Evaluation of Remineralizing Potential of White Tea and Green Tea on Artificially Demineralized Dentin (In-Vitro Study)

Mona M. El Sayed*and Reem Z. Roushdy**

Abstract:

Purpose: The aim of this study was to evaluate the remineralizing potential of white tea and green tea pretreatment on microhardness of demineralized dentin. **Materials and Methods**: A total number of 21 dentin samples were divided into three main groups of 7samples each according to the utilized treatment after demineralization. In the first group, dentin samples were immersed in 10% white tea extract solution followed by casein phosphopeptide amorphous calcium phosphate application. In the second group, samples were immersed in 10% green tea extract solution followed by casein phosphopeptide amorphous calcium phosphate application, while in the third group ,casein phosphopeptide amorphous calcium phosphate(CPP-ACP) only was applied on dentin surface. Microhardness was evaluated at baseline, after demineralization and after treatment for all dentin samples. **Results:** Microhardness mean values results revealed statistically significant increase in all groups after treatment .The highest percent of change of microhardness was found in white tea group, followed by green tea, while the control group revealed the lowest percent of change. **Conclusions**: Tea extracts increased dentin microharness when used before application of remineralizing agent. However, white tea extract showed better microhardness values indicating stabilization of dentin collagen network resulting in functional remineralization.

Key words: White tea, green tea, microhardness, remineralization**.**

* Lecturer Conservative Dentistry Ahram Canadian University, Egypt.
** Lecturer Conservative Dentistry Ahram Canadian University Egypt.

Introduction:

Dental caries is the most frequent chronic oral disease and is considered a worldwide oral health problem. It is also considered an irreversible microbial disease that negatively affects the inorganic and organic portions of the tooth, leading to cavitation and possible tooth loss.[1]Dentin occupies a significant portion of the tooth, with 70% inorganic matrix(hydroxyapatite), up to 20% organic matrix and 10% fluid. These organic components mainly consist of NCPs and type I collagen.[2]

Because of the littler amount of dentinal crystallites and presence of dentinal fluids within ,dentin exhibits higher affinity towards demineralization than enamel. It occurs at a critical pH higher than that for enamel (critical pH≈6.7) [3]

In pathological conditions, demineralization becomes dominant over remineralization; in dentin, demineralization initiates with dissolution of its inorganic components (HA) by organic acid, mainly lactic acid. After the mineral disintegration, exposed organic matrix is invaded by proteolytic enzymes, leading to degradation of the supporting collagen matrix and decrease in the mechanical properties of dentin. [4]

Dentin remineralization is clinically significant for treating dentin hypersensitivity and dentin caries and increasing dentin–resin bond durability. [5]True functional remineralization involves stabilization of both inorganic and organic components during the remineralization process. [6]

Different remineralizing agents and techniques have been researched to achieve newer strategies for remineralization of dentin. In recent years, there has been an interest in active compounds that are derived from natural products which might have therapeutic potential in the field of oral health.[7]The major advantages of using herbal preparations are easy availability, cost-effectiveness, increased shelf life, lack of microbial resistance and low toxicity.[4]

Tea is a widely consumed beverage and is popularised throughout the world. The growing interest in the potential health benefits of tea, together with its popularity as a beverage have lead to numerous investigations on the chemical constituents of tea and their biological properties such as antimutagenic, anticarcinogenic, antioxidant and antibacterial. [8]

Tea is well known to contain catechins like epigallocatechin gallate (EGCG), epicatechin gallate, epigallocatechin, epicatechin. Both white and green teas are

known to have these catechins in large volumes. Isolated green tea catechins such as epigallocatechin gallate (EGCG) have been proven to have inhibitory effect on elastase and collagenase. In comparison, white tea has a significantly higher anti collagenase and anti elastase effects. [9]

Casein phosphopeptide amorphous calcium phosphate (CPP-ACP) is a bioactive peptide derived from casein (milk protein) combined with amorphous calcium phosphate. It has been extensively studied for its effects on dentin and enamel. One of the advantages of CPP-ACP products over fluoride products is that they do not cause enamel fluorosis and are ingestible as compared to the fluoride products that pose a threat if the patient ingests a significant amount of fluoride.[4]

Accordingly, this study aimed to evaluate and compare the effect of white tea extract and green tea extract pretreatment on microhardness of artificially demineralized dentin using microhardness test.

