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Original Research Article

Preventive effects of α-pinene against ketamine-induced schizophrenia-like behaviors in mice: insight into oxidative stress, neuroinflammatory cytokines, TLR4 and neurotrophin pathways

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Abstract

Objective: Schizophrenia (SCZ) is a chronic neuropsychiatric disease with behavioral and cognitive abnormalities. The pathogenesis of SCZ is associated with oxidative stress, inflammation, and disturbances in toll-like receptors (TLRs) and neurotrophic signaling. α-Pinene, a bioactive phytochemical, reduces schizophrenia-related symptoms in animal models, but the mechanisms are unclear. This study examined the mechanisms underlying α-pinene pretreatment on ketamine-induced schizophrenic-like behavior.

Materials and Methods: Forty male NMRI mice were allocated into five experimental groups (n=8): groups 1 and 2 received Tween-80 10% (10 ml/kg), and groups 3-5 received α-pinene 20, 40, or 80 mg/kg, respectively orally, for 4 weeks. Then, ketamine (20 mg/kg per day, intraperitoneally (i.p.), groups 2-5) or saline (10 ml/kg, i.p., group 1) was administered concomitantly during the final 2 weeks. Behavioral abnormalities were assessed by the open field, Y-maze, social interaction, and forced swimming test. The brain malondialdehyde (MDA) content and superoxide dismutase (SOD) and catalase (CAT) activities were assessed. The expression levels of brain-derived neurotrophic factor *(BDNF)*, nerve growth factor *(NGF)*, *TLR4*, tumor necrosis factor-alpha *(TNF-α),* and *interleukin-6 (IL-6)* were analyzed using real-time PCR in the brain.

Results: α-pinene pretreatment significantly improved behavioral impairments accompanied by attenuated oxidative stress through lowering MDA levels and elevating activities of antioxidant enzymes, SOD, and CAT. α-pinene pretreatment increased *BDNF* and *NGF* expression and decreased expression of *TLR4*, *TNF-α*, and *IL-6*.

Conclusion: α-pinene may be considered a promising candidate for ameliorating cognitive deficits and depressive and anxiogenic-like behaviors in SCZ, linked to reductions in oxidative damage, lower *TLR4*/cytokine expression, and enhancement of neurotrophic support. Nonetheless, more mechanistic studies remain needed.

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Introduction

Schizophrenia (SCZ) is a long-term mental disorder that affects about 1% of population, featuring positive (delusions, hallucinations, and disorganized speech/thought) and negative (anxiety, depression, and social withdrawal) symptoms and cognitive deficits (attention, memory, and executive function) (Anwar et al. 2025).

Also, accumulating evidence highlights the significant role of inflammatory and oxidative processes in the pathogenesis of SCZ. Some studies suggests that oxidative imbalance and impaired antioxidant defenses contribute to the disorder's pathophysiology. In a similar vein, repeated ketamine exposure induces oxidative stress via cytokine release and NADPH oxidase 2 (Nox-2) upregulation (Oshodi et al. 2021; Wei et al. 2022). However, antioxidant agents consistently attenuate ketamine-induced behavioral impairments (Ben-Azu et al. 2024; Oshodi et al. 2021). These findings indicate that antioxidants hold promise as both preventive and therapeutic interventions for SCZ.

Inflammation has increasingly been recognized as a central feature of SCZ, with elevated pro-inflammatory cytokines contributing to cellular damage and immune dysregulation. Neuroimaging studies have revealed signs of neuroinflammation, including microglial activation, cortical thinning, and white matter deterioration, changes that are linked to inflammatory mediators and cognitive decline. Increased levels of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), tumor necrosis factor alpha (TNF-α), and interleukin 6 (IL-6) have been detected in brain tissue following the onset of psychosis, and adjunctive anti-inflammatory therapies have shown clinical benefits, particularly in early stages of the disease (Gober et al. 2024, Goldsmith et al. 2018). Cytokine production is primarily driven by pattern recognition receptors (PRRs), particularly Toll-like receptors (TLRs), which sense pathogen- and damage-associated signals. TLRs, expressed in both neural and glial cells, activate NF-κB signaling and influence plasticity (Weickert et al. 2024). Human and experimental studies implicate TLRs—especially TLR4—in SCZ pathophysiology, showing elevated expression and heightened cytokine production in affected individuals (Patlola et al. 2025, Ahmadi et al. 2025).

Neurotrophic factors, essential regulators of neuroplasticity and neurogenesis, have also been implicated in SCZ. According to the neurotrophin hypothesis, SCZ is associated with impaired neurogenesis, with brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) playing central roles. Recent studies have shown that SCZ patients exhibit lower BDNF and NGF levels compared to healthy individuals (Turkmen et al. 2021). Reduced expression of these neurotrophins has also been observed in the hippocampus and prefrontal cortex of ketamine-induced rodent models (Valvassori et al. 2023), as well as in the blood of drug-naïve and unmedicated patients, with partial restoration following antipsychotic treatment (Jena et al. 2019). These results highlight the essential role of neurotrophic factor dysregulation in the pathophysiology of SCZ and its potential relevance to therapeutic strategies.

