Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

A Dual-Mechanism Approach to Opioid Adaptation: Clomipramine Reverses Morphine-Induced Tolerance and Withdrawal

Tahereh Eteraf-Oskouei
Tabriz University of Medical Sciences
Adel Mahmoudi Gharehbaba
Tabriz University of Medical Sciences
Samar Nobavar
Tabriz University of Medical Sciences
Bohloul Habibi Asl
habibiaslbohloul@gmail.com
Tabriz University of Medical Sciences

Research Article

Keywords: Morphine tolerance, Physical dependency, Clomipramine, Opioid withdrawal, Mice

Posted Date: October 1st, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10874510/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Abstract

Background: Chronic morphine use is limited by the development of tolerance and physical dependence, processes influenced by neuroadaptive signaling, oxidative stress, inflammation, and efflux transporters such as P-glycoprotein (P-gp).

Objective: This study investigated the effects of clomipramine, which has been reported to have anti-inflammatory properties and to inhibit P-gp, on morphine-induced tolerance and physical dependence in a mice model.

Methods: Male mice received morphine to induce tolerance and dependence. Clomipramine (12.5, 25, or 50 mg/kg, i.p.) was administered during the final week of morphine treatment, 30 minutes prior to morphine injections. Withdrawal behaviors were assessed following naloxone administration, and tolerance was evaluated using the hot-plate test. Body weight changes were monitored throughout the protocol.

Results: Clomipramine significantly attenuated naloxone-precipitated withdrawal behaviors in a dose-dependent manner. All doses also reversed morphine tolerance, with the highest dose demonstrating the most pronounced effect across multiple time points in the hot-plate assay. Additionally, clomipramine administration partially reversed morphine-induced suppression of body weight gain.

Conclusion: Clomipramine effectively reversed both tolerance and dependence induced by chronic morphine exposure, likely via combined modulation of P-gp function and oxidative/inflammatory pathways. These findings suggest that clomipramine may serve as a potential adjunctive therapeutic strategy to improve opioid analgesia and reduce adverse neuroadaptive effects. Further mechanistic and clinical studies are needed.

1. Introduction

Drug addiction represents a chronic and relapsing neuropsychiatric condition, defined by a compulsive pattern of drug-seeking and use (1, 2). This behavior persists even in the face of significant negative consequences and is accompanied by physiological states of tolerance and dependence (3). The scale of this public health issue is substantial, with millions in the United States affected by substance use disorders, including a growing crisis of addiction to opioids such as heroin and prescription analgesics (4). This has precipitated a sharp rise in overdose fatalities involving substances like fentanyl and oxycodone (5, 6).

Morphine, a potent opioid, is a cornerstone in the clinical management of severe pain, valued for its rapid and effective relief (7). However, its therapeutic utility is critically limited by the development of tolerance, where escalating doses are required to achieve the same analgesic effect, and physical dependence (8, 9). Dependence involves neuronal adaptations that manifest as a characteristic withdrawal syndrome upon drug cessation or administration of an antagonist like naloxone (10, 11). This

syndrome can encompass a range of distressing symptoms, from gastrointestinal distress and autonomic disturbances to anxiety, which severely impair quality of life and can be life-threatening (12). In preclinical research, these states are modeled in animals through observable behaviors such as jumping and tremors, providing a system to study these complex phenomena (13, 14).

Tolerance, a key facet of adaptation to chronic morphine exposure, is not a unitary phenomenon (15). It can be categorized as pharmacokinetic, involving changes in the drug's metabolism; pharmacodynamic, involving cellular and receptor-level adaptations such as receptor desensitization; and learned, which involves behavioral compensation. At a molecular level, pharmacodynamic tolerance often involves complex intracellular signaling pathways, including the upregulation of second messengers like Cyclic adenosine monophosphate(cAMP), leading to altered neuronal excitability (16–18). Learned tolerance describes a reduction in drug effect attributable to acquired compensatory mechanisms (19).

Growing evidence indicates that efflux transporters at the Blood-Brain Barrier (BBB), particularly P-glycoprotein (P-gp), are implicated in opioid tolerance development (7, 20). P-gp (ABCB1), a well-characterized member of the ATP-binding cassette (ABC) transporter superfamily, is recognized for extruding xenobiotics from cells (21–23). While its substrate specificity is broad, typically encompassing lipophilic compounds under 500 molecular weight with hydrogen-bonding capacity, its precise mechanism is not fully defined (24). Critically, chronic opioid exposure can upregulate P-gp expression. This is mediated via toll-like receptor (TLR) activation and inflammatory signaling, which enhances P-gp activity and reduces central opioid efficacy, thereby contributing to tolerance (22).

Animal models employing specific behavioral tests such as tail immersion test (25), tail-flick test and hot-plate test (26) are standard tools for evaluating tolerance and screening potential therapeutic compounds.

The interplay of tolerance and dependence often drives a cycle of escalating use and can precipitate addictive behaviors, particularly during attempts at discontinuation. Consequently, a detailed understanding of the molecular mechanisms that underpin these adaptive processes is imperative. Such knowledge is a prerequisite for the identification of novel therapeutic targets and the development of future pharmacological interventions that can mitigate these detrimental effects, thereby enhancing the clinical safety profile of morphine.

The tricyclic antidepressant (TCAs) clomipramine, a serotonin-noradrenaline reuptake inhibitor, has been consistently demonstrated to possess anti-inflammatory properties at therapeutic concentrations across in vitro, in vivo, and human studies (27). Peripherally, it modulates cytokine release by reducing pro-inflammatory agents (IL-6, IL-1β, TNF-α) and elevating the anti-inflammatory IL-10. Furthermore, research indicates that clomipramine exerts comparable anti-inflammatory effects within the central nervous system (CNS) (27).

Furthermore, clomipramine has been identified as a substrate for the P-gp efflux pump (28, 29). By competing for this transporter, clomipramine may inhibit the P-gp-mediated efflux of other substrates,

such as morphine, from the CNS, thereby potentially increasing morphine's central bioavailability and analgesic efficacy (30).

Based on the documented anti-inflammatory properties of clomipramine and its potential to act as a P-gp substrate, this study aims to investigate the effects of clomipramine on the development of morphine-induced tolerance and physical dependence in a mice model.

2. Materials and Methods

2.1. Materials

All pharmacological agents were obtained commercially. Morphine sulfate, Clomipramine, naloxone and sodium thiopental were sourced from Iranian suppliers (Darou Pakhsh, Amin, Tolid Daro and Exir Pharmaceutical companies, respectively).

