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Cover Page Footnote

This paper would not have been possible without the exceptional support of my supervisors. Their enthusiasm, knowledge and exacting attention to detail have been an inspiration and kept my work on track. Last but not least, my special thanks for all staff members of operative dentistry in Future University, for standing by my side all through the course of this study.

Comparative Evaluation for the Effect of Green tea, Aloe vera and Chlorhexidine on Dentin Erosion

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Abstract

Purpose: This study aimed to compare the anti-collagenolytic effect of Green tea, Aloe vera and chlorhexidine on artificial erosion in human dentin using microhardness test and environmental scanning electron microscope (ESEM). Materials and Methods: For this study, 60 extracted sound human maxillary premolars were subjected to artificial erosion and randomly divided into three equal groups (20 premolars each) according to immersion in the tested anti-collagenolytic agents; group (A1) Green tea, (A2) Aloe vera and group (A3) Chlorhexidine (CHX). The microhardness analysis and (ESEM) photomicrographs were taken for the surface of dentin specimens three times, at base line, after the artificial erosion and after immersion in each treatment solution. Bonferroni's post-hoc test was used for pair-wise comparisons. Statistical analysis was performed with IBM SPSS Statistics for Windows. Results: Results showed no statistically significant difference in microhardness mean values between Aloe vera and (CHX), However, both showed statistically significant increase in microhardness values than Green tea. These results were also supported by (ESEM) photomicrographs. Conclusion: Chemical gold standard CHX could be substituted by natural safe herbal sources and hence Aloe vera opens a new perspective in management of dental erosion.

Keywords: Green tea, Aloe vera, Chlorhexidine, Dentin, Erosion, Microhardness

1. INTRODUCTION:

Teeth erosion is a complicated process. It does not affect the enamel surface only, in its severe cases it is associated with dentin exposure, this might be accompanied by hypersensitivity[(1)].

Matrix metalloproteinases (MMPs) are suggested to play a critical role in the organic matrix destruction of dentin followed by demineralization[(2)]. Therefore, MMP inhibitors were considered as potent curative agents in the prevention and treatment of many diseases and to reduce dentin organic part destruction[(3)].

Chlorhexidine is considered as the most remarkable dentin enzymes inhibitor. It possesses an inhibitory action on dentin MMPs 2, 8 and 9 and could reduce erosion [(4,5)]. Nevertheless, it also has many side effects, as teeth staining and taste alteration. Consequently, folk medicine and drugs from plant origin have gained great attention.

Green tea is the most consumed natural beverage that is obtained from tea plant (Camellia Sinensis). It consists of high levels of polyphenol

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(catechins). Tea polyphenols (TP) are vigorous antioxidants and considered as caries preventive agent and supposed to be the crucial component in tea in reduction of dental caries, specifically (EGCG) epigallocatechin-3-gallate which has distinct inhibitory action on MMP 2 and 9 [(6)].

Aloe Barbadensis Miller commonly referred to as Aloe vera, has been used as natural therapeutic agent due to its antioxidant, anti-inflammatory, antimicrobial and curing properties. Aloe vera gel has MMP inhibition action on MMP-2 and MMP-9 and it is very effective in stabilizing dentin collagen and preventing its destruction through its potent MMPs inhibition[(7)]. Thus this study was done to compare the anti-collagenolytic effect of Green tea, Aloe vera and chlorhexidine on erosive like lesions in human dentin.

2. MATERIALS AND METHODS:

Materials preparation:

(a) **Green tea:**

Solution was prepared as stated in the manufacturer's instructions, by adding a ready prepared tea bag which contains 2 gm of crushed dried leaves of Cammelia Sinensis to 100 ml of boiled deionized water (100°C) for five minutes. The tea was cooled off for four minutes at room temperature (37°C)[(8)].

(b) **Aloe vera**:

The Aloe vera solution was prepared by dissolving 2 gm of Aloe vera powder (Procured From Indigo Herbs Ltd Products, Wells Rd, UK) of 99% purity A. Barbadensis Miller measured by analytical balance in 100 ml of deionized water[(9)].

2.1. Chlorohexidine:

100 ml of 0.12% chlorhexidine hydrochloride, (Hexitol mouth rinse, ADCO The Arab Drug Company, Cairo- Egypt LOT:21918) was used without dilution.

