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Original Article

Effects of Isoniazid on Expression and Acquisition of Tolerance to High-Dose Morphine-Induced Hyperlocomotion in Male Mice

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Abstract

Isoniazid, a hydrazine derivative, can influence the GABAergic system, which plays an important role in modulating morphine tolerance. This study aimed to assess how isoniazid effects on both the expression and acquisition of tolerance to morphine-induced hyperlocomotion in mice. Nineteen groups of male mice (n=8 per group) were used. The locomotor activity of the animals was measured for a duration of 20 minutes using an actimeter after administration of morphine (1-30 mg/kg, s.c.) or isoniazid (25-75 mg/kg, i.p.). Tolerance was induced by administering morphine (30 mg/kg) twice daily for three consecutive days. The same dose on the fourth day served as a challenge to assess tolerance development. To investigate how isoniazid affects tolerance expression and acquisition, mice were divided into eight groups. Four groups received saline or isoniazid one hour before the final morphine dose (expression test). The remaining four groups were administered isoniazid prior to each morphine injection during the tolerance induction phase (acquisition test). Morphine produced two distinct effects on locomotor activity: at a low dose (1 mg/kg, s.c.) it reduced locomotion in mice ($P<0.05$), while at a high dose (30 mg/kg, s.c.) it increased locomotor activity ($P<0.01$). Isoniazid administration one hour before the test significantly increased locomotor activity. Isoniazid administration before morphine suppressed both the expression ($P<0.01$) and the acquisition ($P<0.01$) of morphine-induced hyperlocomotion. However, isoniazid's effect on tolerance expression may be due to its ability to increase locomotor activity. Isoniazid could be a promising candidate for attenuating morphine tolerance development.

Keywords: Morphine, Adverse Effects, Drug Tolerance, Isoniazid, Pharmacology, Locomotion, Mice.

Introduction

Morphine is widely recognized in clinical practice as a major opioid prescribed for managing pain ranging from moderate to severe [1]. The use of opioids in medicine has consistently been a contentious topic due to their side effects, which limit their applicability [2-4]. A major drawback of prolonged morphine use is the development of tolerance [5]. This adaptive response may appear in different aspects of morphine action, such as pain relief, locomotor stimulation, or its rewarding properties [6]. Tolerance reflects a neurobiological adjustment that diminishes the drug's efficacy after repeated exposure [5]. When tolerance to morphine develops, increasing the drug dose is necessary to compensate for reduced effectiveness. Dose escalation underlies other adverse effects, some of which may threaten users' lives [7]. Experimental findings indicate that while elevated doses of morphine increase locomotor activity in animals [8], repeated dosing eventually results in tolerance to this effect [9].

Isoniazid, a long-established antimicrobial agent, exhibits bactericidal effects against Mycobacterium and remains a cornerstone in both the treatment and prevention of tuberculosis [10]. Due to its hydrophilic nature, isoniazid can easily penetrate the blood–brain barrier and influence certain neurotransmitter pathways, particularly the GABAergic and catecholaminergic systems [11,12]. Recent molecular studies have shown that genetic variations can influence isoniazid metabolism and its pharmacological effects. For example, mutations in the NAT2, katG, and rpoB genes have been linked to changes in drug structure, blood concentration, and antimicrobial activity [13,14]. Such findings suggest that molecular factors may also contribute to isoniazid's neuromodulatory effects on the central nervous system. Due to its weak inhibition of monoamine oxidase [15], isoniazid has negligible effects on catecholamines. However, several studies have specifically demonstrated that isoniazid modulates the GABAergic system in the brain. High doses of isoniazid may induce convulsions by decreasing GABAergic tone in the brain [16], an effect exploited in screening antiepileptic drugs. In contrast, some studies have suggested that low doses of isoniazid may elevate GABA [17].

The GABAergic system plays a critical role in morphine's central nervous system effects, including reward, dependence, reverse tolerance, and tolerance [18,19]. As noted above, isoniazid can modulate GABAergic neurotransmission and may, in turn, affect various neurobehavioral processes. However, despite extensive research on morphine tolerance and its underlying mechanisms, no previous studies have specifically examined whether modulation of the GABAergic system by isoniazid can influence the development or expression of morphine-induced locomotor tolerance. Therefore, the present study was designed to explore this potential interaction and to clarify the role of isoniazid in morphine tolerance–related behavioral responses, focusing on both the development and expression phases of tolerance.

