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REMINERALISATION EFFECT OF NANO-HYDROXYAPATITE AND FLUORIDE CONTAINING TOOTHPASTES ON ARTIFICIAL ENAMEL CARIES (AN IN VITRO STUDY)

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ABSTRACT

Aim: This investigation was carried out to assess and evaluate the remineralization ability of artificial enamel carious lesions by (n-HAP) tooth paste in comparison with (F) tooth paste.

Material and Methods: sixty healthy extracted teeth, the roots were cut and sectioning crowns. The samples were placed in acrylic resin blocks, covered with nail varnish except a 3x3 mm of exposed enamel. The samples were divided into three groups based on the toothpaste used: GA1 (n-hap), GA2 (F) and GA3 (n-hap+ F). The initial enamel lesion was produced using a demineralizing solution. Applying toothpaste for 30 cycles twice daily, and washing them under running water. In pH-cycling models, samples were demineralized twice daily for three hours, followed by a two-minute treatment with a remineralizing agent slurry twice daily over a 24-hour cycle. After two weeks and one month of brushing, specimens were examined utilizing (EDAX) and (SEM).

Results: At baseline and after demineralization, there was no statistically significant difference found between the three tested toothpastes, respectively, while after 2 weeks, the statistical analysis showed G A1 (n-hap) was significantly higher than of GA2 (F) and GA3 (n-hap+ F). After 1 month, there was a statistically significant difference as G A1 (n-hap) was significantly higher than GA2 (F) and GA3 (n-hap+ F).

Conclusions: Nanohydroxyapatite-containing toothpaste exhibits superior remineralizing potential as compared to fluoride-containing toothpaste

KEYWORDS: nano-hydroxyapatite, enamel remineralization, fluoride

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INTRODUCTION

Dental caries is a multifactorial, progressive illness that affects the calcified tissues of teeth and is defined by the organic portion of the tooth disintegrating and the inorganic component demineralizing.It is regarded as a dynamic disease process in which protective factors that cause remineralization and pathogenic variables that cause demineralization are in balance.[1]

Modern preventive dentistry has entered a new era thanks to the early recognition of carious lesions and their subsequent remineralization. The use of topical Dentifrices, mouthwash solutions, gels, and varnishes that include fluoride can aid in the remineralization of early enamel carious lesions. [2]

Given that the procedure of remineralization involves providing calcium and phosphate ions from an external origin to the tooth to encourage ion depositing into crystal gaps in demineralized enamel, dental products that include calcium, phosphate, and, compared to products that just contain fluoride, fluoride are said to improve remineralization. As a result, goods with bioavailable forms of calcium, phosphate, and fluoride have been released onto the market. [1]

Often referred to as "nanomaterials," nanostructured materials are solid systems with nanodomains contained in a large, dense matrix. It is important to distinguish this kind of arrangement from nanoparticles that are "free", which are usually produced as solid, dispersed colloidal elements. They are used in dental personal hygiene items that contain liquid dispersions like mouthwash or solid materials like toothpaste, where the stability of each component over time and throughout manufacture is crucial. [3]. Nanocrystalline HA exhibits superior mechanical, biocompatibility, and bioactivity compared to microcrystalline and bulk HA. [4]

In this study, three different tooth pastes were used to compare enamel remineralization power. First, the Apagrd royal toothpaste paste, utilizing a concentration of 10% nano-HA, is ideal for the remineralization of initial carious lesions. The nano-size of the particles and improved functionality of the nano-HA may allow for the availability of either calcium or phosphate ions to be more effective than fluoride at penetrating the enamel surface and continuing to fill the porosities of artificial carious lesions. [5] Because the hydroxyapatite nano crystals are smaller than 100 nm, the hydroxyapatite nanoparticles' surface area increases, increasing the agent's bioactivity. [6]

The second toothpaste used was Sensodyne toothpaste, which contains 1450 ppm fluoride. It has been demonstrated that using topical fluoride, which is found in toothpaste formulations and other professional applications, is an effective way to prevent and regulate carious processes. Fluoride is regarded as the most effective substance for preventing demineralization processes. [7] Fluoride has been utilized to encourage the development of fluorapatite, which improves dental enamel's ability to withstand acid, and it has been identified as a major promoter of remineralization. [5]

The third toothpaste used was Curaprox, be you yellow contains low-concentration nano-HAP modified Sodiummonofluorophosphate.[8] More calcium and phosphate ions from the surrounding remineralizing fluid may be drawn to the nano-HAP particles and lodged in the outermost layer of enamel. By doing this, they could bridge the spaces between the calcium crystals in the enamel, creating a homogeneous crystalline structure with a high mineral content and fluoride to help the enamel layer recalcify and mend. By solidifying the current layer, it serves to safeguard the teeth. This might be explained by the fact that NaF and n-HAP worked in concert throughout the remineralization process. [9]

Given the numerous benefits of nano-hydroxyapatite, many studies have been conducted to evaluate its efficacy in enamel remineralization. Nevertheless, limited studies have directly compared its effectiveness with fluoride or explored the synergistic effects of combining nano-hydroxyapatite and fluoride on early caries remineralization.

