Remineralizing Effect of White Tea, Green Tea and Casein Phosphopeptide Amorphous Calcium Phosphate (CPP-ACP) on Artificially Demineralized Enamel (In-Vitro Study)

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Abstract:

Purpose: The aim of this study was to evaluate the remineralizing effect of white tea, green tea and casein Phosphopeptide amorphous calcium phosphate (CPP-ACP) on demineralized enamel by microhardness testing.

Materials and Methods: A total of 24 enamel samples were divided according to enamel pretreatment after artificial demineralization of 8 samples each, group I (white tea + CPP-ACP, group II (green tea + CPP-ACP) group III (CPP-ACP), microhardness was evaluated at baseline, after demineralization and after treatment for all enamel samples.

Results: there was insignificant difference between groups where group I was insignificantly the highest, followed by Group II & group III.

Conclusions: the use of CPP-ACP with tea increased the remineralization potential.

Key words: White tea, green tea, microhardness, Casein Phosphopeptide amorphous calcium phosphate (CPP-ACP), remineralization.

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Introduction:

Dental enamel is hard, a cellular and avascular tissue, consists of 96% inorganic material (hydroxyapatite nanocrystals), 3% water, and 1% organic portion. The ameloblasts produces the enamel protein matrix which is then mineralized by calcium phosphate crystals. When caries starts to progress ,the enamel crystals spaces starts to widen. Which leads to the formation of chalky white lesion which is distinguished without drying from the surrounding healthy enamel. The enamel starts to lose its translucency as water is replaced gradually by air between the enamel prisms[1].

Remineralization can occur normally for carious lesions to a limit but when bacterial acid challenge is severe, natural remineralization cannot stop or reverse the caries process; Therefore, the development of novel antibacterial and remineralizaing dental materials is essential to fight caries [2].

Tea is the most consumed famous beverage in the world. It is extracted from the leaves of evergreen tree Camellia sinensis, tea is classified into four main categories according to oxidation process, i.e., green (little oxidized), oolong tea (semi-oxidized), black (fully oxidized) and white tea (un oxidized)[3].

White tea is the least processed form of tea, it consists of young buds and leaves which is silvery or whitish in color. White tea is said to have the highest content of catechins, it has an anti-collagenolytic action on the collagen network which may stabilize the collagen and maintain the collagen network in an expanded state so that the intrafibrillar spaces are left open for remineralization[4].

Green tea contains minerals as potassium, phosphorus, calcium, and magnesium. Green tea is characterized by high fluoride content (over 1500 ppm) as well as its catechins content like epigallocatechin gallate (EGCG) which act as an enamel matrix metalloproteinases (MMPs) inhibitor[5]. Green tea has many health benefits which depends on various factors such as plucking season, processing, extraction, storage and drying. Green tea extract has antioxidant, anti-inflammatory and anticarcinogen activity[6].

Combination of Amorphous Calcium Phosphate (ACP) with Caesin Phospho Peptides (CPP) forms a complex (CPP – ACP) known to be helpful in calcium and phosphate ions precipitation and in remineralization of teeth. Caesin phospho peptides stabilizes calcium phosphate in nano complexes which is known to stabilize the

inorganic content of the tooth only. while the true functional remineralization involves the stabilization of both organic and inorganic components. So remineralization may be possible with the aid of anti-collagenolytic agents[7].

Therefore, this study aimed to evaluate the effect of white tea extract and green tea extract pretreatment followed by Casein Phosphopeptide amorphous calcium phosphate (CPP-ACP) application on remineralization of artificially demineralized enamel using microhardness test.

Materials and Methods:

Study design

A total of 24 enamel samples were collected from human molars. Samples were immersed in demineralizing solution for 48 hours to create artificial carious lesions. Then, they were divided into three groups of 8 samples each according to the utilized treatment. In the first group, enamel 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 enamel 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 enamel 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#50).

