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EFFICIENCY OF FLUORIDE-FREE TOOTHPASTES ON THE INHIBITION OF EROSIVE TOOTH WEAR IN PRIMARY AND PERMANENT TEETH

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ABSTRACT: The objective of this study was to evaluate inhibition efficiencies of different fluoride-free remineralization agents against erosive challenge of orange juice on primary and permanent teeth samples using optical profilometer and scanning electron microscope. Forty-five non-carious permanent third molar teeth extracted in the orthodontic treatment and 45 non-carious primary teeth extracted at the exfoliation time were included in the study. After surface preparation for each sample, three different toothpastes which included CPP-ACP (Group-1), organic calendula-xylitol (Group-2), and calcium hydroxyapatite-silica (Group-3), were applied during erosion cycle with orange juice. After the cycle, surface depth and roughness for all samples were evaluated by optical profilometer. According to the analysis of exhibited data, significance level was accepted as p<0.05. Linear surface depth values were significantly higher in permanent teeth compared to primary teeth for Group-1. However, volumetric surface roughness values were statistically higher in Group-2 and Group-3 for primary teeth compared to permanent teeth samples. Erosion inhibition efficiency of fluoride is known. However, parents are very concerned about the toxic nature and prefer using fluoride-free toothpastes. Based on the findings, parents should be informed about insufficient erosion inhibition efficiency of these fluoride-free toothpastes and further studies are needed to inform dentists about the remineralization efficiency of ingredients of toothpastes for both primary and permanent teeth.

Keywords: Casein phosphopeptide-amorphous calcium phosphate; Erosive tooth wear; Fluoride-free toothpaste; Inhibition of erosion; Optical profilometer.

INTRODUCTION

Chemical parameters, such as citric acid containing beverages, and physical parameters can modulate the erosion process on the enamel tissue of teeth. High concentration of acid attacks or acid exposure for a long time or properties of exposed teeth like being a primary or permanent tooth might affect erosion depth on the tooth surface.[1,2] Degradation mechanism in these erosive tooth wear (ETW) lesions can be stopped or inhibited by the accumulation of minerals at neutral or alkaline pH to rebuild a surface on existing crystal residues.[3] Erosion and inhibition of erosion processes should occur in a certain balance. This balance can be affected by factors such as low pH, inadequate salivary function, increased incidence of carbohydrate uptake, insufficient fluoride uptake, and the balance can change rapidly in the direction of erosion.[1,4]

Today's modern dentistry aims to treat or inhibit non-cavitated caries or ETW lesions with preventive agents as non-invasive treatment approaches. The basic principle of these treatments is to prevent the progression and maintain the integrity of healthy tooth structure and remineralizing agents are the most preferred preventive treatment among these approaches.[5] These agents might be toothpaste or mouthwash

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containing fluoride, casein phosphopeptide amorphous calcium phosphate (CPP-ACP), and hydroxyapatites.[6,7]

Initial stage of the erosion area, which is at submicroscopic level, cannot be seen clinically. However, continuity of mineral loss makes it clinically visible.[1,6] In modern dentistry, some non-invasive and non-destructive devices aim to evaluate this erosion and inhibition processes at initial stage at submicroscopic level. These devices, such as scanning electron microscopy (SEM), confocal laser scanning microscopy, quantitative light-induced fluorescence, optical coherence tomography, and surface profilometer, give us quantitative and qualitative information about morphological changes on ETW surface.[2,8,9]

The objective of this study was to evaluate erosion inhibition efficiency of different toothpastes including CPP-ACP, organic calendula-xylitol, and calcium hydroxyapatite-silica as fluoride-free remineralization agents against the erosive effect of orange juice in both primary and permanent teeth by using an optical profilometer. This study hypothesized that (1) primary teeth would be affected by erosive attacks more than permanent teeth and, also (2) CPP-ACP containing toothpaste would inhibit the further progression of ETW more than other agents.

MATERIALS AND METHODS

Study design: Sample size of each group was calculated for binary primary outcome measures as the evaluation of surface depth so, 15 samples per group for primary and permanent teeth with a total number of 90 samples were used to detect a significant difference for a two-sided type I error at 5% and 90% power.[10,11]

This research study was approved by Başkent University Institutional Review Board (Project no: D-KA 18/16) and non-carious primary molar teeth extracted at the exfoliation time and non-carious permanent third molar teeth extracted in the orthodontic treatment from healthy patients with no systemic disorders were collected in six months and included in the study. Also, all teeth were clinically evaluated as being without any hypomineralized areas on enamel surfaces before inclusion.

