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COMPARATIVE EVALUATION OF NANOHYDROXYAPATITE AND FLUORIDE CONTAINING TOOTHPASTES ON DENTINAL TUBULES OCCLUSION

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ABSTRACT

Aim: This investigation was conducted to compare the impact of three distinct toothpastes on mineral deposition and dentinal tubules occlusion.

Material and Methods: Sixty dentin samples were prepared from sixty extracted molar. The samples demineralized by submerging in 6% citric acid for two minutes. Then assigned into three groups (n=20) according to the three different toothpastes utilized: Group A1: Fluoride containing toothpaste. Group A2: Nano-hydroxyapatite containing toothpaste. Group A3: Nano-hydroxyapatite with Fluoride containing toothpaste. For a total of two weeks, brushing was repeated twice a day with a 12-hour interval between each session. Each sample were quantitatively assessed the ion concentrations (atom%) on the dentin surface using Energy-dispersive Analytical X-ray (EDAX) and qualitatively examined the dentinal tubules occlusion using Scanning Electron Microscope (SEM).

Results: The lowest mean value of (Ca) % was found in (A1). The lowest mean (P) % value observed in (A1). The lowest mean value of (F) % was recorded in (A2), while the greatest mean value was identified in (A1 and A3).

Conclusion: Toothpastes containing nano-hydroxyapatite, both with and without fluoride, show similar effects on mineral deposition and blockage of dentinal tubules.

KEYWORDS: Dentin hypersensitivity, Nano-hydroxyapatite, Dentinal tubules occlusion, Fluoride, Mineral deposition

INTRODUCTION

Dentin hypersensitivity (DHS) is a prevalent condition among dental patients, reducing their quality of life. It can be described as a brief, acute pain that arises from uncovered dentin in a reaction to external stimuli, mainly thermal, osmotic, evaporative, chemical, or tactile and it is unrelated to any underlying dental condition or defect [1].

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Although the precise cause of DHS is unclear, the most generally accepted explanation is the theory of hydrodynamic which suggests that the nerve fibers close to the pulp are stimulated by fluid movements within the dentinal tubules, which are caused by numerous stimuli, resulting in nerve activation and sensitivity [2]. Tubule occlusion is therefore regarded as one of the most successful treatment approaches since it is believed to prevent the hydrodynamic process and reduce dentinal sensitivity [3].

Fluorides may occlude dentin tubules by increasing hydroxyapatite mineralization, promoting deposition, decreasing permeability, thus reducing pain[4]. However, fluoride-containing products can form an acid-resistant layer which might restrict remineralization by preventing remineralizing ions from flowing into deeper layers, also excessive fluoride intake cause fluorosis. Consequently, new approaches for treating dental caries must be developed [5].

Toothpastes containing (n-HAp) showed complete dentinal tubules sealing and dentin coating because the nanoparticles' morphology is similar to tooth apatite crystals. Furthermore, their remineralizing effect as they may act as a carrier for missing minerals or physically replace them, providingaprotectivelayerreducing hypersensitivity sensation [6].

Therefore, the aim of this research was to assess the mineral deposition and dentinal tubules occlusion of three distinct toothpastes: fluoride, nano-hydroxyapatite (n-HAp), and nanohydroxyapatite (n-HAp) with fluoride containing toothpastes using Energy dispersive analytical X-Ray (EDAX) and Scanning electron microscope (SEM). The null hypothesis was proposed as there would be no distinction among the three examined toothpastes regarding mineral deposition and dentinal tubules occlusion.

TABLE (1) Toothpastes brand name, specification, composition, manufacturer, and lot No.

