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Effectiveness of a nanoemulsion-containing *Nigella sativa* nanoparticles encapsulated in propolis nanomicelles on dentin tubule occlusion: An scanning electron microscopy study

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ABSTRACT

Background: Dentin hypersensitivity (DH) is a prevalent clinical condition, occurring when exposed dentin reacts to various thermal, chemical, or mechanical stimuli. Although different interventions such as fluoride varnish, adhesives, and natural bioactive compounds have been tested, there is still a demand for more effective and durable solutions. This study aimed to evaluate the ability of a nanoemulsion containing *Nigella sativa* nanoparticles encapsulated in propolis nanomicelles to occlude dentinal tubules and to compare its performance with fluoride varnish under the simulated acidic and mechanical challenges.

Materials and Methods: In this *in vitro* study, hydroethanolic extract of *Nigella sativa* was encapsulated in propolis-based micelles to prepare the nanoemulsion. Thirty-six extracted human third molars were sectioned at the mid-crown and randomly assigned to four groups (n = 9): (1) normal saline, (2) nanoemulsion (15-min immersion), (3) nanoemulsion (30-min immersion), and (4) 5% fluoride varnish. Each group was further divided into three subgroups: control (no challenge), acid challenge, and simulated toothbrushing. Scanning electron microscopy at × 4000 magnification was used to quantify the percentage of occluded dentinal tubules. Data were analyzed with the two-way analysis of variance and least significant difference *post hoc* tests at a significance level of $P \leq 0.05$.

Results: Material type ($P = 0.018$), challenge regimen ($P < 0.001$), and their interaction ($P < 0.001$) significantly influenced occlusion percentage. The highest occlusion was observed with nanoemulsion (30-min immersion) in the acid challenge subgroup (46.78%), followed by nanoemulsion (15-min immersion) after toothbrushing (41.85%), and fluoride varnish in the acid challenge subgroup (37.19%). Acidic and brushing challenges significantly reduced occlusion in all groups ($P < 0.001$).

Conclusion: Nanoemulsion containing *Nigella sativa* nanoparticles within propolis nanomicelles demonstrated superior dentinal tubule occlusion compared to fluoride varnish, with notable resistance to acid and brushing challenges. Given their natural origin, anti-inflammatory, and remineralizing properties, such nanoformulations may offer an effective and biocompatible alternative for managing DH. Clinical studies are recommended to validate these findings *in vivo*.

Key Words: Dentin-desensitizing agents, dentin sensitivity, electron, microscopy, nanoparticles, *Nigella sativa*, propolis, scanning

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INTRODUCTION

Dentin hypersensitivity (DH) is characterized by sharp, short episodes of pain resulting from exposed dentin in vital teeth, triggered by thermal, mechanical, osmotic, or chemical stimuli.[1] The prevalence of tooth sensitivity has been reported to range from 4% to 57%, with higher rates among patients with periodontal diseases, reaching 60%–98%.[2] It has been shown that in insensitive dentin, particularly in the cervical region, nearly all dentinal tubules are occluded. Enamel defects in the cervical area and the use of hand instruments on cervical dentin surfaces during periodontal procedures such as scaling, root planing, and surgery are among the most significant factors exposing dentinal tubules to the oral environment and various stimuli.[2] Additional factors include attrition, abrasion from toothbrushing, chemical erosion, and gingival recession associated with aging or periodontal diseases. Microscopically, the severity of tooth sensitivity is primarily determined by the number and diameter of open dentinal tubules.[3]

Another increasingly recognized cause of dentinal exposure is the use of bleaching agents in esthetic dentistry. Oxidative compounds such as hydrogen peroxide, commonly found in both in-office and at-home whitening products, can induce morphological and chemical alterations in enamel and dentin, including demineralization and increased dentinal permeability. These changes may facilitate the exposure of dentinal tubules and contribute to DH, particularly when high concentrations or repeated applications are used. Recent systematic reviews have confirmed that bleaching procedures can adversely affect the ultrastructure of dental hard tissues and play a significant role in the etiology of sensitivity symptoms.[4]

