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Comparative evaluation of lycopene and grape seed extract in preventing enamel erosion: An atomic force microscopic analysis

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Abstract

Introduction: Erosive tooth wear has been widely studied because of its increasing incidence resulting from the modern society lifestyle and substantial changes in eating habits. Advanced tooth erosion can affect aesthetics and function and induce hypersensitivity. Therefore, it is essential to focus on the initial phases of the erosive process establishing appropriate measure of erosion prevention and control.

Aim: The aim of this study is to evaluate the effect of lycopene and grape seed extract in preventing enamel erosion.

Materials and Method: Eighty freshly extracted mandibular pre molar teeth were used in this study. The enamel blocks of dimension 5x5x3 mm were prepared by cutting at enamel dentin junction from the buccal surface of the tooth, with a high-speed diamond rotary disc with a water air spray. Samples then embedded in acrylic resin and polished with silicon carbide disc to

obtain a smooth flat surface. The samples then randomly divided in to 4 groups (n=20) according to the remineralizing agent as follows; GROUP 1: surface treatment with Deionized water [negative control], GROUP 2: Surface treatment with Casein phosphopeptide- amorphous calcium phosphate (CPPACP) [Positive control], GROUP 3: Surface treatment with 5% Lycopene , GROUP 4: Surface treatment with 6.5% Grape Seed extract. All the specimens were treated with 20 ml of remineralizing agents for 30 seconds twice daily and continue for 28 days. After each application with remineralizing agents, the specimens were rinsed with distilled water and stored in artificial saliva. After the samples were remineralized for 28 days, they were immersed in 5 ml of coca cola for 10 minutes daily for 4days to induce artificial erosive effect. After the erosive cycle, the specimens were observed with an atomic force microscope to evaluate the average surface roughness (Ra).

Results: The result of this invitro study demonstrated that, CPP-ACP has high resistance against enamel erosion, followed by 5% Lycopene , 6.5% Grape seed extract & Deionized water Group.

Keywords: CPP ACP, Lycopene, Grape Seed Extract, Atomic Force Microscope, Average Surface Roughness (Ra)

Introduction

Dental enamel is a crystalline lattice work composed of various minerals, the principal component of which is a complex calcium phosphate mineral called hydroxyapatite. Chronic exposure to extrinsic or intrinsic acids with a low ph leads to dental erosion[1]

Dental erosion is a process involving the dissolution of enamel and dentine by non-bacterial acids that softens the enamel surface, so mechanical factors such as abrasion and attrition may result in further loss of tooth tissue. The prevalence of dental erosion is thought to be increasing, due to the wide availability and frequent consumption of acidic drinks such as soft drinks, sports drinks and fruit juices. The development of erosion involves a chemical process in which the inorganic phase of the tooth is demineralized, thereby reducing the hardness of tooth substrates[2]

Erosive tooth wear has been widely studied because of its increasing incidence resulting from the modern society lifestyle and substantial changes in eating habits. Advanced tooth erosion can affect aesthetics and function and induce hypersensitivity. Therefore, it is essential to focus on the initial phases of the erosive process to establishing appropriate measure of erosion prevention and control[3]

Fluoride and mineral synergistic products having anti erosive properties such as fluoride enriched Casein phosphopeptide- amorphous calcium phosphate (CPP-ACP) and beta tricalcium phosphate have been introduced as improved agents for dental enamel remineralization. These agents affect remineralization of the teeth by various mechanisms[4]

More recently, a variety of naturally occurring vegetables and food supplements has shown to promote health and is now suggested that antimicrobial compounds of plant origin can be considered alternative to the commonly used chemicals for controlling dental plaque and dental diseases.8 Plant extracts such as green tea have proven to have remineralizing property due to the presence of poly phenols which inhibit the collagen degradation due to the presence of matrix metallo proteinases inhibitors. Grape seed extract (GSE) and Lycopene are other natural products containing polyphenols; however, their role in preventing dental erosion is speculative[5]

Therefore, the aim of this study is to comparatively evaluate the role of Grape seed extract and Lycopene in preventing dental erosion using atomic force microscopy.

Materials and Methods

Eighty single rooted mandibular first premolar teeth that are indicated for extraction due to orthodontic reasons and periodontal problems were collected by simple random sampling method. The extracted teeth were scaled to remove debris, calculus, and rinsed with sodium hypochlorite to remove organic tissue and then stored in distilled water.

