The Potential Therapeutic Role of Aphanizomenon flos-aquae Extract Against Clozapine on a Ketamine Rat Model of Psychosis

Original Article

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ABSTRACT

Introduction: Psychotic disorders affect approximately 0.75% of the general population worldwide with a great risk of complications and mortality. Clozapine and current antipsychotic drugs evoke serious complications. Aphanizomenon flos-aquae (AFA) extract has antioxidant, anti-inflammatory and neuroprotective properties.

Aim of Work: The aim of the work was to evaluate possible therapeutic effects of aphanizomenon flos-aquae extract in psychosis based on behavioral, biochemical and histopathological studies of CA1 region of the hippocampus.

Materials and Methods: Sixty adult male albino rats were organized into five groups; control, AFA extract (200 mg/kg/d orally), psychosis (ketamine 25 mg/kg/d intraperitoneal), psychosis treated with clozapine (5 mg/kg/d intraperitoneal), psychosis treated with AFA extract. Ketamine was administered from day 1 to day 14. Clozapine and AFA extract were administered from day 8 to day 21. Behavioral tests including open field test (OFT), sucrose preference test (SPT) and novel object recognition test (NORT) besides biochemical analyses were conducted. After sacrification of rats, the brain was subjected to histological and immunohistochemical studies.

Results: Ketamine induced positive, negative and cognitive symptoms of psychosis with increased brain acetylcholinesterase activity. Ketamine also induced degenerative changes in the hippocampus with increased glial fibrillary acidic protein (GFAP), P53 positive cells and decreased myelin basic protein (MBP) expression. Clozapine and AFA extract reversed the symptomatic and degenerative changes of psychosis, decreased brain acetylcholinesterase activity, decreased GFAP, P53 positive cells and increased MBP expression. However, clozapine promoted weight gain, dyslipidemia, hyperglycemia and agranulocytosis.

Conclusion: AFA extract has therapeutic effects against ketamine induced psychosis without causing the side effects of clozapine.

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Key Words: Acetylcholinesterase, blue green algae, hippocampus, immunohistochemistry, psychotic disorders.

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INTRODUCTION

Psychosis is a neuropsychiatric disorder well known for positive symptoms mainly hallucinations and delusions, negative symptoms such as avolition and anhedonia and cognitive symptoms such as learning and memory impairment[1]. The exact cause of psychosis is unknown but established hypotheses indicate neurochemical dysfunction. Reduced gamma aminobutyric acid (GABA) signaling in the hippocampus leads to hippocampal hyperactivity which upregulates dopamine signaling within the mesolimbic pathway[2]. Acetylcholine dysregulation, neuroinflammation and oxidative stress are growingly entailed in the pathophysiology of psychosis[3,4]. The hippocampus is one of the most affected brain structures in the pathophysiology of psychosis, especially CA1 region[5,6]. Psychotic disorders demonstrate a reduction in hippocampal volume, number of cells and neural stem cell proliferation[7,8]. Clozapine, the drug of choice for refractory psychosis, is associated with diabetes, obesity, dylipidemia and agranulocytosis[9,10,11].

Aphanizomenon flos-aquae (AFA) are blue green algae belonging to the cyanobacteria phylum. AFA extract is rich in proteins, polyunsaturated fatty acids, fibers, sterols, carotenoids, chlorophyll and phycocyanins well known for their antioxidant and anti-inflammatory properties[12]. It also contains GABA, minerals including iron, magnesium, calcium, and zinc together with vitamins such as vitamin D3, E and B group[13]. AFA extract has a neuroprotective effect due to its vitamin B12 content and antioxidant anti-inflammatory components which inhibit neuronal toxicity and protect against neurodegeneration[14]. AFA extract is known to have hypocholesterolemic and antidiabetogenic effects[15].

Therefore, the aim of the current study was to compare the therapeutic outcomes of AFA extract with that of clozapine on adult male albino rats treated with ketamine to induce psychosis; clinically, biochemically and histopathologically (CA1 region of the hippocampus).

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MATERIALS AND METHODS

Drugs and chemicals

Ketamine hydrochloride was purchased as (KETALITE®) 500 mg/ 10 ml vials from Elite Pharma Co. (Cairo, Egypt). Clozapine was purchased as (Clozapine®) 25 mg tablets from El-Ezaby pharmacy (Menoufia, Egypt). AFA extract was purchased as (AFA EXTRACT®) 500 mg capsules from Super Smart Co. (Delaware, USA).

