ORIGINAL STUDY

Remineralizing Efficacy of Green and Black Tea on Artificially Demineralized Primary Molars

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Abstract

Purpose: To assess the remineralizing efficacy of green and black tea in comparison to casein phospho-peptide amorphous calcium phosphate (CPPeACP) on artificially demineralized primary molars. Patients and methods: A total number of 30 human primary teeth were collected to study the remineralizing efficacy of green and black tea in comparison to CPP (tooth mousse) paste on artificial demineralization of primary molars. Teeth were divided into three groups, group I: 10 teeth were treated with black tea, group Π: 10 teeth were treated with green tea, group III: (control group): 10 teeth were treated with CPP (tooth mousse) paste. All groups were assessed using Vickers microhardness test and scanning electron microscope. Results: All three groups show remineralizing effect after demineralization with the highest mean value was recorded in CPP paste followed by black tea, with the least mean value recorded in green tea. Conclusion: Three groups showed their ability for remineralization after demineralization however, the greatest effect was with CPP paste followed by black tea, and finally green tea.

Keywords: Black tea, Casein phospho-peptide amorphous calcium phosphate, Green tea, Primary teeth, Scanning electron microscope, Vickers microhardness

1. Introduction

Dental caries is a common condition that affects people all over the world. It has a serious physical, social, and economic impact on children, which lowers the family's standard of living [1]. Caries begins with the decalcification of the inorganic component of the tooth and progresses to the destruction of the organic matrix, resulting in gradual demineralization of the tooth structure [2]. The earliest sign of dental caries is a white spot lesion (WSL). According to its definition, it is milky white opacity on smooth surfaces that manifests clinically as subsurface enamel porosity brought on by carious demineralization [3]. WSLs have a porous underlying enamel layer underneath the virtually complete, radiopaque outer layer that covers the lesion. To stop lesion activity and enhance aesthetics, WSL management involves avoiding demineralization and enhancing remineralization [4].

Dental caries prevention treatments generally aim to arrest caries and remineralize the afflicted dental surface while it is still in its early stages. Employing preventive materials to treat the first decay and white lesions on the enamel will slow or stop the formation of the cavity and preserve the tooth structure. With specifically designed treatment regimens, initial enamel lesions can be cured and their acidity resistance strengthened. The microhardness of newly formed lesions is lower than that of the sound and caries-free enamel surface. After demineralization, the process of chemically dissolving enamel rods weakens the enamel and creates voids, resulting in a loss of microhardness [5].

Because tea and medicinal plants have fewer side effects than chemical medications in the treatment of many diseases, their use has increased significantly in recent years. Tea is a widely available and frequently used plant extract with benefits that have been known for at least 4000 years [2].

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Based on the processing method in the factory, it is possible to divide the kinds of tea into three categories. In nonfermented tea (green tea), the oxidation process of polyphenols is not performed. In semi -fermented tea (oolong tea), the oxidation process of polyphenols is limited. In fermented tea (black tea), the oxidation process of polyphenols is completely carried out [6].

Black tea is more oxidized than all other types of teas. It contains antioxidants and other substances. Green tea has undergone minimal oxidation during process. It is able to maintain important molecules called polyphenols, which seem to be responsible for many of the benefits of green tea [7].

A beneficial compound called casein phosphopeptide amorphous calcium phosphate (CPP-ACP) is produced from the casein protein present in milk. It stops enamel demineralization by keeping calcium and phosphate in saliva supersaturated, stabilizing the plaque's pH, and increasing the amount of calcium and phosphate ions in the plaque. Since CPP-ACP can raise salivary pH and flow rate, it has been proven to be helpful for tooth remineralization [8].

2. Patients and methods

2.1. Study design

The Faculty of Dental Medicine for Girls, Al-Azhar University, Cairo, Egypt, Research Ethics Committee (protocol code: P-PE-21-02, final code: REC-PE-23-08), has authorized this study.

Through microhardness measurement and electron microscope assessment, this invitro study compares and contrasts the effects of black and green tea and casein phosphopeptide (tooth mousse) on early enamel caries-like lesions of extracted human primary teeth.

To study the remineralizing efficacy of green and black tea in comparison to CPP (tooth mousse) paste (control group) on artificially demineralized primary molars, ANOVA test or an equivalent non-parametric test will be used for comparison between groups. Using G power statistical power Analysis program (version 3.1.9.7) [9] for sample size determination, a total sample size of 30 (10 in each group) will be sufficient to detect an effect size.

