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Pinus gerardiana Nut Supplementation Mitigates Nicotine Withdrawal-Induced Behavioral and Neurochemical Disturbances in Adolescent Rats

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ABSTRACT

Background: We aimed to evaluate the effects of *Pinus gerardiana* nut supplementation on behavioral, neurochemical, oxidative, and neuroinflammatory alterations induced by nicotine withdrawal in adolescent rats.
Methods: Eighty male Wistar rats at postnatal day 21 (PND 21) were randomly assigned to eight experimental groups and treated with nicotine (2 mg/kg, s.c.) and diets supplemented with either 12% or 25% *P. gerardiana* during nicotine exposure and withdrawal. Anxiety- and depression-like behaviors were assessed using the Open Field Test (OFT), Elevated Plus Maze (EPM), Forced Swim Test (FST), and Sucrose Preference Test (SPT). Biochemical analyses of the prefrontal cortex included serotonergic biomarkers [serotonin, 5-hydroxyindoleacetic acid (5-HIAA), and monoamine oxidase A (MAO-A)], oxidative stress markers [malondialdehyde (MDA), total thiol (TT), superoxide dismutase (SOD), and catalase (CAT)], and neuroinflammatory and neurodegenerative biomarkers [glial fibrillary acidic protein (GFAP), brain-derived neurotrophic factor (BDNF), interleukin-10 (IL-10), and neuron-specific enolase (NSE)].
Results: Nicotine withdrawal induced significantly anxiety- and depression-like behaviors, impaired serotonergic function, increased oxidative stress, and neuroinflammatory alterations. Dietary supplementation with *P. gerardiana*, particularly at the 25% level, significantly ($P < 0.05$) improved behavioral performance, restored serotonin metabolism, enhanced antioxidant defenses, reduced MAO-A activity, and normalized neuroinflammatory and neurodegenerative biomarkers.
Discussion: *P. gerardiana* supplementation effectively alleviated the behavioral and neurobiological consequences of nicotine withdrawal in adolescent rats. These findings suggest that its neuroprotective effects are mediated through the combined modulation of serotonergic signaling, oxidative homeostasis, and neuroinflammatory pathways, supporting its potential as a natural dietary strategy for reducing nicotine withdrawal-related neurobehavioral dysfunction during adolescence.

Keywords: Nicotine withdrawal, *Pinus gerardiana*, Anxiety, Depression, Serotonin metabolism, Oxidative stress.

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Introduction

Nicotine consumption during adolescence poses a critical public health issue, primarily due to adolescents' heightened susceptibility to addiction and the prolonged detrimental effects of nicotine exposure at this stage of neurodevelopment (1). This period of neurodevelopment is marked by central nervous system (CNS) maturation and shifts in reward-related neurotransmission, which underlie adolescent tendencies for risk-taking and novelty-seeking (2-4). Repeated nicotine exposure reinforces reward circuitry, thereby enhancing its addictive liability across tobacco products (5). Adolescents also exhibit reduced sensitivity to nicotine's aversive effects, which may further explain its increased usage in this demographic (6). Numerous studies have shown a robust association between nicotine dependence and psychiatric disorders, particularly anxiety (7, 8) and depression (9, 10). Furthermore, the metabolism of nicotine produces reactive oxygen species (7), triggering oxidative stress that results in cellular injury. This cascade of oxidative damage is implicated in neurobehavioral disturbances such as anxiety, depression, neuroinflammation, and disrupted neurotransmission (9, 11, 12).

Adolescents are especially susceptible to the negative consequences of pharmacological therapies, largely due to the ongoing maturation of the brain. Such treatments may contribute to lasting neurobiological alterations and heightened risk of developing addiction (13). Furthermore, pharmacological agents can intensify behavioral disturbances—such as aggression and impulsivity—that are already commonly observed in adolescents struggling with substance use (14). These findings highlight the critical need for safe and targeted interventions that address the distinct neurodevelopmental and psychological vulnerabilities of adolescents. Interestingly, recent evidence suggests that consumption of certain nutrients may hold promise for preventing the development of depressive and anxiety-related psychopathologies, as well as supporting their treatment (15). In this regard, nuts have garnered attention due to their comprehensive nutritional profile. Nuts are known for their high fat content and richness in unsaturated fatty acids, phytosterols, and other health-promoting substances (16, 17). Among the different types of pine nuts, *Pinus gerardiana* stands out (18). *P. gerardiana* nuts are widely consumed in roasted form in many countries and are a rich source of nutrients and bioactive compounds, including flavonoids, phenols, terpenes, saponins, alkaloids, essential fatty acids, proteins, dietary fiber, starch, vitamins, and minerals such as manganese, potassium, calcium, iron, magnesium, zinc, and selenium (19). These diverse nutritional constituents contribute to the remarkable biological and pharmacological properties of *P. gerardiana* (20). Despite its therapeutic potential, the effects of *P. gerardiana* nuts on behavioral and neurochemical alterations associated with nicotine withdrawal have not yet been investigated (21). Therefore, we aimed to determine whether dietary supplementation with *P. gerardiana* nuts could alleviate nicotine withdrawal-induced anxiety- and depression-like behaviors in male rats. Additionally, we examined their effects on serotonin metabolism, oxidative stress, and neuroinflammatory responses to provide insight into the underlying mechanisms involved.

Materials and Methods

Animals

A total of 80 male Wistar rats at postnatal day 21 (PND 21) were obtained from the Animal Research Center of Ghalib University. Upon arrival, the animals were randomly housed in transparent Plexiglas cages, with three to four rats per cage, under standard laboratory conditions. The animal room was maintained at 22 ± 2 °C with a relative humidity of 55–65% under a 12-h light/12-h dark cycle (lights on

at 06:00 h). Throughout the experiment, the rats had free access to food and water. Standard laboratory chow (Javaneh Khorasan, Mashhad, Iran), containing 46% nanofibrillated cellulose, 25% neutral detergent fiber, 19% protein, and 10% fat, was provided ad libitum. All experimental procedures in accordance with the Guide for the Care and Use of Laboratory Animals (22).

Drugs and Experimental Procedure

Nicotine base (Sigma-Aldrich, USA) was freshly dissolved in sterile 0.9% saline immediately before administration and injected at a final volume of 1 mL. To induce chronic nicotine exposure, rats received once-daily subcutaneous (s.c.) injections of nicotine at a dose of 2 mg/kg throughout the experimental period (1, 9). Raw *P. gerardiana* nuts were sourced from the Chardehi district of Laghman Province, Afghanistan. The nuts were ground into a fine powder and thoroughly mixed with standard rodent feed powder. The modified chow was then reformed into its original pellet shape (18) and provided ad libitum to the intervention groups.

The study included eight experimental groups, each consisting of ten adolescent rats, to examine the preventive and therapeutic effects of *P. gerardiana* during nicotine exposure and withdrawal. The experimental protocol comprised two consecutive phases: a nicotine exposure period (PND 21–42) followed by a post-exposure/withdrawal period (PND 42–63). Control animals (Vehicle–Vehicle) received saline throughout the entire study. The nicotine withdrawal group (Nicotine–Vehicle) was administered nicotine (2 mg/kg, s.c.) during the exposure phase and subsequently received saline during the withdrawal phase. To evaluate the therapeutic efficacy of *P. gerardiana* after nicotine cessation, two treatment groups received nicotine during PND 21–42 followed by dietary supplementation with *P. gerardiana* at concentrations of 12% or 25%, respectively, throughout PND 42–63 (Nicotine–*P. gerardiana* 12% and Nicotine–*P. gerardiana* 25%). To investigate the preventive potential of the plant, two additional groups were treated concurrently with nicotine and *P. gerardiana* (12% or 25%) during the exposure period and then maintained on saline alone during withdrawal (Nicotine + *P. gerardiana*–Vehicle). A separate group received only a 25% *P. gerardiana*-supplemented diet during PND 21–42, followed by the standard diet for the remainder of the experiment, allowing assessment of the independent effects of the plant (*P. gerardiana*–Vehicle). Finally, the delayed-treatment group (Vehicle–*P. gerardiana*) consumed the standard diet during the nicotine exposure phase and was switched to the 25% *P. gerardiana*-supplemented diet only during the withdrawal period to determine the effectiveness of post-exposure intervention. The selected dietary concentrations of *P. gerardiana* (12% and 25%) were based on previous studies demonstrating their biological efficacy (23).

Behavioral assessments

Behavioral testing was conducted 21 days after the final nicotine administration, corresponding to the intermediate stage of nicotine withdrawal, which is characterized by marked emotional and cognitive disturbances in adolescent rats. Anxiety-like behaviors are minimal or absent during the first week after nicotine cessation (24). Therefore, this time point was selected to optimally assess withdrawal-induced behavioral alterations. Anxiety- and depression-like behaviors were evaluated using a battery of validated behavioral paradigms, including the Open Field Test (OFT), Elevated Plus Maze (EPM), Forced Swim Test (FST), and Sucrose Preference Test (SPT). Before each test, the animals were allowed to acclimate to the testing room for 30 min to minimize stress associated with handling and transportation. Illumination was adjusted according to the requirements of each behavioral assay, with light intensity maintained at 40 lux for the OFT and 150 lux for the EPM. To minimize potential confounding effects of olfactory and social cues, rats that had completed testing were housed separately from those awaiting assessment, and all apparatuses were thoroughly cleaned with 10% ethanol between trials.

Open Field Test

The OFT is a widely used behavioral assay for evaluating anxiety-like behavior and the efficacy of anxiolytic interventions in rodents (25). The test exploits the natural conflict between an animal's innate drive to explore a novel environment and its aversion to open, exposed spaces. Anxiety-like behavior is primarily assessed by the time spent in the center of the arena, with increased central exploration reflecting reduced anxiety (26-28). The OFT was performed in a square arena (100 × 100 × 40 cm) constructed of opaque, non-reflective material to minimize external visual cues. At the start of each trial, each rat was gently placed in the center of the arena and allowed to explore freely for 5 min. This duration has provided reliable measurements of exploratory and anxiety-related behaviors while minimizing habituation to the testing environment (29). In addition, the initial 5-min exposure is considered optimal for detecting novelty-induced behavioral responses and evaluating the effects of pharmacological or dietary interventions (30). Animal behavior was recorded using an automated video-tracking system, and anxiety-like behavior was quantified by measuring the time spent in the central and peripheral zones of the arena.

