ISSN: 3005-6632

Afghanistan Journal of Basic Medical Sciences

2026 Jan; 3(1): 10-21.

ajbms.knu.edu.af

Aqueous Extract of Saffron (*Crocus sativus* L.) Reduces Anxiety and Depressive-Like Behaviors Induced by Nicotine Withdrawal in Male Rats

Ahmad Wali Ataye [1, 2], Sayed Hussain Amiri[3], *Murtaza Haidary [4, 5], Abdullah Sahar[6]

1. *Department of Public Health, Faculty of Medicine, Afghan International Islamic University of Kabul, Afghanistan.*
2. *Department of Microbiology, Faculty of Allied Sciences, Kabul University of Medical Science, Kabul, Afghanistan*
3. *Department of paraclinic, Faculty of Curative Medicine, shifa University, Kabul, Afghanistan*
4. *Department of Paraclinical Sciences and Laboratory Management, Faculty of Medical Laboratory Technology, Khatam Al-Nabieen University, Kabul, Afghanistan*
5. *Medical Research and Technology Center, Khatam Al-Nabieen University, Kabul, Afghanistan*
6. *Department of Microbiology, Faculty of Medical Laboratory Technology, Spinghar University-Kabul, Afghanistan*

ARTICLE INFO

Type: Original Article

Received: 7 Sept, 2025

Accepted: 1 Dec, 2025

*Corresponding Author:
E-mails: sadramurtaza@gmail.com
murtaza.haidary@knu.edu.af

To cite this article:

Ataye AW, Amiri SH, Haidary M, Sahar A. Aqueous extract of saffron (Crocus sativus L.) reduces anxiety- and depressive-like behaviors induced by nicotine withdrawal in male rats. Afghanistan Journal of Basic Medical Sciences. 2026 Jan;3(1):10–21.

DOI:
https://doi.org/10.62134/khatamuni.122

ABSTRACT

Background: Anxiety and depressive-like symptoms are common during the discontinuation of addictive substances, underscoring the need for effective interventions. We aimed to evaluate whether an aqueous extract of saffron (*Crocus sativus* L.) could attenuate anxiety- and depression-like behaviors induced by nicotine withdrawal in male rats.

Methods: Adolescent male rats received daily nicotine administration for 21 d. After cessation, the animals entered a 21-day withdrawal period during which they were treated with the saffron aqueous extract. Behavioral assessments were conducted using the Open Field Test (OFT), Elevated Plus Maze (EPM), and Forced Swim Test (FST) to evaluate anxiety- and depression-related responses.

Results: Nicotine withdrawal produced marked anxiety- and depressive-like behaviors. Administration of the saffron extract during the withdrawal phase significantly alleviated these behavioral disturbances.

Conclusion: Saffron may have therapeutic potential in mitigating emotional disturbances associated with nicotine withdrawal. Given the high prevalence of nicotine use among adolescents and their susceptibility to withdrawal-induced psychological symptoms, further research into the underlying mechanisms and clinical applicability of saffron is warranted to inform strategies for managing withdrawal-related affective disturbances.

Keywords: Nicotine; Adolescence; Anxiety; Depression; Saffron

Copyright © 2026 Afghanistan Journal of Basic Medical Sciences, and Khatam Al-Nabieen University. All rights reserved.
This work is licensed under a Creative Commons Attribution-Noncommercial 4.0 International License

Introduction

Adolescent engagement in substance use has become a pressing worldwide public health issue (1). This concern has prompted growing scientific attention toward understanding usage trends during adolescence, a period marked by substantial neurobiological maturation and reorganization in the brain (2). Nicotine, a cholinergic receptor agonist exhibiting both parasympathomimetic and stimulating effects, possesses pharmacological actions similar to those of amphetamines an aspect that contributes to its strong potential for dependence and misuse (3).

Its rewarding effects promote continued use across a variety of tobacco products (4). Adolescents are particularly vulnerable, exhibiting increased nicotinic acetylcholine receptor density and enhanced connectivity within reward-related neural circuits, which amplify nicotine's reinforcing properties (5). In addition, adolescents tend to exhibit a reduced sensitivity to the immediate harmful effects of nicotine compared to adults, which contributes to higher intake during this life stage (6). Although short-term nicotine exposure can temporarily yield calming or mood-elevating influences (7), prolonged use beginning in adolescence is closely linked to the development of mental health disturbances such as anxiety (8) and depression, especially upon cessation (9). These detrimental consequences have been associated with alterations in serotonergic pathways as well as heightened oxidative stress (10). Therefore, discovering therapeutic approaches that are both safe and capable of reducing nicotine dependence and withdrawal-related psychological symptoms remains an important focus of ongoing research.

Medicinal plants have attracted considerable attention for the management of drug dependence due to their lower cost and reduced side-effect profile compared with synthetic agents (11). Among these, saffron (*Crocus sativus* L.) is one of the most widely recognized and valued medicinal plants. This perennial herb, often referred to as the "king of medicines," is native to Southwest Asia (12), and is currently cultivated primarily in Afghanistan, Iran, India, Italy, and several other countries. Its pharmacological effects are largely attributed to three major bioactive constituents: crocin, picrocrocin, and safranal (13). Experimental studies across diverse models have demonstrated that saffron extract and its active component crocin exert beneficial effects on morphine dependence, effectively attenuating withdrawal symptoms in morphine-dependent mice (14).

