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Original Article

Antinociceptive and anti-inflammatory actions of curcumin and nano curcumin: a comparative study

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Abstract

Background and purpose: Pain and inflammation can be treated by various therapies that for the most part are not effective and can result in adverse effects. The current study was proposed to compare the antinociceptive and anti-inflammatory actions of curcumin and nano curcumin in rats.
Experimental approach: Rats were randomly allocated into ten groups of six for formalin and tail-flick tests including the control group, curcumin and nano curcumin groups (20, 50, 100 mg/kg), morphine group (10 mg/kg), naloxone + 100 mg/kg curcumin group, and naloxone + 100 mg/kg nano curcumin group. There were nine groups for the carrageenan test. Groups 1-7 were the same as the previous division; groups 8 and 9 received 10 mg/kg diclofenac and 1% carrageenan, respectively.
Findings/Results: All doses of nano curcumin significantly decreased the paw-licking time in both phases of the formalin test. In the tail-flick test, curcumin 100, nano curcumin 100, naloxone + curcumin 100, and naloxone + nano curcumin 100 showed significant analgesic effects compared to the control group. In the paw edema test, at 180 s after injection, curcumin (50 and 100 mg/kg) and all doses of nano curcumin significantly inhibited carrageenan-induced edema. Myeloperoxidase activity and lipid peroxidation decreased at doses of 50 and 100 mg/kg of curcumin but at three doses of nano curcumin (20, 50, and 100 mg/kg).
Conclusion and implication: In conclusion, our results suggest that the nanoemulsion formulation of curcumin can be efficient in reducing pain and especially inflammation in lower doses compared to the native form of curcumin.

Keywords: Antinociceptive effects; *Curcuma longa;* Curcumin; Nano curcumin.

INTRODUCTION

The lifestyle of individuals around the world is meaningfully affected by pain and inflammation. Several treatments exist for the healing of pain and inflammation, but these treatments are not always successful and may result in experiencing adverse effects. Based on these facts, the identification of new compounds for healing pain and inflammation is an essential challenge in biomedical research (1,2). There are countless medicinal plants with possible analgesic activity, such as *Artemisia herba-alba, Saliva hydrangea, Lavandula officinalis, Thymus vulgaris, Melissa officinalis, Menthe pulegium, Salvia sclarea, Moringa oleifera, Passiflora leschenaultia, Tamarindus indica Linn, Vaccinium ashei, etc*. In addition to the use of new compounds, the use of strategies that can increase the effectiveness of the existing ones should also be considered. Among different strategies, using nano-sized carriers is a broadly used method to achieve acceptable bioavailability and suitable metabolism in therapeutic use (3,4).

Curcumin (*Curcuma longa*) [1,7-bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione], also named diferuloylmethane (5), is a natural phenolic complex that can be extracted from turmeric roots (6).

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In recent years, a broad spectrum of exciting bioactivities has been known or attributed to curcumin, particularly antioxidant (7,8), antimicrobials (9), anticancer and antimutagenic (10) properties. This compound is bright orange-yellow thus it can be used as a food colorant (5). Recently, it has been established that most of the properties of *Curcuma longa* belong to curcumin with possible effects against allergies, Alzheimer's disease, diabetes, arthritis (11,12), and other chronic illnesses (13,14). Nevertheless, curcumin treatment in humans is confined by its great metabolic variability as well as low absorption and bioavailability (15).

Some tactics have been adopted to increase oral bioavailability and solubility of curcumin (16,17). It has been shown that curcumin mixtures with nanoparticle-based encapsulation or polymeric micelles display better systemic bioavailability, chemical stability, and antitumoral activities than natural curcumin (18). It has been revealed that polymeric nanomicelles are effective nanocarriers in the development of the bioavailability of hydrophobic drugs. According to these findings, it can be a promising approach in curcumin delivery by increasing stability, aqueous solubility, and bioavailability (19).

Thus, the purpose of the current study was to investigate and compare curcumin and nano curcumin's preventive effects on pain and inflammation in rats.

MATERIALS AND METHODS

Materials and reagents

The chemicals used in the current investigation were as follows: curcumin (Sigma-Aldrich, St. Louis, MO, USA) dissolved in 10% dimethyl sulfoxide (DMSO) with 90% normal saline solution, nanomicelle curcumin (Exir Nano Sina Company, IRAN), diclofenac sodium (Abu Raihan Company, Iran), carrageenan (Sigma-Aldrich, St. Louis, MO, USA), morphine (Alborz Darou Company, Iran), and naloxone (Tolid Daru Company, Iran).