Phytochemicals often exhibit broad pharmacological activities, lower toxicity, and fewer adverse effects compared to synthetic agents. Moreover, they may enhance the efficiency or allow for reduced dosages of conventional drugs (Saleem et al. 2022). However, their neuroprotective mechanisms remain poorly defined.

Among these bioactive compounds, α-pinene, a monoterpene abundantly found in various plant species, including *conifers, Piper nigrum, and Juniperus species* (Allenspach and Steuer 2021), has been established as a viable therapeutic option for SCZ (Moghaddam et al. 2024, Ueno et al. 2019). α-Pinene demonstrates diverse pharmacological properties, including

antidepressant (Ueno et al. 2019), anxiolytic (Johnson et al. 2022), antioxidant, and anti-inflammatory effects (Rahimi et al. 2023). Inhalation of α-pinene has been shown to reduce anxiety- and depression-like behaviors in a dizocilpine-induced rodent model of SCZ (Ueno et al. 2019). More recently, Moghaddam et al. showed that α-pinene attenuated anxiety, depression, and oxidative stress in a ketamine-induced SCZ model (Moghaddam et al. 2024). While these findings highlight the therapeutic potential of α-pinene, its capacity to prevent psychiatric-like behavioral abnormalities and its underlying mechanisms remain to be fully elucidated.

The ketamine model reproduces a range of behavioral abnormalities relevant to the positive, negative, and cognitive symptom domains of SCZ. Ketamine, a noncompetitive NMDA receptor antagonist, induces these behaviors via neuroinflammation, oxidative stress, and neurotrophic dysregulation following prolonged administration (Ben-Azu et al. 2024, Huang et al. 2025). These findings suggest redox imbalance, neuroimmune dysfunction, and neurotrophic deficits as promising therapeutic targets beyond conventional approaches (Ben-Azu et al. 2024).

To evaluate its neuroprotective potential, α-pinene was administered both before and during ketamine exposure, allowing assessment of its preventive and protective effects against SCZ-like deficits. This study therefore aimed to investigate whether α-pinene pretreatment could mitigate ketamine-induced behavioral, cognitive, and neurochemical abnormalities in mice (Figure 1).

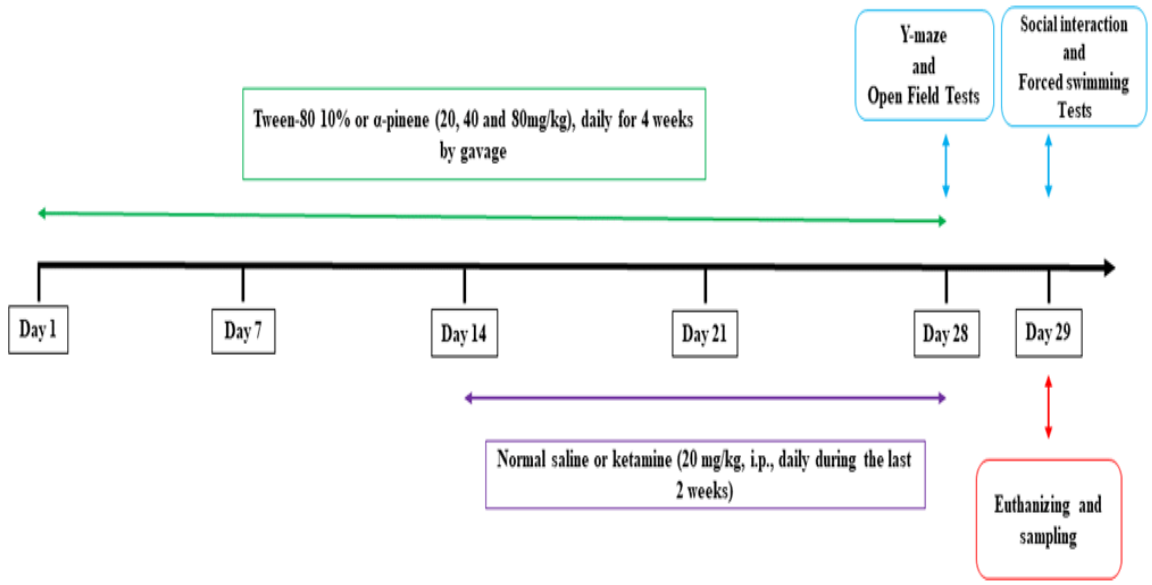

Figure 1. Time line of the experimental study

Materials and Methods

Animals and drugs

Adult male NMRI mice (25–35 g) were obtained from the Pasteur Institute (Amol, Iran). Animals were housed under standard laboratory conditions (22±2°C, 50–55% humidity, 12 hr light/dark cycle) with unrestricted access to food and water. The Animal Ethics Committee of Amol University of Special Modern Technologies approved all experimental protocols to reduce animal use and suffering (IR.ASMT.REC.1403.002).

