ORIGINAL RESEARCH

Assessment of the Remineralizing Efficacy of Grape Seed Extract vs Sodium Fluoride on Surface and Subsurface Enamel Lesions: An *In Vitro* Study

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Abstract

Aim: The aim of this study was to evaluate the efficacy of grape seed extract (GSE) on remineralization of surface and subsurface enamel lesions compared to that of sodium fluoride (NaF).

Materials and methods: A total of 20 intact bovine incisor crowns were separated from their roots and immersed in a demineralizing solution for 96 hours at 37°C to create artificial enamel lesions. The specimens were randomly divided into two groups (n = 10): 6.5% GSE solution and 1000 ppm NaF solution. The specimens were subjected to six daily pH cycles for 8 days. The microhardness test was carried out at three different stages: baseline, after artificial caries formation, and after pH cycling. Raman spectroscopy was used to evaluate the depth of enamel remineralization. Surface morphology and elemental analysis were assessed using a scanning electron microscope (SEM) and an energy dispersive X-ray (EDX) spectroscope, respectively. Statistical analysis was performed using SPSS 22.0 at a significance level of $p \leq 0.05$.

Results: There was a significant increase in the mean values of enamel surface microhardness after pH cycles in the two groups compared to after artificial caries formation, but there was no significant difference between both groups. The B-type carbonate/phosphate (Ca/P) ratio at 10 and 40 µm depth revealed no significant difference between the two groups. Scanning electron microscope micrograph revealed occlusion of porosities and particle precipitation on the enamel surface of the two groups, while EDX results for the Ca/P ratio of the GSE and NaF groups were 1.59 and 1.60, respectively.

Conclusion: Grape seed extract and NaF are equally effective in remineralizing surface and subsurface artificial enamel lesions.

Clinical significance: Grape seed extract can be considered a promising herbal material and a safe alternative to traditional NaF for the noninvasive treatment of enamel lesions.

Keywords: Enamel remineralization, Grape seed extract, Microhardness, pH cycling model, Raman spectroscopy, Sodium fluoride.

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Introduction

Dental caries is one of the most widespread human diseases and is considered as a prime cause of oral pain and tooth loss.[1] It occurs as a result of altering the balance between demineralization and remineralization processes.[2] The demineralization process begins at and below the enamel surface and is caused by acid attacks that diffuse into the tooth structure, leading to the dissolution of calcium (Ca^{2+}) and phosphate (PO_4^{3-}) ions into the surrounding aqueous phase between the crystals. The remineralization process is a natural repair process for non-cavitated carious lesions by restoring lost mineral ions to the hydroxyapatite crystals.[3]

Fluoride is the most commonly used remineralizing agent with an evidence-based anticariogenic effect.[4] On the contrary, it suffers from a major drawback as the remineralization efficacy of fluoride is restricted only to the outer 30 µm layer of the tooth surface; fluoride becomes less effective below a pH of about 4.5; fluoride is highly effective on smooth surface caries, but its effect is limited to pit and fissure caries.[5] Moreover, fluoride has a limited ability to penetrate the biofilm layer on the tooth surface.[6] Finally, the effects of fluoride are dose-dependent, and their effects increase with an increased dose, which can cause fluorosis and toxicity.[7]

Nowadays, there is a tremendous shift toward the replacement of chemical ingredients with natural ones. Natural products have been used in medicine for thousands of years and are thought to be promising sources of novel therapeutic agents, including in oral diseases.[8] Grapes are one of the most consumed fruits in the world, and their seeds are highly rich in polyphenols. Grape seed extract is a crude mixture of several phenolic compounds obtained by the chemical processing of grape seed for different applications. Although there are various types of phenolic compounds in chemistry science, GSEs contain only two types: flavonoids and phenolic acids.[9]

In dentistry, GSE has been used in different fields, including conservative dentistry for bonding of restorations; endodontic dentistry as an endodontic irrigating solution; treatment of periodontal diseases; and treatment of oral cancer. In preventive dentistry, GSEs are used to prevent dental caries by acting on the different stages of dental caries formation.[9] Firstly, as antiplaque agents that prevent biofilm adhesion;[10] secondly, as antibacterial

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agents against cariogenic bacteria;[11] thirdly, as bio-modifier agents that prevent collagen matrix degradation;[12] and fourthly, as remineralizing agents that enhance the natural remineralization process.[13,14]

Several studies have evaluated the efficacy of GSE for remineralization of dentin and cementum lesions. However, there is a deficiency in evaluating its efficacy for remineralizing surface and subsurface enamel lesions. Therefore, the aim of this study was to evaluate the efficacy of GSE on the remineralization of enamel compared to the effect of NaF using the microhardness test. Moreover, Raman spectroscopy was used to evaluate the depth of enamel remineralization. The null hypothesis stated that there is neither difference in enamel surface microhardness nor in depth of enamel remineralization between the GSE and NaF.

