Nobel Med 2025; 21(1): 30-39

EVALUATION OF THE EFFECT OF MORINGA OLEIFERA ON DENTAL HARD TISSUES

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ABSTRACT

Objective: *Moringa oleifera plant is used in both traditional medicine and nutrition due to its beneficial content. This study aimed to evaluate the occluding and remineralization effects of Moringa oleifera.*

Material and Method: *Seventy-five enamel and one-hundred-five dentin samples were prepared. Enamel samples were subjected to demineralization solution (pH=4.5) to create initial caries lesions and divided into five groups (n=15): Control group (no treatment), (S) Sensodyne Repair and Protect, (M) Herbal toothpaste containing Moringa oleifera, (MF) Herbal toothpaste containing Moringa oleifera with fluoride, and (MO) Moringa oleifera oil. To expose the dentin tubules, the dentin samples were immersed in a 6% citric acid solution for 2 minutes and then divided into seven groups (n=15). Control, (S), (M), (MF), (MO), (R) R.O.C.S. Sensitive, and (O) Oral-B Sensitivity and Gum Calm. The agents were applied three times daily for seven days using a pH-cycle model. 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 and fluorescence-based devices (FluoreCam and DIAGNOdent Pen).*

Results: *According to fluorescence and microhardness evaluations, the remineralization effect on enamel samples was similar in all treatment groups, except for MO ($p<0.001$). On dentin samples, after the treatment, microhardness values were significantly lower in the control group following MO ($p<0.001$). Fluorescence results were significantly higher in the treatment groups compared to the MO and control groups ($p<0.001$).*

Conclusion: *Herbal toothpastes containing Moringa oleifera were effective in remineralization and dentin tubule occlusion.*

Keywords: *Herbal toothpaste, demineralization, Moringa oleifera, remineralization, dentin hypersensitivity.*

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DELIVERING DATE: 19 / 02 / 2024 • **ACCEPTED DATE:** 22 / 07 / 2024

MORİNGA OLEİFERA'NIN DİŞ SERT DOKULARI ÜZERİNE ETKİSİNİN DEĞERLENDİRİLMESİ

ÖZET

Amaç: Moringa oleifera bitkisi faydalı içeriği nedeniyle hem geleneksel tıpta hem de beslenmede kullanılmaktadır. Bu çalışmanın amacı Moringa oleifera'nın dentin tübül tıkaçlaması/remineralizasyon etkisini değerlendirmektir.

Materyal ve Metot: Yetmiş beş adet mine ve yüz elli adet dentin örneği hazırlandı. Mine örnekleri başlangıç çürük lezyonlarını oluşturmak için demineralizasyon solüsyonuna (pH=4,5) tabi tutuldu ve beş gruba ayrıldı (n=15). Kontrol grubu (tedavi yok), (S) Sensodyne Onarım ve Koruma, (M) Moringa oleifera içerikli bitkisel diş macunu, (MF) Florürlü Moringa oleifera içerikli bitkisel diş macunu ve (MO) Moringa oleifera yağı. Dentin örnekleri, dentin tübüllerinin açılması için 2 dakika boyunca %6 sitrik asit çözeltisinde bekletildi ve yedi gruba (n=15) ayrıldı: Kontrol, (S), (M), (MF), (MO), (R) R.O.C.S. Sensitivity ve (O) Oral-B Hassasiyet ve Diş Eti Rahatlama. Ajanlar, yedi gün boyunca günde 3 kez bir pH döngüsü modeli kullanılarak uygulandı. Tüm ajanlar örneklerin yüzeyinde 2 dakika bekletildikten sonra elektrikli diş fırçası (Oral-B Test Drive (Genius)) kullanılarak 1 dakika süreyle uygulandı. Örnekler mikrosertlik cihazı ve floresans bazlı cihazlarla (FluoreCam ve DIAGNOdent Pen) değerlendirildi.

Bulgular: Floresans ve mikrosertlik değerlendirmelerine göre, mine örneklerinde remineralizasyon etkisi Moringa oleifera yağı hariç tüm tedavi gruplarında benzerdi ($p<0,001$). Dentin örneklerinde tedavi sonrası mikrosertlik değerleri MO ve kontrol grubunda anlamlı derecede düşüktü ($p<0,001$). Floresan sonuçları tedavi gruplarında MO ve kontrol grubuna göre üstün bulundu ($p<0,001$).

Sonuç: Moringa oleifera içeren bitkisel diş macunları remineralizasyon ve dentin tübül tıkaçlanmasında etkili olmuştur.

Anahtar kelimeler: Bitkisel diş macunu, demineralizasyon, Moringa oleifera, remineralizasyon, dentin aşırı duyarlılığı.

INTRODUCTION

As interest in natural products for oral and dental care grows, the variety of herbal products available on the market continues to expand. For this reason, the pharmaceutical properties of the plants used in these products and their possible effects in treating diseases are important research topics. Herbal products are valuable potential resources for dental disease treatments, yet their effects on dental hard tissues remain inadequately explored.[1,2] Among these plants, Moringa oleifera, called the miracle tree, is used in the treatment of many diseases in traditional medicine. It also contains minerals, fats, vitamins, proteins and vitamins as an important nutritional source.[3] In addition to its nutritional content, the Moringa oleifera plant also has potential health benefits.[4] Due to its high calcium, potassium, and natural protein content, Moringa oleifera is believed to aid in the remineralization of demineralized enamel.[5]

Dentin sensitivity is one of the most common dental problems. Erosion caused by acidic foods and drinks negatively affects the daily life of the individual by exposing the dentinal tubules and causing dentine sensitivity. Sensitive dentin has more and wider dentinal tubules than non-sensitive dentin.[6] Occluding dentin tubules by using daily toothpaste is one of the methods used in the treatment of dentine sensitivity.[7] It has been established that desensitizing toothpastes are useful in the treatment of dentin sensitivity.[8,9]

Studies in the literature showed that Moringa oleifera-containing products show enamel and dentin remineralization.[10-14] It has been reported that Moringa oleifera increases calcium levels in dental tissue and therefore contributes to remineralization.[11,12,14] Moreover, it has been indicated that the use of Moringa oleifera extract is effective in reducing dentin permeability.[15] Moringa oleifera-based toothpaste could be developed as an additional ingredient that could be used in the remineralization and dental calcification processes.