Drugs and adminstration

Ketamine hydrochloride 10% (Bremer Pharma GmbH, Germany) was diluted in saline and administered intraperitoneally

(i.p.) at a dose of 20 mg/kg. α-pinene (Sigma-Aldrich, product number: 147524) was prepared in Tween-80 (10% in saline) (Sigma-Aldrich, product number: 655207) and administered via oral gavage at dose ranges of 20, 40, or 80 mg/kg once daily for 4 weeks, based on previous reports (Moghaddam et al. 2024, Ueno et al. 2019). All treatments were administered in a volume of 0.1 ml/10 g of body weight.

Mice were randomly assigned into four groups (n = 8 per group):

1. Control: Tween-80 10% (daily for 4 weeks by gavage) combined with normal saline i.p. daily during the last 2 weeks.
2. Ketamine (KET): Tween-80 10% (orally for 4 weeks), followed by ketamine (20 mg/kg, i.p., daily during the last 2 weeks).
3. α pinene 20: α-pinene (20 mg/kg)
4. α pinene 40: α-pinene (40 mg/kg)
5. α pinene 80: α-pinene (80 mg/kg)

groups 3-5 received α-pinene by gavage, daily for 4 weeks combined with ketamine (20 mg/kg, i.p., daily during the last 2 weeks). α-pinene and its vehicle were administered 30 min before the ketamine injection (Ben-Azu et al. 2024). Following the final administrations of ketamine and pinene on day 28, behavioral testing was carried out on days 28 and 29 (Figure 1).

Behavioral assessments

Y-Maze test

A three-arm Y-maze (30 × 10 × 20 cm per arm; black Plexiglas; 120° apart) was used to assess spatial memory. The index measured was spontaneous alternation—i.e. sequential entries into each of the three different arms—which was calculated from a 5-min free-exploration session during which each mouse was placed at the central junction (Ben-Azu et al. 2024).

Open-Field test (OFT)

Each mouse was placed in the central zone (30 × 30 cm) of an open-field arena (50 × 50 × 30 cm; opaque white Plexiglas) located in a sound-attenuated room with dim lighting. Behavior was recorded for 5 min using an overhead camera. The number of line crossings, and the frequency of rearing were quantified (Mashayekhpour et al. 2025).

Social interaction test

Social behavior was evaluated in the same OFT arena. Pairs of unfamiliar mice were placed together for 10 min, and interactions (following, adjacent lying, general investigation, and anogenital sniffing) were scored. The cumulative duration of interactions was analyzed (Ramos et al. 2013).

Forced swimming test (FST)

Mice were individually tested in a lucid cylinder (20 cm height × 10 cm diameter) containing water (10 cm deep, 25°C). Total immobility time was recorded during the last 4 min of a 6-min session (Gavzan et al. 2023).

After behavioral tests, mice were anesthetized with ether and euthanized by cervical dislocation. The brains were rapidly harvested for biochemical and molecular analyses (Figure 1).

Preparation of tissue homogenate

The two cerebral hemispheres were carefully separated on an ice-cold plate. Tissues obtained from the right hemispheres were designated for biochemical assays, while those from the left hemispheres were reserved for PCR analysis. From the left hemisphere, brain regions of particular relevance to schizophrenia— the cortex, hippocampus, and midbrain—were dissected and pooled together in microtubes. All dissected tissue samples were rapidly snap-frozen in liquid nitrogen and then maintained at −80 °C for later use.

Biochemical analysis of oxidative stress

Brain tissues were homogenized following the protocols supplied with commercial assay kits (Navand Salamat Co., Urmia, Iran). For the malondialdehyde (MDA) assay, 10 mg of brain tissue was

homogenized in 300 µL of lysis buffer containing 3 µL of butylated hydroxytoluene (BHT). For the superoxide dismutase (SOD) assay, 10 mg of brain tissue was homogenized in 50 µL of lysis buffer. For the catalase (CAT) assay, 50 mg of brain tissue was homogenized in 0.5 mL of lysis buffer. All homogenates were then centrifuged at 12,000 rpm for 5 min, and the clear supernatants were collected for subsequent biochemical analyses. MDA concentrations, as well as SOD and CAT activities, were quantified spectrophotometrically, using the manufacturer's instructions (Navand Salamat Co., Urmia, Iran). Absorbance was read at 550 nm for MDA, 405 nm for SOD, and 550 nm for CAT (Gavzan et al. 2023).

Quantitative Real-Time PCR (RT-qPCR)

Total RNA was first extracted from 20 mg of brain tissue using the RNeasy Mini Kit (QIAGEN, Germany). cDNA was synthesized with Superscript III Reverse Transcriptase (Fermentas, USA) following the manufacturer's protocol. RT-qPCR was conducted to measure mRNA levels of *BDNF*, *NGF*, *TLR4*, *IL-6*, and *TNFα*, with *GAPDH* as the reference gene. Primer sequences are provided in Table 1. Relative expression was determined by the 2^-ΔΔCt method (Mashayekhpour et al. 2025).

