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The effects of extremely low-frequency pulsed electromagnetic fields on analgesia in the nitric oxide pathway

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ABSTRACT

There is growing interest in the effects of extremely low-frequency electromagnetic fields on mechanisms in biological organisms. This study's goal is to determine the role of the Nitiric Oxide (NO) pathway for thermal pain by intentionally interfering with it using a pulsed electromagnetic field generated by an extremely low-frequency alternating current (ELF-PEMF) in combination with BAY41-2272 (sGC activator), NOS inhibitor L-NAME, and NO donor L-arginine. This study included 72 adult male Wistar albino rats (mean weight of 230 ± 12 g). The rats were kept at room temperature (22 ± 2 °C) in a 12-h light/dark cycle and in a room with sound insulation. PEMF (50 Hz, 5 mT) were applied four times a day for 30 min and at 15-min intervals for 15 days. Analgesic effects were assessed with tail-flick and hot-plate tests. Before the tests, NO donor L-arginine (300 mg/kg), sGC activator BAY41-2272 (10 mg/kg), and NOS inhibitor L-name (40 mg/kg) were injected intraperitoneally into rats in six randomly-selected groups. The maximum analgesic effect of a 5 mT electromagnetic field was on day 7. PEMF significantly increased the analgesia effect when the functioning of the NO pathway was ensured with L-arginine, which is a NO donor, and BAY41-2271, which is the intracellular receptor and sGC activator. However, there was no difference between rats treated with PEMF and the NOS inhibitor L-NAME as compared to rats only treated with PEMF. In conclusion, PEMF generate analgesia by activating the NO pain pathway.

1. Introduction

Over the last twenty years, investigation into the positive and negative effects of extremely low frequency (1–300 Hz) pulsed electromagnetic fields (ELF-PEMF) on biological organisms has increased. In the last ten years, studies have been able to show that electromagnetic fields affect biological mechanisms in living creatures. However, these effects have not been clearly explained.

Nitric oxide (NO) is a common signal molecule with some physiological roles such as neurotransmitter, vasodilator, and anti-inflammatory agent. Since the discovery of NO as a biological tool, the importance of NO in the functioning of the central nervous system (CNS) has been discussed by various researchers [1,2]. Experiments have demonstrated that NO, which is derived from different isoforms of nitric oxide synthase (NOS), plays a role in the nociceptive mechanism [3–6]. NO plays a role in many physiological processes, but this study's focus is on its main role as a neurotransmitter in the nociceptive mechanism. NO is a signal molecule that is vital in both pathways (central and peripheral) for acute [3] and chronic [4] pain. Peripheral NO levels are synthesized from L-arginine by sensory neurons and nitric oxide synthase (NOS), which is found in the dorsal horn in the spinal cord [7,8]. Experimental studies show that thermal pain increases when NO formation is reduced with the NOS inhibitor Nx-nitro-L-arginine methyl ester (L-NAME) [9,10].

NO is also involved in pain formation throughout the nervous system. It has been demonstrated that NOS inhibitors reduce hyperalgesic pain formation in acute and chronic neuropathic pain models [11]. Soluble guanylate cyclase (sGC) is the known intracellular receptor for NO. The NO/sGC signaling pathway mediates a large number of NO functions [12]. The nociceptive effect of NO includes sGC stimulation, resulting in the conversion of guanosine triphosphate to cGMP. NO inhibits nociception in the peripheral and central nervous system. NO participation in nociceptive processes has been demonstrated by experiments that used NOS inhibitors to reduce NO

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production. Cury et al.'s review [13] draws attention to the dual effect of NO on both analgesia and hyperalgesia.

