Orginal Article

Antioxidant, Antimicrobial and Antiangiogenic Properties of Corylus Avellana Essential Oil Nanoemulsion (CAEO-NE)

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Abstract

Background & Objective: Corylus avellana essential oil (CAEO) due to its individual phytochemicals and phenolic compounds has been recognized as a potent cell-protective compound against oxidative stresses. In the current study, CAEO was encapsulated in the nanoemulsion drug delivery system to improve its bioactivity properties assuming antioxidant, antimicrobial, and anti-angiogenic potentials.

Materials & Methods: CAEO nanoemulsions (CAEO-NE) were synthesized applying a high energy ultrasonic method for the emulsification processes and were characterized by DLS, AFM, and SEM analysis. Their antioxidant activity was evaluated by measuring the radical scavenging effect on both DPPH and ABTS free radicals. The CAEO antibacterial and antiangiogenic potentials were studied by measuring the non-growth ring diameter of the staphylococcus aureus culture plate and monitoring the blood vessels of the chick chorioallantoic membrane and its length, respectively.

Results: The 45.9-nm CAEO-NE significantly inhibited both DPPH and ABTS free radicals. Meaningful antibacterial and antiangiogenic impacts were detected following increasing CAEO-NE treatment doses (P-value < 0.001).

Conclusion: CAEO-NE exhibited three key medicinal activities (antioxidant, antibacterial, and antiangiogenic), which make it a potentially safe antibacterial compound. It is suggested that CAEO-NE has anticancer potential due to its antioxidant and antiangiogenic effects. However, further in vitro and in vivo studies are required to verify its mentioned bioactivities and define details of its mechanism.

Keywords: Corylus avellana essential oil; nanoemulsion; antioxidant; antibacterial, anti-angiogenesis

Introduction

The focus on herbally-derived bioactive compounds is rapidly increasing due to their lesser toxicity effects on the human body compared with their synthetic counterpart. In this regard, it has been shown that the consumption of nutrients rich in bioactive phytochemicals with affirmed antioxidant activity has the protective potential against various types of disorders such as neurodegenerative, cardiometabolic, and age-related diseases (1-3). Increasing the intake of nut-derived antioxidants affect aging-related risk factors and thus, can improve and extend human lifespan (4). Furthermore, it has been shown that nut

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consumption decreases the risk of colorectal, pancreatic, oesophageal, and gastric cancers (5, 6).

Corylus avellana (hazelnut), due to its individual rich bioactive ingredients such as vitamins, healthy fatty acids, polyphenols, and minerals, can be regarded as a natural antioxidant gift (7-9). Moreover, it has been known to have potent antimicrobial potential, which makes it suitable for treating bacteria-caused intestinal infections such as several types of food gram-positive bacteria (bacillus cereus) (10) as well as gram-positive infections such as staphylococcus aureus, which is considered as one of the most common causes of skin infections, endocarditis, pneumonia, and osteomyelitis (11).

Its bioactive ingredients can be improved in the case of bio-accessibility and structure stability by nano-encapsulating them in organized delivery transporters such as nanoemulsions (12). The 20- to 200-nm formed-droplets in hydrophilic solutions containing lipophilic compounds are named nanoemulsions delivery systems, which are produced by applying high energy-consuming [~ 108 – 1010 watts per kilogram (W/kg -1)] methods such as ultrasound-based emulsification (13, 14). Safety, being cost-effective, and ease of handling the production process of ultrasound-based emulsification are the main reasons for its growing application. Furthermore, improved stability, absorbency, and solubility of the produced nano-emulsified phytochemicals make them suitable to be used in biological applications as safe drug delivery systems (15).

In the current study, Corylus avellana essential oil (CAEO) was encapsulated by the ultrasound-based emulsification method to improve and evaluate its antioxidant, antibacterial, and, anticancer potentials by analyzing its radical scavenging effects on DPPH/ABTS oxidants, non-growth ring diameter on staphylococcus aureus culture media, and antiangiogenic impact on the blood vessels of chick chorioallantoic membrane, respectively.