Materials and Methods

Animals

For this study, male albino NMRI mice weighing 23–28 g was sourced from the Pasteur Institute (Tehran, Iran). After being moved to the University of Maragheh animal facility, the animals were kept in a colony room with controlled standard conditions: 12/12 light-dark cycle, temperature 22±2 °C, and humidity about 45%. The animals were provided with ad libitum access to commercial food pellets and tap water, except when locomotor activity experiments were conducted. To mitigate the stress associated with shipping, the animals underwent a one-week adaptation period before the onset of behavioral tests. All experimental protocols, including housing conditions, handling procedures, and drug administration schedules, were reviewed and approved by the Institutional Ethics Committee at the University of Maragheh (ethics code: IR.UM.1402.008). Throughout the animals' maintenance and experiments, ethical guidelines for working with laboratory animals were strictly followed. All possible steps were implemented to limit any discomfort, pain, and stress at all stages of the study. To ensure unbiased data collection and avoid potential experimenter bias, all laboratory tests were performed under blinded conditions. Specifically, the experimenters responsible for behavioral observations and data recording were unaware of the treatment groups and the specific objectives of the study. Group assignments were randomized, and all data were coded to maintain blinding until statistical analysis was completed. In total, 19 experimental groups were included across all experiments. To prevent potential carryover

effects between experiments, each experiment was conducted on a distinct cohort of animals, with 8 male mice per group, ensuring that prior exposure to drugs or behavioral testing did not influence subsequent results.

Drugs

The study used morphine sulfate (10 mg/mL; Darou Pakhsh, Tehran, Iran) and isoniazid in white powdered form (SolarBio, Beijing, China). Predetermined doses of isoniazid (25-75 mg/kg) and morphine (1-30 mg/kg) were prepared by dissolving the calculated amounts or volumes of the drugs in saline according to the study protocol. Morphine was administered subcutaneously (s.c.), while isoniazid was administered intraperitoneally (i.p.) to the animals.

Tolerance Induction

Tolerance to morphine-induced hyperlocomotion was established following the protocol described by Sahraei *et al.* [9]. In this procedure, mice were rendered tolerant to morphine with six high doses over three consecutive days; each day consisted of two morphine injections at 9:00 a.m. and 3:00 p.m. On the test day, each animal was administered a test dose of morphine (30 mg/kg, s.c.) to assess tolerance induction.

Locomotion Measurement Device

The custom-made actimeter used in the present research consisted of an electronic part with infrared sensors for counting the animals' locomotor activity and a wooden box (32 × 32 × 50 cm, H). While the inner walls of the box were colored white, the floor was black. On each of the two opposite walls, three infrared sensors (transmitters or receivers) were installed two centimeters above the floor, spaced eight centimeters apart. Altogether, the invisible infrared beams divided the floor of the device into 16 squares (8 × 8 cm). When mice interrupted any of the beams, the actimeter counted this as one locomotor activity. After each test session, the floor and walls of the box were cleaned with ethanol (70%) to avoid the influence of cues related to body odor, urination, and defecation on the results of the next test. The experiments were performed under low light conditions. The locomotor activity of each mouse was recorded over a 20-minute period. Before turning on the device, each mouse was allowed to acclimate to the apparatus for one minute before the test began.

Design of Experiments

Dose-Response Assessment of Morphine Effects on Locomotion

Five randomly selected groups of mice received either control solution, saline 10 mL/kg, s.c., or morphine at doses of 1, 3, 10, and 30 mg/kg. The locomotor activity of each mouse, one minute after the injection of morphine or saline (adaptation time), was measured with the actimeter for 20 minutes.

Evaluation of Dose-Response Effects of Isoniazid on Locomotor Activity

The locomotor activity of four randomly selected groups of mice, one hour after the injection of saline (10 mL/kg, i.p.) or isoniazid (25-75 mg/kg, i.p.), was measured using the actimeter for 20 minutes.