Elevated Plus Maze

Following the Open Field Test, anxiety-like behavior was further assessed using the EPM, a widely accepted and extensively validated model for evaluating anxiety-related behavior in rodents (31). The EPM consisted of a plus-shaped apparatus elevated 50 cm above the floor, with two opposing open arms and two opposing enclosed arms surrounded by 40-cm-high walls. The test relies on rodents' natural tendency to avoid elevated, unprotected spaces while simultaneously exploring unfamiliar environments. Consequently, increased exploration of the open arms is interpreted as reduced anxiety, whereas preference for the enclosed arms reflects heightened anxiety-like behavior. In this study, the maze was constructed from matte gray, non-reflective wood to minimize glare and external visual cues. At the beginning of each trial, individual rats were placed on the central platform facing an open arm and allowed to explore the maze freely for 5 min under dim lighting. Animal movements were continuously recorded using an automated video-tracking system, and anxiety-like behavior was evaluated by quantifying the time spent in the open and closed arms. These behavioral measures were used to determine the potential anxiolytic effects of *P. gerardiana* supplementation during nicotine exposure and withdrawal in adolescent rats.

Forced Swimming Test

Depression-like behavior was assessed using the FST, a widely used behavioral assay for evaluating behavioral despair and the antidepressant potential of therapeutic interventions in rodents (32). The test is based on the behavioral responses of rodents when exposed to an inescapable water-filled environment, where the balance between active and passive coping strategies serves as an indicator of emotional state. Each rat was individually placed in a transparent glass cylinder (50 cm high, 20 cm in diameter) filled with water to a depth of 30 cm (24 ± 2 °C), preventing the animals from touching the bottom with their hind limbs. Behavior was video-recorded for 5 min and analyzed offline. Three behavioral parameters were evaluated: struggling, defined as vigorous limb movements accompanied by escape-directed climbing attempts; swimming, characterized by active horizontal movement without attempts to escape; and immobility, during which the animal remained almost motionless, performing only the minimal movements necessary to keep its head above the water. Increased immobility together with reduced active behaviors (struggling and swimming) was interpreted as an indicator of depression-like behavior. These behavioral measures were used to evaluate the potential antidepressant effects of *P. gerardiana* supplementation during nicotine exposure and withdrawal.

Sucrose Preference Test

Anhedonia-like behavior was evaluated using the SPT, a widely used method for assessing reward sensitivity in rodents (33). The test is based on the natural preference of healthy rodents for a sweet sucrose solution over plain water. A decline in sucrose consumption reflects reduced sensitivity to rewarding stimuli and is therefore widely recognized as a behavioral marker of anhedonia, a hallmark symptom of depression (34). Accordingly, the SPT is commonly employed to investigate depression- and addiction-related behavioral changes in experimental animal models (35). Before the preference test, the animals underwent a 4-day habituation protocol to minimize bottle-position bias and acclimate them to the sucrose solution. During the first two days, two identical bottles containing tap water were provided. On the third day, both bottles were replaced with bottles containing a 1% sucrose solution to familiarize the rats with its taste. After an overnight (12 h) period of food and water deprivation, the preference test was performed on the fourth day by simultaneously presenting one bottle containing 1% sucrose solution and another containing plain water. Fluid intake from each bottle was measured after 12 h of free access. Sucrose preference was calculated using the following formula: Sucrose Preference (%) = [Sucrose intake / (Sucrose intake + Water intake)] × 100 (36). Lower sucrose preference values were interpreted as reduced reward sensitivity and increased anhedonia-like behavior.

Euthanasia

Upon completion of the behavioral assessments, the animals were euthanized using a gradual-fill carbon dioxide (CO_2) protocol, in which 95% CO_2 was introduced into the euthanasia chamber at a displacement rate of approximately 30% of the chamber volume per minute, in accordance with established guidelines (37). Death was confirmed before tissue collection. The brains were rapidly removed, and the prefrontal cortex (PFC) was carefully dissected, snap-frozen in liquid nitrogen, and stored at an appropriate temperature until biochemical analyses. PFC samples were subsequently analyzed for a range of neurochemical, oxidative stress, neuroinflammatory, and neurodegenerative biomarkers, including serotonin, 5-hydroxyindoleacetic acid (5-HIAA), monoamine oxidase A (MAO-A) activity, malondialdehyde (MDA), total thiol (TT) content, superoxide dismutase (SOD), catalase (CAT), brain-derived neurotrophic factor (BDNF), glial fibrillary acidic protein (GFAP), interleukin-10 (IL-10), and neuron-specific enolase (NSE).

Biochemical Measurements

Measurement of Serotonin, MAO-A Activity, and 5-HIAA

Neurochemical analyses of the prefrontal cortex focused on key components of the serotonergic system. Serotonin concentrations were quantified using a commercially available ELISA kit (Cat. No. MBS713292; MyBioSource, San Diego, CA, USA). The enzymatic activity of monoamine oxidase A (MAO-A), a major enzyme involved in serotonin metabolism, was determined using a dedicated ELISA kit from the same manufacturer (Cat. No. MBS721413). In addition, concentrations of 5-hydroxyindoleacetic acid (5-HIAA), the primary metabolite of serotonin, were measured with a corresponding ELISA kit (Cat. No. MBS700811; MyBioSource, San Diego, CA, USA). All assays were performed according to the manufacturer's recommended protocols.

Quantification of MDA, SOD, TT, and CAT

Lipid peroxidation in the prefrontal cortex was assessed by quantifying malondialdehyde (MDA) using the thiobarbituric acid reactive substances (TBARS) assay. Briefly, 0.5 mL of tissue homogenate supernatant was combined with 2.5 mL of 20% trichloroacetic acid (TCA) and 1.0 mL of 0.67% thiobarbituric acid (TBA) (Sigma-Aldrich, St. Louis, MO, USA). The reaction mixture was heated in a boiling water bath (95 °C) for 30 min to facilitate formation of the MDA–TBA adduct. After cooling to room temperature, the

samples were centrifuged at 3,000 rpm for 10 min, and the absorbance of the clear supernatant was measured at 532 nm with a UV–Vis spectrophotometer (Shimadzu UV-1600, Kyoto, Japan). MDA concentrations were calculated from the absorbance values and expressed as nmol/mg tissue (38, 39).

Total thiol (TT) content was measured using Ellman's colorimetric assay with 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB; Sigma-Aldrich, St. Louis, MO, USA). In brief, 50 µL of tissue homogenate was mixed with 1 mL of Tris-EDTA buffer (pH 8.6), followed by the addition of 50 µL of 10 mM DTNB. After incubation at room temperature for 15 min, absorbance was recorded at 412 nm. Thiol concentrations were calculated using the DTNB molar extinction coefficient (13,600 M^{-1} cm^{-1}) and normalized to protein content (µmol/mg protein) (40).

SOD activity was determined using a commercial colorimetric assay kit (Cayman Chemical, Cat. No. 706002) according to the manufacturer's instructions. This assay measures the ability of SOD to inhibit the reduction of the tetrazolium salt WST-1 by superoxide radicals, thereby decreasing the formation of a water-soluble formazan product detected at 450 nm. Enzyme activity was calculated from a standard calibration curve, with one unit of SOD defined as the amount of enzyme required to inhibit color development by 50%. Catalase (CAT) activity was evaluated using the spectrophotometric method described by Aebi (1984). Briefly, 50 µL of tissue homogenate was added to 2.95 mL of freshly prepared 30 mM hydrogen peroxide (H_2O_2; Sigma-Aldrich) in 50 mM phosphate buffer (pH 7.0). Catalase activity was determined by monitoring the decrease in H_2O_2 absorbance at 240 nm over a 1-min period and was expressed as µmol H_2O_2 decomposed per minute per milligram of protein

Measurement of Neuroinflammatory and Neurodegenerative Markers

To evaluate neuroinflammatory responses, neuroprotective signaling, and neuronal injury, selected protein biomarkers were measured in prefrontal cortex (PFC) homogenates using commercially available ELISA kits in accordance with the manufacturers' instructions. Glial fibrillary acidic protein (GFAP), an indicator of astrocyte activation, was quantified using a MyBioSource ELISA kit (Cat. No. MBS042687, San Diego, CA, USA). Brain-derived neurotrophic factor (BDNF), a key regulator of neuronal survival and synaptic plasticity, was measured with a rat-specific ELISA kit from CUSABIO (Cat. No. CSB-E04504r, Wuhan, China). Levels of the anti-inflammatory cytokine interleukin-10 (IL-10) were determined using a MyBioSource ELISA kit (Cat. No. MBS2021530, San Diego, CA, USA), while neuron-specific enolase (NSE), a biomarker of neuronal injury, was quantified with the corresponding MyBioSource kit (Cat. No. MBS729794, San Diego, CA, USA). Optical density was recorded at the wavelength recommended for each assay using a UV–Vis spectrophotometer (Shimadzu UV-1600, Kyoto, Japan), and protein concentrations were calculated from the respective standard calibration curves.

Statistical Analyses

All statistical analyses were performed using GraphPad Prism software (version 8.4.3; GraphPad Software, San Diego, CA, USA). Group differences were analyzed by one-way analysis of variance (ANOVA), and significant main effects were further examined using Tukey's multiple-comparison post hoc test. Results are expressed as mean ± standard error of the mean (SEM). A two-tailed *P* value of less than 0.05 was considered statistically significant.