Thus, the aim of this study was to assess and compare the remineralization effect of three distinct tooth pastes: Nano-hydroxyapatite (n-HAP) containing tooth pastes, fluoride containing tooth pastes, and n-HAP with fluoride containing tooth pastes on artificial enamel caries. The null hypothesis was postulated as there is no difference between the three tested tooth pastes regarding their effect on remineralisation of initial enamel caries**.**

MATERIALS AND METHODS

Tree reminiralizing tooth paste were used **table (1) ,** demininralizing solution and artificial saliva. **table (2)**

Ethical regulation :

The current in-vitro research obtained approval for ethics from the Research Ethics Committee's (REC) at the Faculty of Dentistry, Minia University, which authorized the research protocol, with assigned protocol number 857/2023 at the meeting (101).

Sample size calculation:

Sample size calculated depending on a previous by **(Juntavee, A.,et al ., 2021)** [5] as reference. According to this study, the minimally accepted sample size was 15 per group, when mean ± standard deviation of group 1 was 0.017 ± 0.015 while mean ± standard deviation of group 2 was 0.095 ± 0.053, with 1.87 effect size when the type I error probability was 0.05 and the power was 80%. G. power 3.1.9.7 was used for the independent t test. To adequately make up for the 25% dropout rate, the total sample size was raised to 20%.

TABLE (1) Tooth paste brand name, specification ,composition and manufacturer.

Material	Specification	Composition	Manufacturer
Apagard® Royal,	Nano- Hydroxyapatite toothpaste (NHT)	10% nanohydroxyapatite , ß-glycyrrhetinic acid ,sodium lauryl sulfate, sodium, glycerin, hydrolysed conchiolin solution, trimagnesium carboxymethylcellulose Phosphatemedical, sodium saccharin	Sangi Co., Tokyo, Japan
Sensodyne	Fluoride containing tooth paste	Sodium fluoride (1450ppm Fluoride) Potassium nitrat, hydratedsilica, penta sodium triphosphate, sodium triphosphate, sodium lauryl sulphate,	GlaxoSmithKline, UK
Be You Yellow, Curaprox	Toothpaste with Nano-Hydroxyapatite and Fluorine	SodiummonoFluorophosphate , aqua, panthenol, glycerin, xylitol hydrated silica, sorbitol, nano-Hydroxyapatite, aroma, Xylitol , microcrystalline cellulose, decylglucoside,glucose oxidase,mannitole	CuradenAG, Swiss

TABLE (2) Deminiralizing solution and artificial saliva composition

Solutions	pH	Composition	Manufacturer
Demininralizing solution	Adjusted pH of 4.8 with 1M KOH).	2.2 mM CaCl2, 2.2 mM NaH2PO4, 50 mM acetic acid,	Institute Of Drug Development And Innovation research, Assuit University
Artificial saliva,	Adjusted pH of 7.0 by adding NaOH	NaCl 0.400 g, KCl 0.400g,NaH2PO4. H2O 0.69g, CaCl2.H2O 0.795 g, Na2S.9H2O 0.005g, Deionized water (1000 mL)	

Teeth Selection:

Sixty healthy molars and premolars were removed from the oral surgery department in Minia University's Dental Hospital. The teeth were removed for periodontal or orthodontic reasons. Using a sharp scaler, the selected teeth were carefully cleaned of tissue deposits and calculus. A 7X magnifying lens was used to analyze the teeth to prevent the selection of teeth with fractures or other morphological problems. Before testing, the teeth were stored for no more than a month at 4°C in distilled water with a 0.1% disinfectant thymol solution. [5]

Teeth preparation:

After the roots of each selected premolar were removed by utilizing a diamond disk (Dentorium, Germany), which attached to a slow speed straight handpiece(sirona, Germany) with a micromotor spinning at 15,000 rpm that had a continuous water coolant, then the crowns were divided into buccal and lingual portions[10], then each crown was placed in acrylic resin blocks (Acrostone, Cairo, Egypt) by using cylindrical molds with (diameter of 1.5cm and height 0f 2 cm) ,with exposed buccal and lingual surface then all of them were covered with two coats of acid-resistant nail polish (kiko nail varnish, Italy) except for a 3x3 mm a square of self-sticking tap above the CEJ of each tooth's labial and lingual surfaces, tape was adhered , after the nail polish dried, the self-adhesive tapes were taken off, revealing only a 3 x 3 mm window of sound enamel was exposed [11], that was subjected to deminiralizing solution in order to produce an initial enamel lesion. [12]

