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ORIGINAL ARTICLE

The Effect of Epigallocatechin Gallate Extract on Dental Erosion an in Vitro Study

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ABSTRACT

Objective: To evaluate the antierosive effect of epigallocatechin gallate extract (EGCG) on dental erosion and to compare it with potassium nitrate containing mouthwash.

Materials and Methods: This invitro study was conducted in Sardar Begum Dental College and Peshawar dental hospital, Peshawar, KPK. The Scanning electron microscope study was carried out in Centralized Resource Laboratory (CRL), University of Peshawar while Microhardness in University of Engineering and Technology (UET), Peshawar. Total 042 healthy human premolars and impacted third molars were divided into 3 groups: Group I as control (distilled water), Group II was treated with potassium nitrate containing mouthwash while Group III was treated with EGCG. The samples were placed in artificial saliva for six hours. Afterwards teeth were placed in 10ml of the respective test solutions (For each group) for 4 minutes. Samples were kept in commercial beverage for fifteen min with constant stirring at (20-25 °C) afterwards washed with distilled water and then placed in artificial saliva for an hour. The process of erosion was done in four cycles. Finally, the samples were kept in deionized water. The samples were then subjected to Scanning Electron Microscopy (SEM), and micro-hardness.Averages were taken for statistical analysis.LSD post hoc (ANOVA) test was done for analysis of the groups.

Results: There was detectable difference in the dentinal tubule occlusion and hardness between the three groups. EGCG and Potassium nitrate containing mouthwash yielded better results.

Conclusion: It was concluded from the present study that EGCG and potassium nitrate demonstrated antierosive effects.

Keywords: Dentin hypersensitivity, Dentinal Tubule, Potassium nitrate, EGCG

INTRODUCTION

Enamel protects the teeth from everyday uses like mastication, biting and crushing food. Teeth erosion occurs when acids deteriorate the enamel (1). Tooth enamel erosion is caused by: Soft drinks and fruit drinks: (xerostomia): A high sugar and starch diet.Gastroesophageal reflux disease Medications: Genetics: Physical strain on teeth. Environmental factors for tooth erosion are friction, wear and tear, stress (2).Other studies showed that bulimia is an explanation for enamel decay and erosion. A linked disorder with binge eating, vomiting, and acid buildup is bulimia. Vomiting frequently damages tooth enamel and can result in cavities(3).

Saliva is essential for maintaining strong, healthy teeth. Saliva protects tooth enamel by covering teeth with protective layer of Ca^{+2} and other mineral deposits. Saliva has effect on erosive materials like acid, leach out toxic materials from the oral cavity. Plaque is a sticky and slimy film formed on surface of teeth from spit, food particles and bacteria present in mouth (4).

Potassium nitrate is a compound with the chemical formula KNO_3. It is made up of K^+ and NO_3^-, is thus a metal nitrate. It is commonly used to protect against tooth sensitivity (5). Nitrate decreases the flow of fluid through the tubules by obstructing them, decreases the activity of the dental sensory nerves, and prevents or reduces the sensory signals from reaching the brain (5).The action potential in the intradental nerves is blocked by potassium ions. Mouthwash and toothpaste both contain potassium nitrate. There is evidence-in the literature that both preparations-have therapeutic potential for alleviating dentin-sensitivity (6).

EGCG is also known as-epigallocatechin-3-gallate, a catechin, an ofand Polyphenol EGCG, a rich catechin in green tea, is frequently studied in science studies for its likely impact on human health and disorders. Numerous dietary supplements include EGCG. Additionally, traces of it can be found in carob powder, hazelnuts, pecans, plums, and onions (at 109 mg per 100 g) (7).

Teeth consist of a biofilm that contains bacteria and the production of the microorganism in this biofilm is called plaque. There are numerous persuasive data that suggest the EGCG may function as a deterrent to disrupt the process of microorganism attachment to enamel, suppression of glucosyltransferase enzyme activity and inhibition of plaque microorganism multiplication. It is undeniable that the A-amylase activity is reserved by EGCG, indicating EGCG able to prevent tooth decay (4). According to study, EGCG prevents growth of biofilm cultures and Streptococcus mutant organisms. Additionally, numerous cariogenic characteristics of Streptococcus mutans are decreased such as acid tolerance (8).