Results

P. gerardiana Supplementation Alleviates Nicotine Withdrawal-Induced Anxiety-Like Behaviors

Nicotine withdrawal markedly increased anxiety-like behavior in the OFT. Compared with vehicle-treated controls, nicotine-withdrawn rats spent significantly less time in the center of the arena and exhibited greater peripheral exploration ($P < 0.001$; Fig. 1A, B). These behavioral alterations were

substantially attenuated by dietary supplementation with 25% *P. gerardiana* during both the nicotine exposure and withdrawal phases. Relative to the nicotine group, treated animals showed a significant increase in center occupancy together with a corresponding reduction in peripheral activity ($P < 0.01$ and $P < 0.001$, respectively; Fig. 1A,B). Administration of *P. gerardiana* alone from PND 42 to PND 63 also promoted exploratory behavior, as reflected by increased time spent in the central zone compared with vehicle-treated rats ($P < 0.05$; Fig. 1A), without affecting peripheral exploration (Fig. 1B).

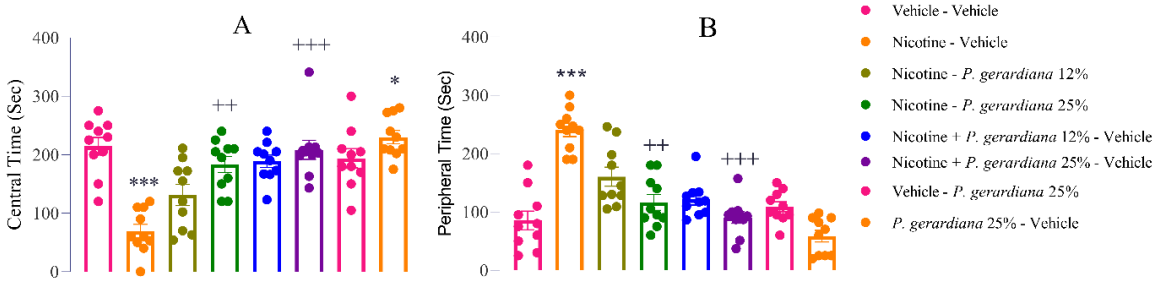


Figure 1: Effects of *P. gerardiana* on Anxiety-Related Behaviors in the Open Field Test (OFT) (A) Time spent in the central area of the arena; (B) Time spent in the peripheral area. Data are expressed as mean ± SEM (n =10). Significant differences: *p < 0.05 and ***p < 0.001 compared to the vehicle–vehicle group; ++*P* < 0.01, and +++*P* < 0.001 compared to the nicotine–vehicle group

A comparable pattern was observed in the EPM. Withdrawal from nicotine significantly reduced open-arm exploration while increasing time spent in the closed arms relative to the vehicle group ($P < 0.001$; Fig. 2A,B), further supporting the development of an anxiety-like phenotype. In contrast, rats receiving 25% *P. gerardiana* during nicotine exposure and withdrawal displayed a significant increase in open-arm occupancy accompanied by a decrease in closed-arm preference compared with nicotine-withdrawn animals ($P < 0.01$ and $P < 0.001$, respectively; Fig. 2A,B). Moreover, treatment with *P. gerardiana* alone enhanced open-arm exploration compared with vehicle-treated controls ($P < 0.05$; Fig. 2A).

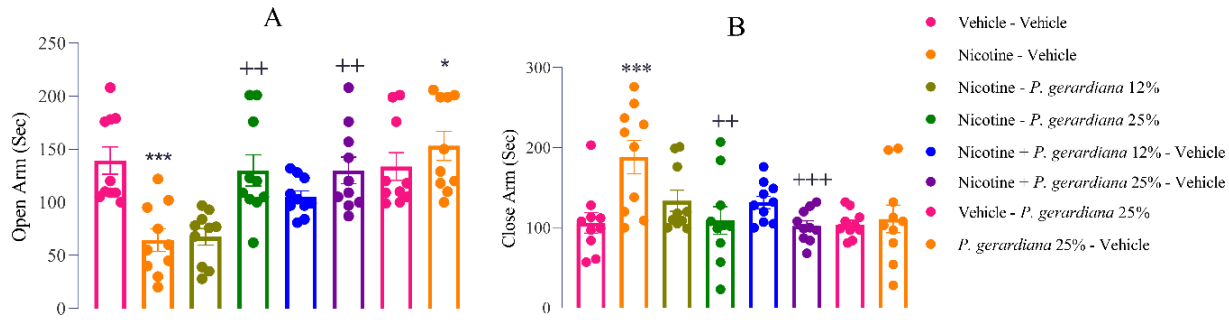


Figure 2: Effects of *P. gerardiana* on Anxiety-Related Behaviors in the Elevated Plus Maze (EPM) (A) Time spent in the open arms; (B) Time spent in the closed arms. Data are shown as mean ± SEM (n =10). Significant differences: **P* < 0.05 and ***P* < 0.001 compared to the vehicle–vehicle group; ++*P* < 0.01, and +++*P* < 0.001 compared to the nicotine–vehicle group

P. gerardiana Supplementation Ameliorates Nicotine Withdrawal-Induced Depression-Like Behaviors

Behavioral performance in the FST revealed a clear depression-like phenotype following nicotine withdrawal. Compared with vehicle-

treated controls, nicotine-withdrawn rats exhibited significantly shorter struggling and swimming durations, accompanied by a marked increase in immobility time ($P < 0.001$; Fig. 3A–C). These withdrawal-induced behavioral impairments were significantly alleviated by dietary supplementation with 25% *P. gerardiana* during both nicotine exposure and withdrawal. Relative to the nicotine group, treated animals displayed greater struggling and swimming activity together with a significant reduction in immobility ($P < 0.01$ and $P < 0.001$; Fig. 3A–C). In contrast, administration of *P. gerardiana* alone had no detectable effect on any FST parameter, with behavioral performance remaining comparable to that of the vehicle-treated group (Fig. 3A–C).

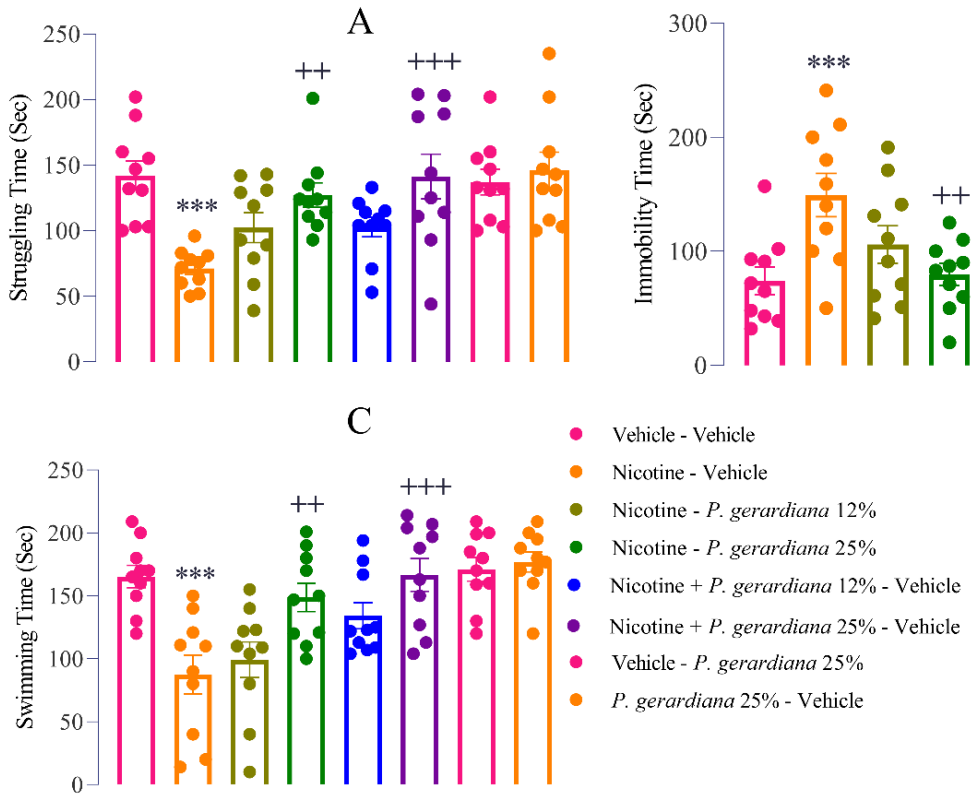


Figure 3: Effects of P. gerardiana on Depressive-Like Behaviors in the Forced Swimming Test (A) Time spent struggling; (B) Time spent immobile; (C) Time spent swimming. Data are represented as mean ± SEM (n =10). Significant differences: ***$P < 0.001$ compared to the vehicle–vehicle group; ++$P < 0.01$, and +++$P < 0.001$ compared to the nicotine–vehicle group

P. gerardiana Supplementation Ameliorates Nicotine Withdrawal-Induced Anhedonia

Findings from the SPT showed that nicotine withdrawal markedly impaired reward-related behavior. Compared with vehicle-treated controls, nicotine-withdrawn rats exhibited a significant reduction in sucrose preference, indicative of an anhedonia-like state ($P < 0.001$; Fig. 4). Supplementation with 25% *P. gerardiana* throughout nicotine exposure and withdrawal significantly reversed this deficit, resulting in higher sucrose preference than that observed in the nicotine group ($P < 0.01$ and $P < 0.001$; Fig. 4). Similarly, rats receiving 25% *P. gerardiana* alone from PND 42 to PND 63 displayed a significant increase in sucrose preference compared with vehicle-treated animals ($P < 0.05$; Fig. 4).