Sample preparation: Firstly, roots were removed by using a diamond saw and remained crown parts of the 45 primary molar and 45 permanent third molar teeth were embedded into acrylic resin blocks (Paladur,® Bad Homburg, Germany). Then, all buccal surfaces of all teeth were ground with water-cooled silicon carbide paper discs from grit 500 to 4000 for five seconds and polished with diamond paste under constant cooling to obtain a flat and highly polished enamel surface (Grinder-Polisher, Metkon,® Germany). This procedure removed a standardized layer of 200 µm of the outer enamel.[2] After surface flattening process, all enamel surfaces of 90 teeth were dyed with nail varnish as 4 mm length and 4 mm width square-shaped area and half of this surface was covered with another acid-resistant nail varnish to create a precycle area (sound area) and the postcycle area (experimental area) that was used to evaluate the inhibition of ETW.

Toothpastes: Fluoride-free toothpastes, which have the ingredients given below, were tested in this study. All toothpastes were applied by the same operator throughout the experiment as a thin layer with a microbrush on the 2 mm length and 2 mm width enamel surfaces.

Group-1: CPP-ACP (GC Tooth Mousse, GC Europe, Leuven, Belgium)

Group-2: Organic calendula-xylitol (Natural Toothpaste, Jack N' Jill Kids, Melbourne, Australia)

Group-3: Calcium hydroxyapatite-silica (Natural Toothpaste, Splat, Moscow, Russia)

Group-4: Control group (not applying any toothpastes)

Erosion cycle: After surface preparation process, erosion cycle was applied to buccal surfaces of all enamel samples with orange juice and artificial saliva. Orange juice (Tropicana,® PepsiCo, Istanbul, Turkey) was chosen with attention to be without any added sugar or acid contents as ingredients and pH value (pH: 3.9) was checked just before every cycle. Artificial saliva was prepared freshly at pH:7 with a suggested formula ($CaCl_2$, KH_2PO_4, KCl, KOH, Hepes).[12,13] The erosion cycle used in this study is shown in Figure 1 and it was performed for five days.[14]

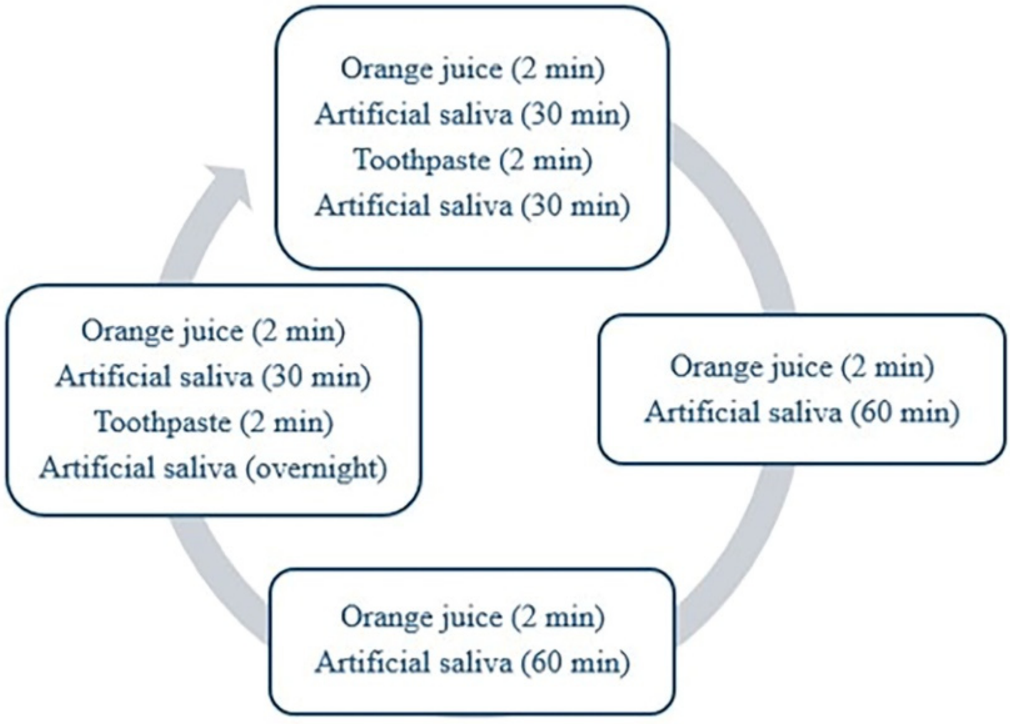

Figure 1. Erosion cycle applied to the teeth samples for five days.