A number of these studies demonstrate anti-erosive effects of various agents, e.g. Mouthwashes containing nitrate (KNO_3) (9) use of halide (10)calcium and phosphate (Somani et al., 2014) and antiseptic solution (9). Due to the erosion of the teeth, there is a mechanical loss of enamel and dentin, which leads to tooth sensitivity (11). Nitrate solution stops tooth hypersensitivity by occluding dentinal tubules (12) and prevents loss of enamel and dentin by remineralizing the mechanical substances of the teeth. Catechin, which plays a wide biological and pharmacological-role, has been proven to stop tooth decay because of its unique-chemical structure and efficient medicinal activity (1)

Ikigai et al. claimed that the generation of oxide by epigallocatechin gallate in the lipid bilayer leads to the damage of the living matter membrane of the bacterium. Various researchers report inhibition of Eubacterium Sobrinus and Eubacterium Mutans by epigallocatechin gallate. Even if it is advised to consume fewer acidic foods, adding bound components, such as dietary tea supplementation (EGCG), will reduce and resist the potential for erosion of enamel and dentin (1).

According to several reports, drinking green tea lowers your risk of developing ailments including heart disease and teeth decay There are four different types of polyphenols in tea: epigallocatechin-3-gallate (EGCG), epigallocatechin (EGC), epicatechin-3-gallate (ECG), and epicatechin (EC). The largest amount present is of EGCG, about 50% (5).

Chlorhexidine cannot be questioned for its cytotoxic effect on marrow cells other than the drug resistance ability. As a naturally occurring substance isolated from tea, EGCG has greater capability, biocompatibility, and antibacterial characteristics when

compared to a conventional bactericide. According to reports, drinking green tea may lower the incidence of cavities in both humans and laboratory animals. studies have shown that a dentrifice with EGCG is beneficial in stopping inflammation in dentistry due to its unique antimicrobial properties. To investigate this mechanism among EGCGs and dentin, Baruch et al. found that the dentinal tubules are completely blocked by the gel containing EGCG, which prevents bacterial spreading in the tubules and prevent hypersensitivity and dental cavities (13)

Objectives: To evaluate the antierosive effect of EGCG on dental erosion.

To compare the antierosive effect of EGCG with mouthwash containing potassium nitrate.

METHODOLOGY

This Experimental study was conducted in Sardar Begum Dental College and Peshawar dental hospital, Peshawar, KPK. The Scanning electron microscope examination part of the study was carried out in Centralized Resource Laboratory (CRL), University of Peshawar while Microhardness examination was performed in University of Engineering and Technology (UET), Peshawar. This study comprised of 42 healthy human premolars and impacted third molars. It was calculated on G-power with effect size of 0.4, alpha 0.05 at power of study 80%.

Extraction of EGCG from green tea: The following method was adopted to produce green tea extract enriched in EGCG. First the green tea leaves were submerged in distilled water for 30 minutes, afterwards the green tea was brewed at 90 °C for 60 min (14).

Tooth specimen's treatment: The collected samples were placed in 30 ml of synthetic saliva (Sigma) for six hours. Teeth specimens were then divided into 3 groups randomly. Group 1: Distilled water (DW) as control. Group 2: Mouthwash containing Potassium nitrate (KNO3). Group 3: Epigallocatechin gallate extract (EGCG). Afterwards teeth were placed in 10ml of the respective test solutions (Group I, II and III) for 4 minutes. Cleaning of specimens with distilled water was done for ten seconds. Finally, samples were kept in artificial saliva for six hours before the erosion of teeth.

Procedure for erosion: Samples were kept in commercial beverage for fifteen min with constant stirring at (20-25 °C). Afterwards they were washed in distilled water for fifteen seconds then placed the samples in artificial saliva for one hour. The process of erosion was done in four cycles. Finally, the samples were kept in deionized water.

Tooth specimen's preparation for further analysis: Teeth specimen were mounted on cold cure acrylic resin, then by using sand paper samples were sequentially polished (silicon carbide paper) on a polishing machine. The enamel removed was not more than 500 µm in depth.

Microhardness Test: Then by using HMV-2 tester (UET Peshawar) we detected the microhardness of the specimens by making five separate indentations at control and experimental sites. Means were taken for statistical analysis.

Scanning electron microscopy evaluation: We observed the erosion of the samples using scanning electron microscopy (CRL Peshawar University). Then immersed the samples in 2.5% cold glutaraldehyde (0.1 mol/L Phosphate Buffer Solution) at the pH of 7.4 for eight hours. Then sequentially dehydrate the specimens in ethanol solutions (from 50% to 100% ethanol) for 45 minutes' duration before examining through SEM.

