

EFFECTIVENESS OF DIFFERENT TOOTHPASTES IN TREATING WHITE SPOT LESIONS: A LABORATORY STUDY

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ABSTRACT

Introduction: Strategy for managing caries has evolved into focusing on early detection of caries lesions and non-invasive treatment of initial lesions using innovative re-mineralizing agents.

Objectives: Aim of the study was to compare the ability of commercially available toothpaste formulations containing zinc-carbonate nano-hydroxyapatite ($ZnCO_3$/n-HAp), 8% arginine and calcium carbonate (Pro-Argin), and fluoride to promote re-mineralization in artificially induced white spot lesions.

Material and methods: A total of thirty-six sound premolars were randomly allocated into four groups according to the assigned toothpaste: G1 – $ZnCO_3$/n-HAp, G2 – Pro-Argin, G3 – positive control (fluoride toothpaste), G4 – negative control (artificial saliva). A scanning electron microscope (SEM) and energy dispersive X-ray (EDX) analyses were conducted at the baseline to assess calcium (Ca) and phosphorus (P) content. Following the induction of white spot lesions, a pH cycle was implemented involving a 7-day application of the toothpastes. Readings were taken both after de-mineralization and at subsequent re-mineralization phases. Collected data were subjected to statistical analysis using SPSS software version 25.0.

Results: The highest mean difference in Ca/P ratio was observed in the G2 group, followed by the G1, G3, and G4 groups in descending order. There were statistically significant differences between all the experimental toothpaste groups (G1, G2, and G3) and the negative control group (G4). However, no statistically significant difference was found among the tested toothpaste groups.

Conclusions: All three toothpastes tested were found to be effective in promoting re-mineralization of initial enamel lesions, with no evidence of superiority of one over the others.

Key words: re-mineralization, toothpastes, zinc-carbonate nano-hydroxyapatite, arginine, fluoride.

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INTRODUCTION

In a well-maintained oral environment, every tooth experiences an ongoing process of de-mineralization and re-mineralization [1]. When the saliva's pH drops below the critical level of 5.5 due to organic acids released by bacteria, it initiates enamel de-mineralization. This leads to dissolution of hydroxyapatite crystals within the tooth enamel, releasing calcium and phosphate ions. Consequently, this phenomenon cause formation of initial caries lesions, commonly known as white spot lesions [2].

Evidence indicates that by effectively altering oral conditions, there is potential for a shift towards an increased emphasis on the re-mineralization process, which can facilitate the repair of lesions [3]. Re-mineralization can take place naturally through the natural saliva buffer-

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ing mechanism, or it can be stimulated biologically using specialized and enhanced re-mineralizing agents [4].

Up to the present time, fluoride treatment has maintained its prominence as the leading method for preventing caries and promoting re-mineralization, and fluoride toothpastes are considered as the most prevalent and user-friendly ways to topically apply fluoride, particularly among children [5].

However, fluoride fails to be a comprehensive solution [5]. Various constraints might stem from fluoride's reduced efficacy when pH drops below 4.5, and the fact that fluoride's efficacy depends on the availability of calcium and phosphate ions in a bio-accessible form, essential elements found in saliva and other sources [6].

Currently, dental studies have been focusing on innovative and minimally invasive methods to create non-toxic anti-cariogenic toothpastes, which provide synergistic and complementary benefits [7, 8]. Contemporary products encompass various types and concentrations of re-mineralizing agents, including calcium, phosphate, and fluoride ions in their bio-available forms. These formulations maintain their capacity to enhance re-mineralization compared with products containing fluoride alone [9].

A novel strategy for caries prevention involves bio-mimetic and bio-inspired agents, which promote re-mineralization and inhibit de-mineralization of dental hard tissues, exceeding the limitations of fluoride. Bio-mimetic hydroxyapatite (HAp) technology, available as micro-clusters or nano-crystals, represents one of the latest advancements in tissue engineering [10]. The concept behind developing bio-mimetic zinc-carbonate hydroxy-apatite technology is based on the idea that materials resembling natural hydroxyapatite found in enamel and dentin have a strong affinity to adhere to these biological tissues; a concept also explored in bone research [11].

Bio-mimetic hydroxyapatite has gained considerable attention for its applications in prevention, therapy, and regeneration, finding its way into various oral care products. Recent studies strongly support its effectiveness in preventing and re-mineralizing dental enamel decay [12]. Experts even proposed that its bionic properties may aid enamel regeneration and the formation of a new enamel layer, highlighting its potential as a promising and innovative approach to dental care [10, 13].

