MERAL Publications and Solutions Pvt. Ltd.

Original Article

The potential therapeutic effect of donepezil on a ketamine rat model of psychosis: behavioral and histological study

Khlood Mohammed Mehdar[1]*

[1]Department of Anatomy, Faculty of Medicine, Najran University, Najran, Kingdom of Saudi Arabia.

Correspondence: Khlood Mohammed Mehdar, Department of Anatomy, Faculty of Medicine, Najran University, Najran, Kingdom of Saudi Arabia. khlood.mehdar02@gmail.com

Received: 27 October 2024; **Revised:** 30 January 2025; **Accepted:** 10 February 2025

ABSTRACT

Mental illnesses like bipolar disorder and schizophrenia often present psychotic symptoms. Current antipsychotic medications, including clozapine, can cause serious side effects. This study investigates the potential therapeutic effects of the cholinesterase inhibitor donepezil compared to clozapine in a ketamine-induced psychosis model in rats. Forty adult male albino rats were divided into four groups: a control group, a psychosis group (given 25 mg/kg ketamine HCl for 14 days), a Clozapine + Psychosis group (received clozapine from days 8 to 21), and a Donepezil + Psychosis group (received donepezil during the same period). Neurobehavioral changes were assessed using the open field test, social interaction test, and Y-Maze test. After the study, the rats were euthanized for histological analysis, including GFAP immunohistochemistry and morphometric evaluation of the hippocampus. Ketamine induced positive, negative, and cognitive symptoms of psychosis and caused hippocampal degeneration, evident through cellular changes and increased GFAP expression. Both donepezil and clozapine mitigated these degenerative and behavioral symptoms associated with psychosis. The administration of ketamine resulted in psychotic symptoms and hippocampal degeneration in rats. Both clozapine and donepezil improved these outcomes, suggesting that donepezil may potentially substitute clozapine in treating ketamine-induced psychosis, addressing both clinical and histological issues effectively.

Keywords: Psychosis, Donepezil, Ketamine, Clozapine, Rat model Hippocampus

Introduction

Psychosis is a mental state where an individual loses all sense of reality, and understanding its behavioral signs can be challenging due to the variety of psychotic disorders [1]. Psychosis symptoms fall into three categories: positive symptoms (hallucinations, delusions), negative symptoms (avolition, isolation, anhedonia), and cognitive decline (issues with attention, concentration, learning, and memory) [2]. Patients with schizophrenia, bipolar disorders, and Parkinson's disease frequently experience psychosis [3].

The exact cause of psychosis is unclear, but evidence suggests it may involve disruptions in the signaling pathways of neurotransmitters like dopamine, glutamate, serotonin, and GABA. These disruptions can lead to abnormal interneuron functioning, resulting in cognitive, behavioral, and social impairments [4]. The common view of the neurobiology of psychosis is that dysfunction in the mesolimbic pathway, caused by a chemical imbalance, leads to the disorder. Decreased GABA signaling in the hippocampus increases its activity, further amplifying dopamine signaling [1].

Additionally, the hippocampus is a vital brain region involved in the pathophysiological changes associated with psychosis. It is essential for forming new memories, regulating emotions, and providing spatial orientation [5]. Notably, impairment in the

Access this article online	
Website: www.japer.in	**E-ISSN:** 2249-3379

How to cite this article: Mehdar KM. The potential therapeutic effect of donepezil on a ketamine rat model of psychosis: behavioral and histological study. J Adv Pharm Educ Res. 2025;15(2):40-50. https://doi.org/10.51847/OdDqDge3fy

hippocampus, especially in the CA1 region, is a significant aspect of the pathophysiology linked to psychosis [6]. Research indicates that neuronal stem cell proliferation and hippocampal volume are reduced in psychotic disorders like schizophrenia [7, 8]. Since hippocampal dysfunction is crucial in the onset of psychosis, addressing it may aid in developing new treatments and preventive strategies.

Animal models play a crucial role in researching the causes of psychosis and finding effective treatments for this and other human illnesses [9]. Ketamine is an FDA-approved anesthetic that acts as an NMDA receptor antagonist. While it has medical applications, increasing evidence indicates it can cause neurotoxicity in animal models [10]. As a glutamate antagonist, ketamine can induce temporary symptoms similar to those of schizophrenia [11] and may worsen schizotypal symptoms in people. Researchers often use sub-anesthetic doses (5–10 mg/kg) in rats to mimic schizophrenia-like symptoms [12, 13].

Antipsychotics are classified into two groups: atypical (second-generation) and typical (first-generation) antipsychotics [14]. Clozapine, authorized by the FDA in 1989/1990, was the first atypical antipsychotic reintroduced to the U.S. market [15, 16], and is considered a key mood stabilizer [17]. While effective in treating symptoms, antipsychotics can cause cognitive impairment and unpleasant side effects [18]. Potential adverse effects include cardiovascular issues (prolonged QT interval), neurological complications, agranulocytosis, and metabolic disorders (type 2 diabetes, weight gain, dyslipidemia) [19]. Thus, developing antipsychotic medications with better efficacy and fewer side effects is essential.

Evidence increasingly links acetylcholine dysregulation to psychosis, particularly in conditions associated with Lewy bodies, such as Alzheimer's disease (AD), Parkinson's disease (PD), and dementia with Lewy bodies (DLB) [20]. Psychotic symptoms significantly burden both patients and caregivers. Acetylcholinesterase inhibitors (AChEIs) may be effective treatments for these symptoms [21]. Research indicates that cholinergic deficits contribute to psychosis in AD, PD, and DLB, suggesting that AChEIs could be more tolerable options than traditional antipsychotics and benzodiazepines [22].

Donepezil, a first-line treatment for AD, enhances cognitive performance and protects neural pathways [23]. It uniquely inhibits both the core and peripheral catalytic sites of acetylcholinesterase, increasing acetylcholine levels by preventing its breakdown [22].

Therefore, the current study aims to demonstrate the potential therapeutic benefits of donepezil compared to clozapine in rats that have been subjected to ketamine-induced experimental psychosis, both clinically and histopathologically.

Materials and Methods

Drugs and chemicals

Ketamine hydrochloride was purchased as (Tekam®) 500 mg/ 10 ml vials from Hikmah Pharmaceuticals (Jeddah, Saudi Arabia). Each 0.1 ml of ketamine HCL was diluted in 0.3 ml of saline (0.9% NaCl) [24]. Clozapine was purchased as (Clozapine®) 25 mg tablets from a local pharmacy, in Jeddah, Saudi Arabia. The intraperitoneal injection (IP) volume for the medicines or solvent was 0.1 mg/kg [25]. Donepezil hydrochloride (cat#120011-70-3) was purchased from Sigma-Aldrich Chemical Co., St. Louis, USA. It was dissolved in saline and intraperitoneally injected in a dose of 0.1 mg/kg [26]. Each dose of Ketamine hydrochloride, Donepezil, and Clozapine was prepared by dissolving the medication in saline (0.9% NaCl). All solutions were freshly prepared before injection. All the drugs or saline were injected intraperitoneally (i.p) 3h before the onset of the behavioral test. Glial fibrillary acidic protein (GFAP) (rat monoclonal antibody, 1/100 dilution, cat# 665759) and streptavidin-biotin peroxidase complex– (cat# 586032) were purchased from Sigma-Aldrich Chemical Co., St. Louis, USA.

