Original Article

Effects of Fe^{2+} Nanoparticles on Pain Responses and Neural Oscillation Following Chronic Neuropathic Pain in Rats

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ABSTRACT

Neuropathic pain, a chronic pain condition caused by nerve damage either of the peripheral or central nervous system, responds poorly to current drug treatments. The present study aimed to investigate the analgesic and anxiolytic effect of Fe^{2+} nanoparticles on chronic constriction injury of sciatic nerve (CCI)-induced neuropathic pain in rats. We also assessed the effects of Fe^{2+} nanoparticles on brain rhythmical oscillation in rats with neuropathic pain. The CCI model was induced by four loose ligations of the left sciatic nerve. Male Wistar rats were divided into four groups: control, sham, CCI, and CCI+Fe^{2+} nanoparticle (1 mg/kg). The Fe^{2+} nanoparticle was administered by gavage on the day of CCI surgery (day 0) and daily (once a day) for 21 consecutive days after CCI surgery. Behavioral studies were conducted on days -1, 3, 7, 14, and 21 after CCI. An acetone test and elevated plus maze were performed to evaluate cold allodynia and induced anxiety-like responses, respectively. A field test was conducted to evaluate innate anxiety-like behaviors. In addition, an electrophysiological study was carried out on day 21 after CCI to assess the effects of drugs on brain wave power. Application of Fe^{2+} significantly reduced cold allodynia in all tested days after CCI, compared to the CCI group. The obtained data demonstrated that Fe^{2+} nanoparticle gavage caused analgesic and anxiolytic effects on all experimental days after CCI, compared to the CCI group. The CCI surgery significantly disturbed theta, alpha, and beta power in the brain. The application of Fe^{2+} nanoparticles could not significantly change brain wave power. It is suggested that Fe^{2+} nanoparticle has analgesic and anxiolytic effects during chronic neuropathic pain in rats. Furthermore, the CCI surgery effectively disturbed brain theta, alpha, and beta power. Nonetheless, the application of Fe^{2+} nanoparticles could not change deregulated brain oscillation in rats.

Keywords: Antioxidant, EEG, EPM, Fe^{2+} nanoparticle, Neuropathic pain

1. Introduction

Neuropathic pain is a complex and severe condition caused by a lesion or disease of the peripheral or central nervous system (1-3). It can last for years or even a lifetime after initial nerve damage, imposing a heavy burden on individuals, societies, and economics. It is well-accepted that some types of peripheral nerve damage, such as sciatic nerve damage, cause chronic neuropathic pain (4, 5). Patients with sciatic nerve-related neuropathic pain demonstrate hyperalgesia (exaggerated pain perception in response to noxious), allodynia (pain perception in response to non-noxious stimuli), and psychological disorders, such as anxiety (6).

It is reported that brain rhythmical oscillations in the low (delta, theta, and alpha) and high (beta and gamma) frequencies of electroencephalography (EEG) are associated with different cognitive and perceptual operations, as well as pain conditions (7). A wide array of studies have shown an increase in brain oscillations in different areas of the brain, including the anterior cingulate cortex, the insular cortex, the thalamus, the frontal cortex, and the somatosensory cortex (7-9). In the same vein, previous studies reported that resting state EEG oscillations in the theta band are increased in neurogenic pain patients (9). LeBlanc et al. pointed out that oscillations within the low-frequency theta (4-8 Hz) band increased in different animal models of pain using spectral analysis of the local field potential, electrocorticography, and EEG (10). Therefore, it would be useful to know how the brain EEG oscillation changes in chronic neuropathic pain conditions. Today, therapeutic options for the treatment of neuropathic pain produce only partial relief, and the treatment of neuropathic pain is still lacking. Therefore, in recent years, new therapeutic options have been developed (11, 12).