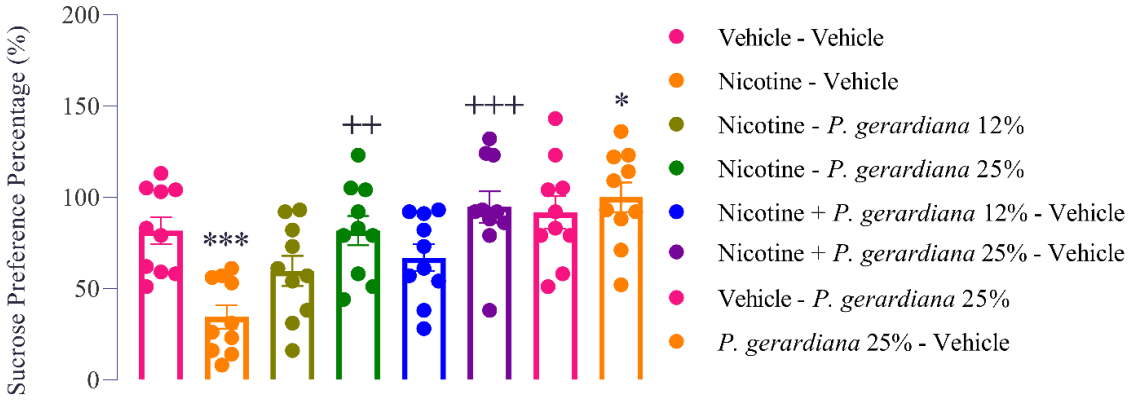


Figure 4: Effects of *P. gerardiana* on Anhedonia-Like Behaviors in the Sucrose Preference Test (SPT). Data are shown as mean ± SEM (n =10). Significant differences: **P* < 0.05 and ****P* < 0.001 compared to the vehicle–vehicle group; ++*P* < 0.01 and +++*P* < 0.001 compared to the nicotine–vehicle group

P. gerardiana Supplementation Restores Serotonergic Neurotransmission Following Nicotine Withdrawal

Analysis of serotonergic biomarkers revealed pronounced neurochemical disturbances following nicotine withdrawal. Compared with vehicle-treated controls, nicotine-withdrawn rats exhibited significantly lower serotonin and 5-hydroxyindoleacetic acid (5-HIAA) levels, accompanied by a marked elevation in monoamine oxidase A (MAO-A) activity (*P* < 0.001; Fig. 5A–C). Supplementation with 12% *P. gerardiana* during the nicotine exposure period partially mitigated these alterations by significantly increasing serotonin concentrations relative to the nicotine group (*P* < 0.05; Fig. 5A).

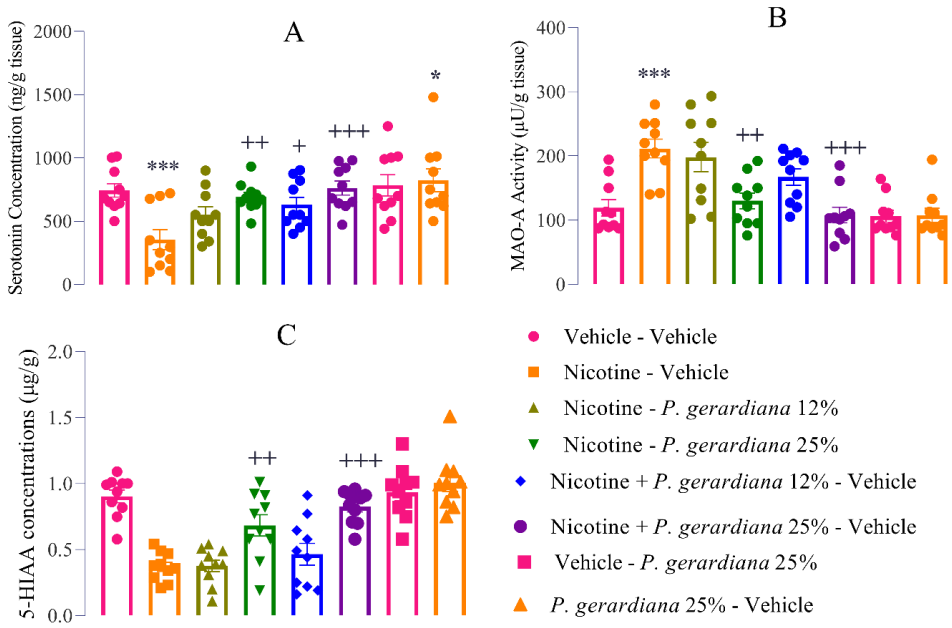


Figure 5: Effects of *P. gerardiana* on Cortical Serotonin Metabolism (A) Serotonin levels; (B) Monoamine oxidase-A (MAO-A) activity; and (C) 5-hydroxyindoleacetic acid (5-HIAA). Data are expressed as mean ± SEM (n =10). Significant differences: **P* < 0.05 and ****P* < 0.001 compared to the vehicle–vehicle group; +*P* < 0.05, ++*P* < 0.01, and +++*P* < 0.001 compared to the nicotine–vehicle group

A more pronounced effect was observed with 25% *P. gerardiana* administered throughout both the exposure and withdrawal phases, which significantly restored serotonin and 5-HIAA levels while suppressing MAO-A activity compared with nicotine-withdrawn animals ($P < 0.01$ and $P < 0.001$; Fig. 5A–C). Likewise, treatment with 25% *P. gerardiana* alone from PND 42 to PND 63 significantly increased serotonin concentrations compared with vehicle-treated rats ($P < 0.05$; Fig. 5A).

P. gerardiana Supplementation Attenuates Nicotine Withdrawal-Induced Oxidative Stress

Assessment of oxidative stress biomarkers demonstrated that nicotine withdrawal profoundly disturbed redox homeostasis in the prefrontal cortex. Relative to vehicle-treated controls, nicotine-withdrawn rats exhibited significantly elevated malondialdehyde (MDA) levels together with marked reductions in total thiol (TT) content, superoxide dismutase (SOD) activity, and catalase (CAT) activity ($P < 0.001$; Fig. 6A–D). Supplementation with 12% *P. gerardiana* during the nicotine exposure period partially attenuated oxidative damage by significantly lowering MDA concentrations compared with the nicotine group ($P < 0.05$; Fig. 7A). A greater protective effect was observed in rats receiving 25% *P. gerardiana* throughout nicotine exposure and withdrawal, which significantly reduced MDA levels while restoring TT content and enhancing the activities of SOD and CAT relative to nicotine-withdrawn animals ($P < 0.05$, $P < 0.01$, and $P < 0.001$; Fig. 6A–D). Similarly, treatment with 25% *P. gerardiana* alone from PND 42 to PND 63 promoted a more favorable redox profile than that of vehicle-treated controls, as reflected by lower MDA concentrations and higher TT levels, SOD activity, and CAT activity ($P < 0.05$; Fig. 6A–D).

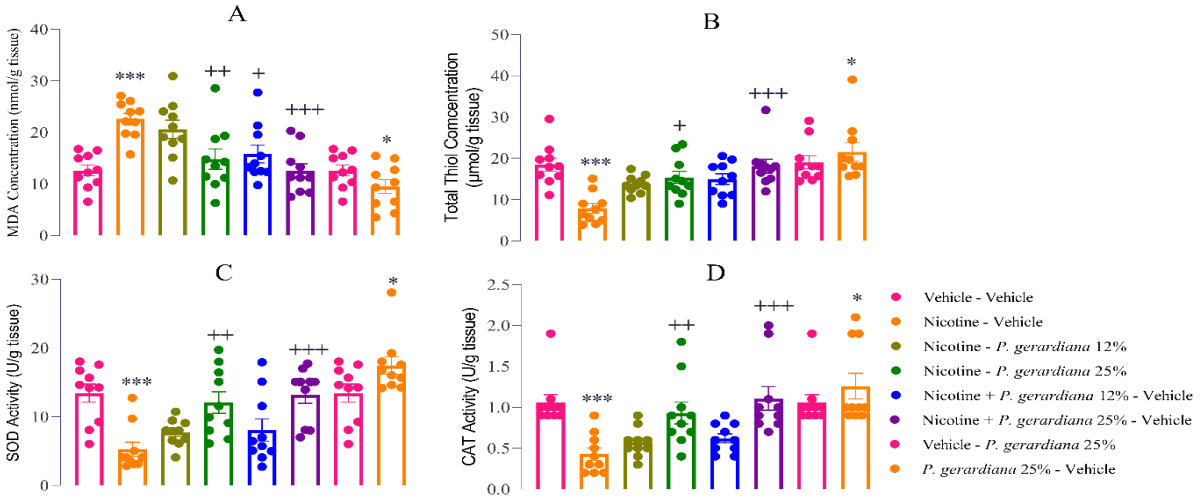


Figure 6: Effects of *P. gerardiana* on Oxidative Stress Markers in the Prefrontal Cortex
(A) Malondialdehyde (MDA) levels; (B) Total thiol (TT) levels; (C) Superoxide dismutase (SOD) activity; (D) Catalase (CAT) activity. Data are presented as mean ± SEM (n =10). Significant differences: *$P < 0.05$ and ***$P < 0.001$ compared to the vehicle–vehicle group; +$P < 0.05$, ++$P < 0.01$, and +++$P < 0.001$ compared to the nicotine–vehicle group

P. gerardiana Supplementation Mitigates Neuroinflammatory and Neurodegenerative Alterations Induced by Nicotine Withdrawal

Evaluation of neuroinflammatory and neurodegenerative biomarkers revealed substantial pathological changes in the prefrontal cortex following nicotine withdrawal. Compared with vehicle-treated controls, nicotine-withdrawn rats exhibited significantly elevated glial fibrillary acidic protein (GFAP) and neuron-specific enolase (NSE) levels,

together with marked reductions in brain-derived neurotrophic factor (BDNF) and interleukin-10 (IL-10) concentrations ($P < 0.001$; Fig. 7A–D). These alterations were significantly ameliorated by supplementation with 25% *P. gerardiana* during both the nicotine exposure and withdrawal phases. Relative to the nicotine group, treated animals showed lower GFAP and NSE levels, accompanied by significant increases in BDNF and IL-10 ($P < 0.05$, $P < 0.01$, and $P < 0.001$; Fig. 7A–D). By contrast, administration of 25% *P. gerardiana* alone did not significantly affect any of the measured biomarkers, with values remaining comparable to those of the vehicle-treated group (Fig. 7A–D).