2.2. Animals and treatment

Adult male albino mice weighing between 20 and 30 grams were obtained from the Pasteur Institute in Iran. For the experiment, these animals were allocated at random into five distinct study groups. Each group consisted of ten mice to ensure statistical reliability. The mice were maintained under standard laboratory conditions with a temperature of 23 ± 1°C, a 12/12-hour light/dark cycle, and free access to food and water. All procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals (NIH Pub. No. 85 – 23, 1985) and were approved by the ethical review board of Tabriz University of Medical Sciences (IR.TBZMED.AEC.1401.037). During drug administration and all behavioral assessments (withdrawal scoring and hot plate testing), animals remained fully conscious and were restrained manually; no anesthesia was administered, as these procedures did not require it. At the conclusion of the experiment (day 22, following completion of the hot plate test), animals were euthanized by an intraperitoneal overdose of sodium thiopental (200 mg/kg), administered until cessation of respiration and cardiac activity was confirmed, in accordance with the AVMA Guidelines for the Euthanasia of Animals and the approved institutional animal care protocol (IR.TBZMED.AEC.1401.037). No tissue or blood samples were collected following euthanasia.

2.3. Experimental groups and drug regimen

The animals were distributed into five experimental groups (n = 10/group) to investigate the effects of clomipramine on morphine dependence and tolerance.

Group 1 (Saline Control): Received a single daily i.p. injection of normal saline (10 ml/kg) for 14 days. From days 15–21, the regimen was changed to two daily saline injections (10 ml/kg, i.p.) spaced 30 minutes apart.

Group 2 (Morphine Control): Underwent a chronic morphine regimen consisting of a single daily i.p. injection of morphine sulfate (25 mg/kg) for 14 days. From days 15–21, a saline injection was

administered 30 minutes prior to the daily morphine (25 mg/kg) injection.

Groups 3–5 (Clomipramine Treatment): Followed the same chronic morphine regimen as Group 2. From days 15–21, they received daily i.p. injections of clomipramine at doses of 12.5, 25, or 50 mg/kg, respectively, 30 minutes before the morphine injection.

2.4. Assessment protocols

All groups were subjected to standardized behavioral tests:

2.4.1. Withdrawal syndrome:

On day 21, two hours' post-final injection, naloxone (5 mg/kg, i.p.) was administered to precipitate withdrawal. Behavioral signs, including standing and jumping, were recorded for 30 minutes (31). To quantify withdrawal behaviors, individual mice were placed in an open plexiglass chamber (25 × 25 × 40 cm) lined with filter paper, and the frequency of jumps and standing was recorded over 30 minutes. An observer blinded to the treatment allocations performed all behavioral scoring. The behavioral data were scored by an observer who was unaware of the experimental group allocations.

2.4.2. Tolerance development (Hot plate test):

On day 22, a test dose of morphine (9 mg/kg, i.p.) was administered. Analgesic response was evaluated using the hot plate test at 15-minute intervals for 60 minutes (26). Animals were placed on a heated (54 ± 1°C), enclosed stainless steel surface, and the latency to a hind paw lick response was recorded. To preclude tissue damage, a 30-second cut-off time was enforced. Pre-treatment baseline latencies were determined 30 minutes before administering the experimental treatments.

2.5. Statistical analysis

Results are expressed as mean ± SEM. Significance was determined using InStat software, employing unpaired t-tests and one-way ANOVA with Tukey's post-hoc analysis. A p-value of less than 0.05 defined statistical significance.

3. Results

3.1. Morphine-induced dependence

Chronic morphine exposure (25 mg/kg, 21 days) produced significant physical dependence, resulting in a robust withdrawal syndrome upon naloxone challenge (5 mg/kg). Standing and jumping behaviors were significantly elevated in morphine-treated animals relative to the saline control group (**Figure 1**).

Figure 1- Naloxone-precipitated withdrawal behaviors in a mice model of morphine dependence. Quantification of (A) jumping and (B) standing episodes over a 30-minute period following naloxone

administration (5 mg/kg, i.p.). Values are expressed as mean ± SEM (n=10). *p < 0.05 indicates a significant difference from the saline-control group (unpaired t-test). (S: saline, M: morphine.)

3.2. Effects of clomipramine on withdrawal symptoms

Administration of clomipramine reversed morphine withdrawal behaviors. As shown in **Figure 2**, pre-treatment with clomipramine (12.5, 25, or 50 mg/kg, i.p., 30 min prior to morphine) from days 15-21 significantly modulated naloxone-precipitated withdrawal. All tested doses of clomipramine significantly decreased both standing and jumping frequencies.

Figure 2- Clomipramine attenuates morphine withdrawal behaviors in a dose-dependent manner.

Administration of clomipramine (12.5, 25, and 50 mg/kg, i.p.) dose-dependently mitigated the severity of naloxone-precipitated withdrawal symptoms. Data represent the mean ± SEM. Significance was determined by one-way ANOVA; *p < 0.05, **p < 0.01, ***p < 0.001 indicates a significant difference between treatment groups. (S: saline, M: morphine, Clo: clomipramine.)

3.3. Comparative analysis of morphine-induced analgesia in control groups

Chronic morphine exposure induced analgesic tolerance. As illustrated in **Figure 3**, mice in the saline + morphine group subjected to chronic morphine treatment (25 mg/kg for 21 days) developed significant tolerance, evidenced by a markedly attenuated analgesic response to a subsequent morphine challenge dose (9 mg/kg) in the hot plate test at 15, 30, and 45 minutes' post-injection, compared to control groups.

Figure 3- Analgesic efficacy of morphine in tolerant versus nontolerant subjects. Values are expressed as mean ± SEM (n=10). Significance was determined by one-way ANOVA; *p < 0.05, **p < 0.01, ***p < 0.001 indicates a significant difference between groups. (S: saline, M: morphine.)

3.4. Effects of administration of clomipramine on morphine-induced tolerance

Clomipramine pre-treatment altered morphine-induced analgesia. As shown in Figure 4, a 7-day regimen of clomipramine significantly modulated the analgesic response to a subsequent morphine challenge (9 mg/kg, i.p.) in the hot plate test. Specifically, the groups receiving 25 and 50 mg/kg of clomipramine exhibited a response profile significantly different from that of the morphine-only control group across multiple post-injection time points.

Figure 4- Impact of clomipramine pre-treatment on established morphine tolerance. Data represent the mean ± SEM. Significance was determined by one-way ANOVA; *p < 0.05, **p < 0.01, ***p < 0.001 indicates a significant difference between treatment groups. (S: saline, M: morphine, Clo: clomipramine.)