Materials & methods

Chemical material

Corylus avellana essential oil, nonionic surfactant tween-80 (T-80), 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals, and the staphylococcus aureus strain was purchased from the Pasteur Institute of Iran (Tehran, Iran). All the chemical materials were purchased from Sigma Aldrich (St. Louis, MO, USA).

CAEO-NE preparation

Three variants of CAEO-NE were produced by ultrasonic-based encapsulating of the CAEO in the presence of water and different T-80 volumes of nonionic surfactants (1, 2, and 3 mL). The hydrophilic–hydrophobic balance was defined at 15 for T-80. The sonication processes were performed at 20 kHz frequency and output power of 750 watts for 60 minutes (16, 17).

CAEO-NE Characterization

The mean hydrodynamic size (Z-average) was measured for all three CAEO-NE variants, which were only different at T-80 volumes (1, 2, and 3 mL). The size of the smallest variant was then verified by performing a high-resolution atomic force microscope (AFM). The emission electron microscopy (FESEM) was then utilized for studying and verifying the morphology of the droplets according to Hongwu Sun et al. Briefly, a thin layer of the CAEO-NE solution was dropped and dried on the microscope slide (gold-coated glass) to use for microscopy studies (18).

Antioxidant measurements

The CAEO-NE antioxidant property was evaluated by measuring its radical scavenging activity. In this regard, both ABTS and DPPH free radicals were utilized based on the following protocols. Briefly, the ABTS and DPPH absorbance was recordable at 734 nm and 517 nm, respectively (19-21).

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ABTS assay

First, the cation radical of ABTS·+ was produced by mixing 7 mM ABTS with deionized water and potassium persulfate (2.45 mM) at a ratio of 1:1. The mixture was kept in dark conditions for 14 hours at 25 °C. The final solution was prepared by diluting with distilled water. The dilution continued until mixture absorbance (at 734 nm) reached 0.700. Then, samples were prepared by adding definite CAEO-NE concentrations with the ABTS final solution and 30-minute incubation. Finally, sample absorbance was recorded at 25 °C in the dark.

DPPH measurement

A DPPH solution was prepared by first dissolving 1 mg DPPH in 17 mL of etanol 96%. Then, the samples were prepared by mixing different CAEO-NE concentrations with DPPH solution.

The sample solutions were kept for 30 min in dark conditions. In the end, sample absorbance was measured at 517 nm.

The antioxidant activity (AA) of CAEO-NE was estimated utilizing the following equation:

$$AA = \frac{S - C}{C} \times 100$$

The S and C refer to “sample absorbance” and “control absorbance”, respectively.

Antibacterial analysis

CAEO-NE bactericidal activity was investigated following the protocol below:

Staphylococcus aureus was inoculated on the Mueller-Hinton agar by carpet–type culturing, antibiogram discs were prepared along with ampicillin and CAEO-NE-smeared discs, and they were placed on the culture plate to be cultured at 37 °C for 24 hours.

CAM assay

AVEO-NE anti-angiogenic activity was checked by applying a chick chorioallantoic membrane (CAM) assay. We prepared 40 fertilized Ross chicken eggs and divided them into five groups of eight treatment groups: one control group: noting for treating, a second lab control group: treated with PBS (0.1 M), and three additional groups: treated with different concentrations of CAEO-NE (25, 50, and 100 µg/ml). Briefly, the 48-hour incubated fertilized eggs (N=40, at 37°C, and 55-70% humidity) were selected to have a square cut (1 cm2) window on their shell. The eggs were sealed under sterilized conditions by paraffin slices and returned to be incubated at previous conditions for the next eight days with twice a day rotation. On the eighth day, the treatment process was performed, and the eggs were resealed to be incubated for a further 72 hours. Stereomicroscope photos were taken and used to measure the length and number of the CAM blood vessels (22).