Materials and Methods:

Study design

A total of 21 dentin samples were used in this study, samples were immersed in demineralizing solution for 72 hours to create artificial carious lesions.Then, they were divided into three groups of 7 samples each according to the utilized treatment. In the first group, dentin samples were immersed in 10% white tea extract solution for 10 minutes followed by casein phosphopeptide amorphous calcium phosphate application for 5 minutes. In the second group, samples were immersed in 10%green tea extract solution followed by casein phosphopeptide amorphous calcium phosphate application for 5 minutes, while in the third group, casein phosphopeptide amorphous calcium phosphate only was applied on dentin surface. All samples were stored in artificial saliva for one week at room temperature. Microhardness was evaluated at baseline, after demineralization and after treatment for all dentin samples.

Research Ethical Approval

The study was approved by the Research Ethics Committee (REC) of the Dentistry Faculty, Al-Ahram Canadian University, Egypt. Research number (IRB00012891#46).

Sample Size Calculation of the study

Sample size was calculated depending on a previous study as a reference [6] if mean and standard deviation was 38 ± 4.88, the

estimated difference is 8, with effect size ratio 1.63, the minimum needed sample size is 7 per each group. T test was used, and the power was set at 80%, and the type 1 error probability (alpha) associated with this test was set at 0.05%. Sample size was performed by using G. power 3.1.9.7.

Materials and Methods

Materials:

1. White tea (manufactured in Fujian Guanglin Fu tea industry co,China).
2. Green tea (Ahmed tea green tea, manufactured in China).
3. CPP-ACP (MI Varnish ^{TM} GC).

Preparation of white tea extract solution

For preparation of 10% White tea extracts solution, 10 g of the white tea powder were weighed using electronic balance and mixed with 100 ml of boiled distilled water in a sterile glass flask for 5 minutes. The extract solution was then filtered and kept in an airtight container. [9]

Preparation of green tea extract solution

10% green tea extracts solution was prepared by adding 10 g of green tea powder to 100 ml of boiled distilled water in a sterile glass flask for 5 minutes .Then, the solution was filtered and kept in an airtight container. [9]

Teeth selection

A total of 21 human sound molars were used in this study. Teeth with fractures, enamel malformations or any other defects were excluded, then the selected molars were stored in distilled water at 4°C till testing where they were used in the study within one month of extraction. [10]

Sample preparation

Coronal part of each tooth was measured, half of it was removed using diamond coated disc under water coolant to expose mid coronal dentin, then the specimen with the roots was embedded in chemical cured acrylic resin blocks, dentin surface of each sample was coated with acid resistant nail varnish, leaving 4 mm × 3 mm window of dentin exposed in the middle of the dentin surfac for application of tested materials (figure 1). [11]

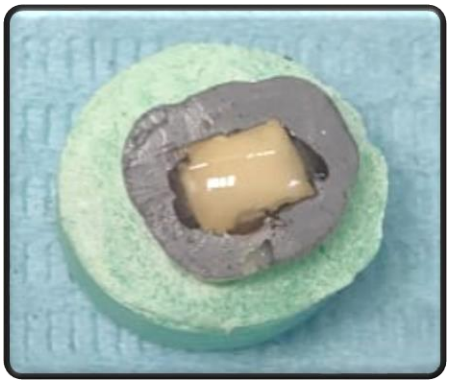

Figure (1) Dentin surface coated with nail varnish except 4 mm × 3 mm window.

Baseline microhardness test

Dentin microhardness was measured at baseline using Vickers microhardness tester (Wilson TukonTM1102, Germany) with Vicker diamond indenter and 20X lens. The surface of each dentin specimen was subjected to a load of 100 gm for 10 seconds (figure 2), three readings were recorded for each sample and their mean was calculated as the Vickers hardness number (VHN).