In terms of ELF-EMF, powerful effects have reported on NO systems in the brain. ELF-EMF can modulate the functional state of various neuron communities. A number of studies found that external ELF-EMF regulate nitric oxidergic, serotonergic, and dopaminergic neural transmission [14]. The analgesic effects of ELF-EMF were first revealed by Ossenkopp et al., in 1985 in a study conducted on mice [15]. Studies by Kavaliers et al. [16] on the land snail Cepaea nemoralis supported these findings. An antinociceptive effect was determined in diabetic rats exposed to a 50 Hz electromagnetic field [17]. Various studies have shown that magnetic fields reduce inflammatory pain caused by rheumatoid arthritis, psoriasis, and tendinitis [18,19]. Cho et al. reported that ELF-EMF exposure could increase NO production through the activation of neuronal NO synthase (nNOS) in the brain and cause physiological reactions through intracellular signaling activation [20]. Other studies have demonstrated that ELF-PEMF can regulate the brain's nitric oxidergic, serotonergic, and dopaminergic neural transmission [21]. Jeong et al. demonstrated that exposure to ELF-EMF might cause NOS activation due to intracellular [Ca^{2+}] and subsequently cause hyperalgesia due to an increase in NO synthesis. They reported that ELF-EMF could create hyperalgesia due to NO synthesis with Ca^{2+} dependent NOS [22].

This study aims to determine the role of the NO pathway by intentionally interfering in the NO pathway for thermal pain with a pulsed electromagnetic field formed by an extremely low-frequency alternating current (ELF-PEMF) in combination with BAY41-2272 (sGC activator), L-arginine, and the NOS inhibitor L-NAME.

2. Materials and methods

After obtaining permission from the Experimental Animals Local Ethics Committee (CU-HEK/2014–67), 72 Wistar albino rats (mean weight of 230 ± 12 g) were procured from the Experimental Animal Center at Cumhuriyet University, Turkey. The rats were kept at 22 ± 2 °C room temperature, in a sound-insulated environment, in a 12 h of light/dark cycle, and with sufficient feed and water in the cages. Before analgesia tests, it was ensured that all animals had adapted to laboratory conditions. All electromagnetic experiments were performed between 10:00–13:00.

2.1. Analgesia tests

Standardized tail-flick test (May TF 0703 Tail-flick Unit, Commat) and hot-plate test (May AHP 0603 Analgesic HP, Commat) devices were used to assess thermal pain. In the tail-flick test, rays from a radiant heat source were applied to a 3 cm distal portion of rats' tails after saline solution or test drugs were administered intraperitoneally. After radiant heat was applied, tail-flick latency (TFL) periods were determined. The basal TFL period of the animals was determined as approximately 2.9 ± 0.5 seconds (s). Rats with basal TFLs under 2.4 s or over 3.4 s were excluded from the study. The test was limited to 15 s (cut-off latency) to prevent tissue damage. Animals that did not respond after 15 s were excluded from the study. The responses to this test reflect pain mechanisms in the central nervous system [23,24].

In the hot-plate test, each rat was placed on a plate heated to 55.0 ± 0.5 °C. The time until the animal's first paw licking or lifting movement marked the latency and hence the pain threshold. The cut-off latency was 30 s to prevent injury. Responses to this test reflect pain in both the peripheral and central nervous system [23].

2.2. Pulsed electromagnetic field application

We used the same pulsed electromagnetic field experiment setup as in one of our previous studies [25,26]. Before ELF-PEMF treatment, animals had one week to adapt to the laboratory environment. During this period, rats were kept in cages within a solenoid at least three times a day for 30 min each time. During the experimental study, rats were exposed to a 50 Hz frequency and 5 mT magnetic field for 2 h and 45 min for 15 days. During exposures, fields were active for 30 min and then inactive for 15 min. This was repeated four times, for a total of 2 h and 45 min.