Statistical analysis

We utilized the software SPSS 21 statistical package (SPSS, Chicago, IL). In order to analyze all the statistical results, the one-way ANOVA test was applied and the statistically significant level was considered at $P< 0.001$.

Results

CAEO-NE characterization

According to the DLS data, a significant relationship was detected between the increasing surfactant (T-80) levels (1, 2, and 3 mL) and decreasing size of the emulsions (Figure 1). The CAEO-NE’s size changed from 81.1 to 45.9 nm following increased T-80 volumes. Also, their PDI values were shifted to lower levels (less than 0.7), which indicates the mono-disperse phase condition during the formation of the emulsions. Therefore, the measured-size values can be considered as a reliable index (23). Moreover, the size of the emulsions was verified by both SEM and AFM microscopy. The CAEO-NE’s size was defined at ~50 and 57.19 nm, respectively (Figure 2A and B).

CAEO-NE antioxidant activity

The radical scavenging effect of CAEO-NE on ABTS and DPPH oxidants reveals its remarkable antioxidant potential compared with glutathione (p-value < 0.001) (Chart 1).

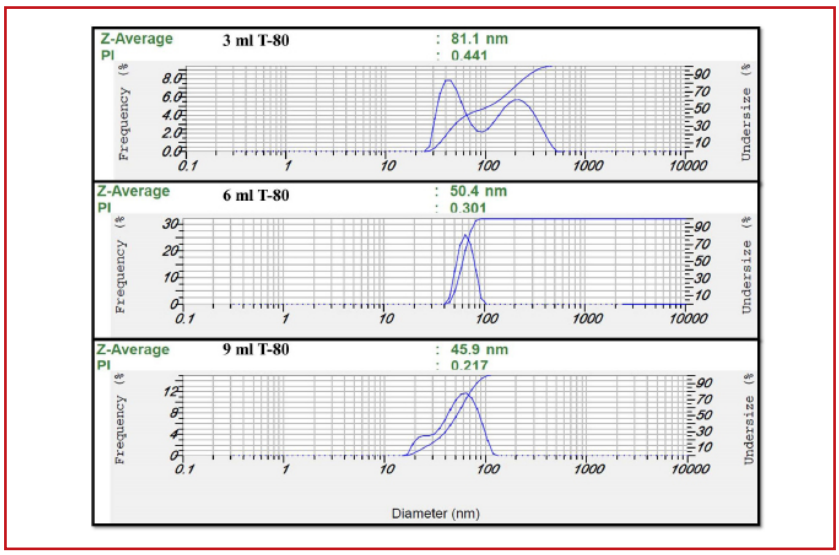

Figure 1. The CAEO-NE's size properties. The DLS data indicate the decreasing CAEO-NE's size following increasing surfactant (T-80) volumes (1, 2, and 3 ml). CAEO-NE: Corylus avellana essential oil nanoemulsion; T-80: tween-80; PI: polydispersity.

