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

M. Mirkarimi¹, L. Toomarian²

¹Assistant Professor, Pediatric Dentistry Department, Children and Adolescence Health Research Center, Zahedan University of Medical Sciences, Zahedan, Iran
²Associate Professor of Pediatric Dentistry Department, Shahid Beheshti University of Medical Sciences, Tehran, Iran

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

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].
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 Al₂O₃ 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 Elec, 9609BNWP) and its pH was 7.0. The teeth were immersed in the green tea solution for 1 minute.
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/mm²) 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 turnover 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, FeSO₄ 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.

ACKNOWLEDGMENTS
This study has been fully granted by Zahedan University of Medical Sciences (grant number 891585). The authors would like to thank Dr Abbas Delazar for providing the plant extract.

REFERENCES
1- Chuajedong P, Kedjarune Leggat U, Kertpon V, Chongsuvivatwong V, Benjakul P. Associated factors of tooth wear in southern Thai lad. J Oral Rehabil. 2002 Oct;29(10):997-1002.
2- Lussi A. Erosive tooth wear a multifactorial condition of growing concern and increasing knowledge. Monogr Oral Sci. 2006;20:1-8.
3- Magalhaes AC, Rios D, Moino AL, Wiegand A, Attin T, Buzalaf MA. Effect of different concentrations of fluoride in dentifrices on dentin erosion subjected or not to abrasion in situ/ex vivo. Caries Res. 2008;42(2):112-6.
4- Honorio HM, Rios D, Jr ES, de Oliveria DS, Fior FA, Buzalaf MA. Effect of acidic challenge preceded by food consumption on enamel erosion. Eur J Dent. 2010 Oct;4(4):412-7.
5- Williams D, Croucher R, Marcenes W, O'Farrell M. The prevalence of dental erosion in maxillary incisors of 14-year-old schoolchildren living in Tower Hamlets and Hackney, London, UK. Int Dent J. 1999 Aug;49(4):211-6.
6- Milosevic A, Young PI, Lennon MA. The prevalence of tooth wear in 14-year-old school children in Liverpool. Community Dent Health.
Effect of green tea on dentin erosion

Mirkarimi and Toomarian

1994 Jun;11(2):83-6.
7- Moslemi M, Khalaji M, Baghaie Anaraki M. Epidemiology of dental erosion in 14-17-year-old girls in Ghazvin; 2004. J Dent Sch Shahid Beheshti Univ Med Sci. 2008 26(1):73-8.
8- Chunmuang S, Jitpukdeebodintra S, Chunnarom C, Benjakul P. Effect of xylitol and fluoride on enamel erosion in vitro. J Oral Sci. 2007 Dec;49(4):293-7.
9- Kato MT, Leite AL, Hannas AR, Oliveira RC, Pereira JC, Tjaderhane L et al. Effect of iron on matrix metalloproteinase inhibition and on the prevention of dentine erosion. Caries Res. 2010;44(3):309-16.
10- Schlueter N, Ganss C, Mueller U, Klimek J. Effect of titanium tetrafluoride and sodium fluoride on erosion progression in enamel and dentine in vitro. Caries Res. 2007;41(2):141-5.
11- Kato MT, Magalhaes AC, Rios D, Hannas AR, Attin T, Buzalaf MA. Protective effect of green tea on dentin erosion and abrasion. J Appl Oral Sci. 2009 Nov-Dec;17(6):560-4.
12- Sulkala M, Tervahartiala T, Sorsa T, Larmas M, Salo T, Tjaderhane L. Matrix metalloproteinase-8 (MMP-8) is the major collagenase in human dentin. Arch Oral Biol. 2007 Feb;52(2):121-7.
13- Tjaderhane L, Larjava H, Sorsa T, Uitto VJ, Larmas M, Salo T. The activation and function of host matrix metalloproteinases in dentin matrix breakdown in caries lesions. J Dent Res. 1998 Aug;77(8):1622-9.
14- Breschi L, Gobbi P, Mazzotti G, Falconi M, Ellis TH, Stangel I. High resolution SEM evaluation of dentin etched with maleic and citric acid. Dent Mater. 2002 Jan;18(1):26-35.
15- Ganss C, Klimek J, Starck C. Quantitative analysis of the impact of the organic matrix on the fluoride effect on erosion progression in human dentine using longitudinal microradiography. Arch Oral Biol. 2004 Nov;49(11):931-5.
16- Chaussain-Miller C, Fioretti F, Goldberg M, Menashi S. The role of matrix metalloproteinases (MMPs) in human caries. J Dent Res. 2006 Jan;85(1):22-32.
17- Tanpure PN, Kuchler EC, Lips A, Costa Mde C, Luiz RR, Granjeiro JM et al. Genetic variation in MMP20 contributes to higher caries experience. J Dent. 2012 May;40(5):381-6.
18- Hannas AR, Pereira JC, Granjeiro JM, Tjaderhane L. The role of matrix metalloproteinases in the oral environment. Acta Odontol Scand. 2007 Feb;65(1):1-13.
19- Ingman T, Tervahartiala T, Ding Y, Tschesche H, Haerian A, Kinane DF et al. Matrix metalloproteinases and their inhibitors in gingival crevicular fluid and saliva of periodontitis patients. J Clin Periodontol. 1996 Dec;23(12):1127-32.
20- Demeule M, Brossard M, Pagé M, Gingras D, Béliveau R. Matrix metalloproteinase inhibition by green tea catechins. Biochim Biophys Acta. 2000 Mar 16;1478(1):51-60.
21- de Souza AP, Gerlach RF, Line SR. Inhibition of human gingival gelatinases (MMP-2 and MMP-9) by metal salts. Dent Mater. 2000 Mar;16(20):103-8.
22- Hebling J, Pashley DH, Tjaderhane L, Tay FR. Chlorhexidine arrests subclinical degradation of dentin hybrid layers in vivo. J Dent Res. 2005 Aug;84(8):741-6.
23- Carrilho MR, Geraldeli S, Tay F, Goes MF, Carvalho RM, Tjaderhane L et al. In vivo preservation of the hybrid layer by chlorhexidine. J Dent Res. 2007 Jun;86(6):529-33.
24- Ostrowska J, Skrzydlewska E. The comparison of effect of catechins and green tea extract on oxidative modification of LDL in vitro. Adv Med Sci. 2006;51:298-303.
25- Jiao HL, Ye P, Zhao BL. Protective effects of green tea polyphenols on human HepG2 cells against oxidative damage of fenofibrate. Free Radic Biol Med. 2003 Nov 1;35(9):1121-8.