Article

Cumin Essential Oil Nanoemulgel as a Topical Analgesic and Anti-Inflammatory Alternative to NSAIDs

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Abstract: *Cuminum cyminum* L. (cumin) essential oil (CEO) possesses documented analgesic and anti-inflammatory properties, but its topical application is limited by volatility, instability and low aqueous solubility. This study aimed to develop a CEO nanoemulgel and evaluate its physicochemical characteristics, dermal safety and antinociceptive/anti-inflammatory effects in rodent models. The optimized formulation exhibited nanometric droplet size, uniform distribution and acceptable physical stability. In a rat skin irritation test, repeated application of the CEO nanoemulgel did not produce visible erythema or edema compared with the gel base. In mice, the formulation increased tail-flick latency and reduced nociceptive behaviors in both phases of the formalin test, and histological analysis revealed attenuated inflammatory cell infiltration. These findings suggest that the CEO nanoemulgel has promising antinociceptive and anti-inflammatory activity and appears to be well tolerated in short-term preclinical models. Further studies, including long-term safety assessment, mechanistic analysis and clinical trials, are required before CEO nanoemulgel can be considered for use in humans.

Keywords: *Cuminum cyminum*; antinociceptive; anti-inflammatory; nanoemulgel; essential oils; nanoemulsion

1. Introduction

Pain is a growing global medical challenge with substantial economic and social impact on patients, healthcare systems, and society [1,2]. The International Association for the Study of Pain defines pain as an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage [3,4]. Some musculoskeletal pain syndromes, such as fibromyalgia, are challenging to manage and continue to present a serious challenge to modern medicine [5,6].

Analgesic medications for topical use are gaining popularity for the management of pain [7,8]. Topical pain relievers and anti-inflammatories that are designed to exert a local therapeutic effect are very popular with the public and are attracting increasing attention from the pharmaceutical industry [9–11]. In recent decades, particular attention has been given to topical nonsteroidal anti-inflammatory drugs (NSAIDs) [12]. However, these agents are associated with gastrointestinal, pulmonary, hematological, cutaneous and other adverse effects, especially with long-term use [13,14]. Consequently, many patients and clinicians consider herbal remedies as complementary or alternative options for pain management. Several reports suggest that, for long-term use, herbal products may offer a safer and better-tolerated approach than conventional NSAIDs [15–18].

The use of plant-derived compounds has a long history, and such products continue to make important contributions to modern pharmacotherapy. Their use in various cultures is well documented, and several medicinal plants have been investigated for the management of chronic pain disorders, including fibromyalgia and cancer-related pain [19–21]. Essential oils (EOs) are complex mixtures of volatile plant constituents, mainly monoterpenes, sesquiterpenes and phenylpropanoids. Numerous EOs have been reported to exhibit larvicidal,

antibacterial, antifungal, anticancer, antimutagenic, antidiabetic, antiviral, anti-inflammatory and antiprotozoal activities, and are also widely used as flavoring agents in food, cosmetic and related industries [22,23].

Cumin (*Cuminum cyminum* L.), a member of the Apiaceae family, is an aromatic spice with documented ethnomedicinal uses [24]. CEO has shown analgesic and anti-inflammatory effects in preclinical studies [25,26] and is commonly used to relieve digestive problems such as flatulence, indigestion and diarrhea [27,28]. Despite these promising properties, several physicochemical characteristics of EOs including volatility, instability, short half-life, low aqueous solubility and potential for dermal irritation, limit their direct incorporation into conventional pharmaceutical dosage forms [29].

Modern drug-delivery systems provide approaches to overcome these limitations. Topical administration of EO-containing emulsions has been explored in creams, ointments and lotions [30,31]. Nanoformulations, particularly nanoemulsions, have emerged as attractive carriers in food, cosmetic, pharmaceutical and agrochemical applications. Nanoemulsions consist of two immiscible liquids stabilized by surfactants, with droplet sizes typically in the 20–200 nm range (often <100 nm), and offer improved kinetic stability, enhanced solubilization of lipophilic compounds and better skin penetration [22,32]. Their small droplets, comprising a lipophilic core surrounded by a surfactant layer, can facilitate faster and more efficient transport across the stratum corneum, acting as penetration enhancers [33–35].

Nanoemulsions are characterized by enhanced absorption, efficient skin penetration, suitability for incorporation into creams and gels, and relatively simple preparation methods [36]. When dispersed into a gel matrix, they form nanoemulgels, an innovative topical delivery system that can improve cutaneous permeability and bioavailability of anti-inflammatory agents and that offers several advantages over conventional topical formulations [37–40]. Encapsulation of EOs in nanoscale drug-delivery systems is a rational strategy that protects these volatile constituents, allows their sustained release in small quantities and prolongs their residence time in the skin, thereby enhancing their biological properties [41–43].

The analgesic and anti-inflammatory effects of CEO are thought to be related to its oxygenated monoterpenes and monoterpene hydrocarbons [44,45] and nanoemulsification has been proposed to further improve the effectiveness of CEO [46]. Accordingly, the present study aimed to develop a CEO nanoemulgel and to evaluate its antinociceptive and anti-inflammatory effects in animal models.

2. Materials and Methods

2.1. Materials

The CEO was obtained from Barij Essence Pharmaceutical Company (Kashan, Iran). Span 80 and Tween 80 were purchased from Merck (Darmstadt, Germany). Diclofenac topical gel (1% *w*/*w*) was obtained from Cipla Ltd. (Mumbai, India). Carbopol 940 was supplied by BF Goodrich Co (Akron, OH, USA). Formalin and triethanolamine were obtained from Sigma-Aldrich (St Louis, MO, USA). Deionized water was purified by Human Power II+ Scholar (Human Corporation, Republic of Korea).

2.2. Formulation of CEO Nanoemulsions

Nanoemulsion was prepared based on our previous study by the high-pressure homogenization (HPH) method in the pharmaceutical laboratory of Mazandaran University of Medical Sciences, Sari, Iran [47]. Briefly, the coarse emulsion was prepared by dispersing CEO into the deionized water and a mixture of Tween 80/Span 80 as a surfactant. Afterwards, the mixture was homogenized by a high-speed homogenizer (Silent Crusher M, Heidolph, Germany) at 8000 rpm for 10 min. The pre-emulsion was homogenized by a high-pressure homogenizer (FBF laboratory, Italy) at 500 bars for 5 cycles to produce nanoemulsions. To prevent EO volatility, the HPH emulsion container was covered with ice bags to keep the temperature constant and prevent overheating [47]. The concentrations of the components are shown in Table 1.

