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ORIGINAL ARTICLE / ARAŞTIRMA MAKALESİ

Assessment of the Remineralization Effect of Hemp-Containing Toothpaste

Kenevir İçeren Diş Macununun Remineralizasyon Etkisinin Değerlendirilmesi

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ABSTRACT

Objective: The study aimed to evaluate the remineralizing effect of hemp on initial enamel caries lesions.

Materials and methods: Initial caries lesions were created on twenty-eight intact enamel samples with demineralization solution (pH=4.5) in a shaking incubator for 72 hours at 37°C. Samples were divided into four groups (n=7) ((1) experimental toothpaste containing hemp (H) (SPC Kozmetik), (2) hemp oil (HO), (3) Sensodyne Repair and Protection toothpaste (S) (GSK), (4) Control (no treatment)). The agents were applied using a pH-cycle model for seven days. All agents were kept on the surface of samples for 2 minutes and then applied for 1 minute using an electric toothbrush (Oral-B Test Drive (Genius)). Samples were evaluated with microhardness device, a fluorescence-assisted imaging device (FluoreCam), laser fluorescence device (DIAGNOdent Pen). Data were statistically analyzed with ANOVA and Kruskal Wallis H tests ($p<0.05$).

Results: According to fluorescence and microhardness evaluations, the remineralization effect was similar in all treatment groups, except for hemp oil ($p<0.01$). Groups S (288.86±9.63), and H (284.86±10.41) showed significantly higher microhardness values compared to HO (238.57±10.89) ($p<0.001$). According to FluoreCam data, lesion size decreased in S (-1.83) and H (-1.07), while an increase was observed in HO (0.75). According to DIAGNOdent Pen data, the mineral increase in S (-8.42±1.18) and H (-8.29±1.03) was significantly higher than in HO (-3.57±2.06) ($p<0.001$).

Conclusions: Herbal toothpaste containing hemp (H) demonstrated a similar remineralization effect to Fluor and NovaMin-containing toothpaste (S).

Keywords: Initial Caries Lesion, Herbal Toothpaste, Demineralization, Hemp, Remineralization.

ÖZ

Amaç: Bu çalışma, kenevirin başlangıç mine çürük lezyonlarına remineralize edici etkisini değerlendirmeyi amaçlamıştır.

Gereç ve Yöntemler: Yirmi sekiz sağlam mine numunesi üzerinde demineralizasyon solüsyonu (pH=4.5) kullanılarak çalkalamalı inkübatörde 37°C'de 72 saat süreyle başlangıç çürük lezyonları oluşturulmuştur. Mine örnekleri dört gruba ayrılmıştır (n=7) ((1) kenevir içeren deneysel diş macunu (H) (SPC Kozmetik), (2) kenevir yağı (HO), (3) Sensodyne Onarım ve Koruma diş macunu (S) (GSK)), (4) Kontrol (tedavi yok)). Ajanlar yedi gün boyunca pH döngüsü modeli kullanılarak uygulanmıştır. Remineralizasyon ajanları diş yüzeyinde 2 dakika bekletildikten sonra elektrikli diş fırçası (Oral-B Test Drive (Genius)) ile 1 dakika süreyle uygulanmıştır. Örnekler mikrosertlik cihazı, floresans destekli görüntüleme cihazı (FluoreCam), lazer floresans cihazı (DIAGNOdent Pen) ile değerlendirilmiştir Veriler ANOVA ve Kruskal Wallis H testleri ile istatistiksel olarak analiz edilmiştir ($p<0,05$).