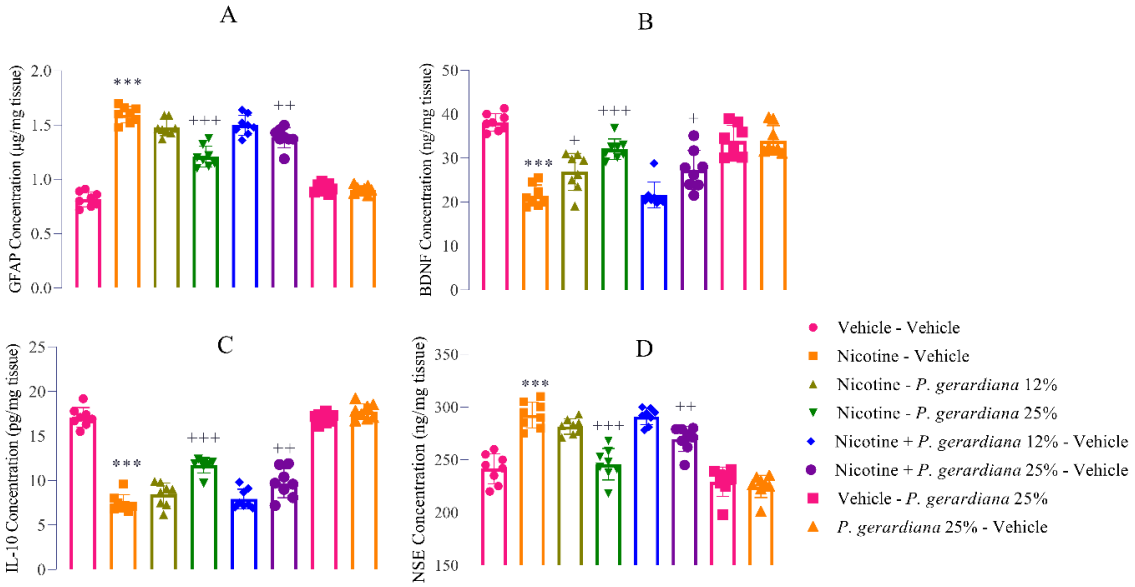


Figure 7: *Effects of P. gerardiana on Neuroinflammatory and Neurodegenerative Markers*
(A) GFAP levels; (B) BDNF levels; (C) IL-10 levels; (D) NSE levels. Data are expressed as mean ± SEM (n = 8). Significant differences: ***$P < 0.001$ vs. vehicle group; +$P < 0.05$, ++$P < 0.01$, +++$P < 0.001$ vs. nicotine group

Discussion

Nicotine exposure during adolescence has profound and lasting effects on brain function, particularly during withdrawal, when behavioral and neurochemical disturbances become most evident (42, 1). Although these adverse consequences are well recognized, nicotine consumption remains highly prevalent among adolescents, making this developmental period a critical target for intervention (42). The intensity of withdrawal symptoms in rodents depends largely on the dose and duration of nicotine exposure, with relatively low doses often failing to elicit pronounced anxiety-like behaviors (24). In contrast, chronic exposure to higher nicotine doses during adolescence produces persistent neurobehavioral alterations that extend into adulthood, increasing vulnerability to addictive behaviors and impairing reward processing (43, 44). The behavioral abnormalities associated with nicotine withdrawal are accompanied by profound neurochemical disturbances. Reductions in serotonergic neurotransmission, impaired serotonin receptor function, and dysregulation of intracellular signaling pathways, including those involving adenylyl cyclase was reported (45). In addition, adolescent nicotine administration has decreased the serotonin concentrations (44) alter serotonin receptor activity, and impair adenylyl cyclase-mediated signaling (46). Beyond these neurochemical alterations, nicotine exposure during adolescence has also been linked to structural changes in the developing brain, including reduced total gray matter volume, atrophy of the medial prefrontal cortex (mPFC), and impaired structural connectivity between the mPFC

and other cortical regions (47). These observations emphasize the heightened vulnerability of the adolescent brain to nicotine-induced neurotoxicity. Disruption of normal neurodevelopment during this critical period may lead to persistent changes in neurotransmission, brain structure, and emotional regulation, thereby increasing the likelihood of developing neuropsychiatric disorders later in life (44).

Here in, adolescent rats were administered nicotine subcutaneously at a dose of 2 mg/kg to establish a robust and reproducible model of nicotine withdrawal. Although this dose may appear relatively high, previous preclinical studies have demonstrated that comparable dosing regimens are necessary to produce consistent behavioral and neurochemical manifestations of nicotine dependence and spontaneous withdrawal in rodents (1, 9, 48). Such dosing differences largely reflect species-specific pharmacokinetic and pharmacodynamic characteristics. Compared with humans, rats metabolize nicotine more rapidly, exhibit a shorter plasma half-life (approximately 1 h versus 2 h in humans), and differ in nicotinic acetylcholine receptor sensitivity, thereby requiring higher nicotine doses to achieve equivalent systemic exposure and receptor occupancy. Consequently, lower doses frequently fail to induce reliable withdrawal-associated phenotypes, including anxiety-like behavior, hyperalgesia, and cognitive deficits, in both adolescent and adult rodents (24). Despite the utility of rodent models for investigating the neurobiological basis of nicotine dependence, nicotine withdrawal in humans is considerably more complex and is shaped by the interaction of biological, psychological, and social factors (26, 49). In addition to neuroadaptive changes within reward-related neural circuits (50), withdrawal symptoms are strongly influenced by emotional distress, mood disturbances, conditioned behavioral responses, and habitual smoking patterns (49, 51). The dynamic interplay among these biological and psychosocial processes ultimately determines the severity of withdrawal symptoms and contributes to continued nicotine use and relapse (52, 53).

Consistent with previous reports, nicotine withdrawal in the present study produced marked anxiety-like behavior, characterized by reduced exploration of the center area in the OFT and decreased time spent in the open arms of the EPM. These behavioral alterations are thought to arise from withdrawal-induced disturbances in stress-regulating neurotransmitter systems, particularly the serotonergic pathway (37). In the OFT, the extent of central zone exploration is widely regarded as an index of anxiety, with greater time spent in the center reflecting lower anxiety and enhanced exploratory motivation. In contrast, anxious animals preferentially remain close to the walls of the arena, a characteristic behavior known as thigmotaxis that represents an innate defensive strategy to avoid perceived threats (54). Likewise, the EPM exploits rodents' natural aversion to elevated and unprotected environments. Increased exploration of the open arms is indicative of reduced anxiety, whereas prolonged occupancy of the enclosed arms reflects heightened anxiety and behavioral inhibition (55). The concordant findings obtained from both paradigms therefore provide robust evidence that nicotine withdrawal induces a pronounced anxiogenic state in adolescent rats.

Notably, dietary supplementation with 25% *P. gerardiana* markedly attenuated these anxiety-like behaviors, as demonstrated by increased exploration of the anxiogenic regions in both the OFT and EPM. These findings suggest that *P. gerardiana* exerts significant anxiolytic effects, which may be attributed to its ability to modulate serotonergic neurotransmission while simultaneously reducing oxidative stress and neuroinflammation. The observation that *P. gerardiana* administered alone also enhanced central zone exploration in the OFT and increased open-arm exploration in the EPM further supports its intrinsic anxiolytic properties. Collectively, these results indicate that the behavioral benefits of *P. gerardiana* are likely mediated through the combined regulation of serotonergic signaling and attenuation of

oxidative and inflammatory processes that contribute to anxiety during nicotine withdrawal.

In the FST, nicotine withdrawal produced a characteristic depression-like phenotype, as reflected by increased immobility together with reduced struggling and swimming behaviors. Increased immobility in the FST is widely recognized as an index of behavioral despair and diminished motivation to escape, whereas reductions in immobility accompanied by greater active behaviors are generally interpreted as antidepressant-like effects (56). Dietary supplementation with *P. gerardiana* throughout both the nicotine exposure and withdrawal periods significantly reversed these behavioral abnormalities by decreasing immobility and restoring active coping behaviors. These findings suggest that *P. gerardiana* exerts antidepressant-like effects, potentially through restoration of serotonergic neurotransmission, attenuation of oxidative stress, and suppression of neuroinflammatory processes, all of which are critically involved in the regulation of mood and emotional behavior. Notably, *P. gerardiana* administered alone did not significantly alter FST performance, indicating that its antidepressant effects may become most apparent under pathological conditions such as nicotine withdrawal rather than in healthy animals.

The SPT further demonstrated that nicotine withdrawal markedly impaired reward-related behavior, as evidenced by a significant reduction in sucrose preference, a well-established indicator of anhedonia. Anhedonia represents a core feature of depressive disorders and is commonly induced in experimental models by chronic stress and substance withdrawal (57, 58). Supplementation with *P. gerardiana* effectively restored sucrose preference in nicotine-withdrawn rats, indicating recovery of reward sensitivity. Moreover, *P. gerardiana* also enhanced sucrose preference in animals not exposed to nicotine withdrawal, suggesting an intrinsic capacity to facilitate reward processing. These behavioral improvements are consistent with the ability of *P. gerardiana* to modulate serotonergic signaling and reduce oxidative stress, both of which are fundamental regulators of mesolimbic reward pathways and hedonic processing (59, 60).

The neuropsychological effects of *P. gerardiana* have received little attention, and its anxiolytic and antidepressant potential has not been extensively characterized. Nevertheless, the beneficial behavioral effects observed in the present study may be attributable, at least in part, to the high content of unsaturated fatty acids in *P. gerardiana* kernels (61, 62). A growing body of evidence indicates that polyunsaturated fatty acids (PUFAs) play a fundamental role in maintaining normal brain function and cognitive performance (63, 64). Deficiency of these essential lipids has been associated with impaired membrane fluidity, disrupted ion channel function, altered synaptic transmission, and reduced synaptic plasticity, all of which can adversely affect neuronal communication. Beyond their structural role in neuronal membranes, PUFA-derived metabolites exhibit diverse biological activities, including anti-inflammatory, antioxidant, anti-apoptotic, and gene regulatory effects that contribute to cellular resilience and neural protection (65). Moreover, long-chain PUFAs, including eicosapentaenoic acid (EPA), have modulated the intracellular calcium homeostasis, thereby facilitating calcium-dependent synaptic vesicle exocytosis and enhancing neurotransmitter release (66).