Teeth selection and grouping:

Sixty freshly extracted sound human maxillary first premolars were selected in the current study. Teeth were randomly divided into three equal groups (20 premolars each) conforming to type of the tested anticollagenolytic agent: group (A1) Green tea, group (A2) Aloe-vera and group (A3) Chlorhexidine. The microhardness analysis and (ESEM) photomicrographs were taken for the surface of dentin specimens three times; at base line, after the artificial erosion and after immersion in each treatment solution

Specimen preparation:

The teeth were sectioned longitudinally, by slow speed saw cutting; ISOMET 4000 (Buehler Ltd., USA) in order to expose the dentin of the buccal aspect of the tooth. The specimens with the roots were embedded in self polymerizing acrylic resin[(10)]. A carbon pencil was used to mark a horizontal small window on the cervical coronal part of the buccal surface of each specimen. Each specimen was coated with dark color acid resistant nail varnish except for (3×3 mm) box for application of tested materials[(9–11)]. Baseline surface microhardness of the dentin specimen was recorded by using Vicker's microhardness tester[(12,13)].

Erosive lesions preparation:

For preparation of artificial erosion, the prepared teeth specimens were immersed in a glass container filled with 250 ml of Coca-cola(R) (The Coca-Cola Company, Cairo – Egypt) at room temperature for 25 hours. The solution was changed each 5 hours. This protocol permits for observing the demineralization in a suitable time period [(14,15)]. Then the teeth were rinsed and kept in de-ionized water (pH 6.0).

Specimens treatment protocol :

The erosive dentin specimens had been soaked in a glass container filled with 100ml of each testing solutions under constant agitation at room temperaturefor one minute. The specimens were kept in de-ionized water at 37°C for next day. This procedure was repeated daily for 7 days[(18,19)].

Micro-hardness assessment:

The dentin microhardness was measured on each specimen three times, at baseline time, after the artificial erosion and after immersion in each treatment solution. Surface microhardness was recorded by using Vicker's microhardness tester with a 25gm load applied for 5 seconds. Vickers Hardness Number (VHN) of three indentations apart from each other by 100 microns were

recorded and the average mean values were evaluated for each specimen[20].

Environmental Scanning Electron Microscope (ESEM) examination:

For the environmental scanning electron microscope (ESEM) examination, a representative specimen from each group was examined three times, at baseline time, after the artificial erosion and after immersion in the treatment solution. The morphologic character was evaluated using back scattered mode of environmental scanning electron microscope with accelerating voltage 30 K.V, at magnifications of 4000X[21].

Statistical analysis :

Numerical data had been investigated for normality through checking the data distribution and utilizing normality tests (Kolmogorov-Smirnov and Shapiro-Wilk tests). Microhardness data exhibited parametric distribution. Data had been presented as mean and standard deviation (SD) values. Bonferroni's post-hoc test had been used for pair-wise comparisons to study the effect of different treatment solutions on microhardness. The significance level had been appointed at P $\leq$ 0.05.

3. RESULTS:

3.1. Microhardness results:

The data in table (1) figure (1) shows the mean, standard deviation (SD) values and results of repeated measures ANOVA test for comparison of dentin microhardness of the three tested materials of each group at different measurement times (base line, after erosion and after immersion in the tested material). It was revealed that, there was a statistically significant difference in microhardness mean values at baseline, after erosion and after immersion in the tested groups; green tea, aloe vera, and chlorohexidine at (P-value <0.001, Effect size = 0.736, 0.793, and 0.773) respectively. pair-wise comparison between the groups showed that**;** there was a statistically significant decrease in microhardness after erosion followed by a statistically significant increase in microhardness after immersion in all the tested materials. However, the mean microhardness after immersion showed statistically significant lower mean value compared to the base line measurement.

The data in table (2) figure (1) shows the mean, standard deviation (SD) values and results of repeated measures ANOVA test for comparison of dentin microhardness of the three tested groups it was revealed that, there was no statistically significant difference between the three materials at base line as well as after erosion, at (P-value =0.998, Effect size = 0.0001) and at (P-value = 0.946, Effect size = 0.002), respectively. However, there was a statistically significant difference between the three materials after immersion at (P-value = 0.001, Effect size = 0.219). Pairwise comparisons between the materials revealed that there was no statistically significant difference between Aloe-Vera and CHX; both showed statistically significantly higher mean microhardness values than Green tea.