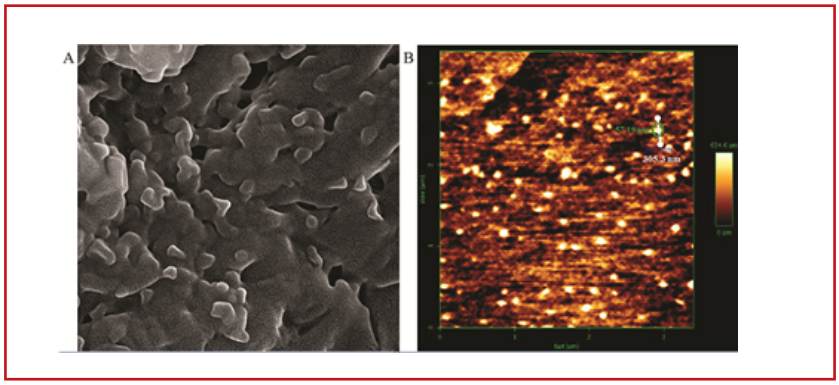

Figure 2. The CAEO-NE's size verification analysis graphs. A) The SEM micrograph of CAEO-NE. The scale bar is defined as 200 nm, and emulsions are estimated at ~50 nm droplets. B) The AFM graph of CAEO-NE. The green ring shows the 45. 9 nm emulsions compared with the white scale line (305.3 nm). CAEO-NE: Corylus avellana essential oil nanoemulsion; SEM: scanning electron microscope; AFM: Atomic force microscope.

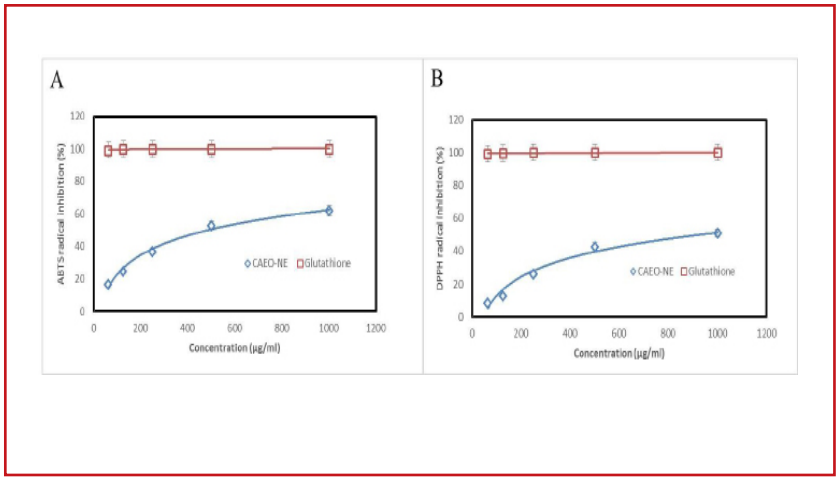

Chart 1. The CAEO-NE antioxidant properties. A and B refer to the CAEO-NE scavenging effect on increasing doses of ABTS and DPPH free radicals, respectively compared with glutathione concentrations. CAEO-NE: Corylus avellana essential oil nanoemulsion.

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The IC50 concentrations of CAEO-NE in both ABTS and DPPH assays were measured at 410 and 560 µg/mL, respectively. The results indicate that CAEO-NE has the potential to be used as a safe natural antioxidant compound.

The antimicrobial activity of CAEO-NE

The bacterial growth pattern around the CAEO-NE's smeared disc reveals a detectable not grown ring (7 mm) compared with the Kanamycin antibiogram disc antigrowth pattern (Figure 3). Therefore, CAEO-NE has significant anti-bacterial potential. However, the MIC (minimum inhibitory concentration) test has to be done to study its strength and specificity of its antibiotic activity.

Anti-angiogenic property of CAEO-NE

The results reveal a significant association among the increasing CAEO-NE doses with decreasing and shortening of the blood vessels of chick embryo chorioallantoic membranes (p-value = < 0.001) (Chart 2), which demonstrates its suppressive impact on angiogenesis induction. Therefore, it can be concluded that CAEO-NE has angiogenic inhibitory potential. However, angiogenic gene expression has to be studied to verify and discover the details of its antiangiogenic activity (Figure 4).

Discussion

Herbally-derived medicinal compounds have long been utilized in human health care. Today, they are presented in various types of formulations, which improve their efficiency. To the best of our knowledge, this is the first study encapsulating CAEO bioactive ingredients by ultrasound-based emulsification technology to study its antioxidant, antibacterial, and antiangiogenic potentials. The 45.9-nm CAEO nanoemulsions significantly scavenged both ABTS and DPPH free radicals, inhibited staphylococcus aureus growth, and suppressed the angiogenesis processes in the chick embryo chorioallantoic membranes.