While teeth were going through the erosion cycle for both orange juice and artificial saliva, samples were under a controlled agitation in a shaking incubator (S16-2 Shel Lab, Sheldon Manufacturing, North Carolina, United States of America) of 75 rpm at 25ºC.[15] After the cycle, the nail varnish on the tooth surface was removed and evaluated as a precycle area while the other area without the nail varnish was evaluated as a postcycle area. So, precycle area was compared with postcycle area to give the difference between sound side and experimental side.

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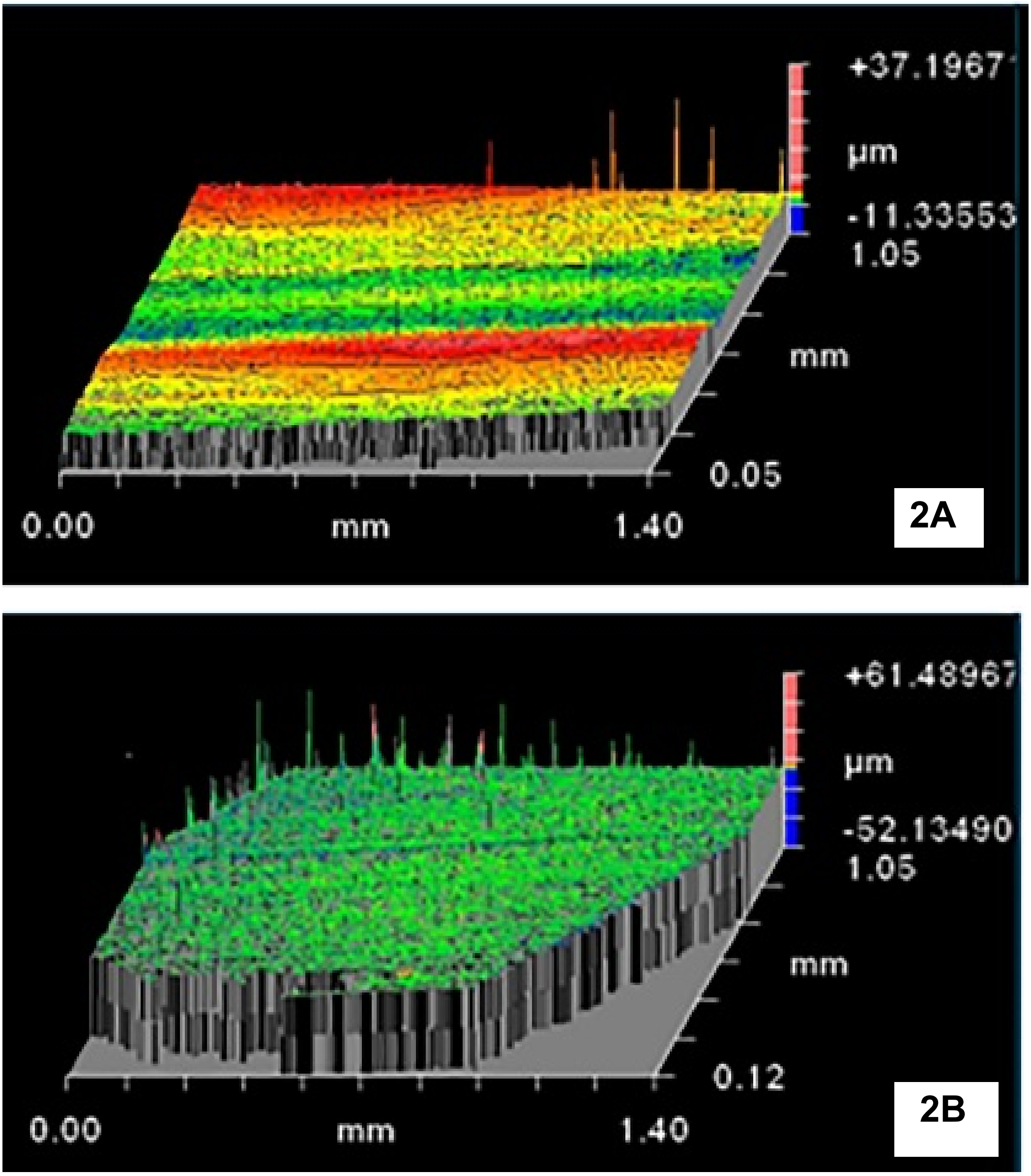
Evaluation with optical profilometer: Following the erosion cycle, erosion inhibition efficiencies of toothpastes for all teeth were evaluated by measuring surface depth (PV) and surface roughness (Ra) values of the enamel surfaces by using optical profilometer device (ZYGO 7200, Zyco Corporation, Middlefield, CT, USA). Figures 2A and 2B show the precycle and postcycle images taken by optical profilometer device for one tooth sample in Group-1.