The erosive pores present on surface of enamel of each tooth in different groups were measured in µm and a mean value was calculated for comparison.

Statistical Analysis: Data was entered and analysed in SPSS software virsion 23. Descriptive statistics like mean and standard deviation for the erosive effect of tooth was calculated for each group. LSD post hoc test (ANOVA) was used for the data analysis.

RESULTS

Comparison of Diameter of Dentinal tubule orifices between the groups: Surface roughness of enamel was greater in Group I (DW) compared to Group II ($KN0_3$) and Group III (EGCG). Graph shows that the mean diameters of the dentine tubular structure openings of Group II (KNO_3) were lesser than Group I (DW) (2 µm). The mean size of the hollow openings was smaller in Group III (EGCG) than Group I (DW) and Group II (KNO_3) (1.5 µm). The differences between Group I and Group II were significant. However, difference between Group II and Group III was not statistically significant. (Table 5.1)

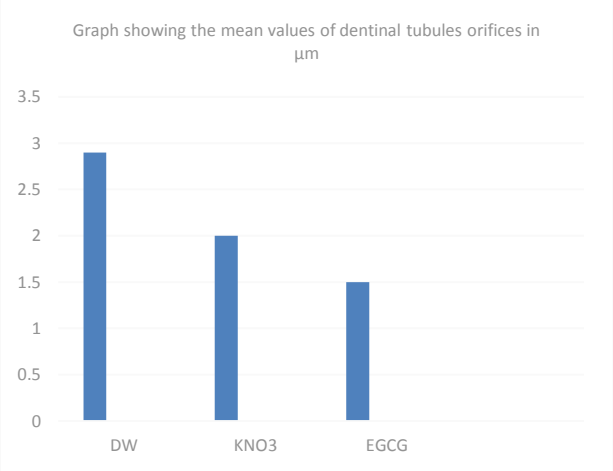
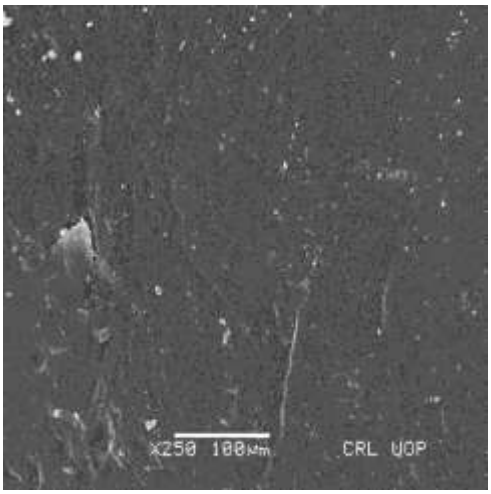


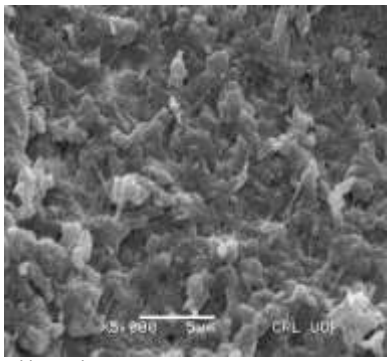
Table 1: Comparison of diameter of dentinal tubule orifices of the groups:

		Mean	Standard Deviation	Significance
Group I (DW)	Group II (KNO_3)	0.94	0.35	.011
	Group III (EGCG)	1.44	0.35	<.001
Group II (KNO_3)	Group I (DW)	-0.94	0.35	.011
	Group III (EGCG)	0.50	0.35	.164
Group III (EGCG)	Group I (DW)	-1.44	0.35	<.001
	Group II (KNO_3)	-0.50	0.35	.164

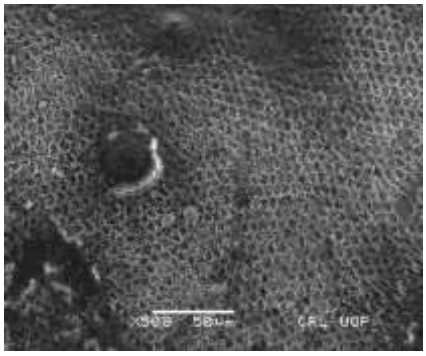
The mean difference is significant at the 0.05 level. Table 5.1

