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The Combined Effect of Two Natural Extracts on Enamel Remineralization (*in vitro* Study)

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Abstract

Objective: Dental caries is a chronic infectious disease that is prevalent worldwide in all age groups. Numerous attempts have been made to develop conservative approaches to halt caries progression and restore enamel defects. This study aimed to investigate the effect of applying grape seed extract and chicken eggshell extract on the microhardness of demineralized enamel in permanent teeth.

Methods: Forty-eight sound upper first premolars were used. Following demineralization with the demineralizing solution for 96 hours, they were distributed into four groups consistent with the treatment agent used: group A was treated with casein phosphopeptide amorphous calcium phosphate (as a control group), group B was treated with grape seed extract, group C was treated with chicken eggshell extract solution, and group D was treated with grape seed extract followed by chicken eggshell extract solution. Vickers microhardness measurements were performed on sound enamel, after demineralization and remineralization.

Results: Paired T-test, one-way ANOVA, and Tukey's HSD test were used for statistical analysis. Enamel microhardness was significantly reduced following demineralization (p=0.000) and significantly increased after remineralization, with group D showing the highest values (mean microhardness=218.99).

Conclusion: Grape seed extract and chicken eggshell extract solutions have a synergistic effect on enamel remineralization which was interpreted from the increase in surface microhardness values.

Keywords: Caseins, Eggshell, Enamel, Grape Seed Extract, Tooth Remineralization.

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Introduction

Dental caries is a dynamic process that is influenced by the balance of pathological and defensive mechanisms [1]. The remineralization of dental tissues is characterized by the re-deposition of minerals, particularly calcium, and phosphate, which are supplied by therapeutic agents into the gaps between hydroxyapatite crystals of demineralized enamel, resulting in mineral gain [2].

Casein phosphopeptide amorphous calcium phosphate (CPP-ACP) is a supplement for calcium and phosphate ions derived from milk protein [3]. The anti-cariogenic mechanisms have been suggested to keep the enamel minerals calcium and phosphate supersaturated in the dental plaque, thereby interrupting biofilm formation, hindering bacterial adhesion on the enamel surface, and buffering pH reduction in the oral environment [4].

Thousands of years ago, therapeutic agents were prepared from natural products that have been considered promising sources for new medicines, particularly in oral diseases, such as dental caries [5].

Chicken eggshell powder (CESP) has various medical applications owing to its elevated calcium concentration (approximately 93%) and increased bioavailability [6]. CESP can be used as a coating material in dental implants [7] and as a remineralizing agent against

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dental caries and enamel erosion with promising results [8-10].

Grape seed extract (GSE) showed a noticeable effect on the prevention and restoration of demineralized teeth by stabilizing the collagen matrix and inhibiting its breakdown. It has several beneficial properties, but high levels of proanthocyanidins are thought to be responsible for its role in caries prevention [11].

Previous studies used the two extracts separately for enamel remineralization. This study aimed to evaluate the combined effect of CESP and GSE on the microhardness of demineralized enamel.

The null hypothesis states that there is no synergistic effect between CESP and GSE in remineralization of enamel.

Material and Methods

Tooth Preparation

Upper first premolars extracted from humans were used, any teeth that had cracks or dental caries, as shown by a magnifying lens, were excluded. Rubber cup and non-fluoridated pumice were used to polish the samples, which were then stored in deionized water containing 0.1% thymol [12]. Each tooth was coated with nail varnish (acid-resistant), except for a window of 2 × 2 mm (in dimension) left in the middle of the buccal surface. These windows were flattened with Sof-Lex Disks (3M ESPE, USA) in an advanced manner [13].

Sample

The sample size was calculated using G power 3.0.10 program [14]. Alpha error of probability=0.05 two-sided was used and 95% was the power of the study [15]. The sample size was 48 teeth (each group consisted of 12 samples).

The samples were randomly distributed into four groups after demineralization. Treatment with different agents was conducted for 14 days.

Group A: Treatment with CPP-ACP cream (GC Tooth Mousse, GC Co, USA) for 3 min daily, then the cream was wiped off with a cotton piece [16] (as control).

Group B: Treatment with grape seed extract solution for 2 min daily [17].

Group C: Treatment with CESP solution for 5 min daily.

Group D: Treatment with grape seed extract solution for 2 min, then with CESP solution for 5 min.

Following the treatment procedure, the samples were washed with deionized water (2 min for each separately) daily and stored until the next day.