Free radicals called oxidant molecules are the origin of cellular oxidative storms, which initiate a harmful abnormality in the metabolism and proliferation of cells. Cells have been programmed to neutralize excessive radicals and prevent or suppress the oxidative stress formation by producing antioxidant molecules (e.g., glutathione and so forth) and enzymes (e.g., CAT and so on.) (24). Therefore, dietary antioxidant intakes improve the health of cells by protecting them against daily oxidative stresses.

There are several types of natural antioxidant compounds extracted in herbal essential oils such as curcumin, Citrus lemon, and dill essential oils, which have been formulated into nanoemulsion-based drug delivery systems (25-27). The antioxidant potential of Corylus avellana is attributed to its individual components of phenolic compounds such as gallic acid and ellagic acid (9, 28, 29). Polyphenols can synergistically improve the neutralization of the oxidants along with other bioactive molecules existing in plant extracts. Moreover, they can reduce age-related chronic disorders and protect us against pathological infections (30). The electron or hydrogen donation by phenolic compounds react with active free radicals and reduce their reactivity with other biomolecules such as lipids, proteins, and DNA (31).

In the current study, CAEO-NE exhibited antioxidant activity by inhibiting both ABTS and DPPH radicals (P-value < 0.001) (Chart 1). The IC50 concentrations of CAEO-NE in both ABTS and DPPH assays were measured at 410 and 560 µg/ml, respectively, which can be attributed to its phenolic ingredient profile such as gallic and ellagic acids. However, further studies are required to verify the exact CAEO-NE antioxidant factor and mechanism of action.

Moreover, the antimicrobial activity of Corylus avellana has been detected in several types of

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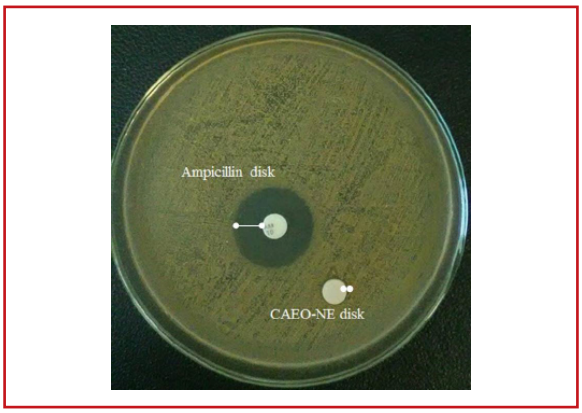

Figure 3. The CAEO-NE antibacterial activity compared with the ampicillin disk as the positive control. The non-grown rings of ampicillin and CAEO-NE smeared-disk have been defined in 21 and 7 mm radius, respectively. CAEO-NE: Corylus avellana essential oil nanoemulsion.

