

Original Article



Anti-Addictive Effects of *Astragalus Fasciculifolius* Extract in a Mouse Stress-Induced Morphine Dependent Model

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Abstract

Introduction: Morphine is a potent analgesic with a high potential for addiction. Emerging evidence suggests that natural products may offer therapeutic benefits in addiction management. Therefore, this study aimed to evaluate the effects of *Astragalus fasciculifolius* extract on morphine-seeking behavior in a mouse model subjected to acute stress. The study also investigated how *A. fasciculifolius* influences morphine-induced conditioned place preference (CPP) and related biochemical changes in stressed mice.

Methods: Forty-two male NMRI mice were randomly assigned to seven groups: control, morphine, morphine + saline, morphine + stress, and *A. fasciculifolius*-treated groups receiving 150, 450, or 800 mg/kg doses. After completing five days of CPP behavioral assessments, the mice were anesthetized via the intraperitoneal administration of ketamine and xylazine. Then, blood samples were collected via cardiac puncture, and hippocampal tissue was isolated. Finally, malondialdehyde (MDA) levels, total antioxidant capacity (TAC), and messenger RNA expression of brain-derived neurotrophic factor (BDNF) and tumor necrosis factor-alpha in the hippocampus were quantified.

Results: Morphine administration significantly increased CPP scores compared to controls ($P < 0.001$). Acute stress further amplified morphine preference in stressed mice relative to non-stressed morphine-treated mice. Moreover, *A. fasciculifolius* at 800 mg/kg markedly attenuated morphine-seeking behavior in stressed mice and substantially enhanced TAC while reducing MDA levels in both hippocampus and serum ($P < 0.001$).

Conclusion: Our findings confirmed the exacerbating effect of stress on morphine-seeking behavior, suggesting that *A. fasciculifolius* may offer protective benefits by modulating oxidative stress and neuroinflammatory markers.

Keywords: Morphine, BDNF, Tumor necrosis factor-alpha, Oxidative stress

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Introduction

Drug addiction is a long-term condition that is marked by persistent drug-seeking behavior despite negative outcomes, frequently resulting in physical and psychological dependence. Several factors increase the risk of addiction, including genetic predisposition, adverse socioeconomic conditions, mental health disorders, and opioid exposure through medical prescriptions. It is noteworthy that the mortality rate due to opioid overdose has risen, particularly from synthetic opioids and polysubstance use, making opioid addiction a critical public health crisis (1). Its prevalence varies across countries, with Afghanistan, Iran, and Russia among those with the highest opioid consumption rates. Treatment options encompass medications such as methadone and buprenorphine, harm reduction programs, and psychosocial interventions, although access to these remains a challenge (2). The social stigma surrounding addiction frequently prevents individuals from seeking help, thereby exacerbating the crisis and increasing the burden on healthcare systems. Future strategies involve improving treatment accessibility, reducing stigma, and

developing innovative therapeutic approaches to combat opioid addiction effectively (3).

Astragalus fasciculifolius is a species within the *Fabaceae* family (4). This plant is native to southern Iran, specifically in Kerman, Sistan and Baluchestan, Fars, and Hormozgan provinces, as well as the Arabian Peninsula. In addition, *A. fasciculifolius* is a tall shrub with short hairs and long thorns, featuring multiple branched stems that end in long clusters. Further, it thrives in desert and arid scrubland ecosystems. Recent studies have highlighted its antioxidant and antibacterial properties, particularly in its gum extract. The methanolic gum extract of *A. fasciculifolius* has demonstrated high levels of phenolics and flavonoids, contributing to its medicinal potential (5). Furthermore, this plant exhibits antimicrobial activities and is effective against *Clostridium perfringens* and *Pseudomonas aeruginosa*. Due to its analgesic and anti-inflammatory effects, along with its bioactive compounds, *A. fasciculifolius* has also potential applications in the pharmaceutical, food, and healthcare industries. In particular, the genus *Astragalus* contains over 140 identified chemical compounds,

including cycloartane triterpene glycosides, phenolic compounds, flavonoids, and various polysaccharides. This species is recognized in global botanical databases, including the “Plants of the World Online” by Kew Science (6).

Brain-derived neurotrophic factor (BDNF) plays a complex role in morphine addiction. This factor enhances neural plasticity and reinforces drug-seeking behavior. Suppression of BDNF in the ventral tegmental area increases dopamine neuron excitability, thus intensifying morphine reward. Conversely, BDNF activation in the nucleus accumbens counteracts morphine-induced neural adaptations and reduces dependence. Histone modifications around the *BDNF* gene promoters influence withdrawal responses. In addition, increased BDNF expression during abstinence may facilitate recovery. These findings underscore BDNF’s dual role in addiction and withdrawal (7, 8).

Tumor necrosis factor- α (TNF- α) significantly contributes to morphine addiction by promoting neuroinflammatory processes. This factor enhances glial activation, leading to increased release of pro-inflammatory cytokines that contribute to morphine tolerance. Likewise, TNF- α interacts with nuclear factor- κ B and interleukin-1 beta, impacting pain sensitivity and opioid efficacy. Additionally, targeted inhibition of TNF- α may provide therapeutic potential for addiction treatment. These data emphasize the dual role of TNF- α in addiction development and withdrawal (9).