Animals

Adult male rats (200-300 g), were kept in suitable cages at 22 ± 2 °C on a 12/12-h light/dark sequence with free access to water and food. Before any testing process animals were permitted to have an acclimation time. Methods used in this study were based on the Guide of Care and were approved by the ethics committee on animal experimentation of the Hamadan University of Medical Sciences (Ethical ID: IR.UMSHA.REC.1396.553). All the nociception tests were approved by the same visual observer. Rats were randomly allocated into ten groups of six for formalin and tail flick tests as follows:

Group 1 received 10% DMSO with 90% normal saline solution by gavage (control group); group 2 received 20 mg/kg curcumin by gavage; group 3 received 50 mg/kg curcumin by gavage; group 4 received 100 mg/kg curcumin by gavage; group 5 received 20 mg/kg nano curcumin by gavagegroup 6 received 50 mg/kg nano curcumin by gavage; group 7 received 100 mg/kg nano curcumin by gavage; Group 8 received 10 mg/kg morphine intraperitoneally (IP); group 9 received 1 mg/kg naloxone IP, 30 min before being treated with 100 mg/kg curcumin; group 10 received 1 mg/kg naloxone IP, 30 min before being treated with 100 mg/kg nano curcumin.

Rats were accidentally allocated into nine groups of six for the carrageenan test as follows:

Group 1 received 10% DMSO with 90% normal saline solution by gavage (control group); group 2 was injected with 1% carrageenan; group 3 received 20 mg/kg curcumin by gavage 30 min before injection of 1% carrageenan; group 4 received 50 mg/kg curcumin by gavage 30 min before injection of 1% carrageenan; group 5 received 100 mg/kg curcumin by gavage 30 min before injection of 1% carrageenan; group 6 received 20 mg/kg nano curcumin by gavage 30 min before injection of 1% carrageenan; group 7 received 50 mg/kg nano curcumin by gavage 30 min before injection of 1% carrageenan; group 8 received 100 mg/kg nano curcumin by gavage 30 min before injection of 1% carrageenan; group 9 received 10 mg/kg diclofenac IP 30 min before injection of 1% carrageenan.

Pharmacological tests

Formalin test

Based on a similar technique designed by Hunskaar and Hole (20), formalin was injected into the right hind paw of the rat (2.5% in 0.9% sterile saline; 50 µL/paw sub plantar) (21). Rats were spotted in the spaces with a mirror and the time spent licking and biting the injected site was considered as an indicator of pain. Reactions were evaluated five (first phase) and 15-30 min (second phase) after formalin administration (22). Curcumin, nano curcumin (20, 50, and 100 mg/kg, gavage), morphine (10 mg/kg, IP), naloxone + curcumin 100, and naloxone + nano curcumin 100 were given 1 h before formalin administration (N = 6 per group). Naloxone (1 mg/kg, IP), an opioid receptor antagonist, was injected 30 min before administration of the curcumin and nano curcumin (100 mg/kg) to evaluate the contribution of opioid receptors in the effect of the curcumin and nano curcumin.

Tail flick test

Tail-flick technique (TFT, Analgesiometer, Insight, Iran) was used to assess the antinociceptive reaction of curcumin and nano curcumin against thermal stimuli (23). Rats were accidentally allocated to ten groups of six. Each rat was located in a ventilated tube with the dorsal surface of the tail situated in the radiant thermal stimuli. The heating was applied to the distal part of the tail at 50 °C. The time of removing the tail in seconds was considered the response time, which was assessed automatically by a recorder joined to the equipment. A termination time of 7 s in each trial was regarded to diminish the possibility of skin damage. The tail-flick latency was evaluated before and 30, 60, and 90 min after administration of curcumin, nano curcumin (20, 50, and 100 mg/kg), and morphine (10 mg/kg, IP).