Animals

Forty mature male albino rats weighing between 200 and 250 grams were enrolled in the experiment. To reduce nonspecific stress on the day of the trial, all the animals were handled for a week before the experiment. They were kept at a constant 22 ± 2 °C ambient temperature and 50–70% humidity with a 12:12 h light/dark cycle (lights on at 7:00 a.m.). During the experiment, water and food were given to them ad libitum. The incinerator was used to dispose of the dead animals' remains.

Experimental design

We randomly divided forty adult male albino rats into four groups, with ten rats in each:

- *Group I (control group): was divided into two subgroups*

  • **Subgroup Ia:** (naive control): Five rats served as the naive control subgroup, and they received no medical treatment.

  • **Subgroup Ib:** (Sham control): Five rats served as the sham control subgroup, and they were given 0.4 ml of intraperitoneal saline once a day for 21 days.

- *Group II (psychosis rat model):* Rats were exposed to intraperitoneal ketamine HCl (25 mg/kg/d) for 14 days, and then they were left untreated for the final 7 days [24].

- *Group III (clozapine + psychosis rat model):* For 14 days in a row, rats were given intraperitoneal ketamine hydrochloride (25 mg/kg/d). This group was given intraperitoneal clozapine (5 mg/kg/d) dissolved in saline from the eighth to the twenty-first day [25].

- *Group IV (donepezil + psychosis rat model):* For 14 days, rats were given intraperitoneal ketamine hydrochloride (25 mg/kg/d). Intraperitoneal injections of donepezil (5 mg/kg/d) were administered to this group from the eighth to the twenty-first day [24].

Behavioral analysis

Under the light period, from 9 a.m. to 1 p.m., each group underwent a separate set of behavioral assessments. On the seventh and twenty-first days of the trial, we conducted all behavioral assessments. There was complete anonymity for the investigator; the rats were tested according to a randomized sequence.

Open field test (OFT)

An open field test (OFT) to assess positive symptoms (locomotor activity) was used in the current study. The OFT apparatus was a 50 × 25 × 50 cm square box, and the field was split into 25 squares. After being put in the middle of the box, each rat was given five minutes to roam around at will. A camera positioned above the arena captured the rat's actions. 70% ethanol was sprayed across the quadrant. The following behaviors were noted for every rat: 1. Locomotion: The quantity of lines a rat crosses in five minutes) [27], 2. Rearing: The tendency of rats to stand on their hind limbs during rearing; and 3. Self-grooming, which is the quick cleaning motions of the forelegs toward the face and/or body) [5].

Social interaction test

A social interaction test was used to evaluate negative symptoms. Before being used in an experiment, each animal was kept in isolation for six hours following the locomotor activity test, with no access to food or water. After that, two animals were chosen at random from the group and given a 15-minute stint in the open field arena (measuring 50 cm x 25 cm x 50 cm). Three parameters were assessed throughout this time: the duration between an animal's first interaction, the number of contacts, and the total duration of time the animals interacted with one another [28].

The Y-Maze test

The Y-maze test was used to assess the signs of cognitive deterioration. The rats' spatial working memory was assessed using the Y-Maze test. To reduce the impact of stress on test behavior, rats in their cages were brought into the test room one hour before the one-session Y maze research began. Using the Y-maze contraption, which measures 30 cm in length, 15 cm in height, and 8 cm in width with arms spaced 120° apart, all animal behaviors were captured by a camera and processed by a video processing system. Every animal was held in the center for eight minutes. Rats frequently switch between the three arms to test their ability to remember. A successful arm entry was demonstrated by the rat fitting all four paws within the arm. The following formula was used to compute the percentage of spontaneous alternation [26].

$$\%\text{Spontaneous alternation (\%SAP)} = \left[\frac{\text{Number of alternations}}{\text{Total arm entries} - 2}\right] \times 100 \quad (1)$$

Brain sample preparation (hippocampus)

Rats were anesthetized and killed by cervical dislocation on the 22nd day of our experiment. After the brain was extracted, it was promptly washed in 0.1 M phosphate buffer saline (pH 7.4). The rat hippocampal region was reached by coronally dividing each left cerebral hemisphere into two halves.

Light microscopic examination

- *Haematoxylin and eosin (H & E)*

After fixing the hippocampus samples in 10% neutral buffered formalin for 24 hours, the next steps were to dehydrate them using graded alcohol, clarify them with xylol, and then embed them in paraffin. In line with Bancroft and Layton's instructions, 5 µm thick sections were cut and stained with haematoxylin and eosin (H&E) in order to study the rat hippocampus region's histological structure [29].

- *Immunohistochemical study for glial fibrillary acidic protein (GFAP)*

After 30 minutes in 0.1% hydrogen peroxide to inhibit endogenous peroxidase, serial sections of paraffin-embedded hippocampal specimens cut at 5 µm were incubated with the primary antibody. Using the modified avidin-biotin-peroxidase technique for GFAP, the astrocytes were examined, and the responses were stained with 3′, 3 Regular diaminobenzidine tetrahydrochloride (DAB), as indicated earlier [30]. After dehydrating and mounting the sections, Mayer's haematoxylin was used as a counterstain. The same procedures were followed to create the negative control sections, but PBS was substituted for the primary antibody.

Morphometric analysis

A light microscope (Leica, Switzerland) with a digital camera attached was used for microphotography. Using the image analysis program "ImageJ" (National Institute of Health, USA), the mean area percentage (%) of GFAP-positive astrocytes was calculated from 10 non-overlapping high-power fields (HPF x 400) on each slide across all experimental groups.

Statistical analysis

The statistical analysis is carried out using SPSS, a program developed and manufactured in Chicago, Illinois, USA, with version 23.00. All data are presented as mean ± standard deviation (SD). To verify normalcy, the Kolmogorov-Smirnov test was utilized. Tukey's multiple comparisons test was utilized to compare the means after one-way analysis of variance (ANOVA) was utilized to analyze the statistical significance of the morphometric data. The clinical behavioral test data was statistically analyzed using two-way ANOVA to compare the means and Tukey's multiple comparisons test to assess for significance. At $P<0.05$, the differences were finally deemed significant. All statistical graphs were created using Graph Pad Prism, version 10.0 (Graph Pad Software, San Diego, CA, USA).

Results and Discussion

Clinical behavioral test results

The effect of donepezil on open field test

These metrics were measured and recorded on days 7 and 22 of the open field test. According to **Table 1** and **Figures 1a-1c**, the frequency of rearing is followed by the number of line crossings, which is the number of times an animal uses all four legs to cross a square. Grooming is the last factor to consider.

Regarding the number of line crossings, on the 7th day, there was a significant ($P<0.0001$) increase in the number of line crossings in each ketamine-treated groups II, III, and IV in contrast with the control group I. However, the administration of Clozapine (group III) and Donepezil (group IV) reversed these outcomes on day 21 as the number of line crossings was significantly ($P<0.0001$) increased in the psychosis rat model (group II) than Clozapine (group III) and Donepezil (group IV). In contrast to group III, which received clozapine, the number of line crossings in the open field test was not significantly different after receiving donepezil ($P>0.99$). Finally, compared to the control group I, rats treated with Clozapine and donepezil had fewer line crossings, but there was also a notable increase ($P = 0.0036$ and $P= 0.0044$, respectively) **(Figure 1a)**.