Iron is an essential element for living organisms, playing a pivotal role in biological functions (13). Furthermore, Iron is an essential cofactor for vital functions, including oxygen transport, DNA synthesis, myelin formation, and synthesis of neurotransmitters. In addition, Iron can promote the consumption of glutathione, which is the most abundant antioxidant agent in the body (14). Today, nanotechnology has emerged as a promising drug delivery strategy and has increasingly been considered an attractive technology in medical studies (15). The length of the synthesized nanoparticle is between 1 nm and 100 nm (16). Nanotechnology increased drug bioavailability and delivery of minerals or drugs to their target (17). Verma et al. reported that decreasing the particle size of elemental iron powder by 50%-60% to a mean particle size of 7-10 mm increased iron absorption by 50% in rats (18). Therefore, the crucial properties of nanoparticles are their passing more easily via the blood-brain barrier, solubility in lipids, as well as greater bioavailability and reactivity. It is reported that particle size reduction is an effective strategy for the improvement of iron bioavailability.

In the present study, we used a rat model of neuropathic pain to assess the analgesic and anxiolytic effects of Fe^{2+} nanoparticles in neuropathic pain in male rats. We also assessed the effects of Fe^{2+} nanoparticles on the brain rhythmical oscillations in chronic neuropathic pain.

2. Materials and Methods

2.1. Animals

Adult Wistar male rats (weight 180-200 g, n =6/group) were obtained from the breeding colony of Baqiatallah University of Medical Sciences, Tehran, Iran. Animals were housed one per cage and placed under a 12-hour light/dark cycle in a room at 22-24℃. Animals had free access to food and water. All experiments were conducted in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals and were approved by the local ethical committee (Ethical code: IR.BMSU.REC.1400.119).

2.2. Experimental design

In the current study, animals were divided into four groups (n=6/group). These groups were as follows: [Group 1: control group], [Group 2: sham group],

[Group 3: neuropathy group (chronic constriction injury) group], and [Group 4: neuropathy+Fe^{2+} nanoparticle (1 mg/kg) group]. Normal saline as vehicle and nanoparticle were gavaged intra-gastric daily from day 1 up to 30 days after the induction of neuropathy. Cold sensitivity and anxiety were obtained 1 day prior to neuropathic surgery and on days 3, 7, 14, and 21 post-surgery, using an acetone test and elevated plus maze (EPM), respectively. Fe^{2+} nanoparticle was dissolved in normal saline 0.9%.

2.3. Induction of neuropathic pain model

Neuropathic pain (chronic constriction injury [CCI] model) was induced, as it was previously introduced by Bennett and Xie (19). In brief, after anesthetizing the animals with chloral hydrate (350 mg/kg, i.p.), the left body of the sciatic nerve (1 cm) was exposed, and then four loss ligatures (4/0 catgut) was tied around the nerve, about 1 mm apart until a brief twitch in the hind limb was observed. In sham animals, only the left sciatic nerve was exposed, but not ligated.

2.4. Cold Allodynia (Acetone test)

To quantify the cold threshold of the neuropathic hind paw, foot withdrawal (as a positive response) in response to acetone drop was evaluated (6). In brief, the rat was placed under a transparent Plexiglas chamber with a metal mesh floor, and an acetone drop was applied to the plantar surface of the hind paw using a syringe. The acetone was applied five times (every 5 min) to each paw. The number of total trials was five. The frequency of paw withdrawal was again expressed as a percent as follows: (Number of positive responses×100) / (Number of total trials).

2.5. Elevated plus maze

The EPM consisted of two open and two closed arms. Animals were placed on the center platform of the maze, facing an open arm for 5 min. The percentage of entries into open arms and the percentage of entries into closed arms were used as an index of anxiety-like behaviors. Fewer entries into open arms and more entries into closed arms were in favor of anxiety (20).

2.6. Open field test

The total distance traveled by rats was examined within 10 min using an open field box. Each rat was placed in the center of the field box, and the total traveled distance in the central zone was monitored by a camera. Total distance was considered an index of anxiety-like behavior (21).