3.5. Morphine-induced alterations in body weight across control groups

As presented in **Table 1**, chronic morphine administration (25 mg/kg/day for 21 days) resulted in a significant attenuation of body weight gain relative to the control (saline + saline) group over the course of the study period.

Table 1-Comparison of body weight progression between morphine-treated and saline-control groups over a 21-day period.

Groups	Weight on day 1(g)	Weight on day 21(g)	Difference in weight between day 1 and day 21(g)
S+S	25.6 ± 1.7	30.1 ± 1.8	4.5 ± 0.1
S+M	25.4 ± 1.6	22.9 ± 1.7	-2.5 ± 0.3***

Data represent the mean ± SEM. Significance was determined by one-way ANOVA; ***p < 0.001 indicates a significant difference between groups. (S: saline, M: morphine.)

3.6. Modulation of body weight by clomipramine compared to a morphine control group

As shown in Table 2, administration of clomipramine (12.5-25-50 mg/kg) from days 15 to 21 significantly altered body weight. Specifically, animals treated with 12.5 and 50 mg/kg of clomipramine exhibited a significant increase in body weight compared to the morphine control group.

Table 2- Changes in body weight following clomipramine treatment (days 15–21) in a morphine-exposed group.

Groups	Weight on day 1(g)	Weight on day 21(g)	Difference in weight between day 1 and day 21(g)
S+M	25.4 ± 1.6	22.9 ± 1.7	-2.5 ± 0.3
Clo(12.5 mg/Kg) + M	26.4 ± 1.2	29 ± 1.5	2.6 ± 0.3***
Clo(25 mg/Kg) + M	27.3 ± 1.8	28.1 ± 1.6	0.8 ± 0.2
Clo(50 mg/Kg) + M	27.8 ± 0.5	30.4 ± 1.1	2.6 ± 0.5***

Data represent the mean ± SEM. Significance was determined by one-way ANOVA; ***p < 0.001 indicates a significant difference between treatment groups. (S: saline, M: morphine, Clo: clomipramine.)

4. Discussion

Opioids are among the primary pharmacological treatments for moderate to severe pain, particularly in patients with advanced cancer who require chronic high-dose opioid therapy (32, 33). Long-term use of opioids such as morphine leads to the development of tolerance and dependence to their analgesic effects (34, 35). Consequently, higher doses of morphine are required to achieve adequate pain relief, which in turn increases the incidence of other adverse effects. Morphine administration also produces numerous side effects, including gastrointestinal disturbances, psychological effects, and the potentially dangerous complication of respiratory depression (36).

Morphine is one of the major alkaloids derived from the opium poppy. It exerts its effects by binding to opioid receptors identified in both the central and peripheral nervous systems (37). The most important of these receptors are the mu, delta, and kappa subtypes (38). Opioid dependence results in the establishment of a new adaptive baseline within the nervous system, triggered by repeated exposure to opioids. Disruption of this adaptive state in the absence of the drug leads to withdrawal, characterized by symptoms such as rhinorrhea, lacrimation, nausea, vomiting, fever, and chills (39). The number and severity of withdrawal symptoms vary depending on the degree of dependence in each individual.

While opioid tolerance arises through multiple pathways (7), the present discussion centers on the mechanism mediated by the P-gp efflux transporter.

The BBB consists of physical and biochemical components. Tight junctions between endothelial cells form the physical barrier, preventing paracellular transport (40, 41). Transcellular transport is regulated by the biochemical component, mediated by efflux transporters. Among these, P-gp is the predominant efflux transporter in the human BBB (20, 42). It uses ATP hydrolysis to move substances against their concentration gradient and thereby transports drugs back into the bloodstream.

Morphine is a substrate of P-gp, and reduced morphine efficacy is directly associated with increased P-gp expression along brain endothelial microvessels (22). As a result, opioids are increasingly transported back into the blood, reducing their penetration into the brain (24).

Recent studies have also demonstrated a strong association between morphine dependence and oxidative stress, including increased production of reactive oxygen species (ROS) and suppression of antioxidant enzymes (43, 44). Even a single dose of morphine has been shown to increase ROS generation and decrease levels of reduced glutathione (GSH) in brain cells (45). Repeated morphine administration depletes GSH, rendering the CNS highly vulnerable to oxidative stress. Chronic morphine use has been reported to increase levels of oxidized glutathione (GSSG), reduce intracellular GSH in the brain, and decrease the activity of catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) (46, 47). It also increases the production of brain catecholamines, amides, and nitric oxide (46, 48). Chronic morphine intake stimulates renal cells to produce inflammatory cytokines and neurotrophic factors such as interleukins, TNF-α, and NO, which facilitate pain transmission and contribute to reduced analgesic efficacy (49, 50).

Inhibition of P-gp markedly alters drug distribution to tissues (51), most notably the brain, in contrast to its effect on plasma concentration levels (52). Morphine is a P-gp substrate in vitro, and P-gp inhibition influences its brain bioavailability and pharmacological effects (24). Studies have shown altered plasma concentrations and clinical responses to oral and intravenous morphine following P-gp inhibition (53).

While the role of P-gp in limiting opioid CNS penetration is well-documented, strategies to modulate it for therapeutic benefit are still being explored. In our previous work, we demonstrated that the antihistamine cetirizine, a putative P-gp inhibitor, effectively reversed established morphine tolerance in mice (26).

The present study aimed to investigate the effects of different doses of clomipramine on dependence and tolerance induced by morphine.

Clomipramine, a TCAs, is used not only to treat depression but also panic disorder (54). According to studies, the severity of morphine dependence can be reduced using intramuscularly injected TCAs (55, 56). Therefore, the anti-inflammatory and antioxidant properties of clomipramine (57), along with its inhibitory effect on P-gp (29, 58), may attenuate tolerance and dependence induced by chronic morphine administration.

In this study, clomipramine was administered to animals at doses of 12.5, 25, or 50 mg/kg. This treatment commenced on day 14 of the morphine injection protocol and continued daily for one week, with each dose given 30 minutes prior to the daily morphine injection. All administered doses resulted in a significant attenuation of withdrawal-related behaviors, including standing and jumping, relative to the control group (Fig. 2). The observed effects demonstrated a dose-dependent relationship, wherein the doses of 25 and 50 mg/kg yielded the most significant reductions, with statistical significance ($p < 0.001$) compared to the 12.5 mg/kg dose.

The results demonstrated that clomipramine reduced the intensity of withdrawal symptoms induced by naloxone in morphine-dependent mice.