Effects of Isoniazid on the Expression of Tolerance to Morphine-Induced Hyperactivity

Mice were allocated at random into five distinct groups: two control groups and three experimental groups. One group, the positive control, received only a single dose of morphine on the test day. After inducing tolerance with a high dose of morphine (30 mg/kg, s.c.), the remaining four groups received either saline (negative control) or isoniazid (25, 50, or 75 mg/kg, i.p.) one hour prior to the final morphine dose (Figure 1).

Impact of Isoniazid on the Development of Tolerance to Morphine-Stimulated Locomotor Activity

The animals were randomly allocated into five groups: two served as controls and three as experimental groups. As the positive control, one group received a single challenge dose of morphine (30 mg/kg, s.c.) on the day of testing. During the process of tolerance development, the other four groups were given saline (negative control) or different doses of isoniazid (25, 50, or 75 mg/kg, i.p.) one hour prior to each morphine injection (30 mg/kg, s.c.). On the fourth day, all mice were administered a single challenge dose of morphine (30 mg/kg, s.c.) (Figure 1).

Statistical Analysis

Locomotor activity data were processed and analyzed using SPSS software, version 16. After confirming that the data were normally distributed and the variances were homogeneous among the groups, a one-way ANOVA was performed to identify any significant differences. Partial η^2 values were obtained directly from the SPSS output (General Linear Model procedure), where they are calculated automatically based on the ratio of the sum of squares for the effect to the sum of squares for the effect plus error. If a significant difference was found, a post hoc Tukey test was conducted. Following the analysis, the results were expressed as mean ± SEM. Differences in average locomotor activity among the groups were considered statistically significant at $p < 0.05$.

Results

Morphine-Induced Changes in Locomotor Activity of Mice

Administration of saline or morphine (1, 3, 10, and 30 mg/kg, s.c.) produced a significant change in locomotor activity in mice, as shown by a one-way ANOVA: $F(4,35) = 14.07$, $p<0.001$, and Partial $\eta^2 = 0.627$. According to Tukey's post hoc analysis, morphine elicited a biphasic locomotor response: a low dose (1 mg/kg, s.c.) suppressed activity, whereas a high dose (30 mg/kg, s.c.) increased activity compared with saline control group (Figure 2). Based on these findings, the 30 mg/kg dose was selected for tolerance induction experiments.

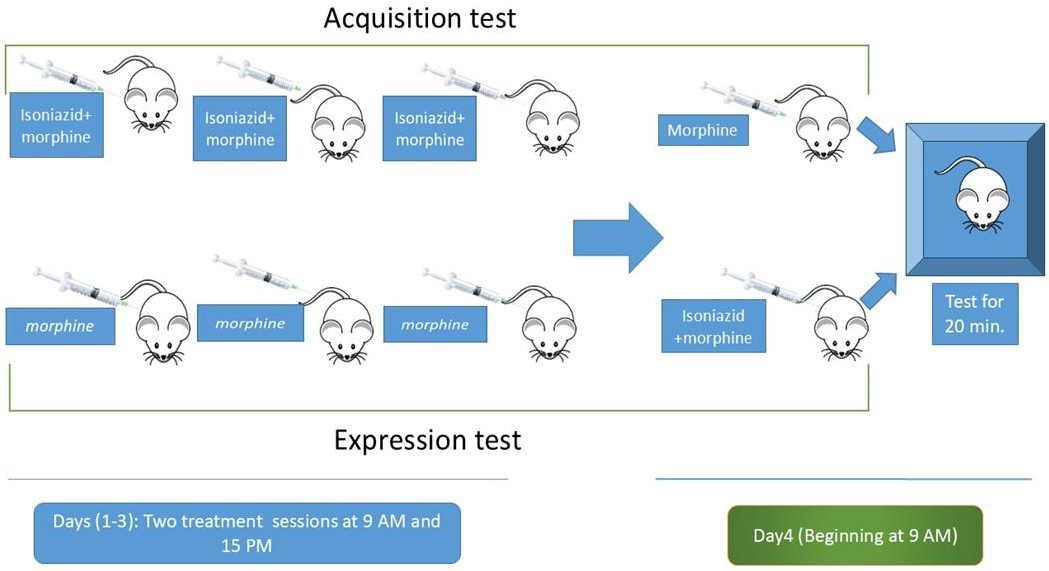

Figure 1 Schematic representation of the steps involved in conducting expression and acquisition tests in mic