Enamel deminiralization

The demineralizing solution is made up of 2.2 mM CaCl2, 2.2 mM NaH2PO4, 50 mM acetic acid, and 1M KOH to bring the pH down to (4.8). Specimens were put in 50ml of demineralizing solution-filled container and incubated in a humidified environment for 12 hours at 37°C in an incubator (Galaxy170-200 RS Biotech, United Kingdom), then rinsed for 60 seconds under running water to create an artificial carious lesion on the surface of the enamel.[12, 5]

Grouping of specimens

Sixty enamel specimens were divided at random into three group of (20) specimen each (n=20) based on the kind of dental paste that was utilized in the study: G1 (A1); samples treated by (n-HAP) containing tooth paste, Group 2 (A2); samples treated by (F) containing tooth paste, Group 3 (A3) ; samples treated by (n-HAP & F) containing tooth paste. SEM & EDAX examination were used to evaluate enamel demineralization and remineralization at 4 time intervals: (T0); before demineralization (baseline), (T1); after demineralization, (T2); treatment for two weeks, (T3); treatment for one month **Tooth paste application and PH cycling**

The remineralization process was achieved by brushing every single tooth with a manual soft toothbrush (oral B 3d brush). The dental paste brushing procedure involved brushing each sample for two minutes, twice daily, with a 12-hour interval[12]. Brushing used a pea-sized amount of chosen toothpaste per sample for 30 cycles, with the bristles kept perpendicular to the enamel surface. This involved submerging the teeth in a toothpaste water-based slurry, and then washing each sample for one minute under running water.[11] Every day, slurries are made before being applied, by using a toothpaste/deionized water in the ratio of 1:3.[13]

The pH-cycling models were utilized to mimic the dynamics of caries formation and were planned to replicate an individual's typical intraoral situation. The samples undergo demineralization twice daily for three hours, followed by a two-minute treatment with the comparable slurry of toothpaste twice daily over a 24-hour cycle[10].

The demineralizing solution and synthetic saliva were replaced every 24 hours [10]. Then the samples were preserved in their respective identified sealed jar containing 50 ml of artificial saliva at 37ºC in an incubator (Galaxy170-200 RS Biotech, United Kingdom), which was replenished every 24h until the subsequent brushing cycle. Following two weeks and one month of brushing, Energy-Dispersive Analytical X-Ray (EDAX) and Scanning Electron Microscope (SEM), were utilized to analyse the samples.

Mineral analysis by Energy-Dispersive Analytical X-ray (EDAX)

All the specimens were examined by energy-dispersive analytical X-ray microanalysis by using (JSM-IT200, JEOL, Tokyo, Japan) to figure out the elemental composition of each enamel surface and to measure the precise quantities of calcium, phosphorus and fluoride ion concentrations (atom% %) performed in different areas of each sample. The samples were evaluated before demineralization, after demineralization and after two weeks and after a month of toothpaste application

Ultra-morphological examination using Scanning Electron Microscope (SEM)

The samples from each group were taken for SEM examination by using SEM (JSM-IT200, JEOL, Tokyo, Japan). First of all, the samples were air dried and then covered by a thin layer of gold by a fine coating sputtering device (JFC-1100 device). Then the images were taken from three different regions on each sample at a magnification of 1000X-2000X. The samples were evaluated before demineralization, after demineralization and after two weeks and one month of treatment cycle.

Statistical analysis

The mean and standard deviation for each group were used to display all of the data. Assessing the given data was accomplished using the Shapiro-Wilk test and Kolmogorov-Smirnov test for normality, which showed that the significance level (P-value) was insignificant as P-value > 0.05, which proved data was developed from a normal distribution. Consequently, a comparison of the three groups was carried out utilizing One Way ANOVA test, followed by multiple comparisons using Tukey's Post Hoc test. Within each group, comparisons between various treatment durations were carried out utilizing Repeated Measures ANOVA test followed by Tukey`s Post Hoc test for multiple comparisons. By using SPSS 16 ® (Statistical Package for Scientific Studies) Statistical analysis was carried out with GraphPad Prism & Windows Excel. The Significant level was set at $P \leq 0.05$.

RESULTS

Calcium (Ca) atom %

The effect of different treatment times on the mean of (Ca) atom % of different tested toothpastes is as follows: Initially, at baseline and after demineralization, there was no significant difference found between the three tested toothpastes. However, after 2 weeks, G A1 showed a significantly higher than G A2 & G A3, while after 1 month, it was significantly higher than G A2 & G A3. **(Table 3 and Figure 1).**

TABLE (3) Mean and standard deviation of (Ca) atom % of different tested toothpastes at different treatment times:

Ca	GI	GII	GIII	P value
	Mean ± (SD)	Mean ± (SD)	Mean ± (SD)	
At baseline	20.77 ^{a}± 2.26	20.97 ^{a}± 2.20	20.84 ^{a}± 2.22	0.95
After demineralization	13.22 ^{a}± 1.53	13.24 ^{a}± 1.53	13.39 ^{a}± 1.49	0.92
After 2 weeks	19.11 ^{a}± 2.58	16.30 ^{b}± 1.48	15.89 ^{b}± 2.72	0.0001*
After 1 month	22.13 ^{a}± 3.19	18.07 ^{b}± 1.44	17.72 ^{b}± 2.60	0.0001*