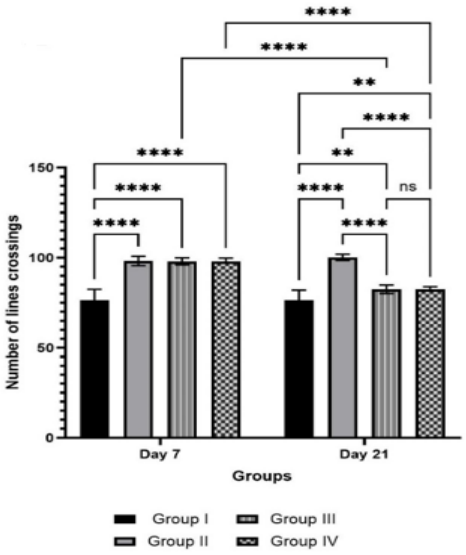
When comparing the rearing and grooming behaviors of the control group I to groups II, III, and IV treated with ketamine, we found that on the seventh day, there was a substantial increase ($P<0.0001$) in the amount of time spent on these activities in all four groups. However, the administration of Clozapine (group III) and Donepezil (group IV) reversed these outcomes on day 21 as the frequency of rearing and grooming time was significantly ($P<0.0001$) increased in psychosis rat model (group II) than Clozapine (group III) and Donepezil (group IV). We found no statistically significant difference between the groups treated with Donepezil and group III treated with Clozapine in terms of rearing time or grooming time in the open field test ($P= 0.99$ and $P= 0.46$, respectively). Finally, in comparison to the control group I, our data showed that rats treated with Clozapine and donepezil had a lower rearing rate, but this was accompanied by a significant rise ($P<0.0001$ for donepezil and $P<0.0001$ for Clozapine) **(Figures 1b and 1c)**. Our findings show that ketamine heightens motor activity indicators like raising and grooming. Positive psychotic-like behaviors are what these are referred to as. This dose-dependent reduction in ketamine-induced rearing and grooming behaviors is indicative of the therapeutic efficacy of Donepezil.

Table 1. Assessment of the Effects of Donepezil on animal behavior analysis in the open field test, Social Interaction Test, and Y-Maze Test in all experimental groups.

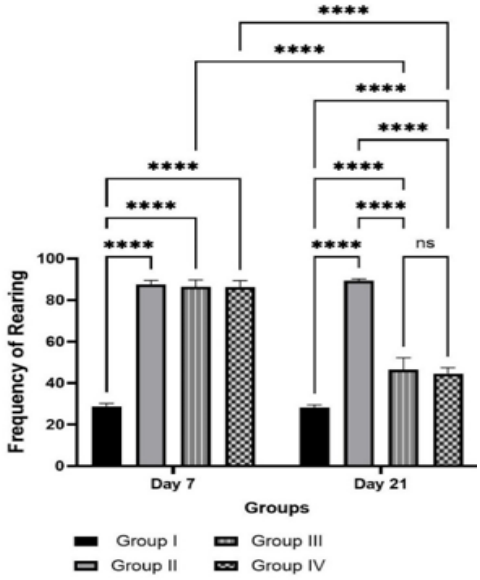
	Group I	Group II	Group III	Group IV
Open Field Test				
A) Number of line crossings				
Day 7	76.4 ± 6.05	98.2± 2.57	98.00± 1.94	97.90±1.91
Day 21	76.4 ± 5.60	100.2± 1.75	82.50± 2.32	82.40±1.50
B) Frequency of rearing				
Day 7	28.60±1.57	87.60±1.95	86.40±3.37	86.20±3.22
Day 21	28.20±1.22	89.40±0.84	46.40±5.75	44.60±2.79
C) Frequency of grooming				
Day 7	7.20±1.22	29.00±2.49	29.40±2.71	29.20±2.14
Day 21	7.60±0.84	32.80±2.44	14.20±1.39	12.40±1.71
Social Interaction Test				
A) Latency time (s)				
Day 7	4.50±0.47	19.20±0.78	18.8±1.22	18.40±1.07
Day 21	4.60± 0.39	19.80±1.39	8.8±1.22	8.40±1.71
B) Number of Interactions				
Day 7	82.80±1.81	58.60±2.06	58.60±1.26	58.80±1.03
Day 21	81.80± 1.68	48.60±1.07	71.00±3.59	72.80±2.44
C) Contact time (s)				
Day 7	316.20±8.59	214.20±7.78	214.00±7.74	214.00±7.74
Day 21	316.00± 8.45	202.80±8.06	308.00±1.76	306.20±3.67
Y-Maze Test				
% spontaneous alternation behavior				
Day 7	64.20± 2.78	37.00± 2.66	36.60± 3.02	36.80± 2.78
Day 21	64.00± 2.90	29.20± 0.78	55.60± 2.71	56.00± 4.57

Statistical analysis was conducted utilizing one-way ANOVA, completed by Tukey's post hoc test. Data are expressed as Mean ± standard deviation (SD), with n representing the number of rats per group. Group I (control group); Group II (Psychosis rat model), Group III (Clozapine + Psychosis rat model), and Group IV (Donepezil + Psychosis rat model).

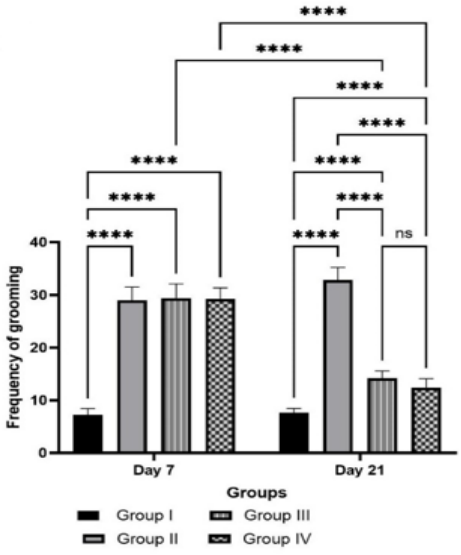
Open Field Test



a)



b)



c)

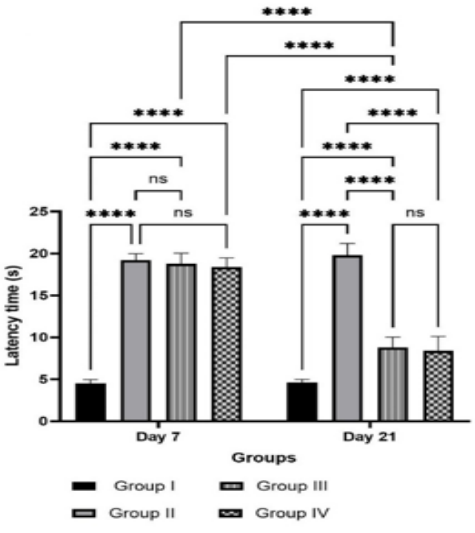
Figure 1. Animal behavior analysis in the open field test. The values are the mean of (a) the number of line crossings, (b) the Frequency of rearing, and (c) the Frequency of grooming. Statistical analysis was conducted utilizing two-way ANOVA, completed by Tukey's post hoc test. Group I (control group); Group II (Psychosis rat model), Group III (Clozapine + Psychosis rat model), and Group IV (Donepezil + Psychosis rat model). ns: nonsignificant, * Significant at $P < 0.05$, ** Significant at $P < 0.01$, **** Significant at $P < 0.0001$.