2.2. Teeth collection and specimen preparation

(a) Inclusion criteria: Teeth free of caries, cracks, or any developmental defects.
(b) Exclusion criteria: Teeth with caries, cracks, or any developmental defects
(c) Specimen preparation:

All teeth were obtained from anonymous patients, at the end of the study teeth were disposed of in medical waste container that uses an incinerator for final disposal. A total number of 30 human primary teeth were collected and stored in a saline solution. Teeth were cleaned and polished from any calculus, plaque, and stains. The crown was separated from the root at the cemento-enamel junction using water-cooled diamond disc (Fig. 1). Teeth were embedded in acrylic resin with their buccal surface facing upwards (Fig. 2).

The buccal and lingual surface 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.

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 (Fig. 3).

Artificial saliva is prepared using albumin, methyl cellulose, sodium carboxymethyl cellulose, hydroxypropylmethyl cellulose, potassium chloride, dipotassium hydrogen phosphate, sodium fluoride, Magnesium chloride, methyl paraben.

Albumin, cellulose, potassium chloride, potassium phosphate, and sodium fluoride were dissolved in a small amount of water. Methyl paraben and magnesium chloride were dissolved in warm water and then cooled down before mixing all the solutions together.

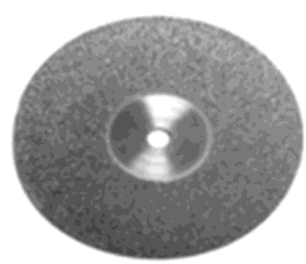

Fig. 1. Diamond disc.

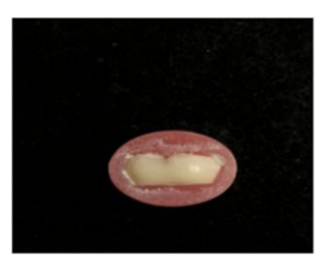

Fig. 2. Teeth embedded in acrylic resin.

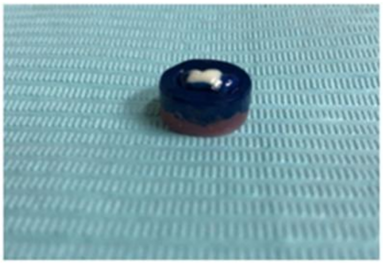

Fig. 3. Acid-proof nail varnish used.

Each acrylic resin block was given a number (1e10) inside the group, being written by an inerasable marker on one surface of the acrylic block together with the symbol of the group.

Such demarcation was made to allow for assessment at baseline, after demineralization, and post treatment. Then the numbered samples were stored in artificial saliva till subjected to scanning electron microscope (SEM) and microhardness assessment, artificial saliva is then changed every 24 h.

2.3. Grouping of the teeth

Teeth were equally divided into three groups, 10 each, according to treatment protocol:

Group I: 10 teeth were treated with black tea.

Group Π: 10 teeth were treated with green tea.

Group III: (control group):10 teeth were treated with CPP (tooth mousse) paste.

2.4. Demineralization of hard dental tissue

Due to the sound enamel's high fluoride concentration, which makes it more acid-resistant than the underlying layer, teeth's surfaces were sanded and polished to create a smooth surface.

Only a tiny, standardized window in the surface of the enamel (4 mm × 4 mm), which will be exposed to 37 % phosphoric acid covered the entire surface exposed from the tooth for 15 s to induce a caries-like lesion after the teeth surfaces were coated with acid-proof nail varnish then was washed for 15 s using water spray and then dried for another 15 s by the air spray [10].

2.5. Remineralization of specimen

One tea bag was added to 250 ml of freshly hot water, which was then stirred for 5 min. The solution was allowed to cool to 37 °C before testing.

Group I: For 7 days, three times per day, a black tea infusion was applied to 10 teeth for 5 min at a temperature of 37 °C. They were then preserved in artificial saliva after being washed with distilled water.

Group II: For 7 days, three times a day, a green tea infusion was applied to 10 teeth for 5 min at 37°. They were preserved in artificial saliva after being washed with distilled water.

Group III: 10 teeth were treated with CPP (tooth mousse) treatment for 5 min, twice daily for 7 days, cleaned with distilled water, and kept in artificial saliva.

2.6. Evaluating the remineralizing efficacy using

2.6.1. Scanning electron microscope

SEM is a type of Electron Microscope that produces images of a sample by scanning it with a focused beam of electrons.