We aimed to investigate whether saffron exerts neuroprotective effects against anxiety- and depression-like behaviors induced by nicotine withdrawal in adolescent male rats, as well as its influence on serotonin regulation, oxidative stress markers, and the interplay between these two pathways. In pursuing this aim, the study contributes to filling an existing gap in the literature concerning the potential role of saffron in alleviating both the behavioral and biochemical disruptions associated with nicotine withdrawal.

Materials and Methods

Animals

Overall, 80 male Sprague–Dawley rats, 21 d old (postnatal day [PND] 21), were enrolled, corresponding to the beginning of the adolescent phase in rodents. In this strain, adolescence typically spans from just after weaning (around PND 21) until approximately PND 60, when animals approach physiological adulthood. The rats were procured from the Laboratory Animal Center of Khatam Al-Nabieen University. They were housed in groups of three to four in open-top Plexiglas cages under controlled laboratory conditions (temperature: 22 ± 2 °C; 12-hour light/dark cycle). Standard laboratory chow and

tap water were made available at all times. The feed, a commercially prepared rat diet (Javaneh Khorasan, Mashhad, Iran), was composed of 46% nanofibrillated cellulose, 25% neutral detergent fiber, 19% crude protein, and 10% fat, designed to meet the nutritional requirements for healthy growth and maturation during adolescence.

All experimental procedures were approved by the Animal Ethics Committee of the author's university (AF, knu.edu.af.rec 28, 7/4/2025) and were adhered to established ethical guidelines for the care and use of laboratory animals (15).

Plant material and extraction

Fresh stigmas of Crocus sativus L. were collected from Herat Province, Afghanistan. The stigmas were air-dried under laboratory conditions and ground into a fine powder. A 4-gram portion of the powdered sample was soaked in 500 mL of distilled water at ambient temperature for 72 h to allow extraction. Following maceration, the suspension was centrifuged, and the resulting supernatant was passed through conventional filter paper. The obtained filtrate was then concentrated by evaporation in a drying oven maintained at 50 °C, producing the final aqueous extract of saffron (16).

Drugs and Experimental Methods

Nicotine (Sigma-Aldrich, St. Louis, MO, USA) was freshly dissolved in 0.9% saline before each injection and administered subcutaneously once daily at 2 mg/kg (9). Eight groups of rats (n = 10 per group) were established to examine the effects of nicotine and saffron treatment during exposure and withdrawal. The control group received saline throughout the entire experimental period (PND 21–63). One group was treated with nicotine for 21 d (PND 21–42) followed by saline during the withdrawal phase (PND 42–63) to evaluate the impact of nicotine cessation. To assess the potential protective or therapeutic effects of saffron, some rats received nicotine during PND 21–42 followed by saffron extract (50 or 100 mg/kg, intraperitoneally) during PND 42–63, while others were co-administered nicotine and saffron extract during the exposure phase and then given saline during withdrawal. Additional groups were treated with saffron alone either during PND 21–42 or during the withdrawal phase (PND 42–63) to determine its independent effects and potential benefits when administered specifically during nicotine cessation.

Behavioral Evaluations

Anxiety-related behaviors in rats typically emerge several d after nicotine cessation (17). Behavioral evaluations were conducted 21 d after the final nicotine exposure to assess emotional and affective disturbances associated with withdrawal. During this period, each rat underwent a series of behavioral paradigms, including the Open Field Test (OFT), Elevated Plus Maze (EPM), Forced Swim Test (FST), and Sucrose Preference Test (SPT), designed to measure anxiety-, depression-, and anhedonia-related behaviors. To reduce variability caused by external stress, animals were permitted a 30-minute habituation period in the testing room prior to each session. All behavioral procedures were performed under controlled laboratory conditions, with illumination levels maintained at 150 lux for the EPM and 40 lux for the OFT to optimize exploratory activity. After testing, rats were separated from those not yet assessed to prevent transmission of stress-related cues. Between trials, all apparatuses were thoroughly wiped with 20% ethanol to remove residual odors and ensure experimental uniformity.

Open Field Test

The OFT is a well-established and widely used behavioral assay for measuring anxiety-like responses in rodents, as well as for investigating the neurobiological and pharmacological mechanisms that regulate anxiety (18). The test relies on the natural conflict in rodents between their

aversion to open, brightly illuminated areas and their innate tendency to explore novel environments. Increased time spent in the central portion of the arena is interpreted as a sign of reduced anxiety, reflecting less avoidance of exposed spaces (19). In this study, the OFT was employed to evaluate anxiety-related behaviors. The apparatus consisted of a square arena measuring 100 × 100 × 40 cm, constructed from opaque, non-reflective material to minimize glare and visual distractions. Each subject was placed gently in the center of the arena and permitted unrestricted exploration for 5 min—an interval sufficient to capture spontaneous locomotor and exploratory patterns without the confounding effects of habituation (20). This setup provides a robust measure of both emotional reactivity and motor activity in response to novelty, thereby offering insights into genetic, neurochemical, or pharmacological modulation of anxiety (21). Behavioral parameters, including time spent in central versus peripheral zones, were automatically recorded and analyzed using a computerized video-tracking system.

Elevated Plus Maze

After completion of the OFT, anxiety-related responses were further examined using the EPM, a widely recognized assay for assessing both anxiety levels and general locomotor function in rodents. The apparatus consisted of a cross-shaped maze positioned 50 cm above the ground, featuring two opposing open arms and two enclosed arms bordered by 40 cm-high walls to create sheltered zones. This design leverages the innate ambivalence in rodents between their curiosity to explore novel environments and their fear of exposed, elevated spaces (22). In the current experiment, the maze was built from matte gray wooden panels to reduce glare and visual distractions, thereby maintaining uniform testing conditions. Each animal was placed gently at the central intersection of the maze, facing one of the open arms, and allowed to move freely for a 5-minute session under low-light conditions. Anxiety-like behavior was quantified by calculating the percentage of time spent in open arms relative to closed arms—greater exploration of open arms being interpreted as an anxiolytic effect. This experimental setup offered an objective behavioral index for evaluating the potential of saffron extract to attenuate anxiety through modulation of avoidance behavior toward exposed environments.

