

Evaluation of the Remineralizing Effect of Elmex Erosion Protection and GC Tooth Mousse Plus on the Eroded Enamel of Primary Teeth induced by Specific Medicinal syrup

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ABSTRACT

Background: Dental erosion is highly prevalent condition not only in adults but also in children and adolescents and pediatric medicinal syrups are thought to be one of the important causes. An aim of modern dentistry is to handle the condition non-invasively by remineralization, using range of remineralizing and protective agents.

Aim: To investigate the remineralizing effect of GC Tooth Mousse Plus and Elmex erosion protection, alone or in combination, on the enamel of primary anterior teeth previously eroded with medicinal syrup using surface microhardness and scanning electron microscope analysis.

Materials and method: Forty primary anterior teeth were used in the study. Enamel blocks were prepared and divided into (5) groups (8) samples for each: group (1) samples were kept in artificial saliva, group (2) samples were exposed to pH-cycle only, group (3) samples were treated with GC-TMP after pH cycle, group (4) samples were treated with Elm-EP after pH cycle and group (5) where samples were treated with combination of Elm-EP and GC-TMP.

Results: High statistically significant differences found among the tested groups after treatment cycle. GC Tooth Mousse Plus, Elmex erosion protection and their combination all showed remineralizing effect expressed in increasing surface microhardness, confirmed by SEM analysis, of the samples previously eroded by medicinal syrups with the best results found in the combination group followed by the group treated with GC-TMP alone then the group treated with Elm-EP alone.

Conclusion: The simultaneous use of GC Tooth Mousse Plus and Elmex erosion protection paste gave better remineralizing effect than the sole use of either GC Tooth Mousse Plus or Elmex erosion protection paste.

Key words: Dental erosion, Casein phosphopeptide amorphous calcium phosphate, Enamel remineralization, GC Tooth Mousse Plus, Elmex erosion protection

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INTRODUCTION

Dental erosion is a pathological process that is chronic and characterized by localized loss of hard tooth structure, which is removed chemically by acid and/or chelation without bacterial involvement. Erosion incidence has been increased in the modern societies especially among children and adolescents as a result of changing in the eating habits. People suffering from erosive wear during period of deciduous dentition are usually at an increased risk of having erosion in the permanent dentition.

Diagnosis and prevention of erosion at early age will aid in avoiding the harm to the permanent teeth [1].

In children, prescriptions in the form of liquid are very common prescribed type of medicine in order to evade the problem encountered in taking the other forms. Acidic preparations are often essential in the formulations of these drugs as they can be added to act as buffering agents for maintaining chemical stability, physiological compatibility, controlling tonicity and enhancing the flavor, thus increasing the palatability to children. Different studies have stated that liquid oral medications can influence the hardness of the enamel and result in morphological alterations [2].

Some morphological differences present between enamel of primary teeth and that of permanent teeth. Deciduous teeth enamel is less mineralized when compared with that of permanent teeth, and the diffusion coefficient is greater in the enamel of deciduous teeth than in case of permanent teeth. Overall mineral density is lesser in the

outermost layers, but shows no substantial differences adjacent to the dentine-enamel junction. It has been found that enamel demineralization of permanent and primary teeth in acidic media exhibits significant differences, with the enamel of permanent teeth showed less susceptibility to demineralization than that of primary teeth. All these variations increase the susceptibility of primary teeth to dental erosion.

To apply an anti-erosive effect, conventional fluorides (such as sodium fluoride (NaF)) seem to need an intensive and impractical fluoridation regimen. Also, the enlarged prevalence of dental erosion among population, in spite of using fluoride-containing toothpastes, indicates the need for more efficient compounds. So, recently, toothpastes and mouth rinses with various novel active ingredients, which are claimed to have anti-erosive properties, have been developed.