Sample Size Calculation of the study

Sample size calculated depending on a previous study[8]. If mean ± standard deviation of control group is 192.33 ± 36.13, the estimated difference is 60, minimally the study needed 7 subjects in each group. Sample size was performed by using independent t test by using G. power 3.1.9.7.

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 solution

10 grams of white tea powder was measured by electronic balance, then 100 ml of boiled distilled water were poured, stirring for 1 minute, then filtered using No(1) filter paper (Whatman, united kingdom)[7].

Preparation of green tea solution

10 grams of tea powder was measured by electronic balance, then 100 ml of boiled distilled water were poured, stirring for 1 minute, then filtered using No (1) filter paper (Whatman, united kingdom)[7].

Teeth selection

A total of 12 Freshly extracted sound human molars were collected, teeth with fractures, cracks, caries, defects and anatomical variations were not included in the study, teeth were cleaned and scaled to remove any remaining soft tissue, and then the teeth were stored in distilled water at 4°C [9]till time of their use (within one month).

Sample preparation

All roots were removed, each tooth was sectioned in mesio distal direction to obtain two halves buccal and lingual with total number of 24 enamel specimen, then each half was embedded in self-curing acrylic resin (Acrostone Dental Factor, England) (Fig 1) .

Figure (1): buccal half of a molar tooth embedded in self cured acrylic resin

Baseline Microhardness testing

Microhardness measurements were taken for each specimen at baseline. Surface microhardness of the specimen was determined using Digital Display Vickers micro-hardness tester with a Vicker diamond indenter and 20X lens. Three indentations were made on the surface of each specimen. These indentations were equally placed over a circle and not closer more than 0.5mm to the adjacent indentation. A load of 25g was applied onto the surface of the specimens for 5 seconds (Fig 2)[10]. A built in scaled microscope was used to measure the diagonals length of the indentations and Vicker values were converted into microhardness values.
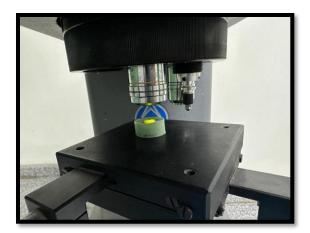
Figure (2): Measuring Microhardness values

Preparation of artificial carious lesion

All specimens were immersed in a demineralizing solution (10 mL for each specimen) for 48 hours the solution was renewed every 12 hours. The demineralizing 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) pH was 4.4. The samples were rinsed thoroughly with distilled water and stored in artificial saliva and evaluated again for surface microhardness [5].

Immersion procedure:

All specimens were randomly divided into three equal groups according to type of treatment group (I) demineralized enamel treated with white tea + Cpp-Acp , group (II) demineralized enamel treated with green tea + Cpp-Acp, group (III)demineralized enamel treated with Cpp-Acp .

Each specimen was immersed in white tea extract solution for 10 minutes followed by casein phosphopeptide amorphous calcium phosphate application for 3 minutes according to the manufacturer's instructions. In the second group, specimens were immersed in green tea extract solution for 10 minutes[11,12] followed by casein phosphopeptide amorphous calcium phosphate application for 3 minutes according to the manufacturer's instructions, while for the third group (positive control) casein phosphopeptide amorphous calcium phosphate will be applied. All samples were stored in artificial saliva for one week which was prepared using Na PO (3.90mM), KCl (17.98mM), NaCl (4.29mM), MgCl2 (0.08mM), CaCl2 (1.10mM), NaHCO3 (3.27mM), H2SO4 (0.50mM) and distilled water at room temperature followed by surface microhardness evaluation.

Microhardness evaluation after treatment

The enamel micro-hardness was tested for sound enamel and after enamel Demineralization then after treatement , then the The percentage of Vickers Micro-hardness value was calculated using the following equation[13]:

(MH%) : <u>(Final MH-demineralized MH)</u>
<u>x   100</u> (Initial MH-demineralized MH)

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 $p \leq 0.05$

Results:

Effect of treatment (comparison between baseline, demineralization, and after treatment):

Mean and standard deviation of all groups at baseline, demineralization and after treatment were presented in table (1) and figure (3).