Brand name	Specification	Composition	Manufacturer (Lot No.)
Sensodyne Fluoride	Fluoride Containing Toothpaste	Sodium Fluoride (1450 ppm F) potassium nitrate, Hydrated silica, Pentasodium triphosphate, sodium triphosphate, Sodium lauryl sulfate,	Sensodyne, GlaxoSmithKline, UK (0826k053)
Apagard Royal	Nano-Hydroxyapatite Containing Toothpaste	10% Nano-Hydroxyapatite (n-HAp), glycerin, Polyethylene glycol (PEG), hydrolysed conchiolin solution, ß-glycyrrhetinic acid, sodium carboxy-methyl cellulose, sodium lauryl sulfate, trimagnesium phosphate, sodium saccharin,	Apagard® Royal, Sangi Co., Tokyo, Japan. (EL02)
Be You Yellow, Curaprox	Nano-Hydroxyapatite and Fluoride Containing Toothpaste	Sodium Mono-fluoro phosphate, Hydrated Silica, Aqua, Sorbitol, Xylitol, Glycerin, Panthenol, Aroma Microcrystalline Cellulose, nano- Hydroxyapatite, Glucose Oxidase, Mannitol, Decyl Glucoside	Curaden AG, Swiss (73315724)

MATERIALS AND METHODS

Three different toothpastes were utilized in the current research, as mentioned in **(Table 1)**.

Ethical regulation:

The current in vitro investigation has received approval from Minia University Faculty of Dentistry's Research Ethics Committee (REC) with protocol number 855/2023 throughout the meeting (102).

Sample size calculation:

Sample size calculated depending on a previous study **(Diab et al., 2022)**[7]. The study found that the minimum acceptable sample size for each group was 18 when group 1's mean ± standard deviation was 22.98 ± 1.5 while estimated mean of other group is 2 was 21.5, with 0.98 effect size when type I error probability was 0.05 and power was 80% . G. power3.1.9.7 was used to conduct the independent t test. The total sample size has been raised to 20 in order to adjust for the 10% drop out rate.

Teeth Selection:

For this study, sixty extracted sound permanent molars were collected from Minia University's Faculty of Dentistry's Oral Surgery Department. The proposed molar teeth used in this study were collected from adult patients in age range group between 40 and 60 years; which had their teeth extracted for periodontal diseases. The teeth were examined with a 7X magnifying lens to ensure that the entire crown was free of caries, wear, fractures, erosion, and fillings. After blood, saliva, debris, tissue fragments, and calculus were removed from the teeth by meticulous scaling, scrubbing, and rinsing, they were maintained in distilled water with a 0.1% disinfectant thymol solution at 4°C and utilized during a month following extraction [8].

Teeth preparation:

Teeth were implanted in acrylic resin blocks (Acrostone, Cairo, Egypt) till the cementoenamel junction using cylindrical molds (measuring 1.5cm in diameter and 2 cm in height), and the dentin surface was exposed by sectioning the occlusal enamel using a water-cooled cutting device (IsoMet 4000 Linear Precision Saw, Buehler, USA). The specimens were then rinsed for 30 seconds with distilled water to get rid of any remaining particles from the cutting process[9].

After that, all specimens were demineralized by immersing them in 6% citric acid for two minutes to eliminate the smear layer, followed by a 30 seconds wash with distilled water [10,11]. Samples were soaked in 10% NaOH solution for six hours, then stored in deionized water for an additional six hours to totally remove the smear layer and organic contents [12].

Teeth Grouping:

The sixty prepared teeth were assigned randomly into three groups (n=20) according to the three utilized toothpastes: Group A1: Samples received fluoride containing toothpaste. Group A2: Samples received nano-hydroxyapatite containing toothpaste. Group A3: Samples received fluoride and nano-hydroxyapatite containing toothpaste.

Treatment Procedures:

Each tooth was manually brushed with a toothpaste slurry made by combining toothpaste and artificial saliva (3:1)[13,14,15] using soft bristled brush (Oral B pro-Expert Max Clean Indicator) per each group, for one minute, moving the bristles in a circular motion perpendicular to the dentine surface. The brushing process was carried out twice a day, with 12 hours interval, for two successive weeks [16,17,5].

After every brushing session, the toothbrushes and samples were cleaned and stored in sealed jars with 50 mL artificial saliva at 37 °C in an incubator (CBM. Torre Picenardi (CR), Model 431/V., Italy), which were refilled daily, until the next brushing cycle [16,18,19]. The brushing process was conducted by the same operator for standardization, and after the final brushing, samples were analyzed using Energy-dispersive analytical X-ray (EDAX) and Scanning Electron Microscope (SEM).