Elmex erosion protection is chitosan-based toothpaste (contains 0.5% chitosan) and enriched with sodium fluoride, amine fluoride (1400 ppm totalF⁻) and SnCl₂ (3500 ppm). Previous studies found that this toothpaste showed major reduction in erosive and erosive/abrasive enamel loss compared to the untreated control as under acidic challenge, stannous salts (Sn₂HPO₄, Sn₃F₃PO₄, Ca (SnF₃)₂) incorporated into the outer layer of enamel in a positive quantity in relation to the tissue loss, possibly due to a complex demineralization and re-precipitation process that modifies the upper layers of the enamel and creates a protective layer against acid attack. The protection of this layer against erosion is improved when stannous is combined with chitosan, which is a natural polysaccharide [3].

The use of casein phosphopeptide amorphous calcium phosphate (CPP-ACP) is among the strategies to control dental erosion. Casein phosphopeptides (CPP) have the cluster sequence of -Ser (P)-Ser (P)-Ser (P)-Glu-Glu from casein. CPP has an incredible capacity to stabilize clusters of ACP into CPP-ACP complexes, throughout these multiple phosphoserine residues, inhibiting their growth to the critical size required for nucleation, phase transformation and precipitation. The CPP- and ACP-formed nanoclusters buffer the activities of free calcium and phosphate ions in the plaque fluid. This will aid in the maintenance of supersaturation with respect to enamel mineral, and perform like a reservoir of calcium and phosphate, thus enhancing enamel remineralization and depressing demineralization. Moreover, adding fluoride to CPP-ACP to form CPP-ACPF, has been studied due to its capacity to improve the protective effect produced by CPP-ACP in the control of dental erosion.

Only limited studies are available regarding the remineralization effect of the combined use of Elmex Erosion Protection and GC Tooth Mousse Plus on the erosive lesions of the primary teeth induced by the use of pediatric liquid medications, and as the use of these medications is inevitable, the acidic preparations are often essential in the drug formulation and maintaining the health of the primary teeth is important for healthy permanent teeth, the significance of this study is to

enhance the remineralization and preserve the health of the primary teeth after exposure to the erosive oral medications.

The aim of the current in vitro study was to evaluate the remineralization capacity of Elmex Erosion Protection (Elm-EP) and GC Tooth Mousse Plus (GC-TMP), alone or in combination, on the enamel of primary teeth previously exposed to erosive challenge.

MATERIAL AND METHOD

Ethical Aspect

This study protocol was conducted in vitro, submitted and approved by the Local Ethics Committee (UoM.Dent/H.L.2/ 21) Research Ethics Committee of Collage of Dentistry, University of Mosul, Nineveh, Iraq.

Sample Collection and Preparation

Upper and lower primary anterior teeth of children living in Nineveh Governorate were collected from the pediatric dental clinic in Al-Noor specialized dental center in addition to some private clinics. Among all the collected teeth, total of (40) deciduous teeth with intact enamel surface (no developmental defects, no cracks, caries, fluorosis, stain, restorations or exposure to chemical agents (i.e., bleaches) were used as the sample of this study after being checked for any structural abnormalities that could possibly interfere with the results.

Teeth were cleaned and polished with rubber cup and non-fluoridated pumice then the roots were cut at the level of cement-enamel junction using straight diamond bur of a high-speed handpiece with continuous water cooling to avoid damaging of the enamel. Then the coronal portions of the specimens were embedded in auto polymerized cold cure acrylic resin blocks using (15 mm height) cylindrical plastic tubes (that were cut and prepared with flat and parallel upper and lower borders) with the outer labial surface facing upward. The enamel surfaces were grinded wet using (400 and 600) grit silicon carbide abrasive paper to produce standardized flat enamel surfaces for surface microhardness test [14].

Study Design

After preparation, teeth Samples were randomly divided into five groups as below:

- Group 1: negative control group No. = 8, these samples were kept in artificial saliva only.
- Group 2: positive control group No. = 8, these samples were exposed to pH- cycling (salbutamol, butadin, syrup) only.
- Group 3: No. = 8, after immersion of teeth samples in the drug cycle, enamel surface was treated with GC Tooth Mousse Plus (GCTMP).
- Group 4: No. = 8, after the immersion in the drug cycle, enamel surface was treated with Elmex Erosion Protection (Elm-EP).