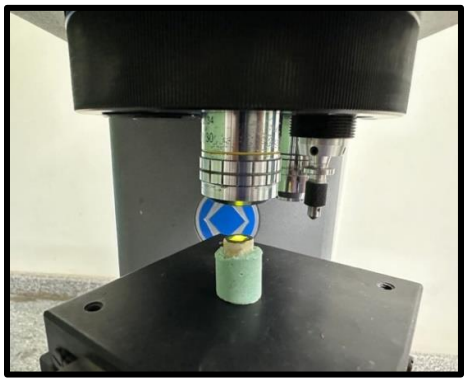

Figure (2) Dentin sample on Vickers microhardness testing machine

Induction of artificial carious dentin

All the samples were immersed in demineralizing solution for 72 hours at room temperature. [4] It consisted of 3 mmol/L Ca^{+2}, 2.2 mM, 3 mmol/L PO_4 and 50 ml/L acetic acid, pH of the solution was adjusted to 4.5 using NaOH. The solution was renewed every 24 hours[10], all the samples were rinsed with distilled water and evaluated again for surface microhardness.

Sample grouping

After demineralization, dentin samples were randomly distributed among three groups according to the applied treatment (n = 7). In the first group, samples were immersed in 10% white tea extract solution for 10 minutes followed by casein phosphopeptide amorphous calcium phosphate application for 5 minutes using bond brush [12]. In the second group, samples were immersed in 10% green tea extract solution for 10 minutes followed by casein phosphopeptide amorphous calcium phosphate application for 5 minutes, while in the third group (control group) casein phosphopeptide amorphous calcium phosphate only was applied for 5 minutes on dentin surface using bond brush.

Microhardness evaluation after treatment

All samples were evaluated for surface microhardness after one week storage in artificial saliva which was prepared using Na PO (3.90mM), KCl (17.98mM), NaCl (4.29mM), $MgCl_2$ (0.08mM), $CaCl_2$ (1.10mM), $NaHCO_3$ (3.27mM), H_2SO_4 (0.50mM) and distilled water.The utilized loads were the same as the initial ones (100 gm for 10 seconds) and the final indentations were approximately 0.1mm far from the past ones. Artificial saliva was replenished every 24 hours.

Three readings of microhardness value of each specimen (MH) in all groups were recorded; initial, demineralized, and final MH values. These readings were used to calculate the percentage of change of microhardness of demineralized dentin (MH%) using this formula:

MH%= (final MH - demineralized MH)/(initial MH – demineralized MH)×100 (10,12)

Statistical analysis:

Statistical analysis was performed with SPSS 16 ® (Statistical Package for Scientific Studies), Graph pad prism & windows excel and presented in 2 tables and 2 graphs. Exploration of the given data was performed using Shapiro-Wilk test and Kolmogorov-Smirnov test for normality which revealed that data originated from normal data. Accordingly, comparison between 3 different groups was performed by One Way ANOVA test followed by Tukey`s Post Hoc test for multiple comparison, while comparison between baseline(initial), demineralization and after treatment(final) was performed by using Repetitive One-Way ANOVA test followed by Tukey`s Post Hoc test for multiple comparisons. The significance level was set at $p \leq 0.05$. Percent of of change of microhardness (MH%) of dentin was calculated using the following formula.(10 ,12)

$$(\text{MH\%}) = \frac{final\ MH - demineralized\ MH}{(Initial\ MH - demineralized\ MH)}\ X100$$

Results:

Mean and standard deviation of microhardness values for all groups at baseline(initial), after demineralization and after treatment(final) are presented in table (1) and figure (3).

Comparison between different intervals was performed using One Way ANOVA test which revealed significant difference in all groups as P<0.0001, = 0.00003, <0.0001 regarding group I, II and III respectively.