Arginine amino acid (Pro-Argin technology) is a component found in commercially available fluoride toothpaste at concentrations of 1.5% and 8%. This toothpaste formulation also includes insoluble calcium carbonate and 1450 ppm sodium mono-fluorophosphate (MFP), all meticulously chosen to optimize re-mineralization of hard dental tissues. Notably, toothpaste variant containing 8% arginine and fluoride, along with an arginine bicarbonate variant, was initially developed to target dentin hypersensitivity [14]. Recent studies have also investigated its capacity as a strong preventive agent against dental caries [15]. There is, however, little data in the literature investigating and comparing the efficiency of toothpastes containing zinc-carbonate nano-hydroxyapatite, 8% arginine, and fluoride in promoting re-mineralization of early caries lesions.

OBJECTIVES

This study aimed to compare the potential of commercially available toothpaste formulations, which included zinc-carbonate nano-hydroxyapatite ($ZnCO_3$/n-HAp), 8% arginine and calcium carbonate (Pro-Argin), and 1450 ppm fluoride, to promote re-mineralization in artificially induced white spot lesions. The current study hypothesis (H_0) suggested that there would be no significant difference in re-mineralization efficacy among the three toothpaste variants.

MATERIAL AND METHODS

This in-vitro study has received approval from the Dental Research Ethical Committee of the Faculty of Dentistry (approval No.: M08040521). Samples used in the study consisted of sound human maxillary premolars extracted for orthodontic purposes. A careful inspection was conducted under proper illumination to identify and exclude teeth, which had previously received chemical treatment, and had cracks, hypoplasia, caries, fluorosis, restorations, or any other developmental anomalies. One single operator performed all procedures to avoid operator bias. Sample size was calibrated using Federer formula: $(t - 1)(n - 1) \geq 15$, with "n" denoting the sample size for each group, and "t" indicating the number of groups [16]. In this case, the study originally required a minimum sample size (n) of 6 in each group. However, to account for potential sample damage and data loss during the study, nine samples were included in each group. Consequently, there were four sample groups, each consisting of nine samples, making a total of 36 maxillary premolars included in the study. A flow chart of the experimental study methodology is represented in Figure 1.

The samples were collected, cleansed, and immersed in physiological saline for two weeks in accordance with the guidelines of occupational safety and health administration (OSHA) [17]. Subsequently, the coronal portion of the teeth was separated from the roots at cement-enamel junction level [9]. This was achieved using a low-speed, double-coated diamond disc attached to a micro-motor straight handpiece (NSK, Japan), with the assistance of a water coolant. Then, the samples were embedded in partially cured acrylic resin (Acrostone, Cairo, Egypt) using custom-made cylindrical plastic molds, ensuring that their buccal surfaces were oriented upwards. An acid-resistant nail varnish (Revlon®, USA) was applied to the enamel surfaces and allowed to dry at room temperature. A standardized 4 × 4 mm window on labial surface of the enamel was left exposed without nail varnish in all samples [15]. To evaluate the calcium (Ca) and phosphate (P) content measured in terms of weight percentage, a baseline assessment was conducted. This evaluation was carried out using energy dispersive X-ray (EDX) analysis. In order to induce white spot lesions, the samples were subjected to a four-day immersion in a freshly prepared de-mineralizing solution [15]. This solution was prepared using 2.2 mM calcium chloride, 2.2 mM sodium dihydrogen phosphate, and 0.05 M acetic acid, adjusted to a pH of 4.4 with 1 M sodium hydroxide at the Analytical Chemistry Department, Faculty of Pharmacy [18]. After the immersion period, the samples were thoroughly rinsed with de-ionized water and kept until additional EDX analysis was performed [19].