Table 1. Components (wt.%) and physicochemical properties of investigated nanoemulsions. The values have been given as Mean ± SD (n = 3).

Nanoemulsion Code	CEO	Tween 80	Span 80	Water	Droplet Size (nm)	PDI	Zeta Potential (mV)
N1	2	1.5	1.5	95	87.533 ± 3.342	0.241 ± 0.029	−3.037 ± 1.115
N2	4	1.5	1.5	93	82.203 ± 5.817	0.199 ± 0.013	−0.496 ± 0.396

2.3. Characterization of Nanoemulsions

The polydispersity index (PDI) of nanoemulsion formulations and droplet size were determined using DLS (dynamic light scattering) via a Zetasizer Nano ZS system (Malvern Instruments, Worcestershire, UK) at an angle of 90° at 25 °C. The zeta potential of the nanoemulsion was determined by laser Doppler electrophoresis [48].

2.4. Transmission Electron Microscopy (TEM)

TEM (Philips EM 208S, The Netherlands) was used for morphological observation and operated at 100 kV. A drop of nanoemulsion was placed on a copper grid and stained negatively with 2 percent phosphotungstic acid and dried at room temperature.

2.5. Stability of CEO Nanoemulsions

2.5.1. Thermodynamic Stability

The optimum nanoemulsions utilized in this research were selected by studying their physical characteristics in a prior investigation [47]. In this investigation, the optimum nanoemulsion was therefore evaluated periodically for its thermodynamic stability according to the droplet size and PDI over 90 days of storage at 25 °C. The Zetasizer Nano ZS system (Malvern Instruments, Worcestershire, UK) was used to observe these variables. The information obtained was useful for estimating instability phenomena associated with Ostwald ripening and coalescence during prolonged storage.

2.5.2. Kinetic Stability

The kinetic stability of the preparation was assessed by the centrifugation method [49]. Samples were centrifuged at 1000, 2000, and 3000 rpm at room temperature (25 °C) for 15 min. To determine kinetic stability, the visible characteristics of the nanoemulsion and any phase separation before and after the centrifugation process were monitored.

2.6. Preparation of Nanoemulgel

The ratios of surfactants and gel components were selected based on established formulation principles and supported by previous nanoemulgel optimization studies. In particular, mixtures of high-HLB and low-HLB surfactants (similar to Tween 80/Span 80 systems) have been shown to produce nanoemulsions with minimal droplet size and optimal stability at HLB ranges around 9–10, providing efficient incorporation into gel matrices [47]. In line with these findings, the selected ratios allowed the formation of a homogeneous and stable dispersion. The gel base concentration (e.g., 0.75% Carbopol) was chosen as this range provides appropriate viscosity, clarity, and spreadability for topical application while maintaining compatibility with nanoemulsion components [47]. The selected concentrations therefore ensured adequate gel structure and favorable release and permeation characteristics.

To prepare the gel base, carbopol 940 (0.75%) was dispersed in deionized water and kept overnight. The next day, the carbopol solution was neutralized by triethanolamine, and pH was adjusted to 6 to form a clear gel. In the final stage, an equal amount of gel base and 2% CEO nanoemulsion or 4% CEO nanoemulsion was mixed under a propeller mixer for 15 min at 700 rpm, to prepare the nanoemulgel. The gel matrix containing 2% CEO nanoemulsion and 4% CEO nanoemulsion will be referred to hereafter as 1% CEO nanoemulgel and 2% CEO nanoemulgel, respectively.

2.7. Animals

According to the research design, male Swiss-Webster mice (20–28 g) and male Wistar rats (260–280 g) were used, provided by Mazandaran University of Medical Sciences Animal Institute. The animals were housed in each plastic cage in the animal room, kept at 22 ± 3 °C on a 12 h light/dark cycle (lights on 06:00–18:00 h), with relative humidity of 50–55% and unlimited access to food and water except during experiments. Each animal was used only once. The Ethics Committee of Mazandaran University of Medical Sciences approved the study. (Approval no: IR.MAZUMS.REC.1398.1383).

2.8. Skin Irritation Studies

Male Wistar rats were used to assess the irritancy of the formulations. One day prior to the test, the dorsal side of each animal was shaved with a clipper. The animals were randomly divided into five groups of five rats each. The control group received no treatment. Group II received 1% CEO nanoemulgel, Group III received 2%

CEO nanoemulgel, Group IV received the gel base, and Group V received 0.8% *v*/*v* aqueous solution of formalin as a typical irritant. The formulations were applied evenly to the shaved area and covered with a gauze patch. At 24 h post-treatment, any signs of hypersensitivity, including edema and erythema, were observed. Skin irritation was measured using the Draize index. The level of irritation was rated on a scale from 0 (no reaction) to 4 (severe reaction). Edema and erythema were the two main skin reactions evaluated [50].

2.9. In Vivo Behavioral Studies

Evaluation of pain threshold was performed by the formalin test and tail-flick methods. This study comprised five groups: (i) Diclofenac topical gel (Dic gel), (ii) 1% CEO nanoemulgel, (iii) 2% CEO nanoemulgel, (iv) Gel base, (v) Negative control. For both the tail-flick and formalin tests, 6 animals were included in each treatment group to ensure adequate statistical power and consistency across experiments.

2.9.1. Tail-Flick Test

This evaluation was designed to measure nociceptive responses through spinal cord stimulation, utilizing the mouse tail as a thermal nociceptive stimulant activated by an intense light beam. Gels were applied to the distal two-thirds of each mouse tail. Then, each mouse was restrained in a plexiglass container while a part of its tail (5 cm from the base) was exposed to the radiant heat source in a tail-flick device (DS20, Hugo Basile, Varese, Italy). During the test, an intense light beam was aimed at the mouse's tail, and the stopwatch started. When the mouse wiggled its tail, the timer was stopped, and the latency time was calculated. The infrared beam was focused on the tail area, 2–3 cm from the base. The time between placing the tail on the heat source and the withdrawal reaction represented the pain threshold. Pain response was measured one hour after topical administration (applied softly 50 times on the dorsal surface of the mouse tail) every 5 min for 60 min. The cut-off time was adjusted to 15 s to prevent tissue damage from the light beam [51–53].