2.7. Electroencephalography experiment

The rats were anesthetized by ketamine (65 mg/kg) and xylazine (15 mg/kg) and fixed on a stereotaxic apparatus. Three stainless steel screw electrodes were implanted in the skull for EEG recording. The EEG data were collected by the electrophysiology recording system (e-probe software, Science Beam Company, Tehran, Iran) at the 3,000 Hz sampling rate. The signals were amplified and filtered (EEG 0-40 Hz, EMG 1-400 Hz). Thereafter, electrodes were placed sub-cranially for EEG recording. The EEG recorder system was a data acquisition system from Science Beam Company, Iran. The system contained the data to distinguish the power of alpha, beta, delta, and theta waves. Waves were filtered in the range of 0-30 Hz. To be precise, 0.1-3.9 Hz waves were counted as delta waves, 4-7.9 Hz waves were theta waves, 8-13 Hz waves were recorded as alpha waves, and 13.1-30 Hz waves were recorded as beta waves (22).

2.8. Statistical analysis

The data were presented as mean ± standard error of the mean (SEM). Data were analyzed in SPSS software (version 24.0). Differences in measured parameters among different groups were analyzed using two- and one-way analysis of variance (ANOVA), followed by the Tukey post hoc test. The differences were considered significant when the probability was less than 0.05.

3. Results

3.1. Analgesic effects of Fe^{2+} nanoparticle following chronic neuropathic pain on cold allodynia (Acetone Test)

As illustrated in figure 1, following CCI, the frequency of paw withdrawal significantly increased

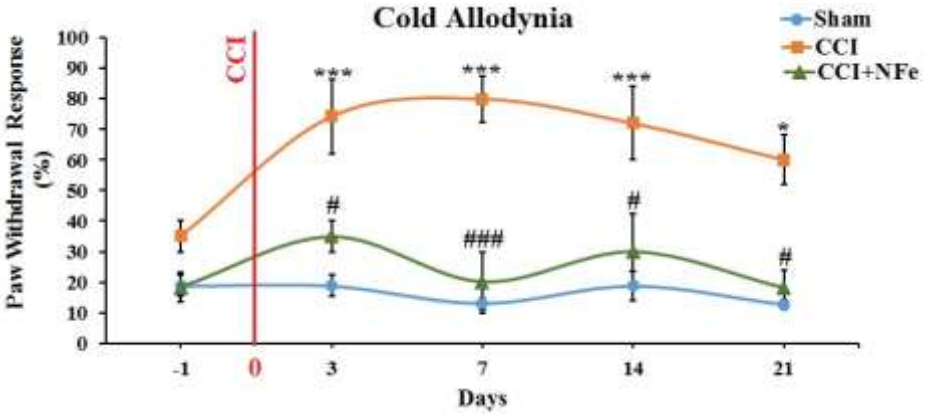

Figure 1. Effects of Fe^{2+} nanoparticle on cold allodynia evaluated. Frequency of hind paw responces to acetone stimulation was assessed on ipsilateral hind paws of experimental groups, at day -1 (baseline), and days 3, 7, 14 and 21 post-neuropathy. Differences in measured parameters among different groups analyzed by using one-way analysis of variance (ANOVA), followed by the Tukey post hoc test. * denote a significant difference with sham animals in each group; # denote a significant difference with CCI animals. CCI; chronic constriction injury; NFe: Fe^{2+} nanoparticle.

in the CCI group ([day 3: 74.28±12.12], [day 7: 80.00±7.55], [day 14: 72.00±12.00], [day 21: 60.00±8.16]), as compared to the sham group ([day 3: 18.75±3.50], [day 7: 12.85±1.84], [day 14: 18.75±4.79], [day 21: 12.50±1.63]) in all experimental days (CCI surgery induced painful behavior) (Figure 1;*P<0.05). In addition, intra-gastric gavage of Fe^{2+} nanoparticles significantly decreased the frequency of paw withdrawal in the CCI+Fe^{2+} nanoparticle group ([day 3: 35.00±05.00], [day 7: 20.00±10.00], [day 14: 30.00±12.24], [day 21: 18.00±5.83]), as compared to the CCI group (Figure 1; P<0.05). There was no significant difference between the control and sham groups. Therefore, we removed the control group data.