Mineral analysis by Energy-dispersive analytical X-ray (EDAX):

For each sample, the energy-dispersive analytical X-ray (JSM-IT200, JEOL, Tokyo, Japan) used to measure dentin surface elemental components and quantities of Calcium, Phosphorus, and Fluoride at; baseline (T0), after demineralization (T1), and after brushing (T2), with elemental weight percentages assessed.

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

The samples were examined utilizing a Scanning Electron Microscope (JSM-IT200, JEOL, Tokyo, Japan) at 1500X magnification at; baseline (T0), following demineralization (T1), and after brushing (T2) to measure dentinal tubules obstruction. After being air-dried, the samples were coated in gold using a gold sputtering machine (JFC-1100, JEOL, Tokyo, Japan), vacuumized, and digitally analyzed.

Statistical analysis:

Within each test, the mean and standard deviation values have been estimated for each group. Using Shapiro-Wilk and Kolmogorov-Smirnov tests, the data were examined for normality and revealed a parametric (normal) distribution. repeated measurement ANOVA was employed. When comparing greater than two groups in related samples. Two groups in related samples were compared using the paired sample t-test. In unrelated samples, comparisons between more than two groups were made using a one-way ANOVA and the Tukey post hoc test. Two groups in unrelated samples were compared using the independent sample t-test. The interactions between distinct variables were examined using three-way ANOVA testing. The level for significance was fixed at $P \le 0.05$. Version 20 of IBM® SPSS® Statistics for Windows was used to conduct the statistical analysis.

RESULTS

I) Minerals analysis by Energy Dispersive Analytical X-ray (EDAX):

1. Effect of different treatment time on mean of (Ca) atom % of different tested toothpastes:

Table (2) and figure (1) showed the impact of different treatment time on mean of Ca atom % of different tested toothpastes as follow:

At base line (T0) and after selective demineralization (T1): Between the three toothpastes that were examined, no statistically significant variations were discovered where (P = 0.476 and 0.781) respectively, while after **toothpaste application (T2):** A statistically significant distinction was identified between (A1) and both of (A2) and (A3)

TABLE (2) Mean and standard deviation of Ca atom % of different examined toothpastes at different treatment times:

Treatment times (T)	Toothpastes (A)			P- Value
	Sensodyne fluoride (A1)	Apagard royal (A2)	Be you yellow (A3)	
	Mean ± (SD)	Mean ± (SD)	Mean ± (SD)	
Base line Sound dentin (T0)	15.241±3.25[a]	14.175±4.53[a]	13.716±3.79[a]	0.476
After selective demineralization (T1)	14.151±4.81[a]	13.28±4.17[a]	13.281±4.51[a]	0.781
After tooth paste application (T2)	11.558±2.65[b]	14.027±3.29[a]	14.638±3.19[a]	≤ 0.001*

At p=0.05, means with the same letter in each row do not differ significantly. **= Significant*

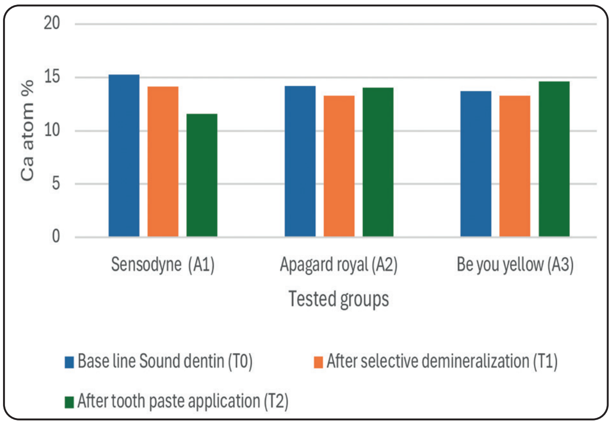


Fig. (1) Bar chart showing the Ca atom % of different tested toothpastes at different treatment times