TABLE (4) Mean and standard deviation of (P) atom % of different tested toothpastes at different treatment times

P	GA1	GA2	GA3	P value
	Mean ± (SD)	Mean ± (SD)	Mean ± (SD)	
At baseline	13.34 ^{a}±1.21	13.38 ^{a}±1.20	13.35 ^{a}±1.20	0.99
After demineralization	9.45 ^{a}±1.29	9.50 ^{a}±1.34	9.56 ^{a}±1.24	0.96
After 2 weeks	12.24 ^{a}±1.28	11.26 ^{ab}±1.46	10.81 ^{b}±1.39	0.006*
After 1 month	13.10 ^{a}±1.89	12.45 ^{ab}±1.74	11.29 ^{b}±1.02	0.003*

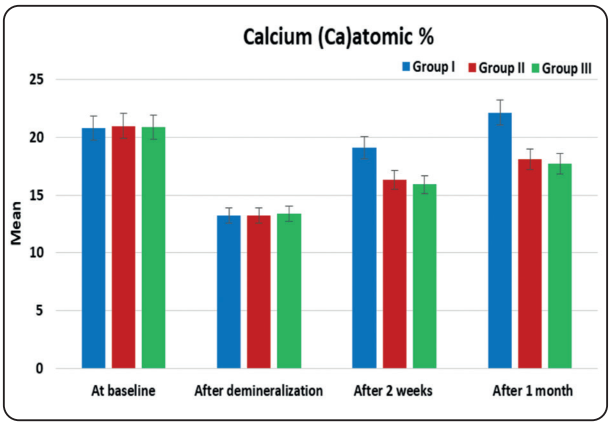

Fig. (1) Bar chart showing Calcium (Ca) atomic %in different tested toothpastes

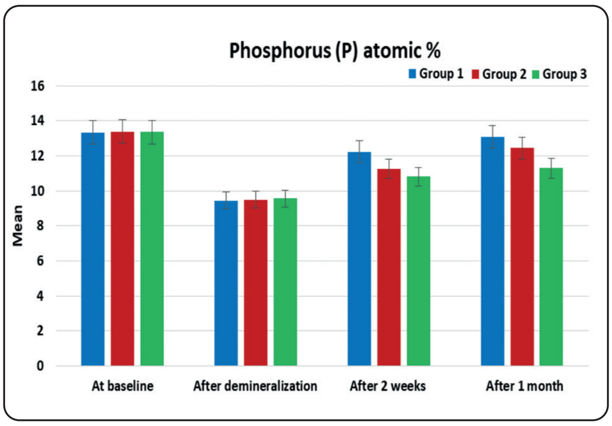

Fig. (2) Bar chart showing Phosphorus (P) atomic % in different tested toothpastes

Phosphorus (P) atom %:

At base line and after demineralization, no significant difference was found. However, after 2 weeks and after 1 month, G A1 was significantly higher than GA3 and GA2**. (Table 4 and Figure 2).**

Ca/P ratio:

At base line and after demineralization: There was no statistically significant difference found between the three tested toothpastes while after 2 weeks and after 1 month, there was a statistically significant difference as G A1 was significantly the highest, while GA2 and GA3 were significantly the least with insignificant difference between them, ($p \leq 0.0001$).

TABLE (5) Mean and standard deviation of Ca/P ratio of different tested toothpastes at different treatment times

Ca /P ratio	**GA1**	**GA2**	**GA3**	**P value**
	Mean ± (SD)	**Mean ± (SD)**	**Mean ± (SD)**	
At baseline	1.56 ^{a}± 0.10	1.57 ^{a}± 0.13	1.56 ^{a}± 0.10	0.95
After demineralization	1.41 ^{a}± 0.15	1.41 ^{a}± 0.16	1.41 ^{a}± 0.13	0.99
After 2 weeks	1.56 ^{a}± 0.11	1.46 ^{b}± 0.09	1.46 ^{b}± 0.08	0.001*
After 1 month	1.70 ^{a}± 0.20	1.49 ^{b}± 0.34	1.57 ^{b}± 0.21	0.04*

TABLE (6) Mean and standard deviation of (F) atom % of different tested toothpastes at different treatment times

F	GA1	GA2	GA3	P value
	Mean ± (SD)	Mean ± (SD)	Mean ± (SD)	
At baseline	1.60 ^{a}± 0.28	1.57 ^{a}± 0.26	1.57 ^{a}± 0.30	0.91
After demineralization	0.93 ^{a}± 0.31	0.94 ^{a}± 0.33	0.94 ^{a}± 0.31	0.99
After 2 weeks	0.66 ^{a}± 0.29	1.97 ^{b} ± 1.23	1.15 ^{a}± 0.46	0.0001*
After 1 month	0.94 ^{a}± 0.53	3.15 ^{b}± 1.63	1.17 ^{a}± 0.36	0.0001*