Herbal medicines offer natural alternatives for managing opioid addiction, thereby reducing reliance on synthetic drugs (10). Some medicinal plants (e.g., kratom and ibogaine) have shown potential in alleviating withdrawal symptoms and cravings. Similarly, these plants may help restore neurochemical balance and support recovery without severe side effects. Unlike conventional opioids, certain herbal drugs have lower addiction risks and can be integrated into comprehensive treatment approaches. Nonetheless, further research is required to ensure their safety and effectiveness in addiction therapy (11).

Species of *Astragalus* are recognized for their neuroprotective and anti-inflammatory properties, which may help mitigate opioid-induced neuronal damage. Research suggests that *Astragalus* extracts can modulate neurotransmitter systems and potentially aid in addiction recovery (12). Considering the aforementioned materials, the present study has been designed to support communities affected by drug addiction, particularly opium dependence, by reducing the harmful effects associated with chemical treatments through the use of medicinal plants. This study primarily aims to investigate the effects of this herbal substance on the process of addiction cessation, following a careful evaluation of its potential side effects in human populations. Ultimately, this research seeks to pave the way for future human studies conducted in full compliance with ethical standards and regulatory requirements.

Materials and Methods

Plant Preparation and Extraction

A 500-g sample of *A. fasciculifolius* gum was purchased from a herbal store and verified by a botanist at Shahrekord University of Medical Sciences. The plant extract was then prepared using the maceration method (13). Next, the dried plant was finely ground in an electric grinder, and the resulting powder was dissolved in 450 mL of ethanol and an equal volume of water. Moreover, the mixture was filtered and dried at 50°C to obtain a hydroalcoholic extract. Eventually, the prepared extract was dissolved in a specific volume of distilled water in order to achieve the required dose for injection (6).

Experimental Animals

This study used 42 male NMRI mice, weighing 25–30 g and aged 8–9 weeks. The mice were maintained under controlled environmental conditions ($23 \pm 1^\circ\text{C}$, 12-hour light/dark cycle) with ad libitum access to food and water.

Animal Grouping and Experimental Interventions

The mice were randomly allocated to seven groups (A-G), with six animals per group.

1. **Control Group:** This group received subcutaneous (SC) injections of normal saline for 3 days during conditioning.
2. **Morphine Group:** It was administered SC morphine injections for three consecutive days during the conditioning phase.
3. **Morphine + Saline Group:** This group was administered SC morphine, along with intraperitoneal (IP) saline injections, for three days during the conditioning phase.
4. **Morphine + Stress Group:** It received SC morphine and 30 minutes of acute restraint stress for 3 days during conditioning.
5. **Morphine + *Astragalus fasciculifolius* (150 mg/kg) + Stress Group:** This group received intraperitoneal injections of *A. fasciculifolius* extract (150 mg/kg), followed by 30 minutes of acute restraint stress. After five minutes, the mice received SC morphine and were then placed in the conditioning chamber.
6. **Morphine + *Astragalus fasciculifolius* (450 mg/kg) + Stress Group:** This group received the same protocol as the previous group but with a higher dose of *A. fasciculifolius* extract (450 mg/kg).
7. **Morphine + *Astragalus fasciculifolius* (800 mg/kg) + Stress Group:** The same protocol was applied to this group, utilizing the maximum dose of *A. fasciculifolius* extract (800 mg/kg).

The impact of a single morphine dose (5 mg/kg) (14) and varying concentrations of *A. fasciculifolius* extract (150 mg/kg, 450 mg/kg, and 800 mg/kg) (6) on the induction of conditioned place preference (CPP) was evaluated through a dose-response curve. Following the experiment, serum malondialdehyde (MDA) levels, total antioxidant capacity (TAC), and hippocampal expression of *BDNF* and *TNF- α* genes underwent analysis (Figure 1).

