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Evaluation of the Effect of Current Herbal Remineralization Agents on Enamel Using Different Methods

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Research Article

History

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ABSTRACT

Objectives: In this study, it was aimed to quantitatively evaluate the remineralization capacity of natural medicaments such as ginger, grape seed extract and rosemary, and materials containing casein and hydroxyapatite of initial caries lesions in vitro.

Materials and Methods: 42 enamel samples obtained from human teeth; were divided into six group as; Group-I (10% Casein Phosphopeptide Amorphous Calcium Phosphate [CPP-ACP], GC Tooth Mousse, Group-II (Rosemary Oil), Group-III (ginger+honey), Group-IV (ginger+honey+cocoa), Group-V (grape seed extract) and Group-VI (control group; remineralization solution). After enamel samples were kept in demineralization solution for 72 hours to create initial caries lesion, grouped remineralization agents were applied to enamel samples with caries lesion during the six-day pH cycle. Measurements were made using surface microhardness and DIAGNOdent at three different stages. The data obtained in the research were analyzed using the SPSS 25.0 program. The results were evaluated at the $p<0.05$ significance level.

Results: In surface microhardness measurements, all test materials showed significantly more remineralization than fluoride tooth cream, and grape seed extract was found to be the most successful group ($p\leq0.05$). When all groups were compared with the positive control group, there were significant statistical differences between the groups ($p\leq0.05$). The remineralization capacities of the groups whose activities were examined; grape seed extract> ginger+honey+cocoa>ginger+honey> rosemary oil > GC tooth mousse.

Conclusions: It is thought that rosemary, ginger + honey + cocoa, grape seed extract materials can be used for remineralization in these working conditions where the initial caries lesions can be remineralized after six days.

Keywords: Microhardness, Remineralization, Grape Seed Extract, Ginger, Rosemary.

Güncel Bitki Özlü Remineralizasyon Ajanlarının Mine Üzerine Etkisinin Farklı Yöntemlerle Değerlendirilmesi

Research Article

Süreç

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ÖZ

Amaç: Bu çalışmada başlangıç çürük lezyonlarının zencefil, üzüm çekirdeği özü ve biberiye gibi doğal medikamentler ile kazein ve hidroksiapatit içeren materyallerin remineralizasyon kapasitesini kantitatif olarak *in vitro* koşullarda değerlendirilmesi amaçlanmıştır.

Gereç ve Yöntem: İnsan dişinden elde edilen 42 adet mine örneği; Grup-I (%10 Kazein Fosfopeptit Amorf Kalsiyum Fosfat [CPP-ACP], GC Tooth Mousse, Grup-II (Biberiye Yağı), Grup-III (zencefil+bal), Grup-IV (zencefil+bal+kakao), Grup-V (üzüm çekirdeği özü) ve Grup-VI (kontrol grubu; remineralizasyon solüsyonu) olarak altı gruba ayrıldı. Mine örnekleri yapay çürük lezyonu oluşturmak amacıyla 72 saat demineralizasyon solüsyonunda bekletildikten sonra, çürük lezyonu oluşturulan mine örneklerine altı günlük pH döngüsü sürecinde, gruplandırılan remineralizasyon ajanları uygulandı. Üç farklı aşamada yüzey mikrosertlik ve DIAGNOdent kullanılarak ölçümler yapıldı. Araştırmada elde edilen veriler SPSS 25.0 programı kullanılarak analiz edildi. Sonuçlar, $p<0,05$ anlamlılık düzeyinde değerlendirildi.

Bulgular: Çalışma sonucunda DIAGNOdent ölçümlerinde tüm gruplarda remineralizasyon gözlenmekle beraber gruplar arasında istatistiksel olarak anlamlı bir fark bulunmamıştır ($p>0.05$). Yüzey mikrosertlik ölçümlerinde ise tüm deney materyalleri florürlü diş kreminden anlamlı derecede daha fazla remineralizasyon göstermiş olup, en başarılı grup olarak üzüm çekirdeği özü bulunmuştur ($p\leq0.05$). Tüm gruplar, pozitif kontrol grubu ile karşılaştırıldığında gruplar arasında belirgin istatistiksel farklılıklar bulunmaktadır ($p\leq0.05$). Etkinlikleri incelenen grupların remineralizasyon kapasiteleri; üzüm çekirdeği özü> zencefil+bal+kakao>zencefil+bal> biberiye yağı > GC tooth mousse şeklinde sıralanmaktadır.