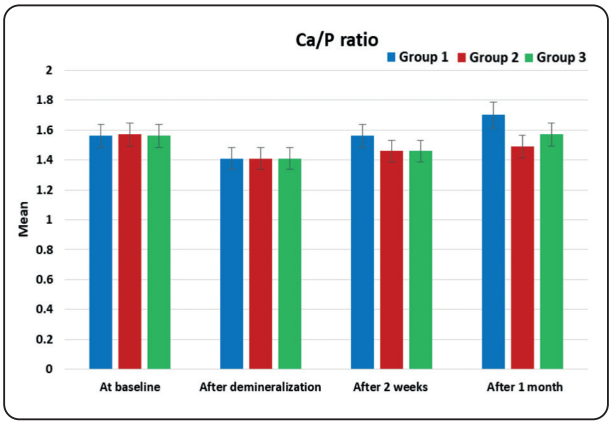

Fig. (3) Bar chart showing Ca/P ratio of different tested toothpastes

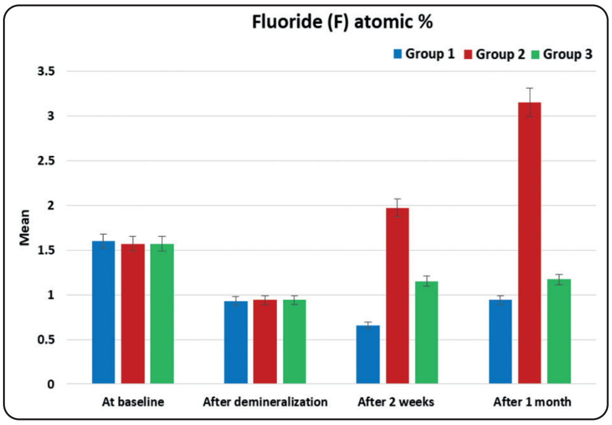

Fig. (4) Bar chart showing Fluoride (F) atomic % in different tested toothpaste.

Fluoride (F) atom %:

Results showed no significant difference between the three tested toothpastes at base line and after demineralization. However, after 2 weeks, G A1 and GA3 showed the least difference, while GA2 showed the highest difference. **(Table 6 and Figure 4).**

Ultra-morphological examination using Scanning Electron Microscope (SEM) :

At baseline the enamel specimen shows a smooth surface without any porosities or irregularities, after demineralization the specimen shows irregular pattern of slits, surface destruction and numerous micro porosities. **Figure (5)**

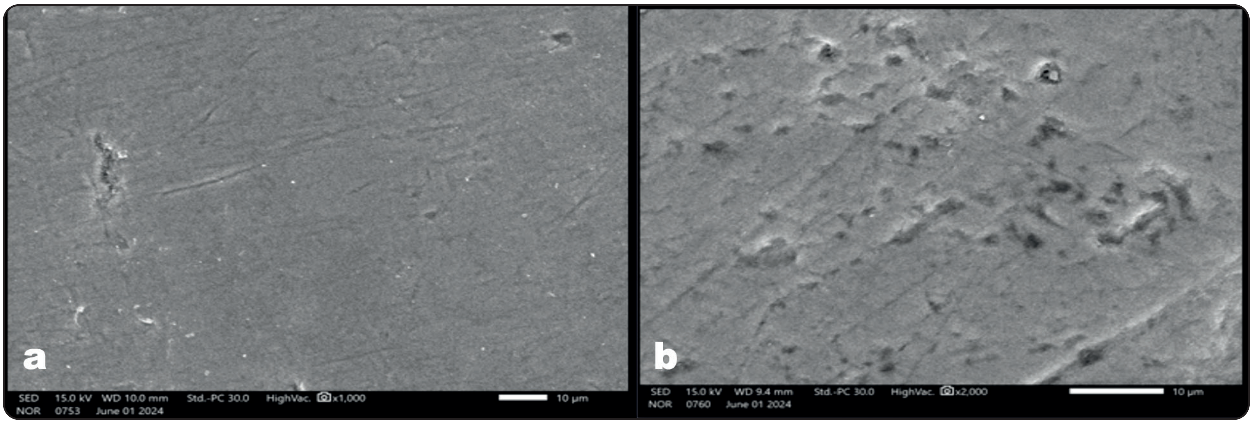

Fig. (5) Enamel samples (A) before deminiralization and (B) after deminiralization.