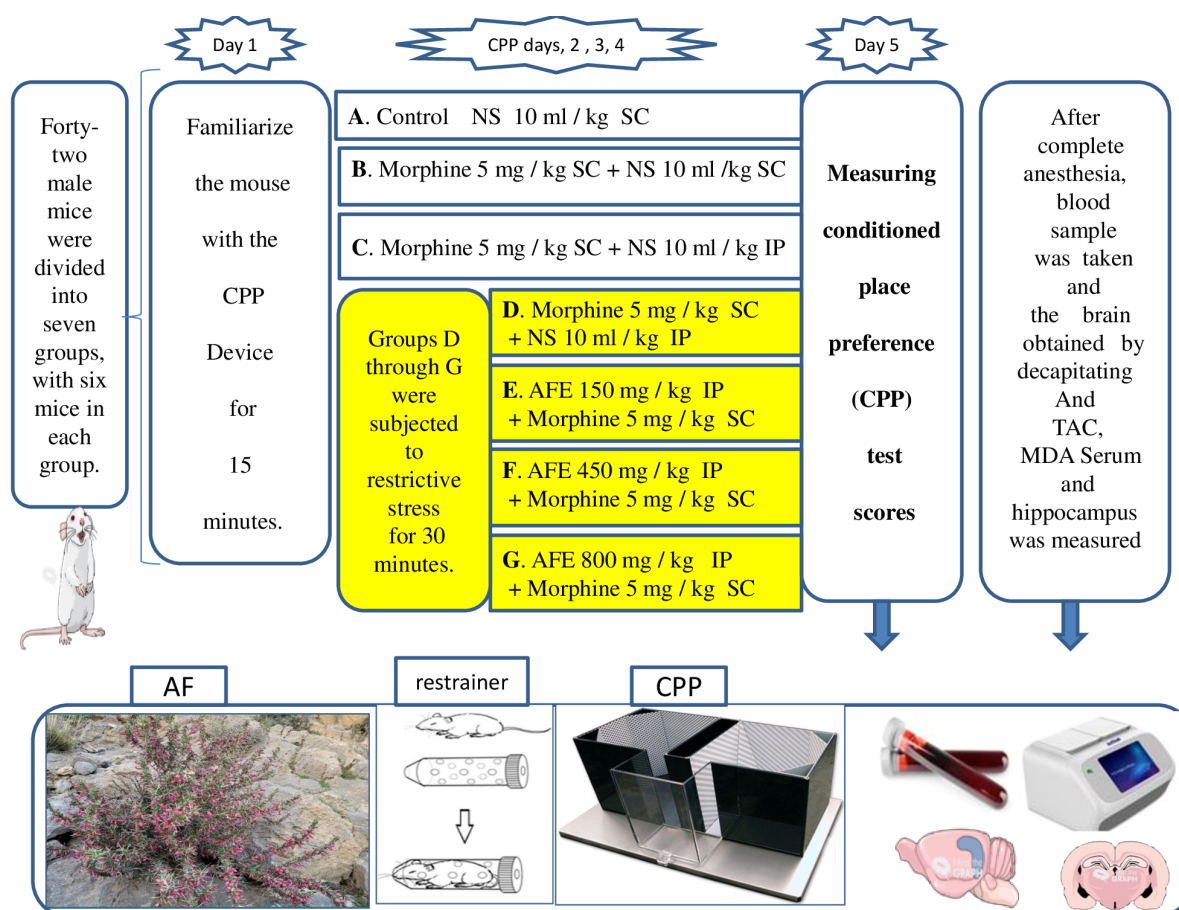


Figure 1. Study Schematic Design

Note. NS: Normal saline; AF: *Astragalus fasciculifolius*; AFE: *Astragalus fasciculifolius* extract; CPP: Conditioned place preference; SC: Subcutaneous; mL/kg: Milliliters per kilogram; mg/kg: Milligram per kilogram; IP: Intraperitoneal; TAC: Total antioxidant capacity, MDA: Malondialdehyde

Locomotor Activity Assessment

During the 15-minute habituation period and on the fifth day of testing, the mice's locomotor activity was recorded using a camera. Then, the number of times each mouse exited compartments A, B, and C or crossed the midline was documented and compared across groups. Ultimately, mice exhibiting fewer than 10 crossings or exits were excluded, although no animals were excluded in this study (15).

Conditioned Place Preference Induction

Conditioned Place Preference Apparatus

The CPP setup comprises three chambers (A, B, and C). Chambers A and B are equal in dimensions (30×30×30 cm), divided by a guillotine door, but differ visually. Further, chamber A features black-and-white striped walls and floors, whereas chamber B has plain white walls and floors. Furthermore, chamber C is smaller (30×15×30 cm) and is laterally positioned, with gray walls. A surveillance camera is installed above the apparatus for behavioral monitoring and is connected to a computer system. Additionally, electronic sensors placed beneath the floor detect the presence of mice and record the duration of their stay in each chamber.

Conditioned Place Preference Experimental Procedure

The CPP study was conducted over five days:

8. **Preconditioning Phase (Day 1):** For environmental acclimatization, mice were introduced into the CPP apparatus for 15 minutes with unrestricted access to all chambers.
9. **Conditioning Phase (Days 2–4):** Across three days, mice were given alternating injections of morphine and saline. On the second day, they received an SC dose of morphine (5 mg/kg) in the morning and saline in the afternoon, followed by conditioned placement in one of the two chambers. On day 3, the injection schedule was, however, reversed (morning saline, afternoon morphine). On day 4, the schedule matched day 2.
10. Across three days, mice were given alternating injections of morphine and saline. On the second day, they received an SC dose of morphine (5 mg/kg) in the morning and saline in the afternoon, followed by conditioned placement in one of the two chambers (16), the details of which are provided in Table 1.

Oxidative Stress and Antioxidant Assays

Total Antioxidant Capacity (Ferric Reducing Ability of Plasma Method)

Serum and brain antioxidant capacity were determined using the FRAP assay. The working solution was prepared with 25 mL acetate buffer, 2.5 mL 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) solution, and 2.5 mL FeCl solution.