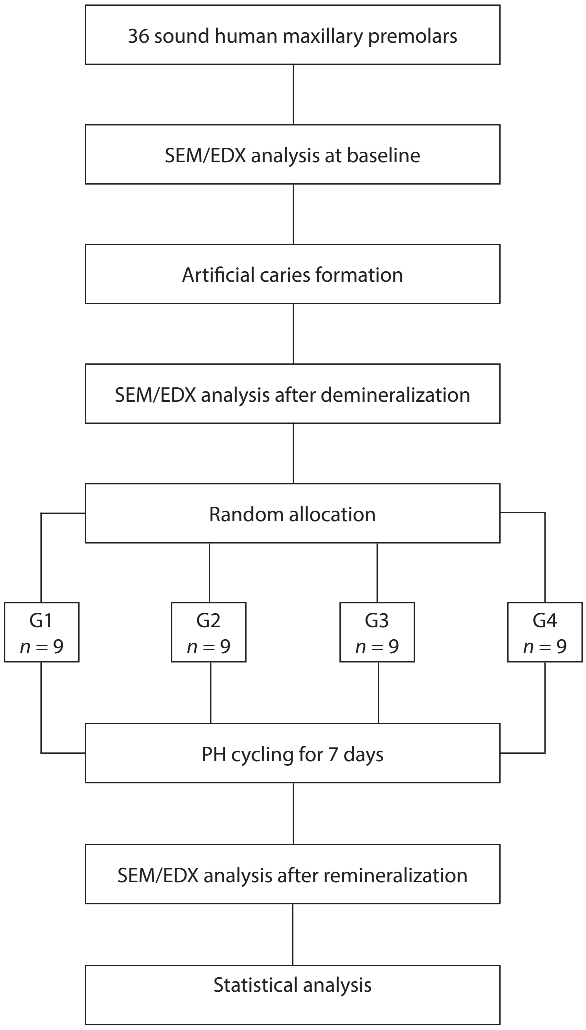

FIGURE 1. Flowchart of the experimental study methodology: G1 ($ZnCO_3$/n-HAp), G2 (Pro-Argin), G3 (fluoride), and G4 (artificial saliva)

Using a simple random sampling technique, the samples were allocated into four distinct groups, each containing nine samples (*n* = 9). The teeth were color-coded in accordance with their assigned groups:

1. G1 ($ZnCO_3$/ n-HAp): samples treated with Biorepair toothpaste.
2. G2 (Pro-Argin): samples treated with Colgate® Sensitive Pro-Relief™ toothpaste.
3. G3 (fluoride): samples treated with Signal Cavity Fighter toothpaste, as positive control.
4. G4 (artificial saliva): samples did not receive any treatment, only immersion in artificial saliva as negative control.

The materials tested in the study and their active components are shown in Table 1.

The samples were subjected to a pH-cycling system for a period of 7 days, aiming to replicate natural pH fluctuations that occur within oral environment (Figure 2). Each cycle involved two instances of 3-hour de-mineralization, paired with 18 hours of immersion in artificial saliva that was prepared using 1.5 mM calcium chloride, 0.9 mM sodium dihydrogen phosphate, and 0.15 M potassium chloride, with a pH meticulously adjusted to 7.0 using 1 M potassium hydroxide [18]. For treatment application, the samples underwent manual brushing using a soft toothbrush with minimal pressure. Each sample was brushed for 5 seconds, and thoroughly mixed toothpaste slurry was applied for additional 115 seconds. As a result, each treatment session had a total time

TABLE 1. Tested materials with their active ingredients and application method

Material	Active ingredient	Application method	Manufacturer
Biorepair toothpaste	20 wt% zinc-carbonate nano-hydroxyapatite ($ZnCO_3$/n-HAp), with a particle size of 20 nm and granular dimensions up to 100-150 nm	Slurry (ratio, 1 : 3) from toothpaste and de-ionized water, brushing for 5 seconds with a total contact time of 120 seconds three times daily in all groups	Dr. Kurt Wolff GmbH & Co. KG, Bielefeld, Germany
Colgate® Sensitive Pro-Relief™ toothpaste	Arginine bicarbonate, calcium carbonate, sodium mono-fluorophosphate (1450 ppm F)		Colgate-Palmolive Manufacturing, Poland
Signal Cavity Fighter toothpaste	Sodium mono-fluorophosphate (1450 ppm F)		Unilever Mashreq-Personal Care Company, Egypt
Artificial saliva	Calcium and phosphate	Immersion only/no treatment	Analytical Chemistry Department at the Faculty of Pharmacy

of 120 seconds [20]. Toothpaste semiliquid mixture was prepared by diluting the toothpaste with de-ionized water at a weight ratio of 1 : 3 to ensure homogeneity [21]. This treatment regimen was carried out before and after the first and second de-mineralization cycles, three times in each day. Following subsequent steps, the samples were thoroughly rinsed with de-ionized water for 60 seconds [22]. As the designated negative control group, G4 group samples underwent the same pH-cycling procedures as the other groups, but did not receive any surface treatment. For each group, distinct containers were used, and daily replacements of all solutions were made throughout each cycle. pH levels were monitored using a pH electrode.

SEM-EDX analysis was conducted on the samples at the Department of Electron Microscopy, Faculty of Agriculture. An environmental SEM (Quanta 250 FEG, FEI Company, Netherlands) equipped with an energy dispersive X-ray analyzer (SEM-EDX) was employed at a magnification of ×600 and a voltage of 20 kV. Prior to analysis, the samples were air-dried, and conductivity was enhanced using graphite tape. Each sample was accompanied by an element table and its corresponding spectrum. Weight percentages of calcium (Ca) and phosphorus (P) were determined enabling calculation of Ca/P ratios at different stages, such as baseline, after de-mineralization, and after re-mineralization.