The effect of donepezil on social interaction test

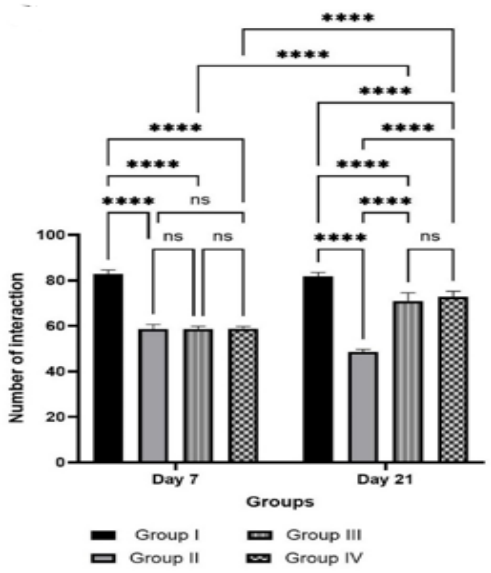
Based on the information in **Table 1** and **Figure 2a**, it was found that on the 7th day, the time it took for animals in treated groups II, III, and IV to form their initial social bond was considerably ($P < 0.001$) longer than in the control group, indicating the presence of social deficits. On the other hand, taken on the 21st day, Clozapine and Donepezil entirely blocked the latency time increase caused by ketamine ($P < 0.0001$ and $P < 0.0001$, respectively). Treatment with these levels of Donepezil counteracts the effects of ketamine, which creates an increase in the delay of the first connection between the animals. Nevertheless, when we compared Donepezil with Clozapine, we found that its administration resulted in a non-significant ($P= 0.99$) change in latency time on the social interaction test. Finally, as compared to the control group I, rats treated with Clozapine and donepezil had a shorter latency duration, although this was accompanied by a significant increase ($P <0.0001$ for donepezil and $P <0.0001$ for Clozapine).

In comparison to the control group, the ketamine group had significantly fewer social contacts and shorter communication durations ($P <0.0001$ and $P <0.0001$, respectively), as demonstrated in **Table 1** and **Figures 2b and 2c**. Contrarily, the rats in the Psychosis group II group that were given ketamine had a significantly lower total time of social contacts after two weeks of treatment with Clozapine and Donepezil, suggesting that the therapeutic effects of these doses of Donepezil reduced the total time of social contacts caused by ketamine.

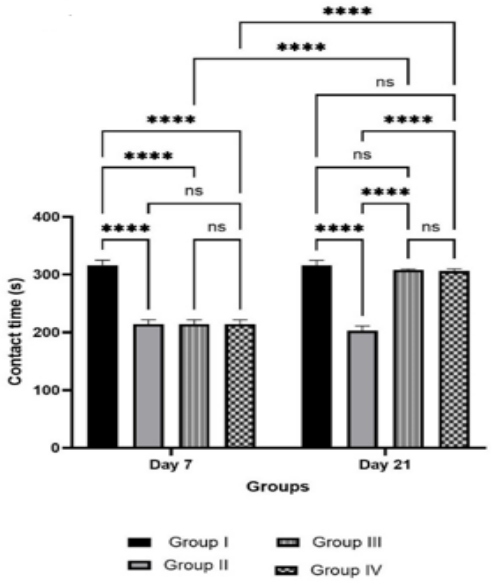
Social Interaction Test



a)



b)



c)

Figure 2. Animal behavior analysis in the social interaction test. The values are the mean of (a) Latency time (s), (b) numbers of interaction, and (c) contact time (s). Statistical analysis was conducted utilizing two-way ANOVA, completed by Tukey's post hoc test. Group I (control group); Group II (Psychosis rat model), Group III (Clozapine + Psychosis rat model), and Group IV (Donepezil + Psychosis rat model). ns: nonsignificant, **** Significant at $P < 0.0001$.

The effect of donepezil on Y-Maze

We found a significant group difference ($P<0.0001$) in the proportion of spontaneous alternation when evaluating the rats' performance in the Y-maze test, which could indicate a notable impact on their short-term memory. On day 7, when the data from the control group was analyzed, it was found that the rats in this group had a considerably greater rate of spontaneous alternation compared to the groups II, III, and IV that were treated with ketamine ($P<0.0001$). The results of the experiment showed that the control group had impaired memory since their alternation behavior was lower.

Alternatively, the cognitive impairment in rats caused by Ketamine (Psychosis group II) was significantly ameliorated after two weeks of treatment with Clozapine and Donepezil, indicating that the treatments enhanced spatial memory and reversed the intellectual impairment ($P <0.0001$). You can view all the mean ± SD results for the percentage of spontaneous alternation in the Y-maze test that was obtained from the two-way ANOVA test in **Table 1** and **Figure 3**.

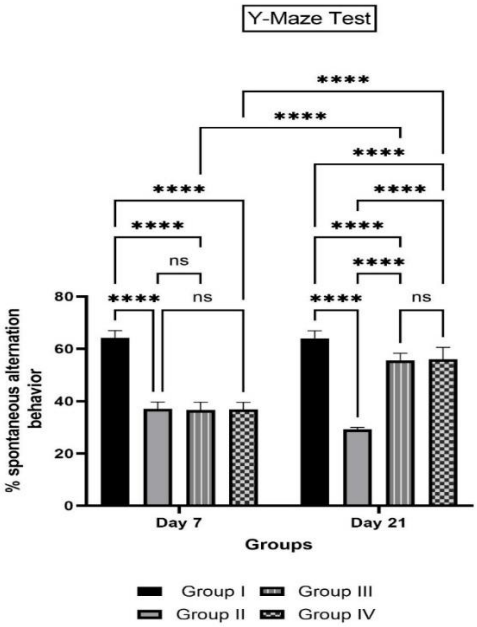


Figure 3. Analysis of animal behavior in the Y-Maze Test. The data represent the average percentage of spontaneous alternation behavior. Statistical analysis was conducted utilizing two-way ANOVA, completed by Tukey's post hoc test. Group I (control group); Group II (Psychosis rat model), Group III (Clozapine + Psychosis rat model), and Group IV (Donepezil + Psychosis rat model). ns: nonsignificant, **** Significant at $P < 0.0001$

Histological results

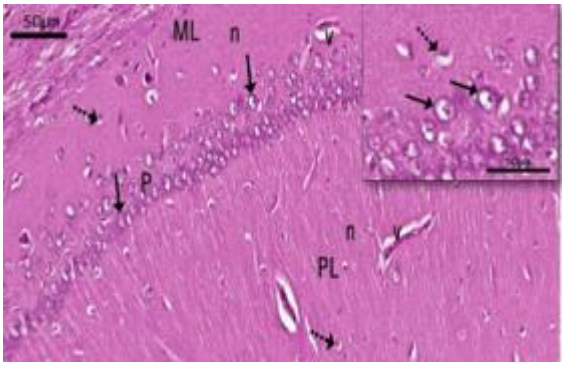
Histological analysis of H&E-stained sections in the Cornu Amminos (CA1) region of the hippocampal region of control rats (group I) revealed three distinct layers: molecular, pyramidal, and polymorphic cell layers. Small, densely packed pyramidal cells with large, rounded vesicular nuclei and conspicuous nucleoli, organized in three or four rows in a pyramidal layer. On an eosinophilic neuropil background, they showed a few scattered nuclei of neuroglial cells, such as next to blood capillaries **(Figure 4a)**.

