Anti-inflammatory and anti-nociceptive effects of Cinnamon and Clove essential oils nanogels: an in vivo study

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Abstract
Background: Cinnamon (Cinnamomum zeylanicum) and Clove (Syzygium aromaticum) essential oils are two medicinally important plant-derived substances with a wide range of biological properties. Besides, nanoemulsion-based gels have been widely used to increase topical drug delivery and effectiveness.

Methods: This study aimed to explore the anti-inflammatory effect (paw edema test) and the anti-nociceptive effect (hot plate and formalin test) of nanoemulsion-based gels containing the essential oils in the animal model. Cinnamon and Clove essential oils nanoemulsions with droplet sizes of $28 \pm 6$ nm and $12 \pm 3$ nm were first prepared. By adding carboxymethylcellulose (3.5% w/v), the nanoemulsions were then gelified. Finally, the nanogels were characterized by ATR-FTIR analysis and were used as topical pre-treatment before induction of inflammation or pain in acute and chronic analgesic experimental studies.

Results: The paw edema and formalin findings showed that the nanogels formulations possess significant anti-nociceptive and anti-inflammatory effects.

Conclusion: The prepared nanogels could be considered as analgesic drugs for inhibiting the inflammation and pain of diseases.

Keywords: Analgesics, Nanomedicine, Painkiller, Paw edema test

Introduction

Inflammation, as a natural reaction at the site of external injurious (events) environments, plays a vital role in the pathogenesis of many chronic diseases such as rheumatoid arthritis, diabetes, and cancer [1, 2]. If the inflammation is severe at the tissue level, damage to nerves can cause pain signals that transmit through neurons to the brain [3, 4]. Nowadays, knowledge about pain and its mechanisms, especially neurophysiological and neuropathic pains, has increased significantly [5]. For example, emerging evidence suggests that inflammation and the release of inflammatory mediators from damaged tissues can cause pain [6]. Anti-inflammatory or painkiller nanodrugs drugs such as steroidal and non-steroidal are commonly used for inflammatory diseases and their related pains while having limited efficiency with side effects [7, 8]. Therefore, attempts to develop green nanodrugs as an important source of novel therapeutics have received more attention recently [9, 10].

Essential oils (EOs) as secondary metabolites of plants have recently been considered to treat inflammation and pain [11, 12]. For instance, Cinnamon EO (Cinnamomum zeylanicum), a spice derived from the inner bark of the
genus Cinnamomum trees, has promising inflammatory
effects [13, 14]. Some reports regarding Cinnamon’s anti-
nociceptive and antipyretic effects in bronchitis, rheuma-
tism, cold, fever, headache, and muscular pain [15, 16].
Also, the Cloves (Syzygium aromaticum) belongs to the
Myrtaceae family, which is a nail-shaped dried flower
bud [17, 18]; its EO has been traditionally applied in aro-
matherapy, relieving headaches, joint pain, toothaches,
and oral antiseptic [19, 20]. Furthermore, Clove EO has
also been used in dental emergencies as an asymptomatic
reliever of toothache and anti-inflammatory in the mouth
and throat [21, 22]. Also, other applications of Clove EO
and Clove extracts have been reported, such as antimuta-
genic, antioxidant, antithrombotic, antiparasitic, antibac-
terial, antiviral, and antifungal activities [23, 24].

Formulating of EOs as nanoformulations is a promising
approach for increasing their efficacy [25, 26]. Among
the nanoformulations, nanoemulsions are more considered
due to their relatively fewer side effects, bioavailability,
and simpler preparation methods [27–29]. Nanoemul-
sions are biphasic transparent dispersions composed of
oil and water phases stabilized by surfactants/co-surfac-
tants. Such systems are stable droplets over aggregation
or creaming processes because of their droplet size (less
than 200 nm) [30, 31]. Nanoemulsions as effective topical
delivery systems have demonstrated favorable character-
istics, including enhanced permeability without skin irrita-
tions [32, 33]. However, transforming nanoemulsions
to gel improves the topical administration and physical
and thermal stability of EOs [34, 35].

To the best of our knowledge, the anti-nociceptive and
anti-inflammatory effects of nanoemulsion-based gels of
Cinnamon and Clove EOs have not been investigated.
Therefore, their efficacy as a topical delivery system
against inflammation and nociception was investigated in
this study.

Materials and methods

Materials

λ-Carrageenan and carboxymethylcellulose (medium
viscosity) were supplied from Sigma-Aldrich (Germany).
Formalin (HCHO) and tween 20 were purchased from
Merck Chemicals (Germany). Cinnamon and Clove EOs
were bought from Green Plants of Life Co. and Zardband
Pharmaceuticals Co. (Iran).

Twenty-four Wistar male rats weighing 180±20 g were
used. They were kept in the Standard Laboratory Ani-
mal Guidelines, and the experimental protocols were
approved by the Tehran University of Medical Sciences
Ethical Committee (code 91–01–87-17,072). Rats were
randomly divided into 4 groups (n = 6). Each group was
treated with 100 mg of the following samples, including
distilled water as negative control (D.W.), blank gel,
Cinnamon-nanogel (cinnamon- NG), and Clove-nanogel
(clove-NG).