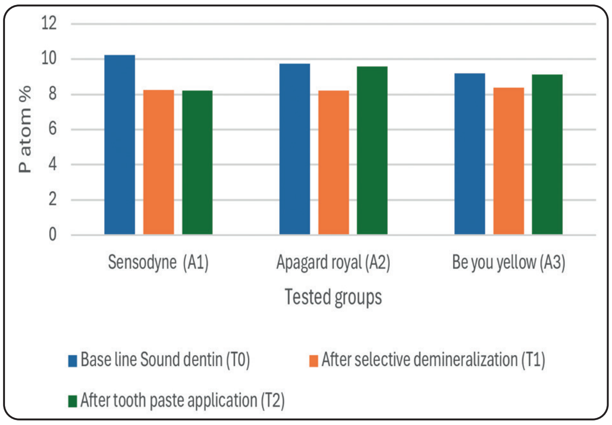


Fig. (2) Bar chart showing the (P) atom % of different tested toothpastes at different treatment times

where (p ≤0.001), and the lowest mean value of Ca % recorded in (A1).

2. Effect of different treatment time on mean of (P) atom % of different tested toothpastes:

Table (3) and figure (2) showed the Effect of different treatment time on mean of (P) atom % of different tested toothpastes as follow:

At base line (T0) and After selective demineralization (T1): Between the three toothpastes that were examined, no statistically significant variations were discovered where (P = 0.317 and 0.197) respectively, while after **tooth paste application (T2):** A statistically significant distinction was identified between (A1) and both of (A2) and (A3) where (p ≤0.001), and the lowest mean value of (P) % recorded in (A1).

3. Effect of different treatment time on mean of (F) atom % of different tested toothpastes:

Table (4) and figure (3) showed the Effect of different treatment time on mean of (F) atom % of different tested toothpastes as follow:

At base line (T0) and After selective demineralization (T1): Between the three toothpastes that were examined, no statistically significant variations were discovered where (P = 0.214 and 0.276) respectively, while after **toothpaste application (T2):** A statistically significant distinction was identified between (A2) and both of (A1) and (A3) where (p ≤0.001), and the lowest mean value of (F) % recorded in (A2), and the greatest mean value of found in (A1 and A3).

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

Treatment times (T)	Toothpastes (A)			P- Value
	Sensodyne fluoride (A1)	Apagard royal (A2)	Be you yellow (A3)	
	Mean ± (SD)	Mean ± (SD)	Mean ± (SD)	
Base line Sound dentin (T0)	10.224±2.13[a]	9.761±3.18[a]	9.184±2.14[a]	0.317
After selective demineralization (T1)	8.237±3.74[a]	8.229±2.75[a]	8.375±3.84[a]	0.197
After tooth paste application (T2)	8.226±3.65[b]	9.597±3.42[a]	9.126±3.56[a]	≤0.001*

*At p=0.05, means that have the same letter within each row do not differ significantly. *= Significant*

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

Treatment times (T)	Toothpastes (A)			P- Value
	Sensodyne fluoride (A1)	Apagard royal (A2)	Be you yellow (A3)	
	Mean ± (SD)	Mean ± (SD)	Mean ± (SD)	
Base line Sound dentin (T0)	0.321±1.67[a]	0.312±1.55[a]	0.485±1.39[a]	0.214
After selective demineralization (T1)	0.322±1.43[a]	0.311±1.62[a]	0.422±1.18[a]	0.2.76
After tooth paste application (T2)	1.213±1.17[a]	0.21±1.44[b]	0.931±1.83[a]	≤0.001*

At p=0.05, means that share the same letter within each row do not differ significantly. *= Significant