Clinical bleaching procedures often result in temporary tooth sensitivity, affecting a significant proportion of patients. This sensitivity is primarily attributed to structural changes in enamel, including increased surface roughness and porosity, which facilitate the penetration of bleaching agents into the dentin and possibly the pulp. These alterations may compromise enamel microhardness and lead to side effects such as hypersensitivity, gingival irritation, and dehydration of the tooth structure.[5]

Several theories have been proposed to explain tooth sensitivity, with the hydrodynamic theory being the most widely accepted. According to this theory, hydraulic changes in the intratubular fluid – induced by thermal, mechanical, chemical, bacterial, or evaporative stimuli – lead to either direct stimulation of pulp mechanoreceptors or indirect stimulation of odontoblasts.[6] In general, desensitizing treatments act through one or both of the following mechanisms: (1) reducing fluid movement within the dentinal tubules, either by decreasing the number of open tubules, narrowing their diameter through coagulation and protein deposition, or forming calcium complexes and (2) reducing dentinal nerve activity by interfering with their electrical conduction via potassium ions.[6]

The current treatment options range from conservative approaches – such as toothpastes containing strontium salts, potassium nitrate, sodium fluoride, monofluorophosphate or amine fluoride, bioactive glass, hydroxyapatite, or Novamin, as well as mouthwashes and adhesive resins – to more invasive interventions, including restorations and root canal therapy. Given the high prevalence of tooth hypersensitivity and the limited efficacy of current treatments, the search for more effective and efficient therapeutic options remains a priority.[7]

In recent years, the therapeutic potential of natural substances in dental applications has gained increasing attention. Propolis and *Nigella sativa* (black cumin seed) have demonstrated promising outcomes in recent studies.[8-10] *Nigella sativa* contains thymoquinone and exhibits antibacterial, anti-inflammatory, and anti-cariogenic properties; it also contributes to the prevention of periodontal and gingival diseases.[11] Propolis, a resinous biomaterial produced by bees, is recognized for its biocompatibility and well-documented benefits to oral health.[12] Evidence suggests that propolis supports the preservation of periodontal connective tissue, enhances healing and regeneration, and promotes bone biomineralization. Chen *et al*. (2015) reported that red propolis extract was capable of occluding dentinal tubules.[13] Propolis possesses antimicrobial, antitumor, anesthetic, anti-inflammatory, antiviral, and regenerative properties. In dentistry, its key applications include alleviating tooth hypersensitivity through tubular occlusion, reducing pulp inflammation, facilitating oral tissue healing, and aiding in the prevention and management of dental caries.[14]

Nanotechnology has driven significant advancements across the various scientific disciplines. Nanoscale

structures, such as nanoparticles, possess a high surface-to-volume ratio, resulting in increased reactivity due to the large number of atoms or molecules relative to their mass. Recently, nanomicelles have attracted attention for their potential in the controlled release of pharmaceuticals. These bipolar structures can simultaneously bind to hydrophilic substances on one side and hydrophobic compounds on the other.[15]

This *in vitro* study aimed to compare the dentinal tubule occlusion produced by a nanoemulsion containing *Nigella sativa* nanoparticles encapsulated in propolis nanomicelles, fluoride varnish, and normal saline under acidic and toothbrush (TB) challenge conditions. The null hypothesis was that there would be no significant difference in occlusion among the treatments, including the nanoemulsion at 15- and 30-min immersion times.

MATERIALS AND METHODS

In this *in vitro* study, the hydroethanolic, hydrophilic extract of Nigella sativa was first produced, enca in bipolar micelle nanoparticles, and placed in a hydrophobic nano-propolis base. This *in vitro* experimental study was approved by the ethics committee under the ethical code IRKMU. REC.1400.63.