Forced Swimming Test

The FST is a classic and extensively validated method for assessing depressive-like behaviors in rodents. In this procedure, subjects are placed in a cylinder filled with water from which escape is not possible, providing a model to examine behavioral adaptation to an acute, unavoidable stressor. The test distinguishes between active coping strategies—including swimming and climbing—and passive coping, manifested as immobility, which is considered an index of behavioral despair and serves as an analogue for depressive symptoms in humans (23). In the current experiment, each rat was carefully introduced into a transparent cylindrical tank measuring 50 cm in height and 20 cm in diameter, containing 30 cm of water maintained at 24 ± 2 °C—deep enough to prevent the animal from supporting itself on the bottom. All experimental sessions were recorded on video for detailed subsequent behavioral scoring and analysis. Rat behaviors were categorized as follows: climbing, involving vigorous forelimb movements against the tank walls; swimming, characterized by horizontal movement around the cylinder; and immobility, defined as minimal motion limited to maintaining the head above water. This protocol provides an objective index of depressive-like behavior and enables evaluation of pharmacological interventions on stress-coping mechanisms.

Sucrose Preference Test

The SPT is a widely utilized method for evaluating reward sensitivity and hedonic behavior in rodents. This test takes advantage of the natural preference of rodents for sweet solutions, typically 1%–2% sucrose, over plain water, providing a reliable measure of positive reinforcement and pleasure-seeking behavior (24). A reduction in sucrose intake or preference serves as an indicator of anhedonia, reflecting a diminished capacity to experience pleasure, which is commonly observed in depressive-like states, substance withdrawal, and various neuropsychiatric disorders (25). Accordingly, the SPT is frequently applied in preclinical studies to investigate the neurobehavioral mechanisms underlying anhedonia. In the present study, rats were first habituated to the testing procedure over three days. On the first two days, each animal was given two bottles of plain water to establish baseline consumption, followed by exposure to two bottles containing 1% sucrose solution on the third day to acclimate them to the sweetened solution. After a 12-hour period of food and water deprivation, each rat was presented simultaneously with one bottle of plain water and one bottle of 1% sucrose solution. To avoid positional bias, the positions of the bottles were switched every 30 min during the 3-hour test period. The following morning, remaining fluid volumes were measured, and sucrose preference was calculated using the formula: sucrose preference percentage = (sucrose solution consumption / (sucrose solution consumption + water consumption)) × 100% (Fig. 1).

Statistical Analyses

All data obtained from the experiments were processed and analyzed using GraphPad Prism (version 8.4.3; GraphPad Software, San Diego, CA, USA). Differences among groups were assessed by one-way analysis of variance (ANOVA), and where significant main effects were observed, Tukey's post hoc test was applied for pairwise comparisons. Data are presented as mean ± standard error of the mean (SEM). Statistical significance was defined as $P<0.05$, corresponding to a 95% confidence interval.

Results

Saffron Attenuates Anxiety- and Depression-Like Behaviors Induced by Nicotine Withdrawal

Nicotine withdrawal caused notable impairments in exploratory behavior in the OFT. Rats undergoing withdrawal displayed a significant decrease in time spent in the center of the arena ($P<0.001$; Fig. 1A) along with a corresponding increase in activity along the periphery ($P<0.001$; Fig. 1B) compared with vehicle–vehicle controls, indicating elevated anxiety and avoidance of exposed areas. Administration of saffron at 100 mg/kg during both the nicotine exposure and withdrawal phases effectively reversed these changes, leading to increased central zone activity ($P<0.01$) and reduced peripheral movement ($P <0.001$; Fig. 1A–B) relative to nicotine–vehicle animals. Rats treated solely with saffron (100 mg/kg, PND 42–63) exhibited a modest but significant enhancement in central exploration compared with controls ($P<0.05$; Fig. 1A). A similar effect was observed in the EPM.

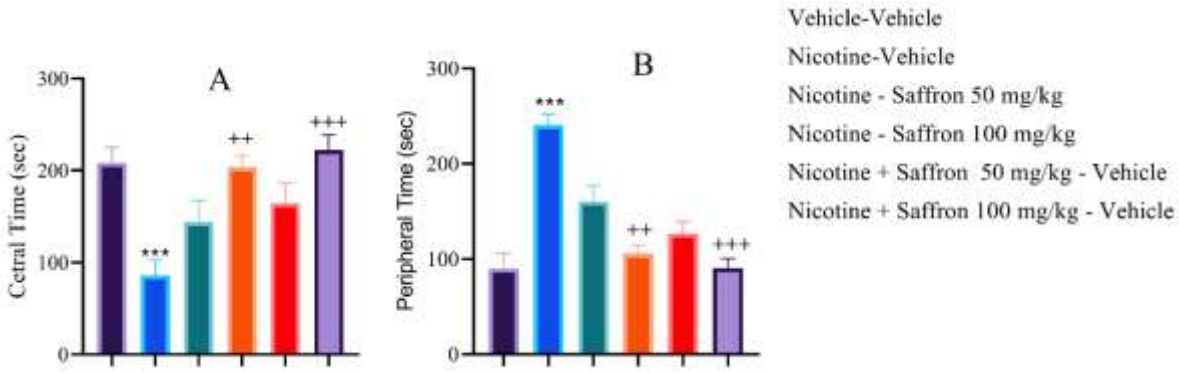

Fig. 1: Effects of saffron on anxiety-like behaviors in the open field test (OFT). (A) Time spent in the central zone; (B) time spent in the peripheral zone across experimental groups. Data are expressed as mean ± SEM. *p < 0.05, ***p < 0.001 vs. vehicle–vehicle group; ++p < 0.01, +++p < 0.001 vs. nicotine–vehicle group