Followed by Tukey`s Post Hoc test for multiple comparisons which revealed that; in group I (White tea), there was a significant decrease from (42.7 ± 7.21) at baseline to (27.19 ±8.68) after demineralization, then there was a significant increase to (32.56 ± 8.96) after treatment .In group II (Green tea), there was a significant decrease from (61.1 ± 8.44) at baseline to (43.06 ±5.91)after demineralization , then there was a significant increase to (47.57 ± 6.38) after treatment. For group III (Control group), there was a significant decrease from (49.29 ± 5.02) at baseline to (36.21 ± 4.54) after demineralization, then there was a significant increase to (39.07 ± 4.68) after treatment.

Table (1): Mean ±SD and P-value of microhardness values for all groups at baseline, demineralization and after treatment and comparison between them using Repetitive One-Way ANOVA test followed by Tukey`s Post Hoc test:

Group	Baseline (initial)		Demineralization		After treatment (final)		P value
	M	SD	M	Sd	M	SD	
group I (White tea)	42.70 [a]	7.21	27.16 [b]	8.68	32.56 [c]	8.96	<0.0001*
group II (Green tea)	61.10 [a]	8.44	43.06 [b]	5.91	47.57 [c]	6.38	0.0003*
group III (Control)	49.29 [a]	5.02	36.21 [b]	4.54	39.07 [c]	4.68	<0.0001*

**Significant difference as P<0.05.*

Means with different superscript letters were significantly different as P<0.05.

Means with the same superscript letters were insignificantly different as P>0.05.

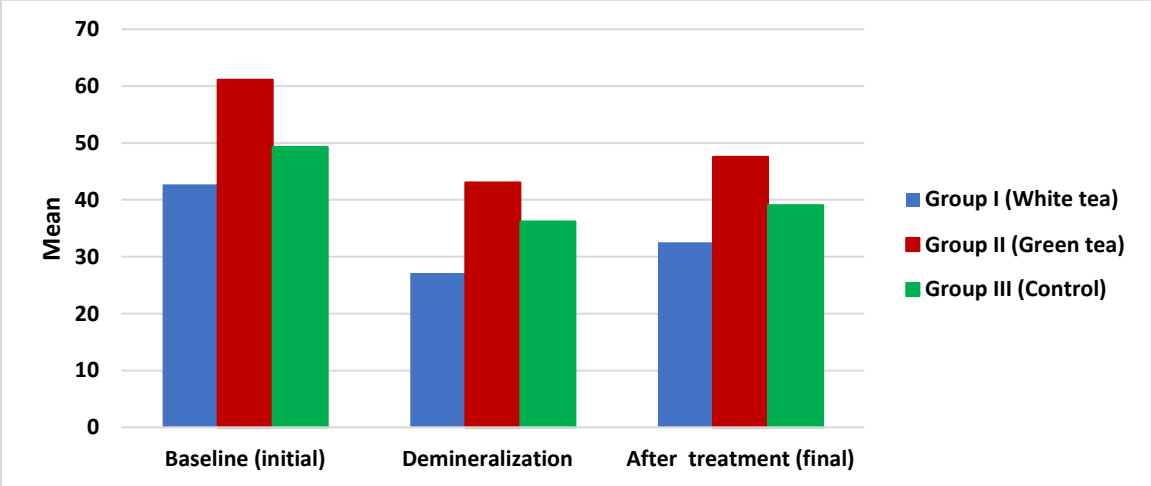

Figure (3): Bar chart showing all groups at baseline, demineralization and after treatment.

Mean and standard deviation of percent of change (MH%) of microhardness of dentin in all groups were presented in table (2) and figure (4). Comparison between different groups was performed using One Way ANOVA test which revealed statistically significant difference among all groups as P=0.04, followed by Tukey`s Post Hoc test for multiple comparisons which revealed that, group I (white tea) yielded the significantly highest mean of percent of change (35.91 ± 0.11), group III (control group) revealed the significantly lowestmean of percent of change (22.37 ±0.09), while group II(green tea) showed insignificant difference with other groups (26.36 ± 0.09).

