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# Effect of experimental moringa and propolis toothpastes on surface microhardness of simulated hypersensitive dentin

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## Abstract

**Objective:** Objective was to investigate the effect of NovaMin toothpaste and two experimental toothpastes on surface microhardness of hypersensitive dentin.

**Methods:** Eighty specimens were prepared and divided randomly into eight groups ( $n = 10/\text{group}$ ) according to the tested toothpastes (NovaMin, moringa and propolis toothpastes) and the acidic challenge (with or without 6% citric acid challenge). Enamel surfaces were removed, and the dentin surfaces were exposed on which the three tested toothpastes were brushed twice a day for 1 week using an electric toothbrush, then a 4-d citric acid cycling model with pH 5 was performed. The prepared specimens were stored in artificial saliva until the microhardness test was performed. Statistical analysis was done using One-way ANOVA followed by Tukey post hoc test.

**Results:** NovaMin-containing toothpaste showed an increased dentin surface microhardness following a week of twice daily brushing compared to other groups. However, NovaMin-containing toothpaste showed the highest microhardness values following pH cycling with citric acid of pH 5 compared to other tested groups.

**Conclusions:** NovaMin-containing toothpaste showed a partial recovery of dentin microhardness at pH 5 during the acidic challenge. Moreover, propolis- and moringa-containing toothpastes had an insignificant effect on dentin surface microhardness with and without citric acid challenge.

**Keywords:** Moringa, Propolis, Toothpastes, Microhardness, Dentin hypersensitivity

## Background

Dentin hypersensitivity is a widely prevalent dental condition that is frequently found within adults with a variable incidence. It can be defined as “short, sharp snapping pain of an exposed dentin surface in response to different stimuli, that might be tactile, evaporative, thermal, chemical or osmotic that cannot be attributed to any other form of dental pathology” (Addy 2005).

For the treatment of dentin hypersensitivity, different maneuvers have been advocated. Recently, NovaMin which is a calcium sodium phospho-silicate product was originally developed to help in case of bone regeneration. It was found to react with saliva releasing sodium, calcium and phosphate leading to formation of hydroxycarbonate apatite that deposits over the dentin surface and inside the dentinal tubules assisting teeth remineralization and acting as a desensitizing agent (Ananthakrishna et al. 2012).

Nowadays, herbal medicine is significantly popular in many dental fields. Herbal medicine employs a wide number of natural herbal products including chitosan, green tea, miswak, propolis, moringa and many more.

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Propolis is naturally produced by bees to help them building and protecting their sophisticated hives. It is a natural, non-toxic material that enjoys the following advantages: antimicrobial, antiviral, antifungal, anti-inflammatory and anticancer activities. In dentistry, propolis assists in dental caries prevention and promotes periodontal health. Furthermore, it can be used as a pulp capping material or as a cavity and root canal disinfectant. Lately, propolis has been considered for its positive effect on dentin hypersensitivity (Khurshid et al. 2017).

*Moringa oleifera* is a plant that grows primarily in the north of India. Nowadays, *Moringa oleifera* has been widely grown worldwide and it is no longer limited to India. Its leaves are enormously nutritious, and it has a widespread diversity for medicinal uses, therefore it is called “the miracle tree.” It was proven to have anticancer, antibacterial, antifungal, antihypertensive and antidiabetic properties. However, in the recent years, *Moringa* showed a significant antibacterial effect against some oral pathogens (Upadhyay et al. 2015).

Determination of the surface microhardness could deliver an indirect indication of the mineral loss or gain to follow up the mineral content in dental hard tissues due to its sensitivity to the changes that occur at the surface, as well as the composition of the tooth structure (Massoud et al. 2017).

Shifting to the alternative herbal medicine represents the most recent inclination in clinical and preventive dentistry, which may have a significant influence on the treatment of dentin hypersensitivity. Therefore, this study was conducted to evaluate hypersensitive dentin surface microhardness after treatment with an experimentally prepared toothpaste containing moringa and propolis extracts. The null hypothesis was that

moringa- and propolis-based toothpastes have the same effect on the surface microhardness of hypersensitive dentin.

## Methods

### Selected material

A commercially available NovaMin-containing toothpaste and two experimental toothpastes containing moringa and propolis are investigated in the present study and presented in Table 1.

