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Original Article

Evaluating the protective effect of a nanoemulsion containing propolis and *Nigella sativa* nanoparticles on enamel surface

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Abstract

Background: Due to the lack of data, the current study aims to determine the protective effect of a nanoemulsion containing *Nigella sativa* (NS) and propolis extract (comparing the macroscale and nanoscale form) on the mineral content of enamel after tooth brushing.

Methods: In this experimental study, six human premolars were sectioned buccolingually, and the specimens were randomly divided into four groups. These groups were treated with nanoemulsion alone, nanoemulsion accompanied by propolis in either bulk or nanoparticle form, and distilled water (as a control). The treatment procedure was seven consecutive days, in which the specimens were etched and brushed daily. The Ca and P content of the enamel surface was quantified by Scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM/EDX), and the surface roughness was recorded by a profilometer. Moreover, the SEM micrographs of the specimens were captured. Ultimately, the data were analyzed using one-way ANOVA with LSD post hoc analysis ($\alpha = 0.05$).

Results: The Ca and P content of the groups were significantly different ($P = 0.000$ and $P = 0.000$), but the difference in the surface roughness of the experimental groups was not significant (0.27).

Conclusion: The beneficial effect of NS + propolis as a barrier on tooth surface was confirmed. However, its effect on the mineral content of enamel needs further assessment.

Keywords: enamel, *Nigella sativa*, Propolis, Nanoparticles, Surface Roughness, Calcium, Phosphate

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Introduction

For a considerable time, public health authorities and official medicine downplayed folk medicine's significance. However, there has been a shift in attitude towards this type of medicine in recent years. It is now recognized as an accumulation of healing knowledge passed down through generations within various ethnic groups. Folk medicine does not disappear entirely in response to modernization but rather undergoes transformations in its manifestations. There is a growing worldwide interest in applying traditional medicine to prevent or treat different diseases.[1]

Furthermore, the World Health Organization (WHO) has actively promoted the utilization of medicinal plants in developing countries. They encourage these nations to harness the potential of their botanical resource to create effective healthcare programs. According to the literature, many people (60%–70%) seek folk or traditional medicine, especially in developing countries. This is primarily due to the economic viability and fewer side effects of herbal products.[2]

Herbs are crucial in preventive dentistry, offering a natural and holistic approach to maintaining oral health. With their inherent medicinal properties, herbs can effectively combat common dental problems, such as gum infection, caries, and bad breath. Incorporating herbs into oral hygiene practices can provide numerous benefits, considering that they can have antimicrobial, anti-inflammatory, and antioxidant properties. Certain herbs such as neem, clove, peppermint, and chamomile exhibit strong antibacterial properties, effectively hindering the proliferation of harmful oral bacteria and minimizing the chances of infections. Moreover, these herbs are known for their calming and curative effects on the gums, fostering general gum well-being.[3]

Since dental caries is one of the most prevalent global infections, its prevention could tremendously affect public health.[4] Moreover, treating dental caries is usually a high-cost process.[5] Therefore, preventive dentistry always attracts much attention from governments, insurance companies, and patients themselves.[6,7] Thus, herbal products could be an excellent help for preventing tooth decay in the population. Previous studies have investigated many herbs that can potentially prevent dental caries.[8-10]

Among various herbs, *Nigella sativa* (NS) has been introduced as a miracle drug for treating or preventing various diseases.[2,11,12] There are numerous reports on the medical effects of NS, including its vasorelaxing, antihypertensive,[13] analgesic, anti-inflammatory, and antibacterial properties.[14] Researchers in dentistry have identified different beneficial effects for NS, including its antibacterial effect against cariogenic bacteria,[2,15] its effectiveness in the treatment of oral mucositis and promotion of differentiation in pulp cells,[16] and its use as pulpotomy medicament.[14] Moreover, NS contains Ca and K.[17] Therefore, due to its calcium content, it could be considered a remineralizing agent for the tooth surface after demineralization.[18] However, the remineralizing effect of NS on enamel samples has not been investigated.

The synergistic effect of NS and honey mixture has been frequently documented in recent and historical documentation.[19-23] Traditional medical texts, such as Avicenna's *The Canon of Medicine* and Haly Abbas's *The Complete Book of the Medical Art*, mention the honey-based formulation of NS.[23-25]

Propolis is a resinous bee product extracted from beehives and used in dentistry.[26-28] Many of its characteristics are similar to those of honey, but it is stickier and more durable on surfaces and has less sugar content.[29] Therefore, NS could be more effective on tooth surfaces in combination with propolis than with honey.