Table 1. How to Perform the Conditioning Protocol and Record Data in Different Groups

Groups	Day	9 AM	3 PM
A: Control	1	The mouse is placed in the CPP apparatus for 15 minutes to explore and acclimate to its environment.	
	2	NS 10 mL/kg SC	NS 10 mL/kg SC
	3	NS 10 mL/kg SC	NS 10 mL/kg SC
	4	NS 10 mL/kg SC	NS 10 mL/kg SC
	5	Recording the duration of stay in each of the three chambers of the device	
B: Morphine	1	The mouse is placed in the CPP apparatus for 15 minutes to explore and acclimate to its environment.	
	2	Morphine 5 mg/kg SC	NS 10 mL/kg SC
	3	NS 10 mL/kg SC	Morphine 5 mg/kg SC
	4	Morphine 5 mg/kg SC	NS 10 mL/kg SC
	5	Recording the duration of stay in each of the three chambers of the device	
C: Morphine + NS	1	The mouse is placed in the CPP apparatus for 15 minutes to explore and acclimate to its environment.	
	2	Morphine 5 mg/kg SC	NS 10 mL/kg IP
	3	NS 10 mL/kg IP	Morphine 5 mg/kg SC
	4	Morphine 5 mg/kg SC	NS 10 mL/kg IP
	5	Recording the duration of stay in each of the three chambers of the device	
D: Stress + Morphine	1	The mouse is placed in the CPP apparatus for 15 minutes to explore and acclimate to its environment.	
	2	Morphine 5 mg/kg SC	NS 10 mL/kg SC
	3	NS 10 mL/kg SC	Morphine 5 mg/kg SC
	4	Morphine 5 mg/kg SC	NS 10 mL/kg SC
	5	Recording the duration of stay in each of the three chambers of the device	
E: Stress + AFE 150 + Morphine	1	The mouse is placed in the CPP apparatus for 15 minutes to explore and acclimate to its environment.	
	2	AFE 150 mg/kg IP + Morphine 5 mg/kg SC	NS 10 mL/kg SC
	3	NS 10 mL/kg SC	AFE 150 mg/kg IP + Morphine 5 mg/kg SC
	4	AFE 150 mg/kg IP + Morphine 5 mg/kg SC	NS 10 mL/kg SC
	5	Recording the duration of stay in each of the three chambers of the device	
F: Stress + AFE 450 + Morphine	1	The mouse is placed in the CPP apparatus for 15 minutes to explore and acclimate to its environment.	
	2	AFE 450 mg/kg IP + Morphine 5 mg/kg SC	NS 10 mL/kg SC
	3	NS 10 mL/kg SC	AFE 450 mg/kg IP + Morphine 5 mg/kg SC
	4	AFE 450 mg/kg IP + Morphine 5 mg/kg SC	NS 10 mL/kg SC
	5	Recording the duration of stay in each of the three chambers of the device	
G: Stress + AFE 800 + Morphine	1	The mouse is placed in the CPP apparatus for 15 minutes to explore and acclimate to its environment.	
	2	AFE 800 mg/kg IP + Morphine 5 mg/kg SC	NS 10 mL/kg SC
	3	NS 10 mL/kg SC	AFE 800 mg/kg IP + Morphine 5 mg/kg SC
	4	AFE 800 mg/kg IP + Morphine 5 mg/kg SC	NS 10 mL/kg SC
	5	Recording the duration of stay in each of the three chambers of the device	

Note. NS: Normal saline; AFE: *Astragalus fasciculifolius* extract, CPP: Conditioned place preference; SC: Subcutaneous; mL/kg: Milliliters per kilogram; mg/kg: Milligram per kilogram; IP: Intraperitoneal.

Then, tissue samples were homogenized and centrifuged at 10,000 rpm. Subsequently, 50 μ L of each sample was mixed with 1.5 mL of freshly prepared FRAP reagent at 37°C, incubated for 10 minutes, and formed a blue complex through the reaction of Fe^{2+} with TPTZ. Finally, the absorbance was recorded at 593 nm, with FeSO serving as the standard (17).

Malondialdehyde Measurement

Plasma and brain MDA concentrations were measured using the thiobarbituric acid reactive substances assay. For plasma analysis, 1 mL of the homogenized sample was incubated at 37°C for 60 minutes in a metabolic shaker. Next, 1 mL of 5% trichloroacetic acid and 1 mL of 67%

thiobarbituric acid were added, thoroughly mixed, and processed for evaluation. Likewise, 1 g of brain tissue from each group was homogenized and subjected to the same procedure (18).

Gene Expression Evaluation by Real-Time Polymerase Chain Reaction

RNA was isolated from hippocampal tissue following a standard protocol. Custom primers were designed, and the expression of *BDNF* and *TNF- α* genes was quantified using a real-time polymerase chain reaction system (Germany), with *B2M* as the reference gene, and calculations performed using the 2- ΔCt method (19). Table 2 provides the primer sets and sequences.

Results

Evaluation of Locomotor Activity in Experimental Animals

The animals' locomotor activity on the first day of conditioning and on the fifth day post-conditioning remained statistically unchanged (Figure 2).