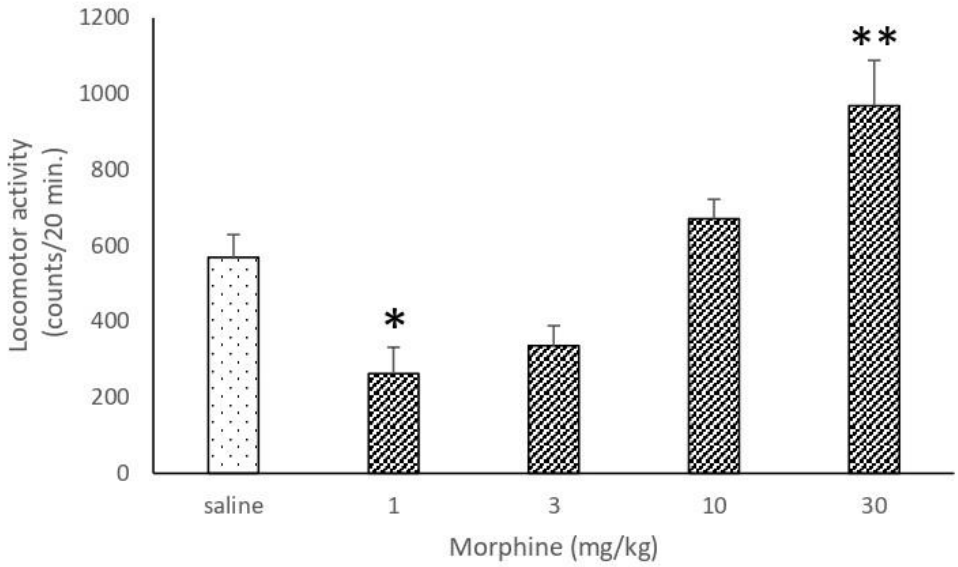

Figure 2 Morphine-induced alterations in locomotor activity in mice: Animals were given either saline (10 mL/kg) or morphine (1–30 mg/kg, s.c.), and their locomotor activity was measured over a 20-minute period. * and ** denote P-values of less than 0.05 and 0.01, respectively, relative to the saline control group

Impact of Isoniazid on Locomotor Activity in Mice

One hour before testing, mice were treated with either isoniazid (25–75 mg/kg, i.p.) or saline. ANOVA indicated that locomotor activity was significantly affected [$F(3,28) = 5.43$, $p < 0.01$, partial $\eta^2 = 0.368$]. Tukey's post hoc test revealed that the 50 and 75 mg/kg doses of isoniazid significantly increased locomotor activity compared with saline (Figure 3).

Influence of Isoniazid on the Expression of Tolerance to Morphine's Locomotor-Activating Effects

Administration of isoniazid (25–75 mg/kg, i.p.) one hour prior to the final morphine dose on the test day significantly influenced locomotor activity [$F(4,35) = 6.28$, $p = 0.001$, partial $\eta^2 = 0.418$]. Post hoc analysis revealed that only the 50 and 75 mg/kg doses produced a significant increase in locomotor activity compared with control (Figure 4).

Impact of Isoniazid on the Acquisition of Tolerance to the Locomotor-Activating Effects of Morphine

During tolerance induction with high-dose morphine (30 mg/kg, s.c.), co-administration of isoniazid (25–75 mg/kg, i.p.) significantly changed locomotor activity on the test day $F(4,35) = 7.49$, $p<0.001$, and Partial $\eta^2 = 0.461$. Tukey's test revealed that 50 and 75 mg/kg doses of isoniazid significantly inhibited the acquisition of tolerance (Figure 5).

