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ORIGINAL RESEARCH

Effect of Green Tea Varnish on Depth of Root Caries

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ABSTRACT

Aim: Root caries is a common, debilitating condition particularly in the elderly population, which can lead to tooth loss. Evidence shows that green tea has cariostatic effects. Considering the gap of information on green tea varnish, this study aimed to assess the efficacy of green tea varnish in the prevention of root caries.

Materials and methods: This *in vitro* experimental study was performed on 42 sound premolars. Two layers of acid-resistant nail varnish were applied on root surfaces except for a window of 1 × 4 mm. The teeth were randomly divided into three groups (n = 14). Group I (control) received no intervention. Group II received green tea varnish applied on the roots every 48 hours for 21 days. Group III received green tea varnish every 24 hours for 21 days. Sections of 40 µ thickness were prepared from the center of the window, and the depth of carious lesion was measured in three points with 500 µ distance from each other using polarized light microscope. Data were analyzed using Statistical Package for the Social Sciences (SPSS) version 16 and nonparametric Kruskal–Wallis and Mann–Whitney U tests (α = 0.05).

Results: The mean (and standard deviation) depth of carious lesion was 54.30 ± 28.64 (µm), 0, and 0 in groups I, II, and III respectively. Control group showed the highest depth of caries. Groups II and III were not significantly different in this respect, but significant differences were noted in depth of caries between groups I and II and also groups I and III ($p < 0.001$).

Conclusion: Based on the results, the green tea varnish may possess anticariogenic effects on root caries when applied every 24 or 48 hours over a 21-day period. Application of green tea varnish every 24 or 48 hours for 21 days may prevent root caries.

Clinical significance: Application of green tea varnish can prevent root caries.

Keywords: Dental caries, Green tea, Root surface, Varnish.

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INTRODUCTION

Root caries is a common, debilitating condition, particularly in the elderly population.[1] This infectious disease is caused by colonization of bacteria and initiated by decalcification of the inorganic part of the tooth structure, which is followed by the destruction of the organic matrix.[2-6] Root caries can also result in tooth loss,[7,8] which negatively affects the quality-of-life of patients, particularly the elderly.[9,10] Tooth decay is a multifactorial disease[11] caused by the complex interaction of cariogenic oral flora and fermentable carbohydrates accumulating on tooth surfaces over time.[12]

Evidence shows that fluoride varnish can cause a 33% reduction in the rate of caries.[13] Furthermore, it has been reported that varnish is more efficient than fluoride gel in prevention of caries due to higher durability and easier and faster application.[14] The use of varnishes made of organic materials is another strategy suggested for caries prevention.

Camellia sinensis, widely known as green tea,[15] has anticancer, antioxidant, anti-inflammatory, and medicinal properties due to having catechin. The anticariogenic mechanisms of green tea have been partly understood.[16-18] Duration of exposure of tooth structure to green tea affects its cariostatic efficacy. Thus, to benefit from its cariostatic effects, green tea must be used in a form with maximum durability on the tooth surface.[2]

Narotzki et al[19] and Araghizadeh et al[20] evaluated and confirmed the inhibitory effects of green tea on cariogenic bacteria. Ferrazzano et al,[21] in their *in vivo* study, evaluated the antimicrobial effect of green tea extract on cariogenic microflora. Since varnish provides longer exposure than solutions and mouthrinses[22,23] and considering the lack of information on the efficacy of green tea varnish on caries formation, this study sought to assess the effect of green tea varnish on the depth of root caries. The null hypothesis was that green tea varnish would have no significant effect on depth of root caries.

MATERIALS AND METHODS

This *in vitro* experimental study was conducted on premolar teeth extracted due to orthodontic or periodontal reasons in the past few months. Immediately after extraction, the teeth were immersed in 10% formalin (Shahid Ghazi Co., Tabriz, Islamic Republic of Iran) at room temperature for 1 week for disinfection. After 1 week, the teeth surfaces were cleaned of soft tissue, debris, and calculus using a periodontal curette (Juya, Kashmir, Pakistan) and polished with a nonfluoridated pumice paste (Golchadent Co., Karaj, Islamic Republic of Iran) and rubber cup (Kerr, California, USA).[24] At 24 hours before the experiment, root surfaces were inspected under a stereomicroscope (Eclipse E 600, Nikon, Tokyo, Japan) at 20× magnification. The inclusion criteria were the absence of cracks, caries, restorations, or congenital anomalies. Totally, 42 sound teeth were chosen. Two layers of acid-resistant nail varnish were applied on root surfaces except for a window measuring 1 × 4 mm (due to limitations in root shape and contour, only one window was considered on the buccal or lingual surface of the roots).[25] The teeth were randomly divided into three groups (n = 15) based on the method of surface treatment:

1. *Group I*: The teeth did not undergo any intervention (control group)
2. *Group II*: Green tea varnish was applied on root surfaces every 48 hours for 21 days
3. *Group III*: Green tea varnish was applied on root surfaces every 24 hours for 21 days[2]

The three groups were marked with three different colors of acid-resistant nail varnish.