### Study design

Eighty extracted bovine anterior teeth were selected for this study, and they were divided randomly into eight groups ( $n=10$ /group) according to the application of the tested toothpastes (NovaMin, moringa and propolis toothpastes) and the acidic challenge (with or without 6% citric acid challenge) [Group A1: no toothpaste (distilled water) + no acidic challenge, Group A2: no toothpaste (distilled water) + acidic challenge, Group B1: NovaMin toothpaste + no acidic challenge, Group B2: NovaMin toothpaste + acidic challenge, Group C1: moringa toothpaste + no acidic challenge, Group C2: moringa toothpaste + acidic challenge, Group D1: propolis toothpaste + no acidic challenge, Group D2: propolis toothpaste + acidic challenge].

### Propolis and moringa total extracts preparation

Air-dried 50 g of moringa and propolis powder were extracted profoundly by reflux using 70% ethanol to form the crude extract. Both extracts were evaporated under vacuum at 40 °C to complete dryness.

**Table 1** Materials, composition and manufacturers

Name	Composition	Manufacturer
SENSODYNE® repair and protect toothpaste	Calcium sodium phospho-silicate (NovaMin), 5.0% w/w, sodium monofluorophosphate 0.788% w/w (Fluoride 0.104% w/w), (alpha) carbomer, DL-Limonene, glycerin, mint flavor, PEG-8, potassium acesulfame, silica, sodium lauryl sulfate and titanium oxide	GlaxoSmithKline
Propolis	30% ethanolic extract of propolis	SmithKline Beecham Ltd, EUCH CQ, U.K
Moringa	30% ethanolic extract of <i>Moringa oleifera</i>	Imtenan, Egypt
Toothpaste base	Sodium lauryl sulfate, Saccharin, Magnesium oxide, Thymol, Peppermint oil, Glycerin, Acacia	Nature's Market, Egypt
17% EDTA solution	Disodium Edetate, Purified water	SDS, France
Artificial saliva	0.40 g NaCl, 0.48 g KCl, 0.795 g CaCl <sub>2</sub> ·2H <sub>2</sub> O, 0.78 g Na <sub>2</sub> S·H <sub>2</sub> O, 0.005 g NaH <sub>2</sub> PO <sub>4</sub> ·2H <sub>2</sub> O, 1 g Urea, 1 L distilled water	Aldrich, South Korea
		Lobachemie, India
		PEVEST Denpro Limited, India
		EI Gomhourya company, Egypt

### **Toothpaste base preparation**

Using a dry and clean mortar, magnesium oxide, sodium lauryl sulfate (SLS), saccharin and thymol were thoroughly mixed. Glycerin and peppermint oil followed by mucilage of acacia were used to levigate the mixture. Distilled water was added drop by drop with trituration until the desired consistency of prepared toothpaste base is obtained.

### **Preparation of the medicated toothpaste**

A 30% of each of moringa or propolis extract was added to the toothpaste base before addition of water (Torwane et al. 2013). Then, mixture was meticulously mixed, and distilled water was added with trituration until the desired consistency is successfully obtained.

### **Specimen preparation**

Eighty caries-free bovine upper anterior teeth were extracted then manually scaled and thoroughly washed under running water to remove any gross debris. The middle and cervical areas of the labial surface of each crown portion were wet ground using 320 grit silicon carbide (SiC) abrasive paper to remove the enamel layer and expose the underlying dentin surface. Then, the exposed dentin surfaces were finished by wet grinding using a sequence of (SiC) papers of 800–1000 grit (Yuan et al. 2012). Afterward, the roots of all teeth were removed 2 mm below the cemento-enamel junction, using a double-side cutting diamond disk mounted on a low-speed handpiece. Then, the specimens were treated for 5 min using 17% Ethylenediaminetetraacetic acid (EDTA) solution (Kunam et al. 2016) and then rinsed with distilled water; to remove the smear layer and to obtain a smooth dentin surface with patent dentinal tubules mimicking the condition of dentin hypersensitivity clinically.

### **Application of the desensitizing toothpastes**

All tested toothpastes were brushed onto the EDTA-treated dentin specimens using an electrical toothbrush (Oral-B®, Procter & Gamble Co., USA) of medium hardness bristles. The toothbrush was applied at an inclination of 90° approximately to the dentin surface under a constant balanced top loading of 150 g (Wang et al. 2011).

For group **A1** and **A2**; the specimens were brushed with distilled water only, without any toothpaste for 2-min (Arrais et al. 2001) twice daily for 7-d (Wang et al. 2011).

