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Original Article

Dose-Dependent Interaction Between Caffeine and Morphine in Analgesia in the Hot-Plate in Mice

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Abstract

Background: Combination analgesic therapy is a widely employed strategy for pain management worldwide. This study investigated the potential synergistic interaction between caffeine and morphine using the hot-plate test and isobolographic analysis in male NMRI mice. The interaction between caffeine and morphine in pain modulation was evaluated, and it was determined whether the combination produces a synergistic effect.

Methods: Mice were treated with morphine (0.135, 1.25, 5, and 20 mg/Kg, s.c.) or caffeine (20, 40, 80, and 160 mg/Kg, i.p.) to obtain the ED50 values of each agent in the hot-plate test, then, an isobolographic analysis was used to evaluate the nature of interaction between the two drugs in mice.

Findings: Morphine significantly increased pain thresholds in a dose-dependent manner ($ED_{50}=2.49$ mg/kg; $P<0.001$). Caffeine at a lower dose (46.13 mg/kg) potentiated morphine's analgesic effect, indicating a synergistic interaction ($P<0.001$). In contrast, a higher caffeine dose (138.3 mg/kg) reversed this effect, demonstrating an antagonistic interaction ($P<0.001$) in the mouse hot-plate test.

Conclusion: A low dose of caffeine synergistically enhanced morphine-induced analgesia in the mouse hot-plate test; this effect was absent at a higher dose. Consequently, only low-dose caffeine administration effectively reduced the required dosage of morphine for acute pain management in the mouse hot-plate test.

Keywords: Caffeine, Morphine, Hot-plate test, Isobolographic analysis

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Introduction

Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage, often leading to significant suffering, functional impairment, and reduced quality of life. Effective pain management remains a central objective in medical practice, driving the need to optimize therapeutic strategies through the combination of analgesics and adjuvants.[1] Among opioids, morphine—a derivative of opium—has been used for millennia for its potent analgesic properties. Nonetheless, its clinical utility is constrained by undesirable side effects, including tolerance and physical dependence.[2] Morphine exerts its analgesic effects primarily by agonizing μ-opioid receptors in the central nervous system. This mechanism inhibits the release of pronociceptive neurotransmitters, reduces neuronal excitability, and alters the affective component of pain perception, thereby decreasing pain sensation and increasing pain tolerance.[3,4] Caffeine, although exhibiting only modest intrinsic analgesic activity, has been identified as a valuable adjuvant in pain management. Evidence suggests that caffeine can enhance the efficacy of opioid analgesics like morphine, allowing for dose reduction and consequently mitigating side effects and delaying the development of tolerance.[5-7] Several preclinical studies report additive or synergistic effects between caffeine and morphine. For instance, caffeine has been shown to enhance morphine-induced analgesia and significantly reduce its ED_{50} value.[8,9] Furthermore, caffeine pretreatment potently potentiates morphine analgesia.[10] However, clinical evidence remains somewhat equivocal, with one study in terminal cancer patients reporting indeterminate interaction outcomes[11], highlighting the need for further mechanistic and dose-response investigations. Preclinical studies demonstrate several facilitatory interactions. One investigation indicated that caffeine increased the spontaneous activity of paragigantocellularis neurons, an effect that was significantly greater in morphine-dependent rats.[12] Furthermore, caffeine has been shown to potentiate morphine's analgesic effects, suppress morphine-induced hyperactivity, and inhibit the development of tolerance in rodents.[13] Conversely, a substantial body of evidence points to antagonistic interactions. Several studies report that caffeine can diminish morphine's effects: repeated caffeine administration was found to reduce sensitivity to the ambulation-enhancing effects of morphine,[14] this is supported by the findings that a caffeine-morphine combination significantly reduced morphine analgesia in the hot-plate test.[15] Chronic caffeine administration decreased the potency ratio of morphine in mice.[16] This inhibitory effect is particularly evident at higher doses, where caffeine has been shown to inhibit the antinociceptive properties of morphine.[5]

Research on the caffeine-morphine interaction reveals a complex and often contradictory pharmacological profile, characterized by both synergistic and antagonistic effects that appear to be dependent on dose, administration paradigm, and species.

