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EFFICIENCY OF THREE DIFFERENT FLUORIDE VARNISHES ON ENAMEL REMINERALIZATION OF PRIMARY TEETH: AN IN VITRO STUDY

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KEYWORDS

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ABSTRACT

Introduction: Fluoride is fundamental in the prevention of dental caries; it enhances the precipitation of calcium and phosphates, forming fluorapatite, which is more acid-resistant than hydroxyapatite. Recently, Nano Silver Fluoride was presented as a new remineralizing agent. **Aim:** This study aimed to compare the remineralization potential of three different fluoride varnishes Fluor Protector varnish (FP), Enamel Pro varnish (EP), and Nano Silver Fluoride varnish (NSF) on the enamel of primary teeth. **Materials and Methods:** 80 samples were gathered, and randomly distributed among three groups: negative control group, demineralized group, and study group which was subdivided into 3 subgroups, subgroup **A:** Fluor Protector, subgroup **B:** Enamel Pro and subgroup **C:** Nano silver fluoride. Pepsi was used for 72 hours to demineralize the Positive control group and the study group. Varnishes were then applied. All teeth were subjected to pH cycle in a demineralization solution/artificial saliva for 10 days. Vicker's microhardness test, Energy Dispersive X-ray Spectroscopy (EDX), and Environmental Scanning Electron Microscope (ESM) were used to measure the extent of enamel mineralization. The data were then, statistically evaluated. **Results:** Enamel Pro showed the highest results followed by Fluor protector and Nano silver fluoride however, there was no statistically significant difference between the three tested groups. **Conclusion:** The study showed that the Enamel Pro had the highest values in microhardness, EDX, and ESM, but there was no significant statistical difference between the three groups. All the materials used proved their efficiency in remineralization of the demineralized enamel.

INTRODUCTION

Dental caries is a serious health problem, despite recent dental care improvements. It is a disease with many factors that result from an imbalance between the pathological and protective factors. The imbalance produced will cause a rupture of the physiological processes of remineralization and demineralization of the tooth structure, causing demineralization [(1-3)].

Management of dental caries should also be focused on understanding the role of remineralization in preventing caries progression and reestablishing a healthy balance when demineralization occurs [(4-6)].

Remineralization helps in regaining the lost calcium and phosphate by introducing fluoride to the tooth structure, to form fluoro-apatite crystals, which are more resistant to acidic dissolution and substantially larger than the original crystals [(7)].

Fluoride is globally used as a remineralizing agent, as it inhibits the demineralization of enamel by changing the critical pH for the dissolution of calcium (Ca^{2+}) and Phosphate (PO_4^{3-}) in bacterial biofilm and adsorbing the apatite crystal surfaces, where it replaces calcium to form acid-resistant mineral fluoroapatite, which is characterized by its low solubility and inhibition of the acid production of Streptococcus mutans [8].

There are several available commercial fluoride varnishes, that are used in caries prevention and arrest, such as; Duraphat (5% sodium fluoride), Enamel Pro varnish (5% sodium fluoride and 2.3% Amorphous Calcium Phosphate, MI paste (CPP-ACP), Clinpro White (Tri Calcium Phosphate) and Fluor Protector (0.1% amine fluoride) [9, 10].

The search for new remineralizing materials has been going on for a long time especially, silver containing formulas, due to their antibacterial activity, on a broad range of microorganisms, which has been known since primordial times, it has proven that at reduced concentrations, silver is safe to human cells [11].

Recently, Nano Silver Fluoride was introduced as a new remineralizing agent to replace Silver Diamine Fluoride (SDF), as the later causes teeth staining and has a metallic taste [12,13].

Punathil et al. [14], executed a study to determine the microhardness changes of enamel surface following fluoride varnish application. Sixty teeth were divided randomly into 3 groups according to treatment modality; group (A) Fluor Protector, group (B): Duraphat varnish, and group(C) Bifluorid 10 varnish. At the beginning of the study and before fluoride varnish application, all samples underwent a PH cycle. Microhardness test was conducted by Vickers microhardness test, which was performed before the application of varnishes (baseline) and on 3rd day of the PH cycle and finally on the 7th day. According to the results, it was found out that Fluor Protector varnish had the highest enamel surface microhardness when compared to other tested varnishes.