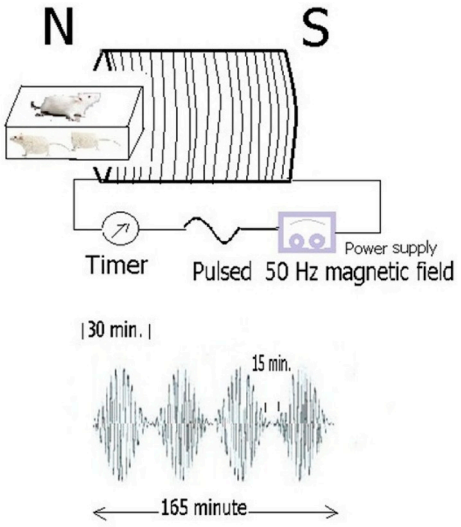

Fig. 1. Schematic representation of placement of the rats in the solenoid with PEMF. The bottom of the figure shows, a schematic of the magnetic field that the rats were exposed to for a total of 165 min, alternating between 30 min of exposure and 15 min of silencing.

To generate the electromagnetic field, a specially designed mechanism was used. A 1.4 mm thick insulated copper wire was wrapped 1400 times around a solenoid measuring 500 mm in length and 210 mm in diameter. A device containing a time relay and alternating electrical current (50 Hz, 120 V) were passed through the solenoid. PEMF exposure was performed at approximately the same time each day (10:00–13:00 in the morning) for 165 min. The intensity of the electromagnetic field was measured and evaluated with an axial probe and a digital teslameter (Phywe, 8010). The direction of the solenoid was north–south and the temperature was 25.0 ± 2 °C. Fig. 1 shows a schematic of the device and the plexiglas cage (40 × 17 × 13 cm in size). Each time, three rats were simultaneously placed in the cage for PEMF exposure. The control group of rats were also placed in cages, but their exposure to the electromagnetic field was blocked. Sufficient rat feed and water were kept in the cages.

2.3. Protocol

All groups except the control group were exposed to PEMF on days 1, 4, 7, 11, and 15. On the same days, the tail-flick and hot-plate analgesia tests were run on all rats. Rats that were subjected to analgesia tests were selected randomly. The rats in each group (n = 6) were exposed to magnetic field for 2 h 45 min and then immediately injected with BAY41-2272, L-arginine, or L-NAME. Then analgesia tests were performed at 0, 30, 60, 90, and 120 min. Prior to tests with drugs, the day with maximum analgesic activity from only the PEMF was determined.

In the first application, prior to the analgesia tests, the soluble guanylate cyclase activator BAY41-2272 (10 mg/kg) was injected to the rats intraperitoneally in order to test the connection between PEMF and the sGC intracellular receptor of NO [27,28]. In the second application, PEMF was applied and NO donor L-arginine (300 mg/kg) was administered [29], and then the analgesia tests were performed. This was done to activate the NO pathway in a controlled manner. The groups were formed by selecting rats randomly into six groups to be treated

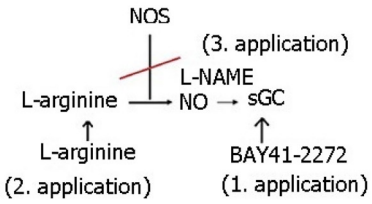

Fig. 2. The locations of three treatments used to evaluate the analgesic effect of PEMF on thermal pain using the NO pathway.

with (1) only BAY41-2272, (2) BAY41-2272 and PEMF, (3) only L-arginine, (4) L-arginine and PEMF, (5) only L-NAME, and (6) L-NAME and PEMF. The last two groups were given the NOS inhibitor L-NAME (40 mg/kg) to ensure that the NO pathway could not function [30,31]. The following groups were formed: Fig. 2 presents the steps for the three different treatments.

2.4. Data analysis

As an estimate of the antinociceptive effect, the percentage of the maximal potential effect (MPE) was calculated based on the latency periods from the tail-flick and hot-plate tests with the following formula:

% MPE = [(test time – basal latency period) / (test cut-off time – basal latency period)] × 100

2.5. Statistical analysis

Two-way analysis of variance (ANOVA) and repeated-measure ANOVA tests were used to analyze % MPE. Tukey's HSD test (post-hoc test), which is a multiple comparison test, was used to determined which group was different, using SPSS 22.0. All data are presented as mean ± standard error. The significance level was $p < 0.05$.