Table1. Forward and Reverse Primer PCR Sequences for Real-time PCR

Gene	Primer	Sequence	Amplicon lengths (bp)
GAPDH	forward	*5´-ATCCTGCACCACCAACTGC-3´*	129
	reverse	*5´-ACGCCACAGCTTTCCAGAG-3´*	
BDNF	forward	*5´-AGCTGAGCGTGTGTGACAGTAT-3´*	239
	reverse	*5´-CCGAACATACGATTGGGTAGTT-3´*	
NGF	forward	*5´-TGCGTTTTTGATCGGCGTA-3´*	190
	reverse	*5´-GTCTACAGTGATGTTGCGGG-3´*	
TLR4	forward	*5´- CCACAAGAGCCGGAAGGTTAT-3´*	130
	reverse	*5´- GACAATGAAGATGATGCCAGAGC-3´*	
TNFα	forward	*5´-GGAGGAGCAGCTGGAGTG-3´*	131
	reverse	*5´-CCTTGAAGAGAACCTGGGAGTAGA-3*	
IL-6	forward	*5´-TCACAGAGGATACCACCCACAA-3´*	146
	reverse	*5´-CAGTGCATCATCGCTGTTCATAC-3´*	

Statistical analysis

Data are presented as means ± standard errors of the mean (SEM). Statistical analyses were performed using SPSS version 24.0. Normality was assessed using the Shapiro–Wilk test, and homogeneity of variances was evaluated with Levene's test. Following confirmation of these parametric assumptions, intergroup differences were analyzed via one way analysis of variance (ANOVA), with Tukey's HSD test applied for post hoc pairwise comparisons. Linear regression was additionally employed to characterize the dose dependent relationship. The significance threshold was set at $p<0.05$.

Results

Effects of α-pinene pre-treatment on behavioral alterations in ketamine-induced SCZ in mice

In the OFT (Figure 2A and B), ketamine increased rearing and line crossings versus control ($p<0.001$). α-pinene pretreatment reduced both measures, with significant

effects at 40 and 80 mg/kg ($p<0.001$ vs. ketamine), indicating alleviation of ketamine-induced hyperactivity (higher dose vs. lower, $p<0.05$ for line crossings). In the Y-maze (Figure 2C), ketamine decreased spontaneous alternation ($p<0.001$ vs. control). All α-pinene doses (20–80 mg/kg) improved alternation ($p<0.001$ vs. ketamine); 80 mg/kg was more effective than 20 mg/kg ($p<0.001$)

As shown in Figure 2D, ketamine reduced social interaction time ($p<0.001$ vs. control). α-pinene at 20, 40, and 80 mg/kg increased interaction time ($p<0.05$, $p<0.05$, $p<0.001$ vs. ketamine, respectively); 80 mg/kg was superior to both lower doses ($p<0.001$). In Figure 2E, ketamine increased immobility time ($p<0.001$ vs. control). α-pinene pretreatment at all doses reduced immobility ($p<0.001$); 80 mg/kg was more effective than 20 mg/kg ($p<0.01$).

Linear regression analysis revealed a statistically significant linear dose-response trend across all behavioral assessments, including line crossing ($R^2 = 0.979$, $p<0.001$), rearing ($R^2 = 0.986$, $p<0.001$), spontaneous alternation ($R^2 = 0.983$, $p<0.001$), immobility time ($R^2 = 0.983$, $p<0.001$), and interaction time ($R^2 = 0.966$, $p<0.001$). Collectively, these results confirm a significant dose-dependent effect of the treatment.