Statistical analysis was performed with SPSS software, version 25.0 (SPSS Inc., PASW Statistics for Windows, version 25.0, SPSS Inc., Chicago, USA). Data were checked for normality using Shapiro-test, and were found to be normally distributed. Means were compared using one-way ANOVA test, while post-hoc Tukey's test was performed for pair-wise comparisons. Significance level was set at $p \leq 0.05$.

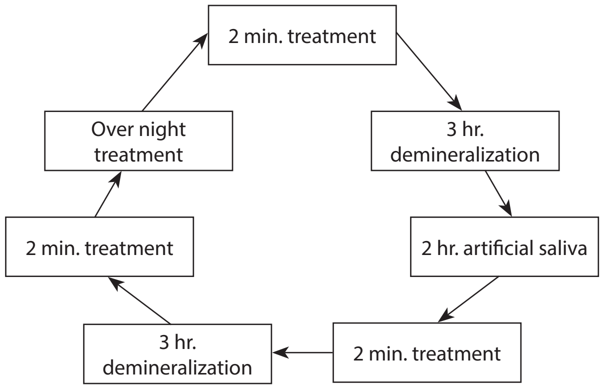

FIGURE 2. PH cycle

RESULTS

The elemental tables and EDX spectra for enamel under different conditions are shown in Figure 3, including baseline (A-B), after de-mineralization (C-D), and after pH-cycling in all groups with treatment using the tested toothpastes (E-J) and artificial saliva (K-L). Table 2 displays the mean and standard deviation results of Ca/P ratio. In this study, the highest mean difference in the Ca/P ratio was observed in the G2 sample group treated with 8% arginine and calcium carbonate (Colgate® Sensitive Pro-Relief™). It was followed by the G1 (Biorepair with 20 wt% zinc-carbonate nano-hydroxyapatite), G3 (Signal with sodium mono-fluorophosphate), and G4 (artificial saliva) sample groups, in descending order.

The results from one-way ANOVA test showed significant differences between all the experimental toothpaste groups and the negative control group (G4). However, when comparing the re-mineralization toothpaste groups, no statistically significant difference was found. Notably, the G2 group exhibited a statistically significant difference in the baseline Ca/P ratio, while the values of other groups did not show any significant difference in the baseline Ca/P ratio.

Based on data from Table 2, a significant reduction in the mean Ca/P ratio after the de-mineralization process was observed. After the pH-cycling, there was a noteworthy increase in the mean Ca/P ratio in all groups ($p < 0.05$). The ability to transition from de-mineralization to re-mineralization was significant in all groups.

Upon evaluating EDX values for calcium (Figure 4), it was evident that all groups using re-mineralizing toothpastes demonstrated a noteworthy increase in calcium content compared with the negative control. However, pair-wise comparisons between these groups revealed no significant differences. Similarly, when examining the EDX values for phosphorus (Figure 5), the results indicated a significant increase in phosphorus content in the G3 and G2 groups when compared with the G4 (negative control) group. However, pair-wise comparisons between the G3 and G2 as well as between the G4 and G1 samples showed no significant differences.

DISCUSSION

Dental caries are considered as one of the most prevalent oral health challenges, impacting primary and permanent teeth throughout a person's lifetime [23]. The current management of dental caries has moved to a more medical, non-invasive model that prioritizes prevention and early intervention. This strategy includes the application of re-mineralizing agents, which provide the teeth with calcium and phosphate ions, encourage ion deposition, generate a net mineral gain, and effectively stop and reverse caries lesions during initial, non-cavitated stage [24].

To gain insights into changes in the mineral content of the samples, SEM-EDX analysis was applied, recognized as a highly reliable and widely adopted method. This was accomplished by measuring the availability of two important enamel constituents, i.e., calcium (Ca)

A

Elem...	Weight%	Atomic%
O K	50.00	69.24
Na K	0.67	0.64
P K	17.30	12.37
Cl K	0.59	0.37
Ca K	31.44	17.38
Totals	100.00	

C

Elem...	Weight%	Atomic%
O K	56.41	74.36
Na K	0.54	0.49
P K	15.82	10.77
Cl K	0.59	0.35
Ca K	26.64	14.02
Totals	100.00	

E

Elem...	Weight%	Atomic%
O K	39.19	59.60
P K	19.25	15.12
Cl K	0.73	0.50
Ca K	40.83	24.78
Totals	100.00	

G

Elem...	Weight%	Atomic%
O K	34.45	54.47
Na K	1.00	1.10
P K	19.56	15.98
Cl K	0.69	0.49
Ca K	44.30	27.96
Totals	100.00	

I

Elem...	Weight%	Atomic%
O K	43.54	63.82
P K	18.03	13.65
Cl K	0.63	0.41
Ca K	37.79	22.11
Totals	100.00	

K

Elem...	Weight%	Atomic%
O K	47.06	66.64
Na K	1.62	1.59
P K	16.04	11.73
Cl K	1.25	0.80
K K	0.35	0.20
Ca K	33.68	19.04
Totals	100.00	

