Original Article

Effect of Green Tea Extract on the Treatment of Dentin Erosion: An in Vitro Study

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Abstract

Objective: The aim of this study was to analyze the impact of green tea on dentin erosion.

Materials and Methods: Twelve extracted sound human premolars were immersed in Coca Cola with a pH of 2.8 for 5 minutes. The surface microhardness was measured with a Knoop diamond under a load of 50gr/10s. The teeth were immersed in green tea (*Camellia sinensis*) solution for one minute. The microhardness values were measured again and compared with pretreatment values by the Wilcoxon test. Three eroded teeth, which were treated with green tea, were evaluated under scanning electron microscope.

Results: The mean ± SD of microhardness values before and after immersion in green tea were 46.5±2.79 and 54.5±4.4, respectively with statistically significant differences between the two measurements (P<0.01). In SEM evaluation there was an improvement in eroded dentin appearance and there were deposits on the dentin surface.

Conclusion: Green tea (*Camellia sinensis*) increased the microhardness of eroded dentin and improved the eroded texture.

Key Words: Herbal Tea; Tooth Erosion; Dentin; Hardness

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INTRODUCTION

Dental erosion is defined as substance loss due to the effect of exogenous or endogenous acids on tooth surfaces without bacterial involvement and with histological changes in the dental hard tissue. Erosion is an ongoing problem that can occur at an early age and is currently believed to be the most common factor of tooth wears [1]. The increased consumption of acidic foods and drinks in modern societies is an important factor in the development of erosive wear.

The acidic attack leads to irreversible loss of dental hard tissue with progressive softening of the surface [2, 3]. Knowledge concerning the etiology of dental erosion is widespread but no generally accepted preventive methods exist [4].

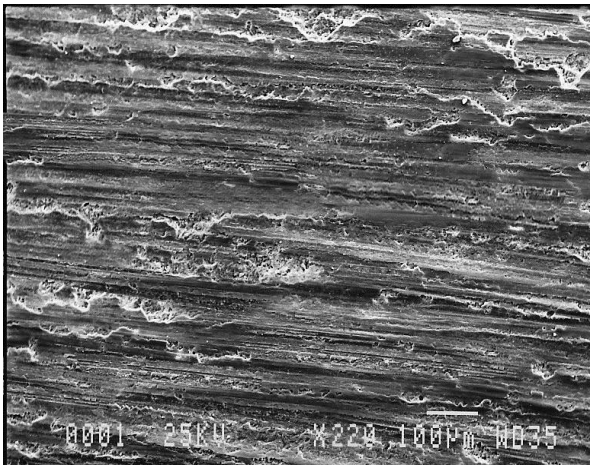

Fig1. Sound dentin structure

The prevalence of erosion in permanent dentition have been reported between 17% [5] and 30% [6], furthermore its prevalence has been estimated as 14.4% among Iranian girls in Qazvin city [7]. It has been shown in numerous studies that some chemical agents or components have anti-erosive potential [8-10]. Natural products are favorable sources for novel therapeutic agents. There are many reports of using these products in medicine for centuries as therapeutic agents; green tea (*Camellia sinensis*) is considered rich in polyphenols. Polyphenols have been reported to have distinct inhibitory activity against matrix metalloproteinases, which are responsible for degradation of the collagen matrix of dentin [11-13].Considering the above instances, the aim of the present study was to investigate the effect of green tea on eroded dentin by measuring microhardness values and scanning electron microscope evaluation.

MATERIALS AND METHODS

Plant material

Green tea leaves (*Camellia sinensis*) were obtained from Lahijan, a city of Gilan province, Iran. They were dried and ground by miller.

Preparation of hydroethanolic total extract of green tea leaves

Shed-dried and grounded green tea leaves (100g) were macerated with ethanol: water 70:30 (v/v); (500 mL). The extract was refrigerated for one hour and filtered through a cellulose paper filter. Extraction was repeated twice, each time by 300 mL of ethanol:water 70:30 (v/v). Then, the filtered extracts were pooled together and dried under vacuum in a rotary evaporator (Büchi, Germany) and stored in the refrigerator.