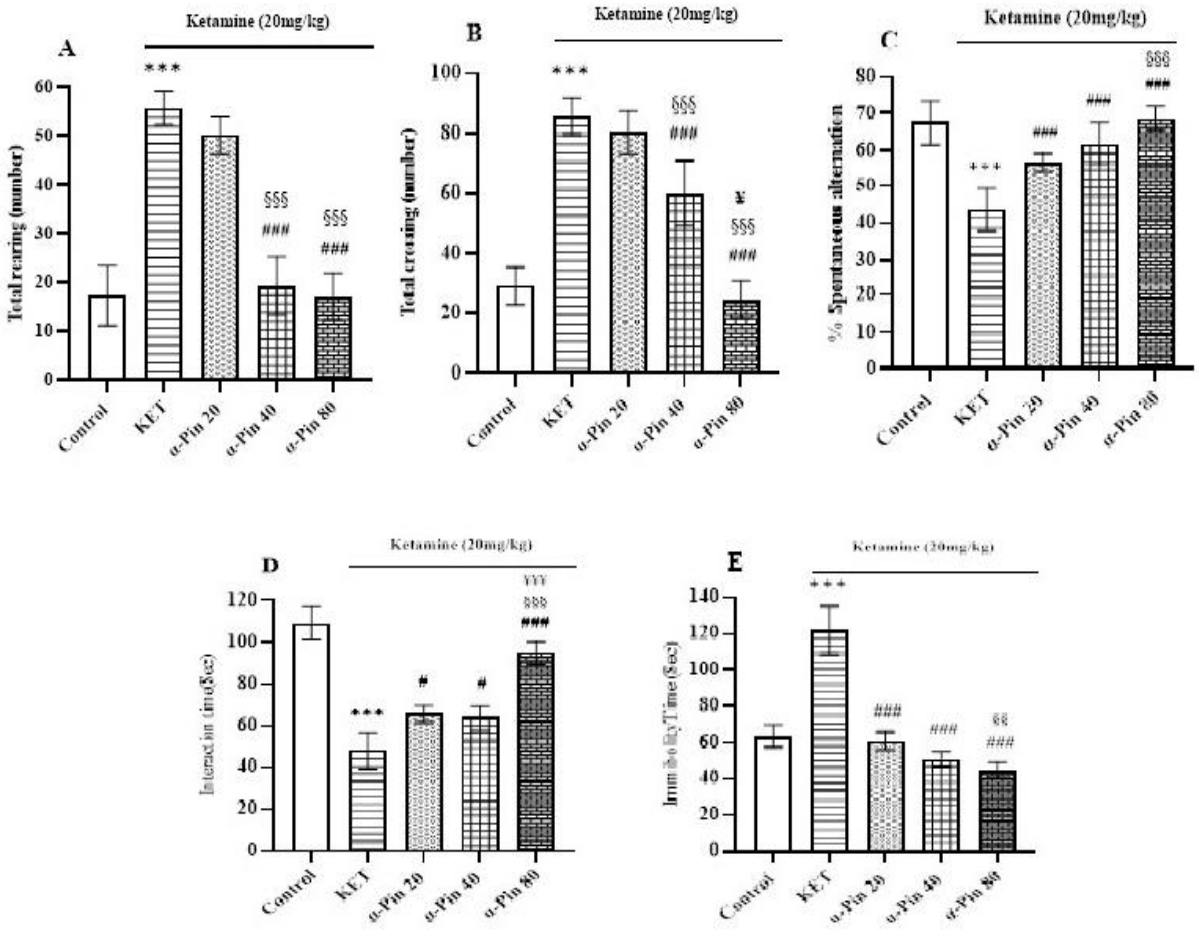

Figure 2. Effects of pre-treatment of α-pinene on schizophrenia like behaviors. (A) Number of line crossings and (B) Number of rearing in open field test .(C) Percentage of spontaneous alternation in Y-maze test, D) Duration of social interaction in social test and E) the immobility time in the forced swim test. KET: ketamine. Data are mean ± SEM. $^{***}p<0.001$ vs. Control; $^{\#}$ and $^{\#\#\#}p<0.05$ and <0.001 vs. KET, respectively; $^{¥}p<0.05$ and $^{¥¥¥}p<0.001$ vs. KET + α-pinene 40; §§ $p<0.01$ and §§§ $p < 0.001$ vs. KET + α-pinene 20.

Effects of α-pinene pre-treatment on antioxidant enzyme activities and oxidative stress biomarker

As shown in Table 2, ketamine significantly decreased SOD ($p<0.001$) and CAT ($p<0.01$) activities while elevating MDA levels ($p<0.001$) compared to controls. α-pinene administration (40 and 80 mg/kg) markedly restored SOD ($p<0.01$ and $p<0.001$, respectively), and CAT activities (20 mg/kg: $p<0.01$; 40 mg/kg: $p < 0.01$; and 80 mg/kg: $p<0.001$), and reduced MDA levels (20 mg/kg and 40 mg/kg: $p<0.05$; and 80 mg/kg: $p<0.01$). The 20 mg/kg α-pinene did not significantly restore MDA levels or SOD activity compared with the ketamine group.

Linear regression analysis demonstrated significant dose-dependent relationships for CAT ($R^2 = 0.968$, $p<0.001$), SOD ($R^2 = 0.985$, $p<0.001$), and MDA ($R^2 = 0.985$, $p<0.003$). Collectively, these findings indicate that α-pinene enhances brain antioxidant defenses and mitigates ketamine-induced oxidative stress in a dose-dependent manner.

Table 2. Effects of α-pinene on oxidative stress biomarkers in ketamine-induced SCZ model—abbreviations: SOD, superoxide dismutase; CAT, catalase; MDA, malondialdehyde. Data are presented as means ± standard errors of the mean (SEM). ***p<0.001 and **p<0.01 vs. control; ###p<0.001, ##p<0.01, and #p<0.05 vs. ketamine.

Groups	SOD (nmol/min/mg tissue)	CAT (nmol/min/mg tissue)	MDA (nmol/mg tissue)
Control	219.32±3.49	1.174±0.03	43.44±1
Ketamine	190.69±3.57***	.928±0.02**	51.75±1.36***
ketamine+α-pinene(20mg/kg)	204.36±2.75	1.171±0.031##	46.26±0.84#
ketamine+α-pinene(40mg/kg)	212.54±2.95##	1.19±0.053##	46.71±0.87#
ketamine+α-pinene(80mg/kg)	216.51±3.70###	1.231±0.04###	45.1 ±1.03##

Effects of α-pinene pre-treatment on pro-inflammatory cytokine, neurotrophins, and TLR4 mRNA expression