This study aimed to investigate the remineralization and tubule occluding effects of two experimental toothpastes containing Moringa oleifera oil and extract, one containing fluoride and one without, and Moringa oleifera oil on dental hard tissues, by comparing them with conventional toothpastes. The hypothesis of the study was designed so that toothpastes containing Moringa oleifera would have similar remineralization and dentin tubule occluding effects to conventional toothpastes.

Table 1. Treatment agents and their content used in the study.

Agents	Content
Moringa oleifera containing experimental toothpaste (M) (SPC Kozmetik, İstanbul, Türkiye)	5% Moringa oleifera oil, 5% Moringa oleifera Extract, Mint oil, Mentol, Polisorbat 20, Xanthan Gum, Aqua, Glyserin, Sorbitol, Phenylpropanol, Capryly Glycol Stevia (Rebaudioside A), Hydrated Silica
Moringa oleifera+Fluor containing experimental toothpaste (MF) (SPC Kozmetik, İstanbul, Türkiye)	5% Moringa oleifera oil, 5% Moringa oleifera Extract, Mint oil, Mentol, Polisorbat 20, Xanthan Gum, Aqua, Glyserin, Sorbitol, Phenylpropanol, Capryly Glycol Stevia (Rebaudioside A), Hydrated Silica, 1450 ppm NaF
Moringa oleifera oil (MO) (Moringantep, Gaziantep, Türkiye)	Moirnga Oliefera Seed Oil
Sensodyne Repair and Protect (Novamin) (S) (Glaxosmithkline (Gsk), Waterford, Ireland)	Gliserin, Peg-8, Hydrated Silica, Calcium Sodium Phosphosilicat (Novamin), Cocamidopropil Betain, Sodium Metyl Cocoyl Taurate, Aroma, Titanium Dioksit, Carbomer, Silica, Sodium Sakkarin, Sodium Flouride (1450 Ppm), Limonene.
Oral-B Sensitivity and Gum Calm (SnF) (O) (Procter & Gamble, Schwalbach, Germany)	Aqua, Sorbitol, Hydrated Silica, Sodium Lauryl Sulfate, Sodium Gluconate, Carrageenan, Aroma, Xanthan Gum, Cocamidopropyl Betaine, Zinc Citrate, Stannous Fluoride, Sodium Hidroxyde, Stannous Chlorite, Sodium Saccharine, CI 77891, Sodium Fluorid, Cinnamal, Benzyl Alkohol, Sucralose, Eugenol, Sodium Benzoate, Citric Acid, Sodium Citrate, Potassium Sorbate (1450 Ppm Fluoride, 0.61% Stannous).
R.O.C.S. Sensitive (%50 Calcium Hydroxyapatite suspension) (R) (R.O.C.S. Trading Gmbh, Munich, Germany)	Aqua, Silica, Glycerin, Xylitol, Hydroxyapatite, Xanthan Gum, Aroma, Calcium Glycerophosphate, Cocamidopropyl Betaine, Sodium Lauroyl Sarcosinate, Hydroxyacetophenone, Sodium Benzoate, Sodium Saccharine, Magnesium Chloride, Sodium Methylparaben, Sodium Propylparaben, O-cymen-5-ol, CI 74160, Limonene.

MATERIAL AND METHOD

Ethical approval of the study was obtained from the Clinical Research Ethics Committee of the Marmara University School of Medicine (Date: 07.05.2021 Protocol no: 09.2021.626).

Sample Preparation

The number of samples was evaluated using G*power version 3.1.9.7 (α=0.05, 1-β=0.95) and the sample size was determined as 15 samples for each group based on a previous study.[16,17] Seventy-five enamel samples and one-hundred-and-five dentin samples were prepared from the extracted teeth using a handpiece (NSK EX-6, Japan) and diamond disc (Sigmadent, Türkiye). Enamel and dentin samples were embedded in acrylic molds, and their surfaces were polished using 1200-grit silicon carbide discs for 5 minutes. The samples were kept in a jar with distilled water at +4°C until the experiments were carried out.

Experimental Groups

Study groups were divided into five groups (n=15) for enamel samples and divided into 7 groups (n=15) for dentin samples. Table 1 represents the agents used in the treatment and their content. Moringa oleifera seed oil and aqueous Moringa oleifera extract obtained from Moringa oleifera leaves along with other ingredients (shown in Table 1) were used to manufacture experimental toothpastes containing Moringa oleifera and Moringa oleifera with Fluor (1450 ppm). No treatment was carried out on enamel and dentin surfaces in control groups.

Demineralization Process on Enamel Samples

Enamel samples were subjected to To create initial enamel lesions, a demineralization solution (pH=4.7) containing 2.0 mmol/L $Ca(NO_3)2.H_2O$, 2.0 mmol/L $NaH_2PO_4H_2O$, and 0.04 µg F/ml (NaF) in 75 mmol/L acetate buffer was used. in a shaking incubator (ZWYR-240, LABWIT, Australia) at 60 rpm, at 37°C for 72 hours, renewing the solution daily.[18]

Treatment Process for Enamel Samples

The treatment agents were applied 3 times a day using a remineralization-demineralization cycle for seven days with 6 hours of demineralization and 18 hours of remineralization each day.[19] Each treatment time, the agents were kept on the enamel surface for 2 minutes and then a rechargeable toothbrush (Oral-B Test Drive (Genius), Germany) was used for one minute to apply the toothpastes. A micro brush was used to apply Moringa oleifera oil (MO). The agents were kept on the enamel and dentin samples for a total of 3 minutes for each treatment time.