To elucidate the biological mechanisms underlying the behavioral effects of *P. gerardiana*, we performed a comprehensive biochemical evaluation of the PFC, a brain region that plays a central role in executive function, emotional regulation, and cognitive processing (67, 68). Dysfunction of the PFC has been consistently implicated in the pathophysiology of anxiety disorders and major depression (69). In the context of nicotine dependence, withdrawal is known to disrupt neurotransmission within the PFC, particularly the serotonergic and dopaminergic systems, thereby contributing to emotional instability, impaired stress coping, and maladaptive behavioral responses (70, 71).

Alterations in serotonergic signaling are of particular importance because this neurotransmitter system is a key determinant of withdrawal severity and vulnerability to relapse in nicotine addiction (72). Consistent with these observations, nicotine withdrawal in the present study markedly reduced serotonin and its principal metabolite, 5-hydroxyindoleacetic acid (5-HIAA), while significantly increasing MAO-A activity in the PFC. Together, these findings indicate impaired serotonergic neurotransmission and accelerated monoamine degradation, reflecting disrupted monoaminergic homeostasis (73). Such neurochemical abnormalities are likely to contribute to the anxiety-like behavior, anhedonia, and depression-like symptoms observed in nicotine-withdrawn animals.

Dietary supplementation with *P. gerardiana* effectively restored serotonergic homeostasis by increasing both serotonin and 5-HIAA concentrations while simultaneously reducing MAO-A activity. The concurrent elevation of serotonin and its primary metabolite suggest normalization of serotonin metabolism and improved serotonergic neurotransmission rather than simple accumulation of the neurotransmitter. One plausible explanation for these effects is the presence of bioactive phytochemicals in *P. gerardiana*, particularly polyphenols and flavonoids, which have been reported to inhibit MAO-A activity and facilitate monoaminergic neurotransmission (74, 75). By limiting serotonin degradation and promoting serotonergic signaling, *P. gerardiana* may help restore neurotransmitter homeostasis disrupted during nicotine withdrawal. These neurochemical changes provide a mechanistic basis for the anxiolytic and antidepressant-like behavioral improvements observed in the present study and further suggest that *P. gerardiana* confers neuroprotective effects through preservation of serotonergic function during nicotine withdrawal.

Nicotine withdrawal also produced profound oxidative stress in the prefrontal cortex, as evidenced by elevated MDA concentrations, depletion of TT content, and reduced activities of the antioxidant enzymes SOD and CAT. Increased MDA levels are a well-established indicator of lipid peroxidation, reflecting excessive oxidative degradation of polyunsaturated fatty acids within neuronal membranes by reactive oxygen species (ROS). This process compromises membrane integrity, disrupts cellular homeostasis, and ultimately contributes to neuronal dysfunction and injury (76, 77). At the same time, the marked reductions in TT, SOD, and CAT demonstrate a substantial impairment of the endogenous antioxidant defense system, resulting in diminished capacity to neutralize ROS and maintain redox homeostasis. Such an imbalance between oxidant production and antioxidant defenses renders neurons highly susceptible to oxidative damage and is likely to contribute to the emotional and cognitive impairments associated with nicotine withdrawal (78, 79).

The disruption of redox homeostasis observed during nicotine withdrawal is closely associated with the emergence of anxiety- and depression-like behaviors. Excessive oxidative stress has been implicated in neuronal dysfunction, impaired synaptic plasticity, and deficits in emotional regulation, all of which contribute to the neurobehavioral manifestations of nicotine withdrawal (1, 9). Consistent with previous reports, our findings demonstrate that chronic nicotine withdrawal induces a pronounced oxidative imbalance characterized by increased lipid peroxidation and impaired antioxidant defenses (18, 20). Importantly, dietary supplementation with *P. gerardiana* effectively restored redox homeostasis by reducing oxidative damage and enhancing endogenous antioxidant capacity. Although the precise antioxidant mechanisms of *P. gerardiana* in nicotine withdrawal have not been fully elucidated, its beneficial effects are likely attributable to its rich phytochemical composition. *P. gerardiana* seeds contain abundant phenolic compounds, including quercetin, gallic acid, vanillic acid, and other bioactive polyphenols, all of which possess well-established free radical-scavenging and antioxidant properties (80). These phytochemicals can

neutralize reactive oxygen species, thereby limiting oxidative damage to neuronal tissues. Previous in vitro investigations have demonstrated strong antioxidant activity of *P. gerardiana* extracts using assays such as DPPH and hydrogen peroxide scavenging tests (19). Furthermore, *P. gerardiana* administration reduces oxidative stress by enhancing endogenous antioxidant defenses, including superoxide dismutase activity and total thiol content, while simultaneously improving metabolic function (18). Interestingly, *P. gerardiana* also improved oxidative stress parameters in animals not exposed to nicotine, suggesting that its antioxidant actions extend beyond the reversal of nicotine-induced injury and might contribute to the maintenance of physiological redox homeostasis under normal conditions.

Nicotine withdrawal is accompanied by a pronounced neuroinflammatory response that exacerbates neuronal dysfunction and increases susceptibility to relapse, with adolescents being particularly vulnerable because of the ongoing maturation of the central nervous system (81). During this developmental period, nicotine exposure and subsequent withdrawal provoke robust activation of the brain's innate immune system, especially microglia, which are the principal mediators of neuroinflammatory processes (81). Excessive microglial activation has been implicated in the development of withdrawal-associated emotional disturbances and cognitive impairments. Moreover, nicotine withdrawal has disrupted the functional connectivity within neural circuits involved in reward processing, cognition, and emotional regulation, thereby contributing to executive dysfunction, intensified craving, and an increased likelihood of relapse (82). In parallel with these neuroimmune alterations, withdrawal-induced dysregulation of monoaminergic neurotransmission, particularly within the serotonergic and dopaminergic systems, further amplifies the inflammatory response. Beyond their established roles in regulating mood, motivation, and cognition, serotonin and dopamine actively participate in neuroimmune signaling. Impaired monoaminergic transmission has been associated with enhanced production of pro-inflammatory cytokines and chemokines, thereby perpetuating central neuroinflammation and worsening the neurobehavioral consequences of nicotine withdrawal (83).

Consistent with the growing evidence linking nicotine withdrawal to neuroimmune dysfunction, our findings demonstrate that withdrawal induces a combination of neuroinflammatory and neurodegenerative alterations within the PFC. These pathological changes were characterized by a marked increase in GFAP, indicating astrocyte activation and an enhanced neuroinflammatory response (84). Concurrently, brain-derived neurotrophic factor (BDNF), a key regulator of neuronal survival and synaptic plasticity, and the IL-10 were significantly reduced, suggesting impaired neurotrophic support together with suppression of endogenous anti-inflammatory mechanisms. In addition, NSE, a well-established biomarker of neuronal injury, was significantly elevated, providing further evidence of ongoing neurodegenerative damage (85). These findings indicate that nicotine withdrawal creates a pathological microenvironment in the PFC characterized by glial activation, diminished neuroprotective and anti-inflammatory signaling, and progressive neuronal injury, all of which are likely to contribute to the emotional and cognitive disturbances observed during nicotine abstinence.

The marked elevation in GFAP expression observed following nicotine withdrawal reflects pronounced astrocyte activation, a hallmark of neuroinflammatory pathology. Reactive astrocytes can amplify neuronal dysfunction by releasing pro-inflammatory cytokines, disrupting synaptic homeostasis, and promoting oxidative stress, thereby contributing to progressive neural damage (86). In parallel, the significant reduction in brain-derived neurotrophic factor (BDNF) indicates impaired neurotrophic support within the prefrontal cortex. As a critical regulator of neuronal survival, synaptic plasticity, neurogenesis, and learning-related processes, BDNF is essential for maintaining structural

and functional integrity of neural circuits (87). Therefore, diminished BDNF availability during nicotine withdrawal may compromise neuronal repair mechanisms and synaptic remodeling, providing a mechanistic explanation for the cognitive deficits and behavioral abnormalities commonly observed during abstinence (88).

A significant reduction in IL-10, one of the principal endogenous anti-inflammatory cytokines, further indicates that nicotine withdrawal shifts the neuroimmune environment toward a pro-inflammatory phenotype (89). Loss of IL-10-mediated immunoregulation is likely to perpetuate inflammatory signaling and oxidative stress, creating a self-sustaining cycle that exacerbates neuronal injury. Consistent with this interpretation, neuron-specific enolase (NSE), a sensitive biomarker of neuronal damage, was markedly elevated following nicotine withdrawal. Increased NSE levels are indicative of neuronal membrane disruption and cellular injury and are thought to result from the combined effects of persistent neuroinflammation, excitotoxicity, and oxidative stress (85). These findings suggest that nicotine withdrawal promotes progressive neurodegenerative changes within the prefrontal cortex, which may underlie the cognitive and emotional disturbances associated with nicotine abstinence (90).

Supplementation with *P. gerardiana* markedly attenuated these neurobiological abnormalities by suppressing astrocytic activation and neuronal injury, as evidenced by reduced GFAP and NSE levels, while simultaneously restoring BDNF and IL-10 expression. These findings indicate that *P. gerardiana* exerts robust neuroprotective effects through modulation of both neuroinflammatory and neurotrophic pathways. Restoration of IL-10 may contribute to re-establishing neuroimmune homeostasis by limiting excessive inflammatory signaling, whereas increased BDNF availability is likely to promote neuronal survival, synaptic plasticity, and structural recovery. Given the central role of BDNF in regulating emotional behavior and cognitive function, its upregulation provides a plausible mechanistic explanation for the marked improvements in anxiety- and depression-like behaviors observed following *P. gerardiana* supplementation.

Collectively, the behavioral benefits of *P. gerardiana* arise from coordinated attenuation of neuroinflammation, preservation of neuronal integrity, and enhancement of endogenous neuroplasticity during nicotine withdrawal.