Nicotine-withdrawn rats spent considerably less time in the open arms (P<0.001; Fig. 2A) and more time in the closed arms (P<0.001; Fig. 2B) than vehicle–vehicle controls, reflecting anxiety-like behavior. Treatment with saffron at 50 mg/kg during nicotine exposure, or at 100 mg/kg across both exposure and withdrawal periods, significantly promoted exploration of the open arms while reducing closed-arm occupancy (P<0.05 and P<0.001, respectively; Fig. 2 A–B). Administration of saffron alone (100 mg/kg) did not significantly alter EPM behavior compared with controls.

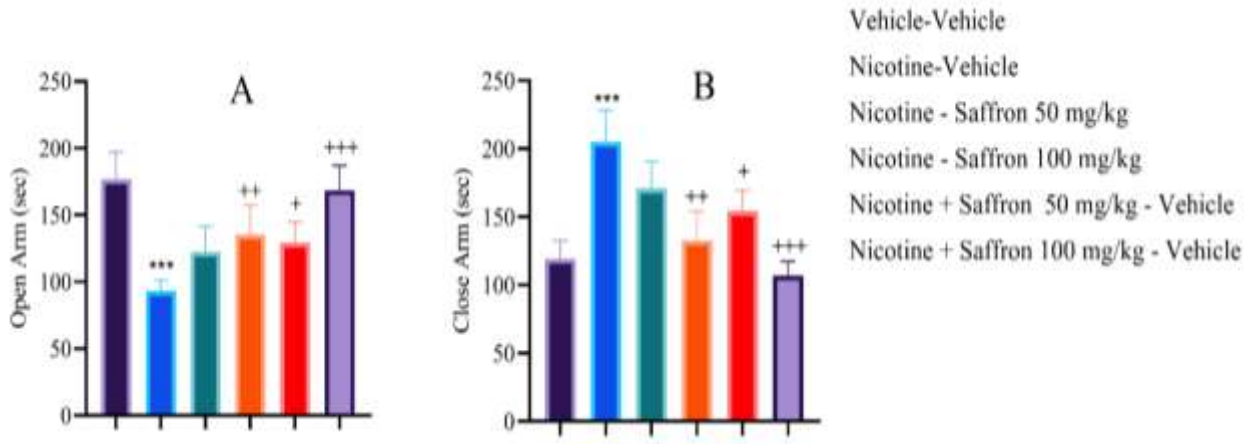

Fig. 2: Effects of saffron on anxiety-like behaviors in the elevated plus maze (EPM). (A) Time spent in open arms; (B) time spent in enclosed arms across experimental groups. Data are expressed as mean ± SEM. ***P<0.001 vs. vehicle–vehicle group; +P<0.05, ++P<0.01, +++P<0.001 vs. nicotine–vehicle group

In the FST, nicotine withdrawal induced depressive-like effects, characterized by reduced struggling (P<0.001; Fig. 3 A), increased immobility (P < 0.001; Figure 3B), and decreased swimming (P<0.001; Fig. 3 C) relative to vehicle–vehicle rats. Saffron at 50 mg/kg during nicotine exposure partially improved these behaviors, increasing active coping behaviors and reducing immobility (P<0.05; Fig. 3 A–C). The strongest antidepressant-like effects were observed with saffron at 100 mg/kg administered throughout both nicotine exposure and withdrawal, significantly enhancing struggling and swimming (P<0.01) and substantially lowering

immobility (P<0.001; Fig. 3 A–C). Saffron alone (100 mg/kg) had no significant effect on FST performance compared with controls.