2.9.2. Formalin-Induced Nociception

The formalin test was conducted to measure antinociceptive activity. This test stimulates a biphasic pain response, including an acute response (via nociceptor activation) and a persistent response (through inflammatory-mediator-induced pain). This model is used to evaluate pain-relieving effectiveness. The formalin test was performed in a clear plexiglass chamber ($30 \times 30 \times 30$ cm^3) with a mirror under it at 45°, providing an unrestricted view of paw responses. One hour after topical administration (applied softly 50 times to the hind paw of the mouse), 20 µL of formalin (2.5%, Mojallali Chemical Complex Co., Tehran, Iran) was injected into the plantar surface of the left hind paw subcutaneously by utilizing a syringe (30-gauge needle). Following injection, each mouse was returned to the test chamber immediately, and pain behaviors were recorded and calculated for the early (0–5 min) phase and late phase (15–60 min). The response included licking, biting, or grooming of the dorsal surface of the injected hind paw [51,54,55].

2.10. Histological Analyses

Mice that received 20 µL of formalin (2.5%) subcutaneously into their right hind paw were euthanized after 24 h. Tissues collected from the right hind paw were preserved in phosphate-buffered formalin at 10% (*w*/*v*). Following dehydration, clearing, and paraffin embedding, routine haematoxylin and eosin (H&E) staining was performed on slices of 5 µm thickness. Infiltrates of leukocytic cells, as well as vasodilation and congestion, were analyzed using H&E staining. The degree of inflammation in the dermis and hypodermis of the skin was evaluated at 100× magnification [56].

2.11. In-Silico ADMET and Toxicity Profiling

We incorporated a complementary computational ADMET and toxicity evaluation to strengthen the safety assessment of the four major compounds of the CEO (cuminaldehyde, p-cymene, γ-terpinene, β-pinene). Predictive platforms including SwissADME [57], ProTox 3.0 [58] and ADMETlab 3.0 [59] were used to estimate dermal permeability, skin irritation potential, systemic toxicity, and metabolic fate. These tools provide mechanistic and machine-learning-based predictions and are widely used when in vivo toxicological testing is not feasible.

2.12. Statistical Analysis

The data were presented as the mean ± SD. Following the Tukey test, the treated groups were compared by analysis of variance (ANOVA). The statistical analysis was carried out using GraphPad Prism 8 (GraphPad, San Diego, CA, USA). The p-value < 0.05 was considered significant.

3. Results

3.1. Characterization of Nanoemulsions

Physicochemical characteristics, including zeta potential, droplet size, and PDI of the CEO nanoemulsion are shown in Table 1.

3.2. Transmission Electron Microscopy (TEM)

The droplet shape of the nanoemulsion in TEM was observed to be spherical, and their sizes were consistent with the values measured by the DLS method (Figure 1).

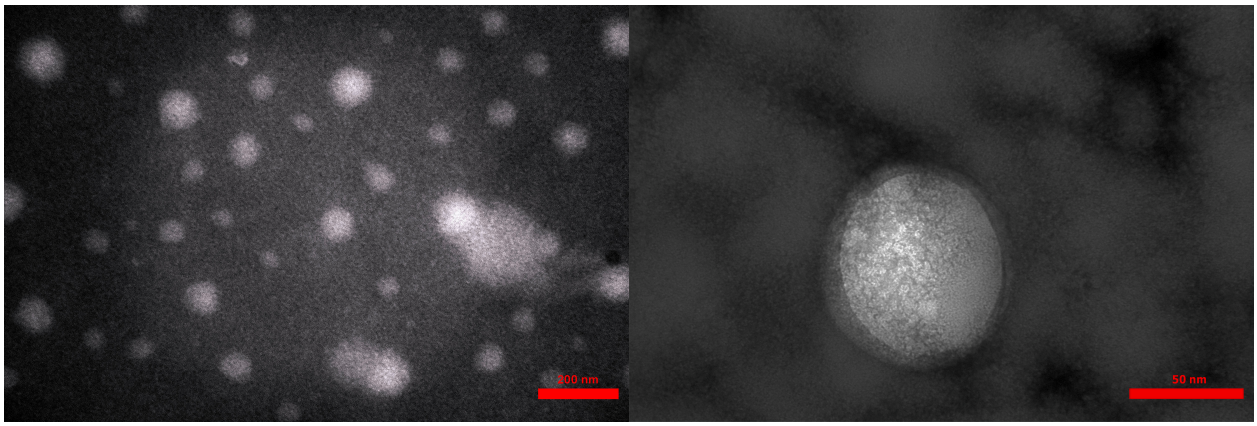
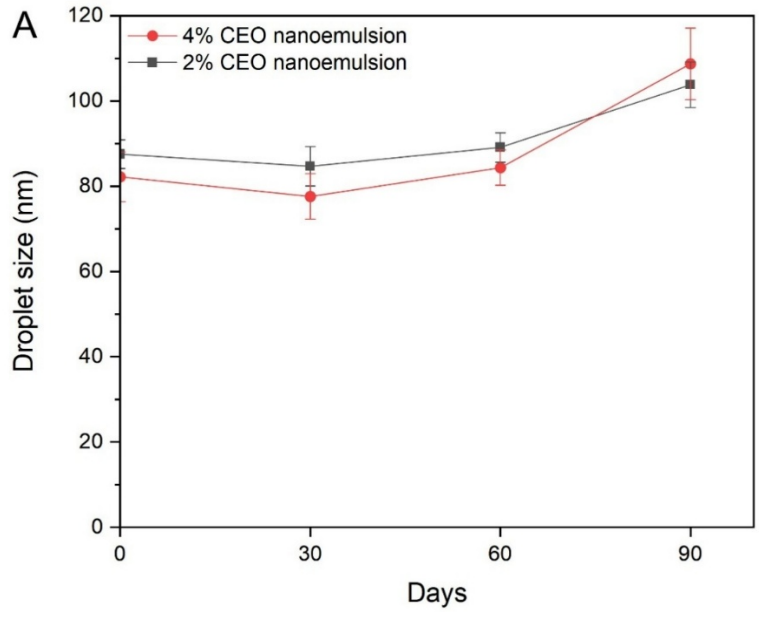


Figure 1. TEM images of 1% CEO loaded nanoemulsion.

3.3. Stability Analysis

3.3.1. Thermodynamic Stability

Nanoemulsion stability is considered important for predicting how long a formulation remains stable in storage. Tracking particle size and PDI during 90 days of storage at 25 °C was essential to assess thermodynamic stability against Ostwald ripening and coalescence [60]. Figure 2 depicts the variations in droplet size and PDI of the CEO nanoemulsion over 90 days of storage at 25 °C.