After two weeks of remineralization showed irregular pattern it indicates the whole carious lesion on the enamel surface has not totally healed in all test groups, and (n-HAP) group showed more remineralization. **Figure (6)**

After one month of remineralization (n-HAP) group showed larger deposits and thick uniform apatitic crystals than the (F) group and the (n-HAP+ F) group, and the the porous were hidden. **Figure (7)**

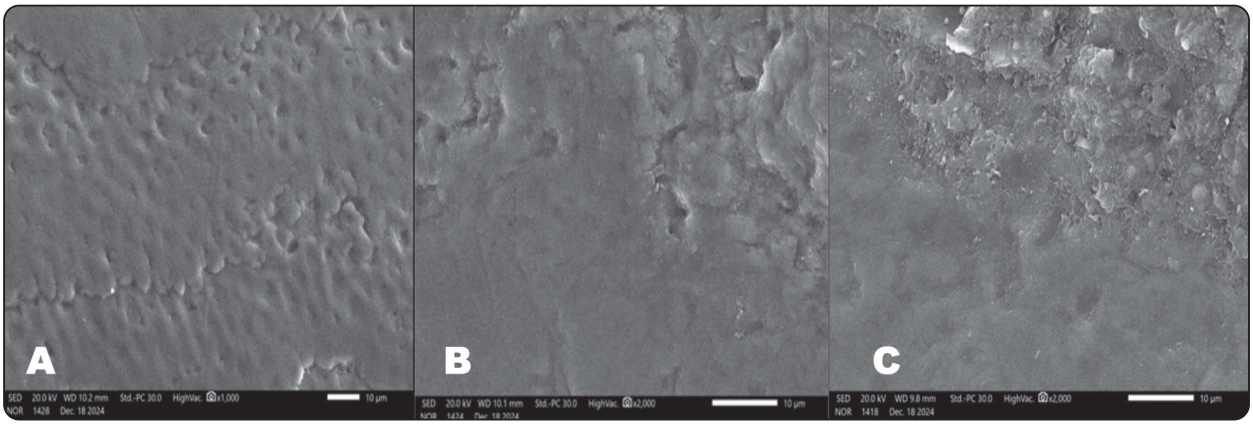

Fig. (6) Treated enamel samples after 2 weeks (A) (n-HAP), (B) (Fluoride), (C) (n-HAP +f).

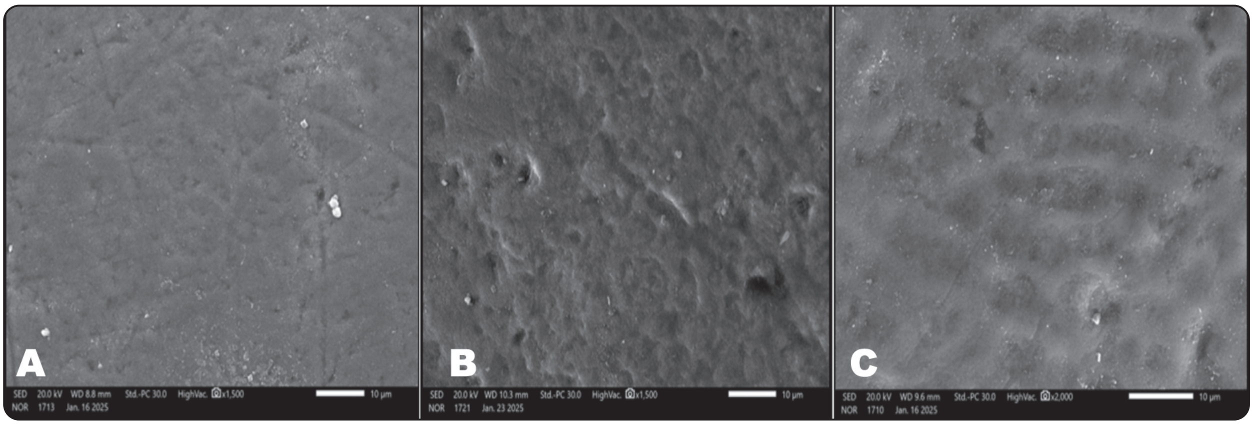

Fig. (7) Treated enamel samples after a month (A) (n-HAP+, (B) (Fluoride), (C) (n-HAP +f).

DISCUSSION

The aim of the study was to assess and compare the remineralisation effect of three distinct tooth pastes, Nano-hydroxyapatite (n-HAP) containing tooth pastes, fluoride containing tooth pastes, and (n-HAP) with fluoride containing tooth pastes on artificial enamel caries.

For our study the null hypothesis of this study was rejected because there was a statistically significant difference, with group A1 (n-HAP), group A1 (n-HAP) containing tooth paste being considerably the highest and group A2 (F) and group A3 (n-HAP +F) containing tooth paste being significantly the lowest, with negligible differences between them.

