ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 19, Issue 1, 2026

Online - 2455-3891
Print - 0974-2441

Research Article

AMELIORATIVE EFFECTS OF SELECTIVE PDE-10 INHIBITORS AGAINST KETAMINE-MEDIATED SCHIZOPHRENIC OUTCOMES IN MICE

RUCHIKA SRIVASTAVA[1], PRABHAT SINGH[2]*, AJEET[3]

[1]Department of Pharmacology, Faculty of Pharmacy, Swami Vivekanand Subharti University, Meerut, Uttar Pradesh, India, [2]Department of Pharmacology, Swami Vivekanand Subharti University, Meerut, Uttar Pradesh, India, [3]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Sanskar College of Pharmacy and Research, Ghaziabad, Uttar Pradesh, India.
***Corresponding author: Prabhat Singh; Email: prabhatsingh509@gmail.com**

Received: 30 June 2025, Revised and Accepted: 14 October 2025

ABSTRACT

Objectives: Schizophrenia (SCZ) is characterized by significant impairments in perception and cognitive flexibility. Making accurate plans for therapy demands a deeper comprehension of the brain mechanisms behind these disorders. The purpose of our investigation is to analyze the protective effects of papaverine, a phosphodiesterase-10 inhibitor, on ketamine-induced SCZ -like behavioral and biochemical alterations in mice.

Methods: For 10 consecutive days, mice were exposed to ketamine (30 mg/kg; *i.p.*) to develop a SCZ-like phenotype. Various behavioral tests, including social interactions, catalepsy, cognitive impairment (Morris water maze), locomotor and anxiety (open field test), and immobility duration (forced swim test), were assessed. Biochemicals (acetylcholinesterase [AChE] activity, glutathione, and lipid peroxides), and histopathological alterations were also investigated. In this study, papaverine (30 mg/kg; i.p.) and clozapine (7.5 mg/kg *p.o.*) served as test and standard, respectively. Results were statistically analyzed and "one-way ANOVA" was performed, and Tukey's multiple comparison test was subsequently applied."

Results: After 28 days of ketamine therapy, significant ($p \leq 0.05$) behavioral alterations have been noted, including increased immobility duration, altered locomotor and anxiety-like behaviors, social interactions, cognitive impairment, and catalepsy. Significant alterations in histopathology, AChE activity, and oxidative stress (increased lipid peroxides and lower glutathione) were also observed in mice treated with ketamine. Treatment with clozapine and papaverine considerably ($p \leq 0.05$) improved the biochemical changes, behavioral problems, and histological changes.

Conclusion: We may conclude that papaverine may have neurodefensive effects against ketamine-induced SCZ in mice based on behavioral, histological, and biochemical observations.

Keywords: Schizophrenia, Phosphodiesterase, Inflammation, Cognition, Social withdrawal, Open field.

 DOI: http://dx.doi.org/10.22159/ajpcr.2026v19i1.55825. Journal homepage: https://innovareacademics.in/journals/index.php/ajpcr

INTRODUCTION

Schizophrenia (SCZ) is a serious mental condition that has terrible effects on individuals who experience it. The epidemiology of SCZ demonstrates that it occurs frequently, in various kinds of settings, and in combination, reducing the quality of life and increasing the risk of dying young [1]. Nowadays, SCZ is thought to be characterized by different symptoms: negative (social withdrawal, alogia, avolition, and anhedonia), positive (hallucinations), and cognitive (deficiencies in attention, verbal and visuospatial learning, memory, problem-solving, and cognitive flexibility) [2-6]. Multiple factors, including genetic makeup, surroundings, and psychological aspects, can cause SCZ. The genesis of this psychological condition is currently not clear; however, it is marked by substantial anatomical and functional alterations in the brain [7]. Ketamine functions by antagonizing N-methyl-D-aspartate (NMDA) to affect the glutamate system [8]. Along with its negative symptoms, such as emotional withdrawal and blunted affect, ketamine also causes positive effects (delusions and abnormal perception) [9,10]. Ketamine (30 mg/kg; *i.p.*) injections for 10 consecutive days were used to provoke SCZ in mice.

Phosphodiesterases (PDEs) are a class of phosphor-hydrolytic enzymes that regulate the strength of intracellular second messenger signaling by converting cyclic guanosine monophosphate (cGMP) (3',5'-cGMP) and cyclic adenosine monophosphate (cAMP) (3',5'- cAMP) to their inactive 5' AMP and 5' GMP forms, respectively [11,12]. Papaverine exhibits notable neuropsychiatric potential through diminishing PDEs-10A (PDE-10A) enzymes in the striatum [13]. PDE-10 is essential to regulate intracellular cAMP and cGMP in the neurons, which serve as key integrators of cortical glutamatergic and dopaminergic signaling [14]. Impaired neurotransmission (glutamatergic and dopaminergic) is strongly linked with SCZ. It was well known that striatal dopaminergic hyperactivity is linked with hallucinations and delusions, while glutamatergic hypofunction is linked with negative and cognitive disability [15,16]. Preclinical evidence shows that PDE-10A inhibitors can normalize striatal output, improve cognitive ability, and attenuate psychosis behaviors in animals [17, 18]. Thus, papaverine (PDE-10A inhibitor) emphasizes its potential as a pharmacological agent for SCZ through targeting dopaminergic and glutamatergic imbalances.

METHODS

Experimental animals and housing

Experiments were carried out on Swiss albino male mice with a body weight of 25±5.0 g. Animals were accommodated under standard laboratory care conditions. (12:00 h light/dark cycle) with free water and a consistent diet. All experimental protocols received approval from the Institutional Animal Ethics Committee (IAEC) (1204/PO/Re/S/08/CPCSEA/24-01). Throughout the whole investigation, all IAEC criteria were strictly adhered to check the suffering of the test subjects.

Experimental protocol

Fresh solutions of each drug were prepared before use. A dosing schedule and dosages were selected based on previously published reports. There were seven groups employed in this study, with six (n=6) mice in each group, to examine the role of PDE-10 receptor modulators in ketamine provoked SCZ.

Group I - Control: Mice were treated with 0.9% normal saline (10 mL/kg; i.p.) for 4 weeks. Group II - Vehicle: 0.5% carboxymethylcellulose (CMC) was administered in mice for 4 weeks. Group III – Papaverine *perse*: Papaverine (30 mg/kg; i.p.) was given to each mouse for 4 weeks to assess the effect of the drug itself on normal mice. Group IV- Ketamine: Ketamine was given to each mouse intraperitoneally at a dose of 30 mg/kg for 10 successive days, followed by behavioral and biochemical assessment for 28 days. Group V - Ketamine+papaverine (15 mg/kg): Each Mouse was treated with ketamine for 10 repeated days, followed by papaverine (15 mg/kg; i.p.) administration from the 10^{th} to 28^{th} day. Group VI- Ketamine+papaverine (30 mg/kg): Each Mouse was treated with ketamine for 10 repeated days, followed by papaverine (30 mg/kg; i.p.) administration from the 10^{th} to 28^{th} day. Group VII- Ketamine+Clozapine (7.5 mg/kg): Mice were treated with ketamine for 10 repeated days, followed by clozapine (7.5 mg/kg; p.o.) administration from the 10^{th} to 28^{th} day.

Behavioral assessment

Forced swim test (FST)

Each mouse was designed to swim (for 6 min) in a circular open glass container measuring (diameter 15 cm, height 25 cm), containing water (25±1°C) as per the published reports. The immobility period during the last 4 min of the 6-min swimming session was recorded and subsequently analyzed [19-21].

Open field test (OFT)

The OFT apparatus (width-50 cm, height-38 cm, length-50 cm) was used to assess the locomotor, anxiety, and exploratory abilities of mice, as per the previous reports. Once the mice had gotten used to each other, they were placed individually at the midpoint of the field and permitted to move around freely for 5 min. The number of crossings with all paws, the latency to move from the center square (in seconds), and the occupancy of the periphery were all recorded [22-24].