Building upon previous findings that caffeine modulates the antinociceptive effects of morphine, the present study employed isobolographic analysis to empirically characterize the pharmacological (synergistic, additive, or antagonistic) interaction between these substances using the hot-plate test in mice.

Materials and Methods

Animals

Male NMRI mice, weighing 20.2 to 25.6 grams, were used in this experiment. The animals were maintained in an environment set at 22 ± 2°C, adhering to a 12-hour light/dark cycle, and were allowed free access to food and water ad libitum. All research and animal care procedures were accomplished according to the guidelines on using laboratory animals and approved by the institutional ethical committee for animal research.

Drugs

Morphine and caffeine were purchased from Merck Pharmaceutical Co. Drugs were dissolved in 0.9% saline. Morphine was administered subcutaneously (s.c.) while caffeine was administered intraperitoneally (i.p.).

Hot-plate test

The procedure outlined by Eddy and Leimbach.[17] It was conducted using a device featuring an electrically heated surface enclosed by a 17 cm high and 22 cm diameter Plexiglas tube, which confined the mice to the elevated temperature area (Ugo Basile, Varese, Italy). The surface temperature was maintained at 55.0 ± 0.1°C. Mice were individually placed on the heated surface, and the latency response was measured in seconds until they exhibited jumping as an endpoint. A latency limit (cut-off) of 60 seconds was implemented to prevent harm to the animals.

Experimental Procedure

Male mice were randomly assigned to several experimental groups (n = 7 per group). Treatment groups received subcutaneous (s.c.) injections of morphine (using a fourfold increasing dosage scheme, 0.31, 1.25, 5, or 20 mg/kg), intraperitoneal (i.p.) injections of caffeine (using a twofold increasing dosage scheme, 20, 40, 80, or 160 mg/kg), or corresponding combinations of both drugs in a fixed ratio. Antinociception was assessed using the hot-plate test. Baseline latency was first established for each animal in the control group. To determine the time-course of effect, analgesic responses were measured at 30, 45, and 60 minutes post-injection. All subsequent drug administrations were timed based on the preliminary time-course data.

Statistical Analysis

The maximum possible antinociceptive effect and its standard error were calculated using the established method of Schmauss and Yaksh (1984).[18] ED_{50} values and their corresponding standard errors were derived from least-squares linear regression analysis applied to a semi-logarithmic dose-response relationship.

To characterize the nature of the drug interaction, an isobolographic analysis was performed. Two fixed-ratio combinations were tested:

A low-dose caffeine combination: 95% of morphine's ED_{50} (2.37 mg/kg) combined with 5% of caffeine's ED_{50} (46.13 mg/kg).

A high-dose caffeine combination: 85% of morphine's ED_{50} (2.12 mg/kg) combined with 15% of caffeine's ED_{50} (138.3 mg/kg).

For each combination, semi-logarithmic dose-response curves were generated using 1/2, 1/4, and 1/8 serial dilutions of the initial fixed-ratio dose to determine the experimental combined ED_{50} value (± 95% confidence interval of ED_{50}). This experimentally derived ED_{50} (± 95% confidence interval of ED_{50}) was then compared to the theoretical additive ED_{50} (± 95% confidence interval of ED_{50}) to determine if the interaction was synergistic, additive, or antagonistic. A p-value of less than 0.05 was considered statistically significant for all analyses.

Results

Antinociceptive effect of morphine

Subcutaneous injection of morphine induced a potent analgesic dose-dependently [one-way ANOVA; $F(3, 28) = 54.851$, $P > 0.001$, ED50 = 2.49 mg/Kg and Tukey´s post-hoc = 0.33, < 0.001 and < 0.001 for 1.25, 5, and 20 mg/Kg of morphine, respectively, in comparison to 0.3125 mg/Kg of morphine] (Figure 1).