The psychosis rat model rats (group II) exhibited a loss of hippocampal tissue integrity in the form of disorganized degenerated pyramidal cells. Loss of the normal arrangement of the pyramidal cells was evident. The degenerated dispersed Pyramidal cells were smaller, the nuclei were darkly elongated pigmented, and pericellular haloes encircled the cells; in some areas, the pyramidal cells had been lost entirely with large lightly

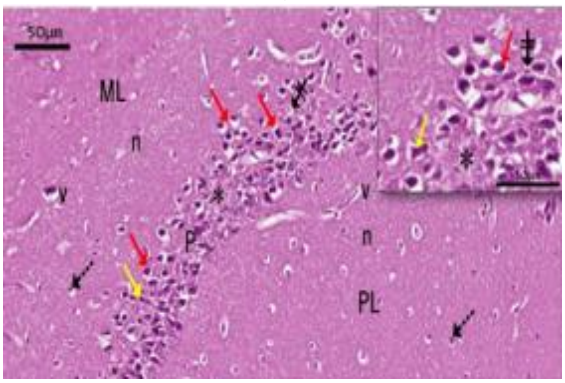
stained nuclei. Perineural glial cells could be observed around the degenerated neurons. Deeply marked nuclei of neuroglia were seen in the molecular and polymorphic layers of CA1, and the neuropil was vacuolated with numerous blood capillaries **(Figure 4b)**.

Group III (Psychosis rat model + clozapine) CA1 samples revealed its three layers on histological slides. The nuclei of glial cells were either strongly stained or only slightly stained in the molecular layer. Some of the pyramidal cells in the layer were degenerated, with shrunken, deeply stained, elongated nuclei or pyknotic nuclei with pericellular vacuolation; the majority of the cells in the layer were normal, and only a small number of cells had big, lightly stained nuclei. The pyramidal and polymorphic layers showed only a small number of vacuolated neuropil regions. **Figure 4c** shows that the molecular and polymorphic layers both have blood vessels.

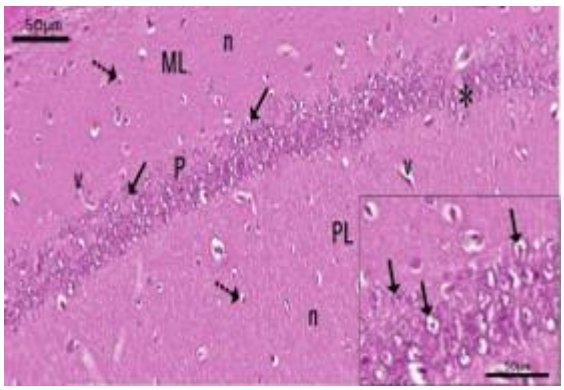
Group IV (the Donepezil-treated psychosis rat model) CA1 also exhibited results comparable to those of the control group. The majority of pyramidal cells were healthy, while a small number of cells showed signs of degeneration, including shrunken cells with heavily stained nuclei. **Figure 4d** shows that when compared to the psychosis group II, the neuroglia and blood capillaries at the molecular and polymorphic layers seemed to serve as a control group.



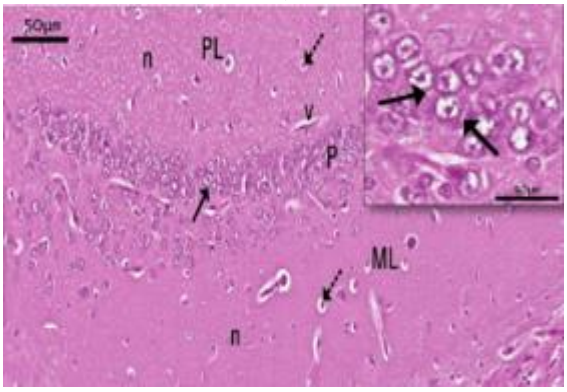
a)



b)



c)



d)

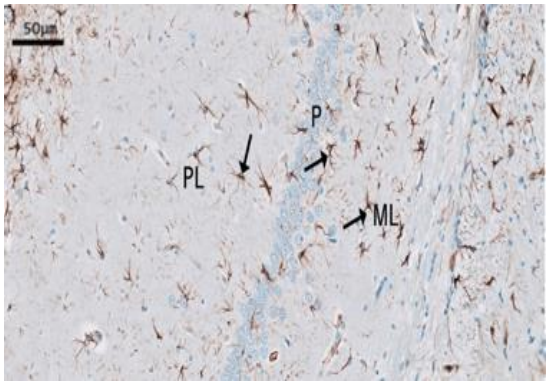
Figure 4. H&E staining of rat hippocampus showing the three layers of the Cornu Ammonis regions (CA1) Polymorphic layer (PL), Pyramidal cell layer (P), and Molecular layer (ML). a) Control rat (Group I) appears with normal architecture are appeared. Closely packed small pyramidal cells (black ↑) arranged in three or four rows in the P layer with large rounded vesicular nuclei and prominent nucleoli. Notice that glial cells (dot arrow) and blood vessels (v) are interspersed within an eosinophilic backdrop of the neuropil (n). b) Psychosis rat model (Group II) shows the degenerated, disorganized CA1 area with loss of normal pattern of pyramidal cell (P) layer arrangement. Some pyramidal cells appear shrunken with darkly stained pyknotic nuclei and wide pericellular spaces (red ↑), whereas others appear with lost nuclear details (yellow↑). Note, areas of neuron loss and vacuolations (*). Perineural glial (double stroke↑) cells can be observed around the degenerated neurons. The neuroglial cells illustrate deeply stained nuclei (dot arrow), and the neuropil (n) is vacuolated. Numerous blood capillaries (v) are also seen. c) Clozapine + Psychosis rat model (Group III) reveals pyramidal cells (black ↑) with large vesicular nuclei have appeared. Few cells with abnormally shaped nuclei and pericellular haloes (red ↑) are also detected. Notice neuroglia (dot arrow) cells are scattered. Few vacuolated neuropil areas (n) in the pyramidal layer (P) and polymorphic layer (PL) are seen. Blood capillaries (v) are noticed in the molecular (ML) and polymorphic (PL) layers. d) Donepezil + Psychosis rat model (Group III) nearly appears as control. Most of the pyramidal cells (P) appear with large vesicular nuclei (↑). A few cells with abnormally shaped deep pyknotic nuclei (red↑) are also detected. Notice that glial cells (dot arrow) and blood vessels (v) are interspersed within an eosinophilic backdrop of the neuropil (n). (H&E. A-D x20, Inset x40)

Immunohistochemical and morphometric results

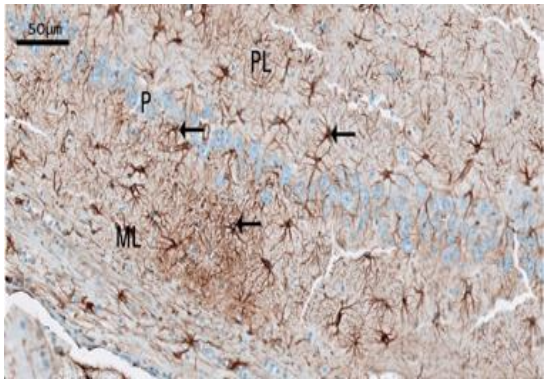
Figures 5 and 6 show the GFAP immunoreactivity and the mean number of positive GFAP cells in CA1 of all experimental groups of rats.