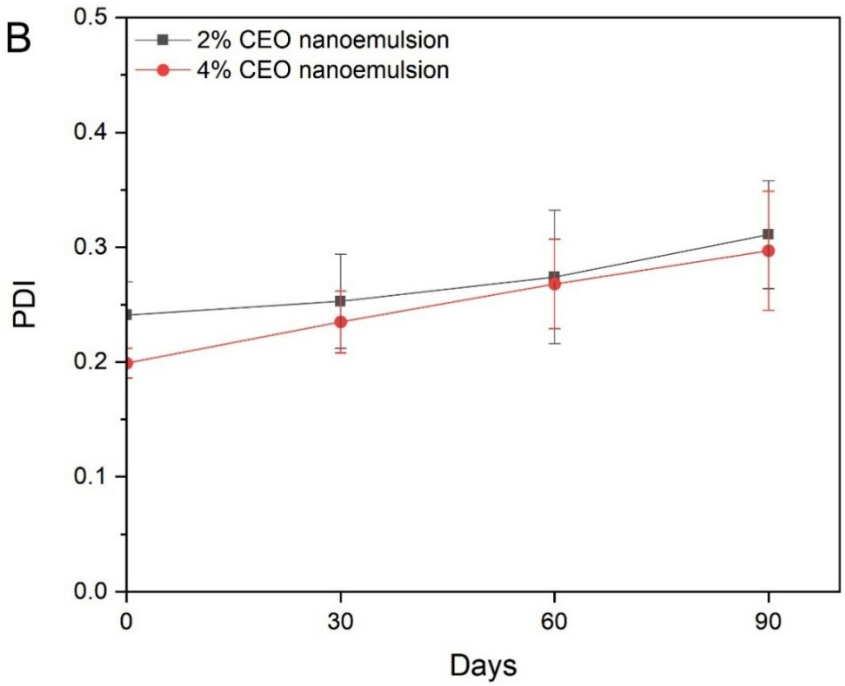


Figure 2. Results of the 90-day storage stability of the optimal nanoemulsion at 25 °C, where plot (**A**) Droplet size and (**B**) Polydispersity index (PDI).

3.3.2. Kinetic Stability

To assess the kinetic stability of the optimized formulation, centrifugation was performed. The product was considered kinetically stable because centrifugation at 1000, 2000, and 3000 rpm did not cause creaming, phase separation, or flocculation.

3.4. Skin Irritation Study

One of the most common drawbacks of topical treatment is skin irritation. In this study, the average skin erythema scores produced by the application of 1% CEO nanoemulgel and 2% CEO nanoemulgel formulations on Wistar rat skin were 0.4 and 0.6, respectively (Table 2). According to Draize et al., substances with a skin irritation score below 2.00 are considered non-irritants. Skin irritation studies showed that none of the nanoemulgel formulations caused skin irritation compared to the control group, even after 24 h of application. Since the CEO formulations yielded scores below 2.00, it was determined that the formulation did not show skin irritation potential and could be applied safely for topical drug administration.

Table 2. The skin irritation study of formulation after topical administration.

	Control		1% CEO Nanoemulgel		2% CEO Nanoemulgel		Gel Base		Formalin	
Rat	Erythema	Edema	Erythema	Edema	Erythema	Edema	Erythema	Edema	Erythema	Edema
1	0	0	0	0	0	0	1	0	3	3
2	0	0	1	0	1	0	0	0	4	3
3	0	0	0	1	0	1	0	0	4	3
4	0	0	0	0	1	1	0	1	4	4
5	0	0	1	0	1	1	1	0	3	3
Total	0.00	0.00	0.40	0.20	0.60	0.60	0.40	0.20	3.60	3.20

(Erythema score: nothing = 0; minor = 1; well defined = 2; moderate = 3; and scar development = 4; for Edema scale: none = 0; slight = 1; well defined = 2; moderate = 3; and severe = 4).

3.5. In Vivo Behavioral Studies

3.5.1. Tail-Flick Test

This test was used to determine baseline nociceptive responses, analgesic activity, and tolerance formation. Figure 3 shows the latency of tail-flick after application of the various preparations across treatment groups at each time interval. All tested formulations (1% CEO nanoemulgel, 2% CEO nanoemulgel, and Dic gel) demonstrated

analgesic activity compared to the control. The difference between the gel base and the control group was not significant ($p > 0.05$). According to the test results, compared with 1% CEO nanoemulgel, Dic gel, and gel base, the 2% CEO nanoemulgel significantly prolonged the response duration to pain stimulation within 60 min of the treatment process ($p < 0.0001$). In addition, 1% CEO nanoemulgel and Dic gel exhibited a similar analgesic effect at each time interval ($p > 0.05$).