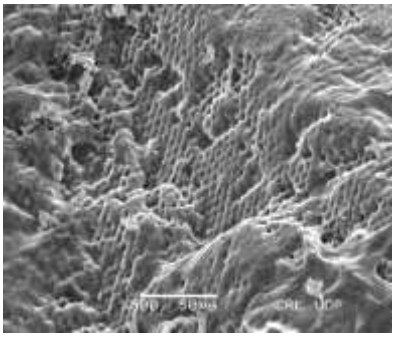


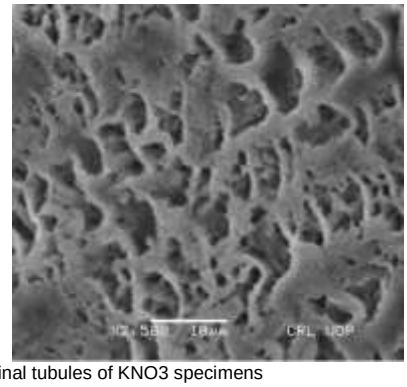
A normal human tooth without erosion



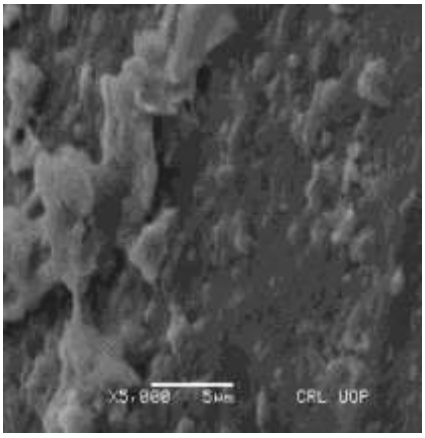
A human tooth with erosion

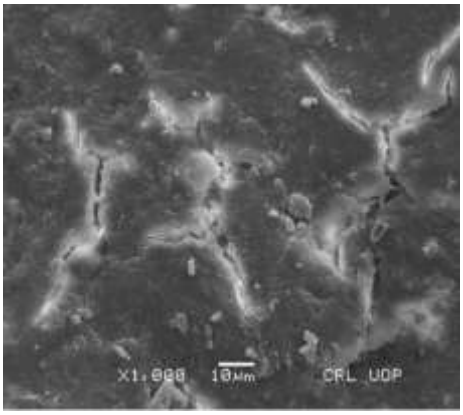


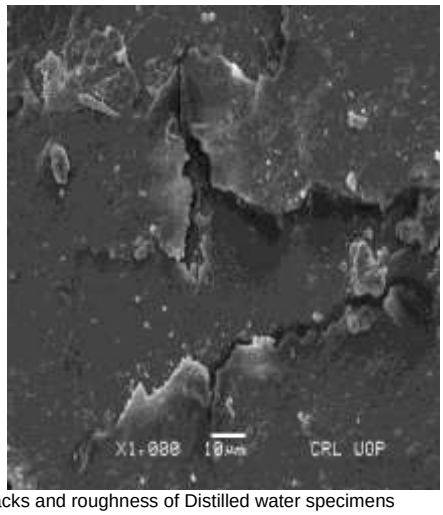




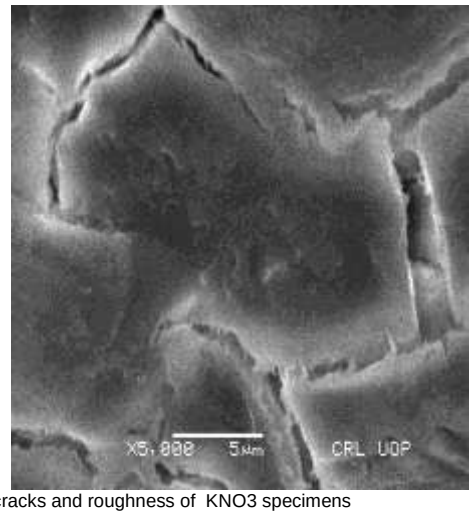
SEM of Dentinal tubules of KNO3 specimens