Table (2): Mean ±SD and P-value of percent of change in all groups and comparison between them using One Way ANOVA test followed by Tukey`s Post Hoc test:

	Percent of change (MH%)		
	M %	SD	P value
Group I (White tea)	35.91 [a]	0.11	0.04*
Group II (Green tea)	26.36 [ab]	0.09	
Group III (Control)	22.37 [b]	0.09	

**Significant difference as P<0.05.*

Means with different superscript letters were significantly different as P<0.05.

Means with the same superscript letters were insignificantly different as P>0.05.

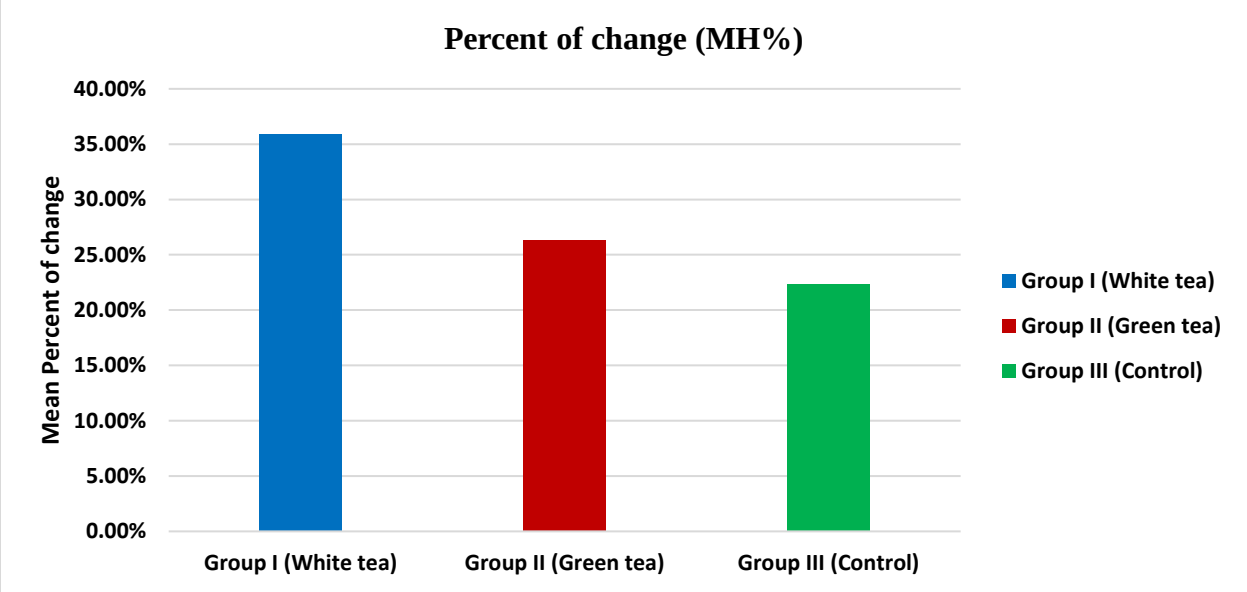

Figure (4): Bar chart showing mean of percent of change (MH%)in all groups.

Discussion:

The concept of minimally invasive dentistry via remineralization of demineralized dentin has a significant importance in preserving the remaining tooth structure.Numerous remineralizing techniques and agents have been extensively researched, and many of them are now being used clinically with highly positive results. (13)

Remineralization of dentin is more challenging, because dentin contains less minerals and subsequently has lower hardness as compared to enamel, which affect its mechanical properties. (14) Dentin collagen matrix acts as a scaffold for precipitations of mineral crystallites, so preservation and maintaining the stability of collagen is essential during the remineralization process. (12)

Caesin phosphopeptides (CPP) stabilizes calcium phosphate in nano complexes because of the presence of multi phosphoseryl sequences in amorphous calcium phosphate (ACP) solutions. Moreover, multiple phosphoseryl sequences aids in binding the CPP to ACP in a metastable solution, thus inhibiting dissolution of phosphate and calcium ions.(9) CPP-ACP is known to stabilize only the inorganic components of the tooth. However, the true functional remineralization involves stabilization of inorganic and organic components, which could be possible by collagen stabilizing agents such as green tea and white tea during remineralization process. (9)

Hence in this study white tea extract and green tea extract pretreatment were comparatively assessed for their effect on remineralization of artificially demineralized dentin, before application of (CPP-ACP) using microhardness test.