Limitations

An important limitation of this study is the lack of analysis of other relevant neurobiological biomarkers that could provide a more comprehensive understanding of the underlying mechanisms. While markers such as serotonin, BDNF, GFAP, and oxidative stress parameters were assessed, the study did not include additional neurotransmitter systems (e.g., dopamine, GABA), inflammatory mediators (e.g., TNF-α, IL-1β), or markers of synaptic plasticity (e.g., PSD-95, synaptophysin) that are involved in nicotine dependence and mood regulation. This narrows the scope of interpretation and may overlook parallel or interacting pathways influenced by nicotine withdrawal or *P. gerardiana* treatment. Furthermore, *P. gerardiana* contains a complex mixture of bioactive compounds—including flavonoids, polyunsaturated fatty acids, terpenoids, and phenolics—yet the present study did not isolate or quantify these components. As a result, the specific compounds responsible for the observed anxiolytic, antidepressant, and neuroprotective effects remain unidentified. Without targeted phytochemical analysis or testing of purified constituents, it is difficult to establish causality or determine dose-response relationships for individual compounds. Future studies employing fractionation, metabolomics, and compound-specific administration will be essential to pinpoint the active agents and elucidate their mechanisms of action.

Conclusion

P. gerardiana supplementation provides substantial protection against the behavioral and

neurobiological impairments associated with nicotine withdrawal in adolescent rats. The observed benefits are likely mediated through the simultaneous regulation of several pathological processes, including normalization of serotonergic neurotransmission, enhancement of endogenous antioxidant capacity, and suppression of neuroinflammatory responses. The greater therapeutic benefit achieved with the 25% *P. gerardiana* diet further suggests a dose-dependent effect, supporting the notion that higher dietary supplementation offers more effective protection against nicotine withdrawal-induced damage. Although *P. gerardiana* also produced mild beneficial effects in healthy animals, these responses were considerably less pronounced than those observed in nicotine-withdrawn rats. This difference suggests that the neuroprotective properties of *P. gerardiana* are most evident under conditions of neurochemical imbalance and oxidative stress induced by nicotine withdrawal. Collectively, these findings highlight the potential of *P. gerardiana* nuts as a promising dietary adjunct for reducing withdrawal-related neurobehavioral disturbances and promoting neural recovery during adolescence.

Data Availability

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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Competing Interests

The authors declare that they have no competing interests.

Reference

1. Hossaini D, Nazari MJ, Ghazanfar KB, Amiri ME, Anwary MT, Jawad MJ, et al. Biotin's protective effects against nicotine withdrawal-induced anxiety and depression: mechanistic insights into serotonin, inflammation, BDNF, and oxidative stress in male rats. Addict Neurosci. 2025; 15:100199.
2. Baum GL, Flournoy JC, Glasser MF, Harms MP, Mair P, Sanders AFP, et al. Graded Variation in T1w/T2w Ratio during Adolescence: Measurement, Caveats, and Implications for Development of Cortical Myelin. J Neurosci. 2022;42(29):5681-94.
3. Bonke EM, Bonfert MV, Hillmann SM, Seitz-Holland J, Gaubert M, Wiegand TL, et al. Neurological soft signs in adolescents are associated with brain structure. Cereb Cortex. 2023;33(9):5547-56.
4. Kim-Spoon J, Lauharatanahirun N, Peviani K, Brieant A, Deater-Deckard K, Bickel WK, et al. Longitudinal pathways linking family risk, neural risk processing, delay discounting, and adolescent substance use. J Child Psychol Psychiatry. 2019;60(6):655-64.
5. 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.
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. 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.
8. Feldner MT, Babson KA, Zvolensky MJ. Smoking, traumatic event exposure, and posttraumatic stress: A critical review of the empirical literature. Clin Psychol Rev. 2007;27(1):14-45.
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. John U, Meyer C, Rumpf H-J, Hapke U. Smoking, nicotine dependence and psychiatric comorbidity—a population-based study including smoking cessation after three years. Drug Alcohol Depend. 2004;76(3):287-95.
11. Paz R, Barsness B, Martenson T, Tanner D, Allan AM. Behavioral Teratogenicity Induced by Nonforced Maternal Nicotine Consumption. Neuropsychopharmacology. 2007;32(3):693-9.
12. Ahmadi-Soleimani SM, Amiry GY, Khordad E, Masoudi M, Beheshti F. Omega-3 fatty acids prevent nicotine withdrawal-induced impairment of learning and memory via affecting oxidative status, inflammatory response, cholinergic activity, BDNF and amyloid-B in rat hippocampal tissues. Life Sci. 2023; 332:122100.
13. Hamed S, Ahmadi-Soleimani SM, Maryam A, Robert FH, Hossein A. Adolescent Substance Abuse, Transgenerational Consequences and Epigenetics. Curr Neuropharmacol. 2021;19(9):1560-9.
14. 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.
15. Murawska-Ciałowicz E, Wiatr M, Ciałowicz M, Gomes de Assis G, Borowicz W, Rocha-Rodrigues S, et al. BDNF impact on biological markers of depression—role of physical exercise and training. Int J Environ Res Public Health. 2021;18(14):7553.
16. Miraliakbari H, Shahidi F. Antioxidant activity of minor components of tree nut oils. Food Chem. 2008;111(2):421-7.
17. Destaillats F, Cruz-Hernandez C, Giuffrida F, Dionisi F. Identification of the botanical origin of pine nuts found in food products by gas-liquid chromatography analysis of fatty acid profile. J Agric Food Chem. 2010;58(4):2082-7.
18. Hosseini SA, Vali M, Haghighi-Zade MH, Siahpoosh A, Malihi R. The effect of Chilgoza pine nut (Pinus gerardiana Wall.) on blood glucose and oxidative stress in diabetic rats. Diabetes Metab Syndr Obes. 2020:2399-408.
19. Bhardwaj K, Sharma R, Cruz-Martins N, Valko M, Upadhyay NK, Kuča K, et al. Studies of phytochemicals, antioxidant, and antibacterial activities of Pinus gerardiana and Pinus roxburghii seed extracts. Biomed Res Int. 2022; 2022:5938610.
20. Hoon LY, Choo C, Watawana MI, Jayawardena N, Waisundara VY. Evaluation of the total antioxidant capacity and antioxidant compounds of different solvent extracts of Chilgoza pine nuts (Pinus gerardiana). J Funct Foods. 2015; 18:1014-21.
21. Singh G, Kumar D, Dash AK. Pinus gerardiana Wallich ex D. Don. -A review. Phytomed Plus. 2021;1(2):100024.
22. Ahmadi-Noorbakhsh S, Mirabzadeh E, Sadighi J, Aldavood S, Abbasi M, Farzad-Mohajeri S, et al. Guideline for the Care and Use of Laboratory Animals in Iran. Lab Anim. 2021:1-3.
23. Alami K, Mousavi SY. Afghan Chehelghoza (Pinus gerardiana L.) pine nut diet enhances the learning and memory in male rats. Nutr Diet Suppl. 2020:277-88.
24. 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.
25. 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.
26. Chellian R, Behnood-Rod A, Bruijnzeel AW. Development of dependence in smokers and rodents with voluntary nicotine intake: similarities and differences. Nicotine Tob Res. 2023;25(7):1229-40.
27. Gould TD. Mood and Anxiety Related Phenotypes in Mice: Characterization Using Behavioral Tests. Vol. II. New York: Springer; 2011.
28. Sturman O, Germain P-L, Bohacek J. Exploratory rearing: a context-and stress-sensitive behavior recorded in the open-field test. Stress. 2018;21(5):443-52.
29. 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.
30. 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.

31. 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.
32. Pumpaisalchai W, Kaewichit S, Taesothikul T, Niwatananun W, Sanichwankul K. The antidepressive effect of barakol in the forced-swimming test. CMU J. 2005;4(2):191-98.
33. 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.
34. Scheggi S, De Montis MG, Gambarana C. Making Sense of Rodent Models of Anhedonia. Int J Neuropsychopharmacol. 2018;21(11):1049-65.
35. Pizzagalli DA. Depression, stress, and anhedonia: toward a synthesis and integrated model. Annu Rev Clin Psychol. 2014; 10:393-423.
36. He L-W, Zeng L, Tian N, Li Y, He T, Tan D-M, et al. Optimization of food deprivation and sucrose preference test in SD rat model undergoing chronic unpredictable mild stress. Anim Models Exp Med. 2020;3(1):69-78.
37. 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.
38. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979;95(2):351-8.
39. Akbari E, Hossaini D, Amiry GY, Ansari M, Haidary M, Beheshti F, et al. Vitamin B12 administration prevents ethanol-induced learning and memory impairment through re-establishment of the brain oxidant/antioxidant balance, enhancement of BDNF and suppression of GFAP. Behav Brain Res. 2023; 438:114156.
40. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys. 1959;82(1):70-7.
41. Haidary M, Nazari MJ, Wasiq SMJ, Amiri ME, Ghazanfar KB. Magnesium sulfate ameliorates nicotine withdrawal-induced anxiety and depression: implications for regenerative psychiatry. Regen Med Rep. 2024;1(2):222-31.
42. Beckmann EA, Pielech M, Parent J. Clinical considerations for adolescents with eating disorders who use nicotine. Brown Univ Child Adolesc Behav Lett. 2024;40(2):1-6.
43. Renda B, Andrade AK, Frie JA, Sgarbossa CL, Murray JE, Khokhar JY. High-dose adolescent nicotine exposure permits spontaneous nicotine self-administration in adult male rats. Drug Alcohol Depend. 2020; 215:108215.
44. Slotkin TA, Card J, Seidler FJ. Nicotine administration in adolescence reprograms the subsequent response to nicotine treatment and withdrawal in adulthood: sex-selective effects on cerebrocortical serotonergic function. Brain Res Bull. 2014; 102:1-8.
45. Slawecki CJ, Gilder A, Roth J, Ehlers CL. Increased anxiety-like behavior in adult rats exposed to nicotine as adolescents. Pharmacol Biochem Behav. 2003;75(2):355-61.
46. Xu Z, Seidler FJ, Cousins MM, Slikker W, Slotkin TA. Adolescent nicotine administration alters serotonin receptors and cell signaling mediated through adenylyl cyclase. Brain Res. 2002;951(2):280-92.
47. Chen X, Long K, Liu S, Cai Y, Cheng L, Chen W, et al. Repeated exposure to high-dose nicotine induces prefrontal gray matter atrophy in adolescent male rats. Neuroscience. 2025; 566:205-17.
48. Beheshti F, Goudarzi M, Kakhki S, Ahmadi-Soleimani SM, Ansari M, Azhdari-Zarmehri H. Vitamin D (3) administration ameliorates the anxiety and depressive-like behaviour induced by nicotine withdrawal: a mechanistic focus on oxidative stress, inflammatory response, and serotonergic transmission. Arch Physiol Biochem. 2025:1-10.
49. Bruijnzeel AW. Reward processing and smoking. Oxford University Press US; 2017. p. 661-2.
50. Bruijnzeel AW. Tobacco addiction and the dysregulation of brain stress systems. Neurosci Biobehav Rev. 2012;36(5):1418-41.
51. Pergadia M, Der-Avakian A, D'Souza M, Madden P, Heath A, Shiffman S, et al. Association between nicotine withdrawal and reward responsiveness in humans and rats. JAMA Psychiatry. 2014;71(11):1238-1245.
52. Torres OV, Gentil LG, Natividad LA, Carcoba LM, O'Dell LE. Behavioral, biochemical, and molecular indices of stress are enhanced in female versus male rats experiencing nicotine withdrawal. Front Psychiatry. 2013; 4:38.