Morris water maze test (MWM)

To investigate memory and learning, MWM tests were used. A 10-cm underwater platform that is submerged in a circular pool of water (45 cm in height and 150 cm in diameter) and painted with white dye. A concealed underwater platform was kept 1 cm below the water's surface, and two cotton strands were used to partition the chamber into four equal quadrants. A single mouse was put through four daily tests with different sinking positions, and the subaquatic platform stayed the same throughout the training stages. Animals were tracked on an immersed platform for 2 min, staying for 20 s. Mice were transported to the platform and allowed 20 s to sit if they were unable to do so. Q4 was an assigned section across every acquisition trial, and learning was measured using the escape latency time (ELT) on the 4^{th} test day. After being taken out of the underwater platform, the mice had 2 min to examine the chamber. Four trials were conducted from various quadrants, with memory being indicated by the period of stay in the assigned quadrant. After assessing ELT, and on the 5^{th} day, they were put through a retrieval test. The experiment was kept at a fixed location in the working area [25-27].

Catalepsy bar test

The bar test measured 12 cm in height. Mice were carefully placed with their forelimbs on the bar and their hind limbs on the floor of the apparatus. To compare the automated measures, a stopwatch was used to measure the amount of time it took the mice to take both paws off the bar. Baseline readings were acquired using the test and standard drug before administration [28].

Social withdrawal test

Equipment for the social withdrawal test consisted of a rectangular, three-chamber box. There was an open central section in each compartment, allowing for unhindered access to each chamber. The dividing partitions were made of clear Plexiglas. One mouse could fit in each of two identical wire-cup-shaped containers with detachable lids. The unfamiliar mouse is held in one of these, which is vertically positioned in each side chamber of the apparatus. Without preventing direct physical contact between an animal inside and an animal outside, each container consisted of tiny metal wires connecting the inside and exterior of the cylinder. The following factors were noted: observation, general lighting in the room, and follow-up. Every chamber was cleaned with 70% ethanol following each experiment [29].

Biochemical estimation

The animal's brain was removed cautiously. Tissue was prepared as a 10% (w/v) homogenate using normal phosphate buffer (0.10 M; pH of 7.4) employing PT 1600 E Polytron homogenizer at 4°C. The aliquots were isolated for biochemistry [26].

Valuation of thiobarbituric acid reactive species (TBARS)

TBARS was assessed spectrophotometrically by employing the technique described by Ohkawa *et al*. in 1979. Once the Pipetting out the supernatant (0.2 mL) into a tube, to prepare the reaction mixture, 1.50 mL of acetic acid, 0.20 mL of 8.1% sodium dodecyl sulfate, and 1.50 mL of 0.8% thiobarbituric acid were combined and thoroughly mixed. 1.50 mL of acetic acid, 0.20 mL of sodium dodecyl sulfate (8.1%), and 1.50 mL of 0.8% thiobarbituric acid were combined. To maintain the content at 4 mL, distilled water was mixed. The combination was then incubated at 95°C for an hour before it was cooled down. 5 mL of a 15:1 v/v butanol-pyridine mixture was added with 1 mL of distilled water once it had cooled. The rotational force of these tubes was 4000 g for 10 min. The emergence of a pinkish color was described as absorbent [26,30].

Valuation of reduced glutathione (GSH)

Reduced GSH was measured using the methods described by Beutler (1963). 10% w/v trichloroacetic acid has been added in a 1:1 ratio to the supernatant. Two milliliters of sodium hydrogen phosphate (0.30 M) and 0.25 mL of 0.001 M DTNB (5,5′-Dithiobis(2-nitrobenzoic acid)) in 1% w/v sodium citrate were added to the supernatant (0.1 mL). A regular plot was created using decreased GSH (10–100 μM) [31].

Estimation of acetylcholinesterase (AChE) activity

AChE activity was measured at 420 nm using the Ellman method. When thiocholine came into contact with dithiobis nitrobenzene, it turned yellow. A 25 mL flask containing 0.5 mL of the supernatant. Dilutions were made by adding the DTNB solution. Two separate test tubes were used to hold 4.0 mL. A single tube was used to mix the eserine solution. After that, 1.0 mL of the substrate solution was added to each tube [32,33].

Histopathological (H&E staining) examination

H&E staining, a common pathologist technique that mixes the two dyes, can be used to diagnose histological sections. Hematoxylin is oxidized to hematein by sodium iodate. Hemalums, a mixture of hematein and aluminum, are responsible for the purple staining of nuclei, mitochondria, and ribosomes. It becomes that color once the stain has been blued. H&E staining was used to assess pyknotic, shrunken, and neuronal damage. To view the slices, a polarized light microscope was used at ×200 magnification [26,34].

Statistical analysis

The results have been presented using the average value with standard error mean (SEM). The results were analyzed statistically by one-way analysis of variance (ANOVA) and subsequently examined with Tukey's multiple comparison test. According to statistics, a result of $p \leq 0.050$ seemed significant. Data analyses were conducted using GraphPad Prism, version 5.

RESULT

Effect of papaverine on immobility in FST

In the FST test, ketamine treatment significantly ($F [6, 35]=97.112$, $^{\#}p \leq 0.050$ vs. control; $^{\$}p \leq 0.050$ vs. ketamine) increased immobility time, an effect reversed by both doses of papaverine and clozapine (Fig. 1).

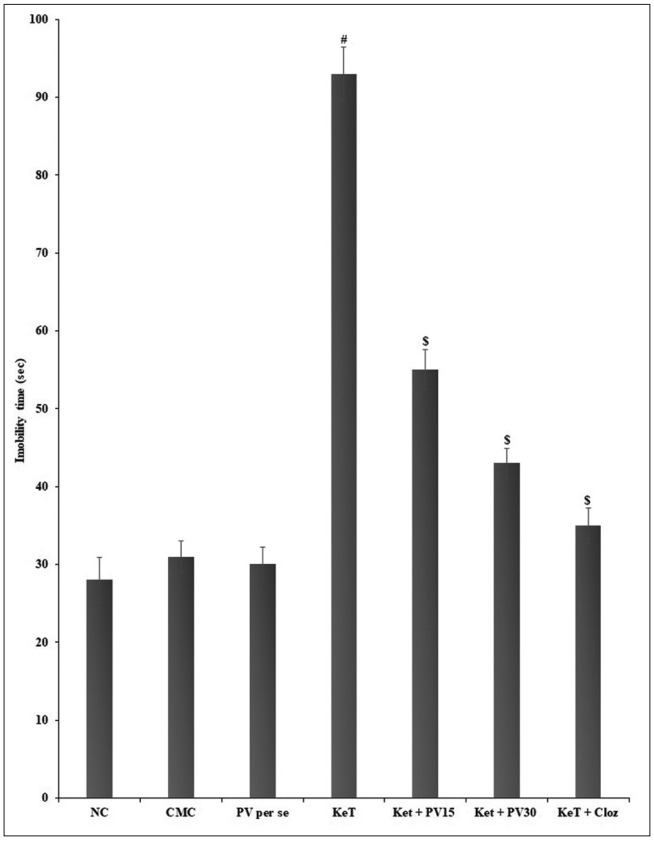

Fig. 1: Effect of Papaverine on immobility in FST. Results are represented as mean±standard error of the mean (n=6); one-way analysis of variance followed by Tukey's Multiple Comparison Test. Immobility time=F (6, 35)=97.112, #p≤0.05 versus control; $p≤0.05 versus ketamine. PV: Papaverine, NC: control (0.9% NaCl); Ket: Ketamine, Cloz: Clozapine, FST: Forced swim test, CMC: 0.5% carboxy methyl-cellulose

Effect of papaverine on locomotory and anxiety behaviors in OFT

In the OFT trials, ketamine exposed animals exhibited considerably higher locomotor (line crossing) activity (F [6, 35]=19.367, #p≤0.05 vs. control; $p≤0.05 vs. ketamine) and decline time spent in the center square (F [6, 35]=67.624, #p≤0.050 vs. control; $p≤0.050 vs. ketamine). In addition, mice treated with papaverine (15 and 30 mg/kg; i.p.) and clozapine (7.5 mg/kg; p.o) reversed the effect of ketamine (Fig. 2a and b).