Carrageenan-induced hind paw edema

A paw edema model induced by carrageenan 1% (injection of 100 µL/animal into the sub-plantar section of the right hind paw of the rat) was used to study the anti-inflammatory effect of curcumin and nano curcumin (24). Rats were allocated to nine groups of six. Rats were pretreated with curcumin and nano curcumin (25, 50, and 100 mg/kg) and diclofenac 30 min before carrageenan injection. Plethysmometer apparatus (P.M4500, Iran) was used to measure the pedal volume of the rat up to the ankle joint before (volume A (VA), baseline) and 1, 2, and 3 h after (volume B (VB)) the sub-plantar administration of carrageenan, as explained formerly (25). Paw edema inhibition was calculated by (VB-VA)/VA formula, where VA stands for the volume of the right hind paw before injection of carrageenan, and VB stands for the volume of the right hind paw after injection of carrageenan. Ultimately, rats were euthanized and right hind paws were separated for biochemical tests (4).

Biochemical tests

Myeloperoxidase test

Migration of neutrophils to the hind paws of rats was appraised by myeloperoxidase (MPO) kinetic-colorimetric assay (26). The measurement was performed by collecting paw tissue in ice-cold phosphate buffer saline (PBS; pH = 6) containing 0.6% hexadecyltrimethylammonium bromide. After homogenization and centrifugation (10000 rpm, 30 min, 4 °C), the resulting supernatant of samples was evaluated for MPO activity spectrophotometrically at 400 nm (UV Spectrophotometer, Analytikjena, Germany). The results were presented as MPO activity.

Lipid peroxidation assay

Lipid peroxidation was measured by evaluating malondialdehyde (MDA) (27). Based on the kit protocol, after tissue homogenizing, 50 µL of supernatant or standard solutions were mixed with 300 µL reagent and heated at 90 °C for 30 min. Following cooling the mixture, the absorbance was evaluated at 532 nm using an Elisa reader (Multi-mode Reader, Botek, USA). The lipid peroxidation was articulated in terms of nmol MDA/mg protein.

Statistical analysis

The data were presented as mean ± SEM. Analysis of variance (ANOVA) followed by the Tukey post-hoc test was used. After obtaining the data from different experimental groups, they were analyzed using GraphPad Prism 8 software, and P values < 0.05 were considered significantly different.

RESULTS

Formalin test

The duration of pain reaction in the early stage (0-5 min) was meaningfully diminished in nano curcumin (20, 50, and 100 mg/kg), naloxone + curcumin 100, naloxone + nano curcumin 100, and morphine-treated groups in comparison with the control group. The duration of pain reaction in the late stage (15-30 min), was meaningfully declined by two doses of curcumin (50 and 100), and three doses of nano curcumin (20, 50, and 100 mg/kg), naloxone + curcumin 100, naloxone + nano curcumin100 and morphine compared to the control group. Moreover, nano curcumin at two doses (50 and 100 mg/kg) was meaningfully better than curcumin (Fig. 1).

Tail flick test

Curcumin 100, nano curcumin 100, naloxone + curcumin 100, and naloxone + nano curcumin100 showed significant analgesic effects (prolongation of the reaction time) compared to the control group. Curcumin and nano curcumin (20 and 50 mg/kg) displayed non-significant results as compared with the control group, whereas morphine showed significant analgesic effects as compared to the control group) (Fig. 2).

Carrageenan-induced hind paw edema

While Carrageenan was applied as an edema inductor (Fig. 3), after 60 min, curcumin (100 mg/kg) and nano curcumin (50 and 100 mg/kg) significantly inhibited carrageenan-induced edema compared to the carrageenan group. Also, at 180 min after injection, curcumin (50 and 100 mg/kg) and nano curcumin (20, 50, and 100 mg/kg) significantly inhibited carrageenan-induced edema compared to the carrageenan group. However, at 120 min after injection, no significant effect was observed. In addition, the control drug, diclofenac, showed significant inhibition of edema volume over 3 h.