Figures 2A and 2B. Optical profilometer images of Group-1 (CPP-ACP group); 2A: shows the reference (precycle) side; 2B: shows the experimental (postcycle) side.

Three measurements were made from precycle and postcycle areas and average of these three measurements was used to determine the difference between sound and experimental sides of each tooth. After the measurements, the difference between

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average values of both areas was considered as the amount of erosion progression that toothpastes induced and, also the significance between the groups.[10]

Evaluation with scanning electron microscope: Randomly selected seven primary and permanent tooth samples from each group were compared visually by using Environmental Scanning Electron Microscope–ESEM (FEIQuanta 200FEG, FEI Company, Hillsboro, Oregon, USA) with 8,000 × magnification in order to estimate the changes of enamel surface after the erosion process.

Statistical analysis: The difference between average values of precycle and postcycle areas were used to determine statistical significancy between groups and tooth types such as primary or permanent. Obtained data were analyzed by using SPSS 21 package program. Kruskall-Wallis H test was used to compare the erosion inhibition efficiency of three different toothpaste groups in order to determine intergroup difference. Also, Mann-Whitney U test was used to compare the erosion inhibition efficiency between primary and permanent teeth in terms of the intragroup difference. Significance level was accepted as $p<0.05$.

RESULTS

Intergroup analysis: Obtained values from all samples showed erosion formation according to the increased linear Ra, volumetric Ra, linear PV, and volumetric PV values between precycle and postcycle areas. When the difference between postcycle and precycle were compared in primary tooth samples, there was no significant difference in linear Ra ($p=0.70$), volumetric Ra ($p=0.28$), linear PV ($p=0.31$), and volumetric PV ($p=0.32$) values between any of the groups. Volumetric PV ($p=0.02$) values were significantly higher in Group-2 than other groups for permanent teeth. However, linear Ra ($p=0.18$), volumetric Ra ($p=0.06$), and linear PV ($p=0.72$) values were not statistically significant for any groups (Table 1).

Table 1. Comparison of Ra and PV values amongst the groups for primary and permanent teeth (SD=standard deviation)

Parameter	Group	Teeth number N	Primary teeth Mean±SD*	Primary teeth Kruskal-Wallis H test p-value*	Permanent teeth Mean±SD*	Permanent teeth Kruskal-Wallis H test p-value*
Linear Ra	Group 1	15	0.33±0.51	0.70	0.26±0.24	0.18
	Group 2	15	0.52±0.71		0.22±0.24	
	Group 3	15	0.31±.046		0.18±0.28	
	Group 4	6	0.24±0.49		0.03±0.15	
Linear PV	Group 1	15	0.10±10.03	0.31	4.40±5.39	0.72
	Group 2	15	0.97±8.86		6.14±11.49	
	Group 3	15	3.35±8.35		1.58±5.14	
	Group 4	6	-11.32±21.05		8.19±14.41	
Volumetric Ra	Group 1	15	0.79±0.88	0.28	0.24±0.18	0.06
	Group 2	15	1.03±0.86		0.30±0.25	
	Group 3	15	0.69±0.78		0.21±0.33	
	Group 4	6	0.38±0.57		0.03±0.20	
Volumetric PV	Group 1	15	-2.00±16.26	0.32	-3.28±29.90[a,b]	0.02*
	Group 2	15	1.59±16.55		14.85±15.19[a]	
	Group 3	15	9.59±15.28		1.70±12.15[a,b]	
	Group 4	6	1.26±22.64		1.35±15.95[a,b]	

*Refers to statistically significant difference, p<0.05; (a) refers the groups with statistically significant difference; (b) refers the groups without statistically significant difference.