Preparation and characteristics of nanoemulsions-based
gels of Cinnamon and Clove EOs

For the preparation of nanoemulsions, at first, Cinnamon
and Clove EOs (2.5% v/v) were added to tubes containing
tween-20 (7.5 and 10% v/v, respectively), which were on
stirrer equipment at 2000 rpm at room temperature (MS-
300HS, Protraction Intertrade Co., Korea). Then, dis-
tilled water was added at room temperature dropwise to
the desired volume, stirring at 2000 rpm for 40 minutes.
Next, nanogels were prepared by adding carboxymethyl-
cellulose (CMC 3.5% w/v) to the as-prepared nanoemul-
sions while mixing in a mild condition (180 rpm) for 4 h.
The prepared gels were abbreviated as cinnamon-NG and
clove-NG. Similarly, a blank gel was also prepared with
distilled water.

The mean droplets size of nanoemulsions was meas-
ured by dynamic light scattering (DLS) at a scattering
angle of 90° using Scatterscope (K-one Ltd. Korea).
Droplet size distribution was calculated using the fol-
lowing equation, D90-D10/D50; D was the diameter of
droplets, and D10, D50, and D90 were the percentile of
droplets with a diameter lower than these values. The
chemical spectra of nanoemulsions containing Cinna-
mon and Clove EOs, CMC powder, and nanogels were
analyzed by Attenuated Total Reflection-Fourier-Trans-
form InFrad (ATR-FTIR) using an infrared instrument in
a wavenumber 400–4000 cm⁻¹ (Bruker, Tensor II,
Germany) without any sample preparation process.

Acute anti-nociceptive studies

The hot plate test was carried out to assess the acute anti-
nociceptive activity of cinnamon-NG and clove-NG [36].
For this purpose, topical pre-treatment was carried out
with 100 mg of the nanogels for each rubbing on the left
hind paw for an hour with intervals of 15 minutes. For
topical adsorption of nanogels, the rats were allowed
1 h after pre-treatment. Then rats were then placed on
a hot plate (53 ± 2°C). The response latency to the ther-
mal stimulus was considered as the sign of nociception.
The response latency was measured as the time taken by
the animal for nocifensive behaviors, including paw lick-
ing, jumping, or flinching. The first nocifensive behaviors
were recorded, and rats were immediately removed from
the hot plate. To prevent tissue damage, a cut-off period
was taken as the 60 s.

Acute and chronic anti-nociceptive studies

Formalin test was used to assess gels’ acute and chronic
anti-nociceptive effects [37]. After 2 weeks after the
hot plate test, the rats were anesthetized, and topical
pre-treatment was carried out with 100 mg of the nanogels (contains 2.5 mg of EOs) for each rubbing on the left hind paw for an hour with intervals of 15 minutes (totally 10 mg EO). For topical adsorption of nanogels, the rats were allowed 1 h after pre-treatment. Then acute pain was induced by subcutaneous injection of formalin 1% into the dorsal surface of the paw. Afterward, the rats were immediately placed into a flat plexiglass chamber (30 x 30 x 30 cm) with a mirror at 45° angles for observing the animals’ response to pain-related behaviors during 15 s periods for 1 h at 1 min intervals based on the numerical scale in Table 1 (Dubuisson, Dennis) [38]. Following formalin injection, anti-nociceptive effects were assessed in two phases: in phase-1 acute pain was recorded 5 min after the injection, and in phase-2 chronic pain was recorded 15–60 min after the injection were assessed. The weighted scores or rating scale method (Eq. 1) was used to quantify the pain [39].

\[
\text{Average weighted score} = \frac{0T0 + 1T1 + 2T2 + 3T3}{T0 + T1 + T2 + T3}
\]  

(1)

The second number of animals in each category is defined as T and multiplied by its given weight score for 5 min test. The total time was 300 s, and the pain scores were generated with a range of 0–3.

In addition to anti-nociceptive effects, the total licking times of the injected paw were recorded in two phases; the first phase 0–5 min (acute) and the second phase 15–60 min (chronic), which represent both neurogenic and inflammatory pain responses, respectively.

**Anti-inflammatory studies**

According to previous studies, the carrageenan-induced model performed the paw edema test to investigate the anti-inflammatory effects of the nanogels [9]. After deep anesthesia of rats, topical pre-treatment was carried out with 100 mg of the nanogels for each rubbing on the right hind paw for an hour with intervals of 15 minutes. For topical adsorption of nanogels, the rats were allowed 1 h after pre-treatment. Then inflammation was induced by injection of 0.1 mL freshly carrageenan solution (1% w/v in normal saline 0.9%) into the sub-plantar region. Then paw edema (average volume of paw swelling) was measured by using a digital caliper up to 5 h with 1 h intervals following carrageenan injection. Finally, the paw edema percentage was calculated by Eq. 2. Where \( V_a \) indicates the paw diameters after injecting carrageenan and \( V_b \) denotes the paw diameters before injecting carrageenan

\[
\text{Paw edema} \% = \frac{V_a - V_b}{V_b} \times 100
\]  

(2)

**Statistical analysis**

One-way analysis of variance and Tukey comparison were performed to assess the statistical significance of differences among groups. Results with a \( p \) value<0.05 were considered statistically significant. Statistical analyses were carried out using the SPSS software, v. 21 (SPSS, Inc., USA).