3. Results

In order to find the day with the greatest analgesic effect from PEMF, we performed tail-flick and hot-plate tests on days 1, 4, 7, 11 and 15. The average latencies were 25.91 ± 3.01 and 62.23 ± 3.11 s, respectively. The latencies were significantly longer on day 7 compared to the control group ($p < 0.01$; Fig. 3). Day 7 was determined to be the best day to use chemical agents that affect the NO pathway (Fig. 2).

3.1. The effects of the soluble guanylate cyclase activator BAY41-2272 on analgesia formed by PEMF

Before analgesia tests, BAY41-2272 was injected into one group of rats (10 mg/kg; Fig. 2). In the rats exposed to the magnetic field, day 7 was the most effective. On this day, the analgesic effect was significantly higher in the group that received BAY41-2272 (tail-flick: 42.11 ± 4.45 s and hot-plate: 62.42 ± 4.67 s) compared to the PEMF-only group (tail-flick: 32.11 ± 3.45 s and hot-plate: 51.13 ± 4.78 s). This was the greatest analgesic effect (% MPE) for 90 min of measurements ($p < 0.05$; Fig. 4).

3.2. The effects of L-Arginine on the analgesia formed by the PEMF

Before the analgesia tests, NO donor L-arginine (300 mg/kg) was injected into another group of rats (Fig. 2). On day 7, the analgesic effect was significantly higher in this group (tail-flick: 42.11 ± 3.45 s; hot-plate: 61.13 ± 4.78 s) compared to the PEMF-only group ($p < 0.05$; Fig. 5).

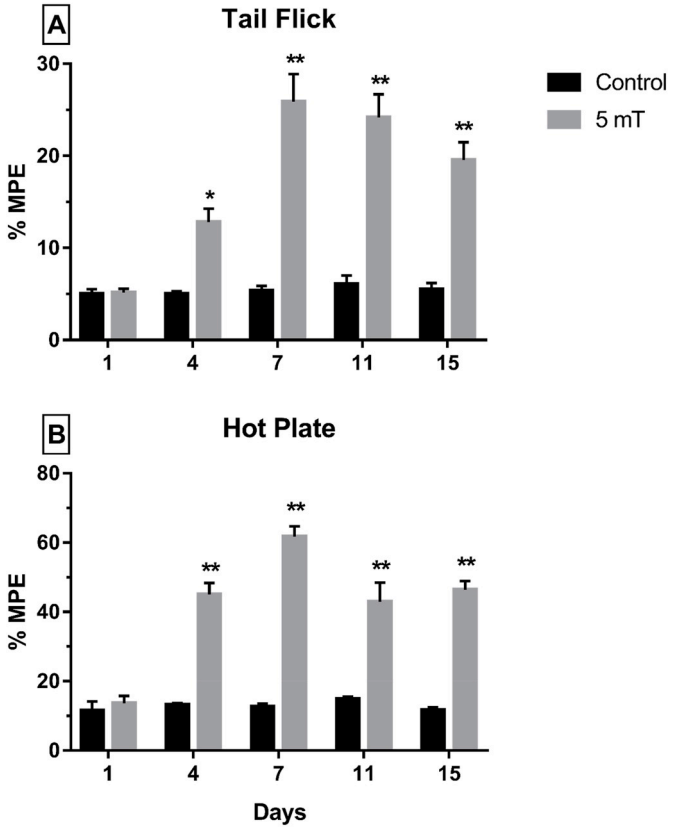

Fig. 3. Analgesic effects of PEMF strength (5 mT) on rats measured by tail-flick test (A) and hot-plate test (B). *$p < 0.01$, when the PEMF effect was compared with the control group; **$p < 0.05$, when the seventh-day effect of the analgesic effects of the 5 mT group was compared with the other days.