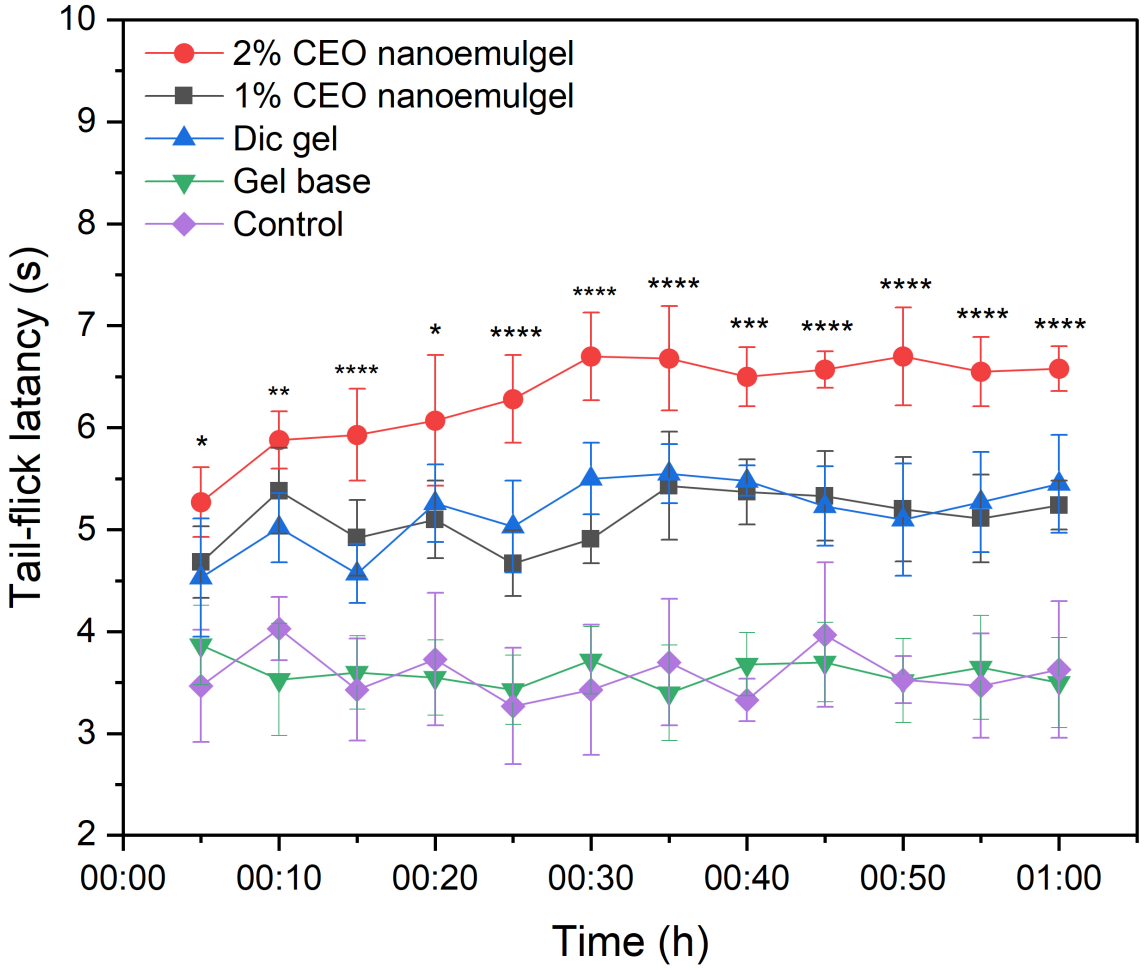


Figure 3. The antinociceptive effect of the investigated formulation in the tail-flick test, expressed as the time-response curve. Data are expressed as mean ± SD (n = 6 per group). Statistical analysis was performed using two-way ANOVA followed by Tukey's post hoc test. Comparison between 2% CEO nanoemulgel and Dic gel. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

3.5.2. Formalin Test

Figure 4A,B show the formalin test results. Compared with the gel base, analgesic effects were observed in both early and late phases for all investigated preparations. According to Figure 4A, in the early phase (0–5 min), 1% CEO nanoemulgel and 2% CEO nanoemulgel showed a higher analgesic effect than Dic gel ($p < 0.05$). The 2% CEO nanoemulgel demonstrated a stronger anti-nociceptive effect than 1% CEO nanoemulgel. This result indicated that the anti-nociceptive effect of CEO was dose-dependent. In the late phase (Figure 4B, 15–60 min), both 1% CEO nanoemulgel and 2% CEO nanoemulgel groups exhibited a greater reduction in pain sensation compared with other treatment groups. A dose-dependent effect of CEO on pain relief was observed ($p < 0.05$).

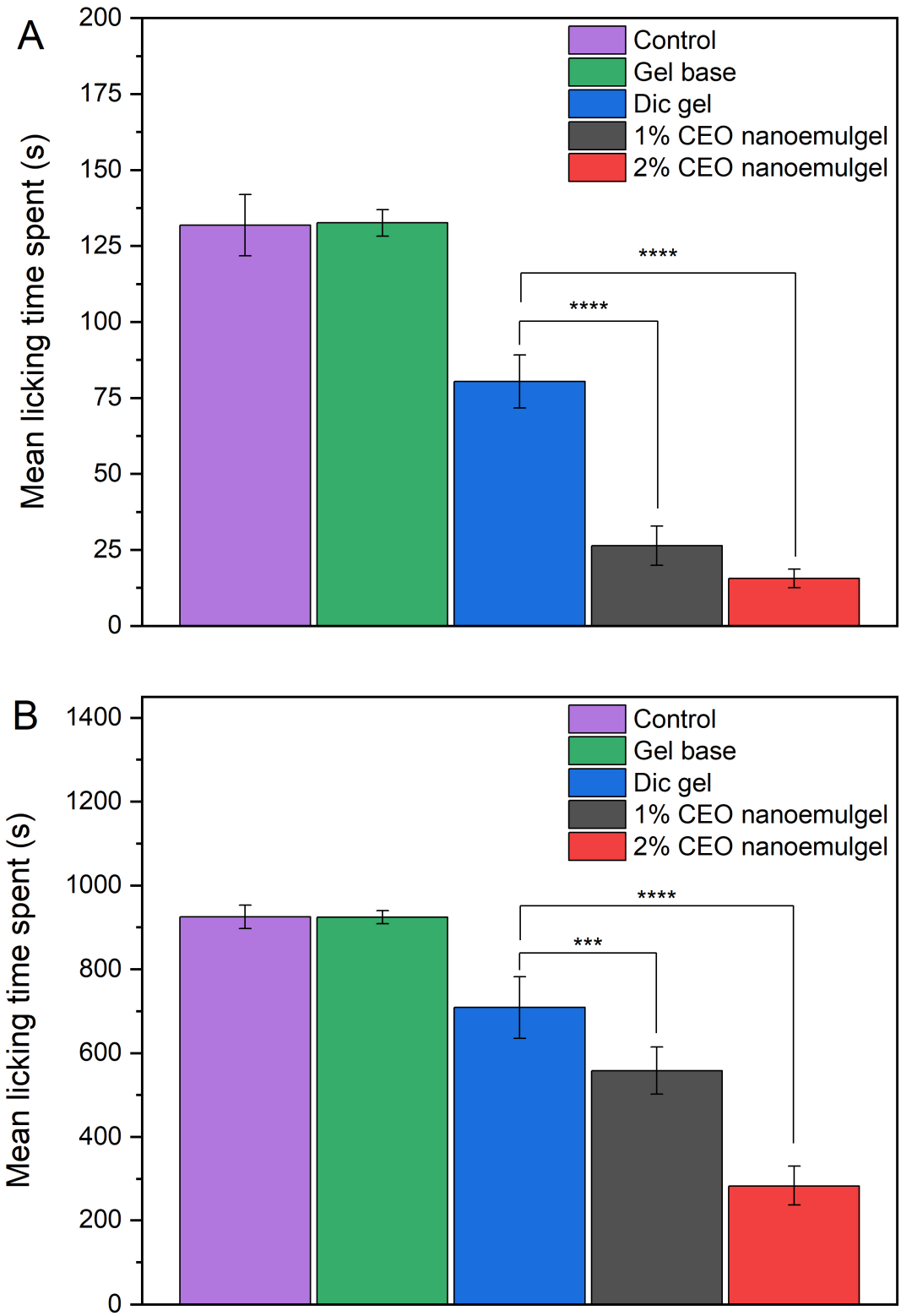


Figure 4. The investigated formulation's antinociceptive effects in the formalin test. (**A**) the early phase and (**B**) late phase. Data are expressed as mean ± SD (n = 6 per group). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. *** $p < 0.001$, **** $p < 0.0001$.