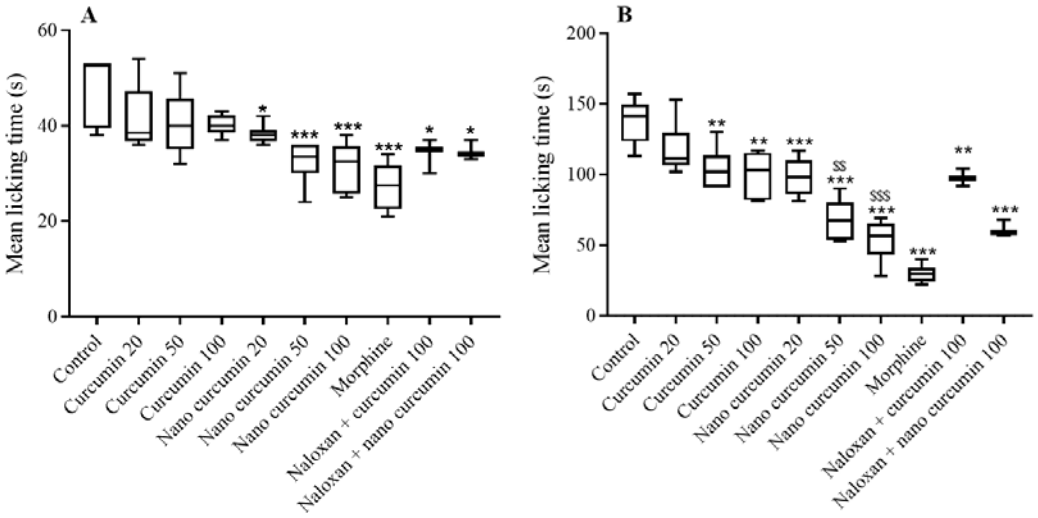


Fig. 1. Comparative graphical representation of analgesic effects of curcumin (20, 50, and 100 mg/kg), nano curcumin (20, 50, and 100 mg/kg), and morphine in formalin-induced paws licking test. Reactions were evaluated after (A) 5 min (first phase) and (B) 15-30 min (second phase) after formalin administration. Each point represents the mean ± SEM. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ indicate significant differences compared to the control group; $^{\$\$}P < 0.01$ and $^{\$\$\$}P < 0.001$ comparisons between curcumin and nano curcumin at the same dose.

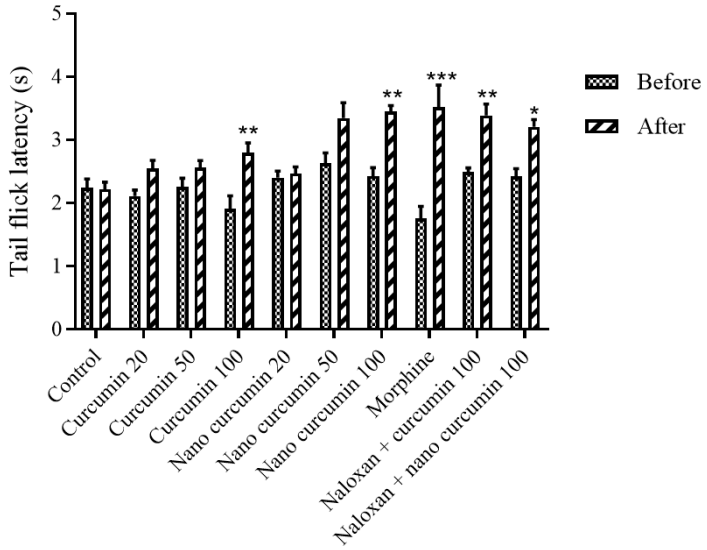
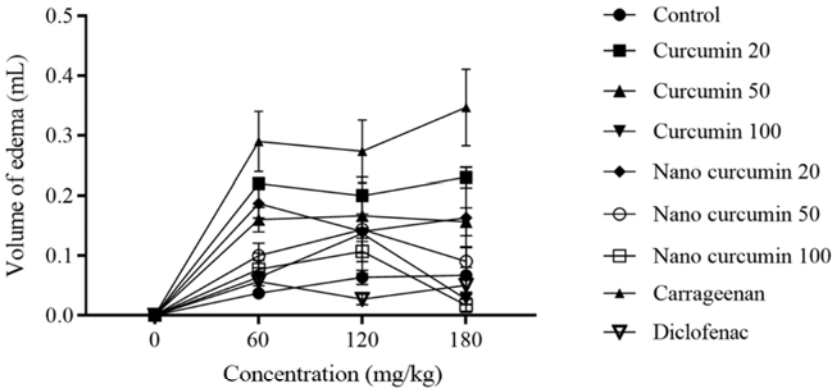


Fig. 2. Comparative graphical representation of analgesic effects of curcumin (20, 50, and 100 mg/kg), nano curcumin (20, 50, and 100 mg/kg), and morphine in tail flick test. Each point represents the mean ± SEM. **P* < 0.05, ***P* < 0.01, and ****P* < 0.001 indicate significant differences compared to the control group.