Effect of papaverine on TSTQ and ELT

It is believed that MWM trials' higher day 4 ELT compared to day 1 ELT indicates a failure in acquisition or learning. But a lower Time spent in target quadrant (TSTQ) on the 5th day is suggestive of memory impairment. In comparison to the 1st day of ELT, the control group demonstrated a notable (p≤0.05) decrease on day 4 ELT, and a notable increase on the 5th day TSTQ (in the Q4 quadrant) as compared to the other Q1, Q2, and Q3 quadrants, which represent normal learning and memory. Ketamine (30 mg kg^{-1}; i.p.) exhibited a notable rise (F [6,35]=43.92, #p≤0.05 vs. control; $p≤0.05 vs. ketamine) in ELT on day 4 and a significant (F [6, 35]=101.45, #p≤0.05 vs. TSTQ in control; $p≤0.05 vs. TSTQ in ketamine) decrease in TSTQ on day 5, indicating impaired learning and memory. While mice treated with both doses of papaverine and clozapine significantly reversed the effect of ketamine (Fig. 3a and b).

Effect of papaverine on social withdrawal (social interactions)

Ketamine significantly (F [6, 35]=83.43, #p≤0.05 vs. control; $p≤0.05 vs. ketamine p≤0.05) decline the number of interactions with other animals, an effect reversed by both doses of papaverine and clozapine (Fig. 4).

Effect of papaverine on catalepsy

The impact of papaverine on cataleptic behavior as measured by descent latency (DL) from the bar is shown in Fig. 5. DL was not improved by papaverine (30 mg/kg), indicating that papaverine did not show anti-psychotic benefits, while clozapine (7.5 mg/kg) showed enhanced DL in contrast to the control.

Papaverine's effect on oxidative stress

Ketamine exposed animals had significantly increased (F [6, 35]=58.32, #p≤0.050 vs. control; $p≤0.050 vs. ketamine) TBARS (Fig. 6a) and decreased (F [6, 35]=41.53, #p≤0.050 vs. control; $p≤0.050 vs. ketamine) GSH (Fig. 6b) content. These effects were reversed by both doses of papaverine and clozapine.

Effect of papaverine on AChE activity

Mice exposed to ketamine exhibited significantly higher AChE (F [6, 35]=91.43, #p≤0.05 vs. control; $p≤0.05 vs. ketamine) activity than controls. While these effects were reversed by both doses of papaverine and clozapine (Fig. 7).

Effect of papaverine on histopathology

The cortex of the ketamine exposed SCZ animals displayed severe vacuolation, and dead cells showed nuclear pyknosis and reduced cell bodies in contrast to the control. In contrast to SCZ, the administration of papaverine and clozapine in conjunction with ketamine reduced the incidence of such structural deformity in the cerebral cortex and showed a nearly normal structural design (Fig. 8).

Illustrative pictures of H&E-stained (magnification ×400) in the cortical area. Ordinary neurons exhibited regularly shaped, round nuclei with bright blue; however, dead cells exhibited pyknotic nuclei and contracted cell bodies. Arrows indicate condensed, shrunken cells that are unevenly shaped and tangled.

Ketamine group: Nuclear pyknosis and reduced cell bodies were observed in dead cells.

Ketamine+papaverine (15 mg/kg; i.p.): Slightly nuclear pyknosis and contracted cell bodies in contrast to ketamine.

Ketamine+papaverine (30 mg/kg; i.p.): There are somewhat smaller cell bodies, fewer dead cells, and less nuclear pyknosis than with ketamine.

Ketamine+clozapine: The cortex's neurons appeared normal, with bright blue nuclei and a regulated, round shape.

DISCUSSION

SCZ is a chronic neurological condition that affects people of all ages by altering their ability to see, anticipate, and behave clearly. Unfortunately, there is currently no cure for SCZ, and the available treatments have unfavorable side effects. It pushes scientists to identify fresh targets for SCZ [35]. Targeting the NMDA receptor, sometimes referred to as a glutamate receptor, is a more modern approach to treating SCZ that has been connected to the genesis of the illness [36]. Since there is evidence that oxidative stress as well as inflammation could contribute to the development of SCZ, research into developing medications that target these two factors is also being conducted [37,38]. Here, we investigate the effects of papaverine (PDE-10 inhibitor), which may shield mice from the symptoms of SCZ caused by ketamine. This study used FST to measure immobility time (depressive behavior) and the open-ended test to measure locomotor and anxiety-like behaviors. In addition, mice's social interaction and spatial learning were evaluated using the withdrawal tests and MWM. The mice that received ketamine displayed symptoms of social isolation, cognitive impairment, increased length of immobility, and impaired locomotor and anxiety behaviors. Reduced GSH content, elevated AChE activity, and TBARS in the brain were also noticed in the ketamine-mediated SCZ -like phenotype in mice. The results verify that mice given ketamine exhibit biochemical and behavioral changes in addition to an increase

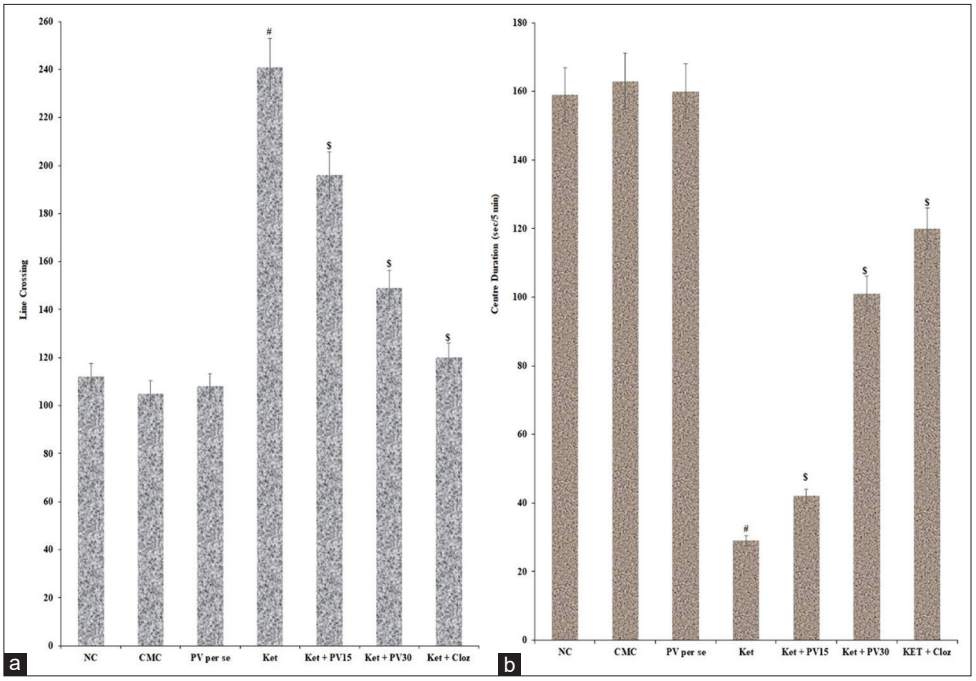

Fig. 2: (a and b) Effect of Papaverine on anxiety and locomotory behaviors in OFT. Results are represented as mean±standard error of the mean (n=6), with statistical significance determined by one-way analysis of variance followed by Tukey's multiple comparisons. Locomotor (line crossing) activity (F [6, 35]=19.367, #p≤0.050 vs. control; $p≤0.050 vs. ketamine) and time spent in the center square (F [6, 35]=67.624, #p≤0.050 vs. control; $p≤0.050 vs. ketamine). PV: Papaverine, NC: Control (0.9% NaCl), Ket: Ketamine, Cloz: Clozapine, OFT: Open field test, CMC: 0.5% carboxy methyl-cellulose