Discussion

This study investigated the influence of isoniazid on the development and expression of tolerance to morphine-triggered hyperactivity in male mice. The main finding was that tolerance to morphine-induced hyperlocomotion was affected by isoniazid. First, the dose–response curve of morphine revealed that the drug had distinct effects on locomotor activity depending on the dose. Specifically, in the present experiment, a low dose decreased locomotor activity, while a high dose produced hyperlocomotion.

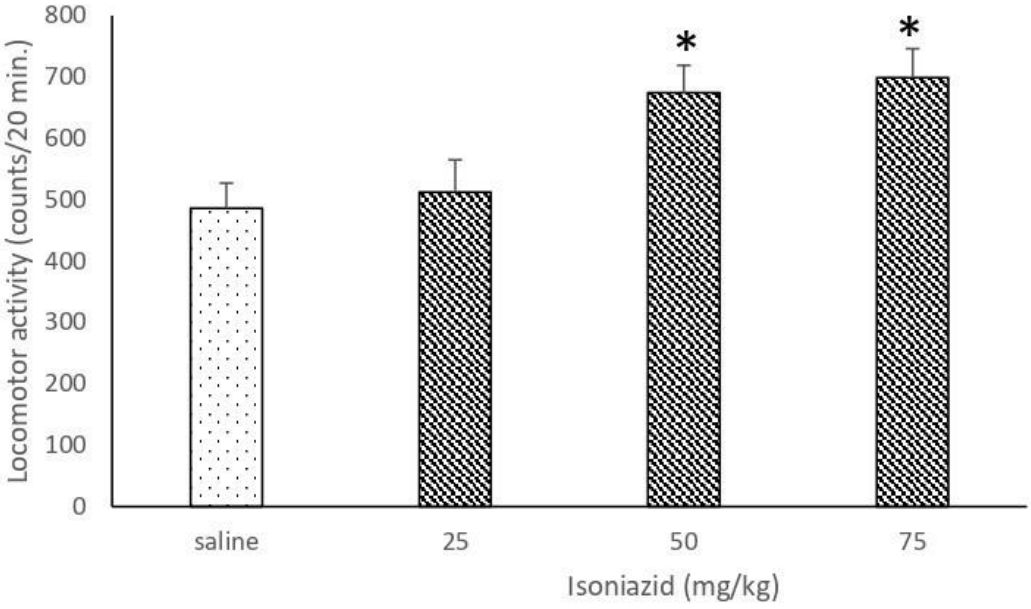

Figure 3 Isoniazid-induced changes in locomotor activity in mice: Animals were given either saline or isoniazid (25–75 mg/kg, i.p.), and the apparatus recorded their locomotor activity for 20 minutes, starting one hour after administration. According to the graph, acute administration of isoniazid increased the locomotor activity at doses of 50 and 75 mg/kg, significantly. * Indicates P<0.05 compared with the saline group