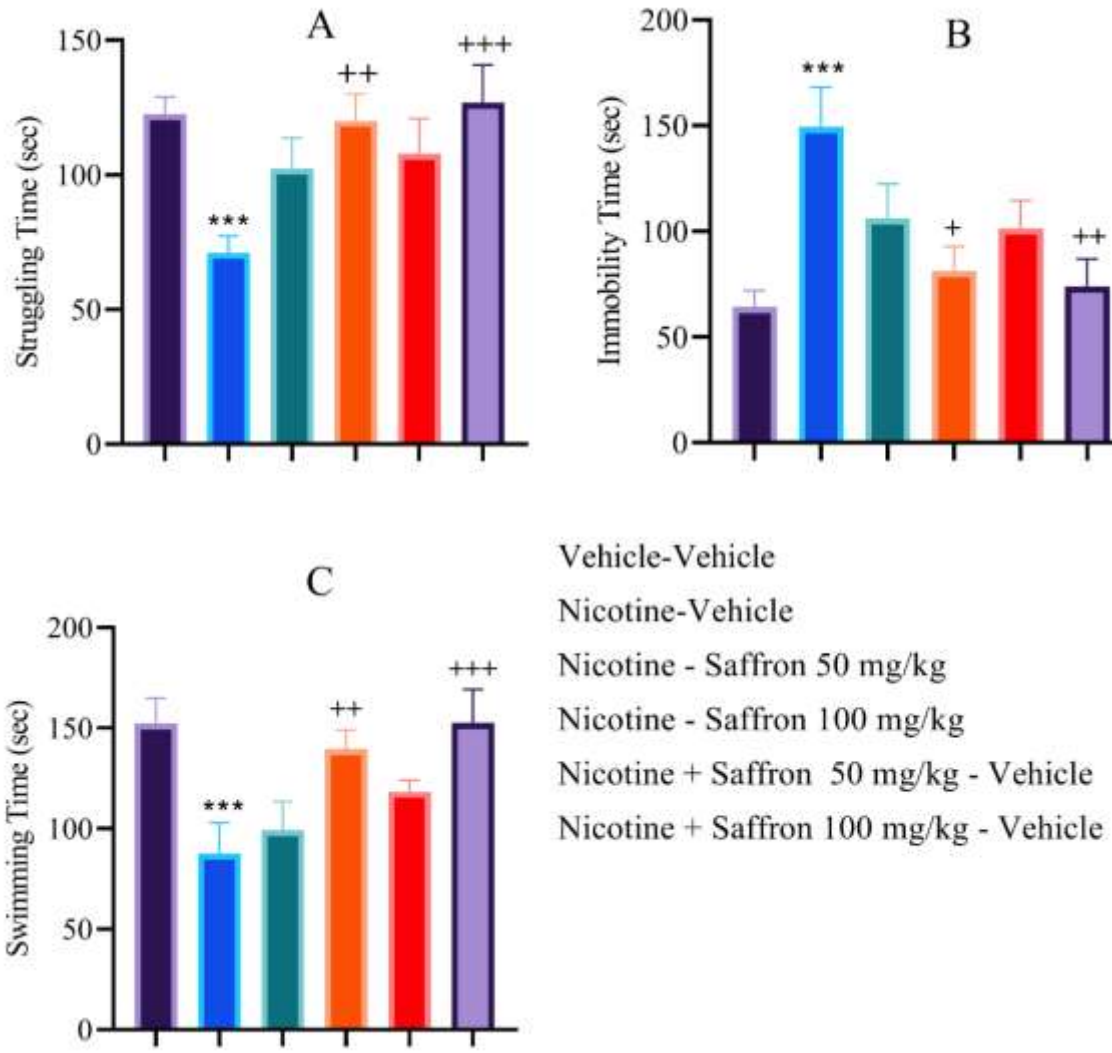

Fig. 3: Effects of saffron on depressive-like behaviors in the forced swim test (FST). (A) Time spent struggling; (B) immobility duration; (C) swimming time across experimental groups. Data are expressed as mean ± SEM. ***P<0.001 vs. vehicle–vehicle group; +P<0.05, ++P<0.01, +++P<0.001 vs. nicotine–vehicle group

Saffron Counteracts Nicotine Withdrawal–Induced Anhedonia

Withdrawal from nicotine caused a substantial reduction in sucrose intake, indicating a strong anhedonic response when compared with the vehicle–vehicle control group (P<0.001; Fig. 4). Treatment with saffron at a dose of 50 mg/kg during nicotine exposure, and at 100 mg/kg across both the exposure and withdrawal phases, effectively reversed this deficit, restoring sucrose preference to levels comparable to controls (P<0.01 and P<0.001, respectively; Fig. 4). In contrast, administration of saffron alone (100 mg/kg, PND 42–63) produced no significant alteration in sucrose consumption relative to untreated controls.

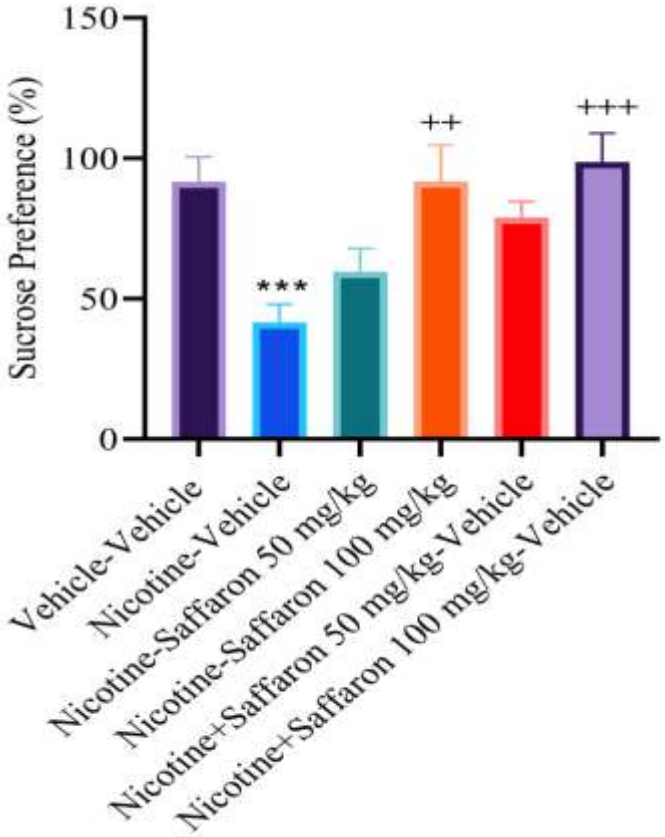

Fig. 4: Effects of saffron on sucrose preference during nicotine withdrawal. Data are expressed as mean ± SEM. ***P<0.001 vs. vehicle–vehicle group; ++P<0.01, +++P<0.001 vs. nicotine–vehicle group