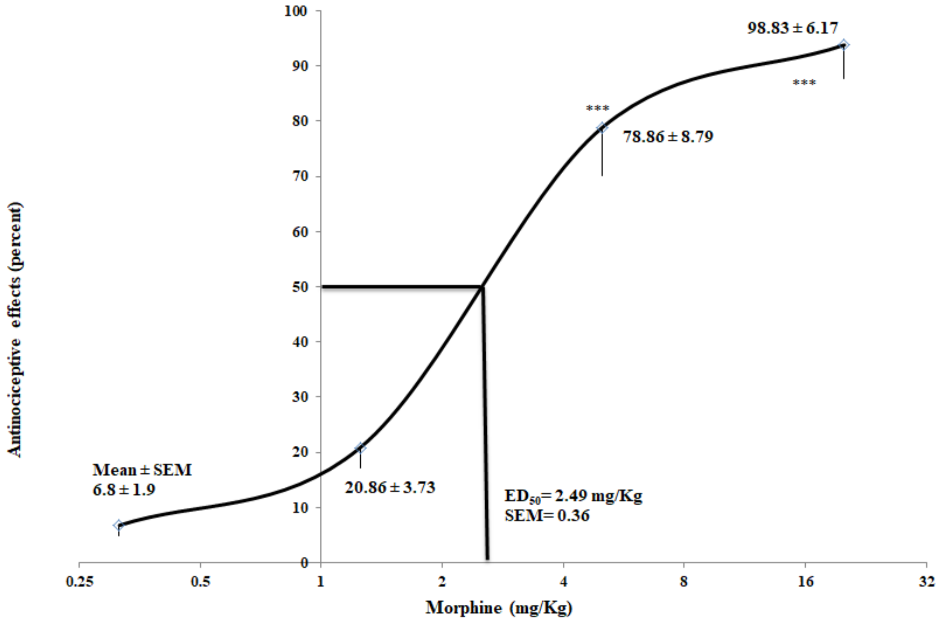

Figure 1. Semi-logarithmic dose response of antinociceptive effect of morphine in the hot-plate test in mice. *** represents P < 0.001, respectively, in comparison with 0.3125 mg/Kg of the morphine group

Antinociceptive effect of caffeine

Intraperitoneal injection of caffeine alone has shown a weak analgesic effect dose-dependently [one-way ANOVA; $F (3, 28) = 8.173$, $P < 0.001$, ED50 = 922.5 mg/Kg and Tukey´s post-hoc = 0.402, 0.01 and < 0.001 for 40, 80, and 160 mg/Kg of caffeine, respectively, in comparison to 20 mg/Kg of caffeine] (Figure 2).

Time-dependent effect of morphine

Sub-cutaneously injection of morphine induced analgesia time-dependently [one-way ANOVA; $F (2, 21) = 12.504$, $P < 0.001$, and Tukey´s post-hoc = 0.007 and < 0.001 for 45 and 60 min after morphine administration in comparison to 30 min, respectively] (Figure 3).

Time-dependent effect of caffeine

Intraperitoneal injection of caffeine induced analgesia time-dependently [one-way ANOVA; $F (2, 21) = 17.457$, $P = 0.034$, and Tukey´s post-hoc < 0.001 and < 0.001 for 45 and 60 min after caffeine administration in comparison to 30 min, respectively] (Figure 4).

Interaction between morphine and caffeine (low-dose)

Combining morphine with a low dose of caffeine (46.13 mg/Kg) significantly enhanced analgesia compared to morphine alone (morphine´s new ED50 ± 95% confidence interval of $ED_{50} = 0.673 \pm 0.031$ mg/Kg, $t = 11.886$, $P < 0.001$), suggesting a synergistic combination effect (Figure 5).

Interaction between morphine and caffeine (high-dose)

Combining morphine with high doses of caffeine (138.3 mg/Kg) significantly reduced analgesia compared to morphine alone (Morphine´s new ED50 ± 95% confidence interval of $ED_{50} = 36.05 \pm 29.92$ mg/Kg, $t = 13.668$, $P < 0.001$), suggesting an antagonistic combination effect (Figure 6).

Discussion

This study investigated the modulatory effects of caffeine on morphine-mediated analgesia, employing the hot-plate test and isobolographic analysis. The results obtained are as follows:

Dose-dependent effect of morphine on pain relief in mice

Our results demonstrate that morphine produced a potent, dose-dependent analgesic effect, significantly increasing latency times on the hot-plate test. This finding is consistent with the well-established antinociceptive properties of morphine. It has been confirmed that administration of morphine induced a dose-dependent antinociceptive effect in the tail-flick test.[19] Moreover, intracerebroventricular (i.c.v.) administration of morphine in mice tail-flick model was investigated, which demonstrated that latencies were prolonged in non-sensitized and morphine-sensitized mice.[20] Furthermore, the magnitude and duration of the analgesic effects of three commonly used opioids [buprenorphine, butorphanol, and morphine (10 mg/Kg for both rats and mice)] were examined, which has been showing that morphine was effective in both the hot-plate and tail flick tests.[21] Morphine (a range of doses from 0.12 to 2.67 mg/Kg) has induced analgesia in the acetic acid writhing test.[22, 23] Consistent with the extensive literature on opioid analgesia, our results further validate the potent antinociceptive efficacy of morphine.