Bulgular: Floresans ve mikrosertlik sonuçlarına göre kenevir yağı hariç tüm tedavi gruplarında benzer remineralizasyon etkisi görülmüştür ($p<0.01$). Grup S (288,86±9,63) ve H (284,86±10,41), HO'ya (238,57±10,89) kıyasla anlamlı derecede yüksek mikrosertlik değerleri göstermiştir ($p<0,001$). FluoreCam verilerine göre S (-1,83) ve H'de (-1,07) lezyon boyutunda azalma görülürken, HO'da (0,75) artış gözlenmiştir. DIAGNOdent Pen verilerine göre S (-8,42±1,18) ve H'deki (-8,29±1,03) mineral artışı HO'ya (-3,57±2,06) göre anlamlı derecede yüksekti ($p<0,001$).

Sonuç: Kenevir içeren bitkisel diş macunu (H), flor ve NovaMin içeren diş macununa (S) benzer remineralizasyon etkisi göstermiştir.

Anahtar Kelimeler: Başlangıç çürük lezyonu, bitkisel diş macunu, demineralizasyon, kenevir, remineralizasyon.

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INTRODUCTION

Dental caries lesions are one of the most common diseases that may lead to tooth loss if it is not treated. The current dentistry aims to improve the aesthetic and function of the tooth by remineralization process and preventing caries lesions (Nagarathana et al., 2015). Demineralization occurs when organic acids produced by acidogenic bacteria in dental plaque dissolve mineral ions from dental hard tissues. This process of returning mineral ions to tooth tissue is called remineralization. There is a continuous

demineralization-remineralization cycle in the oral cavity. Demineralization is a reversible process; therefore, initial enamel caries can be remineralized with plaque control, regulation of diet, and application of antibacterial agents (Guerrieri et al., 2012). Remineralization is a conservative method with short-term, economical, and easy application. Nowadays, herbal extracts have gained special attention as antimicrobial agents by preventing and treating caries due to being not chemical and synthetic (Poureslami, 2012). Some natural plant extracts may likely prevent demineralization and maintain remineralization of the tooth tissue (Philip, 2019).

NovaMin, a commercial form of bioactive glass, rapidly releases calcium, sodium, and phosphorus ions which are essential for remineralization into the saliva, when it is present in the saliva and the pH in the oral cavity increases. The released ions attach to the tooth surface and remineralize the tooth surface forming hydroxycarbonate apatite. (Du et al., 2004).

Hemp (*Cannabis sativa L.*) has been used as many sources, including fiber, food, and medicinal products (Bonini et al., 2018). Especially the seeds of hemp have high oil content (Truta et al., 2009). Hemp containing products may be more effective in reducing the number of bacterial colonies in dental plaque compared to some existing oral hygiene products and may be a safer alternative to conventional oral hygiene products due to antimicrobial resistance (Stahl & Vasudevan, 2020). Previous research in dentistry on hemp oil is limited and the effect of hemp oil on dental hard tissues is not fully discovered.

This study aimed to investigate the effect of hemp-containing herbal experimental toothpaste on remineralization of initial caries lesions. The hypothesis of the study was that herbal toothpaste containing hemp has a similar remineralization effect compared to commercial toothpaste containing Fluor and NovaMin.

MATERIALS AND METHODS

Ethical approval of the study was obtained from the Clinical Research Ethics Committee of Marmara University Faculty of Dentistry (Date: 03.09.2020 Decision no: 2020/59).

Sample preparation

The number of samples was evaluated using G*power version 3.1.9.7 (α=0.05, 1-β=0.80) and the sample size was determined as four samples for each group based on previous study (Şivet et al., 2023). Twenty-eight enamel samples (n=7) were obtained by cutting the middle buccal surfaces of the extracted teeth using a handpiece (NSK EX-6, Japan) and diamond disc (Sigmadent, Turkey) under water cooling. Enamel samples were placed in acrylic molds with the dentin surface in acrylic and the enamel surface exposed. The surfaces of the samples were sanded under water for 5 minutes with 1200 silicon carbide sanding discs and then polished for 2 minutes with polishing pastes (CleanPolish (RDA:43.8) and SuperPolish (RDA:9.8), Kerr, Switzerland). After that, the samples were washed with distilled water and kept in a refrigerator at +4 °C in a container with distilled water until the measurements were made.