Using SEM Model Quanta 250 FEG (Field Emission Gun), with accelerating voltage 30 K. V., magnification 14× up to 1,000,000 and resolution for Gun.1n. (FEI Company, Netherlands).

The electrons interact with atoms in the sample, producing various signals that contain information about the samples' surface topography.

The electron beam is generally scanned in a raster scan pattern, and the beam's position is combined with the detected signal to produce an image. Teeth were dried and applied directly in the microscope without any special precautions. SEM is used to demonstrate the changes that occur in the surface after demineralization and after using black and green tea and compare it with the changes that occur in the control group (tooth mousse).

2.7. Assessing of microhardness [11]

The enamel surface microhardness was assessed using a digital display Vickers microhardness tester before and after demineralization and after treatment with black, green tea, and tooth mousse.

A load of 50 g was applied to the surface of the specimens for 10 s. Three indentations, which were equally placed over a circle and not closer than 0.5 mm to the adjacent indentations, were made on the surface of each specimen. The diagonal length of the indentations were measured by built in scaled microscope and Vickers values were converted into microhardness values. This procedure is repeated after applying demineralizing gel and remineralizing agents, each remineralizing agent will have a separate reading.

Microhardness was obtained using the following equation: $HV = 1.854\ P/d^2$.

Where, HV is Vickers hardness in Kgf/mm^2, P is the load in Kgf and d is the length of the diagonals in mm.

2.8. Data management and statistical analysis

Comparing groups based on normally distributed numerical data (VH) was done using the ANOVA test, and pairwise comparisons were done using the Bonferroni post hoc test. To compare observations made by members of the same group, the Paired *t*-test was utilized. Based on nonparametric numeric variables (percent change in microhardness), the KruskaleWallis test was employed to compare groups. To compare different observations, the Friedman test was used.

3. Results

3.1. Vickers micro-hardness tester (HV) (quantitative evaluation)

3.1.1. Baseline

The mean value was 189.04 ± 4.81 in black tea, compared with 189.01 ± 6.75 in green tea and 183.59 ± 7.07 in tooth mousse. Statistically, there was no significant difference between the groups. ($P = 0.101$).

3.2. Demineralization

The mean value was 167.78 ± 4.96 in black tea, compared with 170.13 ± 6.03 in green tea and 161.62 ± 6.39 in tooth mousse statistically, there was no significant difference between the groups. ($P = 0.09$).

3.3. Remineralization

The mean value was 184.78 ± 4.28 in black tea, compared with 181.64 ± 5.84 in green tea and 180.96 ± 4.44 in tooth mousse. Statistically, there was no significant difference between the groups. ($P = 0.197$) (Table 1 and Fig. 4).

3.4. The percentage changes of Vickers microhardness (HV)

3.4.1. From baseline to demineralization

The mean value of percentage decrease was (—11.24 ± 1.82; median —11.7) in black tea, in

Table 1. Comparison of Vickers micro-hardness (HV) between groups (ANOVA test).

	Mean	SD	Median	Min	Max	F value	*P* value
Baseline							
Black tea	189.04	4.81	189.89	180.41	195.49	2.5	0.101 ns
Green tea	189.01	6.75	189.65	174.32	198.05		
Tooth mousse	183.59	7.07	183.02	174.32	194.32		
Deminer-alization							
Black tea	167.78	4.96	167.58	158.49	174.38	1.57	0.09 ns
Green tea	170.13	6.03	170.32	161.81	178.42		
Tooth mousse	161.62	6.39	162.55	151.37	168.25		
Reminera-lization							
Black tea	184.78	4.28	184.16	179.76	192.54	1.77	0.197 ns
Green tea	181.64	5.84	182.84	172.98	191.72		
Tooth mousse	180.96	4.44	180.98	174.42	187.53		

Significance level *P* less than or equal to 0.05, ns = nonsignificant.
Bold values indicate p > 0.05.