Discussion

In the present study, nicotine withdrawal in adolescent male rats consistently produced anxiety-like, depressive-like, and anhedonic behaviors. Combined and post-treatment with saffron, especially at the higher dose of 100 mg/kg, substantially mitigated the behavioral deficits induced by nicotine withdrawal. In the OFT, withdrawal significantly impaired exploratory behavior, evidenced by decreased time in the central zone and increased peripheral activity, indicative of heightened anxiety and avoidance of exposed areas. Similarly, in the EPM, nicotine-withdrawn rats showed reduced time spent in the open arms and increased occupancy of the closed arms, reflecting an anxiety-like behavioral profile. Administration of saffron at 100 mg/kg across both exposure and withdrawal phases effectively restored normal exploratory patterns in both paradigms, highlighting its potential anxiolytic properties in the setting of nicotine dependence.

Notably, saffron alone during PND 42–63 modestly increased center-zone exploration in the OFT, although it did not significantly affect EPM measures, suggesting some baseline anxiolytic or exploratory-enhancing effects at this dose, albeit not consistently across all paradigms. These results are consistent with previous reports of saffron's anxiolytic properties. For instance, aqueous saffron extract and its constituent safranal have been shown to produce anxiolytic and hypnotic effects in mice, including increased open-arm time in the EPM at low doses (26). More recently, saffron extract (Safr'Inside™) enhanced center-zone activity in an open-field test and improved anxie-

ty-related outcomes in a mouse model of low-grade inflammation, likely via modulation of the gut–brain axis and microbiota-derived metabolites (27). Collectively, saffron exerts anxiolytic effects across different experimental contexts, and our data extend these effects to nicotine withdrawal in adolescent rats.

In the FST, nicotine withdrawal induced a pronounced depressive-like phenotype, marked by reduced struggling, increased immobility, and decreased swimming. Treatment with saffron at both 50 and 100 mg/kg—particularly the latter administered during both nicotine exposure and withdrawal—attenuated these effects, promoting active behaviors and reducing immobility. Saffron given alone, however, did not significantly affect FST performance compared with controls. Similarly, in the SPT, nicotine withdrawal produced significant anhedonia, evidenced by decreased sucrose consumption, which was effectively reversed by saffron treatment, most notably at the 100 mg/kg dose. The absence of a significant effect of saffron alone on sucrose preference suggests that its action is particularly relevant under pathological conditions, such as withdrawal-induced stress, rather than broadly enhancing hedonic behavior in healthy animals. These findings are in line with previous preclinical studies supporting the antidepressant-like properties of saffron and its bioactive components. For instance, saffron extract (Safr'Inside™) reduced depressive-like behaviors in naïve mice in the FST, accompanied by modulation of monoaminergic neurotransmission, including serotonin and dopamine (28). In a chronic unpredictable mild stress (CUMS) model of depression, saffron extract alleviated depressive behaviors, reduced neuroinflammation, and enhanced neuroplasticity, particularly within the hippocampus (29). Additionally, crocin, the main bioactive carotenoid in saffron, has been shown to mitigate stress-induced anxiety and depression, likely through reductions in oxidative stress, inflammatory mediators, and corticosterone levels (30). Collectively, our results extend this body of evidence by demonstrating saffron's protective effects in a nicotine withdrawal context, highlighting its potential as a therapeutic agent for withdrawal-associated depressive and anhedonic symptoms.

Although our study did not directly investigate underlying mechanisms, the behavioral improvements observed with saffron suggest the involvement of multiple neurobiological pathways. Based on existing literature, several plausible mechanisms merit consideration. Saffron and its bioactive constituent crocin have been shown to modulate monoamine neurotransmission. For instance, behavioral improvements following saffron treatment in mice were associated with alterations in serotonergic and dopaminergic systems (28). Aqueous saffron extract in rats increased dopamine and glutamate release, without significantly affecting serotonin or noradrenaline under the experimental conditions (31). While this latter effect may be dose- or timing-dependent, it highlights the potential role of dopaminergic and glutamatergic modulation in saffron's antidepressant and neuromodulatory actions. Another well-established pharmacological property of saffron and crocin is their antioxidative activity. Oxidative stress is a key pathogenic mechanism in nicotine exposure and withdrawal. In a study of nicotine-induced neurodegeneration, crocin treatment ameliorated behavioral deficits and reduced oxidative stress markers—including malondialdehyde levels and increased activities of SOD, GPx, and GR—in the hippocampus and other brain regions (32). Thus, the attenuation of withdrawal-induced mood deficits by saffron may, at least in part, reflect its ability to counteract oxidative damage and restore redox homeostasis in vulnerable neural circuits.

Neuroinflammation is increasingly recognized as a key contributor to the pathophysiology of depression and withdrawal states. Saffron has demonstrated anti-inflammatory effects in multiple models, including stress-induced hippo-

campal dysfunction and colitis (33). In the chronic unpredictable mild stress (CUMS) paradigm, saffron reduced inflammatory cytokines and microglial activation, thereby restoring neuronal health and behavioral outcomes (29). In the context of nicotine withdrawal, saffron attenuates proinflammatory signaling and oxidative stress in mood-relevant brain regions, such as the hippocampus and prefrontal cortex, thereby preventing behavioral deficits.