Preparation of Grape Seed Extract Solution

GSE powder (Bulk Supplements, USA) was dissolved in a phosphate buffer solution (0.025 M KH_2PO_4, pH=7.4) to obtain a concentration of 6.5% [18].

Preparation of CESP Solution

The calcination protocol of chicken eggshells was performed according to the World Property Intellectual Organization [19]. A gram of the resultant powder was dissolved with 20 ml of acetic acid (concentration =4%), and only the clear solution was used as a remineralizing agent [20].

Demineralizing Protocol

The following concentrations were used to prepare demineralizing solution: acetic acid (0.05 M), sodium dihydrogen orthophosphate dehydrate (2.2 mM), and calcium chloride (2.2 mM). The solution pH was 4.4 (modified by the addition of 1M potassium hydroxide). Each tooth was immersed separately for four consecutive days (96 h) to stimulate enamel demineralization

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[21]. The solution was changed every day. Finally, the samples were washed thoroughly and kept in deionized water.

Measurement of Enamel Microhardness

The microhardness was measured using a digital Vickers microhardness tester (Fischer Technology, Inc., USA) for sound enamel, following demineralization, and after treatment with the selected agent. The load was 500g for 30 sec. For each reading, the tooth was subjected to three indentations, and the average was calculated to denote its hardness value.

Statistical analysis

SPSS-26 program was used for analysis. One-way ANOVA, Paired T-test, and Tukey's HSD test were used for statistical analysis.

Results

Baseline surface microhardness (SMH) was not significantly different among the groups (p=0.11, F-value=2.14), as shown in Figure (1). Following demineralization, SMH significantly decreased in all samples (p=0.000). Table 1 describes the difference among the groups at the sound and demineralization stages. Following treatment with the remineralizing agents, enamel SMH significantly increased (p=0.000, F=203.87). SMH increased compared with that after demineralization (p=0.000) (Table 2). Tukey's test revealed that group D had the highest SMH value followed by group C (with a non-significant difference between them p=0.94) then groups A and B (Table 3).

Discussion

Dental tissue remineralization is the preferred preventive measure for dental caries [22]. Many studies have focused on the use of natural products for disease management [5,23]. The present study selected GSE and CESP because they are available and economical and have advantageous properties. Baseline SMH was not significantly different among the groups, indicating a relative approximation in the mechanical appropriates of the samples. Following demineralization, enamel microhardness significantly reduced. Initial enamel lesions have fewer minerals, resulting in a reduced microhardness value [8].

Following remineralization, enamel microhardness significantly increased in all samples. Group D showed the highest SMH indicating that the combination GSE and CESP had a synergistic effect on remineralizing the enamel surface. A possible explanation is that gallic acid, a major constituent of GSE [24], promotes the deposition of CESP minerals, primarily on the enamel surface. This is consistent with a preceding study which concluded that GSE could enhance enamel remineralization by increasing SMH [25].

Mirkarimi et al. (2013) reported the remineralizing property of GSE by forming insoluble deposits on enamel by using a scanning electron microscope and reported elevated microhardness value following demineralization [26]. Yassen and Safy (2018) compared GSE with sodium fluoride (NaF) by VMH and found a non-significant difference between them in dentin remineralization [27]. Amin et al. (2019) found that GSE could increase SMH but the value is lower than that achieved by NaF [28]. The current study demonstrated the GSE remineralizing efficacy as evident by a significant increase in SMH of group B following demineralization. Furthermore, CESP has a high calcium concentration and elevated pH [29], causing a thorough blockage of surface pores and a net increase in enamel

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microhardness[8]. Preceding studies stated that CESP could increase SMH [9] and decrease the surface roughness of enamel affected by erosive lesion [20]. CESP also increases SMH and decreases lesion depth of demineralized enamel [10], in addition to increasing the Ca/P ratio and acid resistance of enamel [30]. This finding can clarify the increase in the enamel microhardness of both groups (D & C with a non-significant difference between them) following exposure to CEPS solution, and the value is higher than those of other groups. No previous study used the same combination to compare with this result.

CCP-ACP has remineralizing properties, which have been proven previously. The CPP/ACP paste treatment of the enamel surface results in filling the inter-prism voids and, to some extent, covering the enamel prisms with a layer-like structure [16], thereby increasing the SMH of group A samples.