Exposing the Dentin Tubules

Dentin tubules were exposed using 6% citric acid solution (pH=2.5) for 2 minutes to imitate the hypersensitive dentin and then washed with distilled water for 30 seconds.[20]

Treatment Process for Dentin Samples

The treatment agents were applied as the same procedure described for enamel samples and an erosive cycle model was carried out for the dentin samples. The samples were kept in artificial saliva (1.5 mmol/l $CaCl_2$, 50 mmol/l KCl, 0.9 mmol/l KH_2PO_4, and 20 mmol/l Tris, pH=7.4) and immersed in 2% citric acid (pH=2.5) solution for 2 minutes 3 times a day. Before applying the treatment agents the samples were immersed in artificial saliva for 30 minutes to avoid the erosive effect of citric acid application.

Assessment Methods

All enamel and dentin samples were evaluated with microhardness device and fluorescence-based devices (DIAGNOdent Pen and FluoreCam) at baseline, after demineralization/exposing dentin tubules, and after treatment stages as described below.

Microhardness Assessment

The surface microhardness values of enamel and dentin samples were evaluated using a microhardness tester (Shimadzu, Corporation, Japan) at baseline, after demineralization/exposing dentin tubules and after treatment stages. Three different measurements were made on enamel and dentin surfaces by applying 200 g of force for 15 seconds. The average of three measurements was recorded for each sample.

DIAGNOdent Pen Assessment

The laser fluorescence-based DIAGNOdent Pen (Kavo, Germany) device was used as recommended by the manufacturer. The device was calibrated before each use and the recommended B-type probe was used held vertically on the samples. Laser fluorescence values were recorded of the samples from 3 different locations at baseline, after demineralization/exposing dentin tubules and after treatment stages. The average of three measurements was recorded for each sample.

FluoreCam Assessment

FluoreCam device (Daraza Corporate Headquarters, USA) was used to assess the fluorescence changes on enamel and dentin samples at baseline, after demineralization/exposing dentin tubules and after treatment stages. Images taken at baseline, after demineralization/exposing dentin tubules and after treatment stages with the FluoreCam system were compared in terms of size and fluorescence values.

Assessment of Scanning Electron Microscopy (SEM) Images

In order to clear the scanned area, the surface of the samples was cleaned with 5% sodium hypochlorite solution and then washed with distilled water. Ethyl alcohol solution was kept on the samples for 10 minutes, then the samples were left to dry for 24 hours before Scanning Electron Microscopy (SEM) examination. The samples coated with platinum-gold alloy were assessed at 10 kV acceleration voltage in the SEM device (ZEISS EVO MA 10, Germany) recording the images of the dentin samples at x2000 magnification at baseline, after exposure of dentin tubules and after treatment stages.

Statistical Analysis

Data were analyzed with IBM SPSS V25. Compliance with normal distribution was examined with the Shapiro-Wilk test. One-Way Analysis of Variance (ANOVA) was used to compare normally distributed data. The Welch test was used for variances that are not homogeneously distributed. Tamhane and Tukey HSD tests were used for multiple comparisons. The Kruskal Wallis H test was used to compare data that did not show normal distribution, and multiple comparisons were examined with the Dunn test. In intragroup comparisons, Repeated Analysis of Variance was used for normally distributed data, and the Friedman Test was used for non-normally distributed data. Analysis results were presented as mean±standard deviation and median (minimum-maximum) for quantitative data. The significance level was taken as $p<0.05$.

RESULTS

Microhardness Assessment

All groups of enamel samples had statistically similar microhardness values at baseline and after demineralization stages ($p=1.000$; $p=0.967$). Following the demineralization process, microhardness values significantly decreased but increased again after remineralization treatment ($p<0.001$). After treatment, the control group showed significantly lower values, while toothpaste-applied groups (M, MF, S) were found to be similar. Although the application of MO did not increase the microhardness values as much as the toothpaste groups, the MO group had higher values than the control group. When the difference between the post-treatment and demineralization values (T3-T2) was examined, the lowest difference was seen in the control group, while the toothpaste groups were found to be similar (Table 2).

Initial microhardness values of dentin samples are similar ($p=0.704$). All values decreased after acid exposure. While microhardness values increased after treatment, the lowest increase was seen in the control group, followed by the MO groups. Toothpaste groups showed a significantly higher increase than MO and control groups ($p<0.001$). The greatest increase in microhardness was observed in the MF group, followed by the S and M groups (Table 3).

Table 2. Microhardness results of enamel samples applied different treatment agents.

	Baseline (T_1)	Demineralization (T_2)	Remineralization treatment (T_3)	(T_3-T_2)	*p***
Moringa TP (M)	304.47 ± 15.88^{A}	191.73 ± 10.57^{B}	288.33 ± 11.15^{Cb}	96.60 ± 8.02^{b}	<0.001
Moringa+Fluor TP (MF)	304.13 ± 14.43^{A}	192.13 ± 9.26^{B}	291.53 ± 8.53^{Cb}	99.40 ± 3.90^{b}	<0.001
Moringa oil (MO)	303.80 ± 11.88^{A}	192.47 ± 9.63^{B}	$248.60\ \pm10.42^{Cc}$	56.13 ± 7.93^{c}	<0.001
Sensodyne TP (S)	304.87 ± 11.73^{A}	192.60 ± 9.18^{B}	291.20 ± 8.89^{Cb}	98.6 ± 6.67^{b}	<0.001
Control	304.33 ± 8.49^{A}	194.07 ± 6.77^{B}	227.13 ± 9.11^{Ca}	33.07 ± 6.35^{a}	<0.001
p*	1	0.967	<0.001	<0.001	

:** One way analysis of variance, *:** repeated measures analysis of variance, same letter codes have no significant difference,±standard deviation, **TP:** toothpaste.

Table 3. Microhardness results of dentin samples applied different treatment agents.