Preparation of propolis nanomicelles

Propolis was collected from beehives in Kerman Province during spring, dried, and stored at 4°C until extraction. For extraction, the dried propolis was immersed in ethanol and blended for 7 days. The mixture was then filtered through Whatman #1 filter paper (pore size 150 µm). After complete evaporation of the solvent, the residue was stored at 4°C until use. At the start of the experiments, the propolis was mixed with pure ethanol to obtain a 70% extract.[16]

Propolis nanomicelles were prepared using the dissolution method. Heated absolute ethanol and isopropanol 400 served as solvents, while Tween 80 and sodium caseinate (dissolved in distilled water) acted as emulsifiers. Specifically, 2 g of propolis extract was added to 20 mL of solvent and heated to 62°C–65°C. Separately, 0.08 g of emulsifiers was dissolved in deionized water and heated to the same temperature as the solvent before mixing.

The solvent phase-containing propolis was poured into the aqueous phase containing the emulsifier and homogenized at 12,800 rpm for 50 s to form propolis nanomicelles. The resulting sample was stored in a closed container in a refrigerator until further use.

Production of *Nigella sativa* hydroethanolic extract

To prepare the hydroethanolic extract, 500 g of *Nigella sativa* powder was macerated in 2 L of 70% ethanol and mixed for 7 days. The mixture was then filtered using Whatmann #1 filter paper (150 µm pore size) and left at the room temperature under negative air pressure for an additional 7 days, until the solvent had completely evaporated, yielding a thick, gum-like concentrated extract. This extract was stored in a glass container at 4°C until it was used.[17]

Preparation of nanoemulsion

To prepare the *Nigella sativa* nanoemulsion, Tween 20, Tween 80, distilled water, *Nigella sativa* extract, and ethanol were mixed in a ratio of 10:2:10:0.5:0.5. The mixture was homogenized at 500 rpm at the room temperature and ultrasonic waves (Hielscher Ultrasonic Technology, Germany) were applied to reduce particle size. Propolis nanomicelles were then gradually added to the black seed nanoemulsion and stirred for 24 h at 30°C until a uniform product was obtained.

Particle size of the suspension was analyzed by dynamic light scattering (DLS) using a nanosizer (Vasco and Wallis Model, Cordouan, France). The final product was freeze-dried (Pishtaz Engineering Co., Iran) to obtain a powder, which was subsequently examined for particle size and morphology using FE-scanning electron microscopy (SEM) analysis.

Preparation of samples

In this study, 36 sound human third molars free of cracks and caries were collected from individuals undergoing extractions for orthodontic or periodontal reasons. Teeth were disinfected in 2.5% sodium hypochlorite for 30 min and rinsed with water. The crowns were sectioned perpendicular to the longitudinal axis at the mid-crown region using a diamond disk (IsoMet® 1000 Precision Saw; Buehler, Lake Bluff, IL, USA) to obtain horizontal dentin sections. Surfaces were polished sequentially with 600-, 1500-, and 2000-grit silicon carbide papers (Softflex, STARCKE, Germany) for 30 s each, followed by ultrasonic cleaning in distilled water for 10 min. Samples were then treated with 17% EDTA (Morvabon, Iran) for 2 min, rinsed in distilled water for 1 min, and ultrasonicated again for 5 min.

Samples were randomly assigned to four groups (n = 9): (1) normal saline; (2) nanoemulsion containing *Nigella sativa* nanoparticles encapsulated

in propolis nanoemulsion (NE) with 30-min immersion; (3) NE with 15-min immersion; and (4) 5% fluoride varnish (ADS, USA). In NE groups, samples were rinsed with saline for 5 min and stored in normal saline after immersion. In the fluoride varnish group, a thin layer was applied for 20 s, air-dried with an air syringe, and then stored in normal saline.