Experimental Groups

Study groups were divided into four groups (n=7) for enamel samples. The agents applied on the enamel samples and their contents are presented in Table 1. Hemp oil was obtained from hemp seeds by cold pressing at Yozgat Bozok University Hemp Institute. Hemp-containing experimental toothpaste was manufactured by SPC Kozmetik using hemp seed oil and aqueous hemp extract obtained from hemp leaves along with other ingredients showed in Table 1. In each group, remineralization agents were applied for a total of 3 minutes (Jo et al., 2014). They were kept on the tooth surface for 2 minutes and then applied for 1 minute with a rechargeable toothbrush (Oral-B Test Drive (Genius) rechargeable toothbrush, Germany). Hemp oil was applied on the enamel surface using a microbrush. In control group no treatment was applied on enamel surfaces. The samples were subjected remineralization-demineralization cycle for seven days with 6 hours of demineralization and 18 hours of remineralization each day (Carvalho et al., 2014). After each demineralization challenge samples were rinsed with distiled water and then placed in remineralization solution. All samples were evaluated with DIAGNOdent Pen, FluoreCam, and microhardness devices at baseline, after demineralization, and after remineralization stages as described below.

Table 1. Agents applied on the enamel samples and their contents.

Remineralization Agent	Content	Manufacturer
Hemp-containing experimental toothpaste (H) (%5 Hemp Oil and %5 Hemp Extract)	5% Hemp oil, 5% Hemp Extract, Mint oil, Mentol, Polisorbat 20, Xanthan Gum, Aqua, Glyserin, Sorbitol, Phenylpropanol, Capryly Glycol Stevia (Rebaudioside A), Hydrated Silica	SPC Kozmetik, Turkey
Hemp Oil (HO)	Hemp Seed Oil	Yozgat Bozok University, Turkey
Sensodyne Repair and Protect (NovaMin) (S)	Gliserin, Peg-8, Hydrated Silica, Calsium Sodium Phosphosilicat (NovaMin), Cocamidopropil Betain, Sodium Metyl Cocoyl Taurate, Aroma, Titanium Dioksit, Carbomer, Silica, Sodium Sakkarin, Sodium Flourid (1450 Ppm), Limonene.	Glaxosmithkline (Gsk), Ireland

DIAGNOdent Pen Analysis

The DIAGNOdent Pen (Kavo, Germany) was used to evaluate the surface changes of the enamel samples at baseline, after demineralization, and after remineralization stages. As recommended by the manufacturer, the instrument was calibrated prior to each measurement using standard ceramic. A recommended B-type probe was used for flat surface detection. The device was held vertically on the samples and measurements were made from 3 different points in each sample. The mean value was then calculated and recorded.

FluoreCam Analysis

Enamel samples were analyzed with a FluoreCam device (Daraza Corporate Headquarters, Indiana, USA) at baseline, after demineralization, and after remineralization stages. Before analysis, each sample was removed from the moist container and dried with a thin paper towel. Images were taken from the surfaces using the intraoral appliance of the FluoreCam system. The FluoreCam system can compare the intact enamel surface and demineralized-remineralized areas in terms of size and fluorescence values with images taken at different times. Analyzes were recorded in the FluoreCam Software program.

Surface Microhardness Analysis

The samples were measured using a Microhardness tester (Shimadzu Corporation, Japan) at baseline, after demineralization, and after remineralization stages. Measurements were made from 3 different areas of the lesion area by applying 200 g of force for 15 seconds on the flat surface of the samples. Then, the average of these three measurements was taken and the average sample value was recorded.