	Baseline (T_1)	Acid exposure (T_2)	Treatment (T_3)	(T_3-T_2)	*p****
Moringa TP (M)	77.00 ± 4.59^{A}	36.33 ± 3.96^{Bab}	$73.00\pm3.46C^{ab}$	36.67 ± 4.30^{ab}	<0.001
Moringa+Fluor TP (MF)	78.73 ± 5.02^{A}	35.87 ± 4.07^{Bab}	74.87 ± 3.83^{Cb}	39.00 ± 1.69^{a}	<0.001
Moringa oil (MO)	77.33 ± 4.51^{A}	34.87 ± 3.81^{Bb}	49.13 ± 2.85^{Cc}	14.27 ± 1.94^{d}	<0.001
Sensodyne TP (S)	79.27 ± 4.90^{A}	$39.13\pm\ 3.94^{Ba}$	75.87 ± 2.97^{Cb}	36.73 ± 3.13^{a}	<0.001
Oral-B TP (O)	78.87 ± 4.08^{A}	37.20 ± 4.3^{Bab}	70.73 ± 2.68^{Ca}	33.53 ± 3.44^{c}	<0.001
R.O.C.S. TP (R)	79.07 ± 3.86^{A}	38.67 ± 3.11^{Bab}	72.40 ± 2.69^{Cab}	33.73 ± 1.28^{bc}	<0.001
Control	79.13 ± 4.36^{A}	37.93 ± 3.73^{Bab}	41.33 ± 3.52^{Cd}	3.40 ± 1.64^{e}	<0.001
p	0.704*	0.032*	<0.001*	<0.001**	

:** One way analysis of variance, *:** Welch test, *****:** repeated measures analysis of variance, same letter codes have no significant difference,±standard deviation, **TP:** toothpaste.

Table 4. DIAGNOdent Pen results of enamel samples applied different treatment agents.

	Baseline (T_1)	Demineralization (T_2)	Remineralization treatment (T_3)	(T_3-T_2)	*p****
Moringa TP (M)	6.13 ± 1.41^{A}	15.87 ± 1.13^{B}	7.40 ± 1.45^{Cbc}	-8.47 ± 0.99^{b}	<0.001
Moringa+Fluor TP (MF)	6.93 ± 1.53^{A}	15.80 ± 1.32^{B}	7.93 ± 1.16^{Ac}	-7.47 ± 1.68^{b}	<0.001
Moringa oil (MO)	6.60 ± 1.45^{A}	15.73 ± 1.67^{B}	8.47 ± 2.07^{Cc}	-7.27 ± 1.62^{b}	<0.001
Sensodyne TP (S)	6.47 ± 1.88^{A}	15.40 ± 1.55^{B}	6.20 ± 1.01^{Ab}	-9.20 ± 1.42^{b}	<0.001
Control	5.53 ± 1.19^{A}	15.87 ± 1.41^{B}	15.27 ± 1.44^{Ca}	-0.60 ± 0.51^{a}	<0.001
p*	0.128*	0.891*	<0.001*	<0.001**	

:** One way analysis of variance, *:** welch test; *****:** repeated measures analysis of variance, same letter codes have no significant difference,±standard deviation, **TP:** toothpaste.

Table 5. DIAGNOdent Pen results of dentin samples applied different treatment agents.

	Baseline (T_1)	Acid exposure (T_2)	Treatment (T_3)	(T_3-T_2)	*p***
Moringa TP (M)	5.40 ± 0.74^{A}	17.87 ± 2.07^{B}	9.47 ± 1.59^{Cd}	-8.40 ± 1.99^{c}	<0.001
Moringa+Fluor TP (MF)	5.40 ± 1.24^{A}	17.20 ± 1.32^{B}	8.87 ± 1.41^{Cad}	-8.30 ± 1.45^{c}	<0.001
Moringa oil (MO)	5.80 ± 1.21^{A}	17.67 ± 0.98^{B}	11.20 ± 1.78^{Cc}	-6.47 ± 1.50^{b}	<0.001
Sensodyne TP (S)	5.80 ± 1.15^{A}	17.00 ± 1.31^{B}	8.53 ± 1.25^{Cad}	-8.47 ± 0.74^{c}	<0.001
Oral-B TP (O)	5.33 ± 0.82^{A}	17.47 ± 1.06^{B}	7.80 ± 0.94^{Ca}	-9.67 ± 0.82^{c}	<0.001
R.O.C.S. TP (R)	5.60 ± 0.83^{A}	16.67 ± 1.23^{B}	8.60 ± 1.18^{Cad}	-8.07 ± 1.16^{c}	<0.001
Control	6.13 ± 1.50^{A}	17.07 ± 1.16^{B}	17.33 ± 1.72^{Bb}	0.27 ± 1.16^{a}	<0.001
p*	0.402	0.211	<0.001	<0.001	

:** One way analysis of variance, *:** repeated measures analysis of variance, same letter codes have no significant difference,±standard deviation, **TP:** toothpaste.

DIAGNOdent Pen Assessment

When the fluorescence values obtained from the DIAGNOdent Pen device were examined, no significant difference was found at the baseline and after demineralization/acid exposure procedures for both enamel and dentin samples (Tables 4, 5). An increase was observed in DIAGNOdent Pen values after demineralization and acid exposure for all samples. After treatment, a significant decrease was observed for all groups except for the control group ($p<0.001$).

FluoreCam Assessment of Enamel Samples

According to the results obtained from the FluoreCam system, the decrease in lesion size after remineralization treatment (T3-T2) was greater in the S group ($p<0.001$). Besides, the decrease in lesion size (T3-T2) of the M and MF groups was similar to the S group (Table 6). A significant worsening in lesion size was observed in the control group compared to the toothpaste-applied groups ($p<0.001$), while a slight decrease was seen in the MO group. The toothpaste-applied groups (M, MF, S) showed "acute improvement" according to the FluoreCam system (Figure 1). According to the FluoreCam "intensity" results which express fluorescence chances on the sample surface, all treatment groups demonstrated significantly greater fluorescence change than the control group after remineralization treatment (T3-T2) ($p<0.001$) (Table 7).

FluoreCam Assessment of Dentin Samples

After the treatment, the lesion size values obtained from the FluoreCam system decreased in the toothpaste groups as being similar to the values obtained at the baseline. The control group and the group treated with MO had an increase in size values after the treatment stage (Table 8). Toothpaste groups showed mild improvement according to the FluoreCam system (Figure 2). The change in fluorescence values after the treatment (T3-T2) was significantly greater in MF, S, and O groups than in other treatment groups ($p<0.001$) (Table 9).