Like fluoride toothpaste, nano-HAP toothpaste can encourage remineralization and hence slow down the demineralization process. It was able to raise the enamel's surface hardness and slow down the progression of caries. The remineralization of demineralized enamel lesions was enhanced when nano-HA was added to mouthwash and toothpaste. **(Juntavee et al 2021)** [5]

The current study examined enamel remineralization using an in vitro model. The caries process and the impact of various anti-cariogenic agents on enamel demineralization and remineralization may be studied using an in vitro model, as commonly used **(Thimmaiah C, et al 2019)**[1]

Just a 3 × 3 mm window of exposed enamel that had been exposed to a deminiralizing solution to create an initial enamel lesion. **(Soni S, et, al., (2021)**[14]**, (Koçyigit, C, et, al., 2020)** [12] **(Kengadaran S,et, al., (2020)**[15] **(Joshi C,et, al., 2019)**[10]The specimens were incubated in a container containing an acidic deminiralizing solution. for 12 hours at 37°C before being washed for 60 seconds under running water **(Juntavee, A., et al ., 2021)**[5].

Unlike in **Nasution AI ,at al., (2017)**[16]**.** They used a different demineralizing solution was a 0.3% citric acid at pH 3.25, maintained by using sodium hydroxide in 10 ml , the possible explanation of short deminiralization period as this produce the same effect of initial demineralized lesion and irregularities on enamel surface.

Each tooth was manually brushed with a soft toothbrush to complete the remineralization process. Each sample was brushed with dental paste for two minutes twice a day, separated by twelve hours. **(Koçyigit, C., et al., 2020)**[12]. **(Joshi C,et, al., 2019)**[10] **(Soni S, et, al .,2021)**[14] **(Kariya et al., 2023)**[13]

The PH cycling was distinguished in **(Koçyigit, C,et al.,2020)**[12] **(Juntavee,A,et al.,2021)**[5]**.** In order to replicate the pH variations that occur in the oral environment throughout the day, the samples were subjected to a demineralization-remineralization cycle that included three hours of demineralization solution, and 16-hour remineralization solution was applied a 24-hour pH cycle the artificial saliva, and the solutions were changed every 24 hours. . **(Juntavee, A, et al., 2021)**[5]**.**

Abdelaziz RH,et al., 2019 [6] **, Sultan SS, et al., 2021**[17]**, Wakwak MA, 2019**[18] and **Sharma A, et al., 2017**[19] in these investigation they did not perform a PH cycling protocols they only did tooth brushing with corresponding reminiralizing agent. For 24 hours at room temperature, the samples were submerged in the artificial saliva, and the solution was changed every 24 hours.

An analytical method for determining the chemical composition of synthetic and biological materials. EDAX can accurately identify even the most minute ion concentrations, causing the method to be applied in research examining enamel's capacity to remineralize. (**Thimmaiah C, et al., 2019)**[1]**.** (**El-Bedewy et al., 2024)**[20]

The results indicate that the material with the greatest rate of deposition is nano-hydroxyapatite. The deposition of calcium and phosphate is extra in the nano-hydroxyapatite group as its composition mainly has calcium and phosphate then it followed by (F), containing tooth paste and GA3 (n-HAP +F), including tooth paste. The possible explanation for these results is due to its affinity for water, nano hydroxyapatite helps create a wetting character that can form a thin but firmly attached coating on the surface of the tooth **(Madhusudanan P, et al, 2018)**[21] and the hydroxyapatite nano crystals' smaller size (less than 100 nm) enhances the surface area of the hydroxyapatite nanoparticles, which in turn improves the bioactivity of the agent. **(Abdelaziz RH,et al 2019)**[6]**.** Which leads to the minerals filling the micropores, and their crystalline deposition mimics nature by covering the demineralized enamel surfaces with a uniform coating of apatite. **(Wakwak MA,2021)**[18] **(Erdilek AD,et al,.2022)**[22]

Fluoride is a key promoter of remineralization and has been used to enhance the formation of fluorapatite, which increases the acid resistance of tooth enamel. **(Juntavee A. et al 2021)**[5]**.**

The availability of calcium and phosphate in saliva and, eventually, in plaque fluid limits fluoride's capacity to encourage remineralization and restrict the development of caries in the oral environment; the rate of remineralization is insufficient to stop the caries process. The calcium and phosphate ions availability limits the capacity to encourage net remineralization. This may consequently constitute the restricting element. **(Thimmaiah C, et al ,. 2019)**[1] **(Khader DA,et , al,. 2023)**[23]

The fluoride addition to the nHAP-containing toothpaste (n-HAP + F) has no remineralization-promoting synergic effect. There are, however, very few changes between the nHAP+F group and the F group, indicating that the nHAP+F group has increased in mineral quantity.(**Koçyiğit C,et al 2020)**[12] **(Quandt DV,et al 2023)**[8]**.**

Our results reported that toothpaste with both fluoride and nano-HAP was not more effective when compared to toothpaste containing fluoride. These results are similar to studies done by **(Ganss C, et al,.2016)**[24]**(Aykut-Yetkiner A, et al ,. 2014)**[25]**, (Ganss C, et al,.2011)**[26] The possible explanation is a potential reaction between nano-HAP and fluoride. Whether the interaction occurs during the manufacturing of the nano-HAP-fluoride mixes or just upon contact with the tooth surface cannot be determined. The simple removal of the remineralization layer suggests that there is no contact with enamel or dentine and that there is an interaction between the two components. It means that the addition of nano-HAP inactivates the solid fluoride binding to the tooth surface.