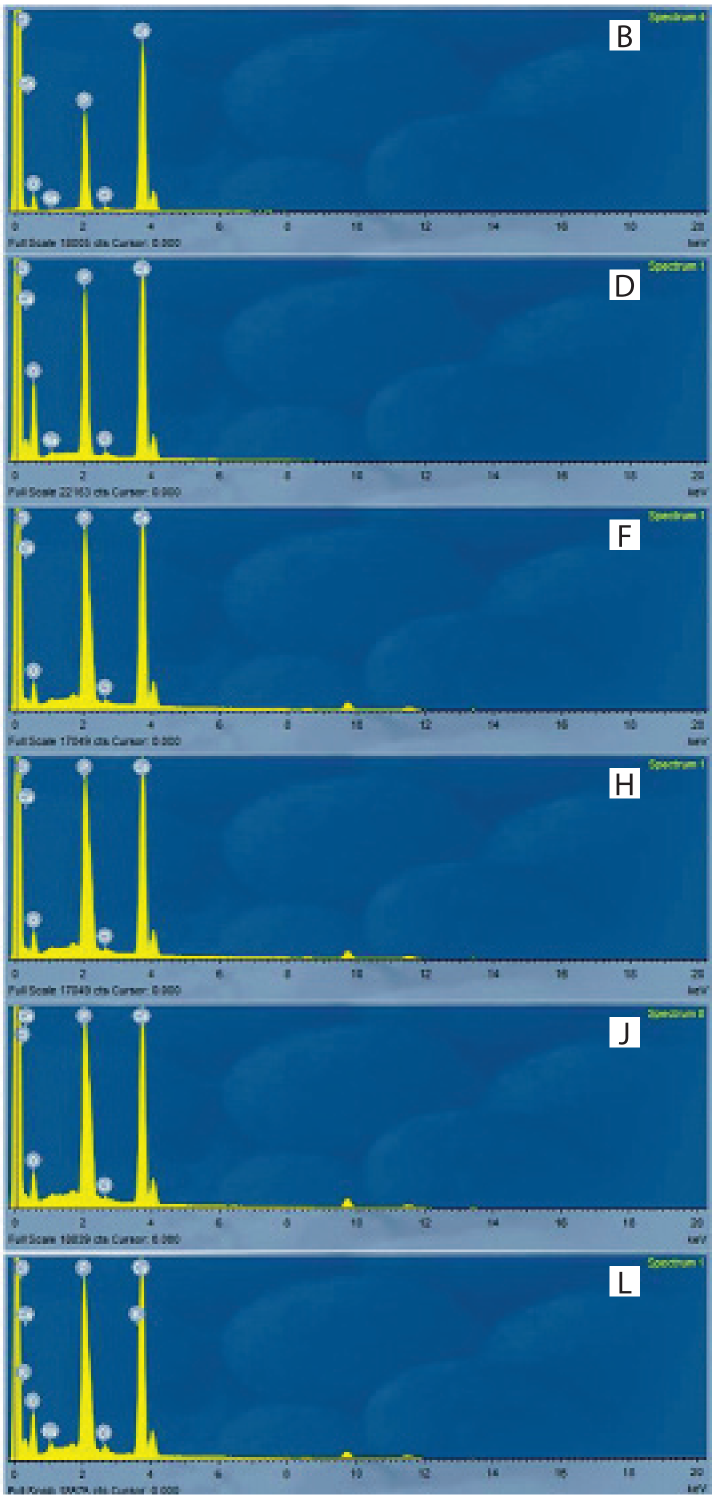

FIGURE 3. Tables of elements and EDX spectra detected by EDX of a sound enamel sample (**A-B**), de-mineralized enamel (**C-D**), after treatment with $ZnCO_3$/n-HAp (**E-F**), after treatment with Pro-Argin (**G-H**), after treatment with fluoride (**I-J**), and artificial saliva (**K-L**)

and phosphorus (P), as markers of enamel health [9, 25]. This study involved analyzing the samples at three stages: baseline (before treatment), post-de-mineralization to confirm the extent of mineral loss, and post-re-mineralization to identify any mineral gain. This enabled a comparison of changes in mineral content before and after treatment.