Intragroup analysis: When all the postcycle and precycle difference values were evaluated, the linear PV values (p=0.04) were significantly higher in permanent teeth compared to primary teeth for Group-1. However, linear Ra (p=0.92), volumetric Ra (p=0.24), and volumetric PV (p=0.40) values were not statistically significant (Table 2). Volumetric Ra values (p=0.01) were higher in primary teeth, while volumetric PV

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(p=0.02) values were significantly higher in permanent teeth for Group-2. However, there was no significant difference between linear Ra (p=0.63) and PV (p=0.40) values for primary and permanent teeth in Group-2 (Table 2). In Group-3, volumetric Ra (p=0.03) values appeared to be significantly higher in the primary tooth but there was no significant difference between the groups in terms of other difference values (Table 2).

Table 2. Comparison of Ra and PV values amongst primary and permanent teeth for each group (SD=standard deviation, Vol RA=volumetric Ra, Vol PV=volumetric PV)

Parameter	Group	Group 1		Group 2		Group 3	
		Mean ±SD	Mann-Whitney U test p-value*	Mean ±SD	Mann-Whitney U test p-value*	Mean ±SD*	Mann-Whitney U test p-value*
Linear Ra	Primary teeth	0.33 ±0.51	0.92	0.52 ±0.71	0.63	0.31 ±0.46	0.42
	Permanent teeth	0.26 ±0.24		0.22 ±0.24		0.18 ±0.28	
Linear PV	Primary teeth	0.10 ±10.03	0.04	0.97 ±8.86	0.40	3.35 ±8.35	0.70
	Permanent teeth	4.40 ±5.39		6.14 ±11.49		1.58 ±5.14	
Vol Ra	Primary teeth	0.79 ±0.88	0.24	1.03 ±0.86	0.01*	0.69 ±0.78	0.03*
	Permanent teeth	0.24 ±0.18		0.30 ±0.25		0.21 ±0.33	
Vol PV	Primary teeth	–2.00 ±16.26	0.40	1.59 ±16.55	0.02*	9.59 ±15.28	0.17
	Permanent teeth	–3.28 ±29.90		14.85 ±15.19		1.70 ±12.15	

*Refers to statistically significant difference, $p<0.05$.

SEM analysis: Figures 3A–3G show the SEM images obtained at 8000 × magnification for primary and permanent teeth. According to the images in Figures 3C and 3D, Group-2 shows the less microporous area when compared with other postcycle images of Group-1 (Figures 3A and 3B), Group-3 (Figures 3E and 3F) and Group-4 (Figures 3G and 3H). Also, the difference between postcycle appearances for primary (3A, 3C, 3E, and 3G) and permanent teeth (3B, 3D, 3F, and 3H) can be seen as upper and lower figures in Figures 3A–3H.