*: Significant at P $\leq$ 0.05, Different superscript letters indicate a statistically significant difference within the same row.

*: Significant at P $\leq$ 0.05, Different superscript letters indicate a statistically significant difference within the same row.

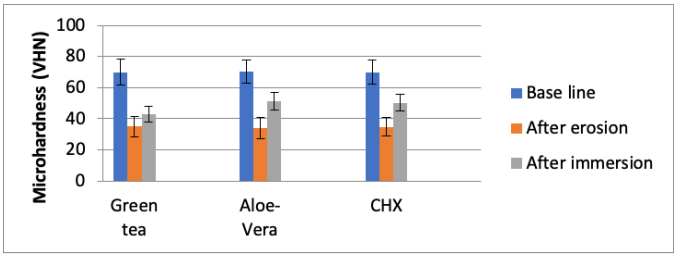

Figure 1: Bar chart representing mean and standard deviation values ofcomparison of microhardness measurements of each tested group in different levels of the study.

4. Environmental Scanning Electron Microscope (ESEM results:

ESEM examination of the dentin specimen morphology at the cervical third of the buccal surface without any treatment (Figure 2), showed the normal surface features of sound dentin. The ESEM examination of the dentin specimen morphology following the artificial erosion with coca

Table 1: The mean, standard deviation (SD) values and results for comparison between microhardness values of dentinin each tested group.

Material	Base line		After erosion		After immersion		P-value	Effect size
	Mean	SD	Mean	SD	Mean	SD		
Green tea	70^{A}	8.3	34.9^{C}	6.4	42.9^{B}	5.3	<0.001*	0.736
Aloe vera	70.2^{A}	7.6	34^{C}	6.6	51.4^{B}	5.8	<0.001*	0.793
CHX	70^{A}	7.5	34.7^{C}	6	50.3^{B}	5.5	<0.001*	0.773

Table 2: The mean, standard deviation (SD) values and results of comparison between dentin microhardness values of the three testedgroups.

	Green tea		Aloe vera		CHX		P-value	Effect size
	Mean	SD	Mean	SD	Mean	SD		
Base line	70	8.3	70.2	7.6	70	7.5	0.998	0.0001
After erosion	34.9	6.4	34	6.6	34.7	6	0.946	0.002
After immersion	42.9^{B}	5.3	51.4^{A}	5.8	50.3^{A}	5.5	0.001*	0.219

cola® beverage (Figure 3), revealed demineralized dentin surface.

On comparing the effect of Green tea (Figure 4), Aloe vera (Figure 5) and chlorohexidine (Figure 6) respectively, on the eroded dentin specimen there was an obvious improvement in the morphological appearance in CHX and Aloe vera groups more than Green tea group.

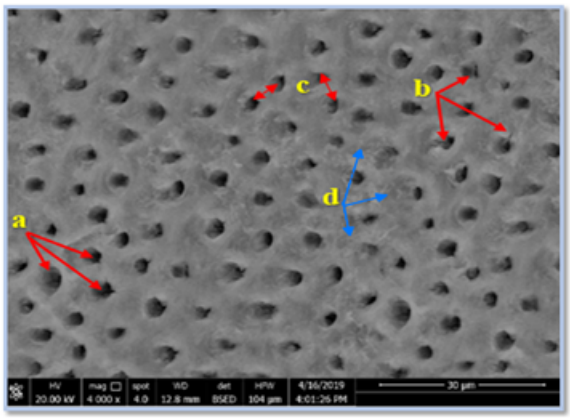

Figure (3): ESEM image of dentin surface treated with coca cola at 4000X showing: (a) Dentinal tubules opened and widened by cola application with different shapes, (circular or oval). (b) Few areas of fine crack some of them radiating from D.T and some present in the intratubular dentin. (c) Intertubular dentin apparently small. (d) Irregular dentin surface with some areas of erosion (blue arrows).