Each group was divided into three subgroups (n = 3): (1) control (no intervention); (2) acid challenge; and (3) TB test. In the acid subgroup, samples were immersed in 6% citric acid (Notron, Iran) for 1 min and rinsed with saline for 2 min. In the TB subgroup, samples were mounted in a TB testing machine (Spadana, Isfahan, Iran) and brushed at 2 cycles/second for 840 cycles, equivalent to 1 month of brushing, using a soft-bristled TB (G. U. M Classic 311, USA) in distilled water.

After treatment, all samples were ultrasonically cleaned for 10 min and rinsed with distilled water. They were then sputter-coated with gold and examined under a scanning electron microscope (SEM; QUANTA 450 FEG, FEI, USA). Four SEM images were taken from each sample at ×4000 magnification, covering mesial, distal, buccal, and lingual areas. Two independent experts assessed each image to determine the number and percentage of open and occluded dentinal tubules [Figure 1a-h].

To evaluate the effects of the desensitizing agent and challenge regimen on dentinal tubule occlusion, data were analyzed using the SPSS software (version 25.0, SPSS Inc., Chicago, IL, USA). A two-way analysis of variance (ANOVA) was performed, followed by the pairwise comparisons using the least significant difference (LSD) test. A significance level of $P < 0.05$ was applied.

RESULTS

Field emission-scanning electron microscope examinations

SEM was performed to examine the size and morphology of the freeze-dried sample. Particle size was measured using the image analysis software, revealing an average diameter of 25 ± 2 nm. The nanoparticles were predominantly spherical [Figure 2].

DLS analysis by a nanosizer

Dynamic light scattering (DLS) analysis was performed using a nanosizer to determine the size of the suspended particles. The average particle size was found to be 81.59 nm, which was consistent with the field emission-SEM results and confirmed the synthesis of particles smaller than 100 nm.