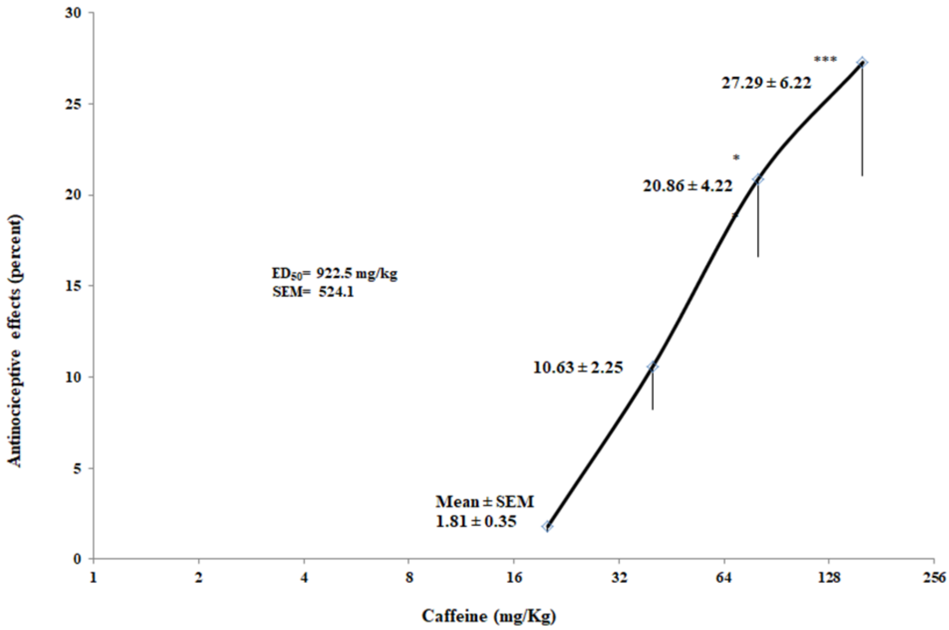

Figure 2. Semi-logarithmic dose response of antinociceptive effect of caffeine in the hot-plate test in mice.
* and *** represent $P<0.05$ and 0.001, respectively, in comparison with the 20 mg/kg caffeine group