Dehailan et al. [15], investigated the efficiency of five, commercially available fluoride varnishes on artificial carious lesions. Ninety bovine enamel specimens were equally distributed among five groups; Group A: Enamel Pro, group B: MI varnish, group C: Flor-Opal varnish, group D: Prevident varnish and group E: Vanish varnish, each group contained 18 samples. Carious lesions were created in the specimens and evaluated using Vickers microhardness test. Fluoride varnish was later applied to each group, and then stored in artificial saliva, which was renewed every 15 min for 6 hours. Artificial saliva samples were examined for fluoride content using an ion-specific electrode for fluoride. Each group was then exposed to a pH cycle for 5 days, after that, surface microhardness was measured again. This study concluded that; Enamel pro had the highest mean value in surface microhardness and there was no substantial difference on amount of fluoride release between the studied groups

Nozari et al.[16], conducted a study to evaluate and compare the remineralization efficacy of Duraphat varnish (5% Sodium Fluoride), nano-Hydroxyapatite Serum (n-HAP) and the newly formulated Nano silver fluoride (NSF) on primary anterior teeth's enamel. Sixty primary sound anterior teeth were randomly selected and immersed in demineralization solution for 72 hours creating initial enamel lesion. They were later divided into four groups of 15 samples each: (group 1) NaF varnish; (group 2) n-HAP repairing serum; (group 3) NSF and (group 4) no treatment (control). Surface microhardness was examined with Vickers micro hardness tester before and after demineralization and following 10 days of pH-cycling.

The study showed that post-surface micro-hardness values (SMH) significantly decreased in all groups. After treatment, SMH values significantly increased in comparison to post lesion ones ($p<0.001$), except, the control group in which there was no difference statistically. The highest SMH values were observed in NSF group. It was concluded that NSF may have the greatest remineralization abilities in comparison to NaF and n-HAP.

Since there were insufficient studies comparing the remineralization efficiency of these 3 varnishes; Enamel Pro, Fluor Protector and Nano Silver Fluoride, this study was conducted to add more data and knowledge in the field of pediatric dentistry.

MATERIALS AND METHODS

This study was waived from the approval of the Research Ethics Committee of the Faculty of Dentistry, Suez Canal University, on 18th of December 2018, code number 143/ 2018, as no living subjects were subjected to any risk during this study.

Sample size calculation

Forty extracted human upper anterior teeth were calculated using, the computer program SPSS software for Windows version 22.0 (Statistical Package for Social Science, Armonk, NY: IBM Corp) at significant levels 0.05 (P- Value ≤0.5). These calculations were based on Carroll and Schneider [18].

A total of 40 sound primary upper anterior teeth extracted due to shedding were gathered from the Outpatient Clinic of Pediatric Dentistry and Dental Public Health Department, Faculty of Dentistry, Suez Canal University, selected primary teeth were cleaned first then, stored in saline at 4˚c prior to preparation. After the removal of the roots of the forty sound primary anterior teeth, the crowns were sectioned labiopalataly using a disc to obtain eighty samples, and then samples were divided as follows (Fig 1):

Negative Control group: no treatment, (16 specimens).

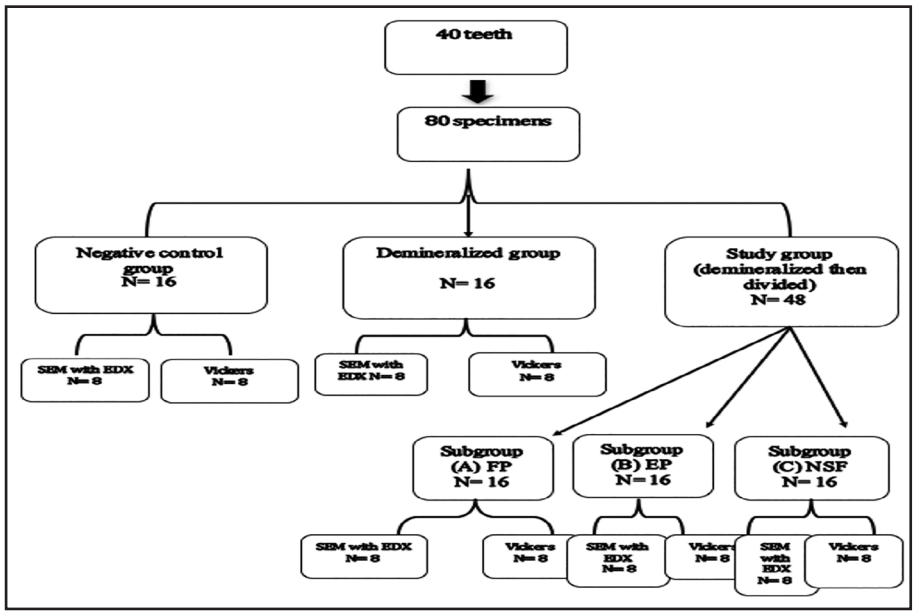

Fig. (1) Sample grouping

Demineralized group: demineralized with no treatment, (16 specimens).