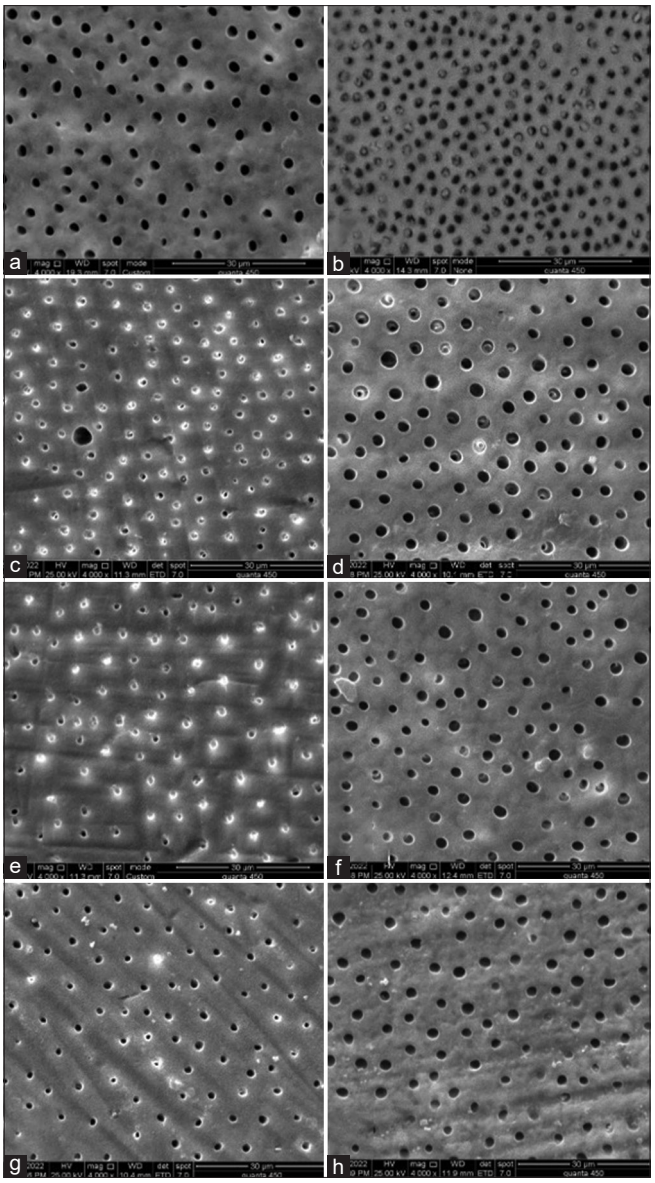

Figure 1: Scanning electron micrograph images of dentinal tubules under different treatment and challenge conditions (×4000 magnification). (a) Acid challenge in the normal saline group; (b) Toothbrushing challenge in the normal saline group; (c) Control regimen in the 15-min nanoemulsion group; (d) Acid challenge in the 15-min nanoemulsion group; (e) Control regimen in the 30-min nanoemulsion group; (f) Acid challenge in the 30-min nanoemulsion group; (g) Control regimen in the fluoride varnish group; (h) Acid challenge in the fluoride varnish group.

Statistical analysis

Table 1 presents the means and standard deviations of dentinal tubule occlusion percentages across the study groups. Two-way ANOVA revealed significant

effects of the material used ($P = 0.018$), the challenge regimen ($P < 0.001$), and their interaction ($P < 0.001$) on occlusion percentage. Table 2 summarizes the LSD *post hoc* test results for pairwise comparisons among materials, whereas Table 3 provides pairwise comparisons among challenge regimens. The control regimen produced significantly greater dentinal tubule occlusion than both the acid and TB challenge regimens.

One-way ANOVA comparing all the combinations of materials and challenge regimens indicated significant differences among groups ($P < 0.001$). In the intra-group analysis [Figure 3], the normal saline group showed no significant difference among its three subgroups. In the fluoride varnish group, the control subgroup exhibited significantly higher occlusion than both the acid challenge ($P = 0.01$) and TB challenge ($P < 0.001$), with the acid challenge also showing significantly greater occlusion than the TB challenge ($P < 0.01$).

In the nanoemulsion group with a 15-min immersion time, occlusion in the control subgroup was significantly higher than in both the acid ($P < 0.001$) and TB ($P = 0.001$) challenge regimens. For the nanoemulsion group with a 30-min immersion time, occlusion in the control subgroup was significantly higher than in the acid ($P = 0.02$) and TB ($P < 0.001$) challenge regimens, and the acid challenge subgroup showed significantly higher occlusion than the TB challenge subgroup ($P = 0.002$).[Figure 4]

Table 1: Occlusion percentage of dentinal tubules (mean±standard deviation) across treatment groups

Material	Challenge regimen	Mean±SD (%)
Normal saline	Acid challenge	30.18±22.55
	Brushing test	37.04±22.19
	No intervention	33.98±16.06
Fluoride varnish	Acid challenge	37.19±15.34
	Brushing test	17.04±3.25
	No intervention	56.96±18.15
Nanoemulsion with 15-min immersion	Acid challenge	29.07±19.86
	Brushing test	41.85±32.57
	No intervention	68.99±18.92
Nanoemulsion with 30-min immersion	Acid challenge	46.78±9.97
	Brushing test	19.74±10.39
	No intervention	66.07±21.39

SD: Standard deviation

DISCUSSION

DH is a prevalent clinical condition, and numerous treatment approaches have been proposed. However, despite extensive research, no universally accepted gold standard for DH management has been established.[1] Under physiological conditions, saliva can contribute to dentinal tubule occlusion by delivering calcium and phosphate ions into the tubules and forming a protective surface layer of salivary glycoproteins combined with these minerals.[18] Nevertheless, this natural mechanism is relatively slow and provides only temporary relief. Therefore, effective treatment strategies capable of achieving rapid and durable tubule occlusion, even under the challenges of the oral environment, are highly desirable. In this context, the present *in vitro* study evaluated the efficacy of a

Table 2: Pairwise comparisons of the materials regarding dentinal tubule occlusion percentages

