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ORIGINAL RESEARCH

Effect of Green Tea and Black Tea on Mineralization of Artificially Demineralized Human Enamel in Comparison with 0.05% Sodium Fluoride: An *in vitro* Study

[1]Amal S Babu, [2]Anulekh Babu, [3]Mali G Nair, [4]JL Jayanthi, [5]Khaleel A Thaha, [6]Joms K George

ABSTRACT

Introduction: The newer preventive approach to caries has resulted in the increased use of remineralizing agents. The remineralizing ability of green tea and black tea is under research.

Materials and methods: Two hundred premolar teeth were collected and a buccal window was exposed on the tooth sample by coating the rest of the teeth with nail varnish. The samples were artificially demineralized using Featherstone pH cycling technique. The test solutions of green tea and black tea were prepared by dissolving 2 gm of the prepared extract in 180 mL of water; 0.05% NaF solution was prepared by dissolving 5 gm of NaF in 1 L of distilled water. A total of 50 samples each were treated with the four test solutions, green tea, black tea, NaF and distilled water (control) for 4 minutes per day for 1 week. The samples were excited using diode laser and the fluorescence spectra were measured using laser-induced fluorescence spectroscopy.

Results: The highest remineralization was shown by samples treated with green tea, followed by NaF. Black tea showed lower remineralization than both green tea and NaF and the least remineralization was shown by the control, distilled water. The difference in remineralization shown by the test solutions was statistically significant except for the difference between black tea and distilled water.

Conclusion: All the three test groups, green tea, black tea, and NaF, showed remineralization. Green tea induced the highest remineralization in the tooth samples, followed by NaF. Black tea produced the least amount of remineralization. Green tea may have a potential role as a remineralizing agent for daily intake.

Keywords: Black tea, Demineralization, Featherstone pH cycling, Green tea, Laser-induced fluorescence spectroscopy, Remineralization, Sodium fluoride.

[1]Senior Resident, [2,5]Assistant Professor, [3]Professor and Head [4]Scientist, [6]Junior Resident

[1,3]Department of Conservative Dentistry and Endodontics Government Dental College, Alappuzha, Kerala, India

[2,5,6]Department of Conservative Dentistry and Endodontics Government Dental College, Thiruvananthapuram, Kerala, India

[4]Department of Crustal Processes Research Group, National Centre for Earth Science Studies, Thiruvananthapuram, Kerala India

Corresponding Author: Amal S Babu, Senior Resident Department of Conservative Dentistry and Endodontics Government Dental College, Alappuzha, Kerala, India, e-mail: amal1221986@gmail.com

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INTRODUCTION

Dental caries has been associated with mankind since time immemorial, and its eradication is considered to be one of the holy grails of dental profession. Various techniques and approaches were introduced to combat caries throughout the years. Some of them were successful and became widely accepted while the vast majority were deemed failures and were long forgotten.[1] In recent years, a subtle shift in the approach to combat caries has occurred with more emphasis being placed on prevention rather than treatment, conforming to the adage "prevention is better than cure."[2]

Prevention of caries involves reducing demineralization and promoting remineralization of the tooth. Among the various preventive agents present today, sodium fluoride is the most widely accepted. Fluoride present is responsible for its caries preventing action. Fluoride prevents caries by making the tooth more resistant to decay and reversing early decay.[3] Risk of toxicity and the need for multiple visits to the dentist are the main disadvantages of sodium fluoride, which led to the search for an alternative to sodium fluoride.[4]

A considerable interest has arisen concerning the health promoting potential of tea (Camellia sinensis), a widely popular beverage, that has been cultivated and consumed for more than 2000 years. Tea leaves have one of the highest amount of fluoride concentration occurring naturally, hence, its possible role as a remineralizing agent. There are various varieties of tea available depending on the method of processing like green tea, black tea, oolong tea, yellow tea, and few others. Green tea and black tea are the most researched upon, among the different varieties.[5]

Over the years, researchers have studied and catalogued the wide range of actions of green tea which include anti-inflammatory, antioxidant, anticancer, anti-

bacterial, antiviral, antihyperglycemic, and antiaging action.

Green tea was found to have numerous oral health benefits, especially because of its potent antibacterial action, which helps to combat periodontitis and halitosis. Remineralization is another possible application of green tea which aids in caries prevention.