The data regarding the synergistic effect of NS and propolis for protecting teeth against dental caries are sparse. By investigating the combined impact of these two natural substances, we can uncover a powerful combination to enhance enamel's protective and reparative properties. NS, known for its therapeutic properties, and propolis, renowned for its antimicrobial and anti-inflammatory effects, may work synergistically to strengthen and fortify tooth enamel against decay, erosion, and bacterial attacks. Understanding the potential synergistic benefits of these substances at the nano level could pave the way for innovative preventive and therapeutic strategies in dental care, contributing to improved oral health outcomes for individuals. Therefore, the aim of the current study includes the protective effect of the mixture of NS and propolis extract (in the macroscale and nanoscale form) on the mineral content of enamel after tooth brushing.

Methods

Our experimental study was approved by the local Ethics Committee (IR.SHAHED.REC.1400.193), and the following steps were performed:

Ethanolic extraction of propolis

After hand-collecting propolis from the beehives (April/May 2019, Jiroft, Iran), it was dried and kept at 4 °C until the study started. Following Bosio and colleagues' method,[30] ethanol 95% (v/v) was added and maintained at 26 °C for seven consecutive days. After that, it was filtered (#4 Whatman paper) and ultimately dehydrated to obtain the pure powder.

Finally, the 70% solution was made by mixing the pure particles and ethanol 95%.

Ethanolic extract of NS

NS was purchased, and an ethanolic extract was prepared using the maceration method. The seeds were mixed with 96% ethanol (equal weight of both) at 26 °C and slowly shaken for 48 hours. The mixture was filtered and dehydrated at 40 °C using air suction. After that, the completely dried sample was kept at 4 °C until the start of the study.

At the beginning of the experiment, a 100 mg/mL solution was achieved by mixing the obtained dried powder with pure ethanol.

Nanoparticle production

In order to produce propolis extract nanoparticles (PEN) and NS nanostructures (NSN), these steps were followed:

Either propolis extract or NS extract (mentioned above) were poured in isolated beakers, shaken vigorously for 30 minutes at 26 °C, filtered via #4 Whatman paper to eliminate any possible impurities, and finally mixed with warm distilled water (1:10 ratio to isolate the pure particles). In order to produce the nanostructures, the whole suspension was put in a homogenizer for 5 minutes and then in an ultrasonic bath for 20–30 minutes. Finally, the mixture received 20 cycles of ultra-prob sonication (each cycle 10 s) (Hielscher, UP100H, Germany). The colloid state was achieved in this stage, and the nanoparticles could be observed. In order to produce the powder form of the nanoparticles, colloid nanoparticles were centrifuged for 20 minutes at 9000 rpm (revolutions per minute). After filtration with filter paper, the mixture was freeze-dried using a freeze dryer (Martin Christ GmbH, Osterode am Harz, Germany) for 24 hours at -70 °C. Subsequently, PEN and NSN samples were obtained in powder form.

Furthermore, the nanoemulsions comprising PEN and NSN were produced using a low-energy titration technique.[31] Formulations were prepared separately, constituted by water, 70% (w/w) propolis, and 5% (w/w) polysorbate 80. The other beaker contained water, 30%

(w/w) NS, and 5% (w/w) polysorbate 80. The extracts were combined at 800 rpm with a surfactant and stirred for 30 minutes using a magnetic stirrer. Following this, water was introduced at a regulated flow rate of 3.5 ± 5 mL/min, and the system was further homogenized for 15 minutes using a homogenizer. Subsequently, the nanoemulsion was stored at room temperature (25 ± 2 °C) in a light-protected environment. The nanoemulsion was diluted in deionized water (1:25) to determine its characteristics, such as droplet size and polydispersity index (PDI), using dynamic light scattering (DLS; Zetasizer ZS, Malvern, UK).

Proteins, enzymes, terpenoids, and flavonoid cofactors in the extract play a dual role in capping and reducing. They act as protective agents and assist in the reduction process. Furthermore, these biomolecules exhibit a strong ability to bind to amino acid residues (carbonyl group), preventing agglomeration and ensuring the stability of the medium. The nanosizer technique was employed for particle size calculations utilizing an SBL analyzer (Statistical Bin Limits). The hydrodynamic radius was excluded from the calculations to minimize agglomeration issues and accurately measure the particle size.

Tooth preparation

A total of six human premolars, which had been extracted for orthodontic purposes, were chosen for the study. The teeth were carefully inspected under a magnification of × 2.5, and any teeth that exhibited cracks or decay were excluded from the sample. The selected teeth underwent a thorough cleaning process using a low-speed headpiece and pumice powder. Each tooth was sectioned mesiodistally after cutting off the root to produce two equal buccal and lingual segments (resulting in 12 specimens). Figure 1 represents the prepared specimens,

Figure 1. The prepared specimens consisting of sectioned premolar teeth and a specified circular area on their surface to be treated by the experimental solutions

each featuring two 1 mm circles painted with nail varnish. These circles served as markers indicating the precise locations where the agent should be applied.