Material 1	Material 2	Mean of differences	*P*	95% CI	
				Lower bound	Upper bound
Fluoride varnish	15-min nanoemulsion	−9.5756%	0.036%*	−18.5127%	−0.6385%
	30-min nanoemulsion	−10.1929%	0.030%*	−19.4050%	−0.9807%
	Normal saline	3.3289%	0.462%	−5.6082%	12.2660%
15-min nanoemulsion	30-min nanoemulsion	−0.6173%	0.895%	−9.8295%	8.5948%
	Normal saline	12.9044%	0.005%*	3.9673%	21.8415%
30-min nanoemulsion	Normal saline	13.5218%	0.004%*	4.3096%	22.7339%

*A significant level. The significance level was $P<0.05$. CI: Confidence interval

Table 3: Pairwise comparisons of treatment regimens based on the percentage of dentinal tubule occlusion

Challenge regimen 1	Challenge regimen 2	Mean of differences	*P*	95% CI	
				Lower bound	Upper bound
Acid challenge	Brushing test	6.0538%	0.133%	−1.8599%	13.9675%
	No intervention	−20.6971%	*0.001%	−28.4368%	−12.9573%
Brushing test	No intervention	−26.7509%	*0.001%	−34.6646%	−18.8372%

*A significant level. The significance level was $P<0.05$. CI: Confidence interval

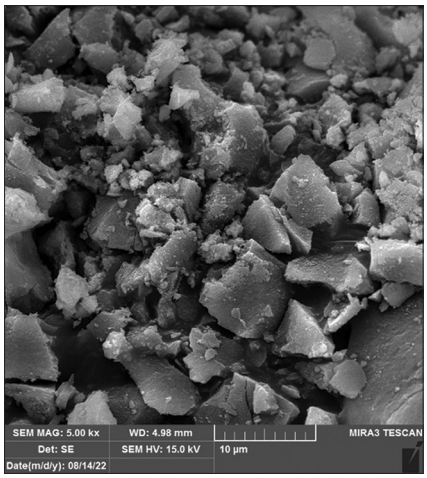

Figure 2: Freeze-dried scanning electron microscopy images of the nanoemulsion sample showing the size and spherical morphology of the nanoparticles (252 nm).

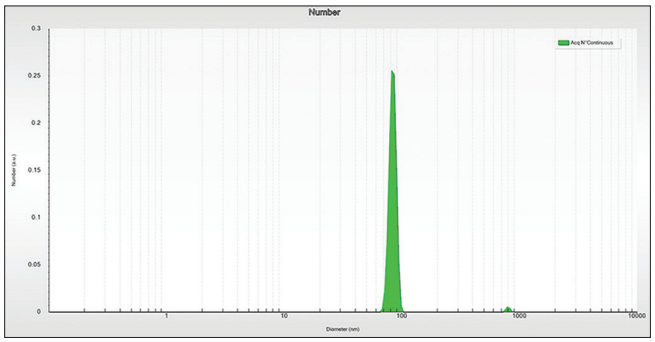

Figure 3: Diagram of dynamic light scattering analysis obtained using a nanosizer, illustrating the particle size distribution of the nanoemulsion.

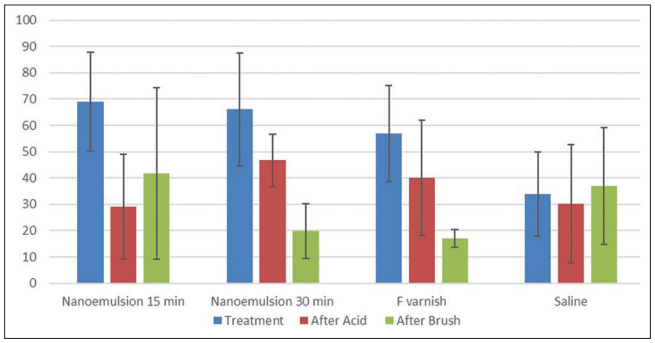

Figure 4: Occlusion percentage of dentinal tubules (mean ± standard deviation) across treatment groups.

nanoemulsion-containing *Nigella sativa* nanoparticles encapsulated within propolis nanomicelles, compared with fluoride varnish, in achieving dentinal tubule occlusion under acidic and TB challenge conditions. studies.