Furthermore, a one-week regimen of intraperitoneally administered clomipramine (12.5, 25, or 50 mg/kg) significantly attenuated morphine tolerance, quantified via the hot-plate test on day 22. The 50 mg/kg dose demonstrated the most potent effect, producing significant reductions at the 30, 45, and 60-minute time points (Fig. 4). While still significant ($p < 0.05$), a more modest reduction in tolerance was observed in the 25 mg/kg group.

Mechanistically, the reduction in tolerance and dependence observed in the present study may be attributed to clomipramine's dual actions on both P-gp function and oxidative stress pathways. By inhibiting P-gp at the BBB, clomipramine may increase morphine's central availability, thereby preventing the compensatory upregulation of efflux transporters that contributes to diminished analgesic efficacy during chronic opioid use (29, 59). At the same time, its reported antioxidant and anti-inflammatory effects may mitigate morphine-induced increases in ROS, depletion of GSH, and elevation of neuroinflammatory mediators (54, 57). This combined modulation of pharmacokinetic and neuroinflammatory pathways provides a plausible explanation for the pronounced attenuation of withdrawal behaviors and tolerance observed in the higher-dose groups.

The present study delineates distinct temporal and pharmacological profiles for body weight modulation in a model of morphine exposure. Consistent with established literature on opioid effects, chronic morphine administration alone significantly attenuated normal body weight gain compared to vehicle controls (Table 1), a finding attributed to the well-documented metabolic and anorexigenic effects of prolonged opioid receptor agonism (60). This establishes a baseline state of impaired weight progression. Intriguingly, the introduction of clomipramine during the final week of morphine exposure produced a divergent outcome. As summarized in **Table 2**, late-stage treatment with specific doses of clomipramine (12.5 and 50 mg/kg) not only counteracted but reversed this trend, leading to a significant increase in body weight relative to the morphine-only group. Future investigations into specific mechanisms, such as food intake monitoring, energy expenditure, and neuroendocrine pathways, are warranted to elucidate how clomipramine exerts this protective or restorative effect on body weight in the context of morphine dependence.

5. Limitations and Future Perspectives

The present study is limited by its reliance on an animal model, which may not fully replicate the complexity of human opioid tolerance and dependence. Molecular analyses, such as direct measurement of P-gp expression, oxidative stress markers, or inflammatory mediators, were not performed and therefore the proposed mechanisms remain inferential. Additionally, clomipramine's potential side effects, pharmacokinetic interactions, and long-term safety were not evaluated within the study period. Future research should incorporate biochemical assays to confirm transporter modulation and antioxidant effects, assess dose–response relationships more precisely, and explore whether

clomipramine interacts synergistically or antagonistically with other opioids. Investigating behavioral, metabolic, and neuroendocrine effects in longer-duration models, as well as validating these findings in clinical populations, will be essential for determining translational potential.

6. Conclusion

The present study demonstrates that clomipramine significantly attenuates both morphine-induced tolerance and naloxone-precipitated withdrawal symptoms in mice. These effects were dose-dependent, with the 25 and 50 mg/kg doses exhibiting the greatest behavioral improvements. The results support the hypothesis that clomipramine's dual actions, its potential modulation of P-gp at the BBB and its known antioxidant and anti-inflammatory properties, may contribute to improved central morphine availability and reduced neuroadaptive stress responses.

In addition, clomipramine administration partially restored normal body weight progression in morphine-treated animals, suggesting possible beneficial effects on metabolic or neuroendocrine pathways disrupted by chronic opioid exposure.

Collectively, these findings indicate that clomipramine may represent a promising adjunctive agent for mitigating morphine tolerance and dependence. Further mechanistic studies and clinical evaluation are warranted to determine its translational potential in opioid pharmacotherapy.

Declarations

Ethics approval and consent to participate

The proposal was approved by the ethical committee of the Faculty of Pharmacy at Tabriz University of Medical Science (IR.TBZMED.AEC.1401.037).

Consent for publication

Not applicable

Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare there is no conflict of interest.

Declaration of generative AI and AI-assisted technologies:

The authors used *ChatGPT (GPT-5, OpenAI)* to assist in refining the language and grammar of the manuscript. Following the use of this tool, the author(s) reviewed and edited the text to ensure accuracy and appropriateness. The authors take full responsibility for the content of this publication.

Funding

The current research was funded and granted by Tabriz Medical Sciences University (grant number: 70287).

Authors' contributions

Tahereh Eteraf-Oskouei: Conceptualization, Supervision, review & editing**. Adel Mahmoudi Gharehbaba**: Writing – review & editing. **Samar Nobavar**: Performed the study. **Bohloul Habibi Asl:** Conceptualization, Supervision, review & editing.

Acknowledgements

This work is a part of a Pharm. D thesis, supported by Tabriz University of Medical Sciences, Tabriz, Iran.

References

1. Su L-Y, Liu Q, Jiao L, Yao Y-G. Molecular mechanism of neuroprotective effect of melatonin on morphine addiction and analgesic tolerance: an update. Molecular neurobiology. 2021;58(9):4628-38.
2. Koijam AS, Singh KD, Nameirakpam BS, Haobam R, Rajashekar Y. Drug addiction and treatment: an epigenetic perspective. Biomedicine & Pharmacotherapy. 2024;170:115951.
3. Hyman SE, Malenka RC. Addiction and the brain: the neurobiology of compulsion and its persistence. Nature reviews neuroscience. 2001;2(10):695-703.
4. Kreek M, Reed B, Butelman E. Current status of opioid addiction treatment and related preclinical research. Science advances. 2019;5(10):eaax9140.
5. Spencer MR, Warner M, Bastian BA, Trinidad JP, Hedegaard H. Drug overdose deaths involving fentanyl, 2011-2016. National vital statistics reports: from the Centers for Disease Control and Prevention, National Center for Health Statistics, National Vital Statistics System. 2019;68(3):1-19.
6. Hedegaard H, Bastian BA, Trinidad JP, Spencer M, Warner M. Drugs most frequently involved in drug overdose deaths: United States, 2011–2016. 2018.
7. Badshah I, Anwar M, Murtaza B, Khan MI. Molecular mechanisms of morphine tolerance and dependence; novel insights and future perspectives. Molecular and Cellular Biochemistry. 2024;479(6):1457-85.
8. Bi K, Lei Y, Kong D, Li Y, Fan X, Luo X, et al. Progress in the study of intestinal microbiota involved in morphine tolerance. Heliyon. 2024;10(6).