Treatment procedure

The prepared specimens were randomly divided into the following four groups ($n = 3$):

1. Nano emulsion
2. PEN + NSN (Propolis Extract Nano + NS Nano)
3. PEB + NSB (Propolis Extract Bulk + NS Bulk)
4. Control (distilled water)

Before the immersion of teeth, each prepared agent (treatment solution) was kept in an ultrasound device to completely de-aggregate the particles.

The specimens were immersed in 3 mL of the assigned solution during the treatment phase for 15 minutes daily. Afterward, they were rinsed with distilled water and stored at 4 °C until the following day. On the next day, the teeth were retrieved from the water, brushed for 1 minute, and immersed in a fresh solution for another 15 minutes. The control group was subjected to a brief etching process of 5 seconds per day, followed by a rinse without any brushing, and then placed in distilled water at the same temperature until the next day.

Scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM/EDX) mapping

After seven days of treatment and brushing, the specimens were analyzed for the weight percent of surface Ca^{2+} and PO_4—by SEM/EDX mapping (EDS/mapping Bruker XFlash6/10, SEM Quanta 45, Netherland).

Surface profilometry

After the seven-day treatment and brushing period, the specimens were subjected to surface roughness analysis (EDS/mapping Bruker XFlash6/10, SEM Quanta 45, the Netherland).

Field emission scanning electron microscopy (FE-SEM) micrograph

In the final stage, the specimens were gold coated, and their surface microstructure was assessed via FESEM (TESCAN MIRA3, Czech Republic).

Statistical analysis

After assessing the normal distribution of data, a one-way ANOVA test was conducted to compare the data. Post hoc analysis was performed using the least significant difference (LSD) method to compare the mineral content within each group before and after acid etching. The significance level was set at 0.05 ($\alpha = 0.05$).

Results

DLS analysis of nanoparticles

Figure 2 (A and B) displays the histogram generated by the

SBL nanosizer, revealing the mean particle size diameter of approximately 98.17 nm for PEN samples and 118.11 nm for NSN samples. The results indicate the nanoparticles' narrow size distribution and homogeneous dispersity, as observed in the figure.

SEM/EDX mapping

Figures 3 and 4 illustrate the mean ± standard deviation (SD) of $Ca^{2+}$and PO4- content in the enamel samples before and after acid etching. One-way ANOVA statistical analysis showed significant differences among the three groups in both Ca and P content (*P*-values of 0.00 and 0.00, respectively). Furthermore, the *P* values corresponding to pairwise comparisons are presented in Table 1.

On the other hand, there was no significant difference among the groups before and after acid etching ($P = 0.91$ and $P = 0.92$ for Ca and P, respectively). As can be seen from Figures 1 and 2, the highest amounts of Ca and P were related to the control group.

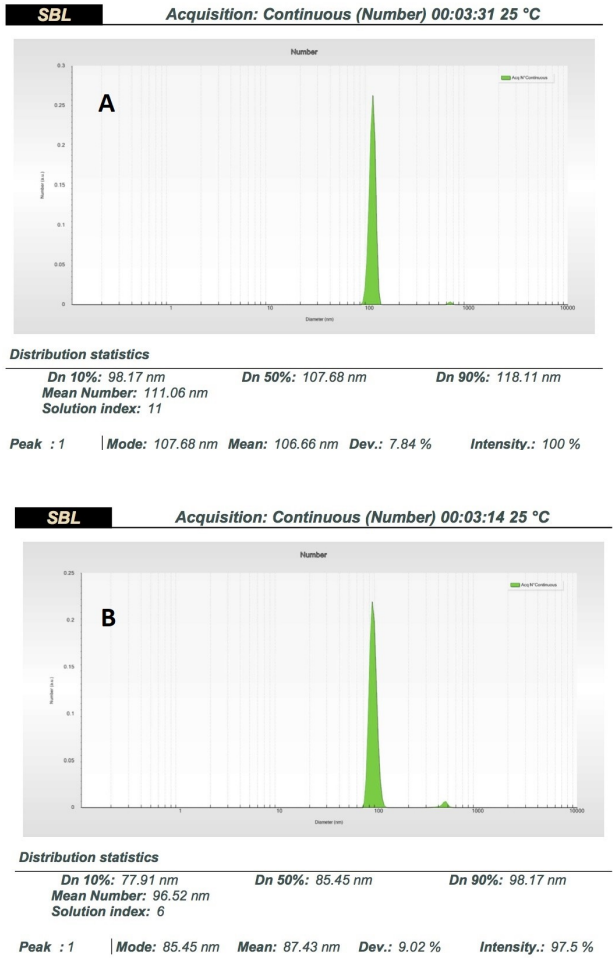

Figure 2. The histogram of the SBL nanosizer related to *Nigella sativa* (A) and propolis (B) nanoparticles