- Group 5: No. = 8, after the immersion in the drug cycle, enamel surface was treated with combination of Elm-EP and GCTMP.

Immersion Cycle

Samples in the first group (negative control group) were kept in artificial saliva during the entire procedure with daily refreshment of the solution that was papered with the following components: NaCl 0.40, KCl 0.40, CaCl₂.2H₂O 0.79, NaH₂PO₄.2H₂O 0.78, Na₂S₂O₈.H₂O 0.005, CO(NH₂)₂ Urea 0.1, in 1000 ml distilled water (concentrations G\L) with pH value 7, while for the samples of group 2, 3, 4 and 5, the following immersion cycling protocol was adopted to simulate the usual number of intakes: Teeth were immersed into 100 ml of undiluted syrup and agitated for 1 min three times per day for 14 days. After each immersion, teeth were washed with distilled water and preserved in artificial saliva with daily change of the solution. The medicine was replaced before each immersion and at the end of the 14 days; teeth were transported to microhardness testing laboratory where their enamel surface microhardness was measured.

Treatment Cycle

Samples in group3, 4 and 5 were subjected to the following treatments for 15 days after the emersion cycle:

- Group 3: samples were covered with pea size amount of GC tooth mousse plus using clean finger for 5 min twice daily (at 10 a.m. and at 10 p.m.). The excess of paste was removed gently with cotton swab.
- Group 4: Elm-Erosion Protection paste was applied to the surface of the teeth using small brush twice daily (at 10 a.m. and at 10 p.m.) and left for two minutes on the surface of the samples before rinsing with water.
- Group 5: samples were first treated with Elmex-Erosion Protection as described before for group4, then pea size quantity of GC Tooth-Mousse Plus was applied to each surface and left for 5 min. The excess of paste was removed with cotton swab gently.

Surface Microhardness Test

The microhardness of the enamel surface of the teeth was tested at Mosul Technical Institute / North Technical University using Vickers microhardness machine (OTTO WOLPERT-WERKE GMBH, V-Tester 2/ Germany). All readings were performed by the same examiner using the same calibrated machine. The microhardness was

determined on the middle third of the labial surface of the teeth by the application of 500 gm load for 15 seconds which were constant for all samples and during the entire study. Three indentations were made on the flattest points of the enamel surface to ensure accuracy of the measurements, the length of each indentation was measured microscopically using 70X lens, and then, the mean value of these 3 indentations was calculated for each sample to obtain one reading.

Indentation result can be seen in the form of shadow forming rhomboid at projector screen, the diagonal lengths of the indentations was measured in micron by microscope. The Vickers values were converted into microhardness values using the following equation:

$HV = 1.854 (p/d^2)$ where:

HV: is microhardness value in Kgf/mm² (Mpa).

P: is the diagonal of the indentation (mm).

d: $d_1 + d_2 / 2$.

Observation with scanning electron microscope

For examination with SEM, the teeth were mounted on the SEM holder using removable adhesive. Under vacuum conditions, each tooth was coated with gold using a sputter coater (Leice EM ce 600 High Vacuum Sputter Coater) and adjusted to be observed under low vacuum using a SEM (Nova Nano SEM 450) at University of Basrah, Collage of Sciences, Department of Physics. The surface morphology of the teeth was examined, and representative photomicrographs were captured and digitally stored.

RESULTS

Indentation Surface Microhardness Test Results

According to the obtained measurements of this study, table (1) shows the descriptive statistics including means, standard deviations, in addition to the numbers of the samples of tested groups at the baseline, after pH cycle and after treatment cycle. Based on the means values for tested groups after treatment cycles, combined application of Elmex-Erosion Protection and GC Tooth-Mousse Plus showed highest increase in surface microhardness mean values and thus considered the most effective when compared with other tested groups regarding the remineralization of the eroded enamel surface of primary teeth.