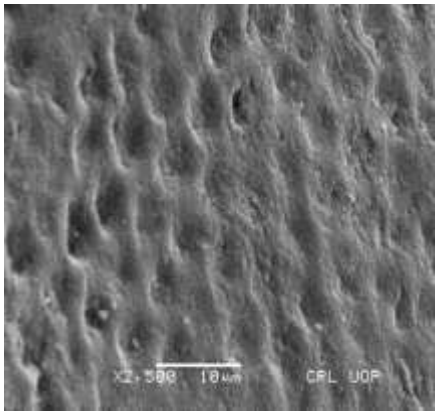


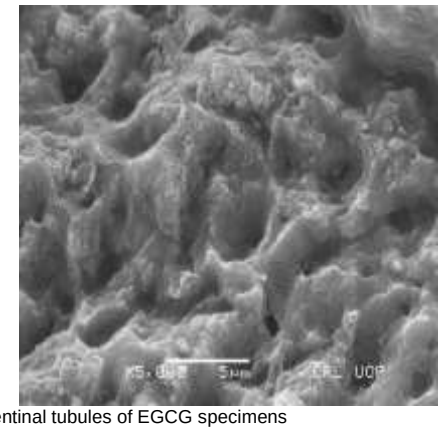


Surface cracks and roughness of Distilled water specimens

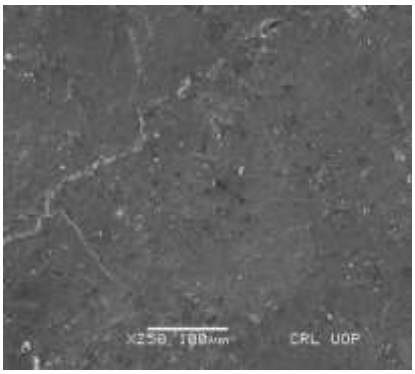


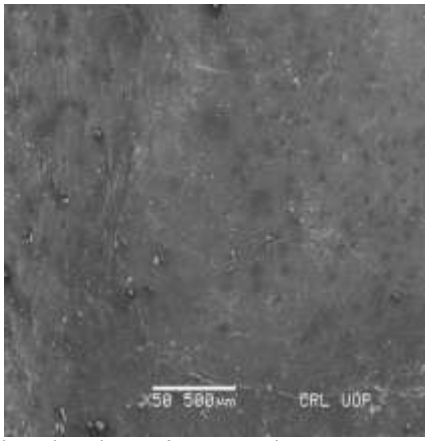
Surface cracks and roughness of KNO3 specimens





SEM of Dentinal tubules of EGCG specimens

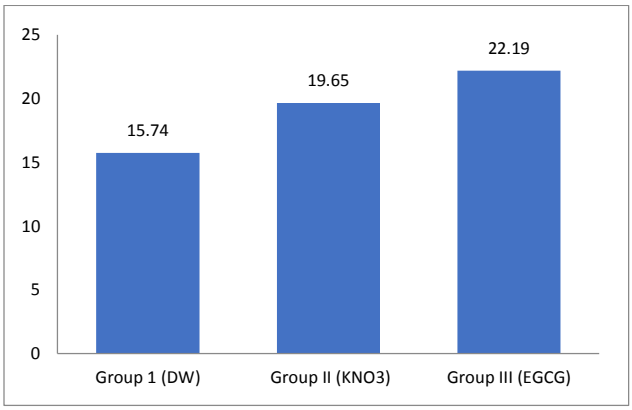




Surface cracks and roughness of EGCG specimens

Comparison of micro hardness between the groups: The micro hardness after the erosive treatment was decreased in all samples. Microhardess was reduced in Group I (DW) in comparsion to Group II (KNO_3) and Group III (EGCG) while microhardness of the samples of Group II (KNO_3) and Group III (EGCG) was reduced to a lesser extent. The difference between the groups was insignificant (p= 0.007) (Table 5.2)

Outcomes of this study showed, the use of both KNO_3 and EGCG may decrease the erosive damage produced by acidic drinks as compared to distilled water. However, the anti erosive effects of EGCG are more effective as compared to KNO_3.



Mean and standard deviation of the 3 groups:

Groups	Mean	Standard Deviation
Group I (DW)	15.74	5.72
Group II (KNO_3)	19.65	5.94
Group III (EGCG)	22.19	3.29

Comparison of surface hardness of the groups:

		Mean	Standard Deviation	Significance
Group I (DW)	Group II (KNO_3)	-3.91	1.94	.051
	Group III (EGCG)	-6.45	1.94	.002
Group II (KNO_3)	Group I (DW)	3.912	1.94	.051
	Group III (EGCG)	-2.54	1.94	.198
Group III (EGCG)	Group I (DW)	6.45	1.94	.002
	Group II (KNO_3)	2.54	1.94	.198

The mean difference is significant at the 0.05 level. Table 5.2.

DISCUSSION

The outcomes of our study specified that immersion of the teeth in KNO_3 and EGCG may reduce the erosion damage as compared to distilled-water. However, the effect of-EGCG was more pronounced as compared to KNO_3. Shielding effects were determined for loss of tooth-material and porosity of the dentinal-tubules.