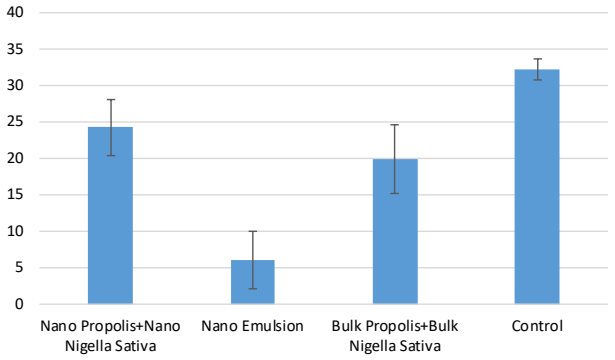

Figure 3. Mean SD of surface Ca in experimental groups before and after acid etching

Surface profilometry

Figure 5 displays surface Ra values' mean ± SD. Despite the variations in mean values among the groups, the one-way ANOVA analysis indicated no significant difference between the experimental groups ($P = 0.273$). Moreover, a sample profilometer of one specimen is presented in Figure 6.

FESEM micrograph

Figure 7 depicts the SEM micrographs of some samples. In the experimental samples, a rough and uniformly distributed layer has formed on the enamel surface. However, the enamel prism orientation, including the head and tail, remains visible beyond this layer.

Discussion

In our study, SEM/EDX showed the effect of NS added to propolis on the calcium content of the enamel. However, due to the stickiness of propolis, the Ca and P content of the samples was reduced. In more detail, the SEM/EDX

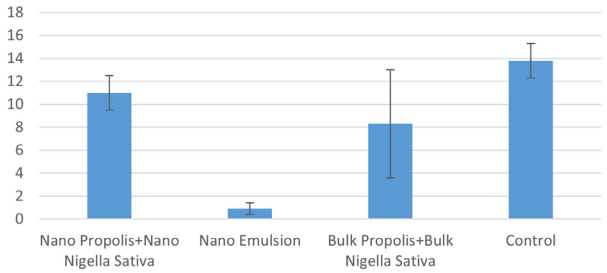

Figure 4. Mean SD of surface P in experimental groups before and after acid etching

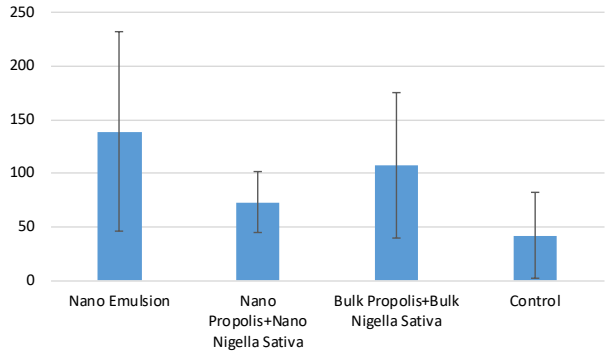

Figure 5. Mean SD of surface roughness in experimental groups (μm)

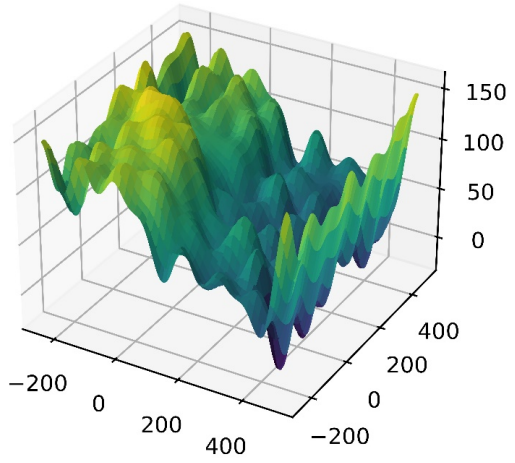

Figure 6. The surface profilometry of a rough sample

probe calculated the Ca and P content of the resinous layer, which was confirmed by the FESEM pictures.

The effect of NS against the most common dental cariogenic bacteria was shown in research done by Nazemi et al.[32] They conducted an experimental study to compare the effects of 0.2% chlorhexidine mouthwash and NS nanoparticle with different dilutions in inhibiting *Streptococcus mutans*, *sobrinus*, and *Salivarius*, *Lactobacillus acidophilus*, and *Enterococcus faecalis* using minimum inhibitory concentration and minimum bactericidal concentration assessment. They showed NS has an inhibitory effect on these bacteria.