3.3. The effects of L-NAME on analgesia formed by PEMF

In a third group, rats were given the NOS inhibitor L-NAME (40 mg/kg). The maximum analgesic effect (% MPE) was obtained in 90 min measurements in all groups (Fig. 6). In PEMF-exposed rats, there was a significant analgesic effect ($p < 0.01$) in both the tail-flick (30.11 ± 2.45 s) and hot-plate (64.15 ± 4.50 s) tests, compared to the saline control group (7.65 ± 0.98 s and 14.26 ± 2.12 s, respectively). As seen in Fig. 6, the test measurements demonstrate that there was a significant increase in latency in the group treated with L-NAME (tail-flick: 37.98 ± 3.35 s and hot-plate: 75.26 ± 5.32 s), compared to the PEMF-only group ($p < 0.05$).

4. Discussion

This study has three significant results. First, the maximum analgesic effect produced during 15 days of 5 mT PEMF treatment was on day 7, as shown by the longer latency in tail-flick and hot-plate tests. The difference between day 7 and 11 was not significant. Based on this, we tested the effect of activator, donor, and inhibitor injections on day 7. Second, the analgesic effects of PEMF increased the binding of NO to intracellular receptor GC. Third, PEMF has greater effects with larger doses of NO donor L-arginine. For groups treated with the NOS inhibitor L-NAME, the PEMF effect was not significantly different than the PEMF-only group.

Extremely low-frequency EMF exposure caused an analgesic effect and some behavioral changes in mice. Similarly, daily repeated magnetic field exposure caused an analgesic effect in earthworms, with a maximum effect on day 4 [31,32].

The literature suggests that electromagnetic fields reduce pain

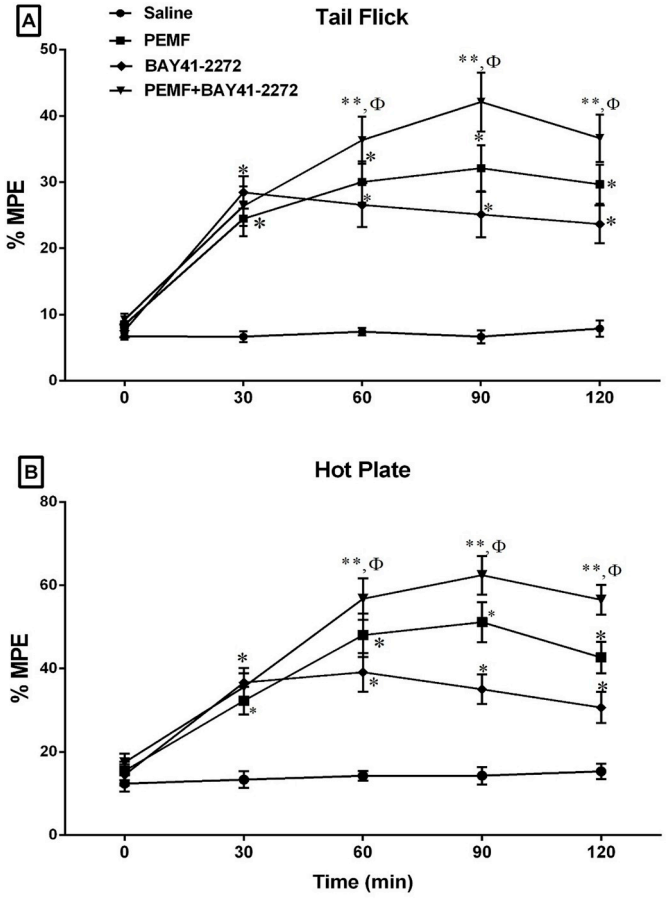

Fig. 4. The effect of BAY41-2272 on PEMF induced analgesia. (A) Shows effect BAY41-2272 on PEMF induced analgesia in the tail –flick, and (B) hot-plate test. Administration of BAY41-2272 to PEMF (5 mT) exposed rats produce a significant increase in % MPE in both TF and HP assays as compared to PEMF group rats ($p<0.05$). The % MPE value of three groups (BA41-2272, PEMF and PEMF + BAY41-2272) was significantly higher than saline ($p<0.01$). Each point represents the mean ± SEM of % MPE for 6 rats. *p < 0.05 and **p < 0.01, when the groups were compared with the Saline. $^{\Phi}$ p < 0.05 compared with the PEMF.