Immunostaining for GFAP in control (group I) animals revealed faint positive reactivity in astrocyte cytoplasm and processes. On the other hand, the CA1 area of the hippocampus of Ketamine-treated rats used to create the psychosis rat model (group II) revealed significantly enhanced GFAP-positive large astrocytes with extensive branching outspreading into the pyramidal. However, rats concomitantly treated with Clozapine (group III) and treated with Donepezil (group IV) displayed moderate reactions compared to Ketamine-treated rats **(Figures 5a-5d)**.

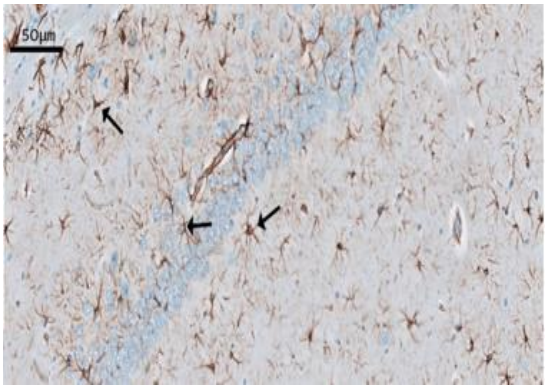
In comparison to the control group, Group II (the rat model of psychosis) had a significantly higher mean number of GFAP-positive cells in the CA1 area ($P < 0.0001$). However, when comparing group III (psychosis rat + Clozapine) to group II ($P < 0.0001$), there was a statistically significant decrease in the mean number of positive immunoreactive cells. The difference between group III and control rats (group I) and rats treated with Donepezil (group IV) was not statistically significant, though. It is worth noting that rats treated with donepezil showed a significantly lower mean area percent of immunoreaction compared to the psychotic rat model (group II), with a p-value of less than 0.0001. When comparing rats given Donepezil to rats in the control group (group I), there was no discernible change ($P=0.7621$). Groups III and IV rats given Clozapine and Donepezil, respectively, showed a marked decrease in responsiveness **(Figure 6)**.



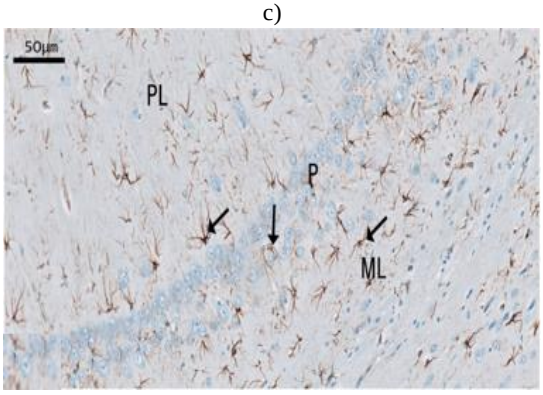
a)



b)



c)



d)

Figure 5. Immunostaining for GFAP-positive cells in the CA1 area of the hippocampus in all experimental groups in the pyramidal layer (P), molecular layer (ML), and polymorphic layer (PL). Control group (Group I; a) reveals faint positive brownish cytoplasmic reactivity (↑) in astrocytes and processes. Psychosis rat model (Group II; b) reveals a strong GFAP-positive brownish (↑) cytoplasmic reaction of large astrocytes with extensive branching outspreading into the pyramidal. Rats concomitantly treated with Clozapine (Group III; c) and treated with Donepezil (group IV; d) display moderate brownish (↑) cytoplasmic reactions. (IHC GFAP. A-Dx20; Scale bar = 50 µm)

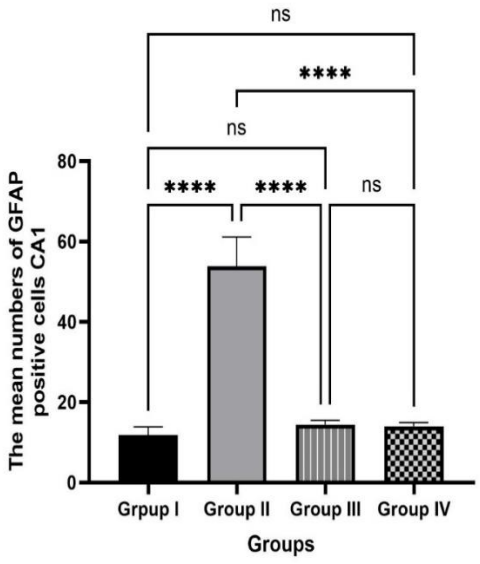


Figure 6. Effect of Donepezil on the mean number of GFAP positive cells in the CA1 area of hippocampus in all experimental groups. Group I (control group); Group II (Psychosis rat model), Group III (Clozapine + Psychosis rat

model), and Group IV (Donepezil + Psychosis rat model). Statistical analysis was conducted utilizing one-way ANOVA, succeeded by Tukey's post hoc test. Values are expressed as the mean ± standard deviation (n = 10). ns= non-significant. Significant difference at **** P < 0.0001.

The results of this trial suggest that donepezil may therapeutic advantage in the treatment of psychosis. Ketamine-induced psychotic symptoms were reversed by clozapine and donepezil, evidenced by decreased locomotor activity and increased social contacts and memory indices compared to a rat model of ketamine-induced psychosis. Research indicated that long-term ketamine treatments can lead to behavioral changes, including anxiety and memory impairment [31]. A study by Gouda *et al*. indicates that a subanesthetic dose of ketamine (25 mg/kg) may induce psychosis-related changes in animals, supporting their use as models for studying psychosis [32]. Teng *et al*. research highlights similarities in symptoms between ketamine users and individuals with schizophrenia, indicating that chronic ketamine exposure can impair cognitive abilities, especially in information processing [33]. Additionally, memory impairment and altered sensory encoding are observed with subanesthetic doses, and ketamine exacerbates symptoms in schizophrenia patients, suggesting that NMDA receptor antagonists impact a brain system already vulnerable to psychosis [33].

Ketamine is a valuable tool for researching the symptoms associated with acute schizophrenia, as demonstrated by recent evaluations [12]. The study assessed various neurobehavioral and schizophrenia-like behaviors using tests such as open-field (for positive symptoms), Y-maze (for cognitive symptoms), and social interaction (for negative symptoms). The results revealed that non-aesthetic doses of ketamine resulted in hyperlocomotion (indicative of a positive symptom) and social deficits (representing a negative symptom), which is consistent with our research [28]. The Y-maze test is essential for exploring spatial working memory in models of schizophrenia [34]. Administering ketamine intraperitoneally (IP) to mice led to a decline in long-term spatial memory, changes in theta and gamma oscillations in the hippocampus, and induced psychosis-like symptoms [12]. Furthermore, the current study indicated that donepezil and clozapine mitigated the psychotic symptoms induced by ketamine [35].