For the rest of the groups; a pea size of the undiluted different desensitizing toothpastes was brushed onto the etched dentin specimens in a wet condition for 2-min, twice daily, for 7-d using the electrical toothbrush. Then, the specimens were rinsed thoroughly with distilled water to remove the remaining toothpaste and the

dentin specimens were stored in the prepared artificial saliva in tightly sealed containers until dentin surface microhardness test is done (Hakeem and Sathyanarayana 2017).

### **Acidic challenge using 6% citric acid**

The assigned groups of dentin specimens (groups: **A2**, **B2**, **C2** and **D2**) were subjected to 6% citric acid challenge. At first, the specimens were electrically brushed twice per day for 2-min for 7-d, and then followed a 4-d schedule of 6% citric acid cycling model (Zaidel et al. 2011) with pH 5. Each citric acid cycle was scheduled for a daily routine which included; brushing the dentin specimens at the beginning of the day with the different tested toothpastes then washing thoroughly with distilled water and storing in the prepared artificial saliva for 1-h. Subsequently, the specimens were immersed in 6% citric acid (pH 5) for 2-min without agitation, washed with distilled water and then stored in the artificial saliva. Specimens were then brushed again with the different toothpastes and incubated for 8-h in the prepared artificial saliva. This daily procedure was continued for four consecutive days.

### **Dentin surface microhardness testing**

The crowns of the treated specimens were embedded horizontally in chemically cured acrylic resin blocks with their labial surfaces facing upward; the labial surface of the crown portion was fixed on a glass slab, a rounded sectional Teflon mold of diameter 4 cm was placed onto the glass slab with the specimen in the center, subsequently, acrylic resin was poured inside the mold, and then another glass slab was placed above the mold after the application of a separating medium to attain a uniform setting of the acrylic resin. Acrylic resin was left to set for 1-h. Dentin microhardness was assessed with Digital Vickers hardness tester (Nexus 4000TM, INNOVTEST, model number 4503, The Netherlands), using a load of 200 g for 15 s (Prabhakar et al. 2013) at magnification  $\times 20$ . Each measurement was the mean value of three readings of three indentations made using Vickers's diamond indenter at three different locations at the center of the dentin surface of each specimen.

### **Statistical analysis**

Standard deviation and mean values were calculated for each group and data were explored for normality using Kolmogorov–Smirnov and Shapiro–Wilk tests and it revealed parametric (normal) distribution. One-way ANOVA followed by Tukey post hoc test was used for comparison between more than two groups in non-related samples. The significance level was set at

$p \leq 0.05$ . Statistical analysis was performed with IBM® SPSS® Statistics Version 20 for Windows.

**Results**

Table 2 shows the effect of different toothpastes application of the mean surface microhardness of dentin with and without citric acid challenge. The data of no citric acid challenge groups revealed that; the highest mean dentin microhardness value was found in NovaMin group (B1) ( $59.62 \pm 0.82$ ), while the least mean dentin microhardness value was recorded for Moringa (C1) ( $47.78 \pm 1.00$ ). There was a statistically significant difference was found between group (A1) and NovaMin group (B1) where ( $p = 0.001$ ), while no statistically significant difference was found between group (A1) and each of Moringa (C1) and Propolis (D1) groups where ( $p = 0.605$ ) and ( $p = 0.530$ ) respectively. A statistically significant difference was found between NovaMin group (B1) and each of Moringa (C1) and Propolis (D1) groups where ( $p = 0.001$ ). No statistically significant difference was found between Moringa (C1) and Propolis (D1) groups where ( $p = 0.077$ ). The data of citric acid challenge groups revealed that; the highest mean dentin microhardness value was found in NovaMin group (B2) ( $50.70 \pm 1.00$ ), while the least mean value was found in group (A2) ( $38.42 \pm 0.50$ ), where distilled water was used instead of the toothpaste. There was a statistically significant difference between group (A2) and each of NovaMin (B2), Moringa (C2) and Propolis (D2) groups where ( $p = 0.001$ ). Moreover, a statistically significant difference was found between NovaMin (B2) and each of Moringa (C2) and Propolis (D2) groups where ( $p = 0.001$ ). In addition, no statistically significant difference was found between Moringa (C2) and Propolis (D2) where ( $p = 0.084$ ).