Sonuç: Altı gün sonunda başlangıç çürük lezyonlarının remineralize olabildiği bu çalışma şartlarında biberiye, zencefil + bal + kakao, üzüm çekirdeği özü materyallerinin, remineralizasyon amacıyla kullanılabileceği düşünülmektedir.

Anahtar Kelimeler: Mikrosertlik, Remineralizasyon, Üzüm Çekirdeği Özü, Zencefil, Biberiye.

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Introduction

Advances in technology significantly reduce the progression of initial caries with early diagnosis and treatment, providing protection to dental tissues to a great extent without invasive procedures.[1] Significant progress has been made on remineralizing agents that can be used within scope of preventive dentistry applications. Fluorine is one of the most commonly used agents.[2] Developed as an alternative to flor; demineralization is prevented and remineralization is supported by binding free phosphate and calcium in dental plaque with casein phosphopeptide-amorphous calcium phosphate complex (CPP-ACP), one of major protective factors in milk.[3] Plant-based medications such as ginger and rosemary can also find a place in current studies with antibacterial effects.[4]

Different methods have been used to examine formation and progression mechanism of caries. The gold standard in these methods; although it is a controlled randomized clinical trial model; conducting such a clinical study is challenging in terms of both cost and time. Therefore, alternative models in which oral environment is imitated have been developed. Studies show that the most frequently preferred model is in vitro models.[5] These simulation models are frequently used because they allow testing of hypotheses that cannot be tested in clinical practice, provide reproducible study data, and practically compare the results obtained from different centers. However, its inability to fully imitate complex biological processes associated with caries and natural oral environment has become disadvantage of this method.[6,7]

pH cycle caries model is an artificial caries model that simulates oral environment by imitating demineralization-remineralization cycle of tooth. The biggest advantage of this method, which is used quite frequently to investigate effect of caries-preventing agents on demineralization-remineralization dynamics, is that it allows the evaluation of changes in outermost enamel layer during development of enamel caries.[8]

Systems used in diagnosis of initial caries and in following remineralization-demineralization cycles of lesions have limited reliability. Microhardness testers and scanning electron microscope are the most frequently preferred devices for this purpose in in-vitro research.[9] It is observed that surface microhardness measurement method is frequently used in studies investigating mechanical properties and efficacy of agents used in treatment of initial caries lesions.[10,11] As a working principle, the diamond tip located at tip of the device applies force to sample surface in certain proportions. The penetration depths obtained from different areas of lesion are recorded as Knoop or Vickers hardness values. These values obtained following the recording phase are compared with the values of healthy surface.[12]

Another method, DIAGNOdent (KaVo, Biberach, Germany), developed to diagnosis possible presence of caries on enamel surface early; it is based on laser autofluorescence technology in principle. The fluorescence intensity diagnosed by the digital display is displayed in quantitative form with numerical values. There is a direct correlation between the depth of caries and number seen on screen.[13]

Although chemicals continue to be widely used today, there has been an increasing interest in natural product diversity lately. Plants such as ginger and rosemary; many studies have been conducted showing its anti-inflammatory, antifungal, antiemetic, antibacterial, antiviral and anticariogenic effects.[14-18] While there are a limited edition of studies investigating effects of these agents on cariogenic bacteria in the literature, there is no study investigating their effects on the remineralization mechanism. The aim of this research; was to compare the effectiveness of CPP-ACP tooth cream, with proven anti-caries effect, with herbal medicines such as rosemary, ginger (a mixture with honey and cocoa), grape seed extract, which have come to the fore with their antibacterial activity.