Influence of *Astragalus Fasciculifolius* Extract on Morphine-Induced Conditioned Place Preference

Statistical analysis using one-way ANOVA followed by Tukey's post-hoc test (Figure 3) revealed that animals in the morphine-treated groups (B, C, D, E, F, and G) spent significantly more time in the morphine-paired compartment compared to the control group (A) ($P < 0.05$, $P < 0.001$). Moreover, administration of *A. fasciculifolius* extract markedly decreased the duration of stay in the morphine-associated chamber relative to the stress + morphine group (D) ($P < 0.05$).

Assessment of Total Antioxidant Capacity in Hippocampus and Serum

One-way ANOVA followed by Tukey's post-hoc analysis demonstrated that TAC in both the hippocampus and serum was sharply reduced in the stress + morphine group (D) compared with the control group (A) ($P < 0.001$). Based on the results (Figure 4), treatment with *A. fasciculifolius* extract substantially enhanced hippocampal antioxidant capacity relative to the stress + morphine group (D) ($P < 0.001$). Moreover, the extract

produced a remarkable elevation in serum antioxidant capacity compared to group D ($P < 0.01$, $P < 0.001$).

Measurement of Malondialdehyde Levels in Hippocampus and Serum

One-way ANOVA followed by Tukey's post-hoc analysis confirmed that hippocampal and serum MDA levels were considerably elevated in the stress + morphine group (D) compared with the control group (A) ($P < 0.001$). The results indicated that administration of *A. fasciculifolius* extract at all three doses could significantly reduce hippocampal MDA concentrations compared with group D ($P < 0.001$). Although the extract also decreased serum MDA levels compared to the stress + morphine group, this reduction did not reach statistical significance (Figure 5).

Expression Levels of *TNF-α* and *BDNF* Genes in the Hippocampus

One-way ANOVA followed by Tukey's post-hoc analysis showed the significant elevation of hippocampal *TNF-α* gene expression in the stress + morphine group (D) compared with the control group (A) ($P < 0.001$). According to the findings, treatment with *A. fasciculifolius* extract at 450 mg/kg markedly decreased *TNF-α* expression relative to group D ($P < 0.001$). In addition, hippocampal *BDNF* gene expression was significantly higher in the stress + morphine group (D) in comparison to the control group (A) ($P < 0.001$); however, administration of *A. fasciculifolius* extract did not produce a remarkable alteration in *BDNF* expression compared with group D (Figure 6).

Discussion

Morphine addiction is a chronic disorder that affects the central nervous system, and prolonged use leads to physical dependence and psychological addiction. Moreover, morphine binds to opioid receptors, triggering dopamine release, thereby reinforcing drug-seeking behavior.

Table 2. Primer Sets and Sequences

Gene	Primer Sequence (5'-3')
<i>B2m</i>	Forward: CGTGATCTTTCTGGTGCTTGTC Reverse: GGAAGTTGGGCTTCCCATCT
<i>BDNF</i>	Forward: GAAATGCCACCTTTTGACAGTG Reverse: TGGATGCTCCCATCAGGACAG
<i>TNF-α</i>	Forward: TCTCGTCATTCTGCTTGTTGGC Reverse: TGATCTGAGCGTGAGGGTCTGG

Note. B2M: β2 microglobulin; BDNF: Brain-derived neurotrophic factor; *TNF-α*: Tumor necrosis factor-α.

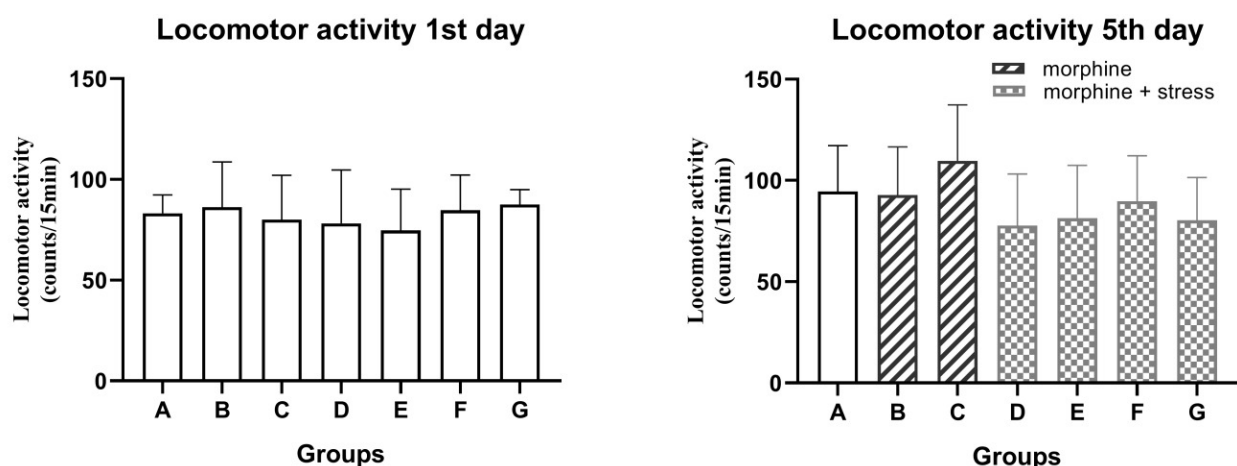


Figure 2. Evaluation of Locomotor Activity in Animals on the First Day of the Conditioning Process and the Fifth Day (Post-Conditioning Phase)