Materials and Methods

This study was conducted in the Biomaterials Department, Faculty of Dentistry, Cairo University, Egypt. The study period was from November 1, 2021 to October 1, 2022.

Preparation and Characterization of the GSE Solution

The solution was prepared in the Analytical Chemistry Department, Faculty of Pharmacy, Cairo University. A 6.5% of GSE solution was prepared by dissolving 6.5 gm of GSE powder (Puritans Pride Inc., Oakdale, NY, USA) in 100 mL of distilled water.[15]

Determination of Total Phenol Content

The total phenolic content was determined using the Folin–Ciocalteu method.[16] In a test tube 1 mL of GSE solution was transferred and mixed with 1 mL of Folin-Ciocalteu reagent. After manually mixing for 3 minutes, 1 mL of 2% sodium carbonate was added. The mixture was agitated with a vortex mixer and kept in the dark for 30 minutes, after which it was centrifuged at 12000 gram-force for 5 minutes. The absorbance of the extracts was measured at 750 nm using a spectrophotometer. The results were compared to the standard curve of the prepared gallic acid (GA) solution.[17] The samples were analyzed in triplicate, and the total phenolic content was calculated using the following formula:[18] $C = C1 \times V/m$, where C is the total phenolic content expressed as milligrams of gallic acid equivalents (GAEs) per gram of extract (mg GAE/1 gm GSE), C1 is the concentration of GA determined by the calibration curve in mg/mL, V is the volume of extract in mL, and m is the extract weight in gram.

Determination of Total Proanthocyanidins (PA) Content

The total amount of PA in the GSE was determined according to the method of Sun et al.[19] The first step was the preparation of the standard solution. The grape seed powder was dissolved in methanol to obtain stock solutions of 120 mg/L. A dilution of the stock solution was made to establish standard curves. Second step, reaction of catechins with vanillin. About 2.5 mL of 1% vanillin solution and 1.5 mL of concentrated HCl were added to 50 mL of diluted stock solution. After allowing the mixture to stand for 15 minutes, the absorbance was measured at 500 nm. A calibration curve of catechin was prepared using the same analyzed sample. The total amount of PA was calculated from the calibration curve and expressed as milligrams of catechin per gram of the extract (mg CE/1 gm GSE). The samples were analyzed in triplicate, and the average quantity of PA was calculated.[20]

Sample Size Calculation

The sample size was calculated by comparing the microhardness of demineralized enamel treated with GSE solution to that treated with NaF solution. As reported in the previous publication by Rubel et al.,[21] the mean ± standard deviation (SD) of microhardness in the GSE group was approximately 357.6 ± 5.53, while in the NaF group it was approximately 336.4 ± 4.1. Accordingly, the minimum proper sample size was calculated to be four samples in each group in order to reject the null hypothesis with 80% power at $\alpha = 0.05$ level using the Student's *t* test for independent samples. Sample size calculation was carried out using PS Power and Sample Size Calculations software, version 3.0.11 for MS Windows (William D Dupont and Walton D, Vanderbilt University, Nashville, TN, USA).

Specimen Preparation

A total of 20 extracted bovine incisors were selected. The teeth were used after the approval of the Institutional Animal Care and Use Committee, Cairo University (No. CU-III-F-C-56-21). The gross debris on the tooth surfaces was removed with an ultrasonic scaler (Siro Sonic, Dentsply Sirona, Germany). The crowns were separated from their roots at the dentino-enamel junction using a diamond disc. The surfaces of the crowns were covered with an acid-resistant nail varnish (Revlon, NY, USA), except the buccal surface, to standardize the area of measurement. The crowns were fixed in an auto-polymerizing polymethyl methacrylate acrylic resin block (Acro Stone, Egypt). After which they were stored in sequentially numbered vials in 0.1% thymol solution.[22] Finally, the specimens were randomly assigned into two groups (10 specimens/group) using the random.org site. The two groups were 6.5% GSE solution group and 1000 ppm NaF solution group.