Groups	60	120	180
Control	***	**	***
Curcumin 20	-	-	-
Curcumin 50	-	-	*
Curcumin 100	***	-	***
Nano curcumin 20	-	-	*
Nano curcumin 50	*	-	***
Nano curcumin 100	**	-	***
Diclofenac	***	***	***

Fig. 3. Comparative graphical representation of anti-inflammatory effects of curcumin (20, 50, and 100 mg/kg), nano curcumin (20, 50, and 100 mg/kg), and diclofenac in carrageenan-induced paw edema test. Each point represents the mean ± SEM. **P* < 0.05, ***P* < 0.01, and ****P* < 0.001 indicate significant differences compared to the carrageenan group.

MPO

MPO activity is indicative of the polymorphonuclear leukocyte recruitment in the organ following any damage. MPO activity was meaningfully higher in the carrageenan-treated group as compared to the control group. Curcumin decreased the MPO activity at doses of 50 and 100 mg/kg but nano curcumin reduced the MPO activity at three doses (20, 50, and 100 mg/kg) in comparison with the carrageenan (Fig. 4).

Lipid peroxidation assay

Since the content of malondialdehyde is meticulously related to lipid peroxidation, the end product of lipid peroxidation was detected in the foot tissues of animals after treatments. Only low quantities of MDA were detected in the control group, but a noteworthy elevation was discovered in the carrageenan-treated group. As expected, MDA contents meaningfully decreased by curcumin at doses of 50 and 100 mg/kg, but nano curcumin reduced the MDA content at three doses (20, 50, and 100 mg/kg compared to the carrageenan (Fig. 5).

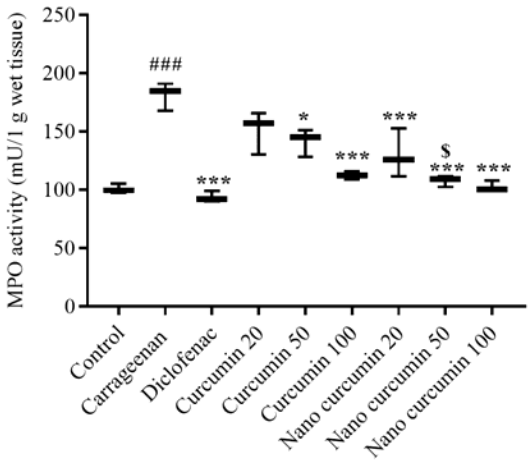


Fig. 4. Comparative graphical representation of curcumin (20, 50, and 100 mg/kg), nano curcumin (20, 50, and 100 mg/kg), carrageenan, and diclofenac on MPO test. Each point represents the mean ± SEM level. $^{###}P < 0.001$ indicate a significant difference compared to the control group; $^{*}P < 0.05$, and $^{***}P < 0.001$ compared to the carrageenan group; $^{\$}P < 0.05$ comparison between curcumin and nano curcumin at the same dose). MPO, Myeloperoxidase.

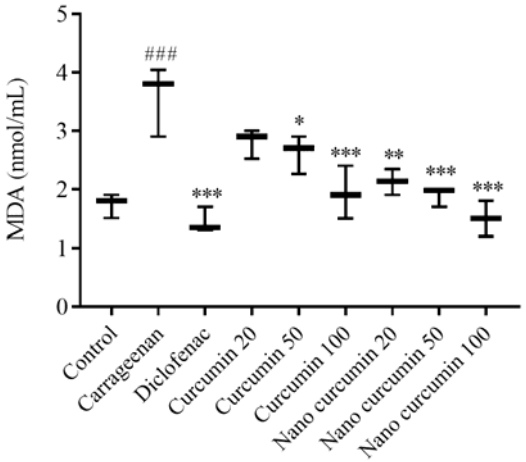


Fig. 5. Comparative graphical representation of curcumin (20, 50, and 100 mg/kg), nano curcumin (20, 50, and 100 mg/kg), carrageenan, and diclofenac on MPO test. Each point represents the mean ± SEM. $^{###}P < 0.001$ Indicates a significant difference compared to the control group; $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ compared to the carrageenan group. MPO, Myeloperoxidase.