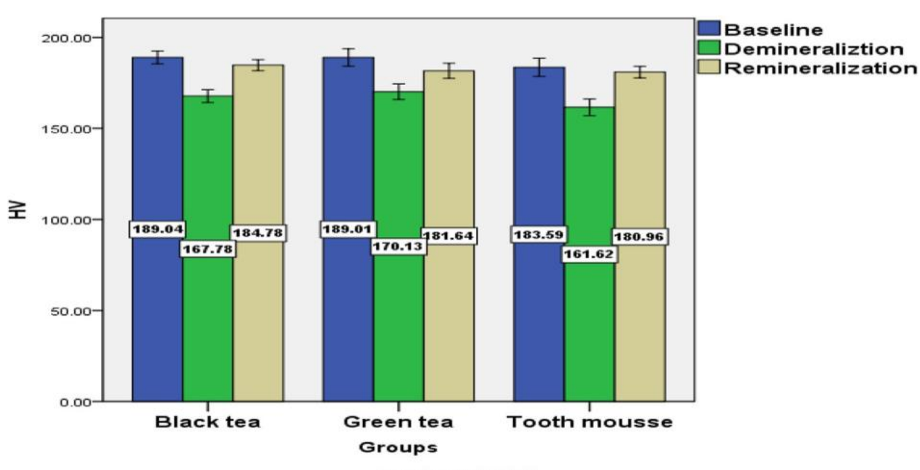

Fig. 4. Mean value of Vickers microhardness (HV) in different groups.

comparison to (—9.97 ± 2.21; median —10.21) in green tea and (—11.95 ± 2.04; median —12.06) in tooth mousse. Statistically, there was no significant difference between the groups (P = 0.098).

3.4.2. From demineralization to remineralization

The great mean value of percentage increase was recorded in tooth mousse (12.04 ± 2.51; median 11.54), followed by black tea (10.15 ± 1.52; median 9.89). The mean and median values recorded in these two groups was significantly greater than the lowest mean value recorded in green tea (6.8 ± 2.49; median 6.97) (P = 0.000).

3.5. From baseline to remineralization

The mean value of percentage decrease was (—2.22 ± 2.37; median —2.47) in black tea, in comparison to (—3.86 ± 2.28; median —4.25) in green tea and (—1.37 ± 2.10; median —1.29) in tooth mousse. No statistically significant difference existed between the groups (P = 0.061) (Fig. 5) Table 2.

3.6. Results of scanning electron microscope (SEM)

Demineralization: Surface integrity was lost, as seen by SEM images (Figs 6e8). A change in the prismatic structure of healthy enamel caused the surfaces to become porous. They may also have various degrees of regions of disintegration, which indicate demineralization.

3.7. Remineralization

On the SEM image under observation (Figs 9e11), the samples showed plugging of the porous defects with a corresponding decrease in the cavities and micro pores.

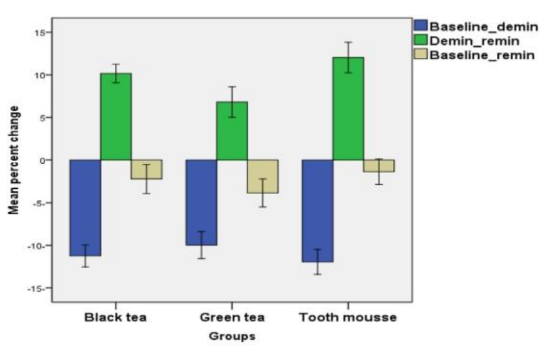

Fig. 5. Percentage change in Vickers microhardness (HV) in different groups.

Table 2. Comparison between groups regarding percentage change in Vickers microhardness (HV) (KruskaleWallis test).

	Mean	SD	Median	Min	Max	P value
From baseline to demin						
Black tea	¡11.24	1.82	—11.70	—13	—8	0.098 ns
Green tea	¡9.97	2.21	—10.21	¡13	—7	
Tooth mousse	¡11.95	2.04	—12.06	¡14	¡7	
From demin to remin						
Black tea	10.15[a]	1.52	9.89	7.98	13.42	0.000*
Green tea	6.80[b]	2.49	6.97	3.25	10.68	
Tooth mousse	12.04[a]	2.51	11.54	8.38	17.19	
From baseline to remin						
Black tea	¡2.22	2.37	−2.47	¡6	1	0.061 ns
Green tea	¡3.86	2.28	−4.25	¡7	¡1	
Tooth mousse	¡1.37	2.10	−1.29	¡5	2	

Significance level P less than or equal to 0.05, * significant, ns = nonsignificant.
Post hoc test: means with different superscript letters are significantly different.
Bold values indicate p > 0.05.