Comparison between different intervals was performed by using One Way ANOVA test which revealed significant difference in all groups as $P<0.0001$, $= 0.0176$, <0.0001 regarding group I, II and III respectively. Followed by Tukey`s Post Hoc test for multiple comparisons which revealed that:

Group I (White tea): there was a significant decrease from (309.29 ± 21.47) at baseline to (238.29 ± 21.66), then there was a significant increase to (281.43 ± 14.18).

Group II (Green tea): there was a significant decrease from (295.71 ± 36.53) at baseline to (232.86 ± 38.77), then there was an insignificant increase to (271.14 ± 6.38).

Group III (Control): there was a significant decrease from (296 ± 6.66) at baseline to (244.14 ± 12.4), then there was a significant increase to (271.29 ± 6.87).

Table (1): Mean and standard deviation of 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)	309.29 [a]	21.47	238.29 [b]	21.66	281.43 [c]	14.18	<0.0001*
Group II (Green tea)	295.71 [a]	36.53	232.86 [b]	38.77	271.14 [a,b]	36	0.0176 *
Group III (Control)	296 [a]	6.66	244.14 [b]	12.4	271.29 [c]	6.87	<0.0001*

*M: mean   SD: standard deviation   *Significant difference as P<0.05.*
Means with different superscript letters were significantly different as P<0.05.
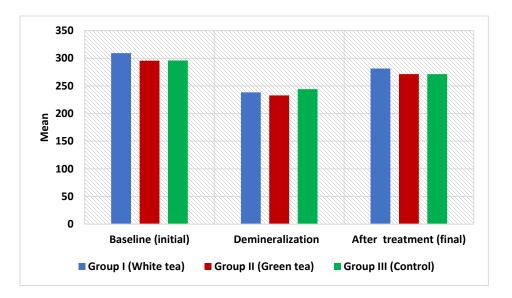*Means with the same superscript letters were insignificantly different as P>0.05.*

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

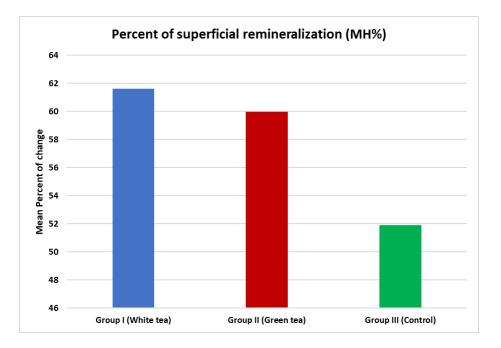**Effect of different materials (comparison between White tea, green tea and control):**

Percent of superficial remineralization (MH%) of enamel of all groups were presented in table (2) and figure (4).
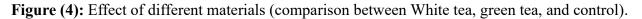
Comparison between different groups was performed by using One Way ANOVA test which revealed insignificant difference between them as P=5735, followed by Tukey`s Post Hoc test for multibed comparisons which revealed that group I (61.61 ± 19.86) was insignificantly the highest, Group III (51.92 ± 18.34) was insignificantly the lowest, while group II (59.96 ± 15.92) revealed insignificant difference with other groups.

Table (2): Mean and standard deviation of % of change in all groups and comparison between them using One Way ANOVA test followed by Tukey`s Post Hoc test:

	% of change		
	M %	SD	P value
Group I (White tea)	61.61 ^{a}	19.86	
Group II (Green tea)	59.96 ^{a}	15.92	0.5735 (NS)
Group III (Control)	51.92 ^{a}	18.34	

M: mean   SD: standard deviation   *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): Effect of different materials (comparison between White tea, green tea, and control).

Discussion

The scope of preventive and minimally invasive dentistry nowadays is guided towards the need for new approaches to remineralize initial enamel lesions. As tooth decay is highly reversible at early stage, enamel and dentin remineralization can be done with the inhibition of biofilm formation and the action of salivary protective factors. In recent years, caries research has been shifted to the development of new methodologies for the non-invasive management of early carious lesions through remineralization to preserve tooth integrity [14].