Kumar et al[33] confirmed NS's remineralizing potential. This in vitro study compared NS's effectiveness with conventional remineralizing agents, sodium fluoride, and

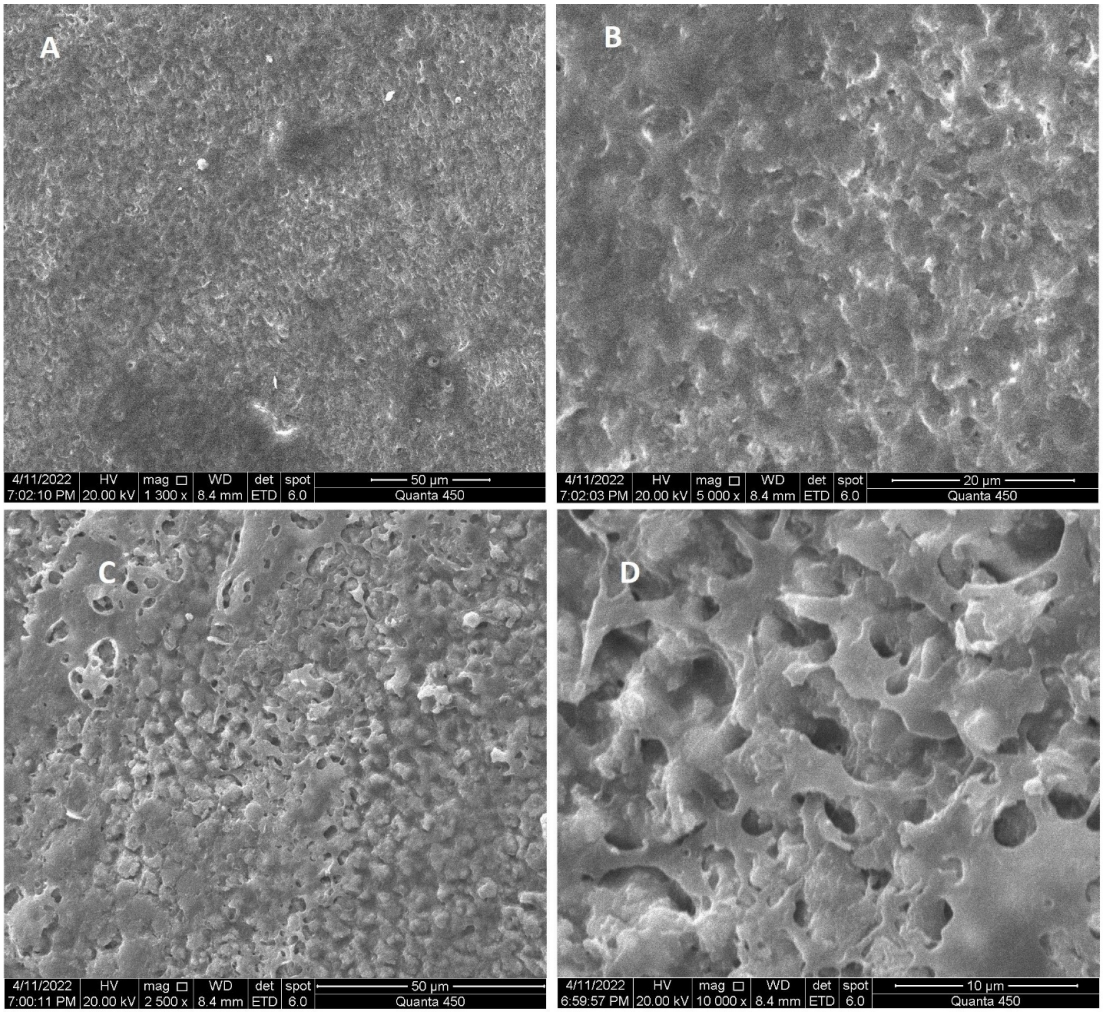

Figure 7. The SEM micrograph shows an enamel prism pattern beneath a sticky, resinous layer

Table 1. *P* values related to pairwise comparison of the groups for Ca (above the diagonal) and P content (below the diagonal)

	Nano emulsion	Nano *Nigella sativa* + nano propolis	Bulk *Nigella sativa* + bulk propolis	Control
Nano emulsion	*	0.000	0.021	0.04
Nano *Nigella sativa* + nano propolis	0.00	*	0.00	0.00
Bulk *Nigella sativa* + bulk propolis	0.09	0.00	*	0.00
Control	0.05	0.00	0.001	*

casein phosphopeptide-amorphous calcium phosphate to assess their ability to promote remineralization. The results showed that the specimens in the NS group had greater depth of remineralization.

The caries protective effect of NS is consistent with Shaker et al,[15] who performed a study on rats. Their results showed that the rats treated with thymoquinone (TQ) (a main active constituent in NS extract) showed statistically lower caries scores and plaque indices although fed a sucrose-rich diet. In their study, the rats received TQ as oral gel or added to their drinking water. Therefore, the researchers concluded that the caries-protective effect of the NS extract was greater than its antibacterial properties.[15] Moreover, Omar et al[34] showed the significant anti-inflammatory effect of NS during pulp cap.