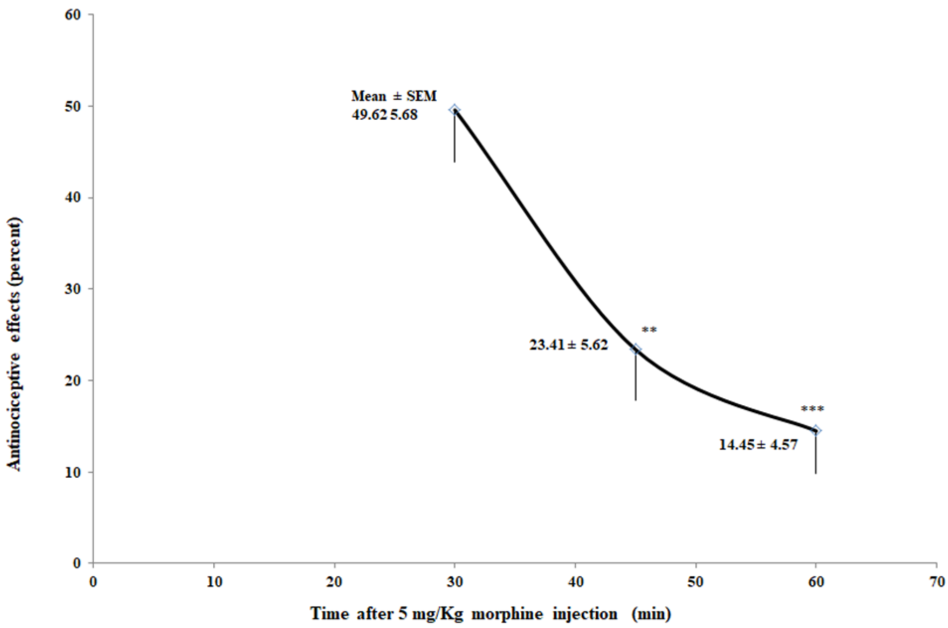

Figure 3. Time course of antinociceptive effect of morphine (5 mg/Kg) in the hot-plate test in mice.
** and *** represent $P<0.01$ and 0.001, respectively, in comparison with the 30 min after morphine injection group

Dose-dependent effect of caffeine on pain relief in mice

Caffeine alone exhibited a weak analgesic effect in the hot-plate test, a finding consistent with prior studies. This activity is likely mediated through its antagonism of adenosine receptors, which are crucial regulators of pain signaling pathways. Previous studies have demonstrated that caffeine elicits antinociceptive effects across various murine models, including the tail-immersion and hot-plate tests.[24] Notably, subcutaneous administration of caffeine (1-5 mg/kg) in mice and intraperitoneal injection (2.5-5 mg/kg) in rats has been shown to elicit significant antinociceptive effects in the hot-plate test.[25] Furthermore, related studies indicate that caffeine administration attenuates neuropathic pain in rat models of nerve injury.[26] Similarly, intraperitoneal administration of caffeine (50-100 mg/kg) produced a dose-dependent reduction in nociceptive responses on the hot-plate test in rats.[27] Finally, it has been proposed that the dose-dependent analgesic effects of caffeine in the hot-plate test are mediated through central adenosine and serotonin systems.[28]

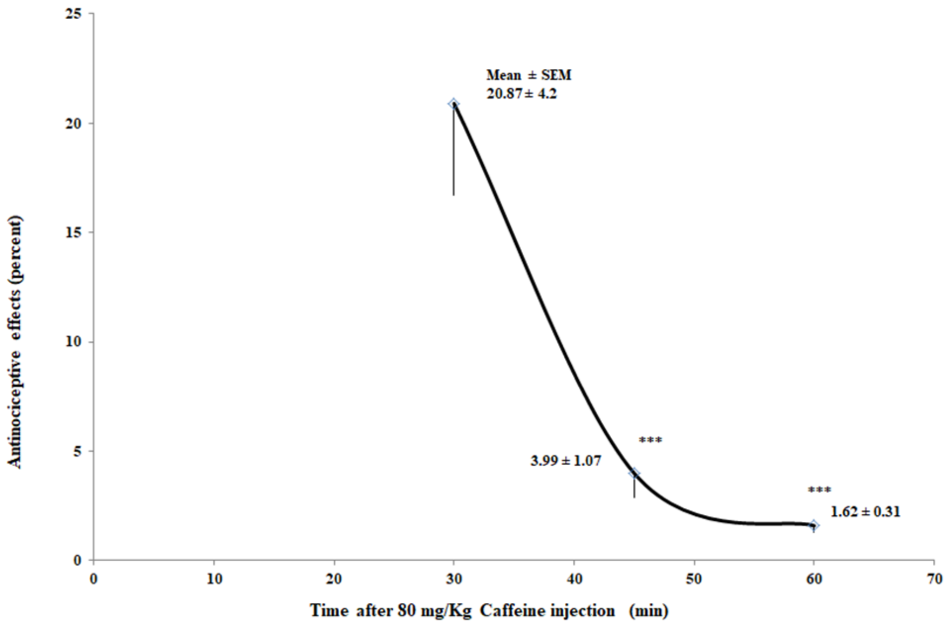

Figure 4. Time course of antinociceptive effect of caffeine (80 mg/Kg) in the hot-plate test in mice. *** represents $P<0.001$, respectively, in comparison with the 30 group after caffeine injection