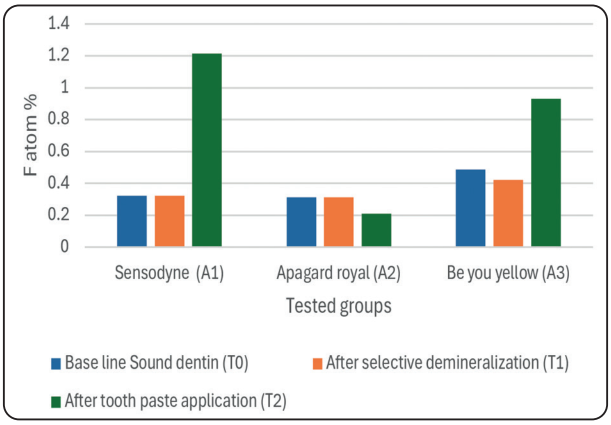
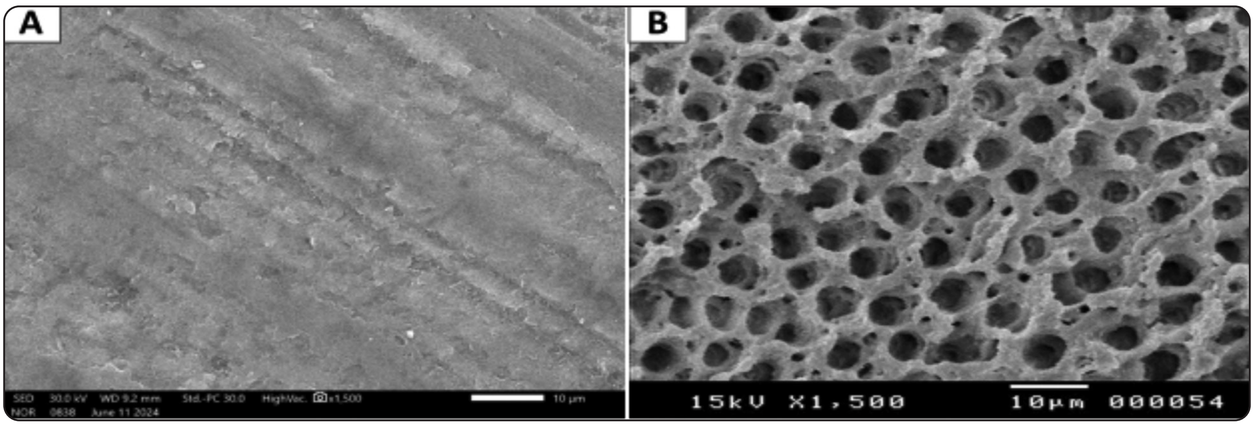


Fig. (3) Bar chart showing the (F) atom % of different tested toothpastes at different treatment times