3.6. Histological Analyses

Figure 5A–F illustrates the histoarchitecture of the tissue from the right hind paw of the rodents at 100× magnification using H&E staining. The section of the normal control group (Figure 5A) displayed irregularly arranged, loose, and dense dermal connective tissue layers. There was no migration of leukocytes from tissue fibers. In contrast, the formalin control group demonstrated severe leukocytic infiltration (Figure 5B). Leukocytosis was predominantly observed in perivenular regions. Some vessels exhibited congestion along with

leukocytic paving and endothelium wall margination. In congested areas, edema was present but obscured by erythrocytic infiltrate. In the diclofenac group (Figure 5C), tissues exhibited slight leukocytic infiltration and relatively small edema. The reference drug (Diclofenac) appeared to inhibit the leukocytic migration pathway, thereby decreasing the infiltration of inflammatory cells. The 2% CEO nanoemulgel group (Figure 5F) showed focal leukocytic infiltrates free of congestion. The 1% CEO nanoemulgel group (Figure 5E) exhibited diffuse leukocytic infiltration and slight edema. Figure 5E,F depict lesions that were significantly less severe than those in Figure 5B (formalin group). Moreover, sections from animals treated with 2% CEO nanoemulgel (Figure 5F) demonstrated slight edema with tissue structure similar to the diclofenac group, indicating that CEO nanoemulgel has a potent anti-inflammatory effect. However, the gel base did not exhibit an anti-inflammatory effect; it revealed focal areas of significant leukocytosis and edema.

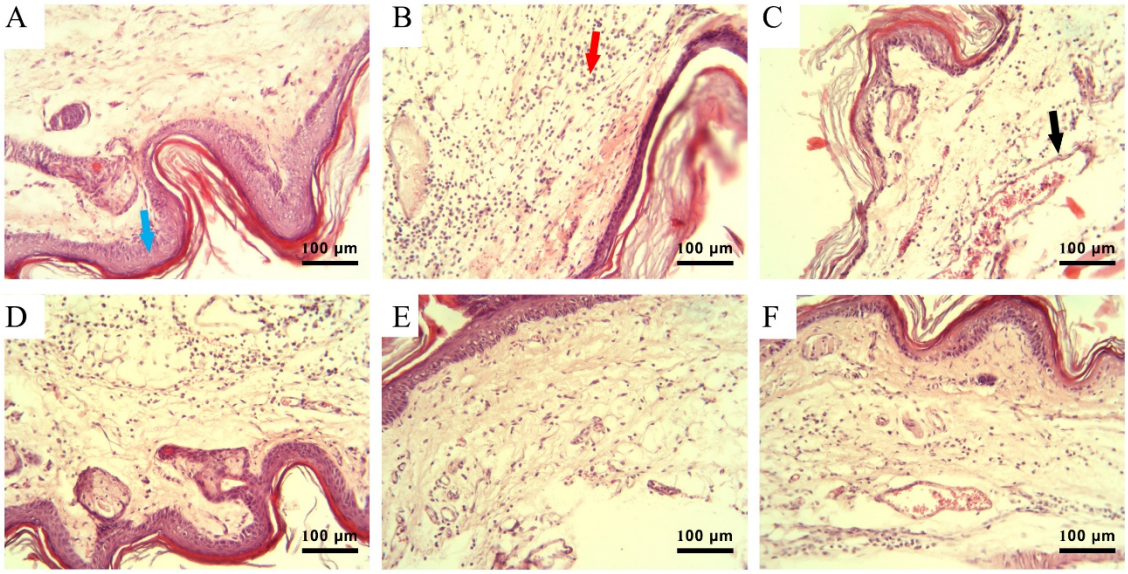


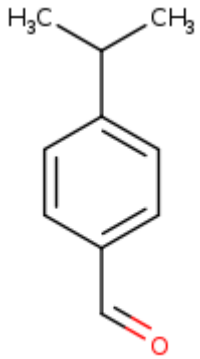
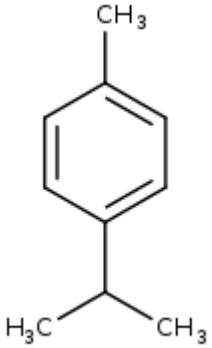
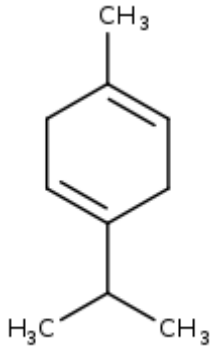
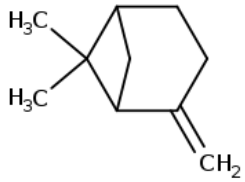
Figure 5. Hematoxylin and eosin-stained tissue portions of the right hind paw in a formalin-induced acute inflammation test. Tissue samples were collected on the 24 h after formalin injection. Blue arrowhead; Epidermis: Red arrowhead; Leukocyte infiltration: Black arrowhead; Congestion. (**A**): control group, with normal structure of dermis and epidermis; (**B**): the group receiving formalin showed severe leukocyte infiltration; (**C**): Dic gel; (**D**): Gel base; (**E**): 1% CEO nanoemulgel; (**F**): 2% CEO nanoemulgel. (Scale bar:100 µm, Mag; ×100, Staining: H&E, CEO: Cumin essential oil, Dic gel: Diclofenac gel).

3.7. In-Silico ADMET and Toxicity Profiling

The in-silico ADMET evaluation of cuminaldehyde, p-cymene, γ-terpinene and β-pinene demonstrated overall favorable dermal safety and permeability profiles. All four compounds exhibited low-to-moderate predicted skin permeation, with Log Kp values ranging from –3.9 to –5.5, indicating diffusion largely restricted to the stratum corneum and upper epidermal layers and a low likelihood of transdermal systemic absorption. Cuminaldehyde and β-pinene showed slightly higher predicted permeation than p-cymene and γ-terpinene, although all remained below thresholds associated with systemic exposure [61].

Toxicity predictions from SwissADME [57], ProTox 3.0 [58] and ADMETlab 3.0 [59] classified all compounds within low acute toxicity categories. No major alerts were identified for hepatotoxicity, cardiotoxicity (hERG inhibition) and mutagenicity (AMES). All monoterpenes exhibited favorable metabolic behavior, undergoing predicted Phase I oxidation primarily via CYP2C9, CYP2C19, and CYP3A4, followed by rapid Phase II conjugation, suggesting low potential for systemic accumulation. Only cuminaldehyde displayed a moderate alert for potential skin irritation, whereas the other compounds demonstrated low irritancy and sensitization potential. The detailed ADMET and toxicity values for the 4 compounds are summarized in Table 3.