Animals

Sixty adult male Sprague-Dawley albino rats weighing 200-250 g were obtained from Theodor Bilharz Research Institute Animal House, Giza, Egypt. Rats were subjected to normal light/dark cycle with ad libitum access to food and water. The procedure was approved by the ethics committee of animal experiment of Faculty of Medicine, Menoufia University, Egypt, 82021 ANAT3.

Experimental design

Rats were randomly divided into five groups (twelve animals each) as follow:

Control group: was divided into two groups:

- Subgroup Ia: (Plain control): consisted of six rats and was kept without any treatment.
- Subgroup Ib: (Sham control): consisted of six rats and received 0.4 ml intraperitoneal saline once daily for 21 days.

AFA extract group: received AFA extract (200 mg/ kg/d) from the 8th day to the 21st day. AFA extract was dissolved in distilled water then given by gastric tube. The dose was calculated according to the equation: Human equivalent dose (mg/ kg) = Animal dose (mg/ kg) × (Animal Km/ Human Km). Km of rat= 6 while km of adult human= 37[16].

Psychosis group: received ketamine HCl (25 mg/kg/d) by intraperitoneal injections once daily for the first 14 consecutive days and was left for the remaining 7 days without treatment. Each 0.1 ml of ketamine HCL was diluted in 0.3 ml of saline[17].

Psychosis group treated with clozapine: received intraperitoneal ketamine HCl (25 mg/kg/d) for 14 consecutive days. From the 8th day to the 21st day, this group received intraperitoneal clozapine (5 mg/kg/d) dissolved in saline[17,18].

Psychosis group treated with AFA extract: received intraperitoneal ketamine HCl (25 mg/kg/d) for 14 consecutive days. From the 8th day to the 21st day, this group received AFA extract (200 mg/kg/d).

Clinical behavioral tests

Open field test (OFT)

To test positive symptoms (locomotor activity), OFT was done on the 7th and 21st days of the experiment. The OFT apparatus was a square box measuring (100 cm x 100 cm x 50 cm). The floor of the box was divided equally into 25 squares. Each rat was placed in the center of the box and allowed to explore freely for 5 minutes. Behavior of rats was recorded by a video-camera. Number of crossed squares was counted manually[19].

Sucrose preference test (SPT)

To test negative symptoms (anhedonia), SPT was conducted twice. The SPT consisted of adaptation and test phases. The adaptation phase lasted for two days. The test phase was conducted on the 7th and 21st days of the experiment and the sucrose preference was measured. Sucrose preference index (%) = sucrose consumption [g] / (sucrose consumption [g] + water consumption [g]) × 100%[20].

Novel object recognition test (NORT)

To test cognitive symptoms (memory), NORT was done on the 7th and 21st days of the experiment. The test apparatus was rectangular and made of plexiglass. The test consisted of familiarization and test sessions. Memory index = N/(N+F). N= time spent by the rat exploring the novel object while F= time spent by the rat exploring the familiar object. Time was collected with a stopwatch[21].

Body and brain weight measurements

Body weight measurements were recorded for all rats on days 1 and 21. Percentage change of body weight = (Weight at day 21 - Weight at day 1)/ Weight at day 1 × 100. The weight of the brain was assessed after sacrification of rats.

Biochemical analysis

By the end of our experiment, rats were anesthetized and sacrificed. We collected blood samples from the rats' retro-orbital venous plexus and measured serum levels of total cholesterol, triglycerides, white blood cells (WBCs) count and blood glucose level[17].

Brain Acetylcholinesterase (AChE) activity: The right cerebral hemispheres were homogenized in 30 mM phosphate buffer (pH 7.6) and centrifuged at 20000 rpm at 4°C for 30 min to measure AChE activity expressed as mol/min/g[22].

Histological examination

Each left cerebral hemisphere was dissected coronally into two parts to reach the rat hippocampus. Specimens were fixed in 10% formalin then dehydrated and embedded in paraffin blocks. 5 µm coronal sections were cut then stained with hematoxylin and eosin and toluidine blue stains[23].