In comparison to the psychosis group treated with Clozapine, the donepezil-treated group exhibited a non-significant decrease in locomotor activity along with an increase in social interactions and memory indices, suggesting donepezil's potential to mitigate positive, negative, and cognitive symptoms similar to Clozapine. Carrasco *et al*. noted that donepezil is generally safe and helps alleviate cognitive symptoms while slowing deterioration in Alzheimer's disease (AD) patients [36].

Previous research evaluated donepezil's effectiveness in an animal model with brain insulin resistance and AD-like features, indicating that donepezil may enhance learning and memory in rats with type 2 diabetes by reversing certain AD-associated changes, such as lowering amyloid-β levels and increasing glutamate receptor expression [37]. The improvement of symptoms in the psychosis group receiving donepezil may result from increased acetylcholine release and the activation of muscarinic acetylcholine receptors, which are linked to enhanced learning and memory in various behavioral tests, also, donepezil boosts pyramidal neuron activity in the CA1 hippocampal region [38].

Fundamentally, neurons, like all cells, contain cytoplasm and a central nucleus. Damage is characterized by disrupted cell structure and component displacement. Our findings by light microscopy showed that pyramidal cells in group II (psychosis rat model) displayed degenerative changes in the CA1 area of the hippocampus, including disorganized, shrunken cells and increased vacuolated pericellular space and dark nuclei. Researchers found that [39]. Ketamine injection (25 mg/kg) links to similar degenerative changes, aligning with our findings. Signs of degeneration in pyramidal cells included perinuclear halos and reduced hyperchromatic nuclei. The neuropil showed many vacuoles, dilated blood vessels, and an increase in astrocytes. Cytoplasmic vacuolation and pyknosis suggest neurotoxicity, contradicting earlier studies [39].

Nogo *et al*. recognized vacuolated pericellular spaces to nerve cell shrinkage and cytoskeletal breakdown [40].

The psychosis groups treated with donepezil or clozapine showed histological and immunohistochemical improvements that matched the clinical symptom alleviation. Clozapine's effects align with Liu *et al*. [34], who associated these benefits with neurogenesis and neurotrophic factors like brain-derived neurotrophic factor. Similarly, donepezil's effects echoed findings by de Moura *et al*. indicating comparable histological outcomes with the control group. The mechanisms for these treatments' ability to reduce or reverse morphological issues may relate to immune system modulation and antioxidant effects, respectively [41]. Donepezil inhibits the hydrolysis of acetylcholine by blocking cholinesterase, providing neuroprotective benefits against various toxicities along with oxygen and glucose deprivation. It may also protect against mitochondrial dysfunction in the hippocampus. Currently, acetylcholinesterase inhibitors are used clinically for a variety of brain disorders and brain injury [42].

Astroglia and microglial activity influence hippocampal neurogenesis. Research indicates that reduced astrocyte activity is associated with aging and neurodegenerative diseases [43]. In this study, a subanaesthetic dose of 25 mg/kg of ketamine increased astrocytic activity, as shown by elevated GFAP expression and a significant rise in GFAP-positive cell area. This response may be an attempt to counteract neuron damage caused by ketamine. Also, rats treated with Clozapine or Donepezil showed a decrease in GFAP immunostained cells, and morphometric analysis confirmed a significant reduction in astrocyte numbers. This decline might reflect a return of neurons to their normal structure, which is mirrored in astrocyte counts. Astrocytes are crucial for the brain's antioxidant defense and can have neurotoxic effects when activated by oxidative stress [44]. The positive effects of donepezil indicate that donepezil may inhibit glial activation and enhance cholinergic activity, thus mitigating memory deficits.

Conclusion

Finally, our findings imply that giving adult male albino rats a subanaesthetic dose of ketamine results in psychosis and degenerative alterations in the hippocampus. Administering clozapine or donepezil improves psychosis's degenerative and behavioral alterations. The results of this investigation point to the potential benefit of donepezil in addressing the behavioral and degenerative aspects of psychosis. We, therefore, concluded that donepezil might completely replace clozapine to fully recover from all clinical and histological findings related to ketamine-induced psychosis in rat models. More studies are recommended to increase the use of these findings in clinical practice.

Acknowledgments: None

Conflict of interest: None

Financial support: None

Ethics statement: They were acquired and treated following the protocols following the consent of the Laboratory Animal Center's Ethics Committees in Assiute University, Cairo, Egypt.

References

1. Rawani NS, Chan AW, Dursun SM, Baker GB. The underlying neurobiological mechanisms of psychosis: focus on neurotransmission dysregulation, neuroinflammation, oxidative stress, and mitochondrial dysfunction. Antioxidants. 2024;13(6):709.
2. Yadav M, Kumar N, Kumar A, Jindal DK, Dahiya M. Possible biomarkers and contributing factors of psychosis: a review. Curr Pharmacol Rep. 2021;7:123-34.
3. Holper L, Ben-Shachar D, Mann JJ. Multivariate meta-analyses of mitochondrial complex I and IV in major depressive disorder, bipolar disorder, schizophrenia, Alzheimer disease, and Parkinson disease. Neuropsychopharmacology. 2019;44(5):837-49.
4. Yang AC, Tsai SJ. New targets for schizophrenia treatment beyond the dopamine hypothesis. Int J Mol Sci. 2017;18(8):1689.
5. Sanavi MA, Ghazvini H, Zargari M, Ghalehnoei H, Hosseini-Khah Z. Effects of clozapine and risperidone antipsychotic drugs on the expression of CACNA1C and behavioral changes in rat 'Ketamine model of schizophrenia. Neurosci Lett. 2022;770:136354.
6. Chen F, Bertelsen AB, Holm IE, Nyengaard JR, Rosenberg R, Dorph-Petersen K-A. Hippocampal volume and cell number in depression, schizophrenia, and suicide subjects. Brain Res. 2020;1727:146546.
7. Knight S, McCutcheon R, Dwir D, Grace AA, O'Daly O, McGuire P, et al. Hippocampal circuit dysfunction in psychosis. Transl Psychiatry. 2022;12(1):344.
8. Roeske MJ, Konradi C, Heckers S, Lewis AS. Hippocampal volume and hippocampal neuron density, number and size in schizophrenia: a systematic review and meta-analysis of postmortem studies. Mol Psychiatry. 2021;26(7):3524-35.
9. Forrest AD, Coto CA, Siegel SJ. Animal models of psychosis: current state and future directions. Curr Behav Neurosci Rep. 2014;1:100-16.
10. J. Bosnjak Z, Yan Y, Canfield S, Y. Muravyeva M, Kikuchi C, W. Wells C, et al. Ketamine induces toxicity in human neurons differentiated from embryonic stem cells via mitochondrial apoptosis pathway. Curr Drug Saf. 2012;7(2):106-19.
11. Estaphan S, Curpăn A-S, Khalifa D, Rashed L, Ciobica A, Cantemir A, et al. Combined low dose of ketamine and social isolation: a possible model of induced chronic schizophrenia-like symptoms in male albino rats. Brain Sci. 2021;11(7):917.
12. Beck K, Hindley G, Borgan F, Ginestet C, McCutcheon R, Brugger S, et al. Association of ketamine with psychiatric symptoms and implications for its therapeutic use and for understanding schizophrenia: a systematic review and meta-analysis. JAMA Netw Open. 2020;3(5):e204693-e.
13. Curic S, Andreou C, Nolte G, Steinmann S, Thiebes S, Polomac N, et al. Ketamine alters functional gamma and theta resting-state connectivity in healthy humans: implications for schizophrenia treatment targeting the glutamate system. Front Psychiatry. 2021;12:671007.
14. Torrisi SA, Laudani S, Contarini G, De Luca A, Geraci F, Managò F, et al. Dopamine, cognitive impairments and second-generation antipsychotics: from mechanistic advances to more personalized treatments. Pharmaceuticals. 2020;13(11):365.
15. Grover S, Gupta B, Ahmed K, Singh Y. Clozapine: a scientific analysis of global publications during 1970-2021. Int J Pharm Investig. 2023;13(1).
16. Peitl V, Puljić A, Škrobo M, Nadalin S, Fumić Dunkić L, Karlović D. Clozapine in treatment-resistant schizophrenia and its augmentation with electroconvulsive therapy in ultra-treatment-resistant schizophrenia. Biomedicines. 2023;11(4):1072.
17. Rybakowski JK. Application of antipsychotic drugs in mood disorders. Brain Sci. 2023;13(3):414.
18. Marder SR, Kirkpatrick B. Defining and measuring negative symptoms of schizophrenia in clinical trials. Eur Neuropsychopharmacol. 2014;24(5):737-43.
19. Dragoi AM, Radulescu I, Năsui BA, Pop AL, Varlas VN, Trifu S. Clozapine: an updated overview of pharmacogenetic biomarkers, risks, and safety—particularities in the context of COVID-19. Brain Sci. 2020;10(11):840.
20. Gomperts SN. Lewy body dementias: dementia with Lewy bodies and Parkinson disease dementia. Continuum (Minneap Minn). 2016;22(2):435-63.