Formation of Artificial Initial Caries on Enamel Samples

Demineralization solution (2.0 mmol/lt $Ca(NO3)_2.H_2O$, 2.0 mmol/lt $NaH_2PO^4H_2O$, 0.04 μg F/ml (NaF) in 75 mmol/lt acetate buffer) (pH 4.7) was prepared in the Biochemistry Laboratory of the Marmara University Faculty of Dentistry (de Mello Vieira et al., 2005). Initial enamel lesions were formed on the samples by shaking 60 rpm at 37°C in a shaking incubator (ZWYR-240, LABWIT, Australia) for 72 hours. The solution renewed daily. The samples were removed from the demineralization solution after 72 hours, washed with distilled water, and kept in containers with distilled water in the refrigerator at +4 °C for measurements after demineralization.

Statistical analysis

Obtained data were analyzed with IBM SPSS V23. The conformity to the normal distribution was examined using the Shapiro-Wilk test. One-way Analysis of Variance (ANOVA) was used to compare normally distributed data according to groups, and multiple comparisons were examined with Duncan, Tamhane and Tukey HSD tests. The Kruskal Wallis H test was used to compare the data that were not normally distributed according to the groups, and multiple comparisons were examined with the Dunn test. In order to analyze within-group comparisons, Repetitive Analysis of Variance was used for data suitable for normal distribution, and Friedman Test for data not suitable for normal distribution. Analysis results are presented as mean ± standard deviation for quantitative data. Significance level was taken as $p<0.05$.

RESULTS

DIAGNOdent Pen Evaluation

There was no significant difference between the groups at baseline (T0) and after demineralization (T1) stages according to the data obtained from the DIAGNOdent Pen device. After remineralization process (T2), laser fluorescence values decreased significantly in toothpaste groups (H and S), while no significant decrease was observed in the hemp oil applied group and the control group. Toothpaste applied test groups (H and S) were found to have similar remineralizing effect according to DIAGNOdent Pen data (Table 2).

Table 2. The data obtained from DIAGNOdent Pen at baseline, after demineralization and after remineralization stages (mean ± standart deviation).

	Baseline (T0)	Demineralization (T1)	Remineralization (T2)	T1-T2	P**
Hemp-containing experimental toothpaste (H)	6.86 ± 1.46A	14.29 ± 1.03B	6 ± 0.53Aa	8.29 ± 1.03a	<0.01
Hemp oil (HO)	6.57 ± 1.29A	16 ± 1.2B	12.43 ± 2.77Cb	3.57 ± 2.06b	<0.01
Sensodyne Repair and Protect (NovaMin) (S)	5.86 ± 2.1A	14.43 ± 0.9B	6 ± 0.93Aa	8.43 ± 1.18a	<0.01
Control	5.57 ± 1.4A	15.14 ± 1.46B	14.57 ± 1.59Bc	0.57 ± 0.49b	<0.01
P*	0.686	0.114	<0.01	<0.01	