Assessment of Scanning Electron Microscopy Images

The Scanning Electron Microscopy (SEM) images revealed that after acid exposure the dentin tubules were wide open (Figure 3h). The SEM images taken after the treatment process demonstrated that toothpaste groups were more effective in dentin tubule occlusion than the MO and the control group. A slight tubule narrowing was observed in MO and the control group because they were kept in artificial saliva during the erosive cycle process (Figure 3).

Table 6. FluoreCam 'size' results of enamel samples applied different treatment agents.

	Baseline (T_1)	Demineralization (T_2)	Remineralization treatment (T_3)	(T_3-T_2)	p**
Moringa TP (M)	3.62 (2.73/4.86)^{Aa}	5.27 (3.80/6.43)^{Bb}	3.54 (2.07/4.84)^{Ab}	-1.84 (-2.70/1.04)^{bc}	<0.001
Moringa+Fluor TP (MF)	3.19 (2.42/4.77)^{Aa}	5.38 (3.38/6.94)^{Bb}	3.28 (2.07/4.62)^{Ab}	-1.66 (-3.29/-0.77)^{bc}	<0.001
Moringa oil (MO)	5.33 (4.16/7.67)^{Ab}	6.84 (4.47/8.69)^{Bab}	6.67 (4.03/8.22)^{ABa}	-0.88 (-3.80/2.89)^{ac}	0.002
Sensodyne TP (S)	5.68 (3.01/7.64)^{Ab}	8.08 (4.18/9.54)^{Ba}	4.35 (3.13/6.35)^{Cab}	-2.67 (-4.07/-0.88)^{b}	<0.001
Control	3.67 (3.10/5.01)^{Aa}	5.05 (4.10/8.36)^{Bab}	6.80 (3.69/9.02)^{Ba}	0.76 (-1.24/2.81)^{a}	<0.001
p*	<0.001	<0.001	<0.001	<0.001	

:** Kruskal Wallis test, *:** friedman test, same letter codes have no significant difference, (min/max), **TP:** toothpaste.

Table 7. FluoreCam 'intensity' results of enamel samples applied different treatment agents.

	Baseline (T_1)	Demineralization (T_2)	Remineralization treatment (T_3)	Fark (T_3-T_2)	p**
Moringa TP (M)	-8.02 (-10.79/-6.01)^{Aab}	-15.40 (-18.92/-10.01)^{B}	-5.46 (-8.89/-3.01)^{Cbc}	9.94 (4.18/13.11)^{b}	<0.001
Moringa+Fluor TP (MF)	-8.75 (-10.98/-6.9)^{Ab}	-15.03 (-18.73/-12.74)^{B}	-4.75 (-7.50/-3.11)^{Cb}	10.38 (7.04/15.62)^{b}	<0.001
Moringa oil (MO)	-8.65 (-10.92/-6.75)^{Ab}	-16.17 (-18.67/-11.16)^{B}	-7.31 (-9.77/-5.73)^{Cac}	8.43 (3.85/11.36)^{b}	<0.001
Sensodyne TP (S)	-7.72 (-10.62/-5.73)^{Aab}	-15.62 (-18.79/-10.66)^{B}	-5.3 (-9.03/-3.28)^{Cb}	10.79 (3.51/14.05)^{b}	<0.001
Control	-6.74 (-9.71/-5.11)^{Aa}	-15.32 (-18.57/-10.05)^{B}	-14.58 (-18.31/-9.34)^{Ca}	0.74 (-0.26/2.27)^{a}	<0.001
p*	0.008	0.929	<0.001	<0.001	

:** Kruskal Wallis test, *:** friedman test, same letter codes have no significant difference, (min/max), **TP:** toothpaste.

Table 8. FluoreCam 'size' results of dentin samples applied different treatment agents.

	Baseline (T_1)	Acid exposure (T_2)	Treatment (T_3)	(T_3-T_2)	p**
Moringa TP (M)	2.52 (1.31/3.38)^{A}	3.02 (2.15/3.65)^{B}	2.49 (1.7/2.98)^{Aa}	-0.53 (-1.46/0.49)^{a}	0.001
Moringa+Fluor TP (MF)	2.99 (1.75/4.53)^{A}	3.38 (2.12/4.81)^{B}	2.73 (1.76/4.32)^{Aa}	-0.46 (-1.09/-0.18)^{a}	<0.001
Moringa oil (MO)	2.88 (1.15/4.24)^{A}	3.35 (1.68/5.08)^{B}	4.37 (2.14/5.75)^{Cb}	0.67 (0.11/1.32)^{c}	<0.001
Sensodyne TP (S)	3.71 (1.09/4.76)^{A}	3.81 (1.25/5.23)^{B}	3.65 (1.10/5.24)^{Aab}	-0.27 (-1.08/0.01)^{ab}	0.001
Oral-B TP (O)	2.16 (0.83/3.48)^{A}	2.98 (0.88/4.05)^{B}	2.27 (0.83/3.92)^{Aa}	-0.63 (-1.06/0.00)^{a}	<0.001
R.O.C.S. TP (R)	2.95 (1.15/3.56)^{A}	3.49 (1.08/3.93)^{B}	3.01 (1.09/3.34)^{Aab}	-0.45 (-1.04/-0.09)^{a}	<0.001
Control	2.42 (1.01/4.95)^{A}	3 (1.90/5.62)^{B}	3.49 (1.93/5.57)^{Bab}	0.13 (-0.65/- 1.42)^{bc}	<0.001
p*	0.173	0.142	<0.001	<0.001	

:** Kruskal Wallis test, *:** friedman test, same letter codes have no significant difference, (min-max), **TP:** toothpaste

Table 9. FluoreCam 'intensity' results of dentin samples applied different treatment agents.