DISCUSSION

Several studies in animal models have confirmed the efficiency of curcumin against different diseases (28-31); however, its clinical efficiency is limited because of unfavorable pharmacokinetic assets. So, the widespread attributes of curcumin have not been entered into the clinical experiments. In the following study, we reported for the first time the usefulness of orally administered nano curcumin on experimental models of pain and inflammation in rats.

A wide range of evidence exists to support the pharmacological effects of curcumin. *Curcuma longa* (turmeric) is a rhizomatous plant of the ginger family. The therapeutic properties of turmeric have been recognized for thousands of years, but the mechanism(s) of action and the active ingredients have been examined recently. Curcumin is a natural polyphenol produced in the rhizome of turmeric. Numerous investigations have suggested the various pharmacological usages of curcumin, including analgesic activity, intense antioxidant activity, anticancer properties, and kidney advantages (10).

Among various experimental simulations for pain, the formalin test is the most helpful technique to confirm whether a compound has antinociceptive properties. In the current study, this investigational model was used to test the antinociceptive effect of curcumin and nano curcumin. Moreover, in this investigation, we applied other experiments, such as the tail-flick and the carrageenan test. The formalin test is used to evaluate both antinociceptive and anti-inflammatory reactions. In the mentioned test the nociceptive conduction is detected in two stages (early and late), which shows different kinds of pain (32). Infusion of formalin into the hind claw makes a biphasic pain answer. It has been supposed that the first stage is affected by direct activation of the initial afferent sensory neurons, while the second stage is affected by replication of the collective effects of afferent input along with central sensitization in the dorsal horn (33); therefore, both stages of pain generation (neurogenic and inflammatory) can be covered by information from the formalin technique. The current research investigated the antinociceptive effects of curcumin and nano curcumin on both neurogenic and inflammatory phases in the formalin technique. However, nano curcumin was meaningfully more efficient than curcumin against the nociceptive performance evoked by formalin in the neurogenic phase. Both curcumin (50 and 100 mg/kg) and nano curcumin (20, 50, 100 mg/kg) were significantly more effective compared to the control group in the inflammatory phase. Moreover, the difference between curcumin and nano curcumin was significant at doses of 50 and 100 mg/kg in the inflammatory phase. These outcomes advocate that nano curcumin may have more antinociceptive and anti-inflammatory effects, which are coherent with the findings of a report by Sankar *et al*. in which it was revealed that nanoformulation had a superior protective effect compared to free curcumin against arsenic-induced genotoxicity in rats (34).

The effectiveness of centrally acting analgesics can be measured by the tail-flick technique, which is a particular investigational model of pain. The analgesic action in the tail-flick test reduces the spinal reflex to the painful stimuli (35). In the current research, the oral administration of curcumin and nano curcumin at the dose of 100 mg/kg meaningfully sustained the latency of the nociceptive reaction compared to the control group, suggesting a central analgesic effect of the curcumin and nano curcumin. Indeed, Curcumin has already been shown to have an antinociceptive effect in various studies. Han *et al*. described pain (formalin test) as being eliminated in the rats treated with curcumin (36). More recently, the effectiveness of intrathecal curcumin in reducing both postoperative and inflammatory pain (carrageenan test) was investigated. Results indicated that intrathecal curcumin was helpful in both mentioned pains, but such antinociception of curcumin was antagonized by both GABA and opioid receptor antagonists. Therefore, GABA and opioid receptors may be involved directly or indirectly in the alleviating of postoperative and inflammatory pain (37). Since an opioid receptor antagonist was used in our study and curcumin and nano curcumin still had their effects, it may be possible to state that the antinociceptive effect of curcumin is not only through opioid receptors.

Several researchers have considered the antinociceptive effects of curcumin on diabetes. In a study by Li, Zhang *et al*. the effect of curcumin on diabetic pain was examined in rats. Outcomes indicated that curcumin drastically reduced the diabetes which induced hyperalgesia and allodynia (28). Fattori *et al*. examined the effect of curcumin on pain-like behaviors induced by superoxide anions. Results revealed that curcumin prevents superoxide anion-induced inflammatory pain-like performances and leukocyte employment by affecting oxidative stress and inflammatory molecules (29). Also, Sharma *et al*. examined the effect of the combination of resveratrol and curcumin in decreasing diabetic neuropathic pain. The results showed an antinociceptive effect of curcumin, resveratrol, and the mixture with insulin, perhaps through the contribution of nitric oxide and tumor necrosis factor alpha (30). Moreover, the findings of Banafshe *et al.* revealed that curcumin could be evaluated as a new possible treatment for diabetic neuropathic pain, and the opioid system might be involved in the antinociceptive impact of curcumin (31). Xie *et al*. proved that curcumin has an antinociceptive impact on brachial plexus avulsion in mice. Results of the mentioned investigation proposed that curcumin meaningfully extenuated inflammation and brachial plexus avulsion-induced pain by diminishing the amount of pro-inflammatory

cytokines and pain-associated proteins and preventing the astrocytes' action (38).