The null hypothesis of the present study was rejected, as significant differences were observed among the tested substances – normal saline, fluoride varnish, and nanoemulsion (NE) with immersion times of 15 and 30 min – in terms of dentinal tubule occlusion. Both the type of desensitizing agent, the challenge regimen, and their interaction had significant effects on tubule occlusion. Under acidic challenge conditions, NE with a 30-min immersion achieved the highest percentage of occluded dentinal tubules, whereas under TB challenge conditions, the highest occlusion was observed with NE after 15 min of immersion. Overall, the nanoemulsion demonstrated superior performance compared with fluoride varnish in occluding dentinal tubules, with the most pronounced advantage observed under TB and acid challenges following 30 min of immersion.

Kumar *et al*. demonstrated that *Nigella sativa* promotes the remineralization of non-cavitated demineralized lesions.[18] Similarly, Farooq *et al*. reported an increase in enamel surface microhardness following exposure to thymoquinone, which persisted after immersion and TB challenge tests.[19] Anthoney *et al*. further investigated toothpastes containing thymoquinone and nano-oxide/bioactive glass fluoride, showing that their application resulted in uniform dentinal tubule occlusion.[20] The ability of *Nigella sativa* to occlude dentinal tubules appears to be linked to the anti-inflammatory, immunomodulatory, and antioxidant properties of thymoquinone, its primary phytochemical component.[21]

Propolis has also been extensively studied for its anti-inflammatory activity, with bioflavonoids identified as its principal bioactive constituents. These compounds can stimulate dentin formation and reduce dentin permeability.[22] Based on the hydrodynamic theory, effective management of DH requires closing dentinal tubules to limit fluid movement and nerve stimulation. Accordingly, the nanoemulsion (NE) used in this study may reduce DH by achieving effective tubule occlusion and decreasing dentin permeability.[6]

Several studies have demonstrated the potential of propolis in managing DH through dentinal tubule occlusion. Using SEM, Midha *et al*. showed that toothpaste containing propolis effectively occluded dentinal tubules.[23] Similarly, Gargouri *et al*. reported that xylitol chewing gum enriched with propolis could achieve comparable effects.[24] Kripal *et al*. observed a significant reduction in the number of patent dentinal tubules following the application of propolis varnish.[25] Arabnejad *et al*. found that propolis, similar

to fluoride varnish, could occlude dentinal tubules, with the resulting deposits demonstrating resistance to acid challenge.[26]

Other investigations have supported these findings: Davari *et al*. reported that 40% propolis gel effectively alleviated DH symptoms, suggesting it as a cost-effective treatment option.[27] Chen *et al*. observed that propolis extract maintained a high degree of tubule occlusion even after acid exposure.[13] Madhavan *et al*. also confirmed the clinical effectiveness of propolis in DH management.[28] However, Sales-Peres *et al*. noted that 10% and 30% propolis gels resulted in only partial dentinal tubule occlusion.[29]

Propolis is more effective than 5% potassium nitrate in relieving DH. Its immediate tubule-occluding effect is attributed to flavonoid compounds, while its sustained action is related to the stability of the product.[22] Hussain *et al*. reported that patients who used propolis after bleaching experienced no hypersensitivity compared to the control group.[30] The precise mechanism of adhesion of propolis to the tooth surface remains unclear; however, if this adhesion is primarily mechanical, it may be lost over time, leading to diminished effects.[27]