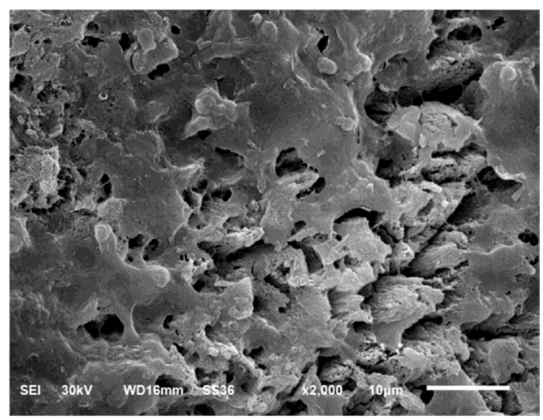

Fig. 6. Demineralization of black tea group. Various degree of regions of disintegration. Scanning electron microscope photomicrograph (×2000).

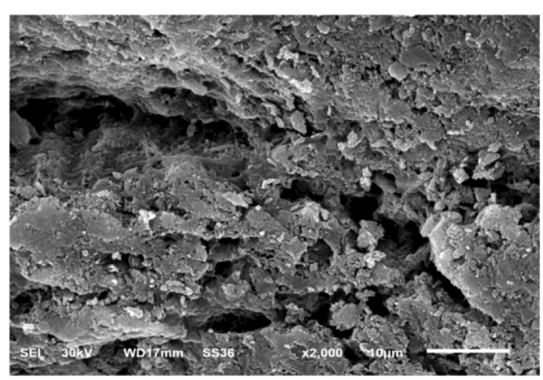

Fig. 7. Demineralization of green tea group. Healthy enamel become porous after demineralization scanning electron microscope photomicrograph (×2000).

4. Discussion

The necessity of remineralizing early enamel lesions is mandated by minimally invasive dentistry.

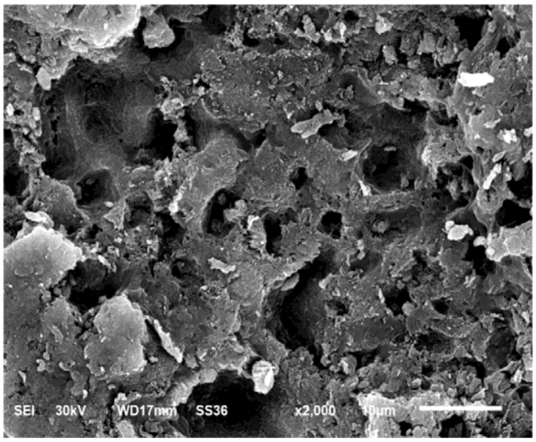

Fig. 8. Demineralization of casein phospho peptide paste group. Loss of surface integrity after demineralization. Scanning electron microscope photomicrograph (×2000).

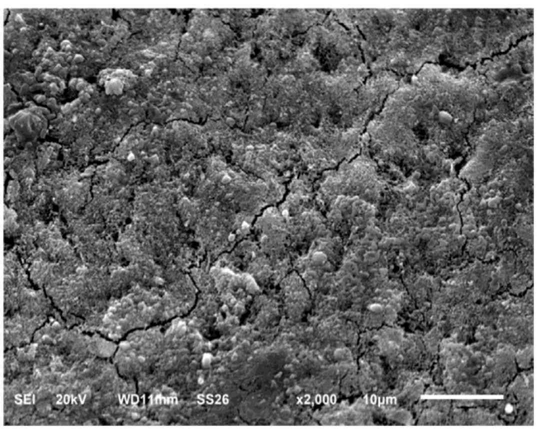

Fig. 9. Remineralization of black tea group. Less micropores was seen in the black tea group compared with green tea. Scanning electron microscope photomicrograph (×2000).

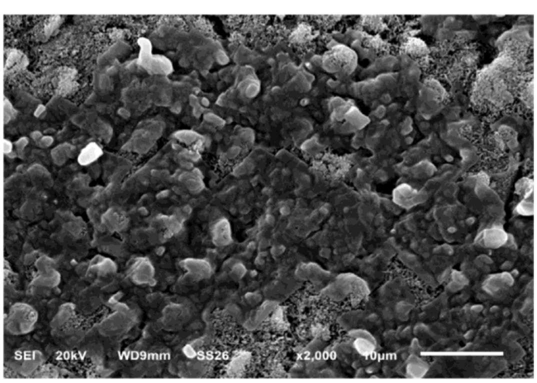

Fig. 10. Remineralization of green tea group. The least remineralization effect was in green tea group compared with black tea and tooth mousse. Scanning electron microscope photomicrograph (×2000).