9. Badshah I, Qazi NG, Anwar M, Shaukat B, Khan MI, Murtaza B. Carveol mitigates the development of the morphine anti-nociceptive tolerance, physical dependence, and conditioned place preference in mice. Heliyon. 2024;10(6).
10. Khan MI, Momeny M, Ostadhadi S, Jahanabadi S, Ejtemaei-Mehr S, Sameem B, et al. Thalidomide attenuates development of morphine dependence in mice by inhibiting PI3K/Akt and nitric oxide signaling pathways. Progress in Neuro-Psychopharmacology and Biological Psychiatry. 2018;82:39-48.
11. Javadi S, Ejtemaeimehr S, Keyvanfar HR, Moghaddas P, Aminian A, Rajabzadeh A, et al. Pioglitazone potentiates development of morphine-dependence in mice: Possible role of NO/cGMP pathway. Brain research. 2013;1510:22-37.
12. Swingler M, Donadoni M, Unterwald EM, Maggirwar SB, Sariyer IK. Molecular and cellular basis of mu-opioid receptor signaling: mechanisms underlying tolerance and dependence development. Frontiers in Neuroscience. 2025;19:1597922.
13. Listos J, Baranowska-Bosiacka I, Talarek S, Listos P, Orzelska J, Fidecka S, et al. The effect of perinatal lead exposure on dopamine receptor D2 expression in morphine dependent rats. Toxicology. 2013;310:73-83.
14. Nicoara D, Zhang Y, Nelson JT, Brewer AL, Maharaj P, DeWald SN, et al. Hyperbaric oxygen treatment suppresses withdrawal signs in morphine-dependent mice. Brain research. 2016;1648:434-7.
15. Zhang X, Jin T, Wang H, Han S, Liang Y. Microglia in morphine tolerance: cellular and molecular mechanisms and therapeutic potential. Frontiers in Pharmacology. 2024;15:1499799.
16. Khan MI, Ostadhadi S, Mumtaz F, Momeny M, Moghaddaskho F, Hassanipour M, et al. Thalidomide attenuates the development and expression of antinociceptive tolerance to μ-opioid agonist morphine through l-arginine-iNOS and nitric oxide pathway. Biomedicine & Pharmacotherapy. 2017;85:493-502.
17. Mitra S, Sinatra RS, Warltier DC. Perioperative management of acute pain in the opioid-dependent patient. Anesthesiology. 2004;101(1):212-27.
18. Hassanipour M, Amini-Khoei H, Shafaroodi H, Shirzadian A, Rahimi N, Imran-Khan M, et al. Atorvastatin attenuates the antinociceptive tolerance of morphine via nitric oxide dependent pathway in male mice. Brain research bulletin. 2016;125:173-80.
19. Huang M, Luo L, Wang W, Xu H, Chen M, Ma X, et al. Targeting Excitatory Glutamate Receptors for Morphine Tolerance: A Narrative Review. CNS Neuroscience & Therapeutics. 2025;31(6):e70468.
20. Tian Y, Lei Y, Wang Y, Lai J, Wang J, Xia F. Mechanism of multidrug resistance to chemotherapy mediated by P-glycoprotein. International Journal of Oncology. 2023;63(5):119.
21. Pote MS, Gacche RN. ATP-binding cassette efflux transporters and MDR in cancer. Drug Discovery Today. 2023;28(5):103537.
22. Gülave B, Lesmana A, de Lange EC, van Hasselt JC. Do P-glycoprotein-mediated drug-drug interactions at the blood-brain barrier impact morphine brain distribution? Journal of Pharmacokinetics and Pharmacodynamics. 2025;52(1):11.

23. Gharehbaba AM, Soltanmohammadi F, Vandghanooni S, Eskandani M, Adibkia K. A comprehensive review on overcoming the multifaceted challenge of cancer multidrug resistance: The emerging role of mesoporous silica nanoparticles. Biomedicine & Pharmacotherapy. 2025;186:118045.
24. Ho H, Zhang E. P-glycoprotein efflux transporter: a key to pharmacokinetic modeling for methadone clearance in fetuses. Frontiers in Pharmacology. 2023;14:1182571.
25. Kotlinska J, Gibula-Bruzda E, Witkowska E, Chung N, Schiller P, Izdebski J. Antinociceptive effects of two deltorphins analogs in the tail-immersion test in rats. Peptides. 2013;39:103-10.
26. Eteraf-Oskouei T, Gharehbaba AM, Asnaashari S, Fazli Z, Asl BH. Reversing Morphine Induced Tolerance: Insights Into Cetirizine and Green Tea Extract Efficacy. Current Therapeutic Research. 2025;102:100783.
27. Baumeister D, Ciufolini S, Mondelli V. Effects of psychotropic drugs on inflammation: consequence or mediator of therapeutic effects in psychiatric treatment? Psychopharmacology. 2016;233(9):1575-89.
28. O'Brien FE, Dinan TG, Griffin BT, Cryan JF. Interactions between antidepressants and P-glycoprotein at the blood–brain barrier: clinical significance of in vitro and in vivo findings. British journal of pharmacology. 2012;165(2):289-312.
29. Schrickx J, Fink-Gremmels J. Inhibition of P-glycoprotein by psychotherapeutic drugs in a canine cell model. Journal of Veterinary Pharmacology and Therapeutics. 2014;37(5):515-7.
30. Ibrahim S, Peggins J, Knapton A, Licht T, Aszalos A. Influence of antipsychotic, antiemetic, and Ca2+ channel blocker drugs on the cellular accumulation of the anticancer drug daunorubicin: P-glycoprotein modulation. The Journal of pharmacology and experimental therapeutics. 2000;295(3):1276-83.
31. Eteraf-Oskouei T, Mahmoudi Gharehbaba A, Majidpour S, Asl BH. Preventive effects of crocin and meloxicam on morphine withdrawal symptoms in mice: behavioral and biochemical insights. BMC Pharmacology and Toxicology. 2025;26(1):159.
32. De Rosa F, Giannatiempo B, Charlier B, Coglianese A, Mensitieri F, Gaudino G, et al. Pharmacological treatments and therapeutic drug monitoring in patients with chronic pain. Pharmaceutics. 2023;15(8):2088.
33. Wang J, Doan LV. Clinical pain management: Current practice and recent innovations in research. Cell Reports Medicine. 2024;5(10).
34. Wang J, Li Z, Tu Y, Gao F. The dopaminergic system in the ventral tegmental area contributes to morphine analgesia and tolerance. Neuroscience. 2023;527:74-83.
35. Lefkof JD, Hill R, Sarantopoulos K. Opioid analgesics, tolerance, dependence and addiction. Anesthesiology In-Training Exam Review: Regional Anesthesia and Chronic Pain: Springer; 2022. p. 367-74.
36. Meissner K, Dahan A, Olofsen E, Göpfert C, Blood J, Wieditz J, et al. Morphine and Hydromorphone Effects, Side Effects and Variability–a Crossover Study in Human Volunteers. Anesthesiology. 2023;139(1):16.