Preparation of Artificial Carious Lesion

The specimens were thoroughly rinsed with deionized water to be cleaned from the thymol solution. Afterwards, the specimens were immersed separately in numbered glass tubes containing 20 mL of demineralizing solution (2.2 mM $CaCl_2$ $2H_2O$, 2.2 mM KH_2PO_2, and 50 mM CH_3COOH with a pH of 4.6) for 96 hours at 37°C.[23]

pH Cycling

The demineralized specimens were then subjected to a pH-cycling model.[24] The specimens in each group were immersed individually in 50 mL of their assigned remineralizing solution [6.5% GSE or 1000 ppm NaF (Merck, Sigma-Aldrich, USA)] for 10 minutes. The specimens were then washed thoroughly using distilled water. Afterwards, the specimens were immersed individually in 50 mL of demineralizing solution for 30 minutes, followed by washing thoroughly using distilled water. Finally, specimens were immersed individually in 50 mL of buffered solution (20 mM HEPES, 2.25 mM $CaCl_2 2H_2O$, 1.35 mM KH_2PO_4, and 130 mM KCl with a pH of 7)[25] for 10 minutes, followed by washing with distilled water. These pH cycles were performed six times a day for 8 days. Between the daily cycling, all the solutions were replenished and the specimens were stored individually in 50 mL of an artificial saliva solution (3.90 mM Na_3PO_4, 4.29 mM NaCl, 17.98 mM KCl, 1.10 mM $CaCl_2$, 0.08 mM $MgCl_2$, 0.50 mM H_2SO_4, 3.27 mM $NaHCO_3$, and 2.87 M distilled water with a pH of 7.2)[26] for 12 hours at 37°C using an incubator (BTC, Egypt).[27,28]

Microhardness Test

Testing of enamel surface microhardness using a Vickers microhardness tester (Tukon™ 1102, Wilson® Micro-Hardness Tester,

Buehler, Germany) was carried out by a blinded assessor at each of the following stages: baseline, after artificial caries formation, and after the pH cycling. In the Vickers test, a 50 gm load was applied smoothly, forcing the indenter into the test specimen. The indenter was held in place for 10 seconds.[29] After the load was removed, the indentation was focused with the magnifying eyepiece, and the two impression diagonals were measured and averaged with a filar micrometer, usually to the nearest 0.1 µm. Vickers hardness number was calculated using the following equation:[30] VHN = 1854.4 L/d^2, where VHN is the Vickers hardness number in gf/µm^2, L is the applied load in gf, and d is the average diagonal length of indentation in µm. Three VHN were recorded for each specimen, and the average value was calculated and recorded.

Depth of Enamel Remineralization

Testing the depth of enamel remineralization using Raman spectroscopy (Lab Ram HR Evolution, Coriba, France) was carried out by a blinded assessor. It was performed on the same-numbered specimens used for the microhardness test after the pH cycling. The specimens were cut in half through the treated area along the direction of the tooth axis using a linear precision saw (IsoMet 4,000, Buehler, Germany). Raman spectroscopy was used to analyze the specimens with a 780 nm laser and a grating of 360 lines per mm. Single points across the cut surfaces of the specimens were measured at 10 and 40 µm from the surface in the direction of the dentino-enamel junction, using an aperture of 100 µm. The measurements at 10 µm were defined as surface areas, and those at 40 µm were defined as subsurface areas.[31]

The heights of phosphate (960 cm^{-1}) and B-type carbonate substitution (1070 cm^{-1}) peaks were measured for each specimen at 10 and 40 µm depth. The ratios of peak intensities of B-type carbonate substitution to phosphate (1070 cm^{-1}/960 cm^{-1}) were calculated and recorded in order to detect variations in carbonate content at 10 and 40 µm of enamel depth in each specimen.[31] Furthermore, Raman spectroscopy provided phosphate maps in form of images. These images were analyzed using an indicative look-up table (LUT), where the yellow hues indicate the highest phosphate intensity, while the dark brown hues indicate the lowest phosphate intensity.[32]

Surface Morphology and Elemental Analysis

Evaluation of the surface morphology using SEM (JSM-IT Schottky Field Emission Scanning Electron Microscope, JEOL, Japan) was carried out by a blinded assessor after the pH cycling. A representative specimen from each group was sputter coated with gold for SEM to evaluate the surface morphology of specimens. The images were captured at a constant magnification of 500× with a 100 µm scale bar from the central portion of each specimen. The spectrometer attached to the SEM was used to determine the chemical elements in the specimens. The spectrum was obtained at 15 Kv. The calcium/phosphorus ratio was calculated by dividing their mass percentages.[33]

Statistical Analysis

The statistical analysis was performed using IBM SPSS Statistics for Windows, Version 23.0 (Armonk, NY: IBM Corp). Numerical data were explored for normality by checking the distribution of data and using tests of normality (Kolmogorov–Smirnov and Shapiro–Wilk tests). All data showed a normal (parametric) distribution. The data were presented as mean and SD values. The repeated measures analysis of variance (ANOVA) test was used to study the effect of two treatment solutions on mean enamel surface microhardness values. The Bonferroni's *post hoc* test was used for pair-wise comparisons when the ANOVA test was significant. The Student's *t*-test was used to compare between different depths of enamel remineralization after application of two treatment solutions. The significance level was set at $p \leq 0.05$.