To prepare 5% green tea varnish, 100 mg of the powdered catechin of green tea (epigallocatechin-3-gallate (EGCG), Sigma-Aldrich, USA) was added to 2 mL of ethyl cellulose and ethanol in a 1:1 ratio. After 1 hour of sonication for homogenization, it was refrigerated at 2 to 5°C in a dark container.

The teeth in each group were placed in three different-coded containers and autoclave-sterilized at 121°C and 15 psi pressure for 15 min. Microbrushes used for varnish application were also sterilized with the same method used for sterilizing the teeth. Next, each tooth was separately placed in brain heart infusion broth containing 1.5×10^8 *Streptococcus mutans* bacteria (equal to 0.5 McFarland concentration), 1.5×10^8 lactobacilli (equal to 0.5 McFarland concentration), and 3 mL of 20% sucrose solution, and was incubated.[2] To ensure nill contamination and assess the growth and proliferation of *S. mutans* and lactobacilli, samples were taken off the plates between 10 and 11 am and cultured on blood agar. No contamination was noted in any group during the study period. In *S. mutans* plates, 10 mL of the culture medium was replaced with 10 mL of fresh medium every other day to provide nutrients for the bacteria.[2]

For application of green tea varnish in group II, the teeth were removed from the medium once every 48 hours, rinsed under distilled water, and dried. One layer of varnish was applied on the root surfaces by a microbrush and allowed to dry for 60 seconds. Next, the teeth were immersed again in the medium. In group III, this process was performed once every 24 hours for 21 days. After completion of this period, the teeth were rinsed with distilled water, mounted in acrylic resin (Acropars, Tehran, Islamic Republic of Iran), and horizontally sectioned at the center of the window into 300 µm slices by a microtome (Hammarlund-Essler, Stockholm, Sweden). Final polishing was done to obtain an ideal thickness of 40 µm using 120 to 1,500 grit paper disks. The thickness of slices was measured by a precise digital caliper (Mitutoyo 12"/300 mm, Japan) with 0.01 mm resolution. The teeth were then immersed in distilled water to remove debris. For histological assessment of sections, distilled water was used as the background substance, and the specimens were observed under a light microscope (SoloMark 900x, Ningbo, China) with a polarized filter at ×10 magnification.[25] Diameter of the field of view was measured to be 1,500 µ using a Neubauer slide under the microscope. This value was used as a reference unit for measurement of the depth of carious lesions. Caries depth in each specimen was measured in three points with 500 µ distance from each other using AutoCAD software (Autodesk AutoCAD 2016) by an oral and maxillofacial pathologist. The mean depth in the three points was calculated and considered as the depth of lesion in each specimen (Figs 1 and 2).[24,26] After calculating the depth of demineralization in the three groups, data were analyzed using SPSS version 16 and nonparametric Kruskal–Wallis and Mann–Whitney test. Level of significance was set at 0.05.

RESULTS

Table 1 shows descriptive statistics in the three groups. As shown in Table 1, the mean depth of carious lesion

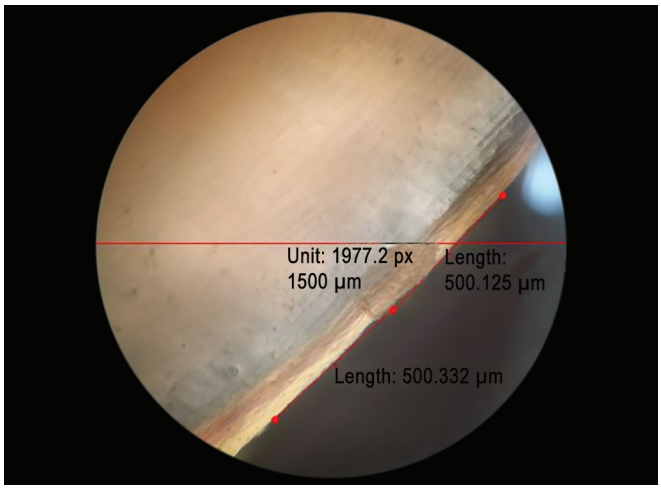


Fig. 1: Polarized microscope view in noncarious specimen indicates no carious lesion, and the surface is intact