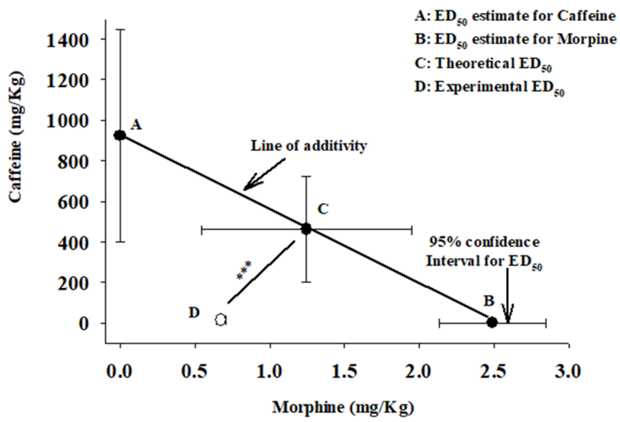

Figure 5. Isoblogram of interaction between morphine and caffeine (46.13 mg/Kg) (Morphine´s new ED50 = 0.673 ± 0.03 mg/Kg, $P<0.001$)

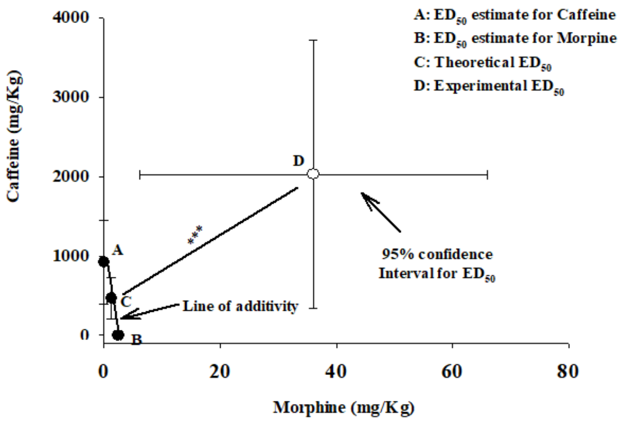

Figure 6. Isoblogram of interaction between morphine and caffeine (138.3 mg/Kg) (Morphine´s new ED50 = 36.05 ± 29.92 mg/Kg, $P<0.001$)

The Time-dependent Analgesic Effects of morphine and caffeine in a murine model

Given the critical importance of timing in analgesic efficacy, the time course of pain relief was assessed at 30, 45, and 60 minutes post-injection. The maximal analgesic effect of morphine was observed 30 minutes post-injection. Consistent with our findings, Fan et al reported a peak analgesic effect approximately 30 minutes after morphine administration in the tail-flick test.[29] Furthermore, intraperitoneal (i.p.) administration of morphine produced a more potent analgesic effect than tramadol (i.p.) at the 30-minute time point in the hot-plate test.[30] Moreover, in a model of morphine tolerance developed using the hot-plate test, the peak analgesic effect was observed 30 minutes post-administration.[31] On the other hand, our finding that intraperitoneal caffeine administration elicited peak analgesic efficacy at 30 minutes is supported by previous work: for instance, a related study demonstrated that a combination of caffeine (5 mg/kg) and nicotine (4 mg/kg) produced a significant antinociceptive effect at the same time point.[32] Besides, supporting the critical window of 30 minutes for caffeine's efficacy, prior research indicates that a combination of indomethacin, caffeine, and prochlorperazine effectively reversed NMDA-induced hyperalgesia when administered 30 minutes before hot-plate testing.[33] Notably, the established timeframe for the peak efficacy of intraperitoneal caffeine (30 minutes post-administration) is consistent with reports that a combination of paracetamol, ibuprofen, and caffeine initiates analgesia within the same window.[34] To assess the impact of acute caffeine administration on acupuncture-induced analgesia, caffeine (administered intraperitoneally) was applied at its time of peak efficacy, which was determined to be 30 minutes post-injection.[35]