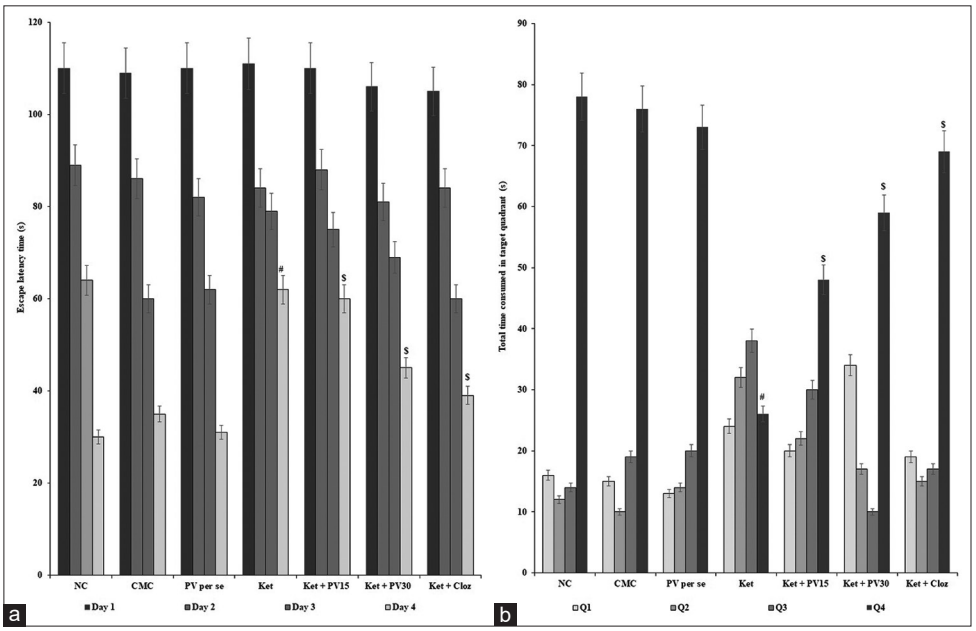

Fig. 3: (a and b) Effect of papaverine on ELT and TSTQ. Results are represented as mean±S.E.M, (n=6); one-way analysis of variance followed by Tukey's Multiple Comparison Test. ELT=(F [6,35]=43.92, p≤0.05) and TSTQ=(F [6,35]=101.45, p≤0.05); #p≤0.05 versus control; $p≤0.05 versus TSTQ in ketamine. TSTQ: Time spent in target quadrant, ELT: Escape latency time, NC: Control (0.9% NaCl), CMC: 0.5% carboxy methyl-cellulose, PV: Papaverine, Cloz: Clozapine, Ket: Ketamine

in brain oxidative damage [39]. Papaverine (15 and 30 mg kg^{-1}; i.p.) was effective in healing ketamine-induced oxidative damage (enhanced GSH activities, decline TBARS), above histopathological and behavioral alterations in mice. The findings of this study confirm that papaverine treatment reduces social interaction, hyper locomotion, and spatial working memory impairment in experimental schizophrenic mice by modifying the antioxidant system and restoring normal cholinergic neurotransmission.

To simulate the symptoms of SCZ, mice were administered ketamine, an NMDA receptor antagonist and D2 receptor agonist. Ketamine provoked SCZ-like symptoms by raising oxidative stress, lowering the activity of antioxidant enzymes, and altering neurotransmitter levels [40]. When ketamine is administered to experimental animals, it causes unpleasant feelings (social withdrawal) as well as cognitive impairments, such as issues with memory and learning [41]. Glutamatergic dysfunction and increased amygdala activity in the brain are caused by ketamine, and

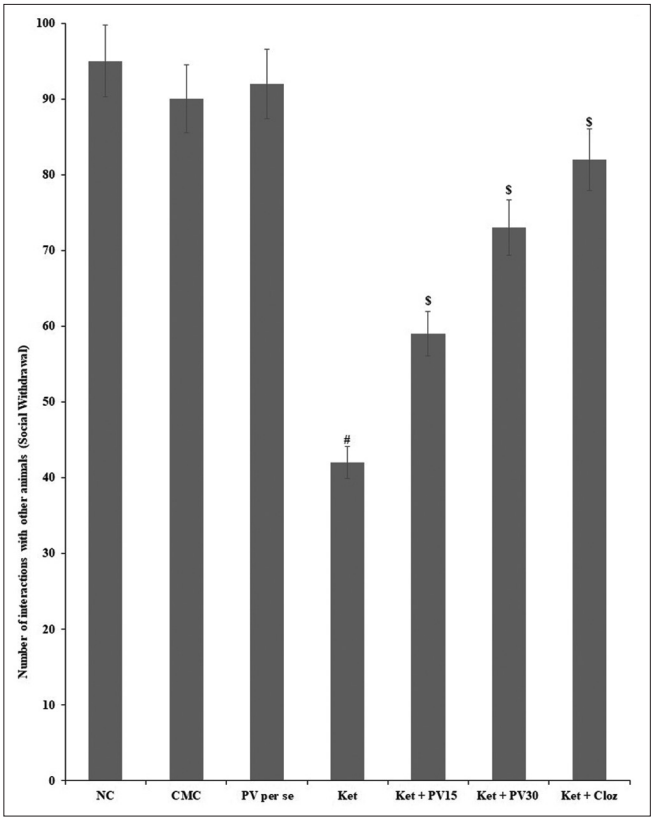

Fig. 4: Effect of papaverine on social withdrawal. Results are represented as mean±standard error of the mean (n=6); one-way analysis of variance followed by Tukey's multiple comparison test. Number of interactions=(F [6, 35]=83.43, p≤0.050); #p≤0.050 versus control; $p≤0.05 versus ketamine. NC: control (0.9% NaCl); CMC: 0.50% carboxy methyl-cellulose, PV: Papaverine, Cloz: Clozapine, Ket: Ketamine

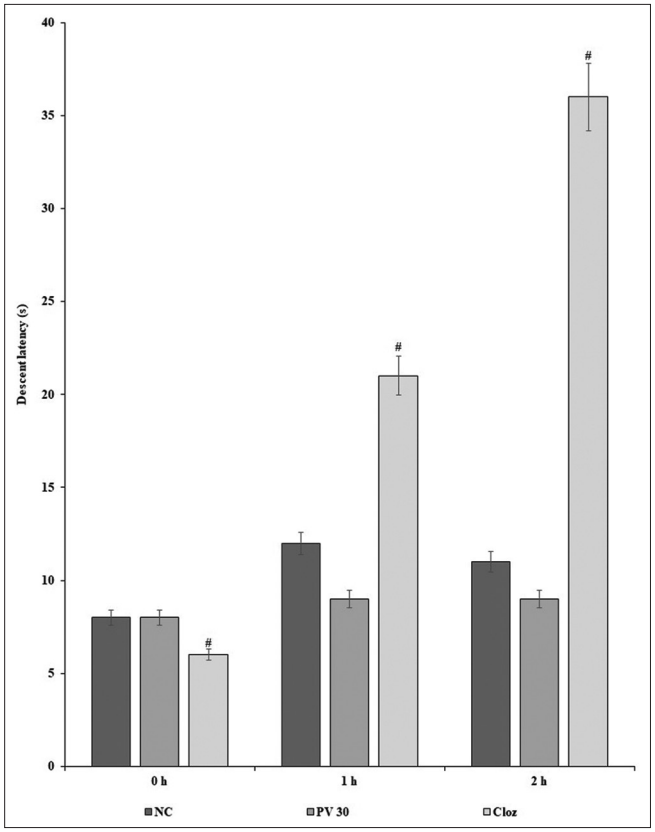

Fig. 5: Effect of papaverine on catalepsy. #p≤0.05 versus control. NC: control (0.9% NaCl), CMC: 0.5% carboxy methyl-cellulose, PV: Papaverine, Cloz: Clozapine, Ket: Ketamine