Previous research has demonstrated that the calcium content of NS is approximately 168 ± 10.9 mg/g, making it the mineral with the highest concentration in NS.[35] This high calcium content enables NS to deposit Ca^{2+} on the enamel surface. On the other hand, propolis has a sticky structure that could form a protective layer on the enamel surface and preserve the Ca^{2+} content of the enamel during acid etching. The health-promoting benefits of NS are attributed to its chemical components, such as fixed oils, proteins, alkaloids, saponins, and essential oils.[2] However, TQ is among its most important active constituents.[2] TQ is an anion scavenger that neutralizes oxygen radicals.[19,36]

Incorporating NS has been strongly recommended in dentistry research due to its natural origin, which has rendered it a biologically accepted agent. It induces a minimal inflammatory response, is inexpensive, and is widely available.[16,34,37]

This study was the first investigation into the potential synergistic effect of NS and propolis. Thus, it can be considered a preliminary study. The combination of NS and honey is also synergistically effective in treating *H. pylori* in clinical studies,[21,23,38,39] COVID-19 treatment,[20] controlling asthma,[19] protecting the liver,[40] and promoting wound healing in rat models.[22]

Javadi et al[22] reported that the combination of NS and honey was significantly more effective in decreasing the wound size than NS or honey alone. Furthermore, El-Kholy et al stated that honey was more hepatoprotective than NS, but their combination was significantly better. In addition, they strongly suggested incorporating this mixture in human food as an additive because of its strong protective effect.[40] On the other hand, Mohtashami et al revealed significant improvement in controlling *H. pylori* in patients suffering from dyspepsia when they received a honey-based formulation of NS compared to the control group. Although they did not compare their results with NS or honey alone, they concluded that NS and honey combination was effective.[23] In a review by Jarmakiewicz-Czaja et al,[39] the hypoglycemic, hypolipemic, and hepatoprotective effects of NS were confirmed. Laboratory tests also show immunomodulatory and anticancer effects in NS.

However, none of these studies has mentioned the mechanism responsible for the synergistic effect of NS and honey.

Strengths and limitations

This study's most important weaknesses include its short-term follow-up (seven days); thus, prolonged durations and exposure to bacterial plaque-induced acid formation are required. Moreover, as this is a laboratory study, its results could not be directly applied to clinical settings.

Conclusion

NS, propolis, and their combination can be considered caries-protective agents that could produce a barrier on the tooth surface. These products are widely available, economically viable, and biologically safe.

Authors' Contribution

Conceptualization: Hedieh Khanabadi, Faeze Hamze.
Data curation: Hedieh Khanabadi.
Formal analysis: Faeze Hamze, Mahnaz Amiri.
Methodology: Faeze Hamze, Hanieh Sadat Emami Razavi.
Project administration: Sepideh Behzadi.
Supervision: Mehrdad Karimi.
Software: Mehrdad Karimi.
Writing–original draft: Hedieh Khanabadi, Faeze Hamze.
Writing–review & editing: Sepideh Behzadi.

Competing Interests

None declared.

Ethical Approval

This experimental study was approved by the local Ethics Committee of Shahed University (IR.SHAHED.REC.1400.193).

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References

1. Shamsutdinova II. Place of folk medicine in the life of the modern population of Russia on the example of villages of the Udmurt Republic. Int J Pharm Res. 2020;12(Suppl 1):2147-50. doi: 10.31838/ijpr/2020.SP1.314.
2. Al-Attass SA, Zahran FM, Turkistany SA. *Nigella sativa* and its active constituent thymoquinone in oral health. Saudi Med J. 2016;37(3):235-44. doi: 10.15537/smj.2016.3.13006.
3. Bigom-Taheri J, Azimi S, Rafieian N, Akhavan Zanjani H. Herbs in dentistry. Int Dent J. 2011;61(6):287-96. doi: 10.1111/j.1875-595X.2011.00064.x.
4. Whelton HP, Spencer AJ, Do LG, Rugg-Gunn AJ. Fluoride revolution and dental caries: evolution of policies for global use. J Dent Res. 2019;98(8):837-46. doi: 10.1177/0022034519843495.
5. Cooray U, Aida J, Watt RG, Tsakos G, Heilmann A, Kato H, et al. Effect of copayment on dental visits: a regression discontinuity analysis. J Dent Res. 2020;99(12):1356-62. doi: 10.1177/0022034520946022.
6. Fraihat N, Madae'en S, Bencze Z, Herczeg A, Varga O. Clinical effectiveness and cost-effectiveness of oral-health promotion