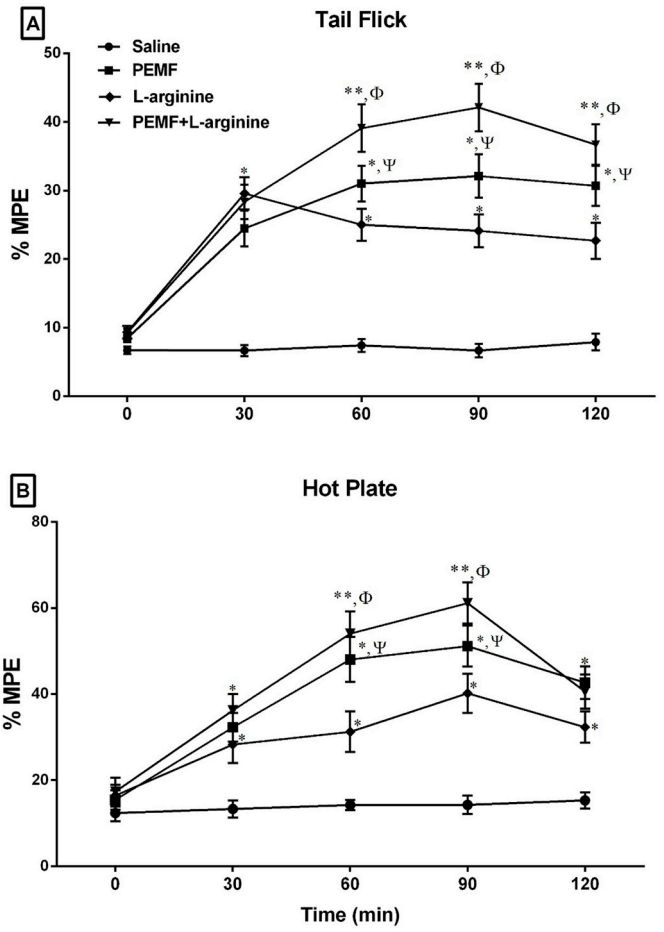

Fig. 5. The effect of L-arginine on PEMF induced analgesia. (A) Shows effect L-arginine on PEMF induced analgesia in the tail –flick, and (B) hot-plate test. Administration of L-arginine to PEMF (5 mT) exposed rats produce a significant increase in % MPE in both TF and HP assays as compared to PEMF group rats ($p<0.05$). The % MPE value of three groups (L-arginine, PEMF and PEMF + L-arginine) was significantly higher than saline ($p<0.01$). Each point represents the mean ± SEM of % MPE for 6 rats. *p < 0.05 and **p < 0.01, when the groups were compared with the Saline group. $^{\Phi}$ p < 0.05, compared with the PEMF; $^{\Psi}$ p < 0.05, compared with the L-arginine.

perception but its mechanism is not yet clear. The present study was carried out to address this. One of the factors in pain perception is the NO pathway. Cury et al. reported that NO had a dual effect on the pain mechanism. NO is a significant physiological mediator which is continuously produced by NOS enzymes from L-arginine [13]. NO relaxes vascular smooth muscles, prevents platelet aggregation, and plays an important role in learning, vision, and inflammatory reactions. In addition, nNO (neuronal NO) participates in the pain formation mechanism throughout the nervous system and acts as a regulator in the neural transmission pathways of pain [33]. Various studies have indicated that electromagnetic field exposure affects NO levels in different tissues. In a study of human cell cultures, extremely low-frequency magnetic fields decreased NO levels by inhibiting the NOS enzyme [34]. A mechanism that explains the analgesic effect of EMF is that they decrease NO levels and induces endogenous opioids [35]. On the contrary, a study conducted on mice found that EMF exposure increased NO formation in the hippocampus [36,37]. Similarly, it was reported that ELF-EMF induced NO synthesis depending on the Ca/CaM (Cam, calmodulin) pathway [38]. Chung et al. reported that EMF increased NO levels in some parts of the brain such as the cortex, striatum, thalamus, and hippocampus. They also demonstrated that while the production of some amino acid neurotransmitters increased, some decreased. The neurotransmitters were glutamate, glutamine, glycine, trozin, and GABA in the thalamus. This means that PEMF may play a role in regulating neurotransmitters in the animal brain. ELF-EMF also induced an increase in NO in seven brain regions [39]. Endogenous NO modulates the release of acetylcholine in the basal frontal brain. One research question has been whether the gaseous precursor affects the release of neurotransmitters as an excitator or as an inhibitor. As a result, serotonin is released in the medial preoptic area and striatum is strengthened by the NO precursor L-arginine and NO donors, respectively [40]. This study demonstrates that PEMF increase analgesia with more NO donor L-arginine in the central nervous system.