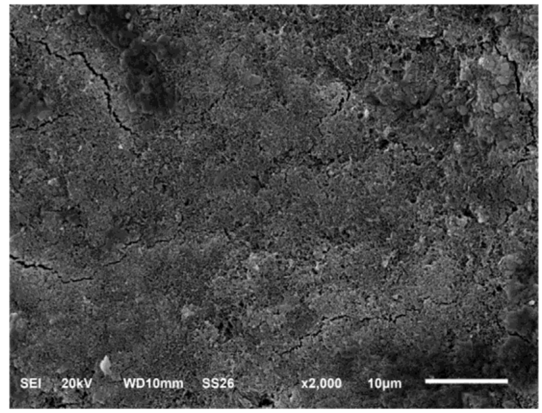

Fig. 11. Remineralization of CPP paste group. Restoring of the surface integrity in tooth mousse group. Scanning electron microscope photomicrograph (×2000).

New toothpaste, mouthwashes, gels, sealants, and lesions have developed to remineralize early enamel lesions. A reorganization of the enamel prismatic structure was claimed due to topical administration of remineralizing agents, which was said to provide ions that encouraged subsurface mineral gain [12].

Natural products are increasingly used due to their lower risk of side effects. Due to the high concentration of polyphenols like epigallocatechin gallate in black and green tea, these two varieties have been the subject of the most investigation. In addition, it has antibacterial qualities since it contains bioactive substances such as polyphenols, minerals, and volatile oils. Its high fluoride concentration aids in remineralizing effect [13]. Enamel caries is increasingly being prevented and treated using CPP-ACP. There is evidence that CPP-ACP stabilizes calcium phosphate in dental plaque close to the teeth. When exposed to an acid challenge, such as immediately following a meal, CPP-ACP helps to keep the minerals in dental enamel supersaturated, preventing mineral loss, and aiding in the remineralization of enamel subsurface lesions [14].

In this in-vitro investigation, after simulating early enamel lesions on primary teeth, green and black tea are utilized as remineralizing agents and contrasted with CPP-ACP. This study investigated the effects of green tea, black tea, and CPP-ACP on the remineralization of artificially demineralized enamel using a microhardness test and a SEM.

There are several ways to examine in vitro demineralization and remineralization. In this study, hardness was measured using the Vickers test three times: at baseline, after the initial enamel lesion induced, and after the administration of remineralizing agents. Each sample's enamel microhardness was assessed three times since any tilt or uneven surface might lead to an excessive indentation and a lesser HV measurement [15].

The samples were equivalent before surface treatment, were in comparable conditions, and could all pass the test according to the identical enamel surface microhardness measurements that were made in the first phase.

Teeth were then etched using 37 % phosphoric acid to produce a demineralized surface that simulates the morphologic changes which occur on the enamel surface when early attacks of caries take place [10].

The remineralization regimen used a 5 min infusion of black and green tea at 37 °C three times a day for seven days; cleaned with distilled water and preserved in artificial saliva. Tea was designed to be prepared like people's habits [11].

The greatest mean value of percentage increase was recorded in tooth mousse, followed by black tea. The mean and median values recorded in these two groups was significantly greater than the lowest mean value recorded in green tea.

This conclusion is in line with the study found that after 5 min of brewing, black tea infusions had fluoride concentrations ranging from 0.32 to 4.54 mg/l and green tea infusions from 0.59 to 1.83 mg/l [16].

When compared with microhardness values following demineralization, it was found that green tea treatment of teeth considerably raised microhardness values. Black tea was used to compare the two values, but no real difference was discovered. Methodologies for making tea vary, which might explain why the outcomes of the current study were different [2].

Green tea has a greater potential for remineralization than black tea. The high catechin percentage, which is what gives the substance its antibacterial properties, was blamed for this outcome. This disparity in our findings might be explained by the fact that commercial tea bags were used in the current study instead of tea leaves, which inactivated or removed some of the elements of black tea required for remineralization during the manufacture of the tea extract [17].

4.1. Conclusion

In this study, the capacity of CPP-ACP and black and green tea to remineralize enamel was examined. Early carious lesions in primary teeth can be treated with black or green tea as remineralizing agents, black tea having a greater effect.