The present study has been designed to evaluate the remineralizing potential of green tea and black tea and to compare with that of sodium fluoride.

MATERIALS AND METHODS

Two hundred extracted maxillary premolars were collected, cleaned, and prepared to expose a circular 6 mm buccal window by coating the rest of the area with nail varnish.[6] The samples were artificially demineralized by Featherstone pH cycling technique.[7] This was done to clinically simulate the demineralization in initial enamel caries. The technique involves two solutions, demineralization solution and remineralization solution. All the teeth specimen were immersed in demineralization solution for 6 hours, rinsed with deionized water, and then kept in remineralization solution for 17 hours. This was repeated for 10 days to induce artificial demineralization. The demineralization was then quantitatively assessed using laser-induced fluorescence spectroscopy.

The teeth were divided into four groups and subjected to the four test solutions respectively: Group I—green tea, group II—black tea, group III—0.05% NaF, group IV—distilled water. Green and black tea were prepared from their respective extracts.

Dried and ground green or black tea leaves (100 mg) without any additives were macerated with 500 mL of ethanol: Water 70:30 (v/v). The extract was then refrigerated for 1 hour and filtered through a cellulose paper filter. Extraction was repeated twice, each time by 300 mL of ethanol: Water 70:30 (v/v). Filtered extracts were pooled together and dried under vacuum.[8] For preparing the test solution, 2 gm of prepared extract (green or black tea) was introduced into 180 mL of boiled water and the solution was allowed to cool off for 5 minutes at room temperature. The test solution for NaF was prepared by dissolving 5 gm of commercially available NaF (Lobachemie, India) in 1 L of distilled water to obtain a freshly prepared 0.05% NaF.

The demineralized samples were immersed in 20 mL of the freshly prepared test solution for 4 minutes, then rinsed and stored in deionized water for the next day. This was repeated for 1 week.[9] The change in mineralization was then quantitatively assessed using laser-induced fluorescence spectroscopy.

The samples of each group were mounted in a wax mold for ease of handling, with buccal window aspect upside. The samples were then excited using diode laser at 404 nm by placing the optical fiber light in the buccal window. The light emitted from the sample was recorded in the spectrometer in nanometers. A set of at least eight measurements was taken from each sample. The fluorescence spectra were collected from the samples. The fluorescence intensity is considered to be inversely related to demineralization. The decrease in fluorescence intensity indicates either an increase in demineralization or decrease in remineralization and *vice versa*.[10]

For comparison of study groups with respect to fluorescence intensity, analysis of variance (ANOVA) was used. A calculated p value less than 0.05 was considered to be statistically significant at 5% level of significance.

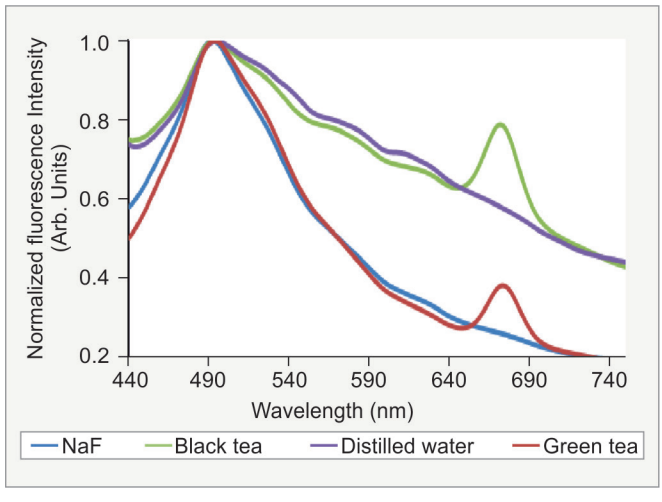
RESULTS

Some difference was noted in the spectral profile of black and green tea as shown in Graph 1. Spectral graph shows another small peak of florescence intensity in the 680 nm range. This suggests a fluorophore component of green tea and black tea which adhered to the tooth and can emit fluorescence.

Distilled water showed the least mean intensity (242 ± 56.3). Black tea showed slightly higher mean intensity (255.6 ± 54.5) than distilled water, but much lower than NaF and green tea. The highest intensity was shown by green tea (414.6 ± 212.5) followed by NaF (377.4 ± 198.1).