Sample preparation and treatment

Twelve sound human premolars extracted for orthodontic or periodontal reasons were selected and thoroughly cleaned of organic debris. The teeth were without cracks as inspected under a stereomicroscope (Magnification ×10, Nikon, Dusseldorf, Germany).

A disk was used in a low-speed handpiece to remove the enamel of the buccal surface until dentin was just exposed.

The teeth were sectioned longitudinally and the sectioned surfaces of the teeth were covered with two layers of acid-resistant nail varnish. The samples were embedded in epoxy resin, grounded flat with water-cooled discs (60 to 3000 grits of SiC papers, Matador, Germany) and polished with 1-µm Al_2O_3 felt papers (Struers, Denmark). The specimens were immersed in Coke (pH=2.8, Coca Cola, Khoshgovar Company, Mashhad, Iran) for 5 minutes at room temperature.

The surface microhardness of the dentin was measured using a microhardness tester (Five HMV 2000, Shimadzu Corporation, Tokyo, Japan) with a Knoop diamond under a load of 50gr/10s. Three indentations were made on the cervical third of each specimen. Two grams of prepared green tea (*Camellia sinensis*) extract were introduced into 180 mL of boiled water; the solution was cooled off for 5 minutes at room temperature. The fluoride content of the green tea solution was 0.6 mg/L measured by a fluoride electrode (ThermoFisher Scientific Orion Ionplus Fluoride Elctrd, 9609BNWP) and its pH was 7.0. The teeth were immersed in the green tea solution for 1 minute.

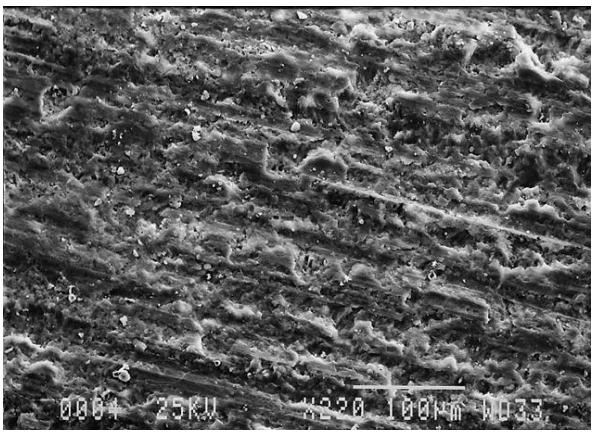

Fig 2. The eroded dentin with a rough surface and significant porosities

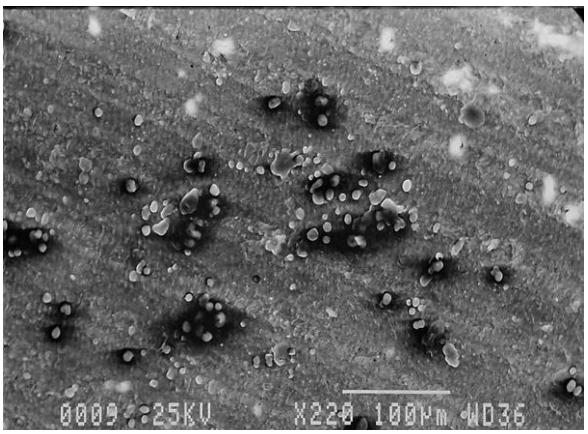

Fig3. The eroded dentin appearance after using green tea extract showing an improved appearance with marked depositions

The microhardness values with three indentations in each specimen were measured with the same procedure in the cervical third of each tooth. The average microhardness values before and after treatment were calculated and compared with each other by Wilcoxon test using SPSS 11.5. Three more samples which were immersed in Coke and treated with green tea solution, as mentioned previously, were lightly gold-sputtered and the morphology of dentin surface was evaluated
under a scanning electron microscope (SEM, JSM-840, Jeol, Japan). In addition, an intact dentin surface of a premolar was observed for better comparison of the samples.