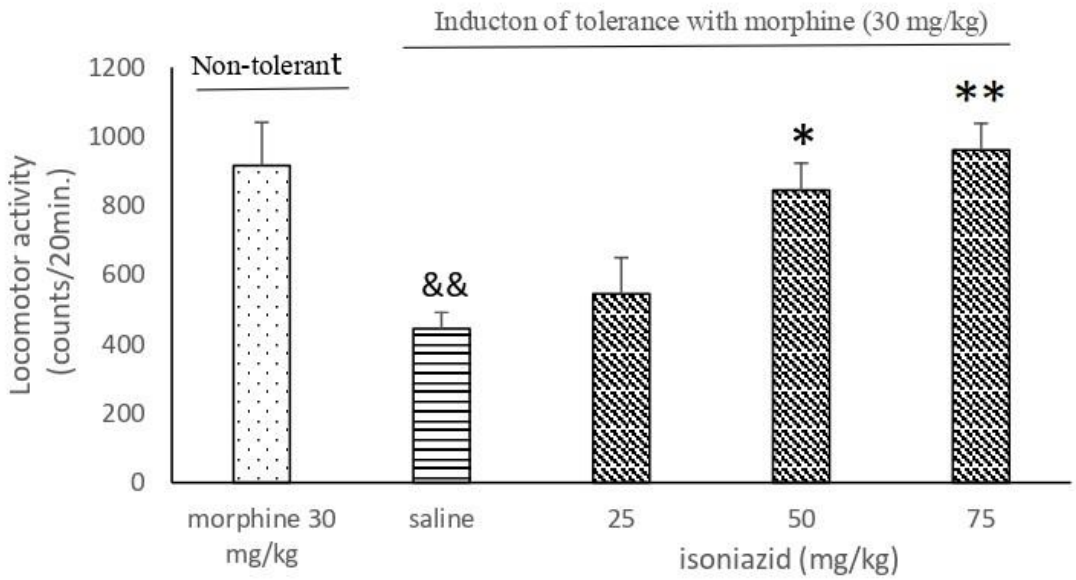

Figure 4 Effect of isoniazid on the expression of morphine tolerance in hyperlocomotion: Tolerance induced in four groups of mice. On the day of testing, the animals were given saline or isoniazid (75–25 mg/kg, i.p.) one hour before the final morphine injection. A positive control group was given a single high dose of morphine (30 mg/kg, s.c.) prior to behavioral assessment. The symbol && indicates a P-value of less than 0. 01 compared to the positive control group. The symbols * and ** indicate P-values below 0.05 and 0.01, respectively, compared with saline-treated animals in the morphine-tolerant groups

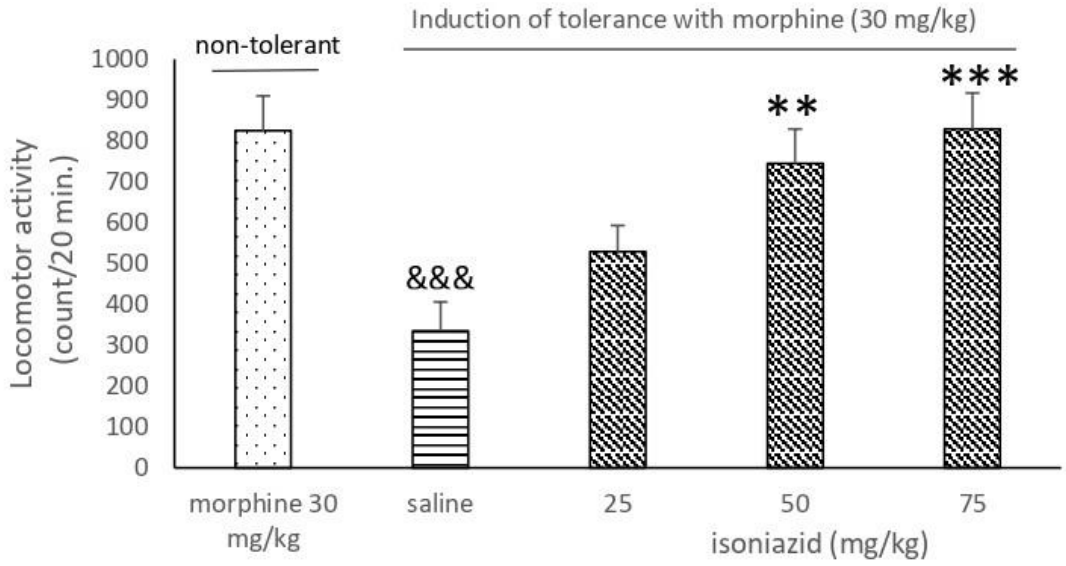

Figure 5 Role of isoniazid in the acquisition of tolerance to morphine-triggered hyperlocomotion: In the course of tolerance induction in four groups of mice, the animals were administered isoniazid (25-75 mg/kg, i.p.) or saline prior to morphine. During test day, one positive control group received only a single high dose of morphine (30 mg/kg, s.c.). The symbols && denote a significance level of $P < 0.01$ compared to the positive control group. The symbols ** and *** represent significance levels of $P < 0.01$ and $P< 0.001$ compared with the saline in morphine-tolerant groups, respectively

The Partial Eta Squared value of 0.627 indicated that approximately 62.7% of the variance in locomotor activity could be explained by the treatment factor (saline vs. different morphine doses). According to Cohen's criteria, this represents a large effect size, suggesting that morphine dosage strongly influenced locomotor behavior in mice. Such bidirectional effects of morphine on locomotion are well documented [8]. However, previous research has also shown that the same dose of morphine may produce divergent effects—ranging from increased locomotion to decreased activity or no significant effect at all—depending on factors such as experimental design, measurement techniques, and instrumentation [20,21]. These inconsistencies may, at least in part, be explained by the involvement of different neurotransmitter systems in modulating morphine's effects on locomotor activity [22-24].