these effects have been closely linked to feelings of social interaction and anxiety reduction [42]. Ketamine also decreased brain-derived neurotrophic factor (BDNF) and released inflammatory cytokines in the cortex, striatum, and hippocampus, according to earlier studies [43]. These findings may be linked to cognitive deficiencies and other abnormalities in animal neurobehavior. Many results indicate that the ketamine-mediated SCZ-like phenotype may be caused by an enhanced cascade of oxido-nitrosative stress, inducible nitric oxide synthase, depletion of the endogenous antioxidant system, and upregulation of the production of inflammatory cytokines [44,45]. In the cerebellum, ketamine also raises proinflammatory cytokines called interleukins, which cause abnormal behaviors like SCZ [46]. In addition to raising TBARS concentrations in the cortex, limbic system, and hippocampus, ketamine also elevated oxidative stress by blocking the synthesis of GSH [43]. Indeed, prolonged inflammation and alterations in the brain can result from excessive oxidative damage [47]. The generation of free radicals and the antioxidant defense system may result in cellular or molecular damage. Long-term microglial activation and dysfunction have been linked to neuronal injury and death in ketamine-induced SCZ [48]. The substantia nigra and ventral tegmental regions of the brain produce dopamine, and alterations in dopamine have been connected to SCZ [49,50]. As reported by "original dopamine hypothesis," Hyperdopaminergic activity underlies the clinical features of SCZ. In rodents, ketamine injection raises dopamine levels in the frontal cortex, striatum, and nucleus accumbens [51]. According to reports, ketamine causes mitochondrial dysfunction in neuronal cells, which may be brought on through the production of reactive oxygen species, mitochondrial enlargement, caspase-3 activation, with the cytochrome c release and other apoptogenic proteins from the mitochondria [52]. In light of the foregoing explanation, ketamine was able to cause SCZ-like behavioral and physiological changes, which could be brought on by heightened cholinergic dysfunctions, elevated oxidative stress, inflammation, and a compromised mitochondrial-mediated cascade of brain death.

PDE inhibitors may be a useful tactic for influencing second messengers linked to mood, memory, and learning [53]. The PDE-10 inhibitor (papaverine) increases cAMP, leading to significant vasodilation, increasing blood flow, and providing anti-inflammatory effects [54]. Papaverine provides neuroprotection against cerebral ischemia and reduces cerebral infarction [55]. As a result, several studies have shown that PDE10 could be a therapeutic target for movement-related neurological conditions, for example, Huntington's and Parkinson's disease, as well as mental illnesses that impact the basal ganglia. Numerous studies indicate that papaverine has a range of pharmacological actions, including anti-oxidative, anti-inflammatory, and anti-apoptotic characteristics [56-58]. It may improve memory and learning abilities by altering the cAMP/cAMP-Response Element-Binding protein (CREB) cascade, possibly as a consequence of an increase in acetylcholine in brain tissue [59]. According to certain theories, cyclic nucleotides (cAMP) have an impact on neuronal synaptic plasticity and memory. PDE10 inhibitors can reduce the breakdown of cAMP and cGMP, making them a promising target for enhancing cognition [60]. PDE10A inhibitors reduce glutamatergic and dopaminergic dysfunction, which is thought to be the cause of SCZ [18]. The Nrf2 signaling pathway may have been activated by papaverine, as it dramatically raised the antioxidant parameters GSH and SOD and decreased the lipid peroxidation marker [61]. In striatopallidal neurons, papaverine-induced PDE10 inhibition triggered cAMP/PKA signaling by inhibiting dopamine D2 receptor signaling and enhancing adenosine A2A receptor signaling, simultaneously. In striatonigral neurons, papaverine-induced PDE10 inhibition triggered cAMP/PKA signaling, which in turn potentiated dopamine D1 receptor signaling

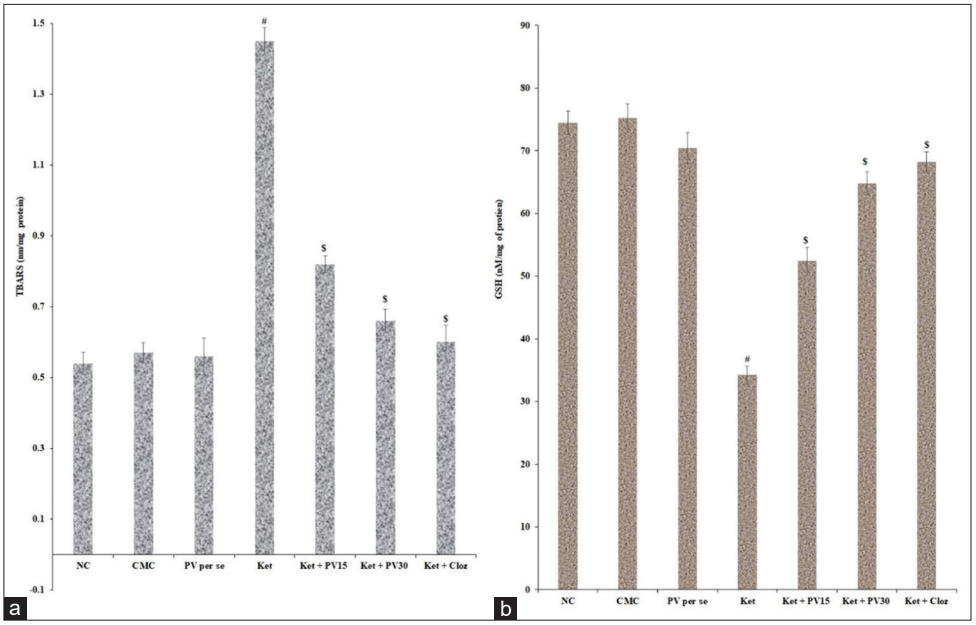

Fig. 6: (a and b) Effect of papaverine on oxidative stress. Results are represented as mean±standard error of the mean (n=6); one-way analysis of variance followed by Tukey's Multiple Comparison Test. TBARS (F [6, 35]=58.32, p≤0.05) and GSH (F [6, 35]=41.53, p≤0.05); #p≤0.05 versus control; $p≤0.05 versus ketamine. GSH: Glutathione, TBARS: Thiobarbituric acid reactive substances, NC: control (0.9% NaCl); CMC: 0.5% carboxy methyl-cellulose, PV: Papaverine, Cloz: Clozapine, Ket: ketamine