Study group, 48 Specimens were demineralized using Pepsi for 48 hours and furtherly, subdivided randomly into 3 subgroups according to treatment modalities:

Subgroup A (FP): Fluor Protector varnish was applied to 16 specimens, with a micro brush on the labial surface for one minute according to manufacturer instructions.

Subgroup B (EP): Enamel Pro varnish was applied to 16 specimens, with micro brush on the labial surface for one minute according to manufacturer instructions.

Subgroup C (NSF): Nano silver fluoride solution was prepared and applied on the last 16 specimens on the labial surface, for each sample, two drops of NSF were applied with a micro brush, and left in contact with the tooth surface for two minutes [11].

pH cycling:

The study group specimens were left in a preserving solution (artificial saliva) for 21 hours and rinsed with distilled water and then put in the demineralizing solution for 3 hours.

This cycle was repeated for 10 days. The de- and remineralizing solutions were changed every third day. Specimens were incubated at 37˚c in an incubator throughout the 10 days of the pH cycle in tightly capped containers to mimic the oral environment [17].

Specimens were examined again with surface microhardness test and Environmental scanning electron microscope with EDX analysis after the 10 days PH. Cycle with no further treatment by the varnish.

Methods of Assessments:

1- Surface microhardness measurements: 8 specimens from each group were taken out of artificial saliva, then examined for microhardness by Digital Micro Hardness Vickers test (Wilson Tukon, USA) at a load of 100 gm for 10 seconds.

% SMHR = Percentage of Surface Micro Hardness Recovery

$$\% \text{ SMHR} = \frac{\text{Treated Enamel (TE)-Demineralized Enamel (DE)}}{\text{Initial Enamel (IE)- Demineralized Enamel (DE)}} \text{X100}$$

2- Energy dispersive X-ray spectroscopy (EDX):

The remaining 8 specimens from each group were taken out of artificial saliva to dry for two hours before examination. Energy dispersive x-ray spectroscopy (Quanta FEG 2500, USA) was used to examine the samples (National Research Center, Dokki, Egypt) to measure mineral content.

3- Enviromental scanning electron microscope (ESEM): The same 8 specimens examined by EDX were subjected to Enviromental scanning electron microscope (Quanta FEG 2500, USA) (National Research Center, Dokki, Egypt) to observe ultra-morphology.

RESULTS

1 Microhardeness and EDX Results

According to the results of the present study, the three fluoride varnishes showed remineralization efficacy when compared to the demineralized group, however, there was no statistically significant difference between the three tested groups regarding the micro hardness test, However, Enamel Pro was

the highest regarding calcium and Phosphorus weight and ratios using EDX and showed higher remineralization efficacy using ESM, followed by Fluor protector and Nano silver fluoride. (Table 1)

Table (1) *Showing data produced from the study*

Groups	Mean ±SD			
	Hardness	Ca	P	Ca/P
Control	271.62a ±25.89	32.568b ±3.174	14.780c ±1.294	2.204a ±272
Demineralized	159.45c ±11.69	14.607c ±1.997	8.227d ±0.817	1.775b ±0.332
Fluor Protector	241.84b ±26.93	35.159b ±5.898	17.148b ±1.238	2.050a ±0.210
Enamel Pro	245.79b ±21.43	40.627a ±7.617	18.267a ±0.916	2.224a ±0.320
Nano Silver Fluoride	233.95b ±24.16	35.197b ±3.629	17.295b ±0.740	2.035a ±0.135
F-test	54.726	61.97	236.06	6.465
P-value<0.05	<0.001	<0.001	<0.001	<0.001

***: a,b,c means significant difference between groups at P value<0.005*

2. Environmental Scanning Electron Microscope Results

Negative control group:

The Scanning Electron micrograph of the negative control group showed normal morphology of the enamel surface layer, smooth and identical structural shape was shown as a whole with bands of normal enamel rods and interprismatic regions. Occasional scratch marks and some foci of indefinable debris were noted (Figure 2).