Subsequently, the effects of isoniazid on locomotor activity was examined in mice. This is the first study to investigate the acute impact of isoniazid on animal locomotion. The dose–response curve revealed that isoniazid administration significantly altered locomotor activity, producing an overall increase. The Partial Eta Squared value of 0.368 indicated that approximately 36.8% of the variance in locomotor activity was explained by the treatment factor (isoniazid vs. saline). According to Cohen's criteria, this corresponds to a medium-to-large effect size, suggesting that isoniazid exerted a meaningful influence on locomotor behavior. Notably, these results contrast with the previous work, where isoniazid did not significantly alter locomotor activity in a CPP experiment [25]. The discrepancy is likely due to methodological differences, as locomotor activity in the earlier study was measured 24 hours post-injection, whereas in the present study assessments were conducted one hour after isoniazid administration. The doses of isoniazid used in this study (25–75 mg/kg, i.p.) correspond to approximately 2–6 mg/kg in

humans when converted by body surface area normalization (Nair & Jacob, 2016). This range is comparable to the clinically used doses of isoniazid (≈ 5 mg/kg/day), suggesting that the effects observed here may have translational relevance to human therapeutic conditions [26].

The results further showed that chronic morphine administration led to tolerance to morphine-induced hyperactivity. Tolerance was demonstrated by a significant reduction in locomotor activity in animals administered a high dose of morphine (30 mg/kg), which had previously shown increased locomotion in the dose–response curve. These results support the validity of the actimeter and the tolerance induction protocol. Overall, the findings are consistent with previous studies reporting tolerance to the locomotor-stimulating effects of morphine [9].

Various mechanisms have been suggested to account for the development of tolerance, including changes in neurotransmitter systems, receptor sensitivity, and intracellular signaling pathways [5,6,27]. Additionally, recent studies highlight the roles of oxidative stress, inflammation, gut microbiota, and epigenetic modifications in tolerance development [5,28]. Finally, evidence suggests that multiple brain regions may contribute to this process [29,30].

The findings of our study indicated that administering isoniazid before the final dose of morphine significantly attenuated the expression of morphine tolerance (partial eta squared, $\eta^2 = 0.418$). This medium-to-large effect size suggests that a substantial proportion of the variance in locomotor activity was explained by isoniazid treatment. However, interpretation should consider that isoniazid itself can increase locomotor activity in mice, meaning that the observed effect might partly result from its direct stimulatory action rather than from the inhibition of tolerance. Most previous studies have focused on the mechanisms underlying the acquisition of morphine tolerance, with research on the expression of tolerance being limited. Among the few available studies, topiramate—which affects both GABAergic and glutamatergic systems—was shown to inhibit the expression of morphine tolerance to its analgesic effects in mice or may have a biphasic effect on it [31,32]. Overall, investigations into tolerance expression may help identify drugs capable of reversing this phenomenon once it has developed. Lastly, the results showed that administering isoniazid prior to morphine during tolerance induction reduced the development of tolerance to morphine-induced locomotor activity (partial eta squared, $\eta^2 = 0.461$). This indicates that approximately 46.1% of the variance in locomotor activity was attributable to isoniazid treatment. The effect is unlikely to result from a direct locomotor-stimulating action of isoniazid due to its short half-life [33]. These results indicate that isoniazid modulates the development of morphine tolerance, extending its previously reported effects on tolerance to morphine's rewarding properties [34] to also encompass the acquisition of tolerance in locomotor activity. Drugs may effect on tolerance differently to different effects of morphine. For instance, Bhargava showed that administration of melanotropin release inhibiting factor could inhibit tolerance to morphine analgesia but not its locomotor stimulating effects [32, 35]. In contrast, the recent findings indicate that isoniazid can inhibit tolerance not only to morphine's rewarding effects [34], but also to its locomotor-stimulating effects**.** These findings suggest that isoniazid likely interacts with multiple effects of morphine, indicating a broad modulatory role on morphine-mediated pathways. The results also raise the possibility that similar neural mechanisms may underlie the development of tolerance to both the rewarding and locomotor effects of morphine. Consistent with these results, other GABAergic agents have been shown to inhibit the