Results

Characterization of GSE

The average quantity of three measurements for the total phenol content of GSE was found to be 267.21 mg GAE/1 gm GSE, while the average quantity of three measurements for PA content of the GSE was found to be 24.56 mg CE/1 gm GSE.

Microhardness Analysis

In the comparison of enamel surface microhardness between two groups, the repeated measures ANOVA test revealed that there was no statistically significant difference in the mean values between both groups at different stages. The mean values of enamel surface microhardness for the GSE and NaF at baseline were 267.1 ± 21.7 and 269.8 ± 21.5 VHN, respectively; after artificial caries formation, they were 75.6 ± 25.5 and 71 ± 26.6 VHN, respectively; and after the pH cycling, they were 103.1 ± 27.2 and 110.5 ± 30.9 VHN, respectively (Table 1). In comparison of enamel surface microhardness between different stages; there was a statistically significant difference ($p < 0.001$) in the mean values between different stages within each group. Pair-wise comparisons between stages revealed a statistically significant decrease ($p < 0.001$) in mean values of enamel surface microhardness after artificial caries formation, followed by a statistically significant increase ($p < 0.001$) after pH cycling. However, the mean values of enamel surface microhardness after the pH cycling showed a statistically significantly lower value ($p < 0.001$) compared with the base line (Table 1).

Raman Data Analysis

The ratios of peak intensities of B-type carbonate substitution to phosphate at 10 and 40 µm depths; a Student's *t*-test revealed that

Table 1: The mean, SD values, and results of repeated measures ANOVA test for enamel surface microhardness

	GSE		*NaF*		*p-value*
Stages	*Mean*	*SD*	*Mean*	*SD*	*(between groups)*
Base line	267.1^{A}	21.7	269.8^{A}	21.5	0.779
After caries formation	75.6^{C}	25.5	71^{C}	26.6	0.693
After pH cycling	103.1^{B}	27.2	110.5^{B}	30.9	0.573
p-value (between stages)	<0.001*		<0.001*		

*Significant at $p \leq 0.05$, uppercase superscripts letters (A, B, C) in the same column indicate the presence of significant differences between stages

there was no statistically significant difference in the mean values of the B-type carbonate/phosphate (Ca/P) ratio between the GSE and NaF groups at both depths (Table 2).

As shown in Figure 1, the Raman spectra of the enamel treated with GSE and NaF solutions revealed different peaks in the 100–2000 cm^{-1} range at 10 and 40 µm depth. Analyses of spectra showed that the peak heights of phosphate (960 cm^{-1}) and B-type carbonate (1070 cm^{-1}) in the GSE group were equivalent to those of the NaF group at 10 µm depth (Fig. 1A), and slightly higher than those of the NaF group at 40 µm depth (Fig. 1B).

As shown in Figure 2, phosphate maps of enamel showed that there was a comparable high phosphate intensity (yellow hues) and a low phosphate intensity (dark brown hues) in most regions of enamel between the GSE (Fig. 2A) and NaF (Fig. 2B) groups.

Table 2: The mean, SD values, and results of the Student's *t*-test for the B-type Ca/P ratio

	GSE		*NaF*		
Depth	*Mean*	*SD*	*Mean*	*SD*	*p-value*
At 10 µm	0.444	0.188	0.391	0.023	0.382
At 40 µm	0.401	0.193	0.384	0.014	0.787