37. Wicks C, Hudlicky T, Rinner U. Morphine alkaloids: History, biology, and synthesis. The Alkaloids: Chemistry and Biology. 2021;86:145-342.
38. Ofoegbu A, B. Ettienne E. Pharmacogenomics and morphine. The Journal of Clinical Pharmacology. 2021;61(9):1149-55.
39. Sheikholeslami MA, Parvardeh S, Ghafghazi S, Sabetkasaei M. Curcumin attenuates morphine dependence by modulating μ-opioid receptors and glial cell-activated neuroinflammation in rat. Neuropeptides. 2023;98:102318.
40. Wu D, Chen Q, Chen X, Han F, Chen Z, Wang Y. The blood–brain barrier: Structure, regulation and drug delivery. Signal transduction and targeted therapy. 2023;8(1):217.
41. Alahmari A. Blood-brain barrier overview: Structural and functional correlation. Neural plasticity. 2021;2021(1):6564585.
42. Juvale IIA, Hamid AAA, Abd Halim KB, Has ATC. P-glycoprotein: new insights into structure, physiological function, regulation and alterations in disease. Heliyon. 2022;8(6).
43. Zeng X-S, Geng W-S, Wang Z-Q, Jia J-J. Morphine addiction and oxidative stress: The potential effects of thioredoxin-1. Frontiers in pharmacology. 2020;11:82.
44. Pysiewicz A, Mazur A, Kotlińska J, Baranowska-Bosiacka I, Fronc K, Łupina M, et al. The Influence of Topiramate on Morphine Dependence in Mice. Biomolecules. 2025;15(5):730.
45. Hejnova L, Hronova A, Drastichova Z, Novotny J. Long-term administration of morphine specifically alters the level of protein expression in different brain regions and affects the redox state. Open Life Sciences. 2024;19(1):20220858.
46. Listos J, Łupina M, Talarek S, Mazur A, Orzelska-Górka J, Kotlińska J. The mechanisms involved in morphine addiction: an overview. International journal of molecular sciences. 2019;20(17):4302.
47. Salehi I, Zarrinkalam E, Mirzaei F, ABBASI OE, Ranjbar K, SOLEIMANI AS. Effects of resistance, endurance, and concurrent exercise on oxidative stress markers and the histological changes of intestine after morphine withdrawal in rats. Avicenna Journal of Medical Biochemistry. 2018;6(2):44-9.
48. Hamdy MM, Elbadr MM, Barakat A. Bupropion attenuates morphine tolerance and dependence: Possible role of glutamate, norepinephrine, inflammation, and oxidative stress. Pharmacological Reports. 2018;70(5):955-62.
49. Xie F, Kitagawa Y, Ogata H, Yasuhara S, You Z, Martyn JJ. Morphine induces inflammatory responses via both TLR4 and cGAS-STING signaling pathways. Cytokine. 2024;183:156737.
50. Malik JA, Agrewala JN. Morphine acts via TLR4 resulting in neuroinflammation and immunosuppression. Medical Hypotheses. 2024;186:111335.
51. Gharehbaba AM, Omidi Y, Barar J, Eskandani M, Adibkia K. Synergistic pH-responsive MUC-1 aptamer-conjugated Ag/MSN Janus nanoparticles for targeted chemotherapy, photothermal therapy, and gene therapy in breast cancer. Biomaterials Advances. 2025;166:214081.

52. Shah D, Ajazuddin, Bhattacharya S. Role of natural P-gp inhibitor in the effective delivery for chemotherapeutic agents. Journal of cancer research and clinical oncology. 2023;149(1):367-91.
53. Nawa A, Fujita-Hamabe W, Kishioka S, Tokuyama S. Decreased expression of intestinal P-glycoprotein increases the analgesic effects of oral morphine in a streptozotocin-induced diabetic mouse model. Drug Metabolism and Pharmacokinetics. 2011;26(6):584-91.
54. Nobile B, Durand M, Olié E, Guillaume S, Molès J-P, Haffen E, et al. The anti-inflammatory effect of the tricyclic antidepressant clomipramine and its high penetration in the brain might be useful to prevent the psychiatric consequences of SARS-CoV-2 infection. Frontiers in Pharmacology. 2021;12:615695.
55. Liu K-S, Chen S-J, Chen Y-W, Sung K, Wang J-J. A dose–response study on the efficacy of tricyclic antidepressants on reducing morphine-withdrawal symptoms. Acta Anaesthesiologica Taiwanica. 2013;51(1):18-21.
56. Habibi-Asl B, Vaez H, Najafi M, Bidaghi A, Ghanbarzadeh S. Attenuation of morphine-induced dependence and tolerance by ceftriaxone and amitriptyline in mice. Acta Anaesthesiologica Taiwanica. 2014;52(4):163-8.
57. Kostadinov I, Delev D, Petrova A, Stanimirova I, Draganova K, Kostadinova I, et al. Study on anti-inflammatory and immunomodulatory effects of clomipramine in carrageenan-and lipopolysaccharide-induced rat models of inflammation. Biotechnology & Biotechnological Equipment. 2014;28(3):552-8.
58. Schäfer J, Klösgen VJ, Omer EA, Kadioglu O, Mbaveng AT, Kuete V, et al. In silico and in vitro identification of P-glycoprotein inhibitors from a library of 375 phytochemicals. International Journal of Molecular Sciences. 2023;24(12):10240.
59. Pariante CM, Hye A, Williamson R, Makoff A, Lovestone S, Kerwin RW. The antidepressant clomipramine regulates cortisol intracellular concentrations and glucocorticoid receptor expression in fibroblasts and rat primary neurones. Neuropsychopharmacology. 2003;28(9):1553-61.
60. Le T, Xia M, Jia M, Sarkar N, Chen J, Li H, et al. Association between initial morphine intake and body weight change, acoustic startle reflex and drug seeking in rats. Psychopharmacology. 2014;231(23):4569-77.