Groups	Variables	Baseline	After PH Cycle
negative control (saliva)	Mean	228	228.125
	Std. Deviation	5.63154	2.85044
	N	8	8
positive control (salbutamol)	Mean	229.75	179.125

	Std. Deviation	5.89794	5.22186
	N	8	8
GC Tooth Mousse Plus	Mean	225	215.875
	Std. Deviation	8.43462	3.31393
	N	8	8
Elmex-erosion protection	Mean	222.875	203.5
	Std. Deviation	9.10945	3.02372
	N	8	8
Combination	Mean	227.125	222.25
	Std. Deviation	9.29574	4.43203
	N	8	8

Table1: Descriptive statistics of microhardness measurements among tested groups at baseline and after PH cycle.

In table (2), ANOVA test explains that there were no significant differences in surface microhardness readings among the tested groups at the baseline at $p \leq 0.01$, while

after PH cycle; high significant differences existed among the tested groups at $p \leq 0.01$.

Microhardness		Sum of Squares	Df	Mean Square	F	Sig.
Baseline	Between Groups	228.65	4	57.163	0.931	0.457
	Within Groups	2149.25	35	61.407		
	Total	2377.9	39			
After PH Cycle	Between Groups	12066.9	4	3016.71	200.684	0
	Within Groups	526.125	35	15.032		
	Total	12593	39			

*highly significant difference at $p \leq 0.01$

Table2: ANOVA test between tested groups at baseline and after PH cycle respectively at $p \leq 0.01$.

In table (3), Duncana multiple analysis range test was done to further explain that there were no significant differences existed before PH cycle at $p \leq 0.01$, as all

groups were arranged in homogenous subset of data representing the surface microhardness means values for each group before PH cycle.

VAR00002	N	Subset for alpha = 0.01
		1
1	8	222.875
2	8	225
3	8	227.125
5	8	228
4	8	229.75
Sig.		0.126

Table3: Duncana Multiple Analysis Range test for tested groups before PH cycle.

While after treatment cycle, Duncana multiple analysis range test was done to further explain that highly significant differences existed in surface microhardness mean values among the tested groups at $p \leq 0.01$. All groups were arranged in nonhomogeneous subsets of data representing the surface microhardness mean

values of each tested group after treatment cycle at which the combination group showed the highest increase in surface microhardness mean values followed by the group treated with GC Tooth mousse Plus only then the group treated with Elmex-erosion protection alone as demonstrated in table (5).

Groups	N	Subset for alpha = 0.01				
		1	2	3	4	5
4	8	179.1				
1	8		203.5			
2	8			215.88		
3	8				222.25	
5	8					228.13
Sig.		1	1	1	1	1

*highly significant difference at $p \leq 0.01$

Table4: Duncana Multiple Analysis Range test for tested groups after PH cycle.

Surface Morphology Evaluation by SEM

The present scanning electron microscope study allowed us to understand qualitatively the demineralization and remineralization processes of enamel surface throughout the observation of specific structural and morphological changes.

In general, the SEM analysis revealed noticeable variations in the tooth enamel among the different experimental groups. The primary teeth samples immersed in artificial saliva (the control group) showed an intact, smooth and relatively flat enamel surface without any morphological irregularities or erosion evidence.

Teeth samples immersed in salbutamol syrup (butadin) clearly exhibited structural loss. They revealed severely irregular surface with hardly identifiable enamel prisms indicating generalized structural loss.

On the other hand, specimens treated with Elmex EP tooth paste after exposure to the drug cycles showed improvement in surface roughness with more compact and homogenous surface compared with enamel surface exposed to the drug cycles but when compared with the negative control samples, teeth treated with Elmex EP were still showed evidence of erosion and roughness.

Regarding the teeth surfaces treated with the CPP-ACP paste after exposure to the drug cycles, the CPP-ACP paste, which is arranged in a globular structure, was able to regenerate a uniform, thick, homogeneous and compact surface layer.