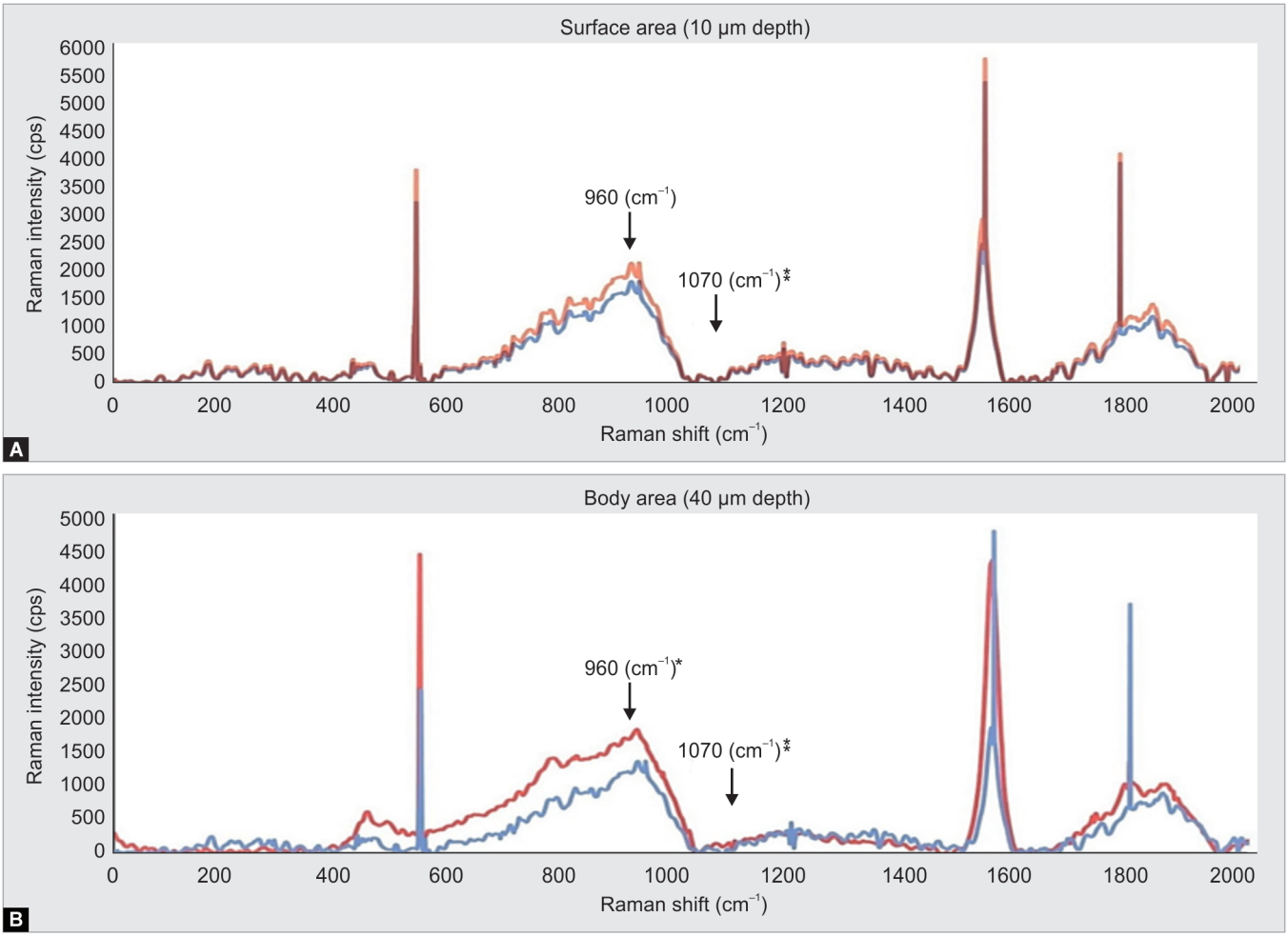
SEM Analysis

The SEM micrographs of the enamel surface for the GSE and NaF groups after pH cycling are shown in Figure 3. The SEM micrograph for the GSE group showed a smooth appearance with the occlusion of most porosities and the precipitation of particles of different sizes and shapes (yellow arrow), whereas the SEM micrograph for the NaF group showed a slightly rough appearance with the occlusion of porosity and the precipitation of numerous deposits, some of which were aggregated to form clumps (yellow arrow).