One-way ANOVA test was used to compare the mean intensity among the four groups and was concluded that there was a statistically significant difference between the remineralizing ability of the four study groups Tables 1 and 2.

Scheffe multiple comparison test compared the mean values of each pair and determined whether the difference was statistically significant. Green tea showed statistically significant difference in fluorescence intensity with all the



Graph 1: Intergroup comparison graph of spectral profile

Table 1: Descriptive statistics of intensity based on group

Groups	*Mean*	*SD*	*Median*	*Minimum*	*Maximum*
I	377.4	198.1	306.7	153.1	780.1
II	414.6	212.5	328.6	159.0	854.7
III	255.6	54.5	262.4	152.1	352.9
IV	242.8	56.3	26.4	151.0	340.1

SD: Standard deviation

Table 2: Comparison between groups using one-way ANOVA

Groups	*Mean*	*SD*	*n*	*f*	*p-value*
NaF (I)	377.4	198.1	884	290.68**	0
Green tea (II)	414.6	212.5	884		
Black tea (III)	255.6	54.5	884		
Distilled water (IV)	242.8	56.3	884		

**Significant at 1% (p-value < 0.01 level); SD: Standard deviation

three groups (green tea, black tea, and distilled water). Sodium fluoride showed statistically significant difference with both black tea and distilled water, whereas there was no statistically significant difference in intensity between black tea and distilled water.

DISCUSSION

The advancement of microbiology and engineering is bringing a medical revolution to dentistry. The ongoing exciting progress in caries research has offered great opportunities to better understand, detect, and monitor the disease. These new biological discoveries and technological developments have led to a new model, the medical approach, to address the etiological cause of the disease (microbial infection) and to more effective treatments and prevention of dental caries.[11] With this new approach gearing more toward the preventive aspects, remineralization is starting to play a major role in combating caries. More studies are being undertaken to introduce new remineralizing agents. With better diagnostic techniques and newer advancements in diagnosis, remineralization is going to become the mainstay in treatment and prevention of dental caries.

Tooth is a mineralized hard tissue which contains multiple layers. It has an inner soft tissue layer of pulp, surrounded by the outer hard tissue layers of dentin and enamel. The enamel contains 96% inorganic and 4% organic content. The inorganic content of the enamel is a crystalline calcium phosphate hydroxyapatite (HA). The susceptibility of these crystals to dissolution by acid provides the chemical basis of dental caries.[12]

Water has the unique ability to dissolve inorganic crystals. In the oral cavity, saliva which is primarily water dissolves the HA crystals of the tooth.[13] As the saliva gets saturated with HA crystals, the tooth dissolution stops. Hence, an equilibrium is maintained in the saliva, saturated with HA crystals.[14] This equilibrium is dynamic, as there is constant exchange of ions. Depending upon the saturation of the HA crystals in saliva, there can be either dissolution or precipitation of HA crystals of tooth. This dissolution and precipitation are two dynamic processes in equilibrium in the oral cavity known as demineralization and remineralization.[15]

Demineralization is the loss of mineral content in the form of mineral ions from the HA crystals in tooth. The main culprits are the acids in the tooth environment, either from the caries bacteria or from dietary sources. The acids lower the pH of the surrounding medium resulting in more dissolution of tooth. This is because, as the pH lowers, more H^+ ions are introduced into the solution, which combine with PO_4^{3-} and OH^- ions (dissolution products of HA) and remove them from the solution. Thus, the solution becomes unsaturated and to regain equilibrium, tooth dissolution, i.e., demineralization, occurs.[16] Research indicates that there is a critical value of pH of saliva below which the equilibrium becomes deranged and the solution becomes unsaturated. This value of pH called critical pH is about 5.5. So when the pH of saliva falls below 5.5, it becomes unsaturated with respect to HA and enamel is at risk of dissolution.[17] At a pH of 5.0, the surface remains intact while the subsurface mineral is lost.