Immunohistochemical examination

The 5-µm brain paraffin sections were deparaffinized then rehydrated in descending grades of alcohol. The 3% H_2O_2 in methanol was used to block endogenous peroxidase activity. A protein blocker was used to block nonspecific

binding sites then the primary antibody anti-GFAP (Rabbit polyclonal, ab7260, 1:300, Midco Trade, Giza, Egypt), anti-P53 (Rabbit monoclonal, ab238069, Sigma-Aldrich, Cairo, Egypt) and anti-myelin basic protein (MBP; Rabbit polyclonal, ab2404, 1:300, Midco Trade, Giza, Egypt) were added with overnight incubation. Biotinylated goat-polyvalent secondary antibody at a concentration of 2% (Vector, Peterborough, UK) was added for 10 minutes (37°C) then the avidin-biotin-peroxidase complex (Vector) was added.

Morphometric and statistical analysis

For histological and immunohistochemical assessment of the pyramidal cell layer thickness, the number of the pyramidal cells, color intensity of toluidine blue, number of GFAP, P53 positive cells and area percentage of MBP expression, ImageJ 1.47v software (National Institutes of Health, USA) was used. The sections from at least six animals/experimental group were examined. Five non-overlapping fields per section were randomly captured by a Leica Microscope DML B2/11888111 equipped with a Leica camera DFC450. The results were analyzed by SPSS version 22.0 and expressed as the mean±standard devia¬tion. Mann Whitney U test was used to analyze data. $P<0.05$ was considered statistically significant.

RESULTS

There was no significant difference in all measured parameters between the plain control and the sham control groups so they were pooled in one group (control). Also, no significant difference was detected in all measured parameters between the control and the AFA extract groups.

Clinical behavioral tests results

Open field test

On the 7th day, there was a significant increase in the number of crossed squares in each ketamine treated group as compared with the control group ($P<0.001$). On the 21st day, a significant reduction was observed in the number of crossed squares in the psychosis group treated with clozapine and the psychosis group treated with AFA extract as compared with the psychosis group (77.5±1.05 vs. 86.5±1.87 and 77.5±1.38 vs. 86.5±1.87 respectively, $P<0.001$). Moreover, no significant difference was found in the number of crossed squares in the psychosis group treated with AFA extract as compared with the psychosis group treated with clozapine ($P>0.05$) (Figure 1).

Sucrose preference and novel object recognition tests

On the 7th day, there was a significant decrease in sucrose preference and memory indices in each ketamine treated group as compared with the control group ($P<0.001$). On the 21st day, a significant increase was observed in sucrose preference and memory indices in the psychosis group treated with clozapine as compared with the psychosis group (54.11±2.41 vs. 19.39±1.36 and 0.55±0.01 vs. 0.25±0.02 respectively, $P<0.001$). In addition, there was a significant elevation in sucrose preference and memory indices in the psychosis group treated with AFA extract as compared with the psychosis group (57.19±1.83 vs. 19.39±1.36 and 0.58±0.01 vs. 0.25±0.02 respectively, $P<0.001$). Furthermore, a significant increase was observed in sucrose preference and memory indices in the psychosis group treated with AFA extract as compared with the psychosis group treated with clozapine ($P<0.05$) (Figure 1).

Body and brain weight results

A significant reduction was observed in percentage change of body weight and brain weight in the psychosis group as compared with the control group (8.83±2.93 vs. 12.03±1.79 and 1.55±0.10 vs. 1.70±0.11 respectively, $P<0.05$). The psychosis group treated with clozapine showed a significant elevation in percentage change of body weight while there was no significant difference in the psychosis group treated with AFA extract as compared with the control group (19.60±1.62 vs. 12.03±1.79 and 11.73±1.91 vs. 12.03±1.79 respectively, $P<0.001$, $P>0.05$ respectively). Percentage change of body weight was significantly lower in the psychosis group treated with AFA extract as compared with the psychosis group treated with clozapine ($P<0.001$). A significant elevation was observed in brain weight in the psychosis group treated with clozapine and the psychosis group treated with AFA extract as compared with the psychosis group (1.72±0.12 vs. 1.55±0.10 and 1.70±0.11 vs. 1.55±0.10 respectively, $P<0.05$). However, no significant difference was found in brain weight in the psychosis group treated with AFA extract as compared with the psychosis group treated with clozapine ($P>0.05$) (Figure 2).