The demineralization procedure used in the current study induced the loss of minerals from the subsurface layer along with preserving calcium and phosphate in the superficial layer [31]. VMH measurement is a quick, nondestructive method for the assessment of surface layer changes in demineralization and remineralization [32]. Flat and polished samples were used for standardization and elimination of naturally occurring variances between teeth that result in different reactions to acid dissolution [33].

Finally, consistent with the obtained outcomes, the null hypothesis was rejected, and the presence of a synergistic effect between GSE and CESP in enamel remineralization was confirmed.

Conclusion

GSE and CESP had a positive role in enamel remineralization. The combination improved the mineral gain and enhanced the mechanical properties of the enamel surface following demineralization, as shown by the increased SMH values. The findings of this study are limited because it was conducted in vitro. Future clinical studies should verify the outcome presented. Additionally, different application techniques, time intervals, and variables are recommended to investigate the combined effect of these extracts.

Ethical Approval

This study was approved by the Research Ethics Committee of the College of Dentistry, University of Baghdad (Reference number: 721, Date 28-12-2022).

Conflicts of interest

The authors declare no competing interest.

References

1. Obaid SF, Munther S, Abbas MH. Relationship between salivary levels of protein carbonyl and total antioxidant capacity and prevalence of dental caries among type 1 diabetic children: An analytical cross-sectional study. Dental Hypotheses. 2023;14(2):59. DOI: 10.4103/denthyp.denthyp_44_23.

2. Arifa MK, Ephraim R, Rajamani T. Recent advances in dental hard tissue remineralization: a review of literature. International Journal of Clinical Pediatric Dentistry. 2019;12(2):139. doi: 10.5005/jp-journals-10005-1603

3. Attar IJ, Ghaib NH. The effect of some remineralization materials and resin infiltration (ICON) on the depth of white spot lesions. J Bagh Coll Dentistry. 2018;30(2). https://doi.org/10.12816/0049755

4. Reema SD, Lahiri PK, Roy SS. Review of casein phosphopeptides-amorphous calcium phosphate. Chin J Dent Res. 2014;17(1):7-14. PMID: 25028684

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Vol 13 No 2 (2024) DOI 10.5195/d3000.2024.712

5. Turki OH, Jafar ZJ. Whitening effect of juglans regia dry husk extract on primary and permanent teeth. International Journal of Biomaterials. 2023;2023.

6. Park JW, Bae SR, Suh JY, Lee DH, Kim SH, Kim H, et al. Evaluation of bone healing with eggshell-derived bone graft substitutes in rat calvaria: A pilot study. Journal of Biomedical Materials Research Part A. 2008;87(1):203-14.

7. Kadhim DR, Hamad TI, Fatalla AA. Use of eggshells as bone grafts around commercially pure titanium implant screws coated with nano calcium sulfate. International Journal of Biomaterials. 2022;2022. https://doi.org/10.1155/2022/8722283

8. Haghgoo R, Mehran M, Ahmadvand M, Ahmadvand MJ. Remineralization effect of eggshell versus nano-hydroxyapatite on caries-like lesions in permanent teeth (in vitro). Journal of International Oral Health. 2016;8(4):435.

9. Yaberi M, Haghgoo R. A comparative study of the effect of nanohydroxyapatite and eggshell on erosive lesions of the enamel of permanent teeth following soft drink exposure: A randomized clinical trial. Journal of International Oral Health. 2018;10(4):176. DOI:10.4103/jioh.jioh_84_18

10. Mohamed EO, Sharaf AA, Talaat DM, Nagui DA. Remineralization effect of eggshell powder and novamine on initial caries-like lesions in young permanent teeth (in-vitro study). Alexandria Dental Journal. 2020;45(2):52-9.

11. Delimont NM, Carlson BN. Prevention of dental caries by grape seed extract supplementation: A systematic review. Nutrition and Health. 2020;26(1):43-52. DOI: 10.1177/0260106019887890

12. Hassan NM, Jafar ZJ, Abdul Latif MH. Nano-hydroxyapatite preparation for the remineralization of primary tooth enamel surface subjected to liquid medication: An observational study. Health Science Reports. 2023;6(4):e1188.

13. Yahya Y, Alrawi N. Remineralizing efficacy of biomimetic self-assembling peptide on artificially induced enamel lesions (in vitro study). J Res Med Dent Sci,. 2020;8 (7): 407-12.