While our findings are promising, several limitations should be acknowledged. We did not directly measure neurotransmitter levels, oxidative stress markers, or inflammatory cytokines; consequently, our mechanistic inferences rely primarily on analogy with prior literature. Future studies should quantify brain monoamines (dopamine, serotonin, norepinephrine), antioxidant enzyme activities, lipid peroxidation (MDA), proinflammatory cytokines (e.g., TNF-α, IL-1β), and neurotrophic factors such as BDNF. Our study focused exclusively on adolescent male rats; sex differences in nicotine sensitivity and saffron efficacy remain possible, and investigations in female subjects are warranted. Furthermore, adolescence represents a developmentally unique period, and whether similar effects occur in adults remains to be determined. Although the doses used here (50 and 100 mg/kg) were effective, the optimal therapeutic range, potential toxicity, and dose–response relationship require further exploration. Future research should aim to elucidate the precise neurobiological mechanisms underlying saffron's protective effects against nicotine withdrawal–induced anxiety, depression, and anhedonia. Mechanistic investigations into neurotransmitter systems, including serotonergic, dopaminergic, and GABAergic pathways, as well as neurotrophic factors like BDNF, will be essential to establish causal links. Given saffron and its bioactive constituents (e.g., crocin, safranal) possess potent antioxidant and anti-inflammatory properties, future studies should examine the contributions of these pathways to the observed behavioral improvements. Dose–response studies and chronic administration protocols will be important to optimize therapeutic efficacy while minimizing potential side effects. Finally, extending research beyond rodent models to other species and ultimately to human clinical trials will be necessary to confirm translational potential. Comparative studies of saffron versus existing pharmacotherapies for smoking cessation may also provide insight into its relative benefits as a stand-alone or adjunctive treatment.

Conclusion

Our findings provide strong evidence that saffron, particularly at 100 mg/kg, effectively mitigates nicotine withdrawal–induced anxiety, depressive-like behaviors, and anhedonia in adolescent male rats. These results complement and extend existing preclinical and clinical literature on saffron's mood-modulating and neuroprotective effects. Although further mechanistic studies and translational research are required, saffron emerges as a promising natural candidate for alleviating the affective components of nicotine withdrawal and may hold potential as an adjunctive intervention to support smoking cessation, especially in adolescent populations.

Acknowledgments

The authors would like to express their sincere gratitude to the Khatam Al-Nabieen University Medical Research and Technology Center for providing the scientific environment, institutional support, and research facilities that enabled the successful development of this study.

Founding

None.

Conflict of interest

The authors declare that there is no conflict of interests.

References

1. Nath A, Choudhari SG, Dakhode SU, Rannaware A, Gaidhane AM. Substance Abuse Amongst Adolescents: An Issue of Public Health Significance. Cureus. 2022;14(11):e31193.
2. Yuan M, Leslie FM. Nicotine and fluoxetine alter adolescent dopamine-mediated behaviors via 5-HT(1A) receptor activation. Front Psychiatry. 2024;15:1380123.
3. Motaghinejad M, Fatima S, Karimian M, Ganji S. Protective effects of forced exercise against nicotine-induced anxiety, depression and cognition impairment in rat. J Basic Clin Physiol Pharmacol. 2016;27(1):19-27.
4. Torabi M, Azizi H, Ahmadi-Soleimani SM, Rezayof A. Adolescent nicotine challenge promotes the future vulnerability to opioid addiction: Involvement of lateral paragigantocellularis neurons. Life Sci. 2019;234:116784.
5. Keeley RJ, Prillaman ME, Scarlata M, Vrana A, Tsai PJ, Gomez JL, et al. Adolescent nicotine administration increases nicotinic acetylcholine receptor binding and functional connectivity in specific cortico-striatal-thalamic circuits. Brain Commun. 2022;4(6):fcac291.
6. von Deneen KM, Hussain H, Waheed J, Xinwen W, Yu D, Yuan K. Comparison of frontostriatal circuits in adolescent nicotine addiction and internet gaming disorder. J Behav Addict. 2022;11(1):26-39.
7. Vázquez-Palacios G, Bonilla-Jaime H, Velázquez-Moctezuma J. Antidepressant effects of nicotine and fluoxetine in an animal model of depression induced by neonatal treatment with clomipramine. Prog Neuropsychopharmacol Biol Psychiatry. 2005;29(1):39-46.
8. Jobson CLM, Renard J, Szkudlarek H, Rosen LG, Pereira B, Wright DJ, et al. Adolescent Nicotine Exposure Induces Dysregulation of Mesocorticolimbic Activity States and Depressive and Anxiety-like Prefrontal Cortical Molecular Phenotypes Persisting into Adulthood. Cereb Cortex. 2019;29(7):3140-53.
9. Amiry GY, Haidary M, Azhdari-Zarmehri H, Beheshti F, Ahmadi-Soleimani SM. Omega-3 fatty acids prevent nicotine withdrawal-induced exacerbation of anxiety and depression by affecting oxidative stress balance, inflammatory response, BDNF and serotonin metabolism in rats. Eur J Pharmacol. 2023;947:175634.
10. Hossaini D, Alipour AK, Sajjadi M, Ansari M, Haidary M. Biotin Mitigates Alcohol Withdrawal-Induced Anxiety and Depression by Regulating Serotonin Metabolism, BDNF, Inflammation, and Oxidative Stress in Rats. Neuropsychopharmacol Rep. 2025;45(1):e12523.
11. Yammine L, Green CE, Kosten TR, de Dios C, Suchting R, Lane SD, et al. Exenatide Adjunct to Nicotine Patch Facilitates Smoking Cessation and May Reduce Post-Cessation Weight Gain: A Pilot Randomized Controlled Trial. Nicotine Tob Res. 2021;23(10):1682-90.
12. Ebrahimie M, Bahmani M, Shirzad H, Rafieian-Kopaei M, Saki K. A Review Study on the Effect of Iranian Herbal Medicines on Opioid Withdrawal Syndrome. J Evid Based Complementary Altern Med. 2015;20(4):302-9.
13. Moshiri M, Vahabzadeh M, Hosseinzadeh H. Clinical Applications of Saffron (Crocus sativus) and its Constituents: A Review. Drug Res (Stuttg). 2015;65(6):287-95.
14. Baba SA, Mohiuddin T, Basu S, Swarnkar MK, Malik AH, Wani ZA, et al. Comprehensive transcriptome analysis of Crocus sativus for discovery and expression of genes involved in apocarotenoid biosynthesis. BMC Genomics. 2015;16(1):698.
15. Committee for the Update of the Guide for the Care and Use of Laboratory Animals. Guide for laboratory animals. 2011. 8th Edition. The national Academic Press.
16. Hosseinzadeh H, Jahanian Z. Effect of Crocus sativus L. (saffron) stigma and its constituents, crocin and safranal, on morphine withdrawal syndrome in mice. Phytother Res. 2010;24(5):726-30.
17. Chellian R, Behnood-Rod A, Bruijnzeel DM, Wilson R, Pandy V, Bruijnzeel AW. Rodent models for nicotine withdrawal. J Psychopharmacol. 2021;35(10):1169-87.