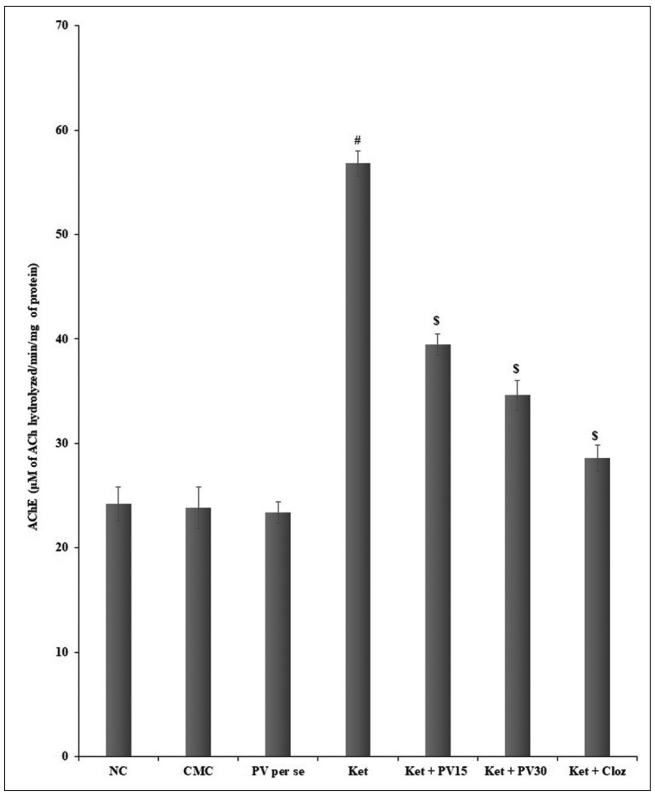

Fig. 7: Effect of Papaverine on AChE activity. Results are expressed as mean±standard error mean (n=6), and statistical analysis was performed using one-way analysis of variance with Tukey's multiple comparison test. AChE (F [6,35]=91.43, p≤0.050) #p≤0.050 versus control; $p≤0.05 versus ketamine. AChE: Acetylcholinesterase; ACh: Acetylcholine; NC: control (0.9% NaCl); CMC: 0.5% carboxy methyl-cellulose, PV: Papaverine, Cloz: Clozapine, Ket: Ketamine

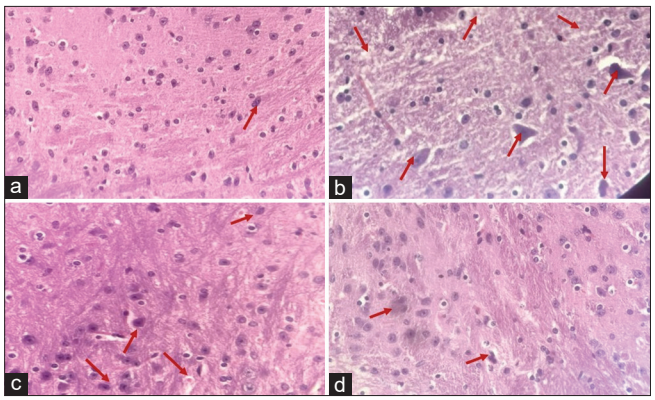

Fig. 8: Effect of cilostazol on histopathology. (a) Control, (b) ketamine, (c) ketamine+papaverine (15 mg/kg), (d) letamine+papaverine (30 mg/kg). The red arrow in the image caption indicates dead cells exhibited pyknotic nuclei and contracted cell bodies that are unevenly shaped and tangled.

[62]. In various brain regions, papaverine injection markedly reduced tumor necrosis factor-alpha-α, IL-6, and TBARS levels while considerably raising BDNF, pCREB/CREB, GSH, and IL-10 levels [59]. Our results imply that PDE-10 inhibition may affect cholinergic activity and oxidative stress, which in turn may affect neurocognitive performance. PDE-10A controls intracellular cAMP and cGMP, which trigger downstream signaling through PKA, CREB, and BDNF [13,63]. However, we only assessed oxidative stress and AChE activity in our work because they are indirect indicators of these processes. There was no direct measurement of cAMP/cGMP levels or PKA, CREB, or BDNF expression. To strengthen the mechanistic evidence supporting the involvement of PDE-10 inhibition in neuroprotection, future research should incorporate these molecular analyses. This study has demonstrated that papaverine has protective benefits against poor cognition, impaired motor coordination, and neuronal death due to its anti-AChE, anti-inflammatory, and antioxidant capabilities.

CONCLUSION

According to the findings of this study and the explanation above, papaverine significantly exhibited the neuroprotective effect in experimental mice treated with ketamine. These results demonstrate that papaverine may improve cerebral biochemical changes, as well as cognition, anxiety, and depression-like behaviors, and social interaction in mice exposed to ketamine.

ACKNOWLEDGMENTS

We are grateful to the management of Swami Vivekanand Subharti University, Meerut, for providing the chemicals and facilities to conduct this research.

AUTHOR CONTRIBUTIONS

The conceptualization and supervision of the study were carried out by Dr Prabhat Singh. Ruchika Srivastava contributed to the implementation of the study, data collection, interpretation of the findings, and manuscript writing. Dr Prabhat Singh and Dr Ajeet contributed to analyzing the data and finalized the manuscript. All authors also made substantial contributions to drafting the article and reviewing.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

FUNDING

This research did not receive any specific grant from any funding agency.