acquisition of morphine tolerance, indicating the involvement of different mechanisms. For example, topiramate, an anti-epileptic drug with positive GABAergic activity, attenuated the acquisition of tolerance to morphine's analgesic effects in mice [31]. Similarly, administration of both GABA-A and GABA-B receptor agonists prevented the acquisition of tolerance to morphine analgesia in Wistar rats [36]. Moreover, a study by Alavian and Ghiasvand highlighted the role of GABA-B receptors in the acquisition of tolerance to morphine's rewarding effects in rats [30]. The exact mechanism by which isoniazid inhibits morphine tolerance remains unclear. As noted earlier, multiple mechanisms are implicated in morphine tolerance, reflecting its complexity. While some studies have linked morphine-induced hyperlocomotion to dopaminergic activation in the mesolimbic pathway [22], others suggest that mesolimbic dopamine release is not universally associated with morphine-induced locomotion [34,37]. Moreover, non-dopaminergic processes may also significantly contribute to morphine's effects on locomotor activity [38-40]. Therefore, the underlying mechanisms of morphine-induced locomotion remain controversial. In this context, isoniazid, by enhancing GABAergic tone, may interact with both dopaminergic and non-dopaminergic pathways involved in morphine-induced locomotor activity. Thus, during tolerance induction, isoniazid may attenuate the development of morphine tolerance by offsetting morphine's effects on these systems. Additionally, recent research highlights the contribution of neuroinflammation to the induction of morphine tolerance [5]. Given that isoniazid has also been reported to exert anti-inflammatory effects [41], its ability to reduce neuroinflammation may represent another pathway through which the drug inhibits morphine tolerance. The present study has some limitations that should be acknowledged. First, only male mice were used, while previous evidence suggests that the mechanisms of tolerance induction may differ between males and females [42]. Thus, the potential influence of sex differences on the effects of isoniazid remains unclear. Second, isoniazid was administered systemically, making it difficult to determine the specific brain regions involved in its modulatory effects on morphine tolerance. Third, although isoniazid is predominantly administered orally in clinical practice, in this study it was delivered intraperitoneally. Considering the suggested role of gut microbiota in morphine tolerance [5], oral administration might have produced different outcomes through potential drug–microbiota interactions. Overall, caution is warranted in drawing conclusions, given the potential constraints inherent in the study design, and future studies are needed to address them. Based on the present findings and considering the relevance of morphine tolerance in pain management, future studies should investigate the role of isoniazid in modulating morphine analgesic tolerance. Given the shared neurobiological pathways underlying addiction to different substances, isoniazid may also influence mechanisms involved in dependence on other addictive drugs. Further experimental and clinical research is warranted to examine these hypotheses.

Conclusion

The present study demonstrates that isoniazid inhibits the development of morphine-induced locomotor tolerance in mice. Considering that isoniazid also increases baseline locomotor activity, its apparent effect on the inhibition of tolerance expression is likely due to this stimulatory action on locomotor activity rather than a true suppression of tolerance. Taken together with previous evidence that isoniazid reduces morphine reward and dependence, these findings suggest that the compound modulates multiple aspects of morphine's

addictive profile. Given isoniazid low cost and ease of administration, further preclinical and clinical studies are warranted to evaluate its potential for repurposing in addiction therapy.

Conflict of interest

The authors declare no conflict of interest.

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Authors' Contributions

Amir Abbas Barzegari designed the study, performed the data analysis, interpreted the results, and wrote the manuscript. Maryam Azaddar and Kamran Shahabi performed the laboratory experiments on the animals.

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