3.2. Anxiolytic effects of Fe^{2+} nanoparticle following chronic neuropathic pain on anxiety-like behaviors (EPM test)

In the EPM test, CCI surgery decreased entries to open arms of EPM on days 3 (16.00±3.92), 7 (12.00±2.00), 14 (14.75±3.20), and 21 (11.66±1.66) post-surgery (displayed increased anxiety-like behaviors), when compared to day -1 (before CCI surgery) or sham group (Figure 2A, *P<0.05). Furthermore, intra-gastric gavage of Fe^{2+} nanoparticles increased entries to open arms of EPM in the CCI+Fe^{2+} nanoparticle group on all experimental days ([day 3: 55.55±9.29], [day 7: 62.00±9.69], [day 14: 57.50±9.97], [day 21: 59.00±11.00]) (anxiolytic effects of Fe^{2+} nanoparticle) (Figure 2A, $^{\#}P$<0.05).

Moreover, our EPM data demonstrated that CCI surgery increased entries to close arms of EPM on days 3 (70.33±10.21), 7 (94.62±5.37), 14 (100.00±0.00), and 21 (94.50±5.50) post-surgery (displayed increased anxiety-like behaviors), when compared to day -1 (before CCI surgery) or sham group (Figure 2B, *P<0.05). Intra-gastric gavage of Fe^{2+} nanoparticle decreased entries to close arms of EPM in the CCI+Fe^{2+} nanoparticle group on days 7 (53.33±3.33), 14 (56.25±6.25), and 21 (55.00±5.00) after CCI surgery (anxiolytic effects of Fe^{2+} nanoparticle) (Figure 2B, #P<0.05).

3.4. Anxiolytic effects of Fe^{2+} nanoparticle following chronic neuropathic pain on anxiety-like behaviors (Open field test)

In the open field test, CCI surgery significantly decreased the distance traveled in the box on days 7, 14, and 21 post-surgery (displayed increased anxiety-like behaviors) when compared to the sham group (Figure 3, *P<0.05). Furthermore, intra-gastric gavage of Fe^{2+} nanoparticles significantly increased the distance traveled in the box on days 3, 7, and 14 post-surgery in the CCI+Fe^{2+} nanoparticle group (anxiolytic effects of Fe^{2+} nanoparticle) (Figure 3, #P<0.05).