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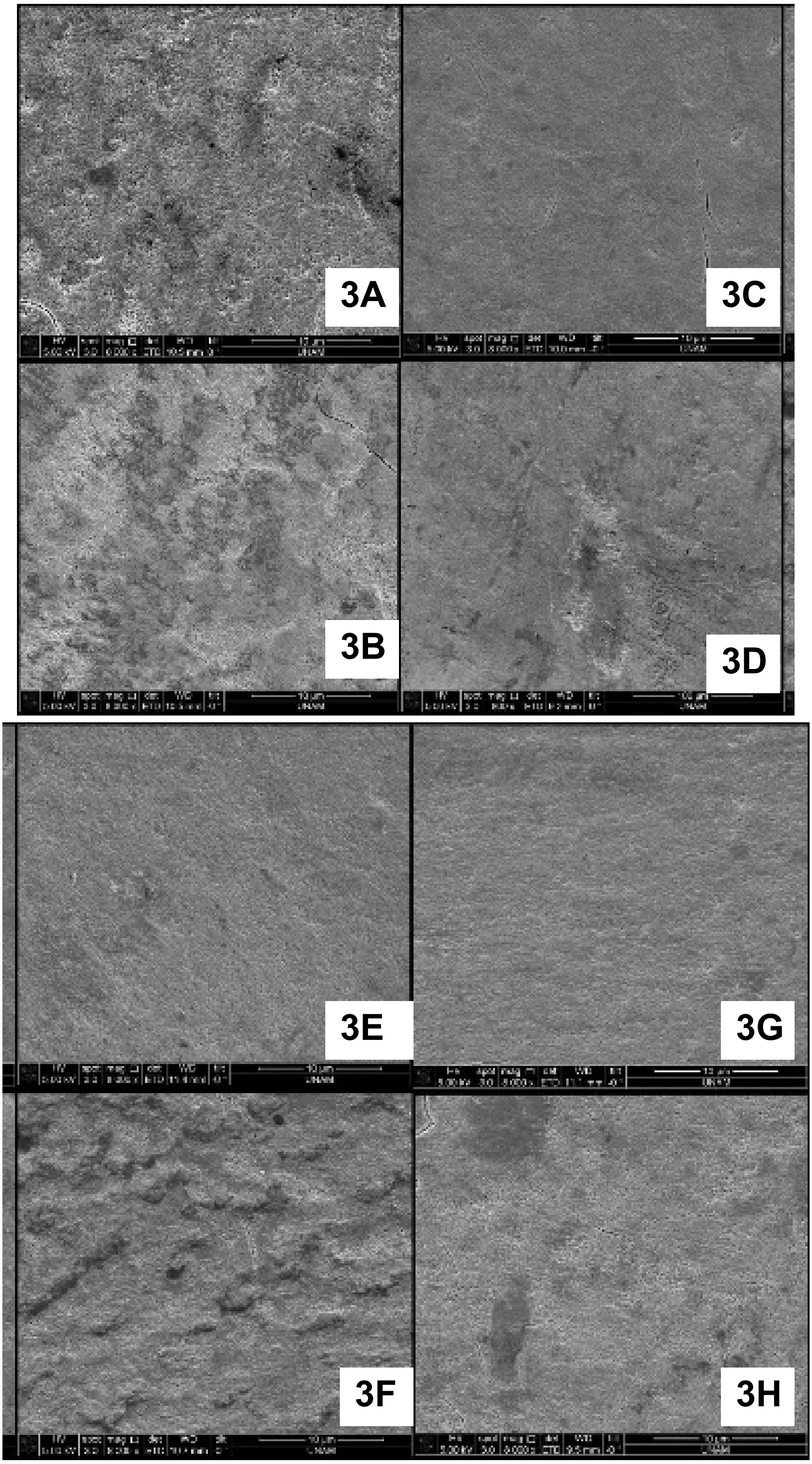

Figures 3A–3H. SEM images of postcycle sides.
3A and 3B: Group-1 (CPP-ACP group): 3A: primary teeth, 3B; permanent teeth;
3C and 3D: Group-2 (Organic calendula-xylitol group): 3C: primary teeth, 3D; permanent teeth;
3E and 3F: Group-3 (Calcium hydroxyapatite-silica group): 3E: primary teeth, 3F: permanent teeth;
3G and 3H: Group-4 placebo group: 3G: primary teeth, 3H; permanent teeth.

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DISCUSSION

Diet, particularly the taking of acidic beverages, is one of the most important etiological factors in the development of excessive tooth wear (ETW) and dental erosion.[16] Acidic beverages can decrease the critical pH level of the tooth surface, in both children and adults.[16] The effect is greater as the acidity and frequency of consumption are increased.[16] Lussi & Carvalho,[17] Lussi & Hellwig,[18] and Lussi et al.[19] analyzed several erosive beverages and foods and the erosion potentials of these on primary and permanent enamel surfaces. According to the results reported by these authors, citric acid in orange juice plays an important role in decreasing the pH level and this induces mineral dissolution from the enamel surfaces for both primary and permanent teeth. As this study was conducted to evaluate the erosion inhibition efficiency of different fluoride-free toothpastes as preventive agents, orange juice was preferred due to the high erosive potential (pH: 3.9) on tooth surfaces and being one of the most preferred healthy beverages in childhood and early adolescence period.