RT-qPCR (Figure 3A–E) showed that ketamine reduced *BDNF* and *NGF*, and increased *TNF-α*, *IL-6*, and *TLR4* versus control ($p<0.001$). α-pinene pretreatment dose-dependently reversed these changes. Specifically, α-pinene at 80 mg/kg elevated *BDNF* ($p<0.01$), and at 40 and 80 mg/kg elevated NGF ($p<0.001$). The effect of α-pinene on *NGF* gene expression was greater at 40 and 80 mg/kg than at 20 mg/kg ($p<0.01$ and $p<0.001$, respectively). α-pinene also reduced *TLR4* overexpression at all doses ($p < 0.001$), and reduced *TNF-α* and *IL-6* at 40 and 80 mg/kg (*TNF-α*: $p<0.001$; and *IL-6*: $p<0.01$). These inhibitory effects were more pronounced at the higher dose (*TNF-α*: $p<0.01$ and $p<0.001$ for 40 and 80 mg/kg; and *IL-6* at 80 mg/kg: $p<0.05$). Moreover, linear regression analysis revealed a significant dose-dependent relationship for all evaluated markers, including *IL-6* ($R^2 = 0.953$, $p<0.001$), *TNF-α* ($R^2 = 0.981$, $p<0.001$), *TLR4* ($R^2 = 0.955$, $p<0.001$), *NGF* ($R^2 = 0.988$, $p<0.001$), and *BDNF* ($R^2 = 0.972$, $p<0.001$).

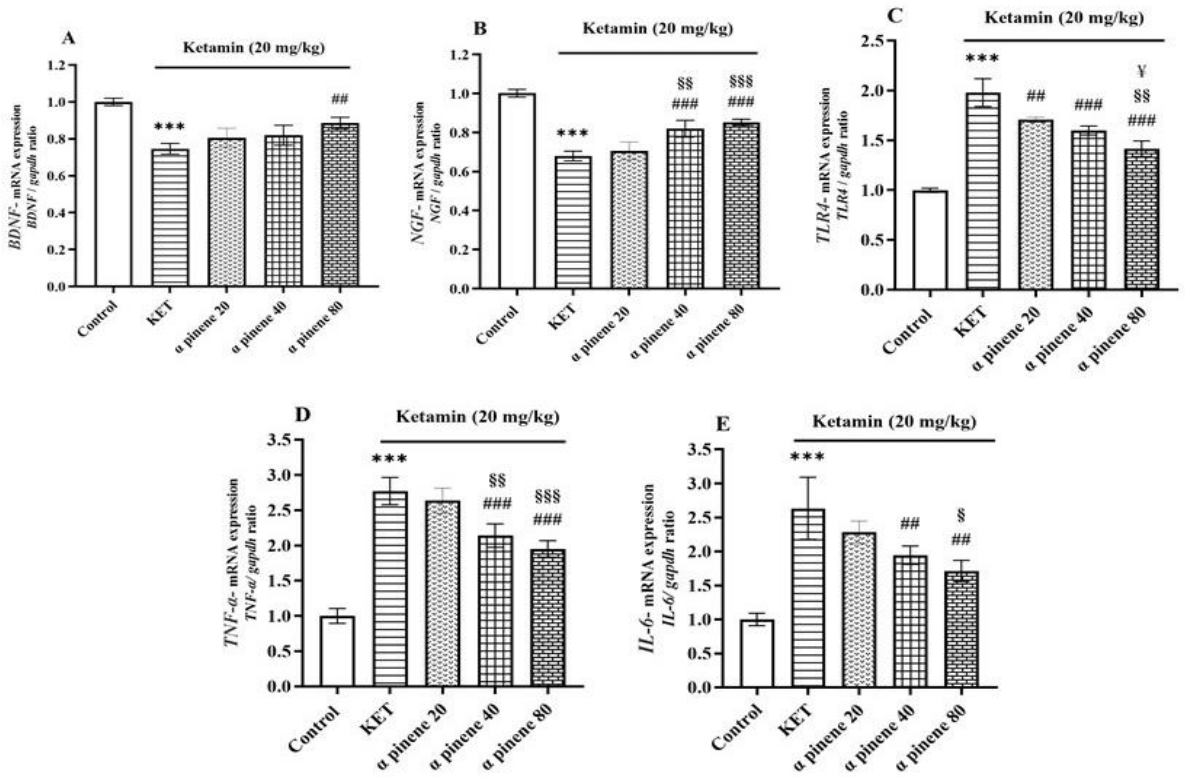

Figure 3. Effects of α-pinene pre-treatment on the expression levels of (A) *BDNF*, B) *NGF*, C) *TLR4*, D) *TNF-α*, and E) *IL-6* genes in the mouse brain. *BDNF*, brain-derived neurotrophic factor*; NGF*, nerve growth factor*; TLR-4*, Toll-like receptor 4; *TNF-α*, Tumor necrosis factor alpha; *IL-6*, Interleukin 6; KET, ketamine. Data are mean ± SEM. $^{***}p<0.001$ vs. control; $^{\#\#}$ and $^{\#\#\#}p<0.01$ and <0.001 vs. KET, respectively, $^{¥}p<0.05$ vs. KET + α-pinene 40; §, §§, and $^{§§§}p<0.05$, p<0.01, and <0.001 vs. KET + α-pinene 20, respectively.