Few studies are directed showning the consequences of-EGCG on the-erosion of enamel of human tooth. Study done-by Yu et al showed similar results to our study that EGCG has more tendencies to block-dentinal tubules and improve hardness thus showing better anti-erosive effects. Yu and his colleagues also showed that once the catechin is applied to a mixture of 2% NaF (pH 5.9), the effect increases by up to an hour (15). The results of Madhan et al were contradictory to our results. The difference may be due to completelety different ratio of catechin and halide and also the increase sample size (Madhan et al., 2007). The results of another study done by Magalhaes et al., goes in the favour of our study. It states that the MMP (matrix metallo proteinases) inhibitors

(chlorhexidine and EGCG) and halides appeared to reduce damage from dentine erosion and abrasion. The actions of medium metalloproteinases (MMPs) are responsible to brought about at a low pH and boosted by neutralization (16).

Maruyama and his co-workers investigated the procedure of EGCG and dentin in averting erosion, they applied gel on the dentin surface. The gel blocked the orifices of dentinal tubules and they observed that EGCG increase the production of acid-resistant organics. These results are not comparable to our study because neither EGCG nor KNO_3 showed complete blockage of dentinal tubules (17).

Another study that goes in the favor of our study was done on rats, in which undeniable effectiveness of KNO_3 in tubules closures was found (18). However, studies have shown further improved clinical efficacy when KNO_3 and EGCG were combined with various active ingredients such as salt and chloride along with monofluorophosphate, nano-hydroxyapatite, antioxidants phloretin, ferulic acid and silymarin. These results are similar to our results in reducing sensitivity by blocking dentinal tubules and improving the anti erosive activity.

Another research lead to-examine actions of fluoride-and EGCG on tooth enamel exposed to acidic drinks-erosion. Soares and his team established that enamel can be protected with EGCG and-fluoride application without alleviating-unevenness or damage of enamel-tissue. These findings are contradictory to our study, as we observed that the irregularity of the enamel tooth surface samples was greatly noticeable in the Group I (DW) than in Group II (KNO_3) and Group III (EGCG). However, EGCG showed better results as compared to Group I and Group II (19).

Dentin-hypersensitivity might increase with the increase in diameter of dentinal tubules. The tubule-orifices diameter in the KNO_3 and EGCG groups were lesser than the DW group. KNO_3 and EGCG reduced dentin-hypersensitivity by occluding dentinal-tubules. When EGCG touches the tooth surface, it forms calcium-precipitates above the tooth surface. Even if the surface of tooth comes across an acidic-setting,-this superficial layer offers resistance to the demineralization-mechanisms and increases the remineralization-procedure (20). This phenomenon is also seen in our study. As EGCG consists of many hydroxygroups, and has a chelating consequence on ions of metal, it makes a shielding surface-layer, and may protect teeth from the process of erosion. EGCG can offer defensive impact in the peritubular place of dentin, as compared with different groups. It may be detected with the SEM images, greater erosive harm was found in Group I (DW).

Softening of teeth is a crucial matter of tooth-injury caused by erosion. Softening cause, the teeth surface more vulnerable to-damage. The tooth having high-mineralization content is susceptible to damge by erosion (Khan et al., 2019). This finding in the above mentioned study is like that of our results, as in our findings all samples were softened following erosive-procedure. Loss of-hardness was present in all samples. The loss of hardness of enamel-samples was less in EGCG group. It is acclaimed; EGCG may deliver more protective-effect from loss of tissue hardness in the teeth-samples than others. However, the -outcome of KNO_3 was similar to that of EGCG.

Another important mechanism of preventing the tooth enamel surface from erosive harm is chelation of metal-ions by -EGCG (21). Though the mechanism was not observed but our findings also showed that EGCG can decrease the erosion initiated by the acidic-beverages, including tooth-structure loss, decrease in surface tissue hardness, and increase in the dentinal tubule orifices diameter. EGCG decrease the hazard of increased hypersensitivity of teeth. EGCG appears to offer more shielding-effect from erosion than KNO_3 at the present-concentrations.

CONCLUSION

Conclusion from the present study is that EGCG and potassium nitrate demonstrated antierosive effects.

Limitations and Recommendation: However, limitations of the study are that it is an in vitro study, hence restricting its capacity to be parallel with the oral health conditions. As dentinal erosion is individual in nature, the results need to be validated against patient's perception of sensitivity and quality of life. In future studies EGCG and potassium nitrate may be given together to see whether they cause synergestic effects or not.

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