**Results**

**Determining the size of the nanoemulsions**

Figure 1 shows the mean droplets size and size distribution of nanoemulsions containing Cinnamon and Clove EOs, which had a size of less than 100 nm and droplet size distribution of less than 1.

**Chemical properties of the nanogels**

Figure 2 shows the ATR-FTIR spectra of the samples in terms of transmittance rate (%). In the Cinnamon nanoemulsions spectrum, the peak at 2922 and 2854 cm\(^{-1}\) are attributed to C-H stretching. The peak at 1732 cm\(^{-1}\) exhibited C=O stretching, and the characteristic absorption at around 1450 cm\(^{-1}\) shows CH\(_2\) bending. In the Clove nanoemulsions spectrum, the broadband at 3358 cm\(^{-1}\) is attributed to O-H stretching vibration due to hydrogen bonding between Clove EO and tween 20 molecules. The peak at 2924 cm\(^{-1}\) is attributed to C-H stretching, and the peak at 1731 and 1631 cm\(^{-1}\) exhibited C=O stretching. The characteristic absorption at around 1463 cm\(^{-1}\) showed CH\(_2\) bending, and the peak at 1003 cm\(^{-1}\) is attributed to C-O stretching.

ATR-FTIR spectrum of CMC showed the broad bands at 3321 cm\(^{-1}\) can be attributed to the stretching of the hydroxyl group O-H due to H-bonding, the strong band at 1589 cm\(^{-1}\) related to COO- group (asymmetric stretching), and 1413 cm\(^{-1}\) related to COO- (symmetric stretching). The characteristic absorption at around 993 cm\(^{-1}\) is attributed to C-O stretching. Cinnamon-NG spectrum peak at about 2922 cm\(^{-1}\) is attributed to C-H stretching due to EO, tween, and CMC. The peak at 1731 and 1674 cm\(^{-1}\) exhibited C=O stretching, representing the carbonyl group in Cinnamon EO with tween molecules. The sharp and strong peak at 1120 cm\(^{-1}\) is attributed to C-O stretching. Noticeably, the COO\(^{-}\) band at
1589 cm\(^{-1}\) in the presence of CMC was shifted toward the lower wavenumber at 1576 cm\(^{-1}\), confirming the association of CMC with tween through intermolecular H-bonding.

Moreover, in the spectrum of clove-NG, the peak at 2922 and 2854 cm\(^{-1}\) are attributed to C-H stretching due to Clove EO, tween, and CMC. The peak at 1735 cm\(^{-1}\) exhibited C=O stretching, representing the overlap carbonyl group in Clove EO with tween molecules. The sharp and strong peak at 1097 cm\(^{-1}\) is attributed to C-O stretching. Noticeably, the COO\(^-\) band at 1589 cm\(^{-1}\) in the presence of CMC was shifted toward a lower wavenumber at 1513 cm\(^{-1}\), confirming the association of CMC with tween through intermolecular H-bonding.

**Acute anti-nociceptive studies**

Figure 3 shows the experimental results of the hot plate test, the thermal anti-nociceptive activity of formulations, and the central pain index. As the details direct, topical administration of cinnamon-NG and clove-NG failed to prolong latency time to the hot plate painful stimulus compared to the control group.

**Acute and chronic anti-nociceptive studies**

Formalin test was used to assess acute and chronic anti-nociceptive responses. The anti-nociceptive results of formulations through chemical stimuli of formalin were reported in Fig. 4 and Table 2. Noteworthy, no anesthesia, movement impairments, and respiratory insufficiency were seen in the animals during the test. In the second phase of the formalin test, pre-treatment rats with cinnamon-NG and clove-NG show significant anti-nociceptive activity and reduced nociceptive
scores. However, this effect was more significant and noticeable in cinnamon-NG. In detail, as the graph shows, no significant difference was observed between groups up to 20 min following formalin injection. However, in the groups pre-treated with cinnamon-NG and clove-NG, an immediate reduction in nociceptive scores was observed at 25 min.

Moreover, these groups remained lower than the blank gel and control groups (D.W.) until the end of the test. Worth mentioning that the significant differences in
nociceptive scores between clove-NG and control were seen from 50 min onwards, while the significant ones between clove-NG and gel were observed at 25, 35, 50, and 60 min. Meanwhile, the significant anti-nociceptive effect in the cinnamon-NG group compared with the control and gel groups was noticed at all-time points after 20 min, indicating a strong anti-nociceptive effect.