Biochemical results

The psychosis group treated with clozapine showed a significant elevation in serum total cholesterol, triglycerides and blood glucose levels and a significant decrease in WBCs count as compared with the control group ($P<0.001$). No significant difference was found in serum total cholesterol, triglycerides, blood glucose levels, and WBCs count in the psychosis group treated with AFA extract as compared with the control group ($P>0.05$) (Figure 3).

Brain Acetylcholinesterase (AChE) activity

The psychosis group showed a significant elevation in brain AChE activity as compared with the control group (0.87±0.01 vs. 0.19±0.02, $P<0.001$). There was a significant reduction in brain AChE activity in the psychosis group treated with clozapine and the psychosis group treated with AFA extract as compared with the psychosis group (0.48±0.01 vs. 0.87±0.01 and 0.50±0.02 vs. 0.87±0.01, $P<0.001$). Moreover, no significant difference was detected in brain AChE activity in the psychosis group treated with AFA extract as compared with the psychosis group treated with clozapine ($P>0.05$) (Figure 3).

Histopathological results

Hematoxylin and eosin brain sections from the control group at CA1 region of the hippocampus revealed the three layers of the hippocampus; molecular, pyramidal and polymorphic layers. The pyramidal cell layer was composed of regular rows of compact pyramidal cells. Pyramidal cells were triangular with basophilic cytoplasm. They had large vesicular nuclei and prominent nucleoli. Molecular and polymorphic layers were mainly formed of eosinophilic neuropil matrix. Different neuroglia were present in the neuropil (Figures 4,5). Toluidine blue stained sections showed multiple Nissl's granules in the cytoplasm of the pyramidal cells of CA1 region of the hippocampus which appeared dark blue in color (Figure 6).

The psychosis group showed a loss of hippocampal tissue integrity. Pyramidal cells were degenerated with shrunken hyperchromatic nuclei and perinuclear halos. Many vacuolations, dilated congested blood vessels and increased astrocytes were noticed in the neuropil (Figures 4,5). There was a significant reduction in the pyramidal cell layer thickness and the number of the pyramidal cells as compared with the control group (P <0.001) (Figure 7). In addition, there was a significant decrease in color intensity of toluidine blue stained sections in the psychosis group as compared with the control group (P <0.001) (Figures 6,7).

In the psychosis group treated with clozapine and the psychosis group treated with AFA extract, most pyramidal cells appeared normal with basophilic cytoplasm, large vesicular nuclei and prominent nucleoli but some cells were degenerated with hyperchromatic nuclei and perinuclear halos. Other cells were swollen and lost their nuclei. The neuropil showed few congested blood vessels and few astrocytes (Figures 4,5). A significant increase was observed in the pyramidal cell layer thickness and the number of the pyramidal cells in the psychosis group treated with clozapine and the psychosis group treated with AFA extract as compared with the psychosis group (P <0.001) (Figure 7). In addition, there was a significant increase in color intensity of toluidine blue stained section in the psychosis group treated with clozapine and the psychosis group treated with AFA extract as compared with the psychosis group (P <0.001) (Figures 6,7). Moreover, no significant difference was found in the pyramidal cell layer thickness, number of the pyramidal cells and color intensity of toluidine blue in the psychosis group treated with clozapine as compared with the psychosis group treated with AFA extract (P >0.05).

Immunohistochemical results

GFAP immunostaining of brain sections from the control group revealed few small star-shaped astrocytes with short processes in CA1 region of the hippocampus. A significant elevation was observed in the number of GFAP positive cells in the psychosis group as compared with the control group (P <0.001). A significant decrease was observed in the number of GFAP positive cells in the psychosis group treated with clozapine and the psychosis group treated with AFA extract as compared with the psychosis group (P <0.001) (Figures 8,9).

Brain sections from the control group stained for P53 protein (a marker of apoptosis) showed negative immunoreaction at CA1 region of the hippocampus. There was a significant elevation in the number of P53 positive cells indicated by brown nuclear staining in the psychosis group as compared with the control group (P <0.001). There was a significant reduction in the number of P53 positive cells in the psychosis group treated with clozapine and the psychosis group treated with AFA extract as compared with the psychosis group (P <0.001) (Figures 8,9).