RESULT

According to the findings of the present study, the mean±SD microhardness value (Kgforce/mm^2) after immersing teeth in coke was 46.5±2.79. Following immersion of the
eroded teeth in green tea solution, the microhardness value was 54.51± 4.46.
Wilcoxon test revealed statistically significant differences between the two groups ($P<0.01$).
In scanning electron microscope evaluation, the findings were as below:
Figure 1 shows the SEM photomicrograph of sound dentin revealing a smooth surface with orderly dentinal tubules.
In figure 2 the eroded dentin with rough and disorganized surface is visible. The surface has marked porosities denoting possible erosion.
Figure 3 demonstrates the eroded dentin treated with green tea solution. Improvement in
the appearance is obvious; moreover, there are likely hard material deposits on the outer
surface of the dentin.

DISCUSSION

This experimental study evaluated the effect of green tea on eroded dentin. The findings revealed increased dentin microhardness values after treatment with green tea. Dentin erosion is not a simple surface process. After acid-induced mineral dissolution, the organic matrix, mainly composed of collagens, is exposed. A thicker matrix slows down erosive mineral loss because the organic components act as a diffusion barrier [14, 15]. Matrix metalloproteinases (MMPs) form a multi-gene family within the metalloproteinase class of endopeptidases, which mediate the degradation of practically all extracellular matrix molecules [16, 17]. Before mineralization of the teeth, MMPs may take part in the organization of enamel and dentin organic matrix or they may control the proteoglycan turn-over and therefore regulate mineralization [18]. MMPs are present in dentin and saliva and are responsible for hydrolyzing the

components of the extracellular matrix during remodeling and degradation processes in the oral environment [7,19]. According to a study performed by Tjaderhane et al., human MMPs 2,8 and 9 activated by bacterial acids have a crucial role in the destruction of dentin by caries; therefore, MMP inhibitors can prohibit or postpone erosion progression [13]. Demeule et al. reported that different biologically active components from natural products such as various catechins isolated from green tea, especially epigallocatechingallate (EGCG) and epicatechingallate (ECG), inhibit MMP activities [20]. Different attempts have been made to justify the role of MMP inhibitors in the inhibition of dentinal degradation. For instance, $FeSO_4$ gel was able to inhibit MMP in vitro based on a study conducted by Kato et al. [9]. Zinc and other divalent metals inhibited MMPs in a study carried out by Souza et al. [21]. In other studies, chlorhexidine, as an MMP inhibitor, reduced the degradation of the dentin hybrid layer [22, 23]. Kato et al. reported that green tea reduces dentin wear under erosive/abrasive conditions measured by wear analysis [11].

The increase in surface microhardness values after immersion in green tea solution reveals an improvement in the demineralized structure of eroded dentin.

Either green tea solution must have allowed the formation of a surface deposit of organic materials on the dentin or it can be attributed to the presence of newly induced collagen crosslinks. Proanthocyanidin is a combination of monomers, oligomers and polymers of flavan-3-ols (catechines), which are widely present in green tea and might interact with the organic portion of dentin [24, 25].

In SEM evaluation, the typical structure of an eroded dentin surface is visible with a rough and demineralized appearance.

After immersion in green tea, there are obvious deposits on the dentin surface and conversion into a more organized feature is detected. Furthermore, preservation of dentin matrix by MMP inhibition might be another probable explanation for these observations. Since there is evidence about inactivity of MMPs in extracted teeth over time, the extracted teeth were used within the first week after extraction. It must be acknowledged that the results of this study should be confirmed with further studies with more close simulation of clinical situations.

CONCLUSION

Based on data obtained from this in vitro study, green tea increases the surface microhardness of eroded dentin and SEM evaluation of surface appearance and obvious depositions confirm the improvement.

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