Figure 2:

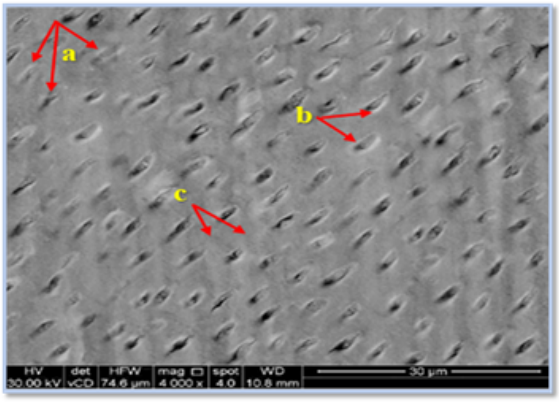

Figure (2): ESEM image of the buccal cervical third of dentin surface for the base line group at 4000X showing: (a)Occluded and partially occluded dentinal tubules. (b)The highly dense mineralized ring peritubular dentin. (c) The smooth regular surface with normal intertubular dentin space.

Figure 3:

5. DISCUSSION:

Over the last few years, erosion has identified as a critical cause of tooth tissue loss. Erosion progression can be prohibited or postponed by matrix metalloproteinases (MMP) inhibitors as human MMPs 2, 8 and 9 which have a key role in the destruction of dentin collagen. Few researches have adopted studying upon the correlation between

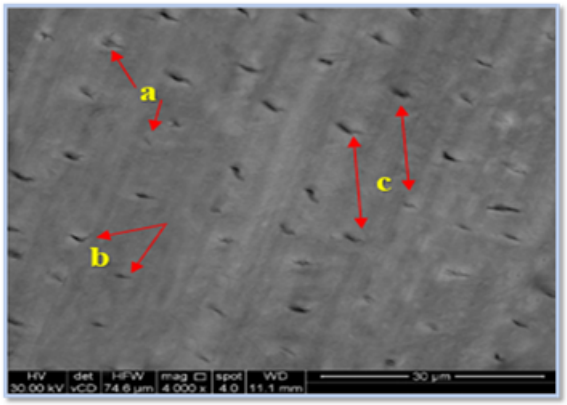

Figure (5): ESEM image of the eroded dentin surface treated with Aloe vera at 4000X showing: (a) Most of dentinal tubules were totally clogged. (b) Some few dentinal tubules were apparently narrow. (c) Intertubular dentin was apparently wide area of almost even dentin surface denoting absence of erosion.

Figure 4:

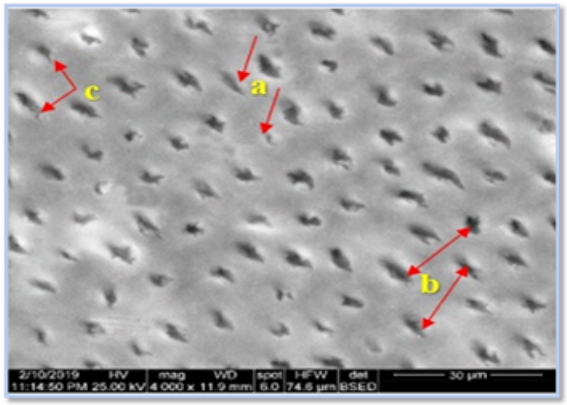

Figure (6): ESEM image of the eroded dentin surface treated with CHX at 4000X showing: (a) Partially and completely occluded tubule orifices were apparent on dentin surface (b) Intertubular dentin was apparently wide area (c) Very few cracks appeard in the D.T.

Figure 6:

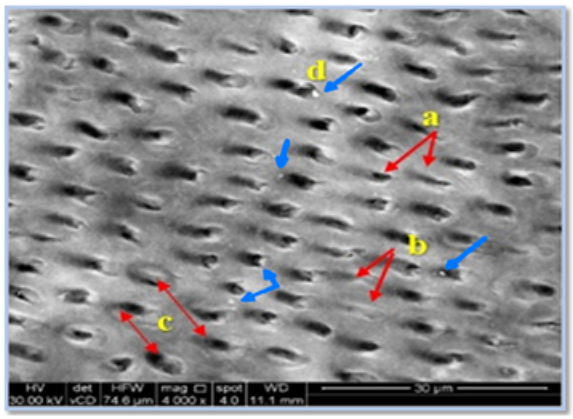

Figure (4): ESEM image of the eroded dentin surface treated with Green tea at 4000X showing: (a) Most of dentinal tubules were narrow. (b) Some few dentinal tubules were totally clogged. (c) Intertubular dentin was apparently wide area. (d) Apparently small spicules of mineral deposition (blue arrows).