This results are similar to a studies done by**.(Koçyiğit C,et al 2020)**[12]**, (Thimmaiah C, et al ,. 2019)**[1]**, (Vitiello F, et ,al ,. 2022)**[27]**, (Hemalatha P, et al,. 2020)**[28]**, (Grewal N, et al ,. 2018)**[29] **(Quandt DV,et al 2023)**[8] who used EDAX analysis reported that nano-hydroxyapatite was better than fluoride in remineralisation of enamel initial carious lesion.

A study by **(Abdelaziz RH, et al,. 2019)**[6]**.** Examine how casein phosphopeptide-amorphous calcium phosphate fluoride paste and nano-hydroxyapatite paste affect the remineralizing ability of early enamel carious lesions in deciduous teeth, reported that Compared to teeth treated with casein phosphopeptide-amorphous calcium phosphate fluoride paste, teeth treated with nano-hydroxyapatite paste had a greater mean surface microhardness. This might be because of the way the paste was applied (10 seconds of friction), and the calcium nanophosphate crystals might have gotten deeper into the carious enamel's defects.

On the other hand, our results didn't agree with **(Elmancy EM, et al,. 2022)**[30], who analyzed the performance of two commercial toothpastes: one that contains HAP-NPs and the other that contains NovaMin, comparing enamel remineralization to fluoridated toothpaste, they found that mean SMH was lowest in the Nano-hydroxyapatite group and highest in the NovaMin group, followed by fluoride. This is contingent upon the fluoride-containing compound's solubility and surface adherence, and the formation of fluoroapatite particles.

According to previous investigations, SEM is among the most reliable and sensitive methods for evaluating the demineralization and remineralization of the carious lesions in vitro**.(Hattab FN, et, al,. 1988)**[31] and quantitatively evaluate the changes in the enamel surface morphology before and after remineralization **(Vijayasankari V, et al.,2019)**[32] **(Thimmaiah C, et al., 2019)**[1]**.**

Our results showed that group A1 (n-HAP) A surface that has been remineralized has less visible microporosities. homogeneous apatitic crystals obscured the porous interprismatic and prismatic enamel structures in the demineralization-created pores. And A2 (F) group revealed a thin layer of mineralization with holes and porosities were not completely filled While A3 (n-HAP+ F) group the specimen's demineralized surface had some apatite crystals, as seen by the surface's mild roughness and micropores in some areas, which suggests partial remineralization.

The reasons which support our results are that nHAP remineralization process operates, by apatite deposition on the demineralized surface creates a layer with a high mineral density, and mineral deposition is used to restore the enamel's crystal structure, while Fluoride dentifrice's remineralization was discovered to be unpredictable, producing a non-homogenous layer and certain micropores that failed to seal. **(Koçyiğit C, et al.,2020)**[12] **(Vijayasankari V,et, al., 2019)**[32] **(Grewal N, et ,al., 2018)**[29]

But our results weren't consistent with a research by **(Nasution AI, et al., 2017)**[16] Their objective was to investigate descriptive analysis and comparative evaluations of enamel surface morphology. They founded that through the formation of fluorhydroxyapatite, fluoride prevents enamel demineralization, as shown by SEM pictures and there is no preservative remineralization potential on the enamel surface offered by n-HAp toothpaste.

LIMITATIONS OF THE STUDY

1. Remineralization in an in vitro model may differ significantly from the dynamic complex in the biological system in the oral cavity in vivo.
2. There are some differences in the formulation of natural and artificial saliva.
3. The lesions created in the study to replicate the demineralized surface may not fully represent the white spot lesion.

CONCLUSIONS

Within the limitations of the present research, the subsequent conclusions could be gained

1. The three tested tooth pastes were efficient at treating and remineralizing the initial enamel carious lesion.
2. HAP dentifrice can be used safely since it works just as well as fluoride dentifrice on early enamel lesions in teeth with less caries-resistant enamel surface.
3. When compared to fluoride paste, nano-hydroxyapatite paste had a superior remineralizing effect on demineralized enamel.
4. The slow-release amorphous form of n-HAP is a good delivery vehicle because it increases the possibility for remineralization and provides a smooth enamel surface.
5. (n-HAP&F) containing toothpaste has the least remineralizing potential as compared to other groups, used in the study, which indicates that there is no synergistic effect of (n-HAP) with (F).

Clinical relevance

1. Application of Apagard® Royal toothpaste (10% nano-hydroxy apatite) is preferred for better remineralization of early enamel carious lesions.
2. Additional clinical research is necessary to fully understand and evaluate the tested material's capacity to remineralize initial enamel carious lesions in a normal oral environment, while adhering to the limitations of our in vitro experiment.
3. It is advised for further study to evaluate the effect of tasted toothpaste on surface roughness and micro-hardness of enamel.

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