Synergistic analgesia induced by low-dose caffeine and morphine combination

Our study demonstrates that the combination of low-dose morphine and caffeine produces a synergistic analgesic effect, where the combined pharmacological outcome exceeds the sum of their individual effects. Consistent with our findings, the concurrent administration of tramadol and caffeine produces a synergistic antinociceptive interaction, suggesting that this combination could permit a reduction in tramadol dosage for managing moderate to moderately severe pain.[36] Furthermore, newer research has demonstrated that co-administration of low-dose caffeine potentiates the analgesic efficacy of nefopam.[37] Also, studies demonstrate that lower doses of caffeine produce a more pronounced synergistic effect when co-administered with morphine (1–10 mg/kg) and diclofenac.[38] In a rat model of pain-induced functional impairment, combinations of paracetamol (316 mg/kg) with caffeine (10-56 mg/kg) produced significantly enhanced analgesia compared to paracetamol alone, whereas caffeine alone exhibited no intrinsic analgesic activity at any tested dose.[39] A distinct study utilizing the formalin test revealed a synergistic antinociceptive interaction between caffeine and metamizole. Metamizole alone produced a dose-dependent effect ($ED_{50} = 329.61$ mg/kg), while caffeine alone was effective at doses ≥ 3.16 mg/kg. Notably, a pronounced synergy was observed when a sub-effective dose of metamizole (100 mg/kg) was combined with caffeine, which significantly enhanced the analgesic response.[40]

The Antagonistic Effect of High-Dose Caffeine on Morphine-Induced Analgesia

Our results demonstrate that high-dose caffeine antagonizes morphine-induced antinociception in the mouse hot-plate test, a finding consistent with previous reports on the inhibitory effects of high-dose caffeine on opioid analgesia. It is reported that high doses of caffeine can inhibit, but low doses of the drug enhance the antinociceptive effect of morphine.[41] Another study demonstrated that chronic caffeine administration (100 mg/kg) in mice significantly decreased the ED_{50} for morphine-induced analgesia.[16] Moreover, studies have demonstrated that caffeine at higher doses inhibited the antinociceptive effects of acetaminophen.[42]

While the present study was limited to an empirical pharmacological assessment of the interaction, future investigations should aim to characterize the associated pharmacokinetic profiles—such as serum concentration and metabolism—and elucidate the exact mechanism of action of this interaction but it seems that the paradoxical effect of caffeine, where low doses enhance while high doses inhibit morphine-induced analgesia, is primarily attributed to its dual and dose-dependent antagonism of adenosine receptors.[43] Adenosine exerts opposing effects on nociception through distinct receptor subtypes. At low doses, caffeine predominantly acts as an antagonist at adenosine A_2A receptors (A_2AR). Under inflammatory conditions or stress, adenosine levels rise and activate G_s-coupled A_2ARs. This activation stimulates adenylate cyclase, increasing cAMP and Protein Kinase A (PKA) activity, which promotes the sensitization of pronociceptive pathways, including TRPV1 potentiation and pro-inflammatory cytokine release.[44, 45] By blocking these A_2ARs, low-dose caffeine suppresses a key pathway of pain sensitization, thereby producing antinociception itself and synergizing with the effects of morphine.[12, 13]

Conversely, high doses of caffeine non-selectively antagonize adenosine A_1 receptors (A_1Rs). A_1R activation, via G_i/o-coupled signaling, mediates potent endogenous antinociception by inhibiting presynaptic voltage-gated Ca^{2+} channels (reducing glutamate release) and opening postsynaptic GIRK potassium channels to hyperpolarize and inhibit second-order neurons in the pain pathway.[45, 46] The antagonism of these inhibitory A_1Rs by high-dose caffeine disrupts a critical endogenous antinociceptive mechanism. This nullifies its beneficial effects at A_2ARs and can directly antagonize morphine's analgesic action, which is partially mediated through the release of adenosine.[47] Furthermore, high-dose caffeine can induce non-specific central nervous system stimulation, including anxiety and restlessness, which may lower pain thresholds and further counteract analgesia.[5]

Conclusion

In the mouse hot-plate test, a low dose of caffeine synergistically enhanced morphine-induced antinociception, whereas a higher dose exerted an antagonistic effect.

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Authors' Contribution
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Data analysis: Mohammad Reza Jafari.
Methodology: Mohaddeseh Ebrahimi-Ghiri, Mohammad Reza Jafari.
Supervision: Mohammad Reza Zarrindast, Mohammad Reza Jafari.
Writing–original draft: Marjan Aminian, Mohammad Reza Jafari.
Writing–review & editing: Mohaddeseh Ebrahimi-Ghiri, Mohammad Reza Jafari.

Competing Interests
There was no conflict of interest.

Ethical Approval
The Vice-Chancellor in Research Affairs, Zanjan University of Medical Sciences (IRB code: IR.ZUMS.AEC.1402.005), approved the study, and the participants filled out the consent form before

conducting the research.

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