18. Kraeuter AK, Guest PC, Sarnyai Z. The Open Field Test for Measuring Locomotor Activity and Anxiety-Like Behavior. Methods Mol Biol. 2019;1916:99-103.
19. Sturman O, Germain PL, Bohacek J. Exploratory rearing: a context- and stress-sensitive behavior recorded in the open-field test. Stress. 2018;21(5):443-52.
20. Delprato A, Algéo MP, Bonheur B, Bubier JA, Lu L, Williams RW, et al. QTL and systems genetics analysis of mouse grooming and behavioral responses to novelty in an open field. Genes Brain Behav. 2017;16(8):790-9.
21. Mesiakaris K, Atsopardi K, Lagoumintzis G, Margarity M, Poulas K. Cannabidiol Modulation of Nicotine-Induced Toxicity: Assessing Effects on Behavior, Brain-Derived Neurotrophic Factor, and Oxidative Stress in C57BL/6 Male Mice. J Neurosci Res. 2024;102(9):e25384.
22. Acikgoz B, Dalkiran B, Dayi A. An overview of the currency and usefulness of behavioral tests used from past to present to assess anxiety, social behavior and depression in rats and mice. Behav Processes. 2022;200:104670.
23. Estrada-Camarena E, Fernández-Guasti A, López-Rubalcava C. Antidepressant-like effect of different estrogenic compounds in the forced swimming test. Neuropsychopharmacology. 2003;28(5):830-8.
24. Liu MY, Yin CY, Zhu LJ, Zhu XH, Xu C, Luo CX, et al. Sucrose preference test for measurement of stress-induced anhedonia in mice. Nat Protoc. 2018;13(7):1686-98.
25. Pizzagalli DA. Depression, stress, and anhedonia: toward a synthesis and integrated model. Annu Rev Clin Psychol. 2014;10:393-423.
26. Hosseinzadeh H, Noraei NB. Anxiolytic and hypnotic effect of Crocus sativus aqueous extract and its constituents, crocin and safranal, in mice. Phytother Res. 2009;23(6):768-74.
27. Pontifex MG, Connell E, Le Gall G, Pourtau L, Gaudout D, Angeloni C, et al. Saffron extract (Safr'Inside™) improves anxiety related behaviour in a mouse model of low-grade inflammation through the modulation of the microbiota and gut derived metabolites. Food Funct. 2022;13(23):12219-33.
28. Monchaux De Oliveira C, Pourtau L, Vancassel S, Pouchieu C, Capuron L, Gaudout D, et al. Saffron Extract-Induced Improvement of Depressive-Like Behavior in Mice Is Associated with Modulation of Monoaminergic Neurotransmission. Nutrients. 2021;13(3).
29. Zhou J, Hu Y, Jing P. Antidepressant-Like Effect of Saffron (Crocus sativus L.) in Mice Exposed to Chronic Unpredictable Mild Stress via Attenuating Neuroinflammation and Recovering Neuroplasticity. Mol Nutr Food Res. 2025;69(21):e70201.
30. Abbaszade-Cheragheali A, Beheshti F, Kakhki S, Khatibi SR, Dehnokhalaji F, Akbari E, et al. Crocin, the main active saffron (Crocus sativus L.) constituent, as a potential candidate to prevent anxiety and depressive-like behaviors induced by unpredictable chronic mild stress. Neurosci Lett. 2022;791:136912.
31. Ettehadi H, Mojabi SN, Ranjbaran M, Shams J, Sahraei H, Hedayati M, et al. Aqueous extract of saffron (*Crocus sativus*) increases brain dopamine and glutamate concentrations in rats. Journal of Behavioral and Brain Science. 2013; 3(3): 315-19.
32. Motaghinejad M, Motevalian M, Sureda A. Preventive effects of crocin, a natural compound from saffron, against nicotine-induced oxidative stress and neurobehavioral disturbances. Acta Neurobiol Exp (Wars). 2024;84(1):59-69.
33. Rashid M, Rashid R, Saroya S, Deverapalli M, Brim H, Ashktorab H. Saffron as a Promising Therapy for Inflammatory Bowel Disease. Nutrients. 2024;16(14): 2353.