Materials and Methods

Our study was carried out in the research laboratories of Çukurova University Faculty of Science Biochemistry Department and Dicle University Faculty of Dentistry, and ethics committee approval was obtained from Dicle University Faculty of Dentistry Unit Ethics Committee (*Decision Protocol No: 2020-1, (Decision Date: 29.01.2020*).

The enamel samples used in our study were selected from caries-free premolar teeth that had no defects. Soft tissue attachments on root surface of teeth were removed with a periodontal curette and cleaned with a polishing brush without damaging surfaces. Teeth were stored in distilled water containing 0.1% thymol at +4 ºC until the study was started.

The middle 1/3 area of enamel on partially flatter buccal surface of teeth was used for the procedures. The crowns of teeth were separated from roots with a micromotor (NSK FX23, JAPAN) device using diamond separation at low speed, under water cooling.

The prepared samples were embedded in acrylic (Imicryl, Turkiye) blocks with the aid of 3cm x 3cm x 2cm silicone molds with their buccal surfaces exposed. To obtain a smooth flat surface and to facilitate the demineralization stage of samples, the outermost layer rich in fluorine was abraded with silicon carbide papers of 200, 400, 600, 800 and 1200 grit, respectively, for ten minutes.

After etching, the samples were washed with deionized water. To create a control area on the healthy enamel surface, 2mm x 2mm tape was adhered to the center of enamel surface of each tooth and two layers of acid-resistant polish (Nail Care, Flormar, Turkiye) was applied to prevent area around the tape from being affected by the caries model to be applied.

After the sample preparation stage, 42 enamel samples of 2mm x 2mm in size were randomly divided into six groups, with seven enamel samples in each group;

Group 1: GC Tooth Mousse (10% CPP-ACP)
Group 2: Rosemary Oil (RO)
Group 3: Ginger - Honey
Group 4: Ginger - Honey - Cocoa

Group 5: Grape Seed Extract (GSE)
Group 6: Control Group (Remineralization Group)

Surface Microhardness measurements (SMH) were made by Buehler Wilson 1202 Mechanical Instrument Hardness Tester (USA). Using Vickers as measuring tip; a total of three measurements were made at the beginning, after demineralization process and after pH cycle. 42 samples were placed on table parallel to the ground and with surfaces to be evaluated upwards. The areas that the Vickers measuring tip will contact were determined from the selected area on surface to be measured. These points, which are determined with help of 400x magnifying eyepieces in the microscope, are placed in the microhardness device for 15 seconds. Throughout, 300 g of force was applied and measurements were made from three different points in each sample. The diagonal length of diagonal area formed by Vickers measuring tip was measured with measuring system on the device, and SMH calculated on the device was recorded. Following registration process, the average of three measurements for each sample was calculated statistically.

In DIAGNOdent measurements of samples, the fissure probe of DIAGNOdent PEN® (KaVo, Zurich) device, which is used on flat surfaces and fissures, was used. The samples were air-dried for five seconds and the measurement process was completed. For each of samples, three different predominantly observed DIAGNOdent values were recorded.

Preparation of Solutions

The solutions were prepared fresh before experiment.
Demineralization solution:
• 1.5 mM CaCl2,
• 0.9 mM KH2 PO4,
• By preparing 50 mM Aceticacid with pH value of 4.8; kept at room temperature.
Remineralization solution:
• 1.5 mM CaCl2,
• 0.9 mM KH2 PO4,
• 130 mM KCl,
• By preparing 20 mM Hepes solution with pH value of 7.0; kept at room temperature.

Creation of Initial Caries Lesion on Samples

The teeth were kept in demineralization solution for 72 hours.

SMH and DIAGNOdent Measurements After Demineralization

Samples with initial caries lesion; after the demineralization process was completed, it was removed from the solution and washed with distilled water. On the dried sample surfaces; the data were recorded by repeating the measurements with the same technique and devices as it was done in the initial stage.