Using a carrageenan-induced claw inflammation model showed the anti-edematous activity of curcumin after 60 min in a dose of 100 mg/kg and nano curcumin after 60 min in both doses of 50 and 100 mg/kg, indicating that nano curcumin has anti-inflammatory activity in a lower dose. Moreover, curcumin showed anti-edematous activity after 180 min in doses of 50 and 100 mg/kg, and nano curcumin after 180 min in all doses of 20, 50, and 100 mg/kg. It can be concluded that nano curcumin can be effective in the management of local inflammation (39). Moreover, these findings propose that nano curcumin may be effective in lower doses which can be explained by the fact that nanoformulations have better structural nature and bioavailability.

The MPO, an enzyme found in neutrophils and macrophages, generates hypochlorite upon the reaction of hydrogen peroxide and Cl (40). In our study, it was found that using curcumin (50 and 100 mg/kg) and nano curcumin (20, 50, and 100 mg/kg) decreased MPO. Moreover, there was a significant difference between curcumin and nano curcumin at the dose of 50 mg/kg. It should be noted that oxidative killing of invasive pathogens is dependent on the regular activity of MPO, but its increased activity may lead to injury of healthy tissues through making free radicals. It has been shown that curcumin can reduce MPO activity in numerous disease models (41). Also, one study has demonstrated that the nanoformulation of curcumin can decrease pro-inflammatory mediators *by* improving nuclear factor-κB signaling. Furthermore, it was revealed that nanoformulation compared to standard curcumin was a better alternative in murine mastitis (42).

MDA is an essential indicator of lipid peroxidation. The current study, in line with prior reports, discovered that MDA decreased significantly after the administration of curcumin (50 and 100 mg/kg) and nano curcumin (20, 50, and 100 mg/kg) (43). Our findings established that nano curcumin could prevent MPO and MDA elevation more than curcumin, proposing that nano curcumin has more favorable effects on inflammation and lipid peroxidation. The impact of polymerized nano curcumin was investigated on an animal model of multiple sclerosis named experimental autoimmune encephalomyelitis. The results revealed an effective therapeutic outcome on the experimental autoimmune encephalomyelitis (44). In a study, natural curcumin and encapsulation of curcumin in nanomaterials were compared in L-thyroxine-induced hyperthyroid rats. It was illustrated that the favorable effects of curcumin might be manipulated by increasing its stability and bioavailability *via* nanoparticle encapsulation (45). These findings are in line with our data on improving the properties of nano curcumin in reducing inflammation and preventing pain. Our research was the first comparative study to determine the promising effects of the nanoemulsion formulation of curcumin in pain and inflammation reversing *in vivo*.

CONCLUSION

The discoveries of current research revealed antinociceptive effects of nano curcumin at lower doses compared to curcumin in the pain models of tail-flick and the formalin-induced reaction. Moreover, nano curcumin showed anti-inflammatory and antioxidant effects at lower doses compared to curcumin. Moreover, there was a significant difference between curcumin and nano curcumin in the late phase of the formalin test at doses of 50 and 100 mg/kg and the MPO test at a dose of 50 mg/kg. We suggest that the nanoemulsion formulation of curcumin can be efficient in reducing pain and especially inflammation in lower doses compared to the native form of curcumin. Nevertheless, more investigations are needed on human subjects to demonstrate its clinical effectiveness and safety as a pain-relieving agent.

Acknowledgments

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Conflict of interest statement

The authors declared no conflict of interest in this study.

Authors' contributions

M. Mohammadi and R. Haddadi conceptualized the study; F. Sangin Abadi conducted the experiments; F. Sangin Abadi prepared the raw data file for analysis; M. Mohammadi and A. Nili-Ahmadabadi analyzed the data and helped in the manuscript writing. The final version of the manuscript was approved by all authors.

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