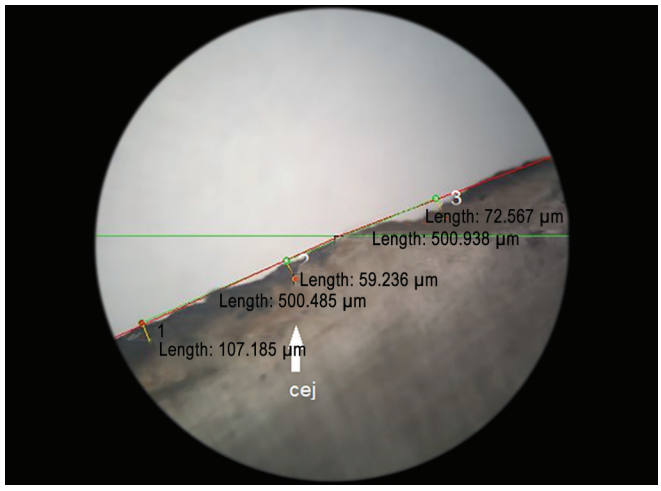
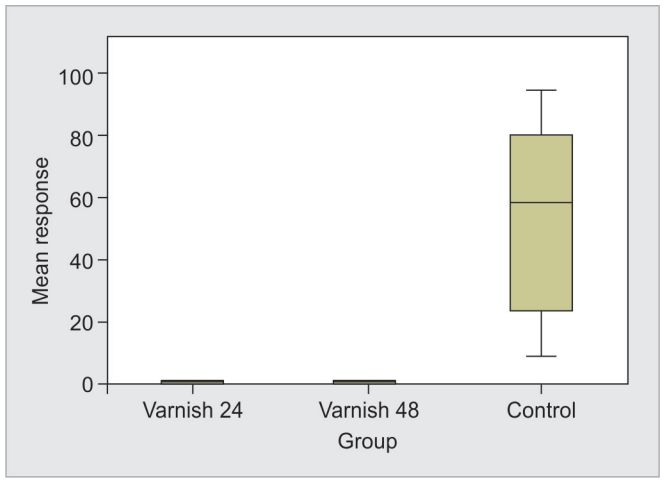


Fig. 2: Polarized microscope view indicates different depths of caries in a carious specimen

Table 1: Descriptive analysis of the carious lesion depth (n = 14)

Groups	*Mean (µm)*	*Standard deviation (µm)*	*Minimum (µm)*	*Maximum (µm)*
Control (group I)	54/30	28/64	8/75	94/30
Varnish every 48 hours (group II)	0	0	0	0
Varnish every 24 hours (group III)	0	0	0	0



Graph 1: Comparison of depth of caries in the studied groups in µm

was the highest in the control group compared with the other groups (Graph 1). In varnish groups, no carious lesion was noted at 24 or 48 hours.

Since data were not normally distributed in the three groups, Kruskal–Wallis test was used for comparison of the three groups, and Mann–Whitney U test was applied for pairwise comparisons. The Kruskal–Wallis test showed that the three groups were significantly different in terms of depth of carious lesion ($p < 0.001$). The Mann–Whitney U test showed that depth of caries was not significantly different between groups II and III. However, the group subjected to varnish application every 24 hours had a significant difference from the control group ($p < 0.001$). Furthermore, group II (varnish application every 48 hours) had a significant difference from the control group (group I) in the depth of caries ($p < 0.001$).

DISCUSSION

Root caries can negatively affect oral health and compromise on the quality-of-life of the elderly population.[9,10] Exposure of cervical dentin due to gingival margin recession commonly occurs as the result of improper toothbrushing, use of abrasive toothpastes, or frequent use of toothpicks. Dental erosion, periodontal procedures, such as gingival surgery, scaling, and root planning, and prosthetic, orthodontic, and restorative treatments, such as tooth whitening, can also increase the risk of root caries.[27-30] Margins of the cavitated root caries are often placed in both enamel and dentin, and restoration of these cavities has a failure rate of 68% in 12 months since there is no restorative material that can provide suitable bond to both enamel and dentin. These carious lesions are often asymptomatic and close to dental pulp.[31] Thus, their prevention is a priority for both dentists and patients. This study evaluated the efficacy of green tea varnish in the prevention of root caries.

Several organic compounds are available for root caries prevention; however, their application is affected by many other factors, such as their stability, odor, taste, and affordability, which must be taken into account.[32-35] Green tea extract is a natural compound that possesses all the aforementioned properties.[33] Of the catechins available in the chemical composition of green tea, EGCG is the most abundant.[34] Tea catechins are water soluble and colorless comprising 25 to 40% of the solid solutes in tea.[36] Green tea

has many beneficial properties for the treatment of cardiac diseases and obesity.[16,17] Anticarcinogenic properties of green tea have also been confirmed.[36-38] Since EGCG is the most active component of green tea extract,[39] it was used as the green tea extract in our study. Furthermore, polarized light was used to assess the depth of caries due to advantages, such as no necessity for slide preparation and high accuracy.[40,41] Since the minimum duration of time required for the development of initial noncavitated carious lesions (first clinical evidence of demineralization) is 21 days,[24,42] the 21-day period was considered for development of caries in this study, and mineralization depth was measured by a polarized light microscope.