	Baseline (T_1)	Acid exposure (T_2)	Treatment (T_3)	(T_3-T_2)	p**
Moringa TP (M)	-7.02 (-9.76/-4.81)^{Ab}	-9.16 (-10.88/-6.00)^{Bc}	-7.47 (-9.64/-5.22)^{Ac}	1.4 (0.54/3.38)^{a}	<0.001
Moringa+Fluor TP (MF)	-9.58 (-11.36/-8.22)^{Aa}	-12.34 (-14.34/-10.12)^{Bb}	-7.64 (-9.87/-5.47)^{Cc}	4.32 (2.15/8.65)^{b}	<0.001
Moringa oil (MO)	-8.70 (-11.12/-4.03)^{Aab}	-9.34 (-14.58/-6.66)^{Babc}	-8.00 (-12.73/-6.00)^{Aabc}	1 (-0.59/6.07)^{a}	<0.001
Sensodyne TP (S)	-8.71 (-11.8/-5.54)^{Aab}	-12.77 (-13.95/-7.96)^{Bb}	-9.47 (-11.93/-5.96)^{Aabc}	3.3 (0.75/5.81)^{ab}	<0.001
Oral-B TP (O)	-7.20 (-10.41/-4.94)^{Aab}	-9.93 (-12.45/-6.42)^{Babc}	-8.03 (-9.09/-5.34)^{Aac}	2.83 (0.11/5.85)^{ab}	<0.001
R.O.C.S. TP (R)	-7.68 (-11.42/-3.06)^{Aab}	-9.40 (-12.19/-4.58)^{Bac}	-6.57 (-11.47/-3.81)^{Aac}	0.80 (0.10/2.94)^{a}	<0.001
Control	-8.88 (-12.02/-6.64)^{Aa}	-11.40 (-14.29/-8.45)^{Babc}	-10.12 (-13.12/-7.96)^{ABb}	0.92 (-1.72/5.44)^{a}	0.005
p*	0.004	<0.001	<0.001	<0.001	

:** Kruskal Wallis test, *:** friedman test, same letter codes have no significant difference, (min-max), **TP:** toothpaste

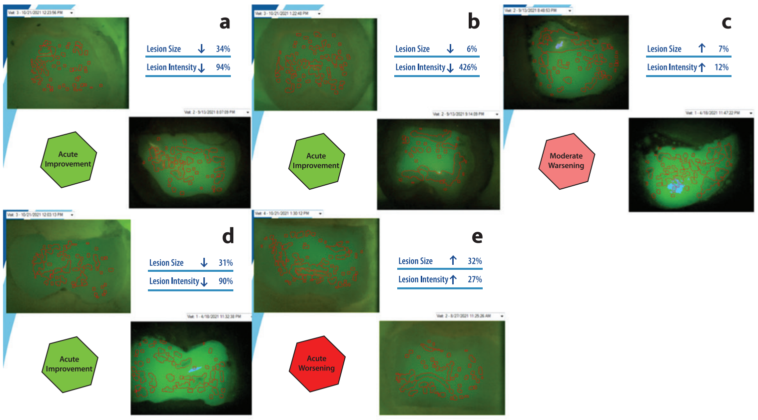

Figure 1. Comparison of fluorescence changes on enamel samples between demineralization and after treatment by FluoreCam system; **a**: Moringa oleifera containing experimental toothpaste (Acute improvement), **b:** Moringa oleifera and Fluor containing experimental toothpaste (Acute improvement), **c:** Moringa oleifera oil (Moderate worsening), **d:** Sensodyne toothpaste (Acute improvement), **e:** Control group (Acute worsening).

DISCUSSION

In this study, the effect of Moringa oleifera on dental hard tissues was investigated. According to the results of this study, herbal toothpastes containing Moringa oleifera extract and Moringa oleifera oil had remineralization effects on enamel and tubule occluding effects on dentin, similar to conventional toothpastes. However, the use of MO alone was not effective.

Moringa oleifera plant has the potential to have positive effects on dental tissues. The role of Moringa oleifera extract in remineralization is not yet fully understood, but several mechanisms may explain its effect. Moringa oleifera enhances remineralization by increasing the pH level. Additionally, the high concentration of minerals and various amino acids it contains may play a role in regulating mineral accumulation and leading to the formation of enamel crystals.[5] A study investigating the remineralization effect of different proteins compared whey protein (45% of all amino acids) with Moringa protein (47% of all amino acids) and reported that Moringa leaf powders with higher protein content had a greater remineralization effect.[5]

Younis *et al.* investigated the remineralization effect of varnish containing 50 mg/ml and 200 mg/ml Moringa oleifera leaf extract on enamel.[11] They stated that the natural proteins contained in Moringa prevent enamel demineralization and increase remineralization by increasing calcium absorption. They argued that the proteins found in Moringa oleifera cause more calcium to be absorbed from artificial saliva and the Moringa oleifera minerals contained in the extract. Researchers also reported that the varnish group containing Moringa oleifera with a high peptide concentration (200 mg/ml) formed a thick, dense, and solid enamel-like mineralized layer. They concluded that Moringa oleifera may accelerate the remineralization of enamel lesions, that Moringa oleifera minerals may chemically interact with enamel minerals, and that peptide-guided remineralization may be the basis for the ability to form a new layer that resembles healthy enamel structure.[11] The results of this study demonstrate that herbal toothpastes containing Moringa oleifera have a remineralization potential comparable to conventional toothpastes containing fluoride and Novamin. Both fluoride-free and fluoridated combinations of Moringa oleifera in toothpaste formulation significantly increased the surface microhardness on demineralized enamel samples (Table 2). Moreover, fluorescence assessment revealed mineral gain increased significantly with the use of these toothpastes (Tables 4,6,7).

Studies have compared the effects of calcium/phosphate solutions with small peptides derived from amelogenin, a natural protein. Doğan *et al.* observed that small peptides derived from amelogenin form a thick (10 µm), densely mineralized layer containing hydroxyapatite on human tooth enamel, resembling a healthy enamel structure.[21] Damle stated that peptides could be used to treat dental caries by designing a product that uses proteins. The researcher concluded that the peptide was able to bind to tooth surfaces and accumulate calcium and phosphate ions, forming 10-50 µm of new enamel and reported that proteins are a good alternative for remineralization therapy.[22] The positive remineralization effects of Moringa oleifera observed in this study can be explained by the high protein content of this plant positively affects remineralization.