The pain response of formulations to both acute and chronic phases of the formalin test is illustrated in Fig. 5. Our results indicated no significant difference between the tested group in the acute phase, while chronic pain intensity was significantly decreased in the cinnamon-NG group 4 h after treatment. The clove-NG inhibitory effect was more than D.W. and blank gel groups at the 1st and 2nd h of the experiment; however, the differences were insignificant. On the other hand, compared to D.W., the cinnamon-NG group was effective against carrageenan-induced inflammation, especially at the 4th and 5th h of the test, suggesting longer biological life cinnamon-NG compared to the carrageenan with the most activity at the 3rd h [41].

Discussions
In the current study, nanoemulsions containing cinnamon and clove EOs were prepared; as obtained droplet sizes were lower than 200 nm, their nanoscale sizes were confirmed. Moreover, a droplet size distribution of less than 1 confirms narrow size distribution and single sharp peaks referred to as monodisperse systems [26]. The low-Energy or spontaneous method is common for preparing EO-based nanoemulsions and preventing evaporating volatile compounds in EOs [42, 43]. This method involves mixing the components of nanoemulsions at room temperature without any external energies as a high-homogenizer or ultrasound [33].

Ingredients of the used EOs in the current study (i.e., Cinnamon and Clove) were identified using Gas Chromatography-Mass Spectrometry and reported in our previous reports. Cinnamaldehyde, with 62.04%, is the major component of Cinnamon EO, and linalool (6.96%), trans-caryophyllene (6.60%), trans-cinnamyl acetate (4.29%), and benzyl benzoate (3.32%) are other major constituents [44]. Besides, eugenol, with 65.41%, is the major component in Clove EO. trans-caryophyllene (12.06%), eugenol acetate (9.85%), caryophyllene oxide (3.00%), and α-humulene (1.73%) are other major constituents [45].

Pro-inflammatory agents, including carrageenan, can affect vascular permeability and blood cells migration through modulated nitric oxide (NO) secretion [46, 47]. On the other hand, the production of pro-inflammatory mediators is stimulated by inducing nitric synthase (iNOS) and cyclooxygenase-2 (COX-2) [50, 51]. It has been reported that eugenol and cinnamaldehyde, the main component of Clove EO and

| Time (min) | Groups                  | P-Value  |
|-----------|-------------------------|----------|
| 25        | D.W. vs. cinnamon-NG    | 0.0194   |
|           | blank gel vs. clove-NG   | 0.0072   |
|           | blank gel vs. cinnamon-NG | 0.0017  |
| 30        | D.W. vs. cinnamon-NG    | 0.0019   |
|           | blank gel vs. cinnamon-NG | 0.0009  |
| 35        | D.W. vs. cinnamon-NG    | 0.0099   |
|           | blank gel vs. clove-NG   | 0.0331   |
|           | blank gel vs. cinnamon-NG | 0.0112  |
| 40        | D.W. vs. cinnamon-NG    | 0.0005   |
|           | blank gel vs. cinnamon-NG | 0.0003  |
| 45        | D.W. vs. cinnamon-NG    | 0.0003   |
|           | blank gel vs. cinnamon-NG | 0.0002  |
|           | clove-NG vs. cinnamon-NG | 0.0241  |
| 50        | D.W. vs. clove-NG       | 0.0007   |
|           | D.W. vs. cinnamon-NG    | 0.0006   |
|           | blank gel vs. clove-NG   | 0.0037   |
|           | blank gel vs. cinnamon-NG | 0.0036  |
| 55        | D.W. vs. clove-NG       | 0.0418   |
|           | D.W. vs. cinnamon-NG    | 0.0007   |
|           | blank gel vs. clove-NG   | 0.0135   |
|           | blank gel vs. cinnamon-NG | 0.0001  |
| 60        | D.W. vs. clove-NG       | 0.0002   |
|           | D.W. vs. cinnamon-NG    | 0.0001   |
|           | blank gel vs. clove-NG   | <0.0001  |
|           | blank gel vs. cinnamon-NG | <0.0001 |

The inhibition of carrageenan-induced paw edema in rats is a model for establishing the efficacy of anti-inflammatory drugs [40]. As shown in Fig. 7, cinnamon-NG suppressed carrageenan-induced rat paw edema more than other groups, including clove-NG, control, and blank gel groups. A maximum inhibitory effect was seen in the cinnamon-NG group 4 h after treatment. The clove-NG inhibitory effect was more than D.W. and blank gel groups at the 1st and 2nd h of the experiment; however, the differences were insignificant. On the other hand, compared to D.W., the cinnamon-NG group was effective against carrageenan-induced inflammation, especially at the 4th and 5th h of the test, suggesting longer biological life cinnamon-NG compared to the carrageenan with the most activity at the 3rd h [41].