53. Isuru A, Rajasuriya M. Tobacco smoking and schizophrenia: re-examining the evidence. BJPsych Adv. 2019;25(6):363-72.
54. Perals D, Griffin AS, Bartomeus I, Sol D. Revisiting the open-field test: what does it really tell us about animal personality? Anim Behav. 2017; 123:69-79.
55. Kraeuter A-K, Guest PC, Sarnyai Z. The Elevated Plus Maze Test for Measuring Anxiety-Like Behavior in Rodents. In: Guest PC, editor. Pre-Clinical Models: Techniques and Protocols. New York: Springer; 2019. p. 69-74.
56. Yankelevitch-Yahav R, Franko M, Huly A, Doron R. The forced swim test as a model of depressive-like behavior. J Vis Exp. 2015;(97):52587.
57. Markov DD. Sucrose preference test as a measure of anhedonic behavior in a chronic unpredictable mild stress model of depression: outstanding issues. Brain Sci. 2022;12(10):1287.
58. Cook JW, Spring B, McChargue D, Hedeker D. Hedonic capacity, cigarette craving, and diminished positive mood. Nicotine Tob Res. 2004;6(1):39-47.
59. Schmidt C, Silberbauer LR, Reed MB, Murgas M, Vraka C, Klug S, et al. Functional dynamics of serotonin synthesis during monetary reward and punishment processing—a hybrid FPET/fMRI study. Int J Neuropsychopharmacol. 2025;28(Suppl 1): i64-i65.
60. Kranz GS, Kasper S, Lanzenberger R. Reward and the serotonergic system. Neuroscience. 2010;166(4):1023-35.
61. Sharma A, Sharma L, Goyal R. A review on Himalayan pine species: ethnopharmacological, phytochemical and pharmacological aspects. Pharmacogn J. 2018;10(4).
62. Thakur N, Gupta A, Chauhan V. Studies on quality characteristics of chilgoza nut and oil. Int J Farm Sci. 2015;5(1):78-82.
63. Yehuda S. Polyunsaturated fatty acids as putative cognitive enhancers. Med Hypotheses. 2012;79(4):456-61.
64. Hajjar T, Meng GY, Rajion MA, Vidyadaran S, Othman F, Farjam AS, et al. Omega 3 polyunsaturated fatty acid improves spatial learning and hippocampal Peroxisome Proliferator Activated Receptors (PPARα and PPARγ) gene expression in rats. BMC Neurosci. 2012;13(1):109.
65. Tallima H, El Ridi R. Arachidonic acid: Physiological roles and potential health benefits - A review. J Adv Res. 2018; 11:33-41.
66. Citri A, Malenka RC. Synaptic plasticity: multiple forms, functions, and mechanisms. Neuropsychopharmacology. 2008;33(1):18-41.
67. Kenwood MM, Kalin NH, Barbas H. The prefrontal cortex, pathological anxiety, and anxiety disorders. Neuropsychopharmacology. 2022;47(1):260-75.
68. Fitzgerald JM, Klumpp H, Langenecker S, Phan KL. Transdiagnostic neural correlates of volitional emotion regulation in anxiety and depression. Depress Anxiety. 2019;36(5):453-64.
69. Shiba Y, Santangelo AM, Roberts AC. Beyond the medial regions of prefrontal cortex in the regulation of fear and anxiety. Front Syst Neurosci. 2016; 10:12.
70. Albert PR, Vahid-Ansari F, Luckhart C. Serotonin-prefrontal cortical circuitry in anxiety and depression phenotypes: pivotal role of pre- and post-synaptic 5-HT1A receptor expression. Front Behav Neurosci. 2014; 8:199.
71. Arzoo P, Ramesh KG. The Potential Role of Dopamine Pathways in the Pathophysiology of Depression: Current Advances and Future Aspects. CNS Neurol Disord Drug Targets. 2025;24(5):340-52.
72. Avelar AJ, George O. How nicotine withdrawal symptoms fight each other: interpeduncular GABA neuron activity dynamically controls negative affect vs. coping behavior. Neuropsychopharmacology. 2022;47(3):617-8.
73. Picciotto MR, Brunzell DH, Caldarone BJ. Effect of nicotine and nicotinic receptors on anxiety and depression. Neuroreport. 2002;13(9):1097-106.
74. Herraiz T, Guillén H. Monoamine Oxidase-A Inhibition and Associated Antioxidant Activity in Plant Extracts with Potential Antidepressant Actions. Biomed Res Int. 2018; 2018:4810394.
75. Khatri DK, Juvekar AR. Kinetics of Inhibition of Monoamine Oxidase Using Curcumin and Ellagic Acid. Pharmacogn Mag. 2016;12(Suppl 2): S116-20.
76. Tirani MM, Haghjou MM. Reactive oxygen species (ROS), total antioxidant capacity (AOC) and malondialdehyde (MDA) make a triangle in evaluation of zinc stress extension. J Anim Plant Sci. 2019; 29:1100-11.
77. Moazamian R, Polhemus A, Connaughton H, Fraser B, Whiting S, Gharagozloo P, et al. Oxidative stress and human spermatozoa: diagnostic and functional significance of

aldehydes generated as a result of lipid peroxidation. Mol Hum Reprod. 2015;21(6):502-15.
78. Salminen LE, Paul RH. Oxidative stress and genetic markers of suboptimal antioxidant defense in the aging brain: a theoretical review. Rev Neurosci. 2014;25(6):805-19.
79. Walker J, Winhusen T, Storkson JM, Lewis D, Pariza MW, Somoza E, et al. Total antioxidant capacity is significantly lower in cocaine-dependent and methamphetamine-dependent patients relative to normal controls: results from a preliminary study. Hum Psychopharmacol. 2014;29(6):537-43.
80. Zulfqar F, Akhtar MF, Saleem A, Akhtar B, Sharif A, Saleem U. Chemical characterization, antioxidant evaluation, and antidiabetic potential of Pinus gerardiana (pine nuts) extracts. J Food Biochem. 2020;44(6): e13199.
81. Mahajan SD, Homish GG, Quisenberry A. Multifactorial etiology of adolescent nicotine addiction: a review of the neurobiology of nicotine addiction and its implications for smoking cessation pharmacotherapy. Front Public Health. 2021; 9:664748.
82. Holliday ED, Nucero P, Kutlu MG, Oliver C, Connelly KL, Gould TJ, et al. Long-term effects of chronic nicotine on emotional and cognitive behaviors and hippocampus cell morphology in mice: comparisons of adult and adolescent nicotine exposure. Eur J Neurosci. 2016;44(10):2818-28.
83. Saggu S, Pless A, Dew E, Ware D, Jiao K, Wang Q. Monoamine signaling and neuroinflammation: mechanistic connections and implications for neuropsychiatric disorders. Front Immunol. 2025; 16:1543730.
84. Li D, Liu X, Liu T, Liu H, Tong L, Jia S, et al. Neurochemical regulation of the expression and function of glial fibrillary acidic protein in astrocytes. Glia. 2020;68(5):878-97.
85. Haque A, Polcyn R, Matzelle D, Banik NL. New Insights into the Role of Neuron-Specific Enolase in Neuro-Inflammation, Neurodegeneration, and Neuroprotection. Brain Sci. 2018;8(2):33.
86. Namba MD, Kupchik YM, Spencer SM, Garcia-Keller C, Goenaga JG, Powell GL, et al. Accumbens neuroimmune signaling and dysregulation of astrocytic glutamate transport underlie conditioned nicotine-seeking behavior. Addiction Biology. 2020;25(5): e12797.
87. Colucci-D'Amato L, Speranza L, Volpicelli F. Neurotrophic Factor BDNF, Physiological Functions and Therapeutic Potential in Depression, Neurodegeneration and Brain Cancer. Int J Mol Sci. 2020;21(20):7777.
88. Begni V, Riva MA, Cattaneo A. Cellular and molecular mechanisms of the brain-derived neurotrophic factor in physiological and pathological conditions. Clin Sci (Lond). 2017;131(2):123-38.
89. Iyer SS, Cheng G. Role of interleukin 10 transcriptional regulation in inflammation and autoimmune disease. Crit Rev Immunol. 2012;32(1).
90. Hong R-H, Zhu J, Li Z-Z, Yuan J, Zhao P, Ding J, et al. red blood cell distribution width is associated with neuronal damage in acute ischemic stroke. Aging (Albany NY). 2020;12(10):9855.