Samples treated with combination of Elmex EP paste and GC Tooth Mousse Plus showed a well mineralized surface with CPP-ACP clusters, which appeared to be larger in size and greater in number compared with those formed by the use of GC Tooth Mousse Plus alone, seemed to occlude large part of the pores produced by the use of medicinal syrups.

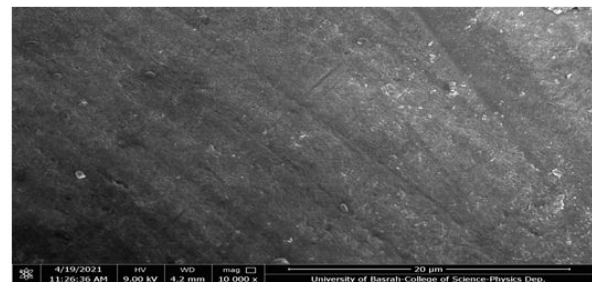


Figure1: SEM image showing normal primary enamel surface.

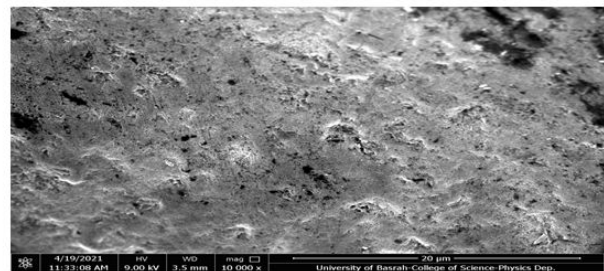


Figure2: SEM showing demineralized primary enamel surface after exposure to salbutamol.

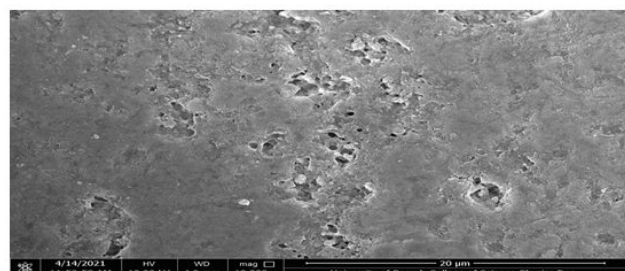


Figure3: SEM image showing primary enamel surface treated with Elmex-erosion protection.

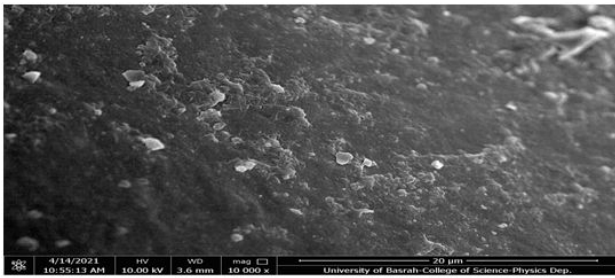


Figure4: SEM image showing primary enamel surface treated with GC Tooth Mousse Plus.

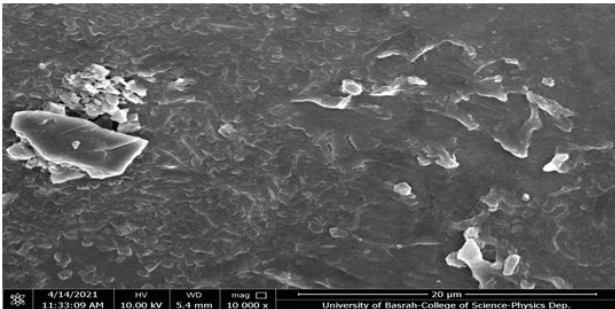


Figure5: SEM image showing primary enamel surface treated with combination of Elm EP and GC-TMP.