### Table 2
Statistical comparison between the groups on formalin test (chronic phase) including distilled water (D.W.: control group), cinnamon-NG, and clove-NG

| Time (min) | Groups                  | P-Value  |
|-----------|-------------------------|----------|
| 25        | D.W. vs. cinnamon-NG    | 0.0194   |
|           | blank gel vs. clove-NG   | 0.0072   |
|           | blank gel vs. cinnamon-NG | 0.0017  |
| 30        | D.W. vs. cinnamon-NG    | 0.0019   |
|           | blank gel vs. cinnamon-NG | 0.0009  |
| 35        | D.W. vs. cinnamon-NG    | 0.0099   |
|           | blank gel vs. clove-NG   | 0.0331   |
|           | blank gel vs. cinnamon-NG | 0.0112  |
| 40        | D.W. vs. cinnamon-NG    | 0.0005   |
|           | blank gel vs. cinnamon-NG | 0.0003  |
| 45        | D.W. vs. cinnamon-NG    | 0.0003   |
|           | blank gel vs. cinnamon-NG | 0.0002  |
|           | clove-NG vs. cinnamon-NG | 0.0241  |
| 50        | D.W. vs. clove-NG       | 0.0007   |
|           | D.W. vs. cinnamon-NG    | 0.0006   |
|           | blank gel vs. clove-NG   | 0.0037   |
|           | blank gel vs. cinnamon-NG | 0.0036  |
| 55        | D.W. vs. clove-NG       | 0.0418   |
|           | D.W. vs. cinnamon-NG    | 0.0007   |
|           | blank gel vs. clove-NG   | 0.0135   |
|           | blank gel vs. cinnamon-NG | 0.0001  |
| 60        | D.W. vs. clove-NG       | 0.0002   |
|           | D.W. vs. cinnamon-NG    | 0.0001   |
|           | blank gel vs. clove-NG   | <0.0001  |
|           | blank gel vs. cinnamon-NG | <0.0001 |
Cinnamon EO, demonstrated anti-inflammatory effects similar to COX inhibitors, including indomethacin and celecoxib [17, 48]. In addition, cinnamaldehyde inhibits lipopolysaccharide-induced chondrocyte inflammation [49]. Besides, eugenol in LPS-stimulated mouse macrophages showed a COX-2 inhibitory effect [50, 51]. This result suggests that cinnamon-NG and clove-NG could be candidates for further developing anti-inflammatory drugs.

It has been proposed that the analgesic effect of EO of Clove and Cinnamon may be due to these main ingredients (eugenol and cinnamaldehyde) [52, 53]. Their administration as analgesic agents in experimental models of pain in mice was reported [54, 55]. Moreover, it has been reported that eugenol demonstrated a significant anti-nociceptive effect against chemical stimuli [56]. Therefore, it is proposed that eugenol predominantly prevents the peripheral pain mechanism.

Two cinnamon-NG and clove-NG block the peripheral pain mechanism and cannot affect central pain through the hot plate test. The formalin (chemical stimuli) results are also inconsistent with these findings.

It is important to note that acute and chronic phases results of the formalin test showed that both cinnamon-NG and clove-NG significantly affect the second phase of the nociceptive response in the formalin test and not in the first phase. Similar observations have also been reported about other nanoemulsions [57]. In addition, it was found that topical application of Clove EO and intra-peritoneal administration of Cinnamon extract significantly decreased acute and chronic pain in formalin tests [58, 59].

According to rat’s paw licking time results, cinnamon-NG inhibited the formalin-induced pain response in both phases, indicating the involvement of both peripheral and central mediated mechanisms. In one study, a significant reduction in pain response was found in Cinnamomum zeylanicum (200 and 400 mg/kg) treated groups during the first phase of the formalin test. However, during the second phase, a significant reduction in formalin-induced pain response was observed in 100, 200, and 400 mg/kg C. zeylanicum extract-treated groups compared to the control group [60]. Moreover, cinnamic...
alcohols (a phenylpropanoid of cinnamon)-treated animals (6.25, 12.5, 25 mg/kg) exhibited reduced paw licking behavior in the first and second phases of the formalin test. Cinnamic alcohol’s anxiolytic and antinociceptive-like effects were suggested to be due to GABAergic system modulation [61]. The clove-NG inhibited the rat’s paw licking time in the late phase. According to the literature, eugenol exhibited an antinociceptive effect more in the inflammatory phase than in the neurogenic phase in a formalin-induced licking pain model [56]. Clove oil also reduces pain response through a mainly peripheral action, as demonstrated by the formalin test and the tail-flick test, which indicated the participation of opioid receptors [62].

**Conclusion**

This study developed cinnamon-NG and clove-NG as a topical delivery system. Our work has led us to conclude that nanoemulsion-based gel formulations, especially cinnamon-NG, could apply as anti-nociceptive
and anti-inflammatory agents or promising therapeutics in relieving diseases accompanied by inflammation and pain.

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Not applicable.

Authors’ contributions
MO and FE conceived and designed the experiments. MO prepared and characterized nanogels. FE and MZ performed the experiments. MZ, YY, and HA rewrote the manuscript and analyzed the data, and performed the analysis with constructive discussions. All authors read and approved the final manuscript.