Demineralized group:

Scanning electron microscope analysis of the demineralized enamel surface revealed porous surface with the appearance of a typical honeycombed pattern of enamel rod (Figure 2).

Subgroup A (Fluor protector):

The Scanning Electron micrograph of the enamel of subgroup FP displayed the interprismatic substances with porosities representing demineralization and areas of remineralization were seen (figure 3).

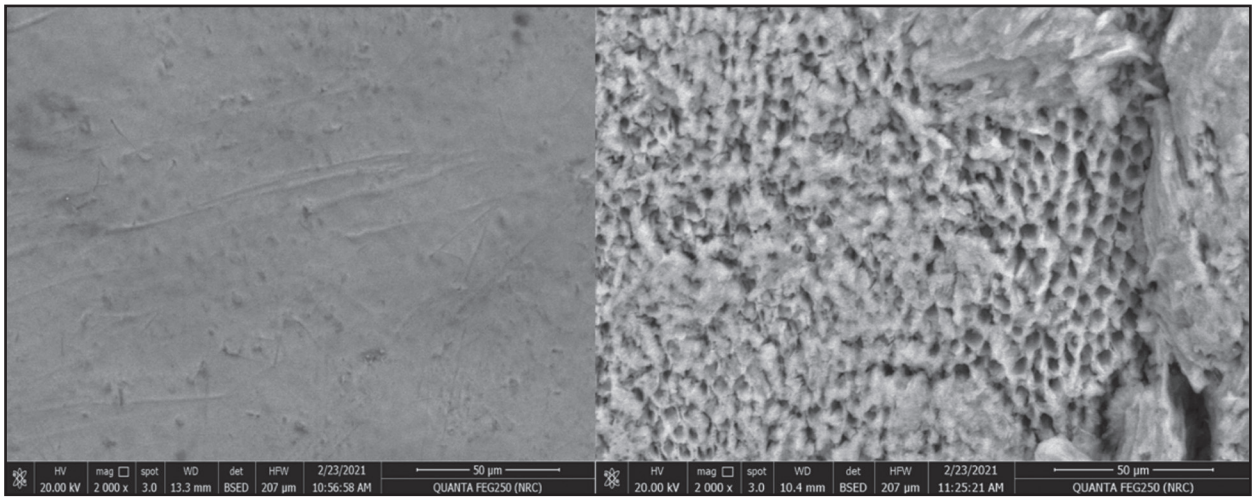

Fig. (2a): Scanning electron micrograph of sound untreated enamel preserved in artificial saliva showing smooth, regular enamel surface with barely evident rod and interterrod (2000xmag) vs **Fig. (2b)**: Scanning electron micrograph of demineralized enamel surface show the rod with honeycombed pattern and interrod (mag. X 2000).

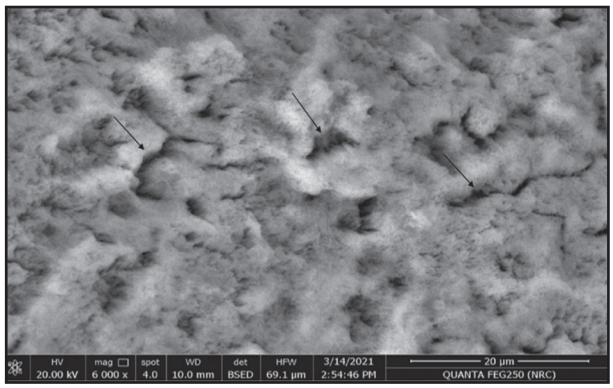

Fig. (3) Scanning photomicrograph of Enamel surface treated with Fluor Protector revealing increased thickness of interrod region (arrows) (mag. X6000).

Subgroup B (Enamel Pro):

The SEM examination of specimens of the subgroup EP discovered healing of the enamel ultrastructure. The specimens showed a smoother enamel surface with Pores obliteration with some surface irregularities (Figure 4).