Note. *A. fasciculifolius*: *Astragalus fasciculifolius*. Data were evaluated using one-way analysis of variance, followed by Tukey's post-hoc test. The results are expressed as means ± standard deviations. Experimental groups included (A) Control, normal saline (10 mL/kg); (B) Morphine (5 mg/kg); (C) Morphine (5 mg/kg) plus normal saline (10 mL/kg); (D) Stress + Morphine (5 mg/kg); (E) Stress + *A. fasciculifolius* extract (150 mg/kg) + Morphine (5 mg/kg); (F) Stress + *A. fasciculifolius* extract (450 mg/kg) + Morphine (5 mg/kg); (G) Stress + *A. fasciculifolius* extract (800 mg/kg) + Morphine (5 mg/kg).

Likewise, chronic use results in tolerance, requiring higher doses to achieve the same effect. OS plays a role in morphine addiction, impacting brain function. Adaptive neuroplastic changes also contribute to compulsive drug-seeking behaviors (20). Treatment approaches include opioid replacement therapy, behavioral interventions, and pharmacological strategies. Emerging research explores novel therapies, such as stem cell treatment and OS modulation. It should be noted that morphine addiction remains a global health concern, requiring multidisciplinary therapeutic strategies (21). Chronic stress alters brain circuits, increasing susceptibility to morphine and drug addiction. It also dysregulates the hypothalamic-pituitary-adrenal axis, promoting opioid-

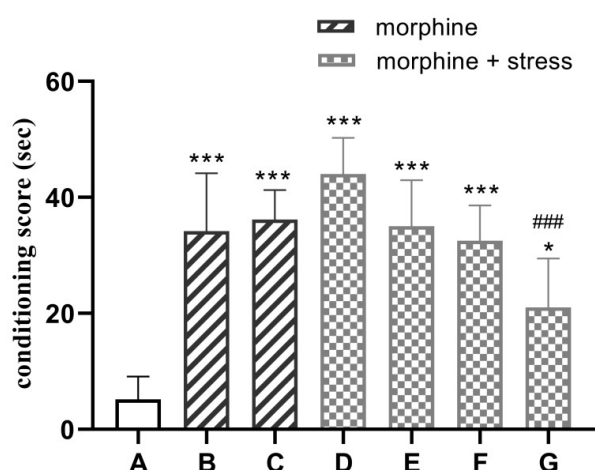
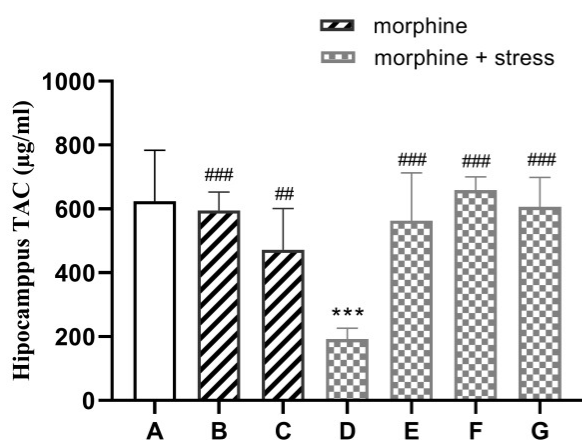


Figure 3. Conditioned Place Preference Scores Following Morphine Conditioning Across the Experimental Groups

Note. *A. fasciculifolius*: *Astragalus fasciculifolius*; ANOVA: One-way analysis of variance. Data were analyzed using one-way ANOVA followed by Tukey's post-hoc test. The results are provided as means $\pm$ standard deviations. Statistical significance is indicated as * $P < 0.05$ and *** $P < 0.001$ versus the control group (A), and ### $P < 0.001$ versus the stress + morphine group (D). The experimental groups were defined as (A) Control, normal saline (10 mL/kg); (B) Morphine (5 mg/kg); (C) Morphine (5 mg/kg) + normal saline (10 mL/kg); (D) Stress + Morphine (5 mg/kg); (E) Stress + *A. fasciculifolius* extract (150 mg/kg) + Morphine (5 mg/kg); (F) Stress + *A. fasciculifolius* extract (450 mg/kg) + Morphine (5 mg/kg); (G) Stress + *A. fasciculifolius* extract (800 mg/kg) + Morphine (5 mg/kg)



seeking behavior. Additionally, neuroinflammation and OS contribute to tolerance and dependence. Effective stress management is crucial for preventing relapse and supporting recovery (22). This study confirmed morphine-induced CPP. Mice exhibited a marked preference for the morphine-paired compartments relative to controls, which is consistent with prior evidence indicating that morphine activates opioid receptors, producing euphoria and reinforcing drug-seeking behavior. OS mechanisms associated with morphine addiction encompass mitochondrial dysfunction, neuroinflammation, and impaired antioxidant defenses. Childhood stress alters stress response pathways and emotional regulation, increasing opioid addiction risk later in life. It is noteworthy that stress management through behavioral therapy, mindfulness, and pharmacological interventions improves addiction treatment outcomes (21). *A. fasciculifolius* is an important medicinal plant whose growth and development are negatively influenced by drought stress (23). *A. fasciculifolius* Bioss, commonly known as Anzaroot, is a medicinal plant from the *Fabaceae* family, which is recognized for its therapeutic properties due to its rich composition of saponins, flavonoids, and polysaccharides. These compounds have been shown to effectively treat heart disease, inhibit cancer cell growth, and alleviate chemotherapy side effects (4). Our findings demonstrated that *A. fasciculifolius* extract reduced morphine-seeking behavior, suggesting its potential effects on brain reward pathways. Finally, these findings indicated that this extract may modulate central neural mechanisms, thereby contributing to reduced morphine preference.