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Availability of data and materials
The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials. Raw data are available from FE upon a reasonable request.

Declarations

Ethics approval and consent to participate
The Ethical Committee of Tehran University of Medical Sciences approved this study (91-01-87-17072), and all methods were carried out per relevant guidelines and regulations. Moreover, all methods are reported per ARRIVE guidelines.

Consent for publication
Not applicable.

Competing interests
All authors declare no conflict of interest.

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References
1. Chen L, Deng H, Cui H, Fang J, Zuo Z, Deng J, et al. Inflammatory responses and inflammation-associated diseases in organs. Oncotarget. 2018;9(6):7204.
2. Zhong J, Shi G. Regulation of inflammation in chronic disease. Front Immunol. 2019;10:737.
3. Jancalek R. Signaling mechanisms in mirror image pain pathogenesis. Ann Neurosci. 2011;18(3):123–7.
4. Shin J, Cho H, Hwang SW, Jung J, Shin CY, Lee S-Y, et al. Bradykinin-12-lipoxygenase-VR1 signaling pathway for inflammatory hyperalgesia. Proc Natl Acad Sci U S A. 2002;99(15):10105–5.
5. Oliveira CC. Understanding pain and human suffering. Rev Biotc. 2016;24:225–34.
6. Ronchetti S, Migliorati G, Delfino DV. Association of inflammatory mediators with pain perception. Biomed Pharmacother. 2017;96:1445–52.
7. Lazzaroni M, Bianchi Porro G. Gastrointestinal side-effects of traditional non-steroidal anti-inflammatory drugs and new formulations. Aliment Pharmacol Ther. 2004;20:48–58.
8. Vongtaw H, Abbah J, Ngaizal J, Kunle O, Chindo B, Otupsa P, et al. Anti-nociceptive and anti-inflammatory activities of the methanolic extract of Parinari polyandra stem bark in rats and mice. J Ethnopharmacol. 2004;90(1):115–21.
9. Esmaeili F, Rajabnejhad S, Partoazar AR, Mehr SE, Faridi-Majidi R, Sahebgharani M, et al. Anti-inflammatory effects of eugenol nanoemulsion as a topical delivery system. Pharm Dev Technol. 2016;21(7):887–93.
10. Subramanian B, Kuo F, Ada E, Kotyla T, Wilson T, Yoganathan S, et al. Enhancement of anti-inflammatory property of aspirin in mice by a nano-emulsion preparation. Int Immunopharmacol. 2008;8(11):1533–9.
11. Aman RM, Hashim IIA, Meshali MM. Novel Clove essential oil nanoemulsion tailored by Taguch’s model and scaffold-based nanoformulators: Phytopharmacological studies. J Ethnopharmacol. 2020;15;2171.
12. Jeengar MK, Rompicharla SVK, Shrivastava S, Chella N, Shastri NR, Naidu V, et al. Emu oil based nano-emulsion for topical delivery of curcumin. Int J Pharm. 2016;506(1–2):222–36.
13. Schink A, Naumskos K, Kitanovski Z, Kampf CJ, Frohlich-Nowosycki J, Thines E, et al. Anti-inflammatory effects of cinnamon extract and identification of active compounds influencing the TLR2 and TLR4 signaling pathways. Food Funct. 2018;9(11):5950–64.
14. Hong J-W, Yang G-E, Kim YB, Eom SH, Lew J-H, Kang H. Anti-inflammatory activity of cinnamon water extract in vivo and in vitro LPS-induced models. BMC Complement Altern Med. 2012;12(1):1–8.
15. Vetal S, Bodhankar SL, Mohan V, Thakurdesai PA. Anti-inflammatory and anti-arthritis activity of type-A procyanidine polyphenols from bark of Cinnamomum zeylanicum in rats. Food Sci Hum Wellness. 2013;2(2):59–67.
16. Ranasinghe P, Pijgera S, Premakumara GAS, Galappaththy P, Constantine GR, Katulanda P. Medicinal properties of true cinnamon (Cinnamomum zeylanicum): a systematic review. BMC Complement Altern Med. 2013;13(1):275.
17. Daniel AN, Sartoretto SM, Schmidt G, Caparozz-Assief SM, Bersani-Amado CA, Cuman RKN. Anti-inflammatory and antinociceptive activities of eugenol essential oil in experimental animal models. Rev Bras Farmacogn. 2009;19(18):212–7.
18. Agra MF, Silva KN, Basilio IJD, Freitas PF, Barbosa-Filho JM. Survey of medicinal plants used in the region northeast of Brazil. Rev Bras Farmacogn. 2008;18(3):472–508.
19. Chaibek H, Hajjaoui H, Zmantar T, Kahla-Nakbi AB, Rouabeha M, Mahdouani K, et al. The chemical composition and biological activity of clove essential oil, Eugenia Caryophyllata (Syzygium aromaticum L. Myrtaceae): a short review. Phytother Res. 2007;21(6):501–6.
20. Cortés-Rojas DF, de Souza CRF, Oliveira WP. Clove (Syzygium aromaticum): a precious spice. Asian Pac J Trop Biomed. 2014;4(2):90–6.
21. Milind P, Deepa K. Clove: a champion spice. Int J Ayurveda Pharm. 2011;2(1):47–54.
22. Prakash P, Gupta N. Therapeutic uses of Ocimum sanctum Linn (Tulsi) with a note on eugenol and its pharmacological actions: a short review. Indian J Physiol Pharmacol. 2005;49(2):125.
23. Valizadeh A, Khaleghi AA, Alipanah H, Zarenezhad E, Osanloo M. Anti-inflammatory and anti-arthritic activities of type-A procyanidines from avocado (Persea americana (L.) Meisn) and black (Persea gratissima (L.) var. negressa (Scribn.) Steenis) as compared with a reference standard of eugenol. Asia Pac J Clin Pharmacol. 2012;8(1):26–32.
24. Zarenezhad E, Abdollahi A, Esmaeili F, Satvati S, Osanloo M. A fast-degradation nano-emulsion preparation. Int Immunopharmacol. 2008;8(11):1533–9.
25. Osanloo M, Arish J, Sereshti H. Developed methods for the preparation of biodegradable nano-dressing with potent antibacterial effect. BioNanoScience. 2021;11(3):678–86.
26. Abedinpour N, Ghanbariasad A, Taghinezhad A, Osanloo M. Preparation of bifunctional nanoemulsions of mentha piperita essential oil and investigation of their cytotoxic effect on human breast cancer cells. BioNanoScience. 2021;11(2):428–36.
27. Maghbool M, Khosravi T, Vojdani S, Chaijan MR, Esmaeili F, Amani A, et al. The effects of eugenol nanoemulsion on pain caused by...
arteriovenous fistula cannulation in hemodialysis patients: a randomized double-blinded controlled cross-over trial. Complement Ther Med. 2020;52:102440.