Anas *et al.* reported that Moringa oleifera-containing paste and CPP-ACP application were effective in increasing calcium levels in teeth.[14] Additionally, no statistically significant difference was found between these two applications in terms of their effectiveness in increasing dental calcium levels. They concluded that Moringa oleifera-based paste can be improved as an additional product in remineralization.[14] In the current study, the microhardness and fluorescence results of experimental herbal toothpastes containing Moringa oleifera showed that remineralization treatment was succesful (Tables 2,4,6,7). Furthermore, although the addition of fluoride increased the remineralization effect of the toothpaste, no significant difference was observed between fluoridated and fluoride-free formulations.

Similar to Anas *et al.*'s study, Khalaf *et al.* indicated that Moringa leaf extract prevents enamel and dentin erosion by localizing calcium and phosphate on the tooth surface and has the same effect as CPP-ACPF paste on enamel and dentin remineralization. In SEM

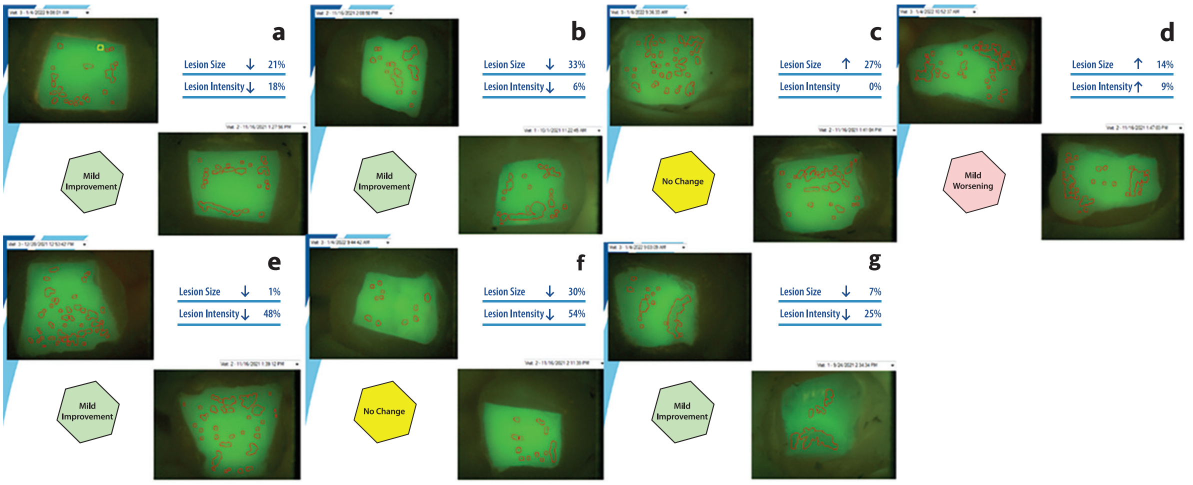

Figure 2. Comparison of fluorescence changes on dentin samples between acid exposure and after treatment by FluoreCam system; **a:** Moringa oleifera containing experimental toothpaste (Mild improvement), **b:** Moringa oleifera and Fluor containing experimental toothpaste (Mild improvement), **c:** Moringa oleifera oil (No change), **d:** Control group (Mild worsening), **e:** Sensodyne toothpaste (Mild improvement), **f:** Oral-B toothpaste (No change), **g:** R.O.C.S. toothpaste (Mild improvement).

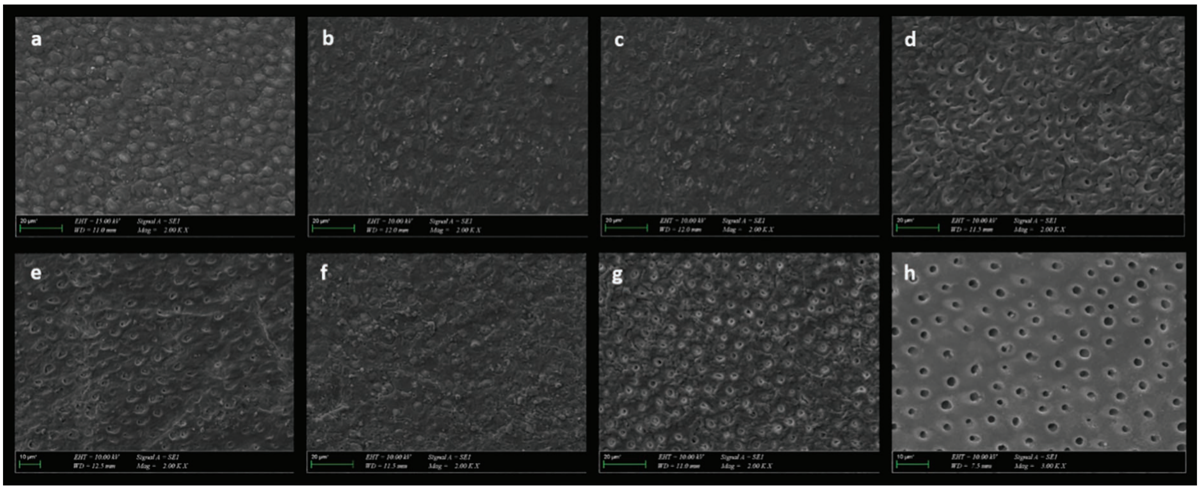

Figure 3. The Scanning Electron Microscopy (SEM) images of dentin samples after treatment; **a:** Moringa oleifera containing experimental toothpaste, **b:** Moringa oleifera and Fluor containing experimental toothpaste, **c:** Moringa oleifera oil, **d:** Control group, **e:** Sensodyne toothpaste, **f:** Oral-B toothpaste, **g:** R.O.C.S. toothpaste, **h:** After acid exposure.

analysis, they reported that calcium deposits were seen in enamel samples applied with Moringa leaf extract and the decrease in the diameter of dentinal tubules was greater than CPP-ACPF.[23] In this study SEM images of dentin samples demonstrated that dentin tubules were occluded after toothpaste application (Figure 3).