**Discussion**

Commercially available desensitizing agents are thriving to meet all ideal requirements; mainly the long-term effect, resistance to acidic and mechanical challenges and the remineralizing ability. On the other hand, research has never gave up on developing an agent that enjoys such properties and earn the title of the “gold standard” in management of dentin hypersensitivity. Recently, a paradigm shift has paved the way for natural products to compete with other chemical agents in offering a reliable agent to treat such condition.

In the current study, the different desensitizing toothpastes were brushed onto EDTA etched dentin surface for 2-min two times per day to mimic a daily oral hygiene routine for 7-d. Then, a 4-d citric acid cycling with pH 5 was established. Citric acid was selected for being one of the major organic acids found in fruits juices, as well as soft drinks. This acid challenge was performed as a post-treatment to simulate the consumption of acidic fruits, juices and soft drinks in the oral environment and to evaluate the resistance of the tested toothpastes to the acidic attack (Chen et al. 2015).

The null hypothesis of the current study was rejected, as the results showed that; NovaMin-containing toothpaste was the only toothpaste that was able to increase dentin surface microhardness following a week of twice daily tooth brushing in comparison with the other tested toothpastes. This finding might be related to the chemistry of NovaMin which consists of calcium sodium phospho-silicate which might lead to the deposition of such minerals onto the dentin surface which consequently will cause hardening of the dentin surface.

These results agreed with the study of Parkinson and Willson (2011) who found that; there was a positive change in dentin surface microhardness of the specimens brushed with NovaMin-containing toothpaste after 4 days of brushing. They related the hardening of the dentin surface to the chemical composition of NovaMin and the subsequent formation of mineral

**Table 2** The mean, standard deviation (SD) values of dentin surface microhardness of the tested groups

Variables	Dentin surface microhardness								
	No toothpaste (A)		NovaMin (B)		Moringa (C)		Propolis (D)		p value
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
No acidic challenge (1)	48.96 aB	1.78	59.62 aA	0.82	47.78 aB	1.00	50.26 aB	2.01	0.001*
Citric acid challenge (2)	38.42 bC	0.50	50.70 bA	1.00	41.56 bB	0.78	43.0 bcB	1.26	0.001*
p value	0.001*		0.001*		0.001*		0.001*		

Means with different small letters in the same column indicate statistically significance difference

Means with different capital letters in the same row indicate statistically significance difference

\*Significant ( $p < 0.05$ ), ns; non-significant ( $p > 0.05$ )

deposits in the dentinal tubules, which are harder than the dentin itself. Furthermore, they claimed that; the increase in dentin microhardness might be also due to the presence of residual unreacted calcium sodium phospho-silicate particles attached to the dentin surface. Therefore, they suggested that; the increase in microhardness is due to a combination of dentin surface mineralization and the presence of partially reacted NovaMin particles on dentin surface.

Correspondingly, Jones et al. (2015) conducted an in situ randomized clinical study to investigate the remineralizing ability of NovaMin. They stated that; under the in situ conditions of the study, NovaMin-containing toothpaste exhibited an increase in dentin microhardness after 5 and 10 days of twice daily brushing. They attributed the dentin hardening to the chemical composition of NovaMin in association with the presence of fluoride in the toothpaste formulation.

On the other hand, a contradicting study conducted by Sauro et al. (2011) revealed that; no change occurred in dentin surface microhardness neither after immediate application of a prophylactic paste containing NovaMin onto demineralized dentin specimens, nor following storage in a remineralizing solution. The authors suggested that; single application of NovaMin-containing paste is not enough to induce dentin remineralization.

The present study showed different results than Aguiar et al. (2017) who reported that; the extended use of a dentifrice containing NovaMin on dentin by simulating 2 years of tooth brushing did not promote an increase in the surface microhardness. They attributed the discrepancies of their results with the other studies results to the different methodological design.

In the current study, brushing dentin specimens for a week with Propolis- and Moringa-containing toothpastes and distilled water did not increase the dentin surface microhardness. These findings might be related to the chemical formulation of these toothpastes.

A study conducted by Bhagawat et al. (2016) compared the effect of 4% Propolis, 17% EDTA, 2% chlorhexidine and 18% etidronic acid as irrigants on root dentin microhardness. They concluded that; 4% Propolis produced a slight decrease in dentin microhardness in comparison with EDTA and chlorhexidine, and they recommended its use as an irrigating solution in root canals.

On the other hand, no data were available in the literature till this very day concerning the effect of Moringa extract on dentin surface microhardness.