It is important to diagnose and treat the first stage of erosive lesions in the primary dentition, which is a predictive factor for ETW in the permanent dentition. Therefore, preventive agents should be applied more in childhood and early adolescence period to decrease ETW prevalence and progression rate in both primary and permanent teeth. In this case, toothpastes are important part of oral hygiene practices during toothbrushing.[20,21] One of the most effective remineralization agents in toothpastes is fluoride and fluoride is the most commonly used preventive agent. However, as we all know, fluoride is one of the chemicals with the potential for toxicity, and its concentration should be kept at a standardized level and not to exceed this.[22] Fluoride enters the body in through drinking water and food and the daily fluoride intake level might be different for every child.[23,24] Therefore, toothpastes including fluoride should be used carefully and with an awareness that an increased level of fluoride might cause problems in general health and developmental disorders in children.[24-26] In recent years, parents have had some confusion and worries about the toxic nature of fluoride and consequences of using an increased level of fluoride intake and have tended to use fluoride-free toothpastes for their children.[27-29] For this reason we planned to compare different fluoride-free toothpastes which included CPP-ACP, organic calendula-xylitol, and calcium hydroxyapatite-silica.

CPP-ACP complex acts as a reservoir for storing bioavailable calcium and phosphate and maintains the solution supersaturated, hence reinforcing the remineralization. Several studies[30-32] have demonstrated the efficiency of CPP-ACP technology in enamel demineralization inhibition and remineralization promotion. According to the exhibited data, the authors stated that CPP-ACP is an excellent remineralization agent to decrease roughness and increase microhardness of enamel surface. So, white spot lesions might be treated, and aesthetics and function might be regained.[31,32] According to the results of the present study, CPP-ACP did not show statistically significant different results in terms of Ra and PV for primary teeth. This might have arisen from the treated area not being a microporous area like a white spot lesion caused by the acid attacks of the cariogenic cycle. The eroded enamel samples had a glazed flat surface which was less microporous than a white spot lesion so that CPP-ACP particles might not be able to settle on the surface of eroded enamel.

Hydroxyapatite, another remineralization agent, is a biomimetic material and has a similar structure to enamel tissue. Remineralizing agents including hydroxyapatite particles reduce initial bacterial colonization to enamel and oral surfaces without killing bacteria. Hydroxyapatite is a promising oral care ingredient due to its high biocompatibility as well as structural and chemical similarities to human enamel.[30,31,33,34] According to the studies based on the hydroxyapatite formation mechanism, if hydroxyapatite was used alone, the results were not statistically significant. However, if it was used with calcium silicate, the hardness of the enamel surface was increased due to the precipitation of hydroxyapatite on the calcium silicate.[35] We might say that the similar erosion inhibition efficiency of calcium hydroxyapatite-silica and CPP-ACP might have arisen for this reason and it might be recommended as an alternative fluoride-free toothpaste instead of CPP-ACP.

Xylitol is a naturally occurring sugar alcohol which has antimicrobial and potentially anticaries effects.[30,36] According to the known literature, xylitol is a promising remineralization agent but there is not enough evidence to assess its potential.[30,37] Dong et al.[37] reported a study about the remineralization effect of sorbitol and xylitol with chewing gum and the results showed that they both have remineralization potential. However, this potential might be related to chewing movements. In the present study, organic calendula-xylitol showed significantly higher volumetric PV values for permanent teeth and volumetric Ra values for primary teeth, which means statistically significant lower erosion inhibition efficiency than other groups. We might say that without chewing movements, xylitol may not be adequate to inhibit erosive effects on the tooth surface.

Alexandria et al.[10] reported a study that evaluated Ra values of bovine teeth after erosion and abrasion cycles with a profilometer while using fluoride, CPP-ACP+fluoride, xylitol+fluoride, and deionized water for remineralization. According to the results, all groups promote significantly lower changes in the enamel compared to the placebo group and there was not any statistical difference between the three treatment groups. These results were similar to the results of the present study. In the present study, there was not any statistical difference between the groups whereas the lowest erosion inhibition efficiency was expected to be at control group, which was a negative control group where no toothpaste was used. So, similar inhibition efficiency results might be caused by including a lower number of tooth samples in the control group than in the other groups. Another reason might be the fact that without the abrasive movements of the toothbrush, the level of erosion progression might be lowered for the control group or by not using any chewing appliance in order to increase the efficiency of applied toothpastes in the experimental groups. So, this result might be considered as a limitation of the study.