The enamel blocks of dimension 5x5x3 mm were prepared by cutting at enamel dentin junction from the buccal surface of the tooth, with a high-speed diamond rotary disc (D 321-300. Kerr dental) with a water air spray. The tooth surface near the enamel dentin junction were ground using silicon carbide paper (3M 401Q grade: 1200) under water irrigation to produce flat enamel surface. The Samples were then embedded in acrylic resin (DPI RR cold cure) using a rubber mould (figure.6) and polished with silicon carbide disc (Toboom DD0001 4A HP) to obtain a smooth flat surface. The sectioned samples were randomly divided in to 4 groups (n=20) according to the remineralizing agents as follows:

Group 1: surface treatment with Deionized water [negative control]

Group 2: Surface treatment with CPP-ACP [Positive control]

Group 3: Surface treatment with 5% Lycopene.

Group 4: Surface treatment with 6.5% Grape Seed extract.

All the specimens were then treated with 20 ml of remineralizing agents for 30 seconds twice daily and continued for 28 days. After each application with remineralizing agents the specimens were rinsed with distilled water and stored in artificial saliva. After 28 days, the samples were immersed in 5 ml of coca cola for 10 minutes daily for 4 days to induce artificial erosive effect. After immersing in the cola solution, the samples were directly stored in artificial saliva.

Atomic Force Microscopy

After 24 hours from the last procedure of demineralization, specimens were observed with an Atomic force microscopy (PARK SYSTEMS,UL 10X/0.23, WD 50.5, Seol,SouthKorea),(IISC Bangalore) equipped with a piezoelectric scanner, which can cover an area of 100 x 100 μm2 with a range of 7 μm in the z direction. The most common topographical parameters were determined, such as average surface roughness (Ra).

The scores obtained were statistically analyzed with One-way ANOVA test followed by Tukey's HSD post hoc analysis to compare mean surface roughness values between 4 groups.

Results

In this study, mean surface roughness values obtained among 4 groups shows statistically significant difference between the control group and the experimental groups (Table 1).

Table 1: Comparison of Surface Roughness Values between different groups using One-way ANOVA Test

Groups	N	Mean	SD	Min	Max	P-Value
Deionized Water	20	266.8021	18.3714	230.515	294.514	<0.001*
CPP-ACP	20	56.6676	7.5957	44.567	69.017	
5% Lycopene	20	90.3575	7.4665	76.597	101.547	
6.5% Grape Seed Extract	20	114.1784	7.8269	104.782	128.820	

* - Statistically Significant

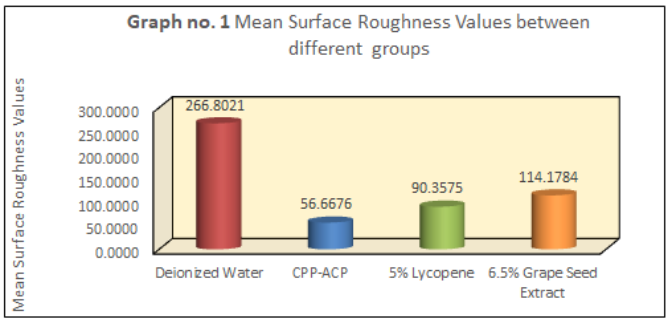
Table 1 represents the comparison of mean Surface Roughness values between different groups. The test results demonstrate that the mean Surface Roughness for Deionized group was 266.8021 ± 18.3714, CPP-ACP group was 56.6676 ± 7.5957, 5% Lycopene group was 90.3575 ±7.4665 and in 6.5% Grape Seed Extract was

114.1784 ± 7.8269. This difference in mean Surface Roughness values between 04 groups was statistically significant at P<0.001. (Refer graph no.1)

Table 2: Multiple comparison of mean difference in Surface Roughness Values between groups using Tukey's HSD Post hoc Analysis

(I) Groups	(J) Groups	Mean Diff. (I-J)	95% CI for the Diff.		P-value
			Lower	Upper	
Deionized Water	CPP-ACP	210.1346	200.7347	219.5344	<0.001*
	5% Lycopene	176.4447	167.0448	185.8445	<0.001*
	6.5% Grape Seed Extract	152.6237	143.2239	162.0235	<0.001*
CPP-ACP	5% Lycopene	-33.6899	-43.0897	-24.2901	<0.001*
	6.5% Grape Seed Extract	-57.5109	-66.9107	-48.1110	<0.001*
5% Lycopene	6.5% Grape Seed Extract	-23.8210	-33.2208	-14.4211	<0.001*

Table 2 represents multiple comparison of mean difference in the Surface Roughness between 4 groups. The results demonstrate that CPP-ACP Group demonstrated significantly least mean Surface Roughness as compared to other study groups at P<0.001.This indicating that CPP-ACP group has most resistance against enamel erosion when compared to other tested groups. This was then followed next by 5% Lycopene Group showing significantly lesser mean Surface Roughness as compared to 6.5% Grape seed extract & Deionized Water groups at P<0.001 respectively. Finally, 6.5% Grape seed Extract group also showed significantly lesser mean surface roughness as compared to Deionized water group at P<0.001. The results infer that CPP-ACP Group showed significantly least mean Surface roughness, followed by 5% Lycopene Group, 6.5% Grape seed extract Group & highest with Deionized water Group.