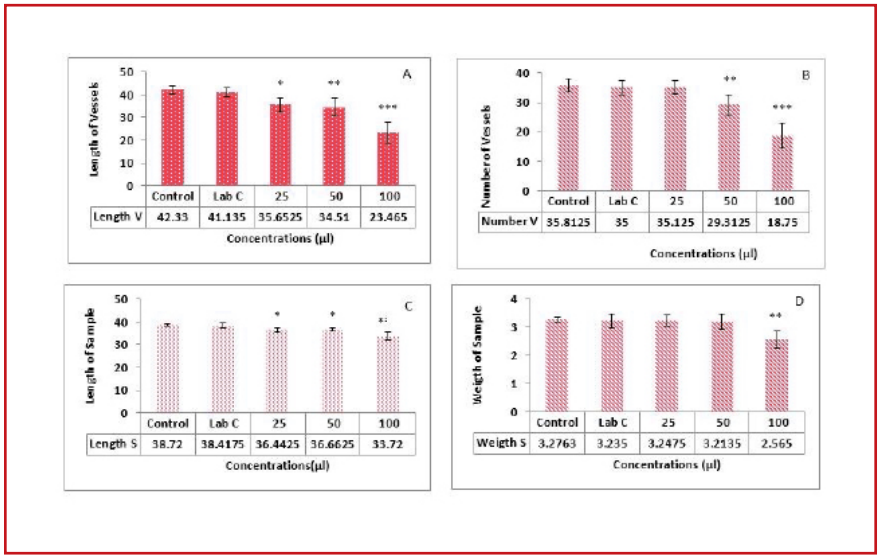

Chart 2 . A and B show the meaningful reduction in the number and length of the blood vessels in response to different treatment doses of CAEO-NE. C and D indicate the significant reduction in the length and weight of the embryos. The control group has not been treated, and lab control group has been treated with 0.1 M PBS. (*P<0.05, **P<0.01, ***P<0.001); CAEO-NE: Corylus avellana essential oil nanoemulsion.

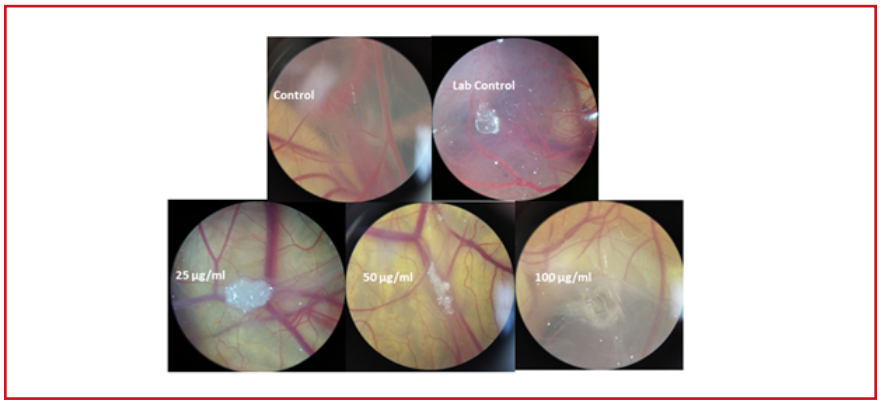

Figure 4. Anti-angiogenic property of CAEO-NEs. A) The stereomicroscopic image of the chorioallantoic membrane of the chick embryo treated with different CAEO-NE treatment doses (25, 50, and 100 µg/ml). The length and number of the CAM's blood vessals have decreased following the different CAEO-NE treatment doses.

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Moreover, the antimicrobial activity of Corylus avellana has been detected in several types of fungi (cryptococcus neoformans and candida albicans), gram-negative (Klebsiella pneumonia, pseudomonas aeruginosa, and escherichia coli), and gram-positive bacteria (10). According to Karolina Pycia et al. the chemical composition of the Corylus avellana seed contains quercetin, which has been proven as an antibacterial compound (9, 32). In the present study, the non-grown Staphylococcus A. ring surrounding the CAEO-NE smeared-disc reveals its antibacterial potential, which can be due to its quercetin composition (Figure 3). However, studying quercetin alone and its synergistic impact on further bacterial strains are required to verify its antibacterial potential on the Staphylococcus A. strain.

Also, there are many studies indicating the role of kaempferol and procyanidin in suppressing angiogenesis, which exist in Corylus avellana essential oil (9, 33, 34), and have been verified in the composition of CAEO as well. Therefore, in the current study, the inhibited angiogenesis on CAM's blood vessels was programmed (Figure 4).

Conclusion

According to the results, the nanoemulsions prepared by Corylus avellana essential oil have the potential to be an efficiently safe alternative to treat diseases caused by oxidative stress. However, more studies are needed to verify its antioxidant capacity. Finally, its suppressive impact on the angiogenesis processes makes it a suitable candidate as an anticancer compound. However, additional investigations on its cytotoxicity on cancer cells and their gene expression profile are required to verify its anticancer potential.

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Conflict of Interest

The authors have declared no conflict of interest.

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