White tea has the least amount of caffeine and less processed compared to green and black tea. The most important ingredient in white tea is polyphenols, which are natural antioxidants. It contains flavanols or catechins, such as epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG), and epicatechin (EC). (15) Moreover, it has an inhibitory activity on collagenases which degrade the organic matrix. Dentin surface pretreatment with white tea is known to inhibit proteolytic degradation, prevents MMP release and enhances the mechanical properties of collagen, thereby producing collagen stabilization.(16)

Green tea is one of the most herbals used in many researches due to their high content of polyphenols (catechins) like epigallocatechin gallate (EGCG) that exhibits profound inhibitory effect on both collagenase and elastase. Additionally, green tea exhibits antibacterial properties attributed to its bioactive compounds like polyphenols, minerals, and volatile oil. The high fluoride concentration in green tea also contributes to its remineralizing effect.[17]

The current study utilized an artificial caries model, in which a demineralization duration of three days was chosen, as a longer period may deplete the collagen matrix of mineral and consequently inhibit the remineralization. Following thisprotocol, a lesion of approximately 150μm depth with a micro hardness being similar to a natural carious lesion of the same depth was obtained.[3]

After treatment, all samples were stored in artificial saliva which was renewed every 24 hours to stimulate, as possible, the oral cavity conditions. It has been proven that the artificial saliva could act as a chemical reservoir for calcium and phosphate ions promoting the remineralizing process. [14]

Knoop and Vickers are common types of microhardness tests which are used for dental purposes. However, the Vickers microhardness test is considered more suitable for assessing the microhardness of the tooth surface because it causes less surface bending.[18]

In this study, Vickers microhardness test was utilized for evaluation of surface hardness of dentin samples initially at baseline, after demineralization and after treatment. This assessment method provides a simple, reliable , non-destructive and rapid method. In addition, it is considered by many researches. [11]

Results of the microhardness mean values demonstrated that, there was statistically significant increase in microhardness after treatment in white tea and green tea groups. This could be related to their main composition of flavonoid (catechins) that stabilized dentin collagen matrix prior to application of casein phosphopeptide amorphous calcium phosphate which resulted in functional remineralization of dentin.

This was in agreement with previous studies [4,9] which revealed that both green tea and white tea increased the microhardness

values when used as pretreatment agents for remineralization of demineralized dentin.

Regarding the control group, statistically significant increase in microhardness mean values was found after treatment which might be due to the capability of CPP-ACP to maintain high concentrations of phosphate and calcium ions on dentin surfaces, thereby promoting remineralization.[19] This was in agreement with previous study which revealed that application of CPP-ACP increased the microhardness of demineralized dentin. [20]

Results of the percent of change (MH%) of microhardness of dentin revealed that dentin samples pre-treated with white tea yielded the significantly highest mean percent of change of microhardness followed by green tea group, this could be due to the superior anticollagenolytic action of white tea. However, the control group revealed the significantly lowest mean percent of change which could be attributed to the superficial mineral deposits rather than functional mineralization.

As the successful repair of demineralized dentin not only relies on the presence of mineral deposits but also on their proper positioning within the organic matrix. The relationship between the organic and inorganic components directly influences the mechanical properties of dentin.[21]

These findings are in accordance with a similar study which concluded that white tea and green tea extracts increased the microhardness values when used as pretreatment regime for remineralization. However, white tea showed better results as compared to green tea indicating stabilization of collagen in dentin resulting in functional remineralization[9].

Conclusion:

According to the results of the study, dentin pretreatment with white tea and green extract increased the microhardness of artificially demineralized dentin which indicates the occurrence of functional remineralization. This effect is likely attributed to the cross-linking of the collagen network, which plays a role in stabilizing the collagen structure and maintains the collagen network in an expanded state that creates open intrafibrillar spaces, enhancing the process of remineralization.

Recommendations:

Further clinical studies are recommended.

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