Remineralizing agents containing fluoride are found in different types and concentrations, calcium and phosphate ions are commercially available as Caesin Phospho Peptides (CPP) which form a complex (CPP – ACP) which release active ions that bond stably to the crystalline enamel structures leading to the formation of new crystals and reassembling the damaged ones [15].

Considering the current tendency in the field of oral health which emphasize the use of natural products for treating diseases rather than conventional way. Several herbal and other natural products have been studied as remineralizing agents. Depending on their specific component which affect mineral saturation and precipitation which could acts as an antimicrobial agent or stabilize collagen which act as a scaffold for mineral deposition [16].

Enamel is the hardest tissue in human body. It is composed of organic and inorganic components that are essential in its natural mineralization processes. Dental enamel consists of intertwined and tightly packed mineral rods (which are called prisms) surrounded by proteins, these rods or prisms consists of protein-bound hydrox- yapatite (HA) fibers which run parallel and located at certain angles to the enamel prisms to ensure a high degree of hardness with certain amount of mechanical elasticity to prevent catastrophic breakdown [17].

White tea origin comes from a plant named Camelia sinensis. Camellia sinensis is a tree belongs to the Theaceae family. Even though its origin is in China mostly from Fujian, Tibet and Northern India, nowadays it is cultivated all over the world. The white tea is covered with a thin layer of silvered hair giving it its unique color, it is not crushed or rolled but is fermented to a certain extent , and the leaves are naturally dried in air to preserve its benefits. It is considered as the

least processed form of tea, and lesser amount of caffeine compared to green and black tea.

The most dominant ingredient in white tea is polyphenols, which is conisered as a natural antioxidant. It consists of flavanols or catechins, such as epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechingallate (ECG), and epicatechin (EC). The catechins has superior anticollagenolytic action. White tea has beneficial effects on many diseases such as neurodegenerative and cardiovascular diseases, obesity and diabetes. [18,19]

Green tea is a short time fermented tea and it has also a plenty of polyphenols, while black tea is fermented for a longer period of time . Tea leaves are rich in polyphenols (catechins) and fluoride which play an important role in combating caries [20].

Regarding Effect of treatment (comparison between baseline, demineralization and after treatment) showed that there was significant difference in all groups as there was a significant decrease from baseline to demineralized then there was a significant increase after treatment which might be due to the effect of CPP-ACP which was used in all groups as The mineralizing mechanism of CPP-ACP (MI)Varnish based on the

increasing level of calcium phosphate which is able to decreases enamel demineralization and enhances remineralization. This is due to the sticky nature of Casein phosphopeptide (CPP) that able to bind to enamel and providing a reservoir of bio-available calcium and phosphate in the saliva and on the surface of the tooth through the release of calcium and phosphate ions from amorphous calcium phosphate (ACP) in response to acid attack[21].

Regarding Effect of different materials (comparison between White tea, green tea and control) Percent of superficial remineralization (MH%) of enamel revealed insignificant difference between them where group I (white tea) was the highest, followed by group II (green tea)while Group III (control) was insignificantly the lowest, which may be due to the presence of polyphenols named catechins in white tea and green tea such as epicatechin (EC), epigallocatechin (EGC), epicatechingallate (ECG) and epigallocatechin gallate (EGCG). Which were considered to be a MMP inhibitor Moreover, white tea is considered as antioxidant which prevents the action of oxidants [22-24].

The results of this study are in agreement with previous studies[25,26] as tea (green

&white) has the ability of protecting the demineralized enamel by increasing the surface micro hardness and due to large amount of catechins in tea which reduces the wear of enamel and enhances their surface quality.

Conclusions:

According to results of this study, all tested remineralizing treatments showed degree of remineralization of white spot lesion and the use of CPP-ACP with tea increased the remineralization potentiality further time is needed for storage in artificial saliva to restore the baseline surface microhardness.

Recommendations:

Further clinical studies are recommended.

Acknowledgement:

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