Remineralization Agents and Application Methods

For the remineralization of the initial caries lesions formed in the enamel samples, a remineralization solution prepared with a pH value of 7.0 for the control group with five different agents was used.
Group 1: Tooth Mousse (GC Corporation, Japan): Dental cream with calcium phosphopeptidamorphic calcium phosphate (CPP-ACP). It was applied to each sample surface with the help of an applicator for four minutes.
Ingredients: water, glycerol, CPP-ACP, D-sorbitol, sodium carboxy methyl cellulose (CMCNa), propylene glycol, silicon dioxide, titanium dioxide, xylitol, phosphoric acid, aroma, zinc oxide, sodium saccharin, ethyl phydroxybenzoate, magnesium oxide, guar gum, propyl p hydroxybenzoate, butyl p-hydroxybenzoate. (lot number: 200908D)
Group 2: Rosemary Oil (RO): (Immu-Nat Herbal Company, Muğla, Türkiye) Standardized oil of rosemary. With the help of an applicator, 1 ml for each sample was applied to the tooth surface for four minutes.
Group 3: Ginger + honey: (Ginger: Arifoglu®, Turkiye/ Honey: Balparmak Plateau Blossom Honey®, Turkiye) Freshly prepared powdered ginger was mixed with honey (8 mg/ml = 8 mg ginger was added for 1 ml honey) to a paste consistency and applied to the surface in the size of a lentil grain for four minutes with the help of an applicator.
Group 4: Ginger + honey + cocoa: (Cocoa: Ülker®, Turkiye) prepared by adding cocoa to the ginger + honey mixture at the same rate as honey (8 mg/ml = 1 mg cocoa was added for 1 ml honey), brought to a paste-like consistency and applied to the surface in size of a lentil grain for four minutes with the help of an applicator.
Group 5: Grape Seed Extract (GSE): concentration amounts 3.75ml/5ml (Immu-Nat Herbal Company, Muğla, Turkiye) 1 ml per sample with the help of an applicator applied to the sample surface for four minutes.
Group 6: Control Group: Only demineralization remineralization procedures were applied to seven sample surfaces in this group, no agent was applied.

Application of pH Cycle to Samples that Simulates Oral Environment

In order to create artificial caries lesions, samples were kept in demineralization solution for 72 hours and then pH cycled at room temperature for a period of six days. The samples were kept in the demineralization solution (30 ml for each sample) for six hours without mixing in one day, washed with distilled water for five seconds, and kept in the remineralization solution (30 ml for each sample) for 18 hours.

Remineralizing agents applied for one minute daily before and after demineralization four times with the help of a small bond brush (Microbrush, medium thickness, USA) daily. After this stage, the sample surfaces were washed with distilled water for five seconds to remove the excess agent. The samples were kept in remineralization solution until they were re-cycled the next day.

Figure 1: The pH cycle applied in the research

SMH and DIAGNOdent Measurements After pH Cycle

After the completion of the six-day pH cycle applied within the scope of study, the samples were washed with distilled water for the last time. On the sample surfaces, which are made ready for analysis by drying; the measurements were repeated with the help of the same technique and devices. All measurements were made by the same specialist dentist (S.G.).

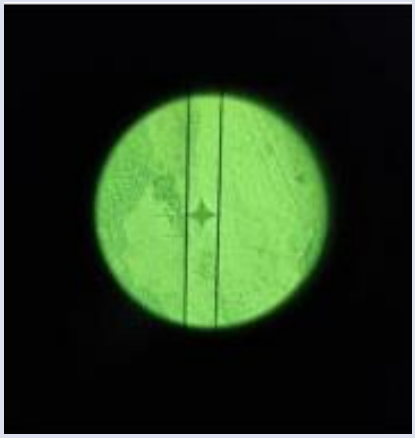

Figure 2: Surface image of the sample examined on the vickers hardness test device

Statistical analysis

The data obtained in research were analyzed using SPSS 25.0 program. Descriptive statistics were used while evaluating data. The results were evaluated at $p<0.05$ significance level.

Results

Results of SMH Analysis

SMH measurements were repeated in three stages, at the beginning of our thesis, after being kept in a demineralization solution for 72 hours and after pH cycle and remineralization agents were applied. Three different measurements were taken from each sample and 126 measurements made from 42 enamel samples were repeated in three stages, and total of 378 SMH measurements were made. The difference between initial and demineralization values was calculated and called as 'delta 1'; the difference between demineralization and remineralization values was calculated and called as 'delta 2'.