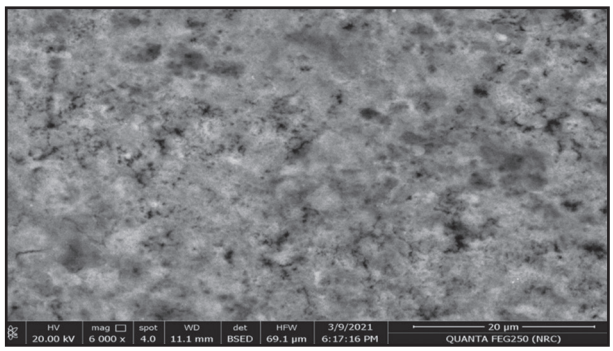

Fig. (4) Amorphous calcium deposit covering the enamel surface treated with Enamel Pro (mag. X6000)

Subgroup C (Nano silver fluoride)

A scanning photomicrograph of Enamel surface treated with Nano Silver fluoride showed an irregular surface with obliteration of pores of enamel rods (Figure 5).

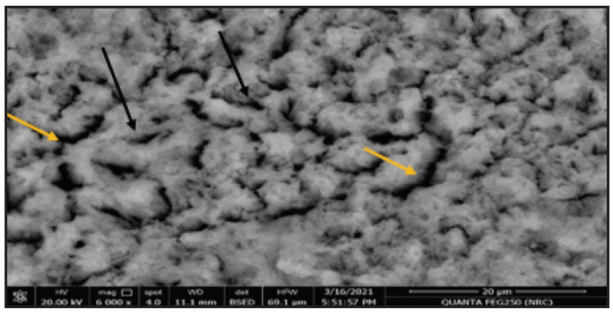

Fig. (5) Scanning photomicrograph of Enamel surface treated with Nano Silver fluoride revealing irregular surface, some deep grooves (yellow arrows) with increased thickness of interrod region (black arrows) (mag. X6000).

DISCUSSION

According to the results of the present study, the three fluoride varnishes showed remineralization efficacy compared to the positive control group, however, regarding the microhardness test there was no statistically significant difference between the three tested groups, despite their different composition.

The results of the present study are similar to the results of a study on Fluor protector by **Punathil et al.** [12] who assessed the microhardness of the enamel surface after Fluor protector varnish, Duraphat varnish and Bifluorid 10 varnish. According to their results, fluorprotector varnish showed higher enamel surface microhardness than Duraphat and Bifluorid 10 varnishes.

Also, the results of the present study agree with the results of a study on Enamel Pro by **Nalbantgil et al.** [19] in which they assessed the effects of Duraflor and Enamel Pro Varnish on enamel demineralization around fixed appliances by Vicker's microhardness test, as their results revealed that, both Enamel Pro Varnish and Duraflor groups exhibited higher enamel surface microhardness than the control group.

In addition, there is a similarity between the results of this study and a study conducted by **Arslan et al.** [20] in which they compared the effects of resin infiltration and sealant type on enamel surface properties and adhesion of S. mutans to artificial enamel lesions and their microhardness. By the end of their study, they proved that Enamel Pro Varnish had the lowest bacterial adhesion and higher enamel surface microhardness, followed by Icon.

Also, there is a similarity with the results of **Dehailan et al.** [15] who studied the effect of five commercially available fluoride varnishes (FV) on caries lesions. This study produced the following results, Enamel pro had the highest mean value in surface microhardness.

There is approvement with a study, which was conducted by **Majithia et al.** [21] in which they evaluated the remineralization potential of three commercially available varnishes and compared between them; Flor-Opal، Enamel Pro and MI effect on artificial enamel lesions using Vicker's microhardness test. This study concluded that all the varnishes were capable of remineralizing initial enamel lesions that were made artificially, but there was no statistically significant difference found between them in enamel surface microhardness despite their different compositions.

Also The results of the present study coincide with the results of a study on Nanosilver fluoride by **Nozari , et al.**[16] who evaluated the remineralization ability of NSF in comparison to Duraphat (5 % NaF)varnish and nano-Hydroxyapatite Serum (n-HAP) on primary anterior teeth enamel, according to their results they were able to conclude that NSF had the greatest remineralization efficacy over both Duraphat varnish and n-HAP serum and had higher enamel surface microhardness. This is also approved by **Teixeira et al.** [22] made an experimental study to compare Nano silver fluoride (NSF) and Duraphat sodium fluoride (NaF) effect in demineralization of enamel, Streptococcus mutans adhesion and the minimal inhibitory concentration and their enamel surface microhardness. The study concluded that there was no statistically significant difference in enamel surface microhardness between NSF and the NaF, both showed the same ability to prevent demineralization in the samples.