Table 3. Predicted pharmacokinetic and toxicity parameters of major cumin essential oil constituents.

	Cuminaldehyde	**p-Cymene**	**γ-Terpinene**	**β-Pinene**
SMILES	**CC(C)C1=CC=C(C=C1)C=O**	**CC1=CC=C(C=C1)C(C)C**	**CC1=CCC(=CC1)C(C)C**	**CC1(C2CCC(=C)C1C2)C**
Structure				
Log Kp (skin permeation)	−5.52 cm/s	−4.21 cm/s	−3.94 cm/s	−4.18 cm/s
Skin Permeation Category	Low	Very low	Low	Low–moderate
Epidermal/Dermal Penetration	Mostly epidermal	Limited to stratum corneum	Mostly epidermal	Upper epidermis, minimal dermal
Lipophilicity (LogP)	2.48	3.50	3.35	3.42
Dermal Irritation Potential	Moderate (aldehyde reactivity)	Low	Low	Low
Hepatotoxicity	No alert	No alert	No alert	No alert
Cardiotoxicity (hERG inhibition)	No alert	No alert	No alert	No alert
AMES Mutagenicity	Negative	Negative	Negative	Negative
Predicted Metabolism	CYP2C9/2C19/3A4	CYP2C9/3A4	CYP2C9/3A4	CYP2C9/3A4
Overall Dermal Safety Assessment	Acceptable with low irritancy caution	Good	Good	Good

Values are based on combined predictions from SwissADME, ProTox 3.0, and ADMETlab 3.0. Log Kp classification: >–3.5 = higher penetration, –3.5 to –4.5 = low penetration, <–4.5 = very low penetration (mostly stratum corneum).

4. Discussion

4.1. Phytochemical Composition and Bioactivity of Major CEO Constituents

According to the literature, a great deal of oxygenated monoterpenes and monoterpene hydrocarbons have been identified in CEO. The major compounds were cumin aldehyde (23.92%), p-cymene (17.15%), γ-terpinene (14.07%), β-pinene (11.73%), and 2-methyl-3-phenyl propanal (8.68%) [47]. The main compounds of EOs often determine their biological properties [62]. Some major terpenes of CEO possess analgesic and/or anti-inflammatory effects. Cumin aldehyde, p-cymene, and γ-terpinene have been reported to exhibit analgesic activity [63,64]. β-Pinene and γ-terpinene demonstrated anti-inflammatory effects [65]. In vitro and in silico studies revealed that cumin aldehyde can act as a competitive inhibitor of lipoxygenase [66].

Santana et al. reported that the reaction time in the tail-flick test was increased when male Swiss mice were treated with p-cymene. The oral administration of p-cymene (25–100 mg/kg) significantly increased the latency time in a dose-dependent manner compared with the morphine-treated group. According to their results, p-cymene pretreatment also decreased carrageenan-induced hyperalgesia. They suggested that the antinociceptive responses may be associated with blockade of neural pain transmission via modulation of the opioid system [67]. In the formalin test, studies showed that p-cymene significantly decreased the licking time in both early and late phases compared with the control group ($p < 0.05$), indicating its ability to modulate the release of inflammatory mediators [68–70].

4.2. Integration with Previous Cumin-Based Analgesic and Antioxidant Datasets

The present findings that CEO nanoemulgel exhibits marked antinociceptive activity in the tail-flick and formalin tests, with efficacy comparable to or greater than diclofenac gel, are consistent with previously published data on cumin and its main constituents. Koohsari et al. demonstrated that systemic administration of cuminaldehyde, the major component of cumin seeds, significantly attenuated nociception in hot-plate, formalin and acetic-acid–induced writhing tests and reduced neuropathic pain behaviors in mice. These effects were shown to be at least partly opioid-dependent and associated with modulation of inflammatory cytokines and the L-arginine/NO/cGMP pathway, highlighting the strong antinociceptive and antineuropathic potential of cuminaldehyde [64].

A recent comprehensive review further highlighted cuminaldehyde's analgesic, anti-inflammatory and antioxidant actions across multiple experimental models, as well as its favourable in silico pharmacokinetic and ADMET profile [71]. Our computational analyses also support that these monoterpenes possess acceptable dermal safety, limited systemic exposure, and no major toxicological liabilities, reinforcing their suitability for topical therapeutic applications. In addition to studies with isolated cuminaldehyde, ethanolic extracts of *Cuminum cyminum* seeds have shown significant analgesic activity in mice, along with an acceptable safety margin in acute toxicity testing [72]. These systemic datasets align with our topical results and suggest that multiple classes of cumin phytochemicals, including cuminaldehyde and monoterpenes such as p-cymene and γ-terpinene, may contribute synergistically to analgesic and anti-inflammatory effects.

From an antioxidant perspective, CEO has also demonstrated strong antioxidant capacity in chemical assays and in food and biological models, often outperforming other commonly used EOs. CEO has repeatedly been reported to display high radical-scavenging and reducing power in vitro, often matching or exceeding standard antioxidants in β-carotene bleaching, DPPH and FRAP assays [73–75]. Recent work has also shown that combinations of CEO with other culinary EOs can produce synergistic enhancements in antioxidant and antibacterial activity [73]. These antioxidant properties are mechanistically relevant because oxidative stress and inflammatory signaling are closely linked in peripheral tissues [76]. Thus, the strong antioxidant background of CEO, as demonstrated in recent cumin-oil nanoformulations, may support the anti-inflammatory and histological improvements observed in our formalin-induced paw inflammation model [77].

Together, these datasets justify the development of advanced topical delivery systems for CEO and provide an external benchmark against which to interpret the present CEO nanoemulgel findings. To better contextualize our findings, we summarised key published datasets on cumin-based analgesic and antioxidant effects alongside the present CEO nanoemulgel results (Table 4). Across different routes of administration and test systems, cumin and cuminaldehyde consistently reduced pain-related behaviors and inflammatory readouts, supporting the robustness of cumin as a pharmacologically active, multi-target analgesic candidate.

Table 4. Summary of selected studies on cumin and cuminaldehyde analgesic/antioxidant activity compared with the present CEO nanoemulgel.