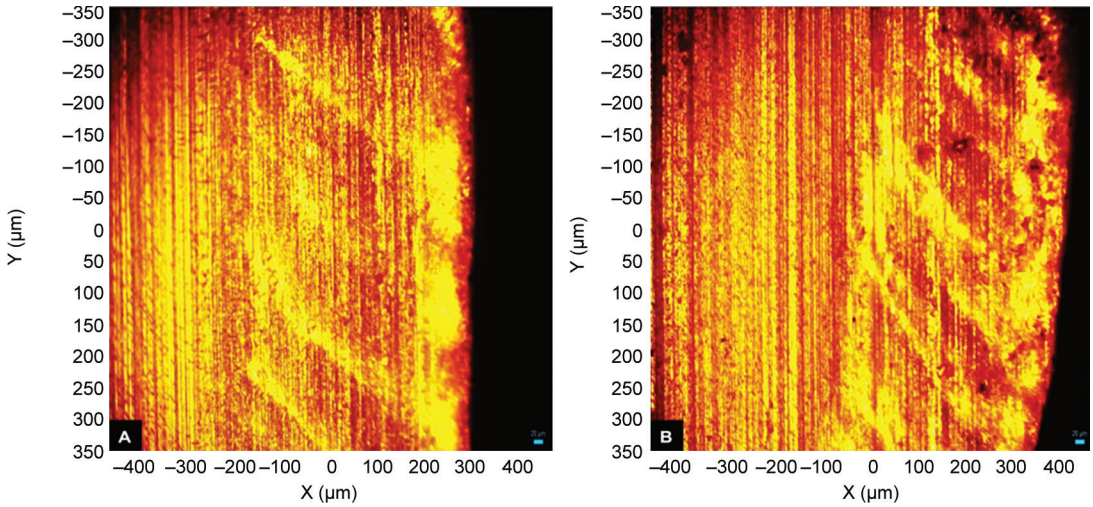
EDX Analysis

The EDX spectrums of the enamel surface for GSE and NaF groups after the pH cycling are shown in Figure 4. Energy dispersive X-ray elemental analysis for the GSE group showed the presence of calcium (40.99 wt.%), phosphorus (25.67 wt.%), carbon (16.46 wt.%), oxygen (16.83 wt.%), and fluoride (0.05 wt.%) with a Ca/P ratio of 1.59, while the EDX elemental analysis for the NaF group showed the presence of calcium (45.01 wt.%), phosphorus (28.00 wt.%), carbon (11.20 wt.%), oxygen (15.70 wt.%), and fluoride (0.08 wt.%) with a Ca/P ratio of 1.60.

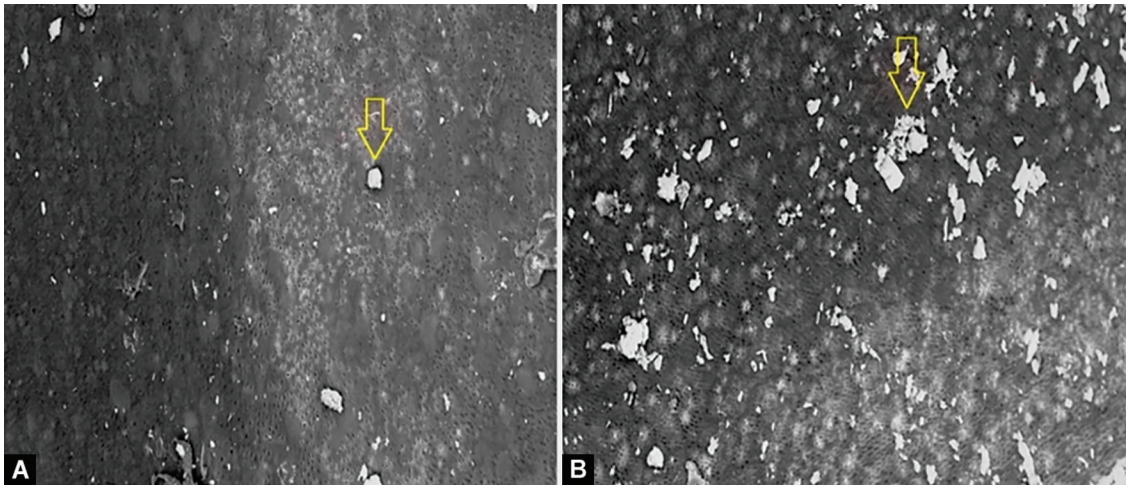
The results of the present study revealed that there was no difference between GSE and NaF regarding their remineralization