A statistically significant difference was found between the initial values, remineralization and demineralization values of each group. There was a statistically significant difference between Vickers delta 1 and delta 2 values according to the groups ($p<0.05$).

While a significant difference was observed between the groups for the initial and post-remineralization Vickers values ($p<0.05$); It was observed that there was no significant difference between the groups for Vickers values after demineralization ($p>0.05$).

It was observed that there was a statistically significant difference between the Vickers values of all groups after demineralization and after remineralization ($p<0.05$). It is

seen that the Vickers values after remineralization are higher than the values after demineralization.

There was a statistically significant difference between Vickers values at baseline, after demineralization and after remineralization in all groups ($p<0.05$). According to results of the multiple comparison test; there is a significant difference between values after demineralization and after remineralization ($p<0.05$).

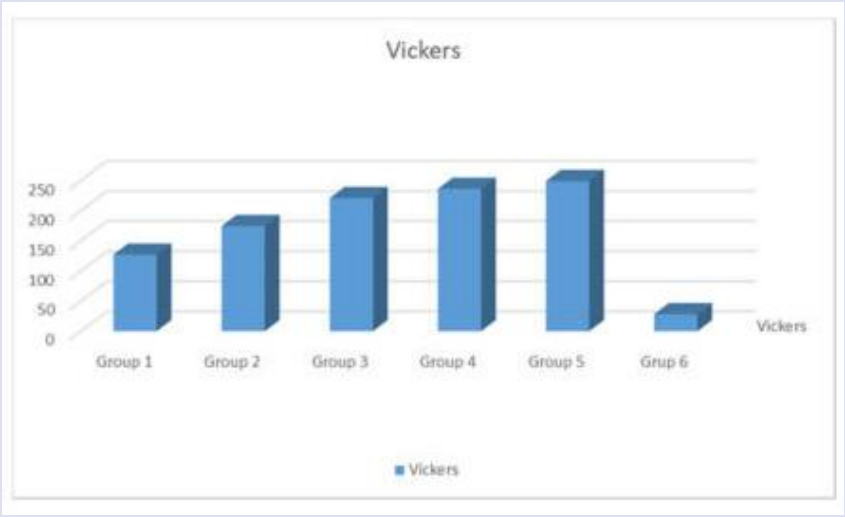

Figure 3: Difference between remineralization and demineralization values (Vickers)

DIAGNOdent Analysis

When Diagnodent delta 1 values of the groups were examined, there was no statistically significant difference between groups, although DIAGNOdent delta 1 values of groups 1, 2, 3, 4 and 5 were lower than the control group ($p>0.05$). It was observed that there was a significant difference between the groups for DIAGNOdent values at baseline and after remineralization ($p<0.05$). It was observed that there was no significant difference between the groups for DIAGNOdent values after demineralization ($p>0.05$).

A statistically significant difference was observed between the initial values of all groups and the DIAGNOdent values after demineralization ($p<0.05$). It is seen that DIAGNOdent values after demineralization are higher than the initial values.

It was observed that there was a statistically significant difference between the DIAGNOdent values of all groups after demineralization and after remineralization ($p<0.05$). It is seen that DIAGNOdent values after remineralization are lower than the values after demineralization.

In all groups; there was a statistically significant difference between the DIAGNOdent values at baseline, after demineralization and after remineralization ($p<0.05$). According to the results of the multiple comparison test; there is a significant difference between the initial, post-demineralization values and post-demineralization and post-remineralization values ($p<0.05$).