in dental caries prevention among children: systematic review and meta-analysis. Int J Environ Res Public Health. 2019;16(15):2668. doi: 10.3390/ijerph16152668.
7. Twetman S. Prevention of dental caries as a non-communicable disease. Eur J Oral Sci. 2018;126 Suppl 1:19-25. doi: 10.1111/eos.12528.
8. Ancuceanu R, Anghel AI, Ionescu C, Hovaneț MV, Cojocaru-Toma M, Dinu M. Clinical trials with herbal products for the prevention of dental caries and their quality: a scoping study. Biomolecules. 2019;9(12):884. doi: 10.3390/biom9120884.
9. Braga AS, Girotti LD, de Melo Simas LL, Pires JG, Pelá VT, Buzalaf MA, et al. Effect of commercial herbal toothpastes and mouth rinses on the prevention of enamel demineralization using a microcosm biofilm model. Biofouling. 2019;35(7):796-804. doi: 10.1080/08927014.2019.1662897.
10. Motallaei MN, Yazdanian M, Tebyanian H, Tahmasebi E, Alam M, Abbasi K, et al. The current strategies in controlling oral diseases by herbal and chemical materials. Evid Based Complement Alternat Med. 2021;2021:3423001. doi: 10.1155/2021/3423001.
11. Almatroodi SA, Almatroudi A, Alsahli MA, Khan AA, Rahmani AH. Thymoquinone, an active compound of *Nigella sativa*: role in prevention and treatment of cancer. Curr Pharm Biotechnol. 2020;21(11):1028-41. doi: 10.2174/1389201021666200416092743.
12. Islam MN, Hossain KS, Sarker PP, Ferdous J, Hannan MA, Rahman MM, et al. Revisiting pharmacological potentials of *Nigella sativa* seed: a promising option for COVID-19 prevention and cure. Phytother Res. 2021;35(3):1329-44. doi: 10.1002/ptr.6895.
13. Cherkaoui-Tangi K, Israili ZH, Lyoussi B. Vasorelaxant effect of essential oil isolated from *Nigella sativa* L. seeds in rat aorta: Proposed mechanism. Pak J Pharm Sci. 2016;29(1):1-8.
14. Saikiran KV, Kamatham R, Sahiti PS, Nuvvula S. Pulpotomy medicaments in primary teeth: a literature review of natural alternatives. SRM J Res Dent Sci. 2018;9(4):181-5. doi: 10.4103/srmjrds.srmjrds_14_18.
15. Al-Wafi H. Benefits of Thymoquinone, a *Nigella Sativa* Extract in Preventing Dental Caries Initiation and Improving Gingival Health [dissertation]. Ann Arbor: ProQuest LLC; 2014. ProQuest LLC. 2014;72.
16. Mendi A. *Nigella sativa* oil could induce osteogenic differentiation of dental pulp mesenchymal stem cells: clinical nutrition for dentistry. Food Health. 2018;4(1):19-24.
17. Nergiz C, Ötleş S. Chemical composition of *Nigella sativa* L. seeds. Food Chem. 1993;48(3):259-61. doi: 10.1016/0308-8146(93)90137-5.
18. Arifa MK, Ephraim R, Rajamani T. Recent advances in dental hard tissue remineralization: a review of literature. Int J Clin Pediatr Dent. 2019;12(2):139-44. doi: 10.5005/jp-journals-10005-1603.
19. Al Ameen NM, Altubaigy F, Jahangir T, Mahday IA, Mohammed EA, Musa OA. Effect of *Nigella sativa* and bee honey on pulmonary, hepatic and renal function in Sudanese in Khartoum state. J Med Plants Res. 2011;5(31):6857-3. doi: 10.5897/jmpr11.1357.
20. Ashraf S, Ashraf S, Ashraf M, Imran MA, Kalsoom L, Siddiqui UN, et al. Honey and *Nigella sativa* against COVID-19 in Pakistan (HNS-COVID-PK): a multi-center placebo-controlled randomized clinical trial. medRxiv [Preprint]. November 30, 2020. Available from: https://www.medrxiv.org/content/10.1101/2020.10.30.20217364v4.
21. Hashem-Dabaghian F, Agah S, Taghavi-Shirazi M, Ghobadi A. Combination of *Nigella sativa* and honey in eradication of gastric *Helicobacter pylori* infection. Iran Red Crescent Med J. 2016;18(11):e23771. doi: 10.5812/ircmj.23771.
22. Javadi SMR, Hashemi M, Mohammadi Y, Mammohammadi A, Sharifi A, Makarchian HR. Synergistic effect of honey and *Nigella sativa* on wound healing in rats. Acta Cir Bras. 2018;33(6):518-23. doi: 10.1590/s0102-865020180060000006.
23. Mohtashami R, Fallah Huseini H, Heydari M, Amini M, Sadeqhi Z, Ghaznavi H, et al. Efficacy and safety of honey-based formulation of *Nigella sativa* seed oil in functional dyspepsia: a double blind randomized controlled clinical trial. J Ethnopharmacol. 2015;175:147-52. doi: 10.1016/j.jep.2015.09.022.