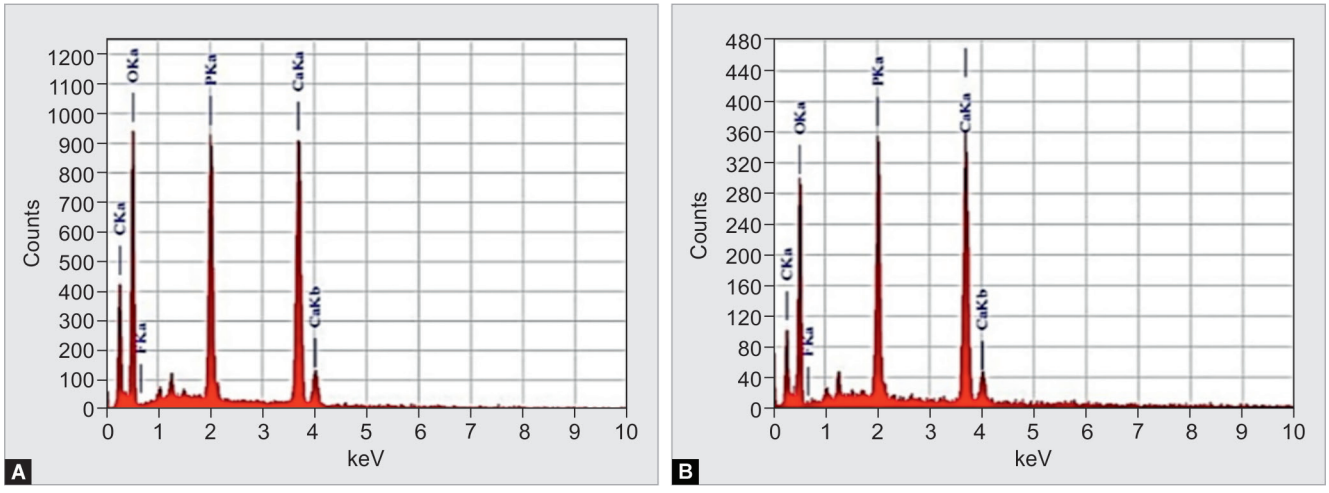
Figs 1A and B: Raman spectra of enamel treated with GSE (red line) and NaF (blue line): (A) Surface area (10 µm depth), (B) Body area (40 µm depth). *Phosphate peak heights; **B-type carbonate peak heights



Figs 2A and B: Phosphate maps of enamel after pH cycling: (A) GSE, (B) NaF. Yellow hues indicate high phosphate intensity, while dark brown hues indicate low phosphate intensity



Figs 3A and B: Scanning electron microscope micrograph of the enamel surface after pH cycling at a 500× magnification: (A) GSE, (B) NaF. Precipitation of particles on the enamel surface (yellow arrow)



Figs 4A and B: Energy dispersive X-ray spectrums of the enamel surface after pH cycling: (A) GSE, (B) NaF

efficacy and depth of remineralization of enamel. Therefore, the null hypothesis of the present study was accepted.

Discussion

Dental caries is a continuous process that progresses slowly and can be stopped in its early stages, but if not treated properly, it will proceed until the tooth is destroyed.[1] Remineralization therapy is the most common way to intervene in this continuing process.[34] Although many types of remineralizing agents have been studied and introduced into the market with generally promising outcomes. However, some of these agents encountered a number of drawbacks that limited their uses.[5] Therefore, novel materials are still required to overcome these drawbacks. Regarding this concept, natural materials may be considered the best choice for their safety, efficacy, availability, shelf life, and low cost.[35]

Interestingly, it has been proven that GSE is a promising material as a bio-modifier, antiplaque agent, anti-bacterial agent, and remineralizing agent for the prevention of dental caries, especially in dentin and cementum.[9] Despite the variation in percentage, there are similarities in the type of organic content among enamel, dentin, and cementum,[3] which in turn could represent the essential component for enamel remineralization. Grape seed extract is a crude mixture of phenolic compounds, especially flavonoids and phenolic acid. Proanthocyanidins form most flavonoid compounds and represent biologically active constituents in GSE.[36] Grape seed extract (*Vitis vinifera*) used in this study contained 267.21 mg GAE/1 gm GSE, which is in agreement with the results documented by Ghouila et al.[37] The total PA content was 24.56 mg CE/1 gm GSE, which is comparable to the results documented by Gu et al.[38]

The application of treatment solutions was carried out through a pH-cycling model. This model is based on subjecting the specimen to alternating demineralization and remineralization periods to mimic the dynamics of mineral loss and uptake that occur during caries formation. A main advantage of the pH-cycling model is that it simulates *in vivo* high caries risk conditions and analyzes the net outcome of demineralization inhibition and remineralization enhancement simultaneously. In addition, it has a high level of scientific evidence, results in lower variability than *in vitro* models, and needs a smaller sample size.[24]

At the baseline stage, there was no significant difference in the mean value of enamel surface microhardness between the GSE and NaF groups. This is in agreement with the results of the study conducted by Al-Salehi et al., who showed that the mean values of enamel surface microhardness at baseline stage for bovine teeth ranged from 250 to 271 VHN.[39] After artificial caries formation stage, the mean value of enamel surface microhardness significantly decreased in both groups compared to the baseline stage. This is mainly attributed to the dissolution of hydroxyapatite crystals in enamel by the loss of mineral ions.[3] Furthermore, at this stage, there was no significant difference in the mean value of enamel surface microhardness between the two groups. This is attributed to the standardized procedure of the artificial caries formation for all specimens in terms of composition, pH level, amount, temperature, and duration of immersion in demineralizing solution.[40]