DISCUSSION

A new risk factor for dental health not only in adults but also in children and adolescents is tooth erosion which is presented by today's lifestyle. In dental erosion, frequent contact between acids and tooth surface occurs which results in demineralization of the tooth surface. Most oral care products contain fluoride, and although some preventive outcome against tooth erosion has been revealed by the fluoridated toothpastes, they don't have the ability to provide full protection by themselves. Thus, additional studies investigating other active ingredients are required.

Pediatric medicinal syrups may have a great erosive potential due to the existence of an acid component in their formulation. Thus, the analysis of their pH is an essential factor when studying dental erosion. The dissolution of enamel is highly dependent on the pH of the substance surrounding it. A lower pH dissolves the hydroxyapatite of enamel more severely and at a faster rate than would a higher pH irrespective of the exact type of acid in a drink. In our study, pH value was measured for salbutamol syrup (butabin) and found to be 3.8, which is below the critical pH of enamel demineralization, and that explains its erosive effect expressed by reduction in the microhardness mean values. Our findings were in accordance with the study of Yilmaz et al., (2019) in which salbutamol syrup also caused reduction in surface microhardness of the teeth.

ANOVA Test demonstrated the difference among the tested groups at base line and after treatment cycle and it was noticed that at base line, no significant differences found among the tested groups, while after exposure of

samples to the treatment cycles, high significant differences were occurred as further noticed at Duncan, a Multiple Analysis Range Test, for test groups after treatment cycles which illustrated that the group treated with a combination of GC Tooth Mousse Plus and Elmex erosion protection paste has shown the maximum recovery of the microhardness values followed by the group treated with GC Tooth Mousse Plus alone then the group treated with Elmex erosion protection paste only and all of them were beneficial in remineralizing the eroded enamel surface of the primary teeth when compared with the group that was kept in artificial saliva which showed the minimum recovery of microhardness values.

GC Tooth Mousse Plus caused an increase in enamel microhardness after the erosive action of the drugs. The remineralization effect of GC Tooth Mousse Plus includes the localization of CPP part of this complex at the enamel so as to supply the enamel rods with calcium and phosphate ions and rebuild the apatite crystals. By maintaining a state of supersaturation with respect to the hydroxyapatite, these ions stimulate the remineralization and decrease the demineralization. The combination of CPP-ACP with fluoride caused colocalization of fluoride ions with calcium and phosphate ions at the surface of enamel. Therefore, it brings all the ions necessary to build fluoroapatite crystals which are more resistant to acid dissolution. Our findings were in agreement with the findings of who all found that CPP-ACPF has remineralizing effect expressed in its ability to increase the values of microhardness of the teeth samples that were previously exposed to demineralization.

Conversely, these findings were in disagreement with the study who concluded that tooth erosion cannot be prevented or repaired neither by the use of MI paste nor by the use of MI paste plus, and the study in which the results obtained showed no remineralizing effect of GC Mousse on early enamel lesions.

Elmex erosion protection on the other hand, is a tooth paste with three main active ingredients, 5 % chitosan, 3500 ppm tin as stannous chloride and 1400 ppm fluoride in the form of sodium fluoride and amine fluoride. Chitosan is nontoxic biodegradable and biocompatible material. The amino groups of chitosan have the ability to catch hydrogen ions when in acidic media, causing an overall positive charge that gives chitosan bioadhesive capability to negatively charged surfaces such as cell membrane, soft tissue and tooth enamel forming a protective layer against tooth wear but it doesn't participate in the remineralization of the already existing lesion. Sn-containing dental products can also offer greater protection against tooth erosion. This protective outcome is owing to the development of amorphous deposits, layer of insoluble mixed tin-salts, on the enamel surface, in addition to the incorporation of Sn into the eroded enamel as found in studies. Fluoride ions, obtained from AmF and NaF, can play double role in the surface-protection process when displace the hydroxyapatite hydroxide groups forming fluoro-hydroxyapatite; the first role, fluoride performs like a

catalyst, aiding in enamel remineralization with phosphate ions dissolved in saliva; the second role, the reduced solubility of fluoro-hydroxyapatite crystals ensures a higher resistance to acid erosion. This was in agreement with a study made who found that AmF and NaF increase enamel microhardness and enhance the process of remineralization throughout speeding up the growth of enamel crystals that have been demineralized.