REFERENCES

1. Volkan K. Schizophrenia: Epidemiology, causes, neurobiology, pathophysiology, and treatment. J Health Med Sci. 2020 Nov;3(4): 487-521.
2. McCutcheon RA, Reis Marques TR, Howes OD. Schizophrenia-an overview. JAMA Psychiatry. 2020 Feb 1;77(2):201-10. doi: 10.1001/jamapsychiatry.2019.3360, PMID 31664453
3. Menon V. Brain networks and cognitive impairment in psychiatric disorders. World Psychiatry. 2020 Sep 15;19(3):309-10. doi: 10.1002/wps.20799, PMID 32931097
4. Shrivastava A, Aggarwal R, Singh RP, Khabiya R. A mini review on properties, mechanism of action, pharmacokinetic and pharmacodynamics and analytical methods of cariprazine. Int J App Pharm. 2023;15(1):31-5. doi: 10.22159/ijap.2023v15i1.46651
5. Moura BM, Isvoranu AM, Kovacs V, Van Rooijen G, Van Amelsvoort T, Simons CJ, *et al*. The puzzle of functional recovery in schizophrenia-spectrum disorders-replicating a network analysis study. Schizophr Bull. 2022 Jul 1;48(4):871-80. doi: 10.1093/schbul/sbac018, PMID 35266000
6. Pannu AS, Parle M. Anti-psychotic activity of *Pyrus communis* juice. Int J Pharm Pharm Sci. 2017;9(4):113-20. doi: 10.22159/ijpps.2017v9i4.14541
7. Yao JK, Dougherty GG, Reddy RD, Keshavan MS, Montrose DM, Matson WR, *et al*. Altered interactions of tryptophan metabolites in first-episode neuroleptic-naive patients with schizophrenia. Mol Psychiatry. 2010 Sep;15(9):938-53. doi: 10.1038/mp.2009.33, PMID 19401681
8. Olney JW, Labruyere J, Wang G, Wozniak DF, Price MT, Sesma MA. NMDA antagonist neurotoxicity: Mechanism and prevention. Science. 1991 Dec 6;254(5037):1515-8. doi: 10.1126/science.1835799, PMID 1835799
9. Krystal JH, Karper LP, Seibyl JP, Freeman GK, Delaney R, Bremner JD, *et al*. Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans. Psychotomimetic, perceptual, cognitive, and neuroendocrine responses. Arch Gen Psychiatry. 1994 Mar 1;51(3): 199-214. doi: 10.1001/archpsyc.1994.03950030035004, PMID 8122957
10. Mendes PF, Simon KA, Hueza IM. Toxic and immunotoxic evaluation of ketamine and/or ethanol in rats during 28 days. Int J Pharm Pharm Sci. 2017;9(9):205-14. doi: 10.22159/ijpps.2017v9i9.20582
11. Samidurai A, Xi L, Das A, Iness AN, Vigneshwar NG, Li PL, *et al*. Role of phosphodiesterase 1 in the pathophysiology of diseases and potential therapeutic opportunities. Pharmacol Ther. 2021 Oct 1;226:107858. doi: 10.1016/j.pharmthera.2021.107858, PMID 33895190
12. Ahmad F, Murata T, Shimizu K, Degerman E, Maurice D, Manganiello V. Cyclic nucleotide phosphodiesterases: Important signaling modulators and therapeutic targets. Oral Dis. 2015 Jan;21(1):e25-50. doi: 10.1111/odi.12275, PMID 25056711
13. Siuciak JA, Chapin DS, Harms JF, Lebel LA, McCarthy SA, Chambers L, *et al*. Inhibition of the striatum-enriched phosphodiesterase PDE10A: A novel approach to the treatment of psychosis. Neuropharmacology. 2006 Aug 1;51(2):386-96. doi: 10.1016/j.neuropharm.2006.04.013, PMID 16780899
14. Threlfell S, Sammut S, Menniti FS, Schmidt CJ, West AR. Inhibition of phosphodiesterase 10A increases the responsiveness of striatal projection neurons to cortical stimulation. J Pharmacol Exp Ther. 2009 Mar 1;328(3):785-95. doi: 10.1124/jpet.108.146332, PMID 19056933
15. Kantrowitz JT, Javitt DC. N-methyl-d-aspartate (NMDA) receptor dysfunction or dysregulation: The final common pathway on the road to schizophrenia? Brain Res Bull. 2010 Sep 30;83(3-4):108-21. doi: 10.1016/j.brainresbull.2010.04.006, PMID 20417696
16. Howes OD, Kapur S. The dopamine hypothesis of schizophrenia: Version III--the final common pathway. Schizophr Bull. 2009 May 1; 35(3):549-62.
17. Schmidt CJ, Chapin DS, Cianfrogna J, Corman ML, Hajos M, Harms JF, *et al*. Preclinical characterization of selective phosphodiesterase 10A inhibitors: A new therapeutic approach to the treatment of schizophrenia. J Pharmacol Exp Ther. 2008 May 1;325(2):681-90. doi: 10.1124/jpet.107.132910, PMID 18287214
18. Grauer SM, Pulito VL, Navarra RL, Kelly MP, Kelley C, Graf R, *et al*. Phosphodiesterase 10A inhibitor activity in preclinical models of the positive, cognitive, and negative symptoms of schizophrenia. J Pharmacol Exp Ther. 2009 Nov 1;331(2):574-90. doi: 10.1124/jpet.109.155994, PMID 19661377
19. Chatterjee M, Jaiswal M, Palit G. Comparative evaluation of forced swim test and tail suspension test as models of negative symptom of schizophrenia in rodents. Int Sch Res Not. 2012;2012(1):595141. doi: 10.5402/2012/595141, PMID 23738205
20. Patel DS, Anand IS, Bhatt PA. Evaluation of antidepressant and anxiolytic activity of phosphodiesterase 3 inhibitor-cilostazol. Indian J Psychol Med. 2012 Apr;34(2):124-8. doi: 10.4103/0253-7176.101776, PMID 23162186
21. Shougrakpam P, Bhattacharjee A, Medhabati M, Gunindro N. Comparative study of antidepressant-like effect of the leaves of *Sapindus emarginatus* and *Acorus calamus* in experimental animal models. Int J Curr Pharm Res. 2021 Jan 15;13:28-31.
22. Kraeuter AK, Guest PC, Sarnyai Z. The open field test for measuring locomotor activity and anxiety-like behavior. Methods Mol Biol. 2019 Dec 11;1916:99-103.
23. Seibenhener ML, Wooten MC. Use of the open field maze to measure locomotor and anxiety-like behavior in mice. J Vis Exp. 2015 Feb;6(96):e52434. doi: 10.3791/52434, PMID 25742564
24. Batool F, Kamal A, Sattar M, Shah HA, Ahmed DS. Evaluation of antidepressant activity of aqueous extract of sea buckhorn fruit in experimental model of depression. Pak J Bot. 2011;43(3):1595-9.
25. Dubey NE, Agrawal OP, Gidwani BI. Optimization, characterization and *in vivo* study of rivastigmine tartrate nanoparticles by using 22 full factorial design for oral delivery. Int J App Pharm. 2023;15(3):80-9. doi: 10.22159/ijap.2023v15i3.47140
26. Singh P, Sharma B. Agonism of histaminergic-H1 receptors in ischemic postconditioning during cerebral ischemia-reperfusion injury is protective. Curr Neurovasc Res. 2020 Dec 1;17(5):686-99. doi: 10.2174/1567202617666201214105720, PMID 33319685
27. Vorhees CV, Williams MT. Morris water maze: Procedures for assessing spatial and related forms of learning and memory. Nat Protoc. 2006 Aug;1(2):848-58. doi: 10.1038/nprot.2006.116, PMID 17406317
28. Luciani KR, Frie JA, Khokhar JY. An open source automated Bar Test for measuring catalepsy in rats. eNeuro. 2020 May 1;7(3):1-5. doi: 10.1523/ENEURO.0488-19.2020, PMID 32198157
29. Kaidanovich-Beilin O, Lipina T, Vukobradovic I, Roder J, Woodgett JR. Assessment of social interaction behaviors. J Vis Exp. 2011 Feb 25;(48):2473. doi: 10.3791/2473, PMID 21403628
30. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979 Jun 1;95(2):351-8. doi: 10.1016/0003-2697(79)90738-3, PMID 36810
31. Beutler RG. Reduced glutathion estimation. J Lab Clin Med. 1963;61:82.
32. Ellman GL, Courtney KD, Andres V Jr., Feather-stone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol. 1961 Jul 1;7(2):88-95. doi: 10.1016/0006-