The reducing potential of each polyphenol compound depends on the number of its hydroxyl groups. Although green and black tea are excellent sources of polyphenols, antioxidant properties of green tea are 5 times higher than those of other types of tea due to it having higher concentrations of polyphenols rich in hydroxyls.[43] Furthermore, it can inhibit matrix metalloproteinase (MMP) enzymes. These enzymes are capable to hydrolyze the organic matrix of demineralized dentin[44,45] such that MMP-2 (gelatinase A), MMP-8 (collagenase), MMP-9 (gelatinase B), and MMP-20 (Enamelysin) are involved in the process of caries, and destruction occurs by activation of MMPs.[39,44,45] Chatterjee et al[46] showed that green tea promotes periodontal health by decreasing inflammation, preventing bone loss, and inhibiting the growth and proliferation of some specific bacteria. Previous studies on the properties of this material and its efficacy for prevention of tooth erosion are limited. Kato et al[47] and Magalhães et al[48] evaluated bovine teeth and concluded that green tea extract can effectively prevent dental caries. Similarly, the present study showed that there is a significant difference between the control and green tea groups in terms of depth of carious lesions.

Some previous *in vitro* and *in vivo* studies on animals and humans have evaluated the effects of green tea on *S. mutans*.[34,35,44,49] Araghizadeh et al,[20] in their *in vitro* study, evaluated the effect of green tea extract on cariogenic bacteria. They showed that *S. mutans* were totally sensitive to green tea and it can decrease their activity and consequently prevent the development of carious lesions to a great extent. In fact, polyphenols present in green tea contain high levels of catechins and theaflavins and have very strong antibacterial properties.[50] Some other studies have evaluated the anticariogenic properties of green tea by assessing its direct effect on microorganisms.[51] *In vitro* studies have shown that catechins present in green tea prevent adhesion of *Streptococci* to tooth surfaces. Otake et al[52] concluded that Sunphenon (a commercial mixture of catechins extracted from green tea leaves) prevents adhesion of *S. mutans* to hydroxyapatite disks covered with saliva. Similar results were reported by Xiao et al,[53] which confirm the favorable properties of green tea. Another suggested mechanism for anticariogenic properties of green tea is by the inhibition of glucosyltransferase and amylase enzymes.[51] These enzymes play an important role in dental caries pathogenesis. Zhang and Shen[54] concluded that green tea extract decreases the activity of amylase enzyme in the human saliva, which leads to cariostatic effects. Another study indicated that drinking one to three cups of green tea per day decreases dental caries and plaque formation on tooth surfaces.[55] No previous study has evaluated the effect of green tea varnish on prevention of root caries. Several studies with different methodologies have evaluated anticariogenic effects of green tea, and almost all of them reported the capacity of green tea to inhibit dental caries. Goenka et al[56] and also Suzuki et al[57] showed that individuals who frequently drink green tea have fewer missing teeth, lower prevalence of caries, and more favorable oral hygiene. You[58] reported that 0.2% green tea used as a mouthwash or with toothbrushing for 5 minutes significantly decreased plaque index. Hattarki et al[59] evaluated over 6,000 children in the United Kingdom and concluded that green tea definitely decreases the prevalence of caries since it inhibits cariogenic microorganisms. This has also been reported in a review article by Jones et al[60]

Suyama et al[61] used this extract for making chewing gums and evaluated the effects of chewing green tea gums on building tooth resistance to acid attacks. They concluded that chewing green tea gums increases the resistance of dentin to acid. Our study showed that application of green tea varnishes every 24 or 48 hours completely prevented the development of caries. The two green tea groups were not significantly different in this respect, which is probably due to the high durability of green tea varnish on the tooth surface for up to 48 hours. The highest depth of caries was noted in the control group.

However, the results of this study were in contrast to those of Rezaei-Soufi et al[2] because they had reported that green tea mouthwash had no significant effect on dental caries. The difference in the results of the two studies may be due to the difference in methodologies since we used green tea varnish, which appears to have more durability than a mouthwash and provides longer exposure. In our study, the effect of toothbrushing during the determined time period[62] was not evaluated on teeth and this was a limitation; this factor should be evaluated in future studies to assess the cariostatic properties of green tea when combined with toothbrushing to obtain more reliable results. Another limitation was the type of study. The present study was designed as an *in vitro* study and could not fully simulate the clinical setting. Thus, clinical studies are required to confirm the obtained results.

Another point to be noted is that a specific concentration of green tea extract was used in this study; future studies are recommended to use other concentrations of green tea varnish to shorten the time interval between applications.

CONCLUSION

Within the limitations of the present study, the green tea varnish may possess anticariogenic effect on root caries when applied every 24 or 48 hours within a 21-day period.

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