In remineralization, the partially demineralized apatite crystals grow back by absorbing the mineral ions. The sources of these ions are saliva and other exogenous sources like topical and systemic fluoride, diet, etc. Remineralization of dental lesions requires the presence of partially demineralized apatite crystals that can grow to their original size as a result of exposure to solutions supersaturated with respect to apatite.[18,19]

In this study, the remineralization potential of the test solutions was assessed in a demineralized sample rather than in a sound intact tooth. The remineralization potential in a partially demineralized enamel is more than in a sound teeth and hence, can be easily assessed in the study.[20] So, in this study, the sound teeth were artificially demineralized using Featherstone pH cycling technique. There are other methods to demineralize the tooth sample like the use of citric acids and other acids,[21] but the Featherstone technique was used in the present study as it clinically simulates the demineralization in initial enamel caries.[20] The pH cycling technique contains two solutions, demineralization and remineralization solutions. Remineralization solution was used in conjunction with the demineralization solution so that the lesion induced by the solutions simulated an initial enamel lesion, i.e., partially demineralized HA crystals, which have more potential for remineralization.[22]

Green tea is a very promising candidate as a remineralizing agent because it can be a cheap, easy to use, and acceptable alternative to sodium fluoride. Apart from

its remineralizing potential, it has various other health benefits, the anticancer and antibacterial actions being prominent ones.[23] Green tea was found to have numerous oral health benefits, especially because of its potent antibacterial action. Catechin fraction of green tea is responsible for its antibacterial action. Horiba et al[24] studied the antibacterial and bactericidal effects of green tea as an intracanal medicament on different bacterial strains and found that extracts of Japanese green tea may be useful as a medicament for treatment of infected root canals. Green tea polyphenols showed statistically significant antibacterial activity against *Enterococcus faecalis* biofilm formed on tooth substrate. Zhu et al[25] investigated the pH value of green tea and found that it has additional anticaries action because of its protective effect on pH. Green tea prevents the pH to fall below critical value.[26]

Kushiyama et al[27] reported that green tea has possible effect against periodontal disease, whereas Lodhia et al[8] determined that green tea was effective against halitosis. Remineralization is another possible application for green tea, and numerous studies had been conducted regarding it. Kato et al[28] studied the protective effect of green tea on dentin erosion and abrasion and found that green tea reduces the dentin wear in erosive/abrasive conditions. It was suggested that the protective effect was due to the remineralizing and antidemineralizing effects of green tea. These findings were supported by Mirkarimi et al[29] who conducted an *in vitro* study to determine the effect of green tea on dental erosion and got similar results.

All three test solutions used in the present study, sodium fluoride, green tea, and black tea, showed increase in intensity of fluorescence of enamel samples, indicating remineralization in the demineralized tooth samples. Green tea showed the highest fluorescence intensity, followed by sodium fluoride and then black tea. Least fluorescence intensity was shown as expected by the control, i.e., distilled water. The result was in consensus with previous studies of remineralization. The high remineralization ability of green tea was supported by previous studies like that of Yoshiharu et al,[30] who showed the increase in mineralization in bovine teeth by green tea, and Suyama et al[31] who determined remineralization potential of green tea in enamel lesions. But the higher remineralization of green tea than sodium fluoride was unexpected and was maybe due to the difference in concentration of the test solutions. There was statistically significant difference in the fluorescence intensity of green tea and sodium fluoride.

Even though black tea produced increase in fluorescence intensity, it was very low and not much more than the control. This result is contradictory to that of Abdulraheam and Garib,[32] who reported that the remineralization by black tea, while lesser than green tea, was still significant. This may be because in the present study tea extract was used, rather than the commercial tea bags used in the study by Abdulraheam and Garib.[32] While preparation of the extract, there might have been inactivation or removal of some of the components of black tea responsible for remineralization. There was a substantial difference in the fluorescence intensity between black tea and both green tea and sodium fluoride, which was statistically significant.

CONCLUSION

It can be concluded from the study that green tea and black tea have the potential to produce remineralization in tooth. This property is evident from the quantitative measurement of remineralization using laser-induced fluorescence spectroscopy. The use of spectroscopic techniques is a fast and precise method to quantify enamel remineralization. Compared with black tea, green tea showed higher amount of remineralization ability, which was more than that of sodium fluoride.

Literature on the mechanism of action of green tea and black tea in remineralization is limited. A complete understanding of the underlying mechanisms is essential. Hence, research on this area has to be expanded with the use of precise tools and technologies. Further *in vitro* and *in vivo* studies are required to pave way for the use of green tea as a cheap, medically beneficial, and viable alternative to sodium fluoride.

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