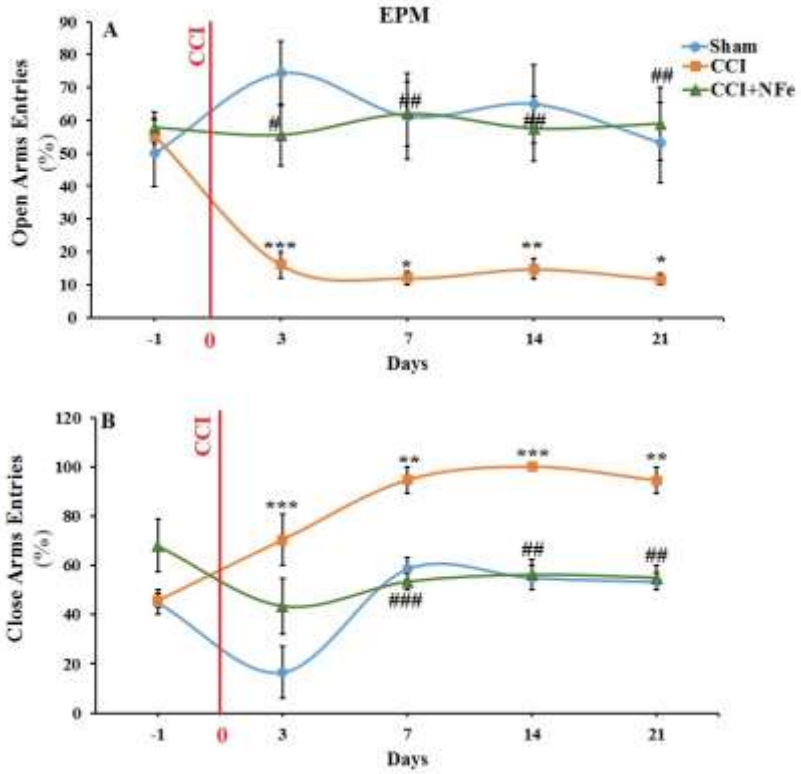

Figure 2. Effects of Fe2+ nanoparticle evaluated on anxiety-like behaviors at days -1 (baseline) and 21 days' post-neuropathy. Percentage of open arms entries (A), and close arms entries (B) were evaluated as an anxiety index. Differences in measured parameters analyzed by using one-way analysis of variance (ANOVA), followed by the Tukey post hoc test. * denote a significant difference with sham animals; # denote a significant difference with CCI group. CCI; chronic constriction injury; NFe: Fe^{2+} nanoparticle.

3.5. Effects of Fe^{2+} nanoparticle following chronic neuropathic pain on EEG power changes

Similar to our behavior data, as described above, both the control and sham groups displayed non-significant EEG power changes; therefore, we selected the sham group as the control. We compared

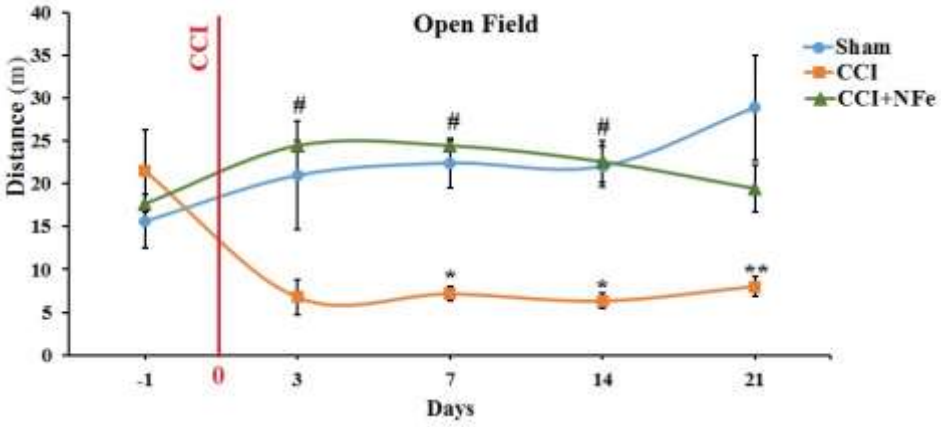

Figure 3. Effects of Fe^{2+} nanoparticle on total traveled distance evaluated in the open field box. Total traveled distance by rats in the open field box was assessed as an anxiety index, at day -1 (baseline), and days 3, 7, 14 and 21 post-neuropathy. Differences in measured parameters among different groups analyzed by using one-way analysis of variance (ANOVA), followed by the Tukey post hoc test. * denote a significant difference with sham animals in each group; # denote a significant difference with CCI animals. CCI; chronic constriction injury; NFe: Fe^{2+} nanoparticle.