28. Ghiasi Z, Esmaeili F, Aghajani M, Ghazi-Khansari M, Faramaz MA, Amani A. Enhancing analgesic and anti-inflammatory effects of capsaicin when loaded into olive oil nanoemulsion: an in vivo study. Int J Pharm. 2019;559:341–7.

29. Sondari D, Tursiloda S. The effect of surfactant on formulation and stability of nanoemulsion using extract of Centella Asiatica and Zingeribe of. In: AIP conference proceedings. 2018. AIP Publishing LLC. 2018. p. 033014. https://doi.org/10.1063/1.5083515.

30. Solans C, Izquierdo P, Nolla J, Azemar N, Garcia-Celma MJ. Nano-emulsions.Curr Opin Colloid Interface Sci. 2005;10(3–4):102–10.

31. Ee SL, Duan X, Liew J, Nguyen QD. Droplet size and stability of nano-emulsions produced by the temperature phase inversion method. Chem Eng J. 2008;140(1–3):626–31.

32. Tadros T, Izquierdo P, Esquena J, Solans C. Formation and stability of nano-emulsions. Adv Colloid Interf Sci. 2004;108:303–18.

33. Abbasifard M, Yousefpoor Y, Amani A, Arababadi MK. Topical bee venom nano-emulsion ameliorates serum level of Endothelin-1 in collagen-induced rheumatoid arthritis model. BioNanoScience. 2021;11:810–5.

34. Hussain A, Samad A, Singh S, Ahsan M, Haque M, Faruk A, et al. Nanoemulsion gel-based topical delivery of an antifungal drug: in vitro activity and in vivo evaluation. Drug Deliv. 2020;27(2):642–57.

35. Ghanbariasad A, Azadi S, Agholi M, Osanloo M. The nanoemulsion-based nanogel of Artemisia dracunculus essential oil with proper activity against Leishmania tropica and Leishmania major. J Nanomed Res. 2021;6(1):89–95.

36. Masocho W, Kombian SB, Edalighio EO. Evaluation of the antinociceptive activities of emomine compounds on the formalin and hot plate tests in mice. Sci Rep. 2016;6(11):1–9.

37. Tjølsen A, Berge O-G, Hunskaar S, Rosland JH, Hole K. The formalin test: an evaluation of the analgesic effect of measurement. Pain. 1993;52(3):259–85.

38. Dubuisson D, Dennis SG. The formalin test: a quantitative study of the nociception and anxiety like behavior in mice. Asian J Pharm Clin Res. 2017;17(2):252–60.

39. Jain S, Gupta S. Effects of Cinnamomum zeylanicum bark extract on nociception and anxiety like behavior in mice. Asian J Pharm Clin Res. 2019;12(9):236–41.

40. de Andréade HHH, Monteiro AB, Braga RM, da Cruz RMD, Salvador MGSS, Scotti MT, et al. Anti-inflammatory and antinociceptive-like effects of cinnamaldehyde in mice. J Biochem Mol Biol. 2016;49(6):713–22.