21. d'Angremont E, Begemann MJ, Van Laar T, Sommer IE. Cholinesterase inhibitors for treatment of psychotic symptoms in Alzheimer disease and Parkinson disease: a meta-analysis. JAMA Neurol. 2023;80(8):813-23.
22. Chen ZR, Huang JB, Yang SL, Hong FF. Role of cholinergic signaling in Alzheimer's disease. Molecules. 2022;27(6):1816.
23. Ali B, MS Jamal Q, Shams S, A Al-Wabel N, U Siddiqui M, A Alzohairy M, et al. In silico analysis of green tea polyphenols as inhibitors of AChE and BChE enzymes in Alzheimer's disease treatment. NS Neurol Disord Drug Targets. 2016;15(5):624-8.
24. George MY, Menze ET, Esmat A, Tadros MG, El-Demerdash E. Naringin treatment improved main clozapine-induced adverse effects in rats; emphasis on weight gain, metabolic abnormalities, and agranulocytosis. Drug Develop Res. 2021;82(7):980-9.
25. Kim H-W, Cheon Y, Modi HR, Rapoport SI, Rao JS. Effects of chronic clozapine administration on markers of arachidonic acid cascade and synaptic integrity in rat brain. Psychopharmacology. 2012;222:663-74.
26. Aykaç A, Özbeyli D, Pekol G, Şehirli AÖ. Evaluation of the effects of donepezil, memantine and α-lipoic acid combined administration in amnesia rats on impaired cognitive functions in terms of behavioural, apoptotic, cholinergic and glutamatergic systems. Clin Exp Health Sci. 2021;11(4):733-41.
27. Ma X-C, Dang Y-H, Jia M, Ma R, Wang F, Wu J, et al. Long-lasting antidepressant action of ketamine, but not glycogen synthase kinase-3 inhibitor SB216763, in the chronic mild stress model of mice. PLoS ONE. 2013;8(2):e56053.
28. Zugno AI, Canever L, Heylmann AS, Wessler PG, Steckert A, Mastella GA, et al. Effect of folic acid on oxidative stress and behavioral changes in the animal model of schizophrenia induced by ketamine. J Psychiatr Res. 2016;81:23-35.
29. Bancroft JD, Layton C. Connective and mesenchymal tissues with their stains. Bancroft's Theory and practice of histological techniques. 2012:187-214.
30. Jackson P, Blythe D. Immunohistochemical techniques. Theory and practice of histological techniques. 2008;7:381-426.
31. 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;27(11):1032-43.
32. Gouda AA, El-Habiby MM, Issa NM, Zaki NG. The potential therapeutic role of aphanizomenon flos-aquae extract against clozapine on a ketamine rat model of psychosis. Egypt J Histol. 2023;46(4):2159-74.
33. Teng Y, Niu J, Liu Y, Wang H, Chen J, Kong Y, et al. Ketamine alleviates fear memory and spatial cognition deficits in a PTSD rat model via the BDNF signaling pathway of the hippocampus and amygdala. Behav Brain Res. 2024;459:114792.
34. Liu Y, Hang X, Zhang Y, Fang Y, Yuan S, Zhang Y, et al. Maternal immune activation induces sex-dependent behavioral differences in a rat model of schizophrenia. Front Psychiatry. 2024;15:1375999.
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;32(4):101994.
36. Carrasco MM, Agüera L, Gil P, Moríñigo A, Leon T. Safety and effectiveness of donepezil on behavioral symptoms in patients with Alzheimer disease. Alzheimer Dis Assoc Disord. 2011;25(4):333-40.
37. Gomaa AA, Makboul RM, El-Mokhtar MA, Abdel-Rahman EA, Ahmed EA, Nicola MA. Evaluation of the neuroprotective effect of donepezil in type 2 diabetic rats. Fundam Clin Pharmacol. 2021;35(1):97-112.
38. Eskandary A, Moazedi AA, Zade HN, Akhond MR. Effects of donepezil hydrochloride on neuronal response of pyramidal neurons of the CA1 hippocampus in rat model of Alzheimer's disease. Basic Clin Neurosci. 2019;10(2):109.
39. Finbarrs-Bello E, Ozor II, OnyinyeAtuadu V, Arinze EM. Neuroprotective effect of chlorpromazine on hippocampus of ketamine-induced psychotic rat model. Ijsr. 2017;6(11):1662-6.
40. Nogo D, Nazal H, Song Y, Teopiz KM, Ho R, McIntyre RS, et al. A review of potential neuropathological changes associated with ketamine. Expert Opin Drug Saf. 2022;21(6):813-31.
41. de Moura FC, Xavier MKF, Rodrigues FEL, dos Santos Pinheiro MF, Machado ECL, de Moura CBC, et al. Behavioral, neurochemical and histological changes in the use of low doses of naltrexone and donepezil in the treatment in experimental model of Alzheimer's disease by induction of β-Amyloid1-42 in rats. World Sci Res. 2019;6(1):5-13.
42. Youn DH, Lee Y, Han SW, Kim J-T, Jung H, Han GS, et al. Therapeutic effect of donepezil on neuroinflammation and cognitive impairment after moderate traumatic brain injury. Life. 2024;14(7):839.
43. Brandebura AN, Paumier A, Onur TS, Allen NJ. Astrocyte contribution to dysfunction, risk and progression in neurodegenerative disorders. Nat Rev Neurosci. 2023;24(1):23-39.
44. Zhou B, Zuo YX, Jiang RT. Astrocyte morphology: diversity, plasticity, and role in neurological diseases. CNS Neurosci Ther. 2019;25(6):665-73.