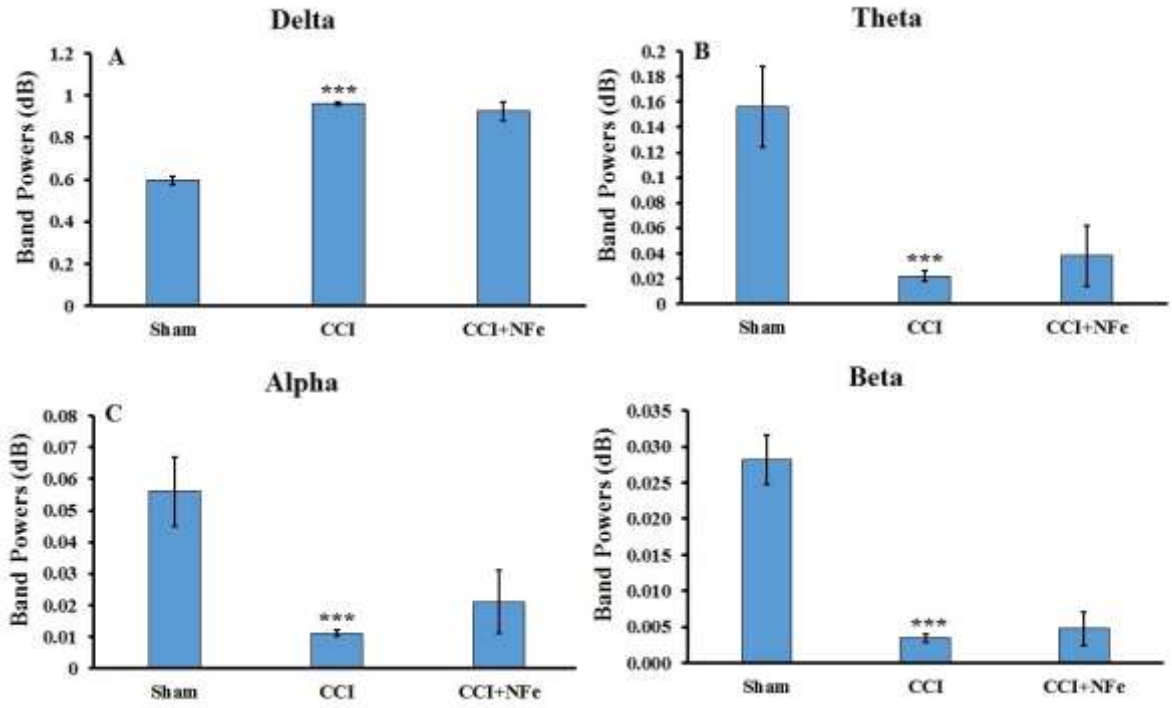

Figure 4. Effects of Fe^{2+} nanoparticle on EEG changes in the absolute powers in 4 frequency bands (delta: 2–4 Hz; theta: 4–8 Hz; alpha: 8–12 Hz; beta: 12–20 Hz; gamma: 20–100 Hz) in rats in the different experimental groups was evaluated on day 21 post-neuropathy. Differences in measured parameters among different groups analyzed by using one-way analysis of variance (ANOVA), followed by the Tukey post hoc test. * denote a significant difference with sham animals in each group. CCI; chronic constriction injury; NFe: Fe^{2+} nanoparticle.

the EEG power changes at different oscillation bands in three groups (Figure 4). Figures 4(A) and 4(B) presented the change rate of the averaged band power of delta and theta, respectively. Figures 4(C) and 4(D) demonstrated the change rate of the averaged band power of alpha and beta, respectively. As illustrated in figure 4, CCI surgery significantly decreased the averaged band power of theta, alpha, and beta in neuropathic rats as compared to that in the sham rats (P=0.001). Nevertheless, CCI surgery significantly increased the averaged band power of delta in neuropathic rats as compared to that in the sham rats (P=0.001). It was observed that intra-gastric gavage of Fe^{2+} nanoparticles could not significantly change EEG power in all parameters (Figure 4).

4. Discussion

Neuropathic pain is a chronic condition affecting almost 20% of the world's population. Nowadays, drugs are ineffective for the treatment of neuropathic pain, largely due to their side effects and poor underlying mechanisms (23). Oxidative stress has also been identified to play a critical role in the development and maintenance of neuropathic pain; therefore, there is a need to understand the analgesic effects of new natural antioxidants (24). Accordingly, the present study was designed to explore the analgesic and anxiolytic effects of Fe^{2+} nanoparticles on chronic neuropathic pain in rats. Based on the obtained results, CCI surgery would lead to the development of cold allodynia and anxiety-like behaviors (from 3 days up to 21 days post-surgery) in the injured hind paws of rats. Cold allodynia and anxiety-like behaviors were identified by an increase in paw withdrawal responses and a decrease in the percentage of open arms entries of the EPM, respectively. Ample documents reported that the CCI model induced cold allodynia and anxiety (25).