Figure 5:

dental erosion and the consumption of natural products(22). Accordingly, the present study was carried on agents extracted from natural products which noted to have anti-MMP action, in particular against MMP-2 and 9 to stabilize dentin collagen in order to assess the influence of Green tea and Aloe vera on eroded dentin in comparison to chlorohexidine.

Green tea was selected for this study due to its rich polyphenols content. The polyphenols are the most critical group in tea components, particularly catechins: epigallocatechin gallate (EGCG) that had strong antioxidant activity and distinct inhibitory activity against matrix metalloproteinases[12,21].

Aloe vera was selected as this material is considered as a miracle plant due to its magical healing property in various human diseases. It is a globally popular natural product used in folk medicine. Aloe vera bioactive components have the ability to inhibit collagenase activity[23].

In this study Vickers microhardness test was chosen because it is considered as one of the most suitable experimental tools for detecting dentin collagen stabilization and indirect way to evaluate dental erosion indicating remineralization or demineralization of the dentin[13,18]. Moreover, environmental scanning electron microscopy (ESEM) was used for confirmation of the organic material on dentin surface[19]. ESEM has proved to be a valuable method for the assessment of dentin remineralization and could accurately evaluate biomimetic collagen stabilization and dentin surface changes[9,20].

The results of this study showed increase in microhardness mean values of dentin after immersion in all the tested groups. However, the mean

microhardness values after immersion showed statistically significant lower mean values compared to base line as it did not completely return to normal values of sound dentin which is in agreement with several studies[6,17]. These results could be explained depending on the fact that, all the tested materials were proved to have potent anti-collagenolytic activity on dentin and inhibitory action on dentin MMP activity. The explanation of microhardness enhancement caused by Green tea might be credited to polyphenols, specifically epigallocatechin-3-gallate (EGCG). These suggestions were in line with other studies[24], which attributed the preventive effect of Green tea on teeth erosion to presence of the active components in Green tea: flavonoids and polyphenols, particularly (EGCG), which possess anti-MMPs ability and can preserve dentin collagen from degradation. These findings also were in line with a previous study study[25].

The increase in VHN value observed with Aloe vera powder could be related to its main composition of flavonoid (anthocyanins) which is almost in agreement with a previous study[24]. Such an increase in dentin microhardness following immersion in Aloe vera goes in the same line with a study[17]which evaluated the inhibitory impact of Aloe vera on MMP-2 and MMP-9 and proved that aloin is the active component in this plant.

The inhibitory impact of chlorhexidine on MMPs is related to it mechanism of chelation. It was similarly reported that chlorhexidine could affect cysteine and sulfhydryl groups in the active site of MMPs and the essential sulfhydryl groups[25]. This results are supported by another study[5] which stated that both CHX and Aloe vera have possible potential for erosion inhibition, due to their inhibition activity on MMPs. Moreover, these findings were totally supported by data of a recent study[25] which noted that, application of CHX and Green tea agents improved the longevity of resin-dentin bonds by inhibiting MMPs, although CHX gave much better results.

On comparing the actions of the three tested materials it was revealed that, there was no statistically significant difference between Aloe vera and CHX; both exhibited statistically significant higher mean microhardness values than Green tea. This could be related to the strong MMPs inhibitory action on dentin of these two materials. These results were in agreement with a previous study[3] which studied and compared the effect of both CHX and Aloe vera as pretreatment agents on acid etched dentin depending on their inhibitory action on MMPs. Their results revealed that, there was no statistically significant difference between CHX and Aloe vera, both could preserve dentin collagen equally[5].

The lower results of green tea in our study could be for the reason of the interference of other components of green tea as we did not use aqueous epigallocatechin gallate extract, while in aloe vera we use aqueous solution of 99% purity A. Barbadensis Miller extract. These results were also confirmed by ESEM findings which showed more improvement in eroded dentin surface treated with Aloe vera and CHX groups compared to Green tea group[1].

6. CONCLUSIONS:

Under the limitations of this study, the followings could be concluded:

1. All the tested materials had a positive effect on eroded dentin surface. However, Aloe vera had comparable effect with CHX concerning dentin microhardness enhancement as well as surface morphological alterations.

2. Chemical gold standard CHX could be substituted by natural safe herbal source and hence Aloe vera opens a new perspective for dental erosion protection.

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