Farouk *et al.* reported that black tea, green tea and Moringa oleifera tea have a remineralization effect on demineralized enamel and dentin, however, the highest remineralization effect was seen in Moringa oleifera tea and stated that these products can be used as natural remineralization agents in daily dental care. They stated that Moringa oleifera leaves are effective in remineralization because they are rich in β-carotene, protein, vitamin C, calcium and potassium and are a good source of natural antioxidants.[24] In this study, the results of enamel samples as well as dentin samples showed that toothpaste application increased the surface microhardness and remineralization (Tables 2-9). However MO application was not effective in remineralization for both enamel and dentin samples.

The use of desensitizing toothpaste is generally preferred as the first treatment option for dentin hypersensitivity due to the advantages such as low cost, easy access and application.[25] In this study, two herbal toothpastes, one containing fluoride and one without, containing Moringa oleifera leaf extract and oil, as well as conventional toothpastes with various active ingredients were used. A previous study noted that the effectiveness of desensitizing toothpastes varies with recurrent acid attacks.[26] Dentin tubules occlude as a result of the use of toothpaste may reopen after a short time due to daily brushing, the washing effect of saliva, and acidic nutrition.[27] For this reason, any particle accumulation on the dentin surface resulting from the use of toothpaste can easily be removed from the surface after erosion attacks and/or contact with saliva. In all groups with herbal or conventional toothpaste application, occlusion was observed in the dentinal tubules, even in an erosive cycle model for 7 days. Therefore, it can be assumed that the active ingredients included in the study have a certain resistance to repeated acid attacks.

Citric acid was used in this study to simulate the oral environment and test the resistance of desensitizing toothpastes to acid attacks, as it has been widely used in previous studies on enamel and dentin erosion and is a common ingredient found in fruit juice and soft drinks.[28] Application of 6% citric acid used in the study for 2 minutes significantly increased dentin permeability (Figure 3h). However, dentin samples treated with the desensitizing toothpastes used in this study showed greater resistance to acid attacks than the control group and the group treated with MO.

Previous studies have shown that brushing with toothpaste causes dentin erosion, causing the dentin tubules to expose.[29,30] On the other hand, some in vitro studies reported that dentin permeability decreased after brushing with toothpaste.[31,32] In this study, dentin samples were kept in saliva solution for 30 minutes after the application of citric acid to minimize the erosive effects of brushing after the acid attack.

Calcium sodium phosphosilicate, known as Novamin, releases calcium and phosphate ions after contact with saliva, forming hydroxyapatite crystals to block exposed dentin tubules.[33] At the same time, residual calcium sodium phosphosilicate particles can also occlude the tubules.[34] Earl *et al.* showed that toothpaste containing Novamin formed a hydroxyapatite-like layer on the dentinal tubules, sealing the tubules, and its effect was more stable than that of toothpaste containing silica.[35] Burwell *et al.* reported that NovaMin is resistant to repeated acid attacks after a single application.[36] A conventional toothpaste containing Novamin and fluoride (Sensodyne Repair and Protect) was used to investigate the effect of herbal toothpastes on dental hard tissues in both the enamel and dentin part of this study. This toothpaste showed successful remineralization and dentin tubule occlusion effects on both enamel and dentin samples.

It is known that high concentration fluoride agents and frequent fluoride applications are effective in preventing dental erosion. It has been expressed that stannous-containing fluoride products will be the most effective approach to preventing dental erosion.[37] In this study the effect of conventional toothpaste (Oral-B Sensitivity and Gum Calm) containing stannous fluoride on dentin tubule occlusion was investigated. This toothpaste was found to be successful in dentin tubule occlusion, similar to other toothpastes included in the study. After application of the toothpaste containing stannous fluoride the surface microhardness and mineral gain increased as well as the lesion size decreased.

In recent years, desensitizing products containing nano-hydroxyapatite have become widespread. Nano-hydroxyapatite, which is similar in structure and composition to the apatite crystal in tooth enamel, is the nanocrystal form of hydroxyapatite, and it has been found that the use of toothpaste containing nano-hydroxyapatite increases the calcium concentration in saliva.[38] Amaechi *et al.* reported that toothpastes containing nHAP and NovaMin were equally effective in occluding of dentinal tubules, while standard 1,500 ppm fluoride toothpaste was not found to be effective.[39] Observations made with SEM showed that nHAp-containing toothpaste blocked the dentin tubules. Additionally, there are several clinical studies support that toothpastes containing nHAp can significantly reduce DH.[40,41] The present study investigated the effects of a conventional toothpaste containing hydroxyapatite (R.O.C.S. Sensitivity) on dentin tissue. As other toothpaste groups included in this study hydroxyapatite-containing toothpaste was found effective in dentin tubule occlusion and remineralization of dentin samples.

Wiegand *et al.* investigated the effect of olive oil and mouthwash containing olive oil on preventing enamel and dentin erosion.[42] They stated that mouthwash containing 2% olive oil protects against enamel and dentin erosion, but the application of pure olive oil alone does not provide protection. In this study, it was determined that herbal or conventional toothpastes had a protective effect against the corrosive effect of citric acid, but the application of MO alone was not effective. The remineralization and tubule occlusion effect of MO was limited and only a slight change was observed in surface microhardness and fluorescence assessments.

CONCLUSION

The use of experimental herbal toothpaste containing Moringa oleifera oil and Moringa oleifera extract had similar therapeutic effects on enamel remineralization and dentin tubule occlusion as conventional toothpastes. The application of Moringa oleifera oil alone was not effective on treatment of dental hard tissues. Herbal toothpastes containing Moringa oleifera could serve as an alternative treatment for remineralizing initial enamel lesions and reducing dentin hypersensitivity.

*The authors declare that there are no conflicts of interest.

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