After application of GSE solution through pH cycling, the mean value of enamel surface microhardness significantly increased compared to that after artificial caries formation stage. This finding is in agreement with the findings of Da Silva et al., Xie et al., Amin et al., and Pavan et al.[22,23,29,41] This is attributed to the capacities of the PA and GA to induce remineralization processes through their participation in the formation of hydroxyapatite crystals, leading to an increase in the hardness of enamel surface. Proanthocyanidins interact with proteins (proline-rich proteins like collagen) to form proline–PA complexes, leading to an increase in collagen cross-links. In addition, the terminal carboxyl and amine groups of cross-linked collagens combine with surface ions of hydroxyapatite crystals, leading to the facilitation of absorption of collagen peptides onto hydroxyapatite surfaces, which results in stimulating the growth of hydroxyapatite.[42] Gallic acids interact with Ca^{2+} ions to form GA–Ca^{2+} complexes that regulate the size and morphology of the crystals, thereby improving the formation of hydroxyapatite crystals.[43]

This finding is confirmed by an analysis of the enamel surface using SEM images in the GSE group, where it demonstrated the occlusion of enamel surface porosities and the precipitation of particles covering the demineralized areas. This is in agreement with SEM results reported by Amin et al.[29] Moreover, the EDX results of the present study for the enamel surface of the GSE group showed that the Ca/P ratio is 1.59. This finding is extremely comparable to the 1.6 ratio, which is consistent with previous literature stating that the optimum rate of enamel remineralization can be achieved with a Ca/P ratio of 1.6 at equal degrees of supersaturation.[44]

These findings were further confirmed by Raman spectroscopy results, which indicate the formation of B-type carbonated hydroxyapatite and the incorporation of phosphate in the demineralized area, which reflects a high degree of crystallinity at 10 and 40 µm in the GSE group. This was in agreement with the results of a study conducted by Xie et al., who observed the formation of the precipitation band of remineralization at 40 µm depth.[23] This finding was also supported by Epasinghe et al. and Pavan et al., who both documented the capacity of GSE to produce subsurface precipitation bands of remineralization.[28,41]

The current study showed no significant difference in enamel surface microhardness between the GSE and NaF groups. These results are in agreement with the study conducted by Xie et al.[23] When the SEM images and EDX analysis of the enamel surface for the GSE and NaF groups were compared, they revealed an obvious similarity regarding the occlusion of porosities, precipitation of particles, and the mineral content between the two groups. This was in agreement with the SEM results reported by Amin et al.[29]

The current study revealed no significant difference in the depth of enamel remineralization between the GSE and NaF groups. These results were in contrast to those published by Xie et al.[23] and Benjamin et al.,[27] who stated that GSE formed a wider mineral precipitation band, indicating a higher depth of remineralization when compared to NaF. This may be attributed to the interaction of PA with the collagen to form a PA–collagen complex. This interaction was higher in the root fragments, which are composed mainly of dentin, and type I collagen accounts for 90% of the dentin organic matrix.[12] In contrast, the enamel used in the present study consists of small amounts of type I collagen and depends mainly on collagen type X for enamel remineralization.[45] Moreover, these results contrasted with those of Pavan et al.,[41] who found that the GSE forms a narrow mineral precipitation band, indicating a lower depth of remineralization when compared to NaF. This may be due to the differences in the duration of the treatment process. The pH cycle was unusually extended to 18 days by Pavan et al., but only 8 days of the pH cycle were used in the present study, as in most previous studies.[22,23,25,27,29]

Although the pH-cycling model is meant to mimic the dynamics of mineral loss and uptake that occur during demineralization and remineralization processes in the oral cavity. However, the major limitation of this study is that the complex intraoral environments cannot be fully replicated as this was an *in vitro* study. Therefore, preclinical and clinical trials are required to evaluate the efficacy of GSE as a remineralizing agent.

Conclusion

Grape seed extract and NaF are equally effective in remineralizing surface and subsurface enamel lesions. Grape seed extract can be considered a promising natural material for remineralization of dental enamel. Future *in vitro* studies for remineralized enamel treated with GSE are required to evaluate acid resistance acquisition and enamel color using spectrophotometric analysis approaches, respectively. Also, clinical studies analyzing the effect of GSE on dental caries are required.

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