II. Ultra-morphological examination of dentinal tubules occlusion by Scanning Electron Microscope (SEM):



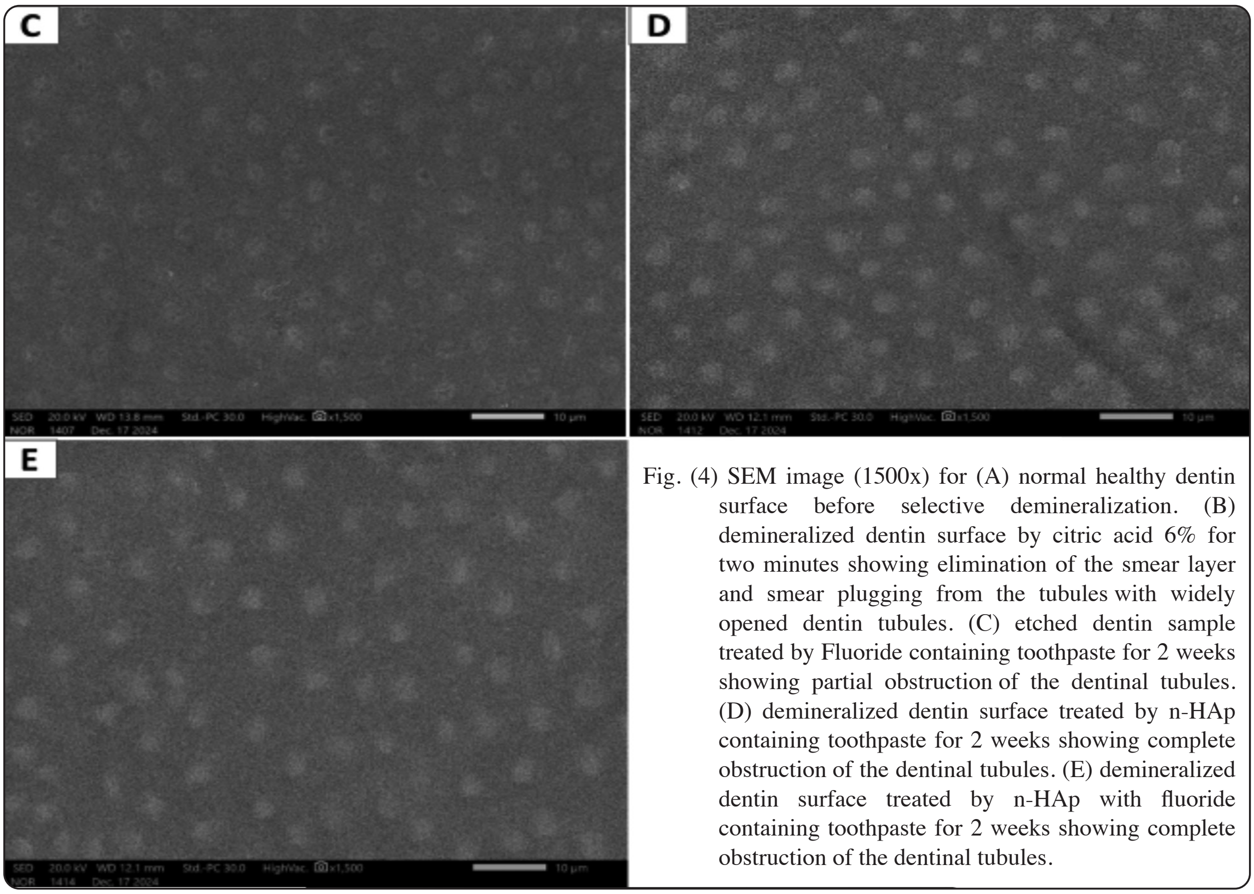


Fig. (4) SEM image (1500x) for (A) normal healthy dentin surface before selective demineralization. (B) demineralized dentin surface by citric acid 6% for two minutes showing elimination of the smear layer and smear plugging from the tubules with widely opened dentin tubules. (C) etched dentin sample treated by Fluoride containing toothpaste for 2 weeks showing partial obstruction of the dentinal tubules. (D) demineralized dentin surface treated by n-HAp containing toothpaste for 2 weeks showing complete obstruction of the dentinal tubules. (E) demineralized dentin surface treated by n-HAp with fluoride containing toothpaste for 2 weeks showing complete obstruction of the dentinal tubules.

DISCUSSION

This research was conducted to assess the mineral deposition and dentinal tubules blockage of three different commercially available toothpastes containing; fluoride, nanohydroxyapatite and nanohydroxyapatite with fluoride.

The study used 1450 ppm fluoride concentration, according to the International Organization for Standardization (ISO 11609). The study used 10% nano-hydroxyapatite, a frequent concentration in RCTs and in vitro studies, and found a significant variation in n-HAp action between 5% and 10% concentrations. The results for 10% and 15% nano-hydroxyapatite were found to be similar [5].

Fluoride promotes remineralization by increasing fluorapatite synthesis, which improves the tooth's ability to tolerate acid, and mineral absorption by absorbing Ca^{+2} and PO_4^{-2} ions from the saliva [20].

Recent research highlights the necessity for dental health products incorporating nano-hydroxyapatite particles (n-HAp), which, due to their small size, efficiently block open dentinal tubules and encourage deposition in irregular areas [21]. Also, numerous studies have documented that fluoride and nano-hydroxyapatite has a synergistic impact on the demineralized lesions' remineralization [22].