14. Faul, F., Erdfelder, E., Buchner, A., & Lang, A.-G. Statistical power analyses using G*Power 3.1: Tests for correlation and regression analyses. Behavior Research Methods. 2009;41: 1149-1160.

15. Cohen j. Statistical power analysis for the behavioral sciences. 1988 (2nd ed) (Hillsdale, NJ:Erlbaum).

16. Poggio C, Lombardini M, Vigorelli P, Ceci M. Analysis of dentin/enamel remineralization by a CPP-ACP paste: AFM and SEM study. Scanning: The Journal of Scanning Microscopies. 2013;35(6):366-74.

17. Tang CF, Fang M, Liu RR, Dou Q, Chai ZG, Xiao YH, Chen JH. The role of grape seed extract in the remineralization of demineralized dentine: micromorphological and physical analyses. Archives of Oral Biology. 2013 Dec 1;58(12):1769-76. DOI: 10.1016/j.archoralbio.2013.09.007

18. Saragih D, Herda E, Triaminingsih S, editors. The influence of topical application of grapeseed extract gel on enamel surface hardness after demineralization. Journal of Physics: Conference Series; 2017: IOP Publishing.

19. Suzuki F, Wang P-Y., Weatherspoon J, Mead L. Method of producing eggshell powder. Patents: US 20060062857 A1. 2004.

20. Feroz S, Moeen F, Haq SN. Protective effect of chicken egg shell powder solution (CESP) on artificially induced dental erosion: An in vitro atomic force microscope study. International Journal of Dental Sciences and Research. 2017;5(3):49-55. DOI:10.12691/ijdsr-5-3-2

21. Itthagarun A, Verma S, Lalloo R, King N, Wefel J, Nair R. Effects of fluoridated milk on artificial enamel carious lesions: a pH cycling study. Journal of Dentistry. 2011;39(12):817-24.

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Vol 13 No 2 (2024) DOI 10.5195/d3000.2024.712

22. Leal SC, Dame-Teixeira N, Barbosa CdB, Kominami PAA, Raposo F, Nakagawa EMT, et al. Minimum intervention oral care: defining the future of caries management. Brazilian Oral Research. 2022;36. DOI: 10.1590/1807-3107bor-2022.vol36.0135.

23. Obeid RS, Khalaf MS. Impact of different types of storage media on enamel surface roughness and granularity distribution of avulsed teeth (in vitro study). J Bagh Coll Dentistry. 2022;34(4):34-43.

24. Cheng L, Li J, Hao Y, Zhou X. Effect of compounds of galla chinensis on remineralization of enamel surface in vitro. Archives of Oral Biology. 2010;55(6):435-40. DOI: 10.1016/j.archoralbio.2010.03.014.

25. Rubel M, Prashant GM, Naveen Kumar PG, Sushanth VH, Imranulla M, Potlia I, Mallick S. Effect of grape seed extract on remineralization of artificial caries: An in-vitro study. Asian J Pharm Clin Res. 2016;9(5):174-6.

26. Mirkarimi M, Eskandarion S, Bargrizan M, Delazar A, Kharazifard MJ. Remineralization of artificial caries in primary teeth by grape seed extract: An in vitro study 2013;7(4):206-10.

27. Yassen AA, Safy RK. Grape seed extract and dentin remineralization. Egyptian Dental Journal. 2018;64:1719-26.

28. Amin RA, Awad SM, Abd El Sattar EA. Evaluation of remineralization of grape seed extract versus sodium fluoride on demineralized primary anterior teeth: An in-vitro study. Egyptian Dental Journal. 2019;65: 1977-84.

29. Tangboriboon N, Kunanuruksapong R, Sirivat A. Preparation and properties of calcium oxide from eggshells via calcination. Materials Science-Poland. 2012;30(4):313-22. https://doi.org/10.2478/s13536-012-0055-7.

30. Elshik AE, Etman WM, Genaid TM. Resistance of chicken eggshell powder-treated demineralized enamel to further acidic challenges. Tanta Dental Journal. 2020;17(2):38.

31. Magalhães AC, Moron B, Comar L, Wiegand A, Buchalla W, Buzalaf MAR. Comparison of cross-sectional hardness and transverse microradiography of artificial carious enamel lesions induced by different demineralizing solutions and gels. Caries Research. 2009;43(6):474-83. doi: 10.1159/000264685

32. Ren Y-F, Zhao Q, Malmstrom H, Barnes V, Xu T. Assessing fluoride treatment and resistance of dental enamel to soft drink erosion in vitro: applications of focus variation 3D scanning microscopy and stylus profilometry. Journal of Dentistry. 2009;37(3):167-76.