Figures

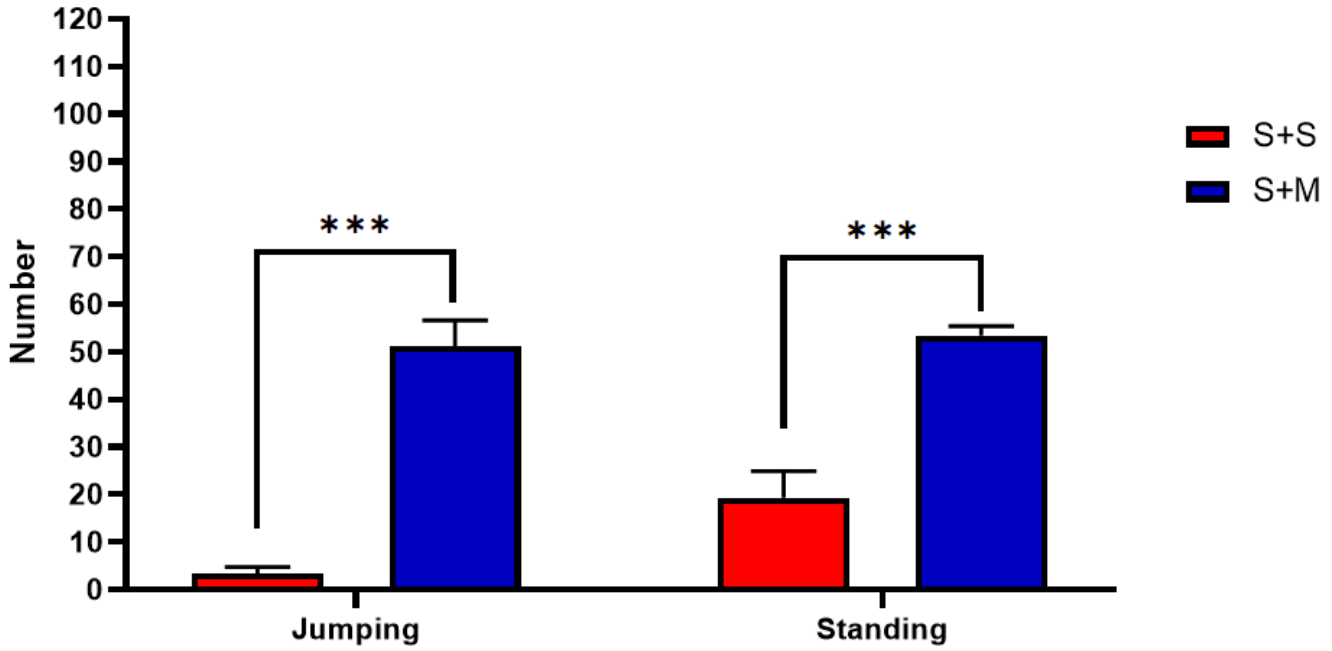

Figure 1

Naloxone-precipitated withdrawal behaviors in a mice model of morphine dependence. Quantification of (A) jumping and (B) standing episodes over a 30-minute period following naloxone administration (5 mg/kg, i.p.). Values are expressed as mean ± SEM (n=10). *p < 0.05 indicates a significant difference from the saline-control group (unpaired t-test). (S: saline, M: morphine.)

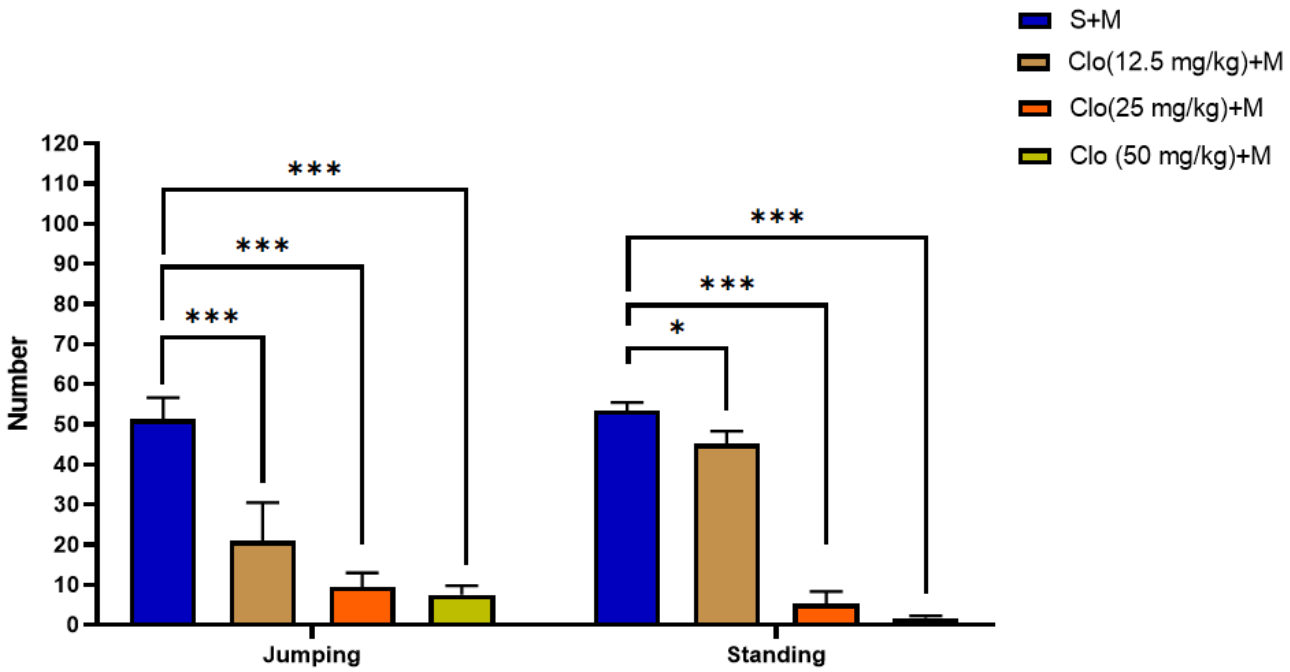

Figure 2

Clomipramine attenuates morphine withdrawal behaviors in a dose-dependent manner.

Administration of clomipramine (12.5, 25, and 50 mg/kg, i.p.) dose-dependently mitigated the severity of naloxone-precipitated withdrawal symptoms. Data represent the mean ± SEM. Significance was determined by one-way ANOVA; *p < 0.05, **p < 0.01, ***p < 0.001 indicates a significant difference between treatment groups. (S: saline, M: morphine, Clo: clomipramine.)