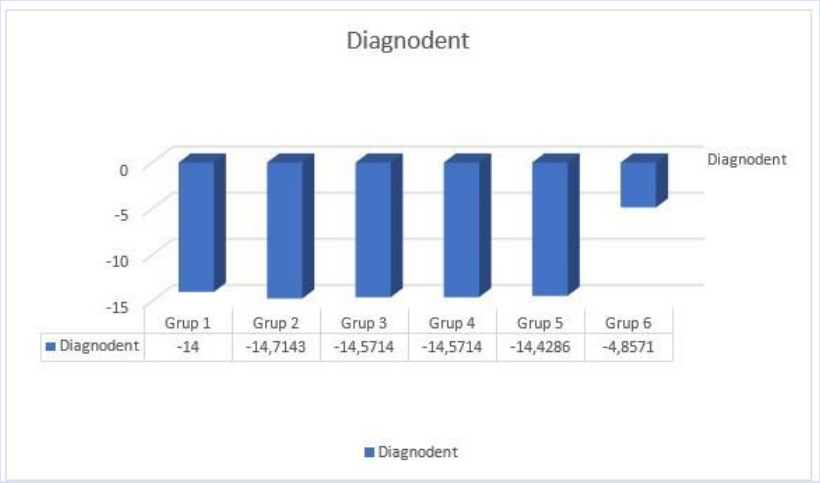

Figure 4: Difference between remineralization and demineralization values (Diagnodent)

Discussion

Current scientific studies are carried out to prevent initial caries lesions with non-invasive methods and to diagnose these lesions with reliable methods at an early stage.[19-22] Different materials such as fluorine, bioactive glasses, theobromine, CPP-ACP and xylitol are used to increase remineralization and prevent demineralization.[23-26]

CCP-ACP; It shows its caries-inhibiting effect by preventing bacterial colonization, increasing the Ca and P ion levels in dental plaque, and binding free Ca and P ions.[27,28] In many studies, it has been reported that CPP-ACP stabilizes Ca and P ions on the enamel surface and provides remineralization.[29,30] Bailey et al., reported that with the use of CPP-ACP for 12 weeks, a significant visible regression of white spot lesions was recorded. In white spot lesions, 27.9% at the end of the four-week period; 65.6% after eight weeks; at the end of 12 weeks, they stated that there was an improvement of 73.8%.[31]

In another recent research examining the surface microhardness of remineralization agents; OCT and SMH measurements were made by applying CPPACP, CPPACP+900 ppm Fluor and agents containing calciumglycerophosphate, magnesiumchloride and 10% xylitol to the enamel surfaces with initial caries, with a 6-day pH cycle. As a result of study; it has been stated that all remineralization agents provide significant remineralization in caries lesions formed in enamel samples.[32,33]

The study, which compared the remineralization capacity of tooth cream containing CPP with ginger, honey, cocoa and rosemary, and toothpaste with NaF, using SMH and FluoreCam methods, draws attention. In this study conducted by Bilgin G, enamel samples with artificial initial caries were used for one minute, three times a day for 21 days; toothpaste with NaF, CPPACP, hydroxyapatite and F, brushed with fluorine varnish, ginger + honey, ginger + honey + chocolate, RO groups. With the statistical evaluation of study; remineralization was detected in all treatment groups, and it was concluded that the remineralization values of the ginger + honey treatment group were more effective than the other groups.[34]

Smullen et al. in their study on RO; the effectiveness of rosemary on plaque inhibition, S. mutans and glycosyltransferase activity was investigated, and it was stated that 80% inhibition of plaque and glycosyltransferase activity was achieved.[35] In a research examining the effect of RO on remineralization; it has been reported that RO significantly increased remineralization on enamel surfaces with artificial initial caries, compared to the positive control group, fluoride toothpaste.[34] Consistent with the results of these researches; it has been concluded that RO we used has a very supportive effect on remineralization according to SMH and DIAGNOdent measurement values, as well as preventing demineralization.

Another material we prefer within the scope of our study is honey. Grobler et al. in their study on oral pathogens, the effect of low pH on enamel is evaluated by SMH and DIAGNOdent. In both of the analysis methods, honey was applied on the enamel for 30 minutes and it was reported that it did not show an erosive effect on the enamel.[36] In the in-situ part of another current study, in which the remineralizing effect of honey was investigated, enamel samples were placed in oral appliances and the participants were given four different types of fluoride toothpaste, fluoride tooth cream, ginger+honey+ chocolate and ginger+rosemary+honey+ chocolate for a 21-day period. group was provided with three minutes of application. The amount of remineralization achieved was measured with FluoraCam and QLF after 21 days. While remineralization was achieved significantly in all groups; it was stated that the highest remineralization was formed by ginger+honey+chocolate mixture and fluoride tooth cream.[34] With the results of these studies; SMH and DIAGNOdent analysis data obtained as a result of the application of honey used in our in-vitro research by mixing it with ginger gives results in accordance with other researches. The fact that this mixture was also found to be a highly effective material on remineralization contributed to the originality of our research.