Limitations of the Study

The limitations of this study were the bureaucracy of obtaining morphine due to the possibility of illegal use of this substance in society, as well as the procurement of mice from Tehran, which was resolved after consultation and coordination with the relevant centers.

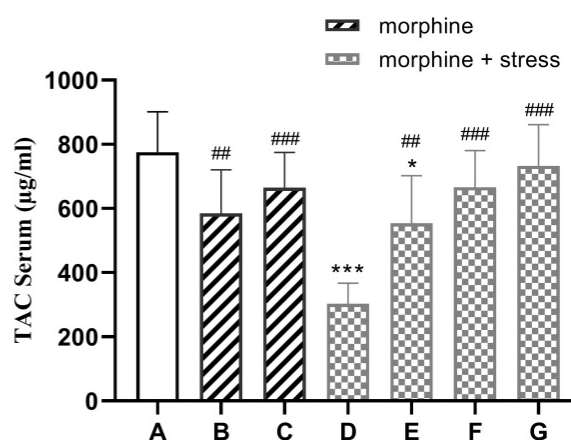


Figure 4. Comparative Total Antioxidant Capacity in Hippocampal and Serum Samples Among the Experimental Groups

Note. *A. fasciculifolius*: *Astragalus fasciculifolius*; ANOVA: Analysis of variance. Data underwent analysis using one-way ANOVA followed by Tukey's post-hoc test. The results are presented as means $\pm$ standard deviations. Statistical significance is represented as * $P < 0.05$ and *** $P < 0.001$ versus the control group (A), and ## $P < 0.01$ and ### $P < 0.001$ versus the stress + morphine group (D). The experimental groups were defined as (A) Control, normal saline (10 mL/kg); (B) Morphine (5 mg/kg); (C) Morphine (5 mg/kg) + normal saline (10 mL/kg); (D) Stress + Morphine (5 mg/kg); (E) Stress + *A. fasciculifolius* extract (150 mg/kg) + Morphine (5 mg/kg); (F) Stress + *A. fasciculifolius* extract (450 mg/kg) + Morphine (5 mg/kg); (G) Stress + *A. fasciculifolius* extract (800 mg/kg) + Morphine (5 mg/kg)

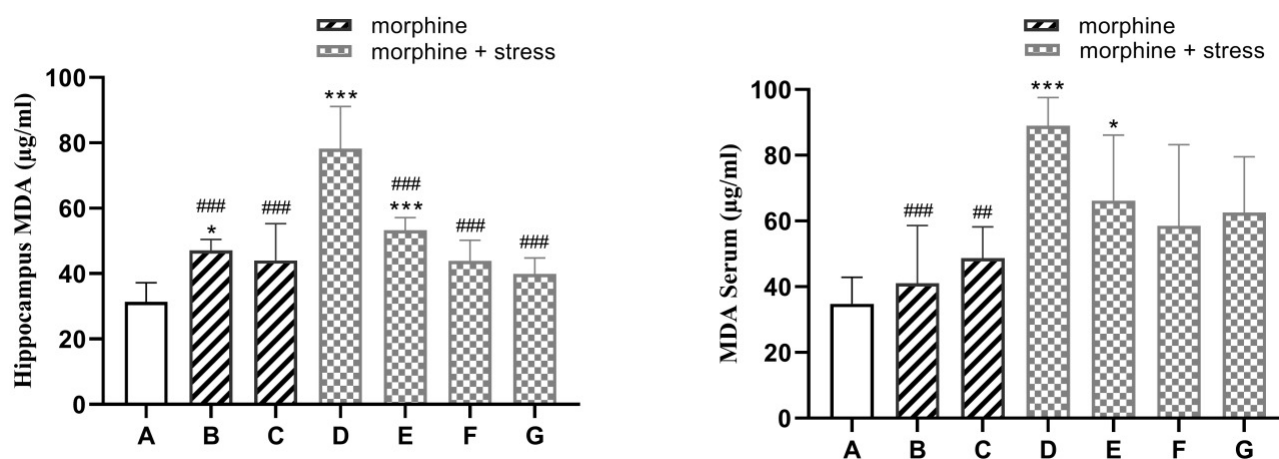


Figure 5. Comparison of Hippocampal and Serum Malondialdehyde Levels Among the Experimental Groups