4.2. Recommendations

(a) The remineralization potential of green and black tea needs to be investigated for a longer duration.
(b) Further in vivo investigation studies are needed to examine the efficacy of the remineralizing agents in the highly challenging oral environment.
(c) Further investigations are recommended for possible applications of black and green tea in the form of materials that can be used in the practice of pediatric and preventive dentistry.

Ethical approval

The study was approved by the Research Ethics Committee in Faculty of Dental Medicine for Girls, Al-Azhar University, Cairo, Egypt, Research Ethics Committee (Approval no.: REC-PE-23-08).

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Biographical information

Study was done at The Faculty of Dental Medicine for Girls, Al Azhar University, Cairo, Egypt.

Conflicts of interest

There are no conflicts of interest.

References

[1] Bayoumi M, Dowidar K, Hanno A. The clinical effect of casein phosphopeptide amorphous calcium phosphate varnish on caries affected dentine in primary teeth (A randomized controlled clinical trial). AlexDentJ 2022;47: 185e91.
[2] Bozorgi M, Ghasempour M, Ahmadi G, Khafri S. Comparison between the effects of green and black tea, and fluoride on micro hardness and prevention of demineralization of deciduous teeth enamel. JBabol Univ Med Sci 2018;20:14e9.
[3] Rodriguez M, Villarreal M, Ramírez EN, Rodriguez R, Ramirez EL, Cepeda M, et al. White spot lesions: pediatric dentistry approach. Int J Appl Decis Sci 2022;8:172e5.
[4] Mashhour A, Allam G, Wassel M. Comparative evaluation of prevention of demineralization of artificial enamel caries treated with two fluoride varnishes and 38 % sdf in primary teeth: an in vitro study. BMC Oral Health 2023;23:1e9.
[5] Al-Mamoori R, Al Haidar A. Effect of resin infiltration and microabrasion on the microhardness of the artificial white spot lesions (an in vitro study). JBCD 2022;34:1817e96.
[6] Bozorgi M, Ghasempour M, Ahmadi G, Khafri S. Comparison between the effects of green and black tea, and fluoride on microhardness and prevention of demineralization of deciduous teeth enamel. J Babol Univ Med Sci 2018;20:14e9.
[7] Shagana JA, Geetha R. Comparative analysis of antimicrobial activity of black tea, green tea and white tea extracts on streptococcus mutans by tube dilution method. J Pharmaceut Sci Res 2017;9:1581e2.

[8] Alkarad L, Alkhouli M, Dashash M. Remineralization of teeth with casein phosphopeptide-amorphous calcium phosphate: analysis of salivary ph and the rate of salivary flow. BDJ open 2023;9:16.
[9] G*Power program (University of Düsseldorf, Düsseldorf, Germany) G* power 3.1 manual October 15. 2020.
[10] Al-Sadek S, Mostafa M, Al-Araby S. Evaluation of moringa oleifera extract on demineralized enamel of primary teeth. Al-Azhar D J 2023;10:185e93.
[11] Talaat D, Omar S. Comparative evaluation of the effect of black and green tea on artificially demineralized permanent enamel: in vitro study. Egypt Dent J 2021;67:61e70.
[12] Rohym S, Essam El Din M, Aidaros N. Effect of green tea, stevia extract solutions, and fluoride-based mouthwash on remineralization of incipient enamel lesion: an in vitro study. ACDJ 2023;2:13e25.
[13] Aidaros N, Mosallam R, Farouk H. Effect of green tea, black tea and moringa oleifera on remineralization of artificially demineralized enamel and dentin: an in-vitro micro hardness analysis. ADJC 2021;3:24e34.
[14] Alattar W, Mostafa M, Hashem S. The effect of remin pro and mi paste plus on induced demineralized enamel in primary molars. Al-Azhar D J 2023;10:239e46.
[15] Nourolahian H, Parisay I, Mir F. The effect of remin pro on the microhardness of initial enamel lesions in primary teeth: an in vitro study. Dent Res J 2021;18:16.
[16] Malinowska E, Inkielewicz I, Czarnowski W, Szefer P. Assessment of fluoride concentration and daily intake by human from tea and herbal infusions. FCT 2008;46: 1055e61.
[17] Babu AS, Babu A, Nair MG, Jayanthi JL, Thaha KA. georgejk. Effect of green tea and black tea on mineralization of artificially demineralized human enamel in comparison with 0.05 % sodium fluoride: an in vitro study. Int J Oral Care Res 2017;5:361e5.