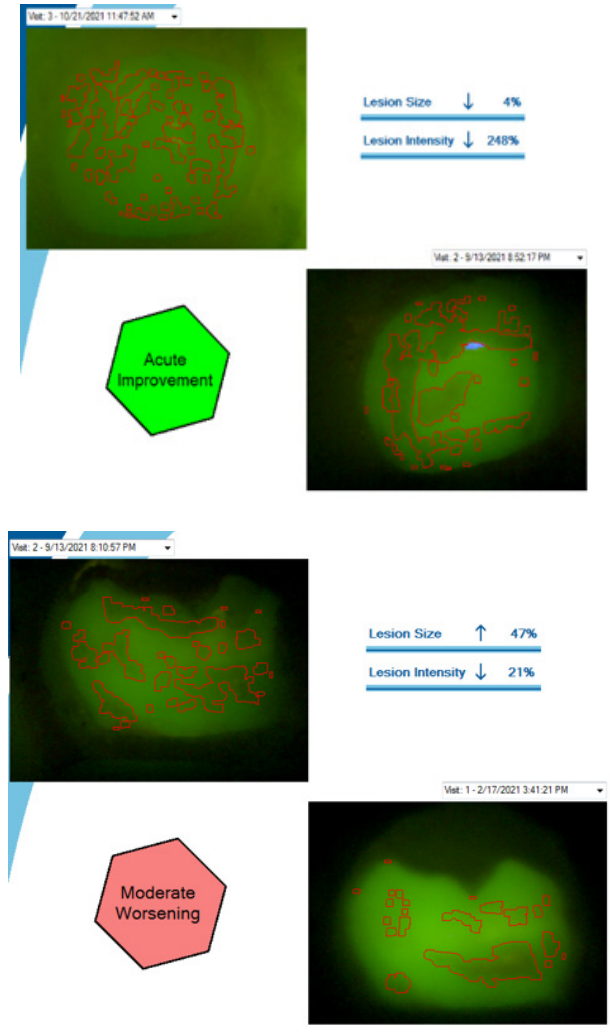
FluoreCam Evaluation

According to FluoreCam data, after remineralization stage (T2-T1), lesion size decreased significantly in both toothpaste applied groups (H and S) which had statistically similar results. There was a slight increase in lesion size values in the hemp oil and control groups. The increase in lesion size was bigger in the control group (Table 3). According to the FluoreCam System, toothpaste applied groups (H and S) had improvement (Fig. 1a and 1c), while Hemp oil applied group and the control group demonstrated worsening (Fig.1b and 1d).

Table 3. The lesion size data obtained from FluoreCam System at baseline, after demineralization and after remineralization stages (mean ± standart deviation).

	Baseline (T0)	Demineralization (T1)	Remineralization (T2)	T2-T1	P**
Hemp-containing experimental toothpaste (H)	5.18 ± 0.78	6.8 ± 1.31	5.13 ± 0.32	– 1.67 ± 1.07a	<0.01
Hemp oil (HO)	2.25 ± 1.55	3.51 ± 1.74	4.07 ± 2.2	0.55 ± 0.75b	<0.01
Sensodyne Repair and Protect (NovaMin) (S)	5.26 ± 0.45	6.77 ± 1.03	4.94 ± 0.54	– 1.83 ± 1.07a	<0.01
Control	3.13 ± 1.06	5.18 ± 2.01	6.2 ± 1.24	1.02 ± 1.34b	<0.01
P*	<0.01	<0.01	0.051	<0.01	