In the present study, NovaMin-containing toothpaste showed a partial recovery of dentin microhardness occurred at pH 5, these results might be related to the partial re-opening of the dentinal tubules and the incomplete washing out of the calcium sodium phospho-silicate

particles from the dentin surface following the citric acid cycling.

The results of the present study were supported by the results obtained by Diamanti et al. (2010, 2011). The authors stated that; after subjecting root dentin specimens brushed with NovaMin toothpaste to a 50 h acid resistance testing, microhardness values were like those of the control toothpaste. Moreover, microhardness values obtained by NovaMin toothpaste were significantly lower in comparison with the other tested fluoridated toothpastes.

Similarly, the data obtained from Jones et al. (2015) study corroborated the current study. When a 20-d in situ study was conducted to evaluate the mineralizing ability of a NovaMin-containing toothpaste, the authors concluded that; after the introduction of an intra-oral acid challenge with orange juice from day 11 to 20 of the study, dentin surface showed decreased surface microhardness values; however, the results obtained were greater than the placebo control group. The authors explained that; NovaMin maybe providing a degree of protection from demineralization due to the presence of an acidic medium that may accelerate the chemical decomposition of NovaMin which would allow a local buffering effect and prevent more demineralization of the dentin surface.

The results of the current study disagreed with the results obtained by Burwell et al. (2009). The authors claimed that; after subjecting the bovine root dentin specimens to a 10-day pH cycling model; which included immersion of specimens in a demineralizing solution twice daily followed by brushing with 7.5% NovaMin, a partial recovery of dentin surface microhardness occurred, which might be due to the formation of a tenacious surface layer protecting dentin from acidic challenge.

On the other hand, the results of the current study were in contradiction with the data revealed by Parkinson and Willson (2011). They found that; the dentin surfaces treated with NovaMin-containing toothpaste exhibited a relative resistance to twice daily citric acid challenge with pH 3.2 for 2 days in a 4-d study. They related their results to the chemistry of NovaMin in acidic medium, where the frequency of calcium and phosphate release increases.

The data obtained from the current study showed a significant decrease in dentin surface microhardness values of the Propolis and the Moringa groups after citric acid cycling in comparison with the NovaMin group. On the other hand, the group that received no treatment at all (A2) presented the least microhardness values following the citric acid challenge. This decrease in the microhardness values might be related

to the chemical composition of Propolis and Moringa extracts. However, no data were available in the literature till the day concerning the effect of acid challenge on dentin surface microhardness following the application of neither Propolis- nor Moringa-containing products.

Several studies demonstrated the high efficiency of a NovaMin-containing toothpaste in occluding dentinal tubules and maintaining its occlusive effect following acid challenge, in addition to its capability to increase dentin surface microhardness. However, other studies revealed contradicting results. The different results are related to the diversity in methodological designs used for evaluation.

On the other hand, natural products were introduced as a clinically safe and low-cost alternative treatment to compete with the chemical agents in treating dentin hypersensitivity. Some investigations proved the ability of Propolis to obliterate the dentinal tubules and to resist acid attack, while other investigations found the opposite. The contradicted results are related to the different botanical origins, extraction, preparations and delivery methods of Propolis.

## Conclusions

Under the limitations of the in-vitro study, it can be concluded that NovaMin-containing toothpaste was able to increase the dentin surface microhardness and it was able to partially recover after citric acid cycling at pH 5. Both Propolis and Moringa-containing toothpastes had an insignificant effect on dentin surface microhardness with and without acidic challenge.

## Abbreviations

SLS: Sodium lauryl sulfate; SiC: Silicon carbide; EDTA: Ethylene-diamine-tetra-acetic acid.

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Not applicable.

## Authors' contributions

HR and MI had developed the original idea and main conception. MI constructed the study design. LM executed the study methodology and conducted the mechanical tests of the study, interpreted the results, and revised the final presented manuscript. HR was responsible for teeth preparation, application of the investigated materials and pH cycling, writing of the manuscript, extraction of the raw data and initial revision of the manuscript. AS developed the experimental toothpastes and wrote the part of the methodology related to the preparation of the toothpastes. OM, MZ and HN had revised the final manuscript. All authors read and approved the final manuscript.

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## Availability of data and materials

The authors declare that the data supporting the findings of this study are available within the article.

## Declarations

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Competing interests

There are no competing interests.

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