Following the ligation of the sciatic nerve, excitation of spinal cord projection neurons occurs, and subsequently, axons get degeneration and demyelination. All of these events lead to the sensitization of nociceptors and increased ectopic firing of afferent neurons, which, in turn, results in increased generation and release of pain-related neurotransmitters, such as substance P (26). The sensitization of spinal cord neurons leads to the maintenance of neuropathic pain (26).

Iron is involved in various biological processes in the brain, such as oxygenation of brain parenchyma, mitochondrial respiration, myelin synthesis, neuronal cell function, as well as neurotransmitter synthesis and metabolism. In addition, iron deficiency results in attenuation of myelination and monoamine metabolism (27). It has been shown that glutamate and gamma-aminobutyric acid metabolism is changed by oscillation of the brain iron levels (27). Therefore, Iron has a protective role in normal brain function.

The present study examined the analgesic and anxiolytic effects of Fe^{2+} nanoparticles in rats with chronic neuropathic pain. The results of our study demonstrated that Fe^{2+} nanoparticles had both analgesic and anxiolytic effects up to 21 days after the induction of neuropathic pain.

Brain oscillations play a critical role in the integration of brain information, such as pain information. Disturbances in brain oscillation are related to several diseases, such as chronic pain. It has been shown that chronic pain is related to maladaptive reorganization of neural activity in different brain regions (28). Indeed, the disturbance of brain oscillation in the thalamocortical pathways plays a crucial role in the pathophysiology of pain.

In the current study, we observed that CCI-induced neuropathic pain disturbed delta, theta, alpha, and beta oscillations as compared with the sham group. The CCI surgery effectively decreased theta, alpha, and beta oscillations in comparison to the sham group. However, this pain model significantly increased delta oscillations. Our results were in line with previous reports regarding the effects of chronic pain on the brain oscillation. For example, Alshelh et al. (2016) reported that neuropathic pain was related to increased infra-slow brain oscillation in the ascending pain pathway (29). Furthermore, Fu et al. (2018) found that acute pain effectively increased alpha oscillation and decreased beta and gamma oscillation of the medial prefrontal cortex in freely moving rats (30). They also observed that chronic neuropathic pain decreased delta oscillation, and both low gamma and high gamma oscillations also decreased with time. They suggested that the acute pain would be a negative stress stimulation and affected mPFC alpha, beta, and gamma oscillations, leading to adverse emotional experiences (30). However, the association of mPFC and hippocampus or other brain areas decreased in chronic pain. Indeed, patients with chronic pain suffer from negative mood disorders, including anxiety. It is suggested that the changes in delta and gamma oscillations may be responsible for the comorbidity of pain and anxiety. Additionally, it has been reported that pain intensity is associated with neural oscillation (26). Moreover, Simis et al. (2022) identified that patients with less knee osteoarthritis intensity and less pain had higher theta oscillations (31).

We also observed that the application of Fe^{2+} nanoparticles could not change deregulated brain oscillation in rats with neuropathic pain. The present findings represented the analgesic and anxiolytic effects of Fe^{2+} nanoparticles in rats with neuropathic pain. Therefore, it is suggested that Fe^{2+} nanoparticle has analgesic and anxiolytic effects during chronic neuropathic pain in male rats. Furthermore, the CCI surgery effectively decreased theta, alpha, and beta power in the brain. Nevertheless, the application of Fe^{2+} nanoparticles could not change deregulated brain oscillation in rats with neuropathic pain.

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Authors' Contribution

Study concept and design: Z. B.
Acquisition of data: M. H. N.
Analysis and interpretation of data: Z. B.
Drafting of the manuscript: H. G. and M.T. M.
Critical revision of the manuscript: M. T. M and Z. B.
Statistical analysis: M. S. and A. G.
Synthesis of Fe 2+ nanoparticle: M. R. N.

Ethics

It is declared that all ethical considerations were taken into account in the preparation of the submitted manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest.

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