**Kruskal Wallis Test; **Friedman test; same letter codes have no significant difference*



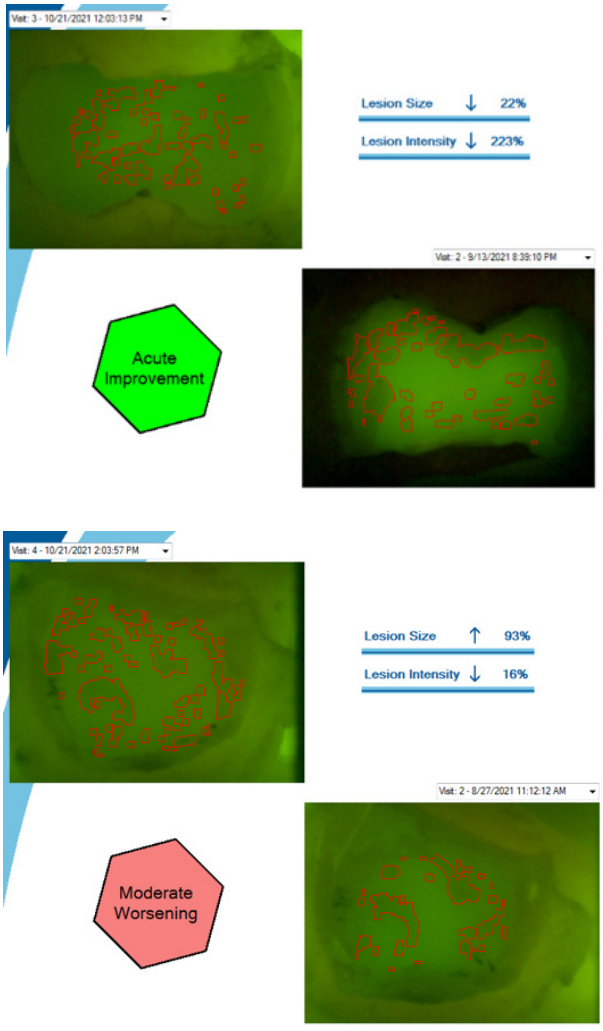


Figure 1: a) FluoreCam image of the sample applied experimental herbal toothpaste containing hemp. b) FluoreCam image of the sample applied hemp oil. c) FluoreCam image of the sample applied toothpaste containing Fluor and NovaMin. d) FluoreCam image of the sample from the control group.

Microhardess Evaluation

There was no statistically significant difference between all test groups at the baseline (T0) and after demineralization (T1) stages. After remineralization (T2), herbal hemp containing toothpaste and Sensodyne Repair and Protect toothpaste showed similar remineralization effects with increase in microhardness values, while hemp oil applied group had lower values. The hemp oil and control group showed similar results, with significantly lower values than the toothpaste applied groups (Table 4).

Table 4. The microhardness values obtained at baseline, after demineralization and after remineralization stages (mean ± standart deviation).

	Baseline (T0)	Demineralization (T1)	Remineralization (T2)	P**
Hemp-containing experimental toothpaste (H)	301 ± 14.95A	188.43 ± 9.32B	284.86 ± 10.41Ca	<0.01
Hemp oil (HO)	302.29 ± 13.77A	187.86 ± 8.66B	238.57 ± 10.89Cb	<0.01
Sensodyne Repair and Protect (NovaMin) (S)	303.57 ± 1.49A	194 ± 8.52B	288.86 ±7.95Ca	<0.01
Control	303.57 ± 10.62A	194.14 ± 7.18B	230.57 ± 9.81Cb	<0.01
P*	0.985	0.465	<0.01	

*One Way Analysis of Variance; **Repeated measures analysis of variance; same letter codes have no significant difference.

DISCUSSION

The process of tooth decay depends on biological factors present in saliva and dental plaque. The amount of cariogenic bacteria present in saliva and plaque is an important determinant in caries formation. The concentration of bacteria in saliva and plaque is highly related to the oral hygiene of the individual, as well as the type of carbohydrate consumption and the frequency of carbohydrate intake (Hicks et al., 2004). Initial enamel lesions can remineralize by applying appropriate antibacterial agents and controlling plaque formation (Guerrieri et al., 2012).

In remineralization of dental tissues, fluoride is regarded as the gold standard. Fluoride prevents demineralization by creating fluorapatite crystals which are larger in size and more resistant to acid attacks compared to hydroxyapatite (Arifa et al., 2019). Additionally, fluoride prevents caries-causing bacteria from producing acid (Soi et al., 2013). High concentrations of fluoride is toxic, and even levels slightly above therapeutic levels may cause fluorosis and therefore its use is limited (Ullah et al., 2017).

Because of the possible toxic effects of fluoride, researchers have sought alternative materials that can provide remineralization effects. NovaMin (calcium sodium phosphosilicate) is a synthetic and highly biocompatible product developed for the regeneration of bone (Layer, 2011). NovaMin has been used as a remineralization agent by adding it to toothpaste and prophylaxis pastes. When NovaMin is

present in the oral cavity, calcium and phosphate ions are released with a rapid increase in pH. The release of the ions lead to the formation of a hydroxycarbonate apatite layer which is similar to natural hydroxyapatite in chemical and structural composition, for a potential replacement for hydroxyapatite (Haghgoo et al., 2016). In this study, Sensodyne Repair and Protection toothpaste containing NovaMin and Fluor was used to compare its positive effects with the herbal products containing hemp.