Preparation/Main Compound	Route/Model	Dose (Example)	Main Outcome vs. Control	Ref.
Cuminaldehyde isolated from *C. cyminum*	i.p.; hot-plate, formalin, acetic-acid writhing, CCI neuropathy	12.5–100 mg/kg	Significant inhibition of writhing and formalin pain; reversal by naloxone; reduction of cytokines; antineuropathic effect	[64]
Crude ethanolic seed extract of *C. cyminum*	Oral; acetic-acid writhing and other analgesic tests	e.g., 200–400 mg/kg	Dose-dependent reduction of writhing; acceptable acute toxicity profile	[72]
CEO	Oral; hypnotic and pain tests	25–100 mg/kg	Significant reduction in nociceptive scores and prolongation of sleep time in rodents	[78]
CEO nanoemulsion/nanogel	In vitro antioxidant, anticancer, antibacterial, larvicidal	EO nanoemulsion droplet size ~121 nm	High antioxidant and antimicrobial activity; supports nanoformulation strategy, though not a pain model	[79]
CEO nanoemulgel (1% and 2%)	Topical; tail-flick and formalin tests; histology	1% and 2% (*w*/*w*)	Significant, dose-dependent increase in nociceptive latency and reduction in formalin pain score vs gel base and diclofenac; improved histology with reduced leukocyte infiltration	Present study

4.3. Comparison with Other Essential-Oil Nanoemulgels

Several previous studies have evaluated nanoemulsion- or nanoemulgel-based delivery of various EOs for analgesic and anti-inflammatory applications, supporting the relevance of the present CEO nanoemulgel findings.

El Asbahani reported that encapsulation of EOs in novel pharmaceutical forms (Such as liposomes, SLNs, and microparticles) is an appropriate approach to overcome volatility and improve stability and controlled release [80]. Mohammadifar et al. *demonstrated* that rosemary and peppermint EO nanoemulgels significantly increased antinociceptive response in an *osteoarthritis rat model* [36]. Borges et al. showed that rosemary EO nanoemulsion exhibited analgesic and anti-inflammatory responses, associated with camphor, which plays a crucial role [22]. Sayah et al. reported that intraperitoneal injection of the CEO emulsion produced significant analgesic action in the formalin test in rats. However, it did not show a significant response to acute pain in the tail-flick test [25].

Aid et al. found that Swietenia macrophylla (SM) oil solution showed poor anti-inflammatory activity compared with positive controls. However, the anti-inflammatory activity of SM oil increased when applied as a nanoemulgel. SM oil nanoemulgel exhibited more effective anti-inflammatory action than positive controls [81]. Jeengar et al. demonstrated that curcumin nanoemulgel in a transdermal drug delivery system is an appropriate technique to overcome the first-pass effect and improve anti-inflammatory and analgesic activity in the joint synovium, alleviating arthritis [82]. Overall, these studies indicate that nanoemulgels have tremendous potential as novel drug delivery systems for enhancing the percutaneous permeability and bioavailability of anti-inflammatory agents.

The analgesic and anti-inflammatory effects observed with the CEO nanoemulgel in the present study are consistent with earlier investigations using nanoemulgels or nanogels prepared from other EOs. Clove and cinnamon essential-oil nanogels have shown marked suppression of formalin-induced nociceptive behavior and paw edema, effects attributed largely to the synergistic action of eugenol, cinnamaldehyde and associated phenolics [83]. Eucalyptus essential-oil nanoemulsions and nanoemulgels have demonstrated enhanced dermal permeation and superior anti-inflammatory activity compared with conventional gels, particularly in carrageenan- or croton-oil–induced inflammation [84,85].

These studies collectively support the view that essential-oil nanoemulgels constitute an emerging class of natural topical anti-inflammatory systems. The present CEO nanoemulgel follows this pattern by producing significant increases in tail-flick latency and reductions in formalin pain scores along with improved histological outcomes. Unlike many earlier formulations that combine EOs with synthetic drugs (e.g., meloxicam or etoricoxib) [85,86], our CEO nanoemulgel uses CEO as the sole active ingredient, highlighting its intrinsic analgesic efficacy. This positions the CEO nanoemulgel as a promising member of this formulation class, while also emphasizing the need for future comparative studies with other EO-based nanoemulgels and conventional NSAID gels.

4.4. Translational and Regulatory Considerations

From a translational perspective, CEO is a relatively inexpensive and widely available natural product used in the food, flavoring and cosmetic industries. Its incorporation into a simple carbopol-based nanoemulgel may offer a cost-effective alternative to more complex topical analgesic formulations, although detailed pharmacoeconomic analyses would be required to substantiate this [87]. Patient-centric attributes are also important in determining clinical acceptability of topical formulations. EOs have characteristic aromas, and CEO possesses a strong, spicy odor that some individuals may find pleasant while others may consider intense. Sensory factors such as odor, spreadability, skin feel and non-greasiness can influence patient adherence and should be evaluated systematically in future human studies [88]. Regarding regulatory aspects, several EOs and their major constituents are classified as GRAS (Generally Recognized as Safe) by the U.S. Food and Drug Administration when used within specified limits as flavoring agents [89]. Essential-oil–containing herbal preparations are also addressed in WHO guidance documents on quality control, safety assessment and regulatory standards for traditional medicines [90]. Nevertheless, a CEO nanoemulgel intended for therapeutic use must comply with national regulatory frameworks governing topical pharmaceutical or cosmeceutical products. This includes requirements for stability testing, preservative efficacy, long-term dermal safety and potential systemic exposure, which remain avenues for future research. Overall, while the present findings support the biological potential and short-term dermal safety of the CEO nanoemulgel, further translational work is necessary before considering its clinical application.

5. Conclusions

In this study, a CEO nanoemulgel was successfully developed and characterized, and its antinociceptive and anti-inflammatory effects were demonstrated in the tail-flick and formalin tests, together with an acceptable profile in a short-term skin irritation model. Overall, the CEO nanoemulgel emerges as a promising topical formulation for the management of acute inflammatory pain, and its antinociceptive activity suggests potential as a topical option alongside existing analgesic therapies. Nevertheless, this work has several limitations, including the absence of detailed mechanistic assays and evaluation in additional pain models such as the hot-plate test, carrageenan-induced paw edema, acetic-acid-induced writhing, or neuropathic pain models (e.g., chronic constriction injury). Future research should clarify the molecular mechanisms underlying the effects of CEO constituents through in vitro enzyme inhibition assays, receptor-binding studies and molecular docking simulations, and should include direct comparisons with standard topical NSAID formulations. In addition, comprehensive preclinical safety studies and well-designed clinical trials will be essential to validate efficacy and to determine whether CEO nanoemulgel can ultimately be established as a safe and effective therapeutic option for pain management in humans.

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