33. Hemingway C, Parker D, Addy M, Barbour M. Erosion of enamel by non-carbonated soft drinks with and without toothbrushing abrasion. British Dental Journal. 2006;201(7):447-50.
PMID: 17031352.

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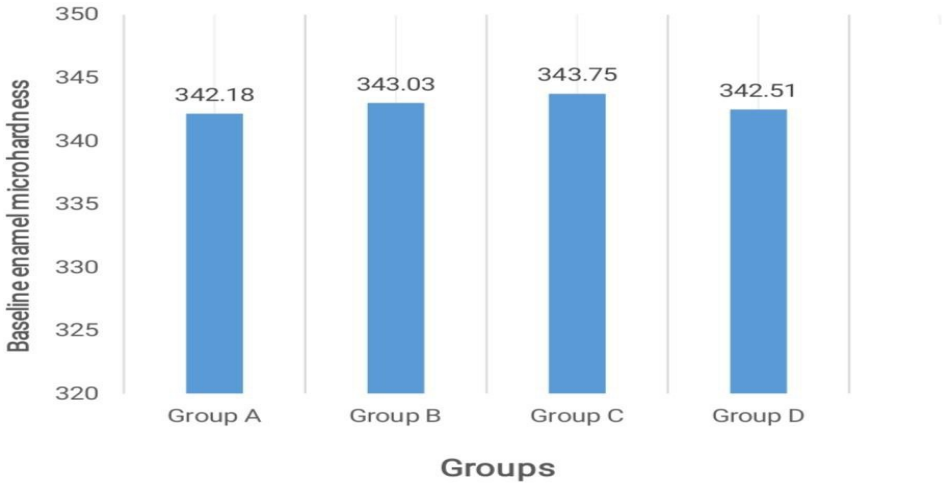

Figure 1. Baseline enamel microhardness.

Table 1. Microhardness at baseline and after demineralization.

Groups	Baseline Mean ± SD	After Demineralization Mean ± SD	Mean Difference ± SE	95% Confidence Interval of the Difference		t-Test	Correlation	P-Value
				Lower	Upper			
A	342.18 ± 0.54	175.62 ± 4.83	166.56 ± 1.42	163.43	169.68	117.23	-0.104-	0.000
B	343.03 ± 2.85	174.50 ± 3.34	168.53 ± 0.93	166.47	170.58	180.46	0.464	0.000
C	343.75 ± 1.31	176.22 ± 1.75	167.53± 0.52	166.37	168.68	319.89	0.326	0.000
D	342.51 ± 0.52	176.31 ± 3.24	166.20 ±0.98	164.03	168.37	168.41	-0.248-	0.000

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Table 2. Microhardness after demineralization and after remineralization .

Study Group	Demineralization Mean ± SD	Remineralization Mean ± SD	Mean Difference ± SE	95% Confidence Interval of the Difference		t-Test	Correlation	P-Value
				lower	Upper			
A	175.62 ± 4.83	198.68 ± 4.09	-23.05±1.92	-27.28	-18.82	-12.003-	-.104-	0.000
B	174.50 ± 3.34	180.94 ± 2.95	-6.44±1.25	-9.20	-3.68	-5.138-	0.053	0.000
C	176.22 ± 1.75	217.84 ± 2.19	-41.62±0.90	-43.60	-39.64	-46.239-	-0.238-	0.000
D	176.31 ± 3.24	218.99 ± 6.80	-42.68±1.18	-45.28	-40.07	-35.992	0.904	0.000

Table 3. Post hoc test (Tukey HSD) for groups microhardness at the remineralization stage.

Groups	Mean Difference ± SE	P-Value	95% Confidence Interval	
			Lower limit	Upper limit
A & B	17.73 ± 1.78	0.000	12.9660	22.5073
A& C	19.16 ± 1.78	0.000	23.9373	14.3960
A& D	20.31 ± 1.78	0.000	25.0806	15.5394
B & C	36.90 ± 1.78	0.000	41.7640	32.1327
B & D	38.04 ± 1.78	0.000	42.8173	33.2760
C & D	1.14 ± 1.78	0.918	5.1940	3.6273