Meanwhile, the results of this study disagree with the finding of **Wang et al.** [23] who compared between Oravive gel and fluor protector on remineralization and resisting sugar drinks erosion. According to their results, Oravive gel effect in enhancement of the resistance of young permanent teeth and the remineralization effect on demineralized enamel is better than fluorprotector. The discrepancy between the results of the present study and this study may be related to the different compositions between Oravive and Fluor Protector. There are also discrepancies between the results of this study and the study by **Akyildiz** and **Sönmez** [24] as they evaluated the remineralization efficacy of NSF in comparison with SDF and sodium fluoride varnish (NaF). NSF was the least effective in remineralization of artificial carious lesions as it had lower enamel surface microhardness, this may be due to different sample sizes.

5-2: EDX:

According to the results of the present study, the three fluoride varnishes showed remineralization efficacy in comparison with the positive control group, however, Enamel Pro was the highest regarding calcium and Phosphorus weight and ratios using EDX, followed by Fluor protector and Enamel Pro, but there was no statistically significant difference found between the two groups.

There is a similarity in the findings of the present study with those of **Majithia et al.**[21] also in which

they compared and evaluated the remineralization efficiency of three commercially available varnishes on artificial enamel lesions in Flor-Opal، Enamel Pro, and MI using EDX. This study concluded that all three commercially available varnishes were successful in remineralizing initial enamel lesions that were induced artificially, with no difference in the calcium and Phosphorus weight and ratios despite their different compositions. This conclusion is similar to the conclusion of this study. There is also agreement with **Soekanto et al.** [25] in which they analyzed the remineralization potentials and fluoride, calcium, and phosphate release from Silver diamine fluoride (SDF), Nano Silver Fluoride (NSF), and propolis fluoride (PPF), similar to the results of the present study. According to their results, fluoride, calcium, and phosphate ions levels increased significantly in groups NSF and PPF when compared to SDF.

3. Environmental Scanning Electron:

According to the results of the present study, the three fluoride varnishes showed remineralization efficacy when compared to the positive control group, however, there was no statistically significant difference between the three tested groups regarding the ultrastructure morphology by ESEM.

The findings of this study are similar to the findings of **Liu et al.** [26] as they evaluated the effect of Fluor Protector in the demineralization of bovine enamel as a result of exposure to beverages. All the enamel specimens were exposed to beverages 10 times daily for five days. The study concluded that Fluor Protector had the ability to inhibit the demineralization of enamel caused by beverages.

Also, there is correspondence with **Xu et al.** [27] who studied the effect of fluor protector varnish against demineralization of deciduous teeth enamel surface by milk beverages, as they stated that, fluor protector had significantly higher Ca (2+) and P(3+) and that the fluor protector can be effective in inhibition of enamel demineralization in the milk beverages.

However, the results of this study disagree with the finding of **Akyildiz** and **Sönmez** [24] they evaluated the remineralization potentials of NSF in comparison with SDF and sodium fluoride varnish (NaF) also using ESEM. There was a statistically significant difference between all groups after remineralization. However, NSF was not as effective as sodium fluoride varnish and SDF in the remineralization of artificial enamel caries lesions.

CONCLUSIONS

The enamel pro group had the highest mean SMR % (Surface microhardness recovery) followed by the fluor protector and then the nano silver fluoride, however, there was no statistically significant difference between the three groups.

The enamel pro group showed the highest mean calcium weight percentage followed by the nano silver fluoride and the fluor protector, there was no statistically significant difference between the three groups.

The enamel pro group showed a statistically higher mean phosphorus weight percentage than the nano silver fluoride and the fluor protector groups. There was no statistically significant difference between the fluor protector and the nano silver fluoride.

The enamel pro group showed the highest mean calcium phosphorous ratio followed by the fluor protector and the nano silver fluoride, but there was no statistically significant difference between the three groups.

RECOMMENDATIONS

In-vivo studies are recommended to determine the efficiency of Fluor Protector, Enamel Pro and Nano silver Fluoride in the remineralization of demineralized enamel and caregivers should be educated about the efficacy of enamel varnishes to remineralize demineralized enamel and prevention of caries.

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