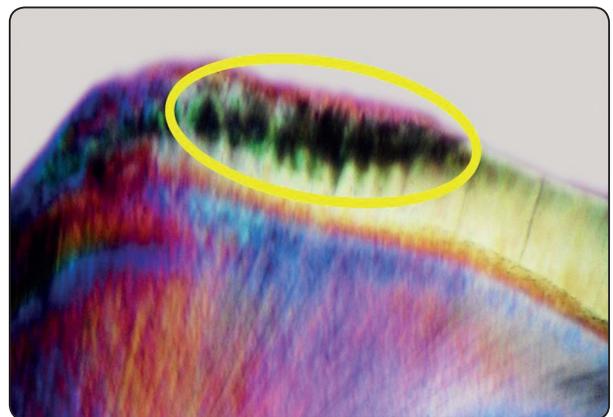


Fig. (4) Polarized light photomicrograph of a longitudinal ground section of a specimen with demineralized zone showing a high degree of positive birefringence with loss of the typical enamel structure within the lesion (circle), green tea group, original magnification x40.

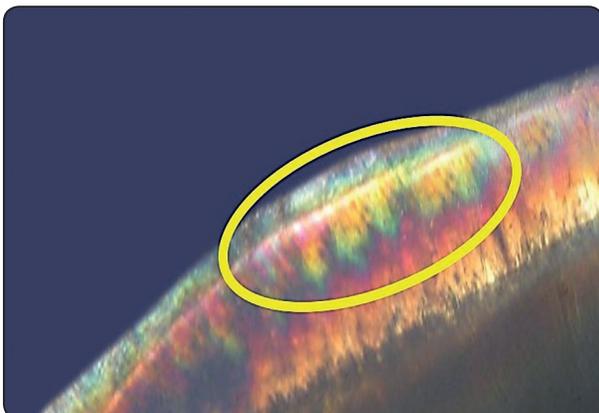


Fig. (5) Polarized light photomicrograph of a longitudinal ground section of a specimen treated with green tea infusion showing a dramatic decrease in the extent of the body of the lesion and noticeable negative birefringence (circle). Note that the degree of negative birefringence in this group is less than that of the black tea group. Original magnification x40.

DISCUSSION

The main objective of the present study was to investigate and compare the effect of black and green tea on initial caries like lesions of human permanent teeth. As the numerical hardness values present a direct proportion to the amount of minerals in the tooth structure, therefore the analysis of microhardness has been applied to detect the loss and gain of minerals into dental tissue⁽²⁸⁾. Based on the results of this study the null hypothesis was rejected. Both black and green tea were able to remineralize the demineralized enamel with a significant increase in the percentage of surface microhardness recovery after remineralization using black tea. Within-group comparison showed a significant increase in the microhardness values as compared to the demineralization phase. Several studies were in agreement with the result of the present study as they reported the remineralizing effect of black and green tea using different methods of assessment^(4,29-31). This effect could be due to the availability of fluoride content in the black and green tea infusions. Moreover, Yu et al⁽³²⁾ demonstrated that tannin and catechins rather than fluoride contribute to the tea anti-cariogenic effect. By contrast, Wu et al⁽³³⁾ reported that 7 days of treatment in black tea extract was not sufficient to remineralize the artificial caries lesions in human extracted teeth. This contradiction may be due to the use of tea extract in their study which has lower fluoride concentration as compared to tea infusion that was used in the present study. Additionally, Rezaei et al⁽³⁴⁾ found that the anti-cariogenic effect of green tea was similar to that of normal saline. This difference from our result could be related to the immersion period of the treated teeth as in their study teeth were placed one day in a solution of the polyphenol extract while in the present study teeth were placed in the tea infusion for 7 days. By comparing the two groups, results showed a significant decrease in the microhardness value of demineralized enamel surfaces, indicating loss of minerals, in both groups with no significant difference between them as all teeth were immersed

in the same demineralizing solution for equal time for standardization. Following the intervention, the percentage of microhardness recovery was more significant in teeth treated with black tea than those treated with green tea. This could be referred to the higher fluoride concentration in black tea infusion as compared to green tea infusion⁽³⁵⁾. This finding is consistent with Malinowaska et al⁽³⁶⁾ who reported that the fluoride content in black tea infusion after 5 minutes of brewing was 0.32- 4.54 mg/L while it was 0.59-1.83 mg/L in green tea infusion. On the contrary, Bozorgi et al⁽²⁹⁾ stated that microhardness values increased significantly after treating the teeth with green tea as compared to the microhardness values following demineralization. However, no significant difference was recorded between the two values following the use of black tea. This difference in the outcome from the present study could be due to different methodology in tea preparation. In addition, in their study, they worked on primary teeth while the current study was conducted on permanent teeth. Similarly, Babu et al⁽⁴⁾ concluded that green tea has a higher amount of remineralization ability as compared to black tea. They referred this result to the high catechin fraction which is responsible for its antibacterial properties. This contradiction from our result could be due to inactivation or removal of some of the components of black tea responsible for remineralization while preparing the tea extract while in the present study the commercial tea bags were used.

A qualitative evaluation was performed using a polarized light microscope as it is the most sensitive and descriptive-analytical technique for evaluating the histological changes in white spot lesions⁽³⁷⁾. In the current study, results from histological evaluation go in line with the changes in the microhardness values at the different phases. They revealed a noticeable decrease in the extent of the lesion with a reduction in the positive birefringence of the body of the lesion in the specimens treated with black and green tea in comparison to their control. This result contradicts with Jazaeri et al⁽³⁸⁾ who stated that

washing the teeth with green tea polyphenol extract did not lead to a significant reduction in cavity depth. This contradiction could be due to a different methodology in tea preparation. In the present study, some specimens that were treated with black tea showed a dramatic decrease in the lesion extension with an increase in the negative birefringence to a degree similar to that of normal enamel. The current result is inconsistent with Abdulaheam and Garib⁽³¹⁾ who reported that green tea produced complete remineralization while black tea resulted in partial remineralization (only remineralization of surface area) although the higher fluoride level in black tea than in green tea. This can be explained that raising the fluoride level does not result in greater degrees of mineralization only free exchangeable one can react with calcium ion⁽³⁹⁾. Also, black tea contains elements other than calcium and phosphorous such as Al, K, Mg, Mn, which may substitute calcium ion of hydroxyapatite crystals (decrease Ca/P molar ratio) and forming other crystals with various sizes and orientation of the heterogenous chemical structure, which could be seen as hypomineralized area in limited quantities⁽³¹⁾. The multiple advantages of tea regarding its stability, acceptable odor and taste, economic feasibility, antibacterial activity on *S. mutans* and ability to remineralize caries like lesions, offer it a valuable measure in controlling dental caries. However, excessive intake of black tea should be avoided in regions with fluoride exposures approximating the upper limit threshold to not exceed the safety level. A possible limitation of the present study was using only two types of commercially available tea of the same brand, on the other hand using different tea types of different brands might have revealed a wider range of results. Another limitation is that although the study methodology attempted to simulate the oral condition as closely as possible, the controlled environment of the study lack some of the natural oral condition such as the changes in pH value or the presence of other probable sources of fluoride. Within the limitations of the study, both

black and green tea proved their effectiveness in the remineralization of initial caries like lesions with better black tea effect, however, further in vivo studies are necessary to assess the effect of oral rinsing with tea infusion on the remineralization of white spot lesions.

CONCLUSION

Both black and green tea proved to have a potential role to remineralize the initial enamel caries lesions in young permanent teeth with better effect of black tea.

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