Note. *A. fasciculifolius*: *Astragalus fasciculifolius*; ANOVA: One-way analysis of variance Data were analyzed using one-way ANOVA followed by Tukey's post-hoc test. The results are expressed as means $\pm$ standard deviations. Statistical significance is indicated as * $P < 0.05$ and *** $P < 0.001$ versus the control group (A), and ** $P < 0.01$ and *** $P < 0.001$ versus the stress + morphine group (D). The experimental groups were defined as (A) Control, normal saline (10 mL/kg); (B) Morphine (5 mg/kg); (C) Morphine (5 mg/kg) + normal saline (10 mL/kg); (D) Stress + Morphine (5 mg/kg); (E) Stress + *A. fasciculifolius* extract (150 mg/kg) + Morphine (5 mg/kg); (F) Stress + *A. fasciculifolius* extract (450 mg/kg) + Morphine (5 mg/kg); (G) Stress + *A. fasciculifolius* extract (800 mg/kg) + Morphine (5 mg/kg)

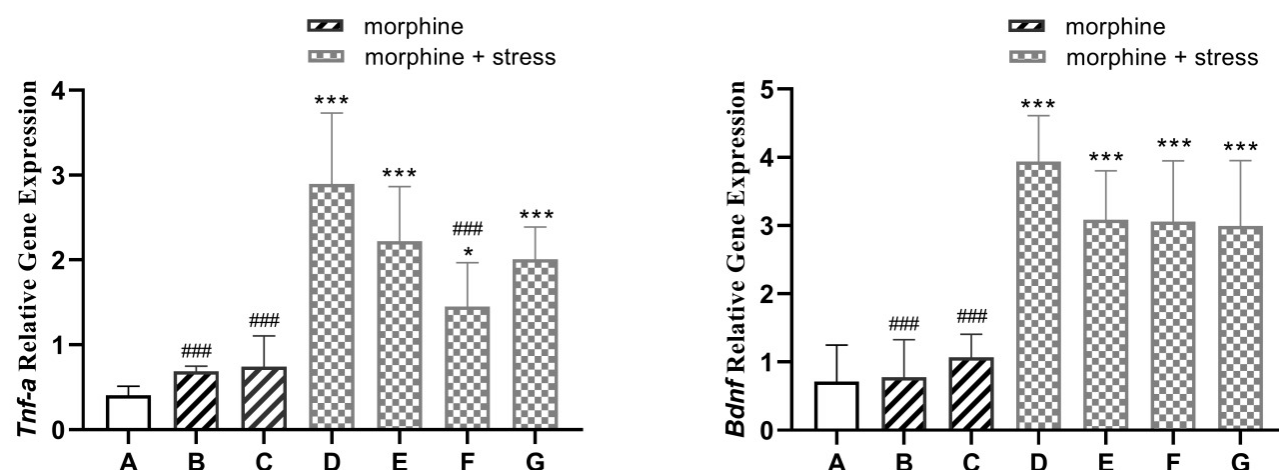


Figure 6. Hippocampal Expression of *TNF-α* and *BDNF* Genes Across Experimental Groups

Note. *A. fasciculifolius*: *Astragalus fasciculifolius*; ANOVA: Analysis of variance. Data were analyzed using one-way ANOVA followed by Tukey's post-hoc test. Results are expressed as mean $\pm$ standard deviation. Statistical significance is shown as * $P < 0.05$ and *** $P < 0.001$ versus the control group (A), and *** $P < 0.001$ versus the stress + morphine group (D). The experimental groups were defined (A) Control, normal saline (10 mL/kg); (B) Morphine (5 mg/kg); (C) Morphine (5 mg/kg) + normal saline (10 mL/kg); (D) Stress + Morphine (5 mg/kg); (E) Stress + *A. fasciculifolius* extract (150 mg/kg) + Morphine (5 mg/kg); (F) Stress + *A. fasciculifolius* extract (450 mg/kg) + Morphine (5 mg/kg); (G) Stress + *A. fasciculifolius* extract (800 mg/kg) + Morphine (5 mg/kg)

Conclusion

Our findings revealed that stress has exacerbating effects on morphine-seeking behavior, supporting the potential therapeutic application of *A. fasciculifolius* in mitigating morphine preference following stress exposure.

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Artificial Intelligence Disclosure Statement

During the preparation of this manuscript, the authors did not use artificial intelligence. They assume full responsibility for the integrity and final content of the published articles.

Authors' Contribution

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Project Administration: Maryam Anjomshoa
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Software: Diana Shahrani, Mohamad Shahrani
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Validation: Najmeh Asgharzadeh
Visualization: Najmeh Asgharzadeh
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Writing–Review & Editing: Mehrdad Shahrani Korrani

All authors commented on previous versions. Moreover, they all read and approved the final manuscript.

Competing Interests

The authors declare that they have no conflict of interests related to

the content of this article.

Ethical Approval

All experimental procedures were conducted in full compliance with the ethical principles of animal research and the relevant international guidelines, including the Declaration of Helsinki and institutional standards for laboratory animal care and use. Moreover, the study protocol was reviewed and approved by the Ethics Committee of Shahrekord University of Medical Sciences (approval No. IR.SKUMS.AEC.1402.014). All efforts were made to minimize animal suffering and reduce the number of animals.

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