In the current study, three distinct toothpaste formulations were investigated: zinc-carbonate nano-hydroxyapatite toothpaste, arginine toothpaste, and fluoride-

TABLE 2. Comparison of Ca/P ratio between studied groups

	Baseline	Demin.	G1	G2	G3	G4	Test of significance
	$n = 36$	$n = 36$	$n = 9$	$n = 9$	$n = 9$	$n = 9$	
Ca/P ratio	2.02 ± 0.18^{abc}	1.67 ± 0.06	2.07 ± 0.04^{bde}	2.17 ± 0.15^{df}	2.05 ± 0.048^{cef}	1.9 ± 0.15^{a}	$F = 19.38$ $p < 0.001$*

F – one-way ANOVA test; similar superscripted letters denote non-significant difference between groups within same row by post-hoc Tukey's test; n – number of samples; Demin. – after de-mineralization; G1 – ZnCO$_3$/n-HAp; G2 – Pro-Argin; G3 – fluoride; G4 – artificial saliva

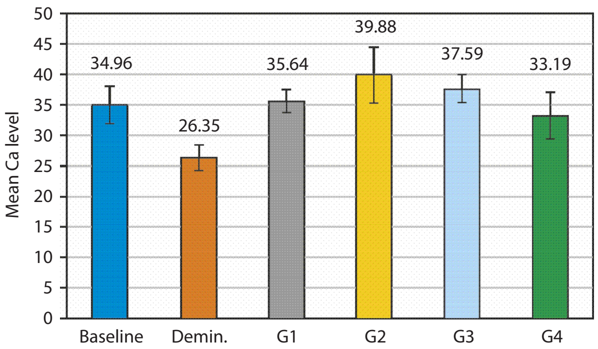

FIGURE 4. Comparison of mean of calcium (Ca) in weight percentage for all groups. Demin. – after de-mineralization; G1 – ZnCO$_3$/n-Hap; G2 – Pro-Argin; G3 – fluoride; G4 – artificial saliva

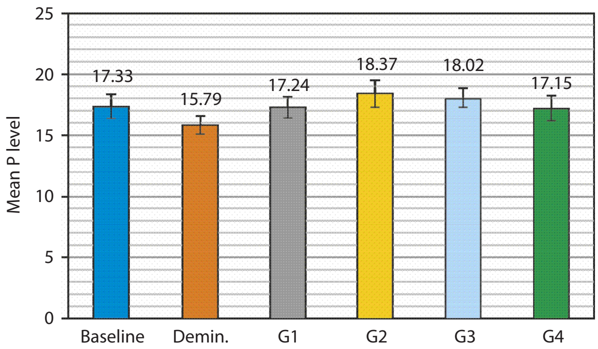

FIGURE 5. Comparison of mean of phosphorus (P) in weight percentage for all groups. Demin. – after de-mineralization; G1 – ZnCO$_3$/n-Hap; G2 – Pro-Argin; G3 – fluoride; G4 – artificial saliva

containing toothpaste. To ensure consistency, all fluoride-containing toothpastes used in the study had a standardized 1450 ppm of sodium mono-fluorophosphate. Although the tested toothpastes employ diverse mechanisms of action, the study outcomes revealed a common feature: all three toothpastes effectively promoted enamel re-mineralization by providing ions that facilitated mineral gain and treated de-mineralized enamel surface.

A thorough analysis was performed to compare the Ca/P ratio values among all groups, including the three experimental toothpastes, both in relation to each other and in comparison with the negative control group as well as baseline values of sound enamel. The findings showed no statistically significant differences among the toothpastes, indicating their equal ability to promote enamel re-mineralization within a short 7-day period.

However, the results demonstrated a significant difference between each experimental toothpaste group and the negative control group (artificial saliva). It was clear from comparing the results at the baseline and post-treatment points that the G2 toothpaste demonstrated a notable and statistically significant difference from sound enamel, suggesting a distinctive and unique impact on the enamel. On the other hand, neither G1 nor G3 samples displayed Ca/P ratio values that were significantly different from those seen in sound enamel.

In the current study, there was no significant difference in Ca/P ratios between zinc-carbonate hydroxyapatite toothpaste and fluoride toothpaste after a 7-day pH-cycling period. These results clearly establish the zinc-carbonate n-HAp toothpaste as non-inferior to the fluoride toothpaste. In fact, the Ca/P ratios for the zinc-carbonate hydroxyapatite toothpaste were slightly higher, indicating potential benefits over fluorides.

Evidence from an earlier study has shown that zinc-carbonate hydroxyapatite nano-particles present in a toothpaste have the ability to actively promote re-mineralization. Their penetration into enamel pores created during the progression of caries and the subsequent filling of these empty spaces help achieving re-mineralization. As a result, they act as anchor points for further mineral deposition, leading to elevated mineral content [26].

Different concentrations of n-HAp have been investigated previously, ranging from 1% to 20% [27]. In this current investigation, Biorepair toothpaste with a high concentration of 20 wt% zinc-carbonate n-HAp was employed.