Propolis is a sticky, lipophilic substance with low water solubility, which increases its contact time with dental tissues and enhances resistance to acid dissolution. Tubule occlusion by propolis has been attributed to interactions among its components, particularly between flavonoids and dentin, resulting in crystal formation that reduces dentinal fluid flow and consequently hypersensitivity, as proposed by Sabir *et al*.[31] These effects are further supported by the correlation between high flavonoid content and crystal deposition on the dentin surface.[29] Additionally, natural resin compounds in propolis confer bonding properties similar to dental adhesives and varnishes, enabling attachment to the tooth surface through diffusion and mechanical interlocking within dentinal microporosities. This process creates a surface coating and occludes the tubules, thereby preventing fluid movement.[32] Owing to its ability to penetrate deeply into dentinal tubules, propolis can provide long-lasting pain relief.[33] Flavonoids within propolis may also stimulate reparative dentinogenesis, which further contributes to tubule occlusion.[31] In the present study, incorporating propolis at the nanoscale alongside *Nigella sativa* nanoparticles appeared to enhance its occlusive efficacy.

Previous studies have shown that fluoride varnish can effectively occlude dentinal tubules.[26] Sales-Peres *et al*. demonstrated that potassium oxalate, fluoride gel, and propolis all reduced dentin permeability through tubule occlusion.[29] Fluoride is thought to decrease dentin permeability by precipitating insoluble calcium fluoride within the tubules, and its role as a desensitizing agent is further supported by its ability to increase resistance to acid-induced demineralization.[28]

In the present study, citric acid was applied following the desensitizing agents to simulate the acidic challenge associated with dietary acids from foods and beverages, while TB abrasion was reproduced using a brushing machine. An effective desensitizer should withstand both acid and mechanical abrasion while maintaining its functional properties. Citric acid, an organic hydroxyl acid present in fruits, juices, and soft drinks, was used at a 6% concentration for 2 min, followed by a 1-min rinse with saline.[34]

The present study demonstrated that nanoemulsion containing *Nigella sativa* nanoparticles encapsulated in propolis-based nanomicelles achieved higher dentinal tubule occlusion than fluoride varnish, particularly under acid and toothbrushing challenges. Statistical comparisons revealed that extending the immersion time from 15 to 30 min improved acid resistance, while the 15-min immersion group exhibited slightly better resistance to mechanical wear. These findings suggest that the nanoemulsion formulation not only promotes immediate tubule sealing but also maintains a degree of durability under conditions simulating the oral environment. Compared with fluoride varnish, the superior performance of the nanoemulsion may be attributed to the synergistic action of *Nigella sativa* and propolis, both known for their anti-inflammatory, antimicrobial, and remineralizing effects, as well as the enhanced penetration and surface interaction provided by their nano-scale delivery.

In light of these findings, the use of a nanoemulsion containing *Nigella sativa* nanoparticles encapsulated in propolis-based nanomicelles offers a promising natural alternative for DH management. The nano-formulation enhances the delivery and penetration of active compounds into the dentinal tubules, improving occlusion efficacy and resistance to acid and mechanical challenges. Unlike conventional desensitizers, this natural biomaterial-based approach combines multiple therapeutic actions in a biocompatible system. Furthermore, the synergistic

interaction between *Nigella sativa* and propolis in nanoform may result in more durable tubule sealing. However, as this was an *in vitro* study, further clinical trials are required to confirm the long-term effectiveness, safety, and patient-centered benefits of this innovative treatment in real-world dental practice.

Strengths and limitations

Propolis and *Nigella sativa*, as natural agents with minimal side effects, demonstrated effective dentinal tubule occlusion, suggesting potential for DH management. The primary limitation of this study is that the findings are based on *in vitro* conditions; the oral environment presents dynamic factors such as salivary flow, pH fluctuations, and bacterial activity, which may influence clinical performance. Consequently, well-designed *in vivo* and clinical trials are required to confirm these results in real-world settings.

CONCLUSION

Nanoemulsion, particularly with 30-min immersion under acid challenge, achieved the highest dentinal tubule occlusion. Acid and TB challenges reduced occlusive efficacy across all materials, with normal saline outperforming fluoride varnish. Propolis and normal saline show potential as natural agents for DH management, warranting further investigation into optimal application parameters.

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Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or nonfinancial in this article.

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