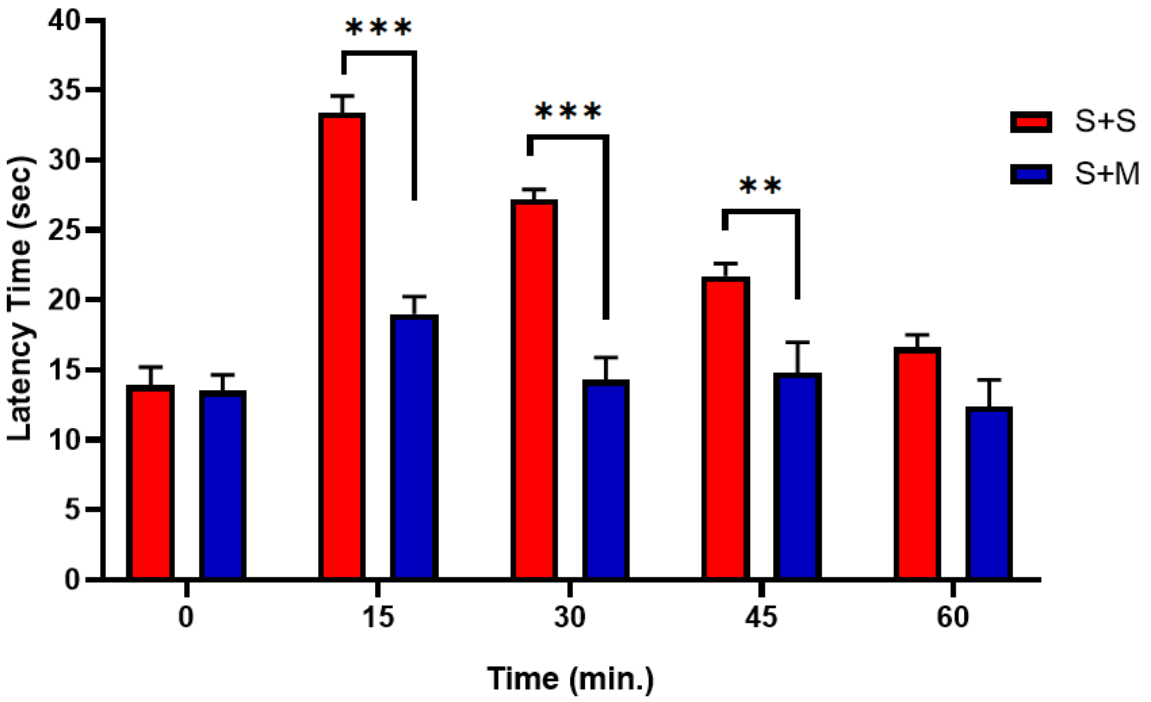

Figure 3

Analgesic efficacy of morphine in tolerant versus nontolerant subjects. Values are expressed as mean ± SEM (n=10). Significance was determined by one-way ANOVA; *p < 0.05, **p < 0.01, ***p < 0.001 indicates a significant difference between groups. (S: saline, M: morphine.)

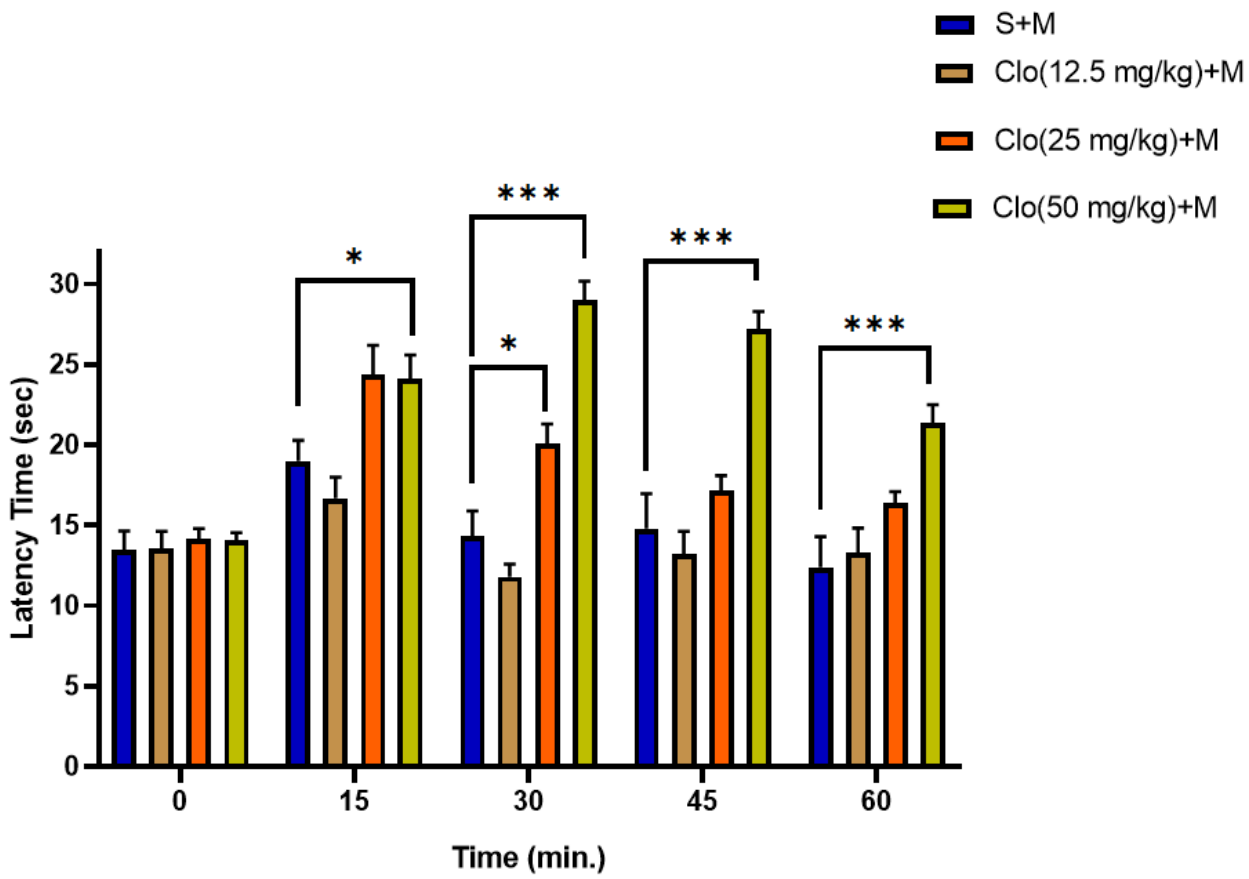

Figure 4

Impact of clomipramine pre-treatment on established morphine tolerance. Data represent the mean ± SEM. Significance was determined by one-way ANOVA; *p < 0.05, **p < 0.01, ***p < 0.001 indicates a significant difference between treatment groups. (S: saline, M: morphine, Clo: clomipramine.)