41. Daroogani S, Parandin R, Yousofozand N, Shakiabeie D. The analgesic effect of topical clove oil using formalin test in male mice. J Ardabil Univ Med Sci. 2017;17(2):252–60.

42. Jain S, Gupta S. Effects of Cinnamomum zeylanicum bark extract on nociception and anxiety like behavior in mice. Asian J Pharm Clin Res. 2019;12(9):236–41.

43. Danesh A, Talebi F, Fernandes PM, Ferreira RMF, Mota GC, et al. Antinociceptive effects of essential oils from Zingiber officinale and Alpinia galanga on animal models of inflammatory pain. Pain Res Manag. 2014;2014:789241.

44. Moemenbellah-Fard MD, Abdollahi A, Ghanbariasad A, Osanloo M. Antibacterial and leishmanicidal activities of Syzygium aromaticum essential oil versus its major ingredient, eugenol. Flavour Fragr J. 2020;35(9):534–40.

45. Costa JFQ, David JP, David JM, Gulietti AM, Queiroz LP, Santos RR, et al. Immunomodulatory activity of extracts from Cordia superba Cham. and Cordia rufescens A. DC.(Boraginaceae), plant species native from Brazilian Semi-arid. Rev Bras Farmacogn. 2008;18:11–5.

46. Markov AV, Senkova AV, Babich VO, Odaenko KV, Talstykh VA, Salomatina OV, et al. Dual effect of solosolone methyl on LPS-induced inflammation in vitro and in vivo. Int J Mol Sci. 2016;17(2):18767.

47. Liao J-C, Deng J-S, Chiu C-S, Hou W-C, Huang S-S, Shie P-H, et al. Anti-inflammatory activities of Cinnamomum cassia constituents in vitro and in vivo. Evid Based Complement Alternat Med. 2012;2012:429320.

48. Chen P, Ruan A, Zhou J, Huang L, Zhang X, Ma Y, et al. Cinnamic aldehyde inhibits lipopolysaccharide-induced chondrocyte inflammation and reduces cartilage degeneration by blocking the nuclear factor-kappa B signaling pathway. Front Pharmacol. 2020;11:949.

49. Kimalo SS, Oh O-J, Min H-Y, Park E-J, Kim Y, Park H-J, et al. Eugenol suppresses cyclooxygenase-2 expression in lipopolysaccharide-stimulated mouse macrophage RAW264. 7 cells. Life Sci. 2003;73(3):337–48.

50. Huss U, Ringbom T, Perera P, Bohlin L, Väisång M. Screening of ubiquitous plant constituents for COX-2 inhibition with a scintillation proximity based assay. J Nat Prod. 2002;65(11):1517–21.

51. Hosseini M, Kamkar M, Rahshandeh H. Analgesic effect of clove essential oil in mice. Avicenna J Phytomed. 2011;1(1):1–6.

52. Churhar R, Solanki P, Vyas S, Hernant Tanwani H, Shubham Atal S. Analgesic activity of cinnamaldehyde per se and it’s interaction with diclofenac sodium and pentazocine in swiss albino mice. Int J Pharmocog. 2016;3:97–102.

53. Kamkar AS, Nazirbournah A, Hosseini M. Analgesic effect of the aqueous and ethanolic extracts of clove. Avicenna J Phytomed. 2013;3(2):186–92.

54. Kamkar A, Amini M, Nazirbournah A. Microwave-assisted extraction of cinnamaldehyde using microwave-assisted extraction of cinnamaldehyde using microwave-assisted extraction of cinnamaldehyde using microwave-assisted extraction of cinnamaldehyde using ultrasound. Avicenna J Phytomed. 2013;3(2):186–92.

55. Sharma M, Rauniar G, Das B. Experimental study of various central nervous system effects of eugenol in mice and rats. Health Renaissance. 2012;10(3):208–14.

56. Kurian R, Arulmozhi D, Veeranjaneyulu A, Bodhankar S. Effect of eugenol on animal models of nociception. Indian J Pharmacol. 2006;38(3):341–5.

57. Ashtari S, Esmaeili F, Pardoazar A, Etemeaei Mehr S, Amani A. Efficacy of nano- and microemulsion-based topical gels in delivery of ibuprofen: an in vivo study. J Microencapsul. 2017;34(2):195–202.

58. Dashitti-Rahmatabadi M, Vahidi Merjadi A, Pilvaran A, Farzan F. Antinociceptive effect of cinnamon extract on formalin induced pain in rat. J Shahid Sadoughi Univ Med Sci. 2009;17(2):190–9.

59. Daroogani S, Parandin R, Yousofzand N, Shakiabeie D. The analgesic effect of topical clove oil using formalin test in male mice. J Ardabil Univ Med Sci. 2017;17(2):252–60.

60. Jain S, Gupta S. Effects of Cinnamomum zeylanicum bark extract on nociception and anxiety like behavior in mice. Asian J Pharm Clin Res. 2019;12(9):236–41.