Discussion

SCZ is a long-term psychiatric illness that disrupts cognition, emotion, and behavior. Current antipsychotic treatments provide limited efficiency for negative and cognitive symptoms and often cause adverse effects. Ketamine, a non-competitive NMDA receptor antagonist, is commonly employed to model SCZ in rodents, as it induces neurotransmitter imbalances, oxidative stress, impaired neurotrophin signaling, and neuroinflammation—processes central to SCZ pathophysiology (Moghaddam et al. 2024, Valvassori et al. 2023, Wei et al. 2022). Furthermore, this induction protocol represents a pharmacological model of chronic schizophrenia features, although the accompanying hyperlocomotion partially indicates acute-like states (Malik et al., 2023). Administration of α-pinene prior to and concurrently with ketamine injection significantly mitigated ketamine-induced behavioral deficits in SCZ mice by reducing hyperlocomotion and stereotypy in the OFT, improving social interaction, decreasing immobility in the FST, and enhancing spatial memory in the Y-maze. Overall, these findings indicate that α-pinene exerts a broad-spectrum effect, ameliorating chronic (negative symptoms and memory impairment) and partially acute schizophrenia-like behaviors (hyperlocomotion)—induced by this subchronic protocol. Moreover, α-pinene prevented oxidative stress, restored the expression of *BDNF* and *NGF*, and counteracted the ketamine-induced upregulation of *TLR4*, *TNF-α*, and *IL-6*.

The findings of our study are consistent with those reported in prior investigations. Earlier researches showed that the administration of α-pinene (50–100 mg/kg), along with ketamine injection, alleviated ketamine-induced SCZ-like

behaviors through its antioxidant effects (Moghaddam et al. 2024). For this investigation, we measured the pretreatment using lower α-pinene doses (20, 40, and 80 mg/kg) over a longer treatment period (four weeks). Moreover, we examined its effects on ketamine-induced social deficits as negative symptoms and spatial memory impairment—features that had not been previously addressed—and explored multiple underlying mechanisms of its potential antipsychotic action. Also, two weeks of α-pinene administration improved memory impairment and anxiety-like behaviors in an Alzheimer's rat model, which was correlated with reduced MDA and NO (Nitric oxide) levels, increased glutathione content and catalase activity, downregulation of pro-inflammatory cytokines (*TNF-α, IL-6*, and *IL-1β*), and enhanced expression of *BDNF* and NMDA receptors in the hippocampus (Khan-Mohammadi-Khorrami et al. 2022). Additionally, α-pinene improved recognition and spatial memory in a capsaicin-induced pain model through GABAergic modulation (Rafie et al. 2022). Consistently, α-pinene alleviated scopolamine-induced memory impairment in the Y-maze and Morris water maze tests and upregulated antioxidant proteins in the hippocampus (Lee et al. 2017). Furthermore, α-pinene inhalation ameliorated dizocilpine (MK-801-) induced SCZ-like behavioral abnormalities in tasks such as the Y-maze and OFT (Ueno et al. 2019).

In contemporary research, the pathophysiology of schizophrenia is significantly promoted by the accumulation of reactive oxygen species (ROS), a process that directly correlates with disease advancement. (Bai et al. 2018). In our study, treatment with α-pinene significantly diminished MDA levels and enhanced the activities of SOD and CAT in the brain tissue of mice subjected to ketamine. These findings suggest that α-pinene pretreatment prevented SCZ-like behaviors through mitigating the elevated oxidative stress induced by ketamine, which align with prior studies (Moghaddam et al. 2024, Rahimi et al. 2023). Unlike previous studies, we focused on α-pinene neurotrophic and neuroinflammatory mechanisms via TLR dependence in SCZ-like behavior.

BDNF and NGF are critical neurotrophins that regulate neuronal viability, synaptic plasticity, and cognitive function. In SCZ, BDNF dysregulation impairs cortical plasticity and increases neuronal vulnerability. Numerous investigations have established a significant association between the pathophysiology of SCZ and altered *BDNF* expression (Turkmen et al. 2021). Furthermore, poorer cognitive function in SCZ has been linked to higher levels of negative symptoms and lower levels of serum BDNF (Xu et al, 2021). Also, antipsychotic agents have been shown to improve cognitive impairment and psychotic symptoms in individuals with SCZ, potentially through increasing serum BDNF levels (Zhao et al. 2024). In animal models, NMDA receptor antagonists such as ketamine have been shown to induce cognitive deficits accompanied by reduced BDNF expression (Valvassori et al. 2023). In this work, we confirmed that ketamine administration resulted in the suppression of BDNF and NGF expression in the mouse brain. Conversely, treatment with α-pinene dose-dependently restored BDNF and NGF expression. A previous study showed that α-pinene treatment improved memory impairment by increasing cortical and hippocampal *BDNF* expression levels in a 3-nitropropionic acid (3-NP) rat model of Huntington's disorder (Hashemi et al. 2025).

Immune dysregulation and the resulting neuroinflammation are recognized as key contributors to the pathophysiology of SCZ (Gober et al. 2024, Goldsmith et al. 2018, Patlola et al. 2025). The enhanced microglia activity and upregulated production of pro-inflammatory cytokines are correlated with neuroinflammation

observed in SCZ (Gober et al. 2024). Furthermore, repeated ketamine administration leads to elevate cytokine release in murine models (Wei et al. 2022). In the present study, the elevated levels of *IL-6* and *TNF-α* after ketamine administration was also consistent with previous research findings and treatment with α-pinene significantly mitigated the overexpression of *IL-6* and *TNF-α* in brain tissue. The neuroimmune hypothesis of SCZ proposes that gliosis intensifies neuroinflammatory signals, consequently exacerbating psychopathological manifestations and cognitive impairments (Huang et al. 2025) and reductions in TNF-α and IL-6 are associated with improvements in negative symptoms in SCZ (Goldsmith et al. 2018).