A prior study by Huang *et al.* [28] demonstrated substantial re-mineralization effects with a 15% n-HAp concentration, whereas lower concentrations showed reduced efficacy. However, it was reported that nano-hydroxyapatite concentrations exceeding 15% did not provide additional re-mineralization benefits, and could result in particle buildup on the enamel [28]. In response to this concern, zinc is often incorporated into such toothpaste formulations due to its ability to inhibit calculus formation and crystallization. This deliberate inclusion of zinc enabled Biorepair toothpaste to achieve even higher concentrations of hydroxyapatite, reaching up to 20 wt% [29].

Despite the lack of in-vitro studies directly comparing zinc-carbonate hydroxyapatite toothpaste with fluoride toothpastes for re-mineralizing dental caries using EDX, other in-vitro research has explored this comparison using different evaluation methods [30]. The current study is in agreement with previous research by Kamath *et al.* [31] and Thimmaiah *et al.* [9] who examined the re-mineralizing efficacy of bio-mimetic hydroxyapatite after artificial de-mineralization. Findings from these studies indicated that toothpaste containing n-HAp exhibited a comparable re-mineralizing effect with fluoride-containing toothpastes. Consequently, the researchers proposed n-Hap-containing dentifrice as a promising and effective substitute for fluoride toothpaste.

It is important to emphasize the crucial role of nano-hydroxyapatite (n-HAp) particle size in the mineralization process. The natural enamel structure consists of HAp nano-particles ranging between 20 and 40 nm. Consequently, there is a suggestion that nano-particles around the size of 20 nm demonstrate notable proficiency in repairing damaged enamel [32]. The selection of toothpaste containing 20 nm nano-hydroxyapatite (n-HAp) particles in this study associates perfectly with dimensions considered optimal for effectively promoting enamel re-mineralization.

Compared with the current findings, a previous study reported contrasting results, indicating that nano-hydroxyapatite toothpaste, even in combination with fluoride, did not effectively promote re-mineralization of caries lesions. This disparity could potentially be attributed to the larger size of nano-hydroxyapatite particles employed in that study (measuring 100 nm), which exceeded the maximum size allowed for nano-materials [33].

Several studies have also produced contradictory findings regarding the effectiveness of zinc-carbonate nano-hydroxyapatite toothpaste in re-mineralization of the enamel. Contrary to the current study that used pH-cycling to simulate actual brushing conditions in clinical practice, a recent investigation with a constant re-mineralization model involving 3-minute brushing sessions repeated three times daily for 15 days, failed to enhance enamel re-mineralization as expected [34].

Furthermore, Nasution and Gani [35] reported conflicting results, potentially due to distinctive methodologies that involved immersing the specimens in various solutions and subsequently subjecting them to centrifugation. The variations in both methodologies might have impacted the overall findings of their studies. Despite these variations in results, the reliability and safety of hydroxyapatite-based toothpaste, including zinc-carbonate formulations, continue to stand out for re-mineralization and caries prevention. Numerous studies findings not only position it on par with fluoride toothpaste, but also suggest its superiority, surpassing the performance of fluoride toothpaste [26, 36].

This study found that among the tested toothpastes, Colgate® Sensitive Pro-Relief™ with Pro-Argin™ technology exhibited the highest Ca/P ratio. Originally formulated to address dentin hypersensitivity [37], this toothpaste has demonstrated potential for both preventing caries and supporting re-mineralization [15]. Its mechanism involves forming a protective layer on the dentin/enamel surface, effectively shielding it from acidic triggers, concealing imperfections, and sealing dentinal tubules to alleviate sensitivity [38].

This toothpaste adheres to the principle of organic-to-inorganic interactions seen in tissue mineralization (bio-mimetic approaches) by containing key elements, such as 8% arginine, a naturally occurring amino acid found in saliva, and insoluble calcium in the form of calcium carbonate. It is believed that positively charged amino acids, such as arginine, serve as an organic nucleus for mineralization by directly interacting with hydroxyl groups in hydroxyapatites via electrostatic forces and hydrogen bonding [39]. Charged amino acids in collagen also bind Ca^{2+} and PO_4^{3-} ions, leading to crystal formation. Furthermore, the combination of arginine and calcium carbonate creates an alkaline environment, promoting the deposition of natural calcium and phosphate ions [40].

The findings of the present study are in alignment with the outcomes of Yu *et al.* [41] and Nanwal *et al.* [42]. Both studies highlighted that the combination of 8% arginine and fluoride within a calcium carbonate base yields enhanced re-mineralization effects. Nanwal *et al.* [42] conducted an investigation on the impact of various agents, including n-HAp particles, NovaMin, Pro-Argin, and calcium sucrose phosphate, on the process of re-mineralization. EDX analysis revealed that all tested re-mineralizing agents effectively restored the mineral content lost due to inter-proximal stripping. Notably, Pro-Argin and n-HAp did not differ statistically from one another.