24. Heydari M, Dalfardi B, Ej Golzari S, Mosavat SH. Haly abbas and the early description of obstructive jaundice. Iran J Public Health. 2014;43(8):1161-2.
25. Heydari M, Hashempur MH, Zargaran A. Medicinal aspects of opium as described in Avicenna's Canon of Medicine. Acta Med Hist Adriat. 2013;11(1):101-12.
26. Abbasi AJ, Mohammadi F, Bayat M, Gema SM, Ghadirian H, Seifi H, et al. Applications of propolis in dentistry: a review. Ethiop J Health Sci. 2018;28(4):505-12. doi: 10.4314/ejhs.v28i4.16.
27. Parolia A, Thomas MS, Kundabala M, Mohan M. Propolis and its potential uses in oral health. Int J Med Med Sci. 2010;2(7):210-5.
28. Zulhendri F, Felitti R, Fearnley J, Ravalia M. The use of propolis in dentistry, oral health, and medicine: a review. J Oral Biosci. 2021;63(1):23-34. doi: 10.1016/j.job.2021.01.001.
29. Rezvani MB, Hemmati MA, Norouzi M, Bakhtiari R, Azinpour F, Hamzeh F. Evaluation of the effect of Kandovan propolis against *Streptococcus mutans*. J Oral Health Oral Epidemiol. 2018;7(2):94-8. doi: 10.22122/johoe.v7i2.407.
30. Bosio K, Avanzini C, D'Avolio A, Ozino O, Savoia D. In vitro activity of propolis against *Streptococcus pyogenes*. Lett Appl Microbiol. 2000;31(2):174-7. doi: 10.1046/j.1365-2672.2000.00785.x.
31. Ostertag F, Weiss J, McClements DJ. Low-energy formation of edible nanoemulsions: factors influencing droplet size produced by emulsion phase inversion. J Colloid Interface Sci. 2012;388(1):95-102. doi: 10.1016/j.jcis.2012.07.089.
32. Nazemi Salman B, Sallah S, Abdi F, Salahi S, Rostamizadeh K, Basir Shabestari S. The comparison of antimicrobial effect of *Nigella sativa* nanoparticle and chlorhexidine emulsion on the most common dental cariogenic bacteria. Med J Islam Repub Iran. 2021;35:149. doi: 10.47176/mjiri.35.149.
33. Kumar NK, Naik SB, Priya CL, Merwade S, Brigit B, Guruprasad CN, et al. Evaluation of the remineralizing potential of *Nigella sativa*, Sodium fluoride and casein phosphopeptide-amorphous calcium phosphate on enamel: an: in vitro: study. J Indian Assoc Public Health Dent. 2020;18(4):313-7. doi: 10.4103/jiaphd.jiaphd_130_20.
34. Omar OM, Khattab NM, Khater DS. *Nigella sativa* oil as a pulp medicament for pulpotomized teeth: a histopathological evaluation. J Clin Pediatr Dent. 2012;36(4):335-41. doi: 10.17796/jcpd.36.4.n6674435856q86w8.
35. Rasoli MS, Khalili M, Mohammadi R, Soleimani A, Kohzadi R, Ilkhanipour M, et al. The chemical composition of *Nigella sativa* L. and its extract effects on lipid peroxidation levels, total antioxidant capacity and catalase activity of the liver and kidney in rats under stress. Gene Cell Tissue. 2018;5(1):e61323. doi: 10.5812/gct.61323.
36. Razmpoosh E, Safi S, Abdollahi N, Nadjarzadeh A, Nazari M, Fallahzadeh H, et al. The effect of *Nigella sativa* on the measures of liver and kidney parameters: a systematic review and meta-analysis of randomized-controlled trials. Pharmacol Res. 2020;156:104767. doi: 10.1016/j.phrs.2020.104767.
37. Setiawatie EM, Gani MA, Rahayu RP, Ulfah N, Kurnia S, Augustina EF, et al. *Nigella sativa* toothpaste promotes anti-

inflammatory and anti-destructive effects in a rat model of periodontitis. Arch Oral Biol. 2022;137:105396. doi: 10.1016/j.archoralbio.2022.105396.

38. Abdi S, Ataei S, Abroon M, Majma Sanaye P, Abbasinazari M, Farrokhian A. A comprehensive review of the role of complementary and dietary medicines in eradicating *Helicobacter pylori*. Iran J Pharm Res. 2022;21(1):e127030. doi: 10.5812/ijpr-127030.
39. Jarmakiewicz-Czaja S, Zielińska M, Helma K, Sokal A, Filip R. Effect of *Nigella sativa* on selected gastrointestinal diseases. Curr Issues Mol Biol. 2023;45(4):3016-34. doi: 10.3390/cimb45040198.
40. El-Kholy WM, Hassan HA, Nour SE, Abe Elmageed ZE, Matrougui K. Hepatoprotective effects of *Nigella sativa* and bees' honey on hepatotoxicity induced by administration of sodium nitrite and sunset yellow. FASEB J. 2009;23(S1):733.2. doi: 10.1096/fasebj.23.1_supplement.733.2.