AFM images of CPP-ACP group showed a uniform enamel surface pattern with more homogenous structure, which could be suggestive of less demineralization of enamel surface after the erosive effect.(fig.1) Whereas in lycopene group an irregular enamel surface with homogenous structure was observed, with projections and depressions in fewer areas , suggestive of relatively less demineralization of enamel surface after the erosive effect(fig.2).In grape seed extract group, after erosion, the enamel surface was appeared more non homogenous in structure, with projections and depressions in irregular manner , which is demonstrating relatively less anti erosive property of grape seed extract when compared to CPP-ACP and lycopene(fig.3). Deionized water, the negative control group showed extremely irregular surface with peaks and depressions, suggestive of excessive demineralization of enamel surface after the erosive effect.(fig.4).

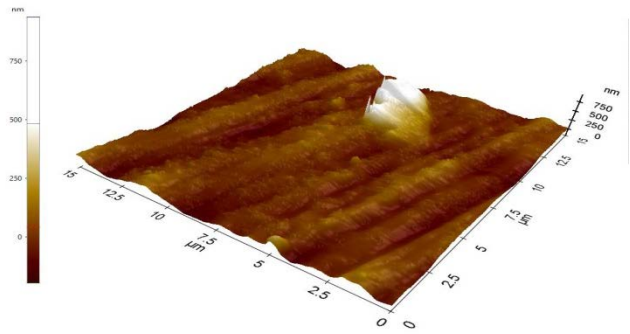


Figure 1: AFM image of enamel surface in CPP-ACP group

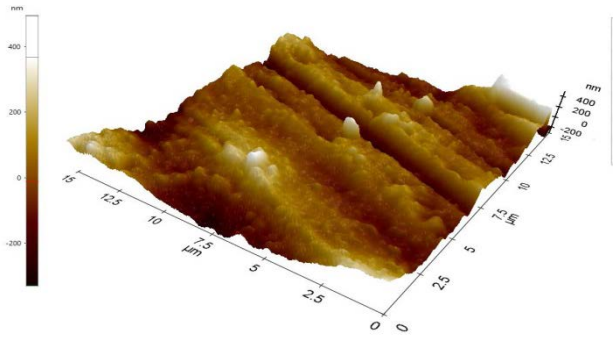


Figure 2: AFM image of enamel surface in lycopene group

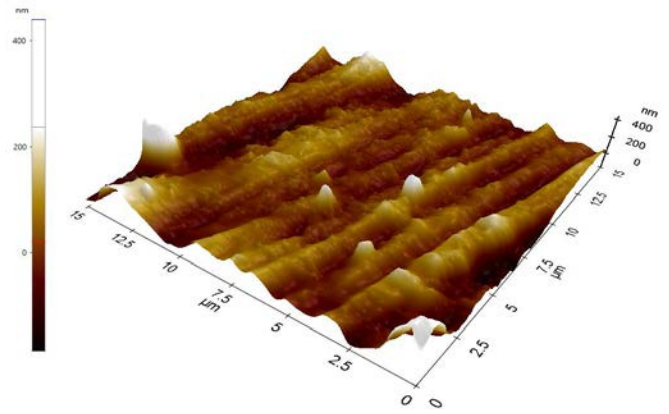


Figure 3: AFM image of enamel surface in grape seed extract group

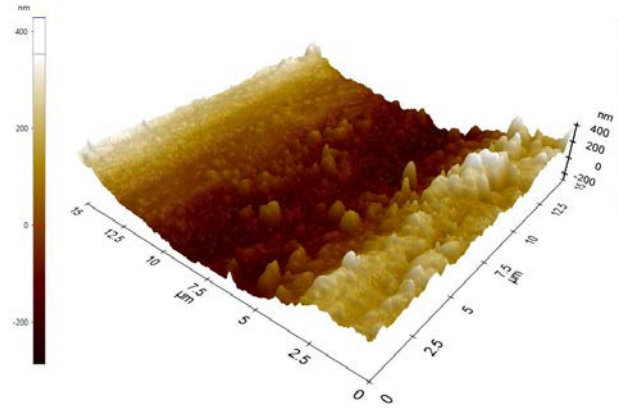


Figure 4: AFM image of enamel surface in Deionized water group

Discussion

The degree of demineralization of tooth structure caused by erosion depends primarily on the amount and frequency of the consumption of acidic drinks, type of acid and the way these drinks are ingested. To prevent the occurrence of tooth erosion, resources for guidance regarding diet may be used, as well as the application of products that minimize demineralization and promote remineralization of the tooth structure[6].