A study conducted by Chandru *et al.* [26] corroborates the current findings, as it also demonstrated that Colgate® Sensitive Plus toothpaste with Pro-Argin™ formula exhibited the highest re-mineralization effect on enamel blocks, followed by Biorepair toothpaste. Interestingly, there were no significant differences in enamel re-mineralization between Colgate® Sensitive Plus and Biorepair toothpastes on the 6th, 9th, and 12th days of pH-cycling. On the other hand, in their study, Regenerate Enamel Science® toothpaste containing 1450 ppm F- of sodium mono-fluorophosphate showed minimal re-mineralization potential. These results strongly suggest that Colgate® Sensitive Plus toothpaste with Pro-Argin™ formula holds significant promise as an effective re-mineralizing agent and a non-invasive approach for managing early enamel carious lesions.

In a study by Gandolfi *et al.* [39] comparing the efficiency of toothpaste containing 8% arginine and calcium carbonate with zinc-carbonate hydroxyapatite in promoting re-mineralization of de-mineralized dentin, the former showed a significant amount of adhesion to dentin, and had the highest Ca/P ratio after treatment. The toothpaste containing zinc-carbonate hydroxy-apatite may have less noticeable effectiveness because of its higher concentration (30%) and relatively larger size (range, 20-200 nm) of nano-hydroxyapatite particles. These factors are presumably major contributors to the study's observed results.

It is worth noting that Bijle *et al.* [21] reported conflicting results, emphasizing the necessity for careful assessment. Their research indicated that the incorporation of 4% and 8% arginine (Arg) into sodium fluoride (NaF) toothpastes did not notably enhance enamel re-mineralization. Furthermore, the median Ca/P ratio of solutions containing additives as well as NaF toothpastes alone, did not show significant differences when compared with the de-ionized water group. However, they concluded that inclusion of 2% arginine in NaF toothpaste demonstrated a remarkable enhancement in enamel re-mineralization for caries-like lesions compared with NaF toothpaste alone. The limited success in promoting re-mineralization observed for 8% Arg-NaF in their study could be attributed to their emphasis on the combined effect of a single arginine variant, namely L-arginine mono-hydrochloride with NaF toothpaste. This specific variant differs from the arginine formulation utilized in the present study, potentially influencing the mechanism of action, pH of toothpaste mixture, and availability of free fluoride. These variations might have had an impact on the outcomes obtained.

The current study outcomes indicated that even though the artificial saliva (negative control) exhibited significantly lower Ca/P ratio values compared with the toothpastes, it still managed to trigger a degree of re-mineralization in the initial caries lesion. This observation aligns with earlier literature on well-documented re-mineralization of white spot lesions through immersion in super-saturated calcium and phosphate solutions [9, 43]. This outcome could be explained by the study routine of replenishing the artificial saliva every 24 hours throughout PH cycle, which ensured ionic equilibrium and preserved a neutral pH of 7.

As a result, the artificial saliva employed a profile more resembling natural saliva of individuals at lower risk for developing caries. This natural saliva tends to have a higher resting pH and greater super-saturation with calcium and phosphate ions, thus enhancing the potential for re-mineralization compared with those with lower resting pH values [1]. However, it is important to note that the present study findings are somewhat restricted in comparison with earlier studies, due to the absence of a negative control group (artificial saliva) in many of those investigations.

While the current study offers valuable insights, it is important to acknowledge its limitations. The utilization of in-vitro methodologies may not entirely replicate the intricate oral environment and personalized responses seen in-vivo. Additionally, the relatively short duration might not capture the complete spectrum of long-term effects of the tested toothpastes. Although the sample size was appropriate for analysis, larger sample groups could enhance statistical robustness. Moreover, the diverse formulations of commercial toothpastes could impact the direct comparability of the tested products with those available on the market. Further investigations are needed to validate the clinical significance of the findings, and provide a more realistic evaluation of the tested toothpastes' re-mineralization potential within the dynamic oral environment.

Nonetheless, the null hypothesis was confidently accepted, supporting the idea that all three toothpaste formulations were successful in promoting re-mineralization with comparable effectiveness.

CONCLUSIONS

Based on the findings from EDX analysis, this study indicates that all the tested experimental toothpastes exhibited the capability to promote re-mineralization of initial enamel lesions, with no evidence of superiority of one over the others. Notably, among the various re-mineralizing toothpastes, the most substantial enhancement in mineral content was observed with Colgate® Sensitive Pro-Relief™ containing 8.0% arginine and calcium carbonate (Pro-Argin™ technology). This was followed by Biorepair containing zinc-carbonate nano-hydroxyapatite and Signal toothpaste with sodium mono-fluorophosphate, although the differences were non-significant.

DISCLOSURES

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