2952(61)90145-9, PMID 13726518
33. Gupta VK, Kumar A, Siddiqi NJ, Sharma B. Rat brain acetylcholinesterase as a biomarker of cadmium induced neurotoxicity. J Toxicol. 2016 Jan 8;1(1):001-7.
34. You W, Zuo G, Shen H, Tian X, Li H, Zhu H, *et al*. Potential dual role of nuclear factor-kappa B in experimental subarachnoid hemorrhage-induced early brain injury in rabbits. Inflamm Res. 2016;65(12): 975-84. doi: 10.1007/s00011-016-0980-8, PMID 27554683
35. Khalid I, Saleem U, Ahmad B, Hawwal MF, Mothana RA. NMDA receptor modulation by Esculetin: Investigating behavioral, biochemical and neurochemical effects in schizophrenic mice model. Saudi Pharm J. 2024 Apr 1;32(4):101994. doi: 10.1016/j.jsps.2024.101994, PMID 38405040
36. Adell A. Brain NMDA receptors in schizophrenia and depression. Biomolecules. 2020 Jun 23;10(6):947. doi: 10.3390/biom10060947, PMID 32585886
37. Hope S, Hoseth E, Dieset I, Mørch RH, Aas M, Aukrust P, *et al*. Inflammatory markers are associated with general cognitive abilities in schizophrenia and bipolar disorder patients and healthy controls. Schizophr Res. 2015 Jul 1;165(2-3):188-94. doi: 10.1016/j.schres.2015.04.004, PMID 25956633
38. Hajizadeh Moghaddam A, Malekzadeh Estalkhi F, Khanjani Jelodar S, Ahmed Hasan T, Farhadi-Pahnedari S, Karimian M. Neuroprotective effects of alpha-pinene against behavioral deficits in ketamine-induced mice model of schizophrenia: Focusing on oxidative stress status. IBRO Neurosci Rep. 2024 Jun 1;16:182-9. doi: 10.1016/j.ibneur.2023.12.012, PMID 38318342
39. Abdel-Salam OM, El-Shamarka M, Omara EA. Brain oxidative stress and neurodegeneration in the ketamine model of schizophrenia during antipsychotic treatment: Effects of N-acetylcysteine treatment. React Oxygen Species. 2018 Jul 1;6(16):253-66. doi: 10.20455/ros.2018.839
40. Vasconcelos GS, Ximenes NC, De Sousa CN, Oliveira T, Lima LL, De Lucena DF, *et al*. Alpha-lipoic acid alone and combined with clozapine reverses schizophrenia-like symptoms induced by ketamine in mice: Participation of antioxidant, nitrergic and neurotrophic mechanisms. Schizophr Res. 2015 Jul 1;165(2-3):163-70. doi: 10.1016/j.schres.2015.04.017, PMID 25937462
41. McCutcheon RA, Abi-Dargham A, Howes OD. Schizophrenia, dopamine and the striatum: From biology to symptoms. Trends Neurosci. 2019 Mar 1;42(3):205-20. doi: 10.1016/j.tins.2018.12.004, PMID 30621912
42. Sobota R, Mihara T, Forrest A, Featherstone RE, Siegel SJ. Oxytocin reduces amygdala activity, increases social interactions, and reduces anxiety-like behavior irrespective of NMDAR antagonism. Behav Neurosci. 2015 Aug;129(4):389-98. doi: 10.1037/bne0000074, PMID 26214213
43. Ben-Azu B, Adebayo OG, Fokoua AR, Oritsemuelebi B, Chidebe EO, Nwogueze CB, *et al*. Antipsychotic effect of diosgenin in ketamine-induced murine model of schizophrenia: Involvement of oxidative stress and cholinergic transmission. IBRO Neurosci Rep. 2024 Jun 1;16: 86-97. doi: 10.1016/j.ibneur.2023.12.008, PMID 38282757
44. De Araújo FY, Chaves Filho AJ, Nunes AM, De Oliveira GV, Gomes PX, Vasconcelos GS, *et al*. Involvement of anti-inflammatory, antioxidant, and BDNF up-regulating properties in the antipsychotic-like effect of the essential oil of *Alpinia zerumbet* in mice: A comparative study with olanzapine. Metab Brain Dis. 2021 Dec;36(8):2283-97. doi: 10.1007/s11011-021-00821-5, PMID 34491479
45. Monte AS, De Souza GC, McIntyre RS, Soczynska JK, Dos Santos JV, Cordeiro RC, *et al*. Prevention and reversal of ketamine-induced schizophrenia related behavior by minocycline in mice: Possible involvement of antioxidant and nitrergic pathways. J Psychopharmacol. 2013 Nov;27(11):1032-43. doi: 10.1177/0269881113503506, PMID 24045882
46. Schiavone S, Tucci P, Trabace L, Morgese MG. Early celastrol administration prevents ketamine-induced psychotic-like behavioral dysfunctions, oxidative stress and IL-10 reduction in the cerebellum of adult mice. Molecules. 2019 Nov 5;24(21):3993. doi: 10.3390/molecules24213993, PMID 31694174
47. Watkins CC, Andrews SR. Clinical studies of neuroinflammatory mechanisms in schizophrenia. Schizophr Res. 2016 Sep 1;176(1): 14-22. doi: 10.1016/j.schres.2015.07.018, PMID 26235751
48. Monji A, Kato T, Kanba S. Cytokines and schizophrenia: Microglia hypothesis of schizophrenia. Psychiatry Clin Neurosci. 2009 Jun;63(3):257-65. doi: 10.1111/j.1440-1819.2009.01945.x, PMID 19579286
49. Bogerts B, Häntsch J, Herzer M. A morphometric study of the dopamine-containing cell groups in the mesencephalon of normals, Parkinson patients, and schizophrenics. Biol Psychiatry. 1983 Sep 1;18(9): 951-69. PMID 6640008
50. Wang YC, Ho UC, Ko MC, Liao CC, Lee LJ. Differential neuronal changes in medial prefrontal cortex, basolateral amygdala and nucleus accumbens after postweaning social isolation. Brain Struct Funct. 2012 Apr;217(2):337-51. doi: 10.1007/s00429-011-0355-4, PMID 22002740
51. Kokkinou M, Ashok AH, Howes OD. The effects of ketamine on dopaminergic function: Meta-analysis and review of the implications for neuropsychiatric disorders. Mol Psychiatry. 2018 Jan;23(1):59-69. doi: 10.1038/mp.2017.190, PMID 28972576
52. Debatin KM, Poncet D, Kroemer G. Chemotherapy: Targeting the mitochondrial cell death pathway. Oncogene. 2002;21(57):8786-803. doi: 10.1038/sj.onc.1206039, PMID 12483532
53. Hebb AL, Robertson HA. Role of phosphodiesterases in neurological and psychiatric disease. Curr Opin Pharmacol. 2007 Feb 1;7(1):86-92. doi: 10.1016/j.coph.2006.08.014, PMID 17113826
54. Kim JH, Yi HJ, Ko Y, Kim YS, Kim DW, Kim JM. Effectiveness of papaverine cisternal irrigation for cerebral vasospasm after aneurysmal subarachnoid hemorrhage and measurement of biomarkers. Neurol Sci. 2014 May;35(5):715-22. doi: 10.1007/s10072-013-1589-0, PMID 24297765
55. Sabir M, Gupta R, Sindhu RK, Gupta AK. A review on papaverine: A potential neuroprotective agent for cerebral ischemia-reperfusion injury. Biochem Cell Arch. 2024 Oct 2;24(1):3361. doi: 10.51470/BCA.2024.24.1-S.3361
56. Lee YY, Park JS, Leem YH, Park JE, Kim DY, Choi YH, *et al*. The phosphodiesterase 10 inhibitor papaverine exerts anti-inflammatory and neuroprotective effects through the PKA signaling pathway in neuroinflammation and Parkinson's disease mouse models. J Neuroinflammation. 2019 Dec;16(1):246. doi: 10.1186/s12974-019-1649-3, PMID 31791357
57. Beker MC, Altintas MO, Dogan E, Bayraktaroglu C, Balaban B, Ozpinar A, *et al*. Inhibition of phosphodiesterase 10A mitigates neuronal injury by modulating apoptotic pathways in cold-induced traumatic brain injury. Mol Cell Neurosci. 2024 Dec 1;131:103977. doi: 10.1016/j.mcn.2024.103977, PMID 39437931
58. Li J, Chen JY, Deng YL, Zhou Q, Wu Y, Wu D, *et al*. Structure-based design, synthesis, biological evaluation, and molecular docking of novel PDE10 inhibitors with antioxidant activities. Front Chem. 2018 May 15;6:167. doi: 10.3389/fchem.2018.00167, PMID 29868568
59. Luhach K, Kulkarni GT, Singh VP, Sharma B. Effect of papaverine on developmental hyperserotonemia induced autism spectrum disorder related behavioural phenotypes by altering markers of neuronal function, inflammation, and oxidative stress in rats. Clin Exp Pharmacol Physiol. 2021 Apr;48(4):614-25. doi: 10.1111/1440-1681.13459, PMID 33480092
60. Ashrafi S, Alam S, Sultana A, Raj A, Emon NU, Richi FT, *et al*. Papaverine: A miraculous alkaloid from opium and its multimedicinal application. Molecules. 2023 Mar 31;28(7):3149. doi: 10.3390/molecules28073149, PMID 37049912
61. Atshan DA, Zalzala MH. Papaverine attenuates the progression of alpha naphthylisothiocyanate induce cholestasis in rats. Curr Res Pharmacol Drug Discov. 2024 Jan 1;6:100177. doi: 10.1016/j.crphar.2024.100177, PMID 38322817
62. Nishi A, Kuroiwa M, Miller DB, O'Callaghan JP, Bateup HS, Shuto T, *et al*. Distinct roles of PDE4 and PDE10A in the regulation of cAMP/PKA signaling in the striatum. J Neurosci. 2008 Oct 15;28(42): 10460-71. doi: 10.1523/JNEUROSCI.2518-08.2008, PMID 18923023
63. Birjandi SZ, Abduljawad N, Nair S, Dehghani M, Suzuki K, Kimura H, *et al*. Phosphodiesterase 10A inhibition leads to brain region-specific recovery based on stroke type. Transl Stroke Res. 2021 Apr;12(2): 303-15. doi: 10.1007/s12975-020-00819-8, PMID 32378029