EDAX quantitatively assessed calcium, phosphorus, and fluoride amounts, while SEM qualitatively analyzed dentinal tubules closure. Both tests were selected for this study due to their non-destructive analysis, ability to provide topographical information, high resolution, and three-dimensional images for element deposition and dentinal tubules [9].

To open the dentinal tubules and simulate sensitive teeth, samples were demineralized by immersing them in 6% citric acid for two minutes, then washed with distilled water for at least 30 seconds [23,24,11].

This approach differs from **(Arnold et al., 2015)**[4], which utilized lemon juice for 30 seconds, and **(Farooq et al., 2015)**[19], who applied ethylene-diamine-tetraacetic acid (EDTA) for one minute.

For two weeks, each sample was brushed twice a day for one minute each, with 12 hours interval, for a total of two minutes each day using a manual soft toothbrush as it causes less abrasion [25,19]. This procedure differs from that of **(Kim et al., 2007)**[22], who submerged the specimens in the remineralizing solution for 24 and 48 hours. This prolonged exposure raised the total amount of minerals, which is unusual for a typical lifestyle.

Samples were preserved, between brushing cycles, in artificial saliva to simulate the oral environment [16,26,27], which were produced according to the suggested formula by **(Farooq et al., 2015)**[19] and refreshed daily.

Following toothpaste application, the mean atom % for calcium and phosphorus elements in nano-hydroxyapatite and nano-hydroxyapatite with fluoride dentifrices showed insignificant variation, this outcome was in line with the conclusions of **(Mahmoud et al., 2015)**[28], who demonstrated that fluoride-containing and fluoride-free nanohydroxyapatite have the same capacity for remineralization.

This contrasted with **(Tschoppe et al., 2011)**[13], which found that n-HAp free of fluoride had a much higher mineral content than n-HAp with fluoride. They explained these results by noting that amine fluoride's PH was lower than n-HAp's.

Additionally, fluoride toothpaste and both nano-hydroxyapatite and nano-hydroxyapatite with fluoride showed a significant difference in the mean of atom % for calcium and phosphorus elements, with the group treated with fluoride having lower Ca and P atoms than the other groups. The study supports the research by **(Tschoppe et al., 2011)**[13], which showed that nano-hydroxyapatite toothpaste is better at remineralizing early defects compared to fluoride toothpaste.

This disagreed with the results of **(Ali., 2013)**[29], who revealed no significant variation among the groups received nanoflurohydroxyapatite solution and sodium fluoride. Due to the deposition of remineralizing materials, control of dentin erosion was equally effective with both treatment strategies.

While the fluoride element's mean atom % results indicated that there was no noticeable difference between fluoride and nano-hydroxyapatite, according to **(Diab et al., 2022)**[7], both fluoride toothpaste and nano-fluorhydroxyapatite are equivalent.

This was in opposed to the findings of **(Jian- Ping et al., 2012)**[30], who claimed that the group treated with fluoride nano-hydroxyapatite had a greater amount of fluoride ions, which explained their superior sealing effect over the sodium fluoride group.

Furthermore, the mean atom % of fluoride element differed significantly between both fluoride and nano-hydroxyapatite with fluoride toothpastes and nano-hydroxyapatite toothpaste, as showed by **(Kim et al., 2007)**[22], who noted that the remineralization of demineralized lesions is enhanced when nano-hydroxyapatite is added to sodium fluoride mouthwash.

Although this contrasted with **(Leal et al., 2020)**[31], who claimed that there is no additive effect and using high-fluoride toothpaste in conjunction with nano-hydroxyapatite would not offer further protection against dentin caries.

CONCLUSIONS

Considering the restrictions of the current investigation, it is feasible to say that:

1. Toothpastes containing nano-hydroxyapatite, both with and without fluoride, have similar effects on mineral deposition and blockage of dentinal tubules.

2. Toothpaste containing fluoride has inferior remineralizing efficiency than toothpaste containing nano-hydroxyapatite, with or without fluoride.
3. Dental products containing nano-hydroxyapatite, either alone or combined with fluoride, can act as an efficient desensitizing agent.

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