Among the materials investigated in our study, the in-vitro study in which the effect of theobromine obtained from the cocoa bean on SMH was investigated is remarkable. In this study, enamel sections taken from premolar teeth were used; Distilled water, theobromine 100 mg/L, theobromine 500 mg/L and theobromine 1000 mg/L were administered to four groups for 15 minutes. Samples that were tested for microhardness using SMH; an increase in enamel microhardness was observed after being treated with four theobromine concentrations, and it was stated that the highest increase was demonstrated by theobromine with a concentration of 1000 mg/L.[37] Ooshima et al. evaluated the relationship between cocoa bean shell and S. mutans in their study with rats. In all streptococci species tested within the scope of study, growth decreased and as a result, acid production decreased and in parallel, surface hardness increased as remineralization was supported. Sucrose-dependent cell adhesion of S. mutans was suppressed with using of cocoa; inhibition of insoluble glucan synthesis due to glycosyltransferase activity was achieved.[38] Ooshima et al. following the research of Osawa et al. in their study, they stated that cocoa bean shell can show antibacterial activity even at a concentration of 30µg/mL.[39] SMH and DIAGNOdent analysis data of our research show that the cocoa has a significant effect on remineralization.

Another material we prefer to increase the originality value of research is; grape seed extract. Benjamin et al. reported that collagen matrices treated with GSE were non-toxic and inhibited the enzymatic activity of glucosyltransferase, F-ATPase and amylase in their study.[18] When the studies on GSE were examined, it was observed that there were a limited number of studies investigating the remineralization capacity.[40,41] Mirkarimi et al. applied GSE to demineralized primary teeth and reported a statistically significant increase in SMH.[42] In another recent study investigating the remineralization

capacity of GSE, Jawale et al., on enamel surfaces with artificial initial caries in 96 hours; three different materials, namely CPP-ACP, calcium glycerophosphate and GSE, were applied six times a day for eight days and examined with polarized light microscopy. As a result of research; a significantly thick band of mineral precipitation appeared in surface layer of lesions treated with GSE compared to other groups; it has been reported that GSE has a supportive effect on remineralization process.[43] In a similar study; samples whose dentin surface was etched for 30 seconds; different concentrations of GSE; it was applied for two minutes and subjected to a 15-day remineralization procedure, SMH analysis were performed. Tang et al reported that the most GSE concentration, which provides remineralization is 15%.[44] Bolty et al. in 2023, concluded that chicken egg shell, GSE and propolis had a promising effect on remineralization of initial caries in their study.[45] Another recent study, the remineralizing effect of initial caries was observed in patients using GSE and NaF mouthwash in 1, 3, and 6-month diagnostic measurements.[46] In a recent study in which the remineralization capacity of CPP-ACPF, tricalcium phosphate, and GSE was measured by Vickers, SEM-EDS, and CBCT, it was noted that GSE group showed significantly greater remineralization compared to other groups.[47] With all these reseraches; SMH and DIAGNOdent analysis data of remineralizing effect of GSE obtained as a result of our research show parallelism.

As a result, it was observed that among the materials whose remineralization capacities we examined, CPP-ACP, ginger + honey, ginger + honey + cocoa formula, rosemary oil and grape seed extract showed positive reparative activities on initial enamel lesions.

Conclusions

This research effectively analyzed initial caries can be cured chemically by stop-preventived procedures. According to the study's findings GSE was found to be more effective than other agents. We report that the use of tooth cream containing herbal formulas that repair caries at the initial stage is a non-invasive and supportive method that can be added to routine oral hygiene practices, and we recommend their use.

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Conflict of interest

There is no conflict of interest among participants in this study.

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