This present study has compared the action of 5% lycopene and 6.5% grape seed extract with well-established remineralizing agent CPP-ACP at experimental periods of time in which these products are known to be effective.

In group 1, Distilled Deionized water was used and the results showed its least effectiveness in preventing demineralization of enamel among all 4 groups. This is in accordance with the study conducted by DJ Manton et al. showed that distilled Deionized water does not have any effect on remineralization of enamel surface[7].Another study conducted by Franciny Querobim Ionta et al, demonstrated artificial saliva formulations with deionized water did not promote any remineralization potential whereas other evaluated formulations have the ability to remineralize initial softened erosive lesion, since they presented greater re hardening effect than deionized water control group[8]

CPP-ACP was used in group 2 and showed least enamel surface roughness value after erosive effect. This indicating it's excellent property in preventing demineralization of enamel. This is in accordance with Rui Vitorino et al. who evaluated that, Casein phosphopeptide- amorphous calcium phosphate can provides all the elements which are necessary for dental remineralization (calcium, phosphate, fluoride, and water) on the tooth surface as well as in the dental biofilm. It was also demonstrated that, plaque enzymes such as phosphatases and peptidases were partially degrade casein phosphopeptide-based products and consequently increasing pH due to the production of ammonia. Adding fluoride to casein phosphopeptide limits phosphatase action by extending the action of molecular complexes[9]

According to a study conducted by Rees J et al. have shown lower enamel erosion due to citric acid when enamel is previously treated with a CPP- ACP paste[10]

Another study done by Tantbirojn et.al demonstrated significant increase in hardness of enamel treated with

CPP-ACP paste after application with cola derivatives, which reduce its hardness[11].

5% lycopene was used in group 3 showed relatively lesser mean Surface Roughness as compared to 6.5% Grape seed extract which is statistically significant at P<0.001.This can be due to the MMP -2 and MMP- 9 inhibiting property of lycopene which was explained by Hwang et al[12]. Various researches conducted by costa a r et al[13]. and Tjaderhane L et al[14]. also demonstrated the MMP inhibiting property of lycopene in their respective studies. Study done by Hannas et.al evaluated the preventive effect of MMP inhibitors on teeth erosion and abrasion. They used mouth rinses with MMP inhibitors like chlorhexidine, and green tea extract and assessed the remineralizing potential using profilometer[15]. More recently, it was reported by Li et al in their study that, the antioxidant carotenoid lycopene, inhibited fluoride induced ameloblast apoptosis and enamel fluorosis in rats by combating oxidative stress[16].

In group 4, grape seed extract showed significantly lesser mean surface roughness value than Deionized water (control group) and higher roughness value when compared to CCP-ACP and Lycopene. study done by Nandakumar et al. who evaluated the effect of grape seed extract and cranberry extract in preventing enamel erosion, and demonstrated Grape seed extract as a phytotherapeutic agent which contain polyphenols, mainly proanthocyanidins (PAs), which may cause superficial mineral deposition over the lesion by forming insoluble complexes[5]. This finding was similar to the studies done by Rubal et al[17]. Another mechanism of PAs was explained by Kosasi et al. who identified the effect of PAs in dentin erosion as a dentin biomodifier, which react with the exposed organic matrix and stabilize the collagen by inducing collagen cross-linking[18].

Mirkarim et al. studied the remineralization effect of grape seed extract on artificial caries in primary teeth and observed mineral deposition predominately on the surface layer, induced by gallic acid, which is one of the major constituents of grape seed extract and galla chinensis[19]. Ketaki Dinkar et al.done a study to determine remineralizing potential of grape seed extract compared to CPP-ACP and calcium glycerophosphate (CaGP), and found that highest mineral deposition with grape seed extract as against CPP-ACP and calcium glycerol phosphate. This is because grape seed extract treatment increased the ultimate tensile strength at demineralized dentin, indicating the potential of grape seed extract to induce cross-links in the dentin collagen[20]

Conclusion

- All the three type of remineralizing agents showed significant resistance against enamel erosion when compared to the control group (Deionized water).
- Among the 2 types of natural plant extracts, lycopene was showing excellent resistance against enamel erosion when compared to grape seed extract.
- Grape seed extract also showing less surface roughness value when compared to the control group, which indicating its remineralizing property and potential to resistance against enamel demineralization
- Further in vitro and in vivo investigations wanted for higher appreciation of the remineralizing property of lycopene and grape seed extract and its effect on preventing enamel erosion.

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