Hashemi et al. reported that In the kainic acid rat model of temporal lobe epilepsy, α-pinene treatment elicited a significant reduction in hippocampal IL-1β and TNF-α concentrations, alongside a reversal of the suppressed IL-10 levels (Hashemi et al. 2025).

In line with this study, Noroozi et al. demonstrated that α-pinene enhanced the antioxidant enzyme activity (SOD, CAT, and Glutathione (GSH), reduced MDA activity, and downregulated the expression of pro-inflammatory genes in the liver of rats exposed to CCl4 (Noroozi et al. 2025). Moreover, low doses of α-pinene administration for 7 days reduced IL-6, TNF-α, SOD, GSH, CAT, and MDA levels in the skin of formalin-injected hind paws and moderated TNF-α and IL-1β expression in the spinal cord (Rahimi et al. 2025, Rahimi et al. 2023).

In murine models, α-pinene reduced oxidative and inflammatory damage, particularly in the brain, by lowering cytokine levels (e.g. TNF-α, IL-1β, and IL-6) and enhancing antioxidant markers and suppresses ROS generation (Khoshnazar et al. 2019, Moghaddam et al. 2024, Rahimi et al. 2025, Rahimi et al. 2023).

Persistent low-grade inflammation, characterized by elevated cytokines, chemokines, and TLRs, has been observed across SCZ stages and tends to decrease following antipsychotic treatment (Patlola et al. 2025). TLR4, expressed predominantly in glial cells, mediates innate immune responses to pathogen- and damage-associated signals, connecting immune activation to mechanisms including synaptic reorganization, myelination, and neurogenesis. Dysregulated TLR4 signaling contributes to chronic neuroinflammation and neuronal damage, while peripheral TLR4-mediated cytokines (IL-1β, IL-6, and TNF-α) may traverse a disrupted blood–brain barrier, amplifying neuroinflammatory cascades and impairing cognition. Modulating TLR4 with antipsychotics suggests a potential therapeutic target in SCZ (Weickert et al. 2024).

The present result demonstrated that *TLR4* expression was elevated in the brain of mice following ketamine injection, and pretreatment with α-pinene dose-dependently reversed these changes. Our results are in accordance with previous evidence implicating TLR4 activation in morphine-induced neuroinflammation, which demonstrated that morphine dependence and withdrawal upregulated hippocampal TLR2, TLR4, and TLR10. α-pinene treatment partially normalized these changes, indicating that the neuroprotective properties of α-pinene may involve suppression of TLR-mediated cascades (Ahmadi et al. 2025). Overall, α-pinene may mitigate ketamine-induced neuroinflammation and neural dysfunction by modulating TLR-dependent neuroimmune responses.

Constraints of this investigation include the lack of multiple antipsychotic positive controls and region-specific analyses in key areas (hippocampus, and prefrontal cortex), both constrained by cost and low tissue yield. These should be addressed in future studies to better elucidate α-pinene mechanisms. Although linear regression confirmed a significant dose-dependent effect, the limited dose range (n = 3)

precluded reliable ED50 calculation. Thus, further pharmacological studies to determine the therapeutic index, evaluate potential side effects, and establish the safety margin of the effective dose of α-pinene are necessary for its clinical application as an alternative therapy.

Overall, our results demonstrate that α-pinene effectively prevents and reverses ketamine-induced SCZ-like behaviors in mice, likely via antioxidant, anti-inflammatory, TLRs-mediated, and neurotrophic mechanisms. These results underscore α-pinene as a capable therapeutic agent for mitigating behavioral and molecular disturbances associated with SCZ, warranting further validation in clinical studies.

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Conflicts of interest

No conflicts of interest are declared by the authors.

Ethical Considerations

The Animal Ethics Committee of Amol University of Special Modern Technologies approved all protocols (IR.ASMT.REC.1403.002)

Authors' Contributions

H.G: Writing – original draft, Supervision, Writing – review & editing, Validation, Resources, Funding acquisition, Project administration, , Data curation, Formal analysis Conceptualization; M.A.M; M.H and M.R.J: : Investigation, Methodology, Project Administration, Formal Analysis;

Abbreviations

SCZ Schizophrenia
KET Ketamine
OFT Open Field Test
FST Forced Swim Test
MDA Malondialdehyde
SOD Superoxide Dismutase
CAT Catalase
TLR4 Toll-Like Receptor 4
IL-6 Interleukin-6
TNF-α Tumor Necrosis Factor-Alpha
BDNF Brain-Derived Neurotrophic Factor
NGF Nerve Growth Factor
PCR Polymerase Chain Reaction
NOX-2 NADPH Oxidase 2
NO Nitric Oxide
NF-κB Nuclear Factor Kappa B
GSH Glutathione

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