5. Conclusion

Our findings show that when the NOS inhibitor L-NAME was administered to rats treated with PEMF, pain perception was similar to rats that did not receive L-NAME. However, pain perception decreased in the PEMF group and even more so when the NO pathway was activated.

In terms of clinical conclusions: (1) it is possible to use lower doses of analgesic drugs in combination with magnetic fields in drug studies that involve the NO pathway; (2) using PEMF and lower doses could help avoid drug's adverse effect. Clearly, the pain mechanism does not

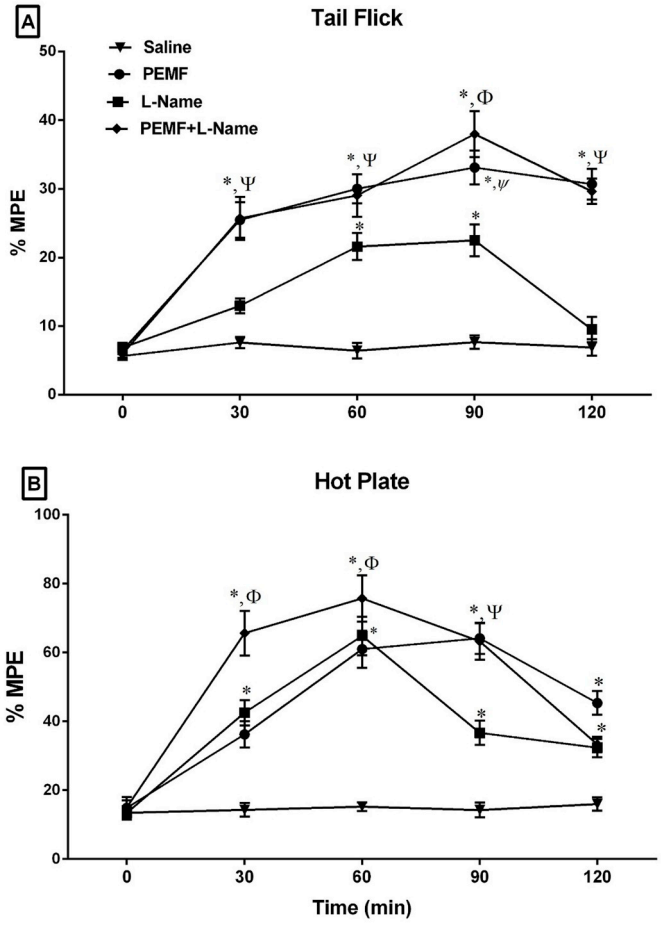

Fig. 6. The effect of L-NAME on PEMF induced analgesia. (A) Shows effect L-NAME on PEMF induced analgesia in the tail –flick, and (B) hot-plate test. Each point represents the mean ± SEM of % MPE for 6 rats. *p < 0.01 when the groups (PEMF, L-NAME and PEMF + L-NAME) were compared with the saline group, Φ p < 0.05, compared with PEMF; Ψ p < 0.05, compared with the L-NAME.

work only through the NO pathway, so further investigations are needed on other pain pathways.

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