In the present study, the re-hardening capacity of GC Tooth Mousse Plus was found to be higher than that of Elmex erosion protection tooth paste as in a study made they found that The remineralizing effect of CPP-ACP paste was better than that of NaF because CPP-ACP repaired the enamel microstructure, including prism and interprism, by increasing the size of hydroxyapatite crystals significantly (12.06 nm) and Ca/P molar ratios (1.637 nm) when compared with NaF (8.56 nm crystal size and 1.397 Ca/P, $p < 0.01$) so NaF doesn't have the ability to repair the structure of the enamel, but it can smooth its surface.

On the other hand, these findings were in disagreement with the study of Oliveira et al., (2014) who found that 1.1% NaF dentifrice (5000ppm) revealed greater remineralization ability than the topical application of CPP-ACP paste [4].

In a study made to investigate the correlation between micro-hardness and mineral content in healthy human enamel, it was found that the lower concentration of minerals which may be expressed in increased porosity was associated with lower enamel hardness values while the high hardness values were related to the high mineral content measurements obtained with the Raman microscope. So, we expect that the highest recovery in microhardness values observed in the combination group of this study can be related to the high amount of minerals came from the two pastes, GC Tooth Mousse Plus and Elmex erosion protection, that were used to treat the samples in this group.

The morphology of enamel surface was viewed by scanning electron microscope at baseline, after exposure to the medicinal syrups and after treatment cycle to understand qualitatively the demineralization and remineralization processes of enamel surface throughout the observation of specific structural and morphological alterations. Regarding the erosive effect of the medications, our findings were in accordance with the findings and the findings of who found that salbutamol syrup caused loss of enamel structure with crater formation and hardly identifiable enamel prisms.

On the other hand, our findings in case of the remineralization were in line with the findings of who found that the CCP-ACP paste, which is organized in a globular structure, was able to recreate a thick, uniform, homogeneous and compact surface layer, and to the findings of Dai et al., (2019) in which they found that after treatment of the demineralized enamel surface with CPP-ACP agent + fluoride varnish, the precipitation of newly formed minerals made the original porous and rough surface flattened and smooth with the clefts and

pores became shallower due to the remineralization effect.

While in case of Elmex erosion protection, alone or in combination with GC Tooth Mousse Plus, our results were in agreement with those of Fanfoni et al., (2020) who found that the enamel specimens previously eroded with acid showed more compact and homogeneous surfaces after treatment with Elmex erosion protection, whereas samples treated with combination of Elmex erosion protection and GC Tooth Mousse Plus showed well remineralized surfaces with CPP-ACP clusters can be recognized onto the surface [5].

The SEM findings of this study, as we conclude, were in line with our surface microhardness and surface roughness results as the loss of enamel surface and the porosity seen in the SEM images of the enamel surface exposed to the medicinal syrups were in accordance with the reduction in surface microhardness mean values and the increase in surface roughness mean values obtained in this study. Also, the smooth, homogenous, flat and well mineralized enamel surfaces obtained after using the various treatment regimens in this study were due to the deposition of minerals that compensate the surface loss, occlude the pores and therefore, caused a reduction in surface roughness and increase in surface microhardness of the teeth samples.

CONCLUSION

Within the limitations of the current study, it was found that both GC Tooth Mousse Plus and Elmex erosion protection tooth paste showed remineralizing action expressed in increasing surface microhardness and decreasing surface roughness, confirmed by SEM analysis, of the primary teeth samples previously exposed to erosive challenge with GC Tooth Mousse Plus showed better results when compared with Elmex erosion protection tooth paste, whereas the combination of GC Tooth Mousse Plus and Elmex erosion protection tooth paste showed the best remineralizing effect when compared with the sole use of GC Tooth Mousse Plus or Elmex erosion protection tooth paste.

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