One of the most preferred non-invasive and non-destructive methods for surface loss assessment in laboratory studies is the surface profilometer. This method quantifies the loss of dental tissue concerning with a non-treated reference area. The profilometer uses a probe with different types of light, which has high precision, detecting surface loss which exceeds about 0.4 µm.[9,38,39] Therefore, flattening the enamel surface during the surface preparation process is important for obtaining the correct measurements. According to several authors, SEM which is another non-invasive and non-destructive method should be used as a supportive method which can give quantitative results.[2,40]

In the present study, a profilometer was used to determine the surface properties with qualitative results and hence, SEM images were used as quantitative results for visual data to support these results. SEM images of precycle and postcycle areas are shown in Figures 3A and 3B. In these figures, the less microporous areas in the organic calendula-xylitol group than in the other groups and similar porous areas in the CPP-ACP, the calcium hydroxyapatite-silica, and the control groups can be seen. These different appearances indicate that there is a lower erosion inhibition efficiency of organic calendula-xylitol than with other groups due to the less microporous structure in comparison to the other postcycle appearances.

According to the literature, primary and permanent teeth have some differences in enamel composition and structure. Besides, there are some differences in chemical composition at different enamel depths, which will impact the solubility of the enamel tissue.[1,16,19,30] This information was verified by Carvalho and Lussi,[41] showing that different tooth types and enamel depths have different susceptibility to the erosive process. They stated that internal enamel presents progressively lower hardness and is more susceptible to erosive dissolution than its outermost layers.[41] So, in the present study, the surface preparation was standardized to remove approximately 200 μm to eliminate differences in the enamel layers and, also in the tolerance level during the surface roughness measurements via a profilometer.

Primary teeth are known to be more prone to wear forces or lower pH level of acidic beverages or drinks than permanent teeth and, also ETW progression is faster due to the structural differences for primary and permanent teeth such as a thinner enamel layer and lower level of inorganic compounds in primary tooth enamel which can therefore can erode more easily than permanent tooth enamel.[38,42] Several studies[42,43] have already evaluated the association of erosive and abrasive challenges in permanent and primary teeth. According to the results of the present study, there were significant differences in all groups. We might say that the structural differences between primary and permanent teeth caused a difference between the effectiveness of the toothpastes by Ra and PV values and, also SEM images. Our first hypothesis, that primary teeth would be affected by erosive attacks more than permanent teeth, was accepted hence the different erosion inhibition features of primary and permanent teeth. According to the exhibited results, primary teeth samples were more eroded than permanent teeth due to the higher PV results in CPP-ACP and organic calendula-xylitol groups. However, based on the results of the comparison between different fluoride-free toothpastes, our second hypothesis, that CPP-ACP containing toothpaste would inhibit the further progression of ETW more than other agents, was rejected since the erosion inhibition efficiency of CPP-ACP did not significantly differ from other groups as expected.

CONCLUSIONS

In recent years, parents have been very worried about the use of fluoridated toothpaste and prefer using fluoride-free toothpastes. However, according to the present study, the results of the erosion inhibition efficiencies of fluoride-free toothpastes were not satisfactory, especially with xylitol. So, although we might

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recommend to parents the use of fluoride-free toothpastes, especially CPP-ACP and calcium hydroxyapatite-silica. However, we should emphasize that they might be inadequate for the efficient inhibition of the erosion and remind them that the correct application of remineralizing agents, at the right dosage, would be more efficient for erosion inhibition.

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CONFLICT OF INTEREST

The authors report no conflict of interest.

AUTHOR CONTRIBUTIONS

The experiments were conceived and designed by D Sakaryalı Uyar and B Memiş Özgül. Experiments were performed and obtained data was analyzed by D Sakaryalı Uyar and B Memiş Özgül. The paper was written by D Sakaryalı Uyar and revised by B Memiş Özgül and RE Tirali. All authors read and approved the final version of the manuscript.

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