Herbal extracts have been considered therapeutic agents in traditional medicine. Nowadays, herbal extracts attract special attention because they are not chemical and synthetic. If the effects of herbal products are supported by evidence from scientifically based studies, they may be safer alternatives for the treatment of initial enamel lesions (Shaheen et al., 2015). Kanth et al. showed plant extracts and essential oils can be used in oral and dental care products, mouthwashes, topical gels, etc. However, they stated that herbal products should be converted into a suitable form for regular use in oral hygiene products and future research studies are needed to evaluate the quality and effectiveness of these products (Kanth et al., 2016).

Various studies have investigated various aspects of hemp, such as its components, biological activities and industrial applications. Hemp may have a therapeutic effect on the treatment of dental caries because of its antibacterial properties (Bellocchio et al., 2023). Hemp-containing mouthwashes, when applied to dental plaque, showed bacterial inhibition with the same efficacy as chlorhexidine, which is a common agent for disinfection. In addition, hemp-fortified tooth polishing powder has been expressed to limit bacteria present in dental plaque (Vasudevan & Stahl 2020). Despite the large number of hemp-based oral products available, the scientific evidence on the safety, toxicity, and efficacy of these products is restricted.

In the present study, herbal hemp-containing experimental toothpaste showed a similar effect in the remineralization of initial enamel lesions as a conventional toothpaste containing fluoride and NovaMin. According to the microhardness data obtained in this study, the group in which hemp toothpaste was applied was the group whose microhardness values increased the most after the remineralization process (T2-T1) (Table 4).

Fluorescence techniques may be used in the diagnosis of initial enamel caries lesion in modern dentistry (Sudjalim et al., 2007). Structurally, enamel tissue is highly mineralized and semi-transparent. Enamel tissue with different degrees of mineralization shows different fluorescence effects. Based on laser fluorescence technique (DIAGNOdent Pen) and FluoreCam device, the mineral loss of the tooth can be expressed visually and quantitatively. For this reason, in the present study, the FluoreCam device was used to measure the remineralization amount of demineralized enamel tissue. It has been suggested that the FluoreCam device is a clinically applicable, non-invasive, repeatable and reliable method (Korkut et al., 2017). According to the results of this study, the FluoreCam and DIAGNOdent Pen data were consistent with the microhardness data.

For thousands of years, natural products have been considered a promising therapeutic, especially for oral diseases such as dental caries (Ancuceanu et al., 2019; Cruz Martinez et al., 2017; Jawale et al., 2017). Fluoride-containing toothpastes were used as positive controls to evaluate the remineralizing activity of herbal agents. Recently, oral care products containing bioactive substances obtained from extracts of plants have been launched. Similar to the this study, Janakiram et al. reported that herbal toothpaste was as effective as non-herbal toothpaste but was not superior to fluoride toothpaste (Janakiram et al., 2020). The results of the present *in vitro* study showed that herbal toothpastes have similar positive effects on remineralization as fluoride containing conventional toothpaste.

Using natural and fluoride-free systems for remineralization may decrease the risk of fluoride toxicity. Herbal-based products may maintain remineralization of the initial enamel lesions. Because of that, they may be preferable for individuals than fluoride-based systems used in caries prevention.

The present study has its limitations as being a short-term and an *in vitro* study. This study was conducted in *in vitro* conditions although a demineralization-remineralization cycle was used to imitate the oral environment. Agents were used for seven days to evaluate their remineralizing effect. The number of days agents were applied may be increased in further studies.

CONCLUSIONS

The use of experimental herbal toothpaste containing hemp oil and hemp extract had similar positive effects on enamel remineralization as traditional toothpaste containing Fluor and NovaMin. The application of hemp oil alone was not effective on enamel remineralization. All treatment groups except hemp oil demonstrated a remineralization effect on initial enamel lesions. The effect of hemp oil was found similar to the control group.

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Conflict of Interest

The authors declare no conflict of interest related to this study.

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