Brain sections from the control group stained for MBP revealed a strong positive immunoreaction at CA1 region of the hippocampus. There was a significant reduction in the area percentage of MBP expression in the psychosis group as compared with the control group (P <0.001). A significant increase was observed in the area percentage of MBP expression in the psychosis group treated with clozapine and the psychosis group treated with AFA extract as compared with the psychosis group (P <0.001). Moreover, no significant difference was detected in the number of GFAP positive cells, number of P53 positive cells and area percentage of MBP expression in the psychosis group treated with AFA extract as compared with the psychosis group treated with clozapine (P >0.05) (Figures 8,9).

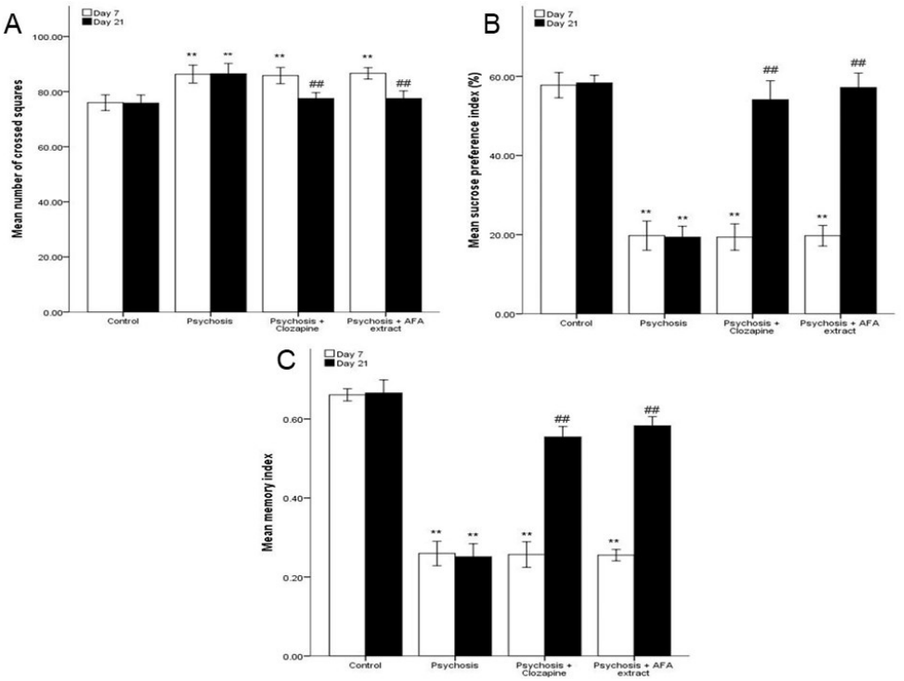

Fig. 1: (A, B, C) Mean number of crossed squares, sucrose preference index (%) and memory index on the 7th and 21st days. ** *P*<0.001 as compared with the control group, ## *P*<0.001 as compared with the psychosis group.

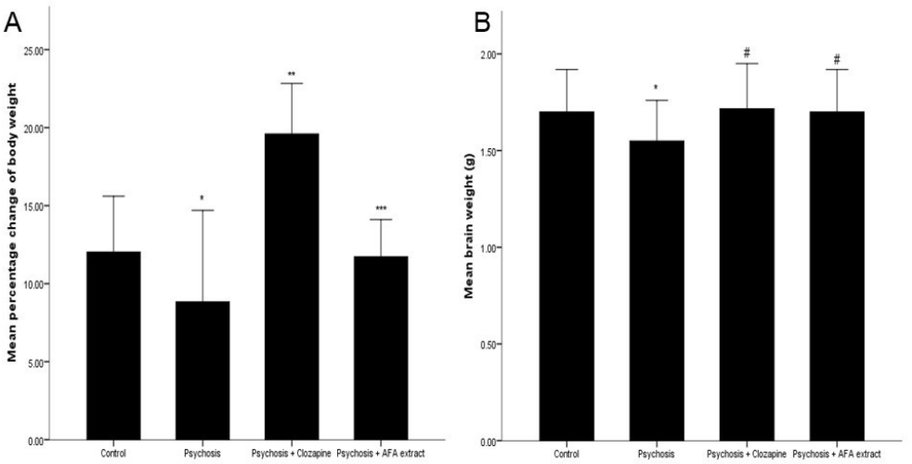

Fig. 2: (A) Mean percentage change of body weight, (B) Mean brain weight (g). * *P*<0.05 as compared with the control group, ** *P*<0.001 as compared with the control group, *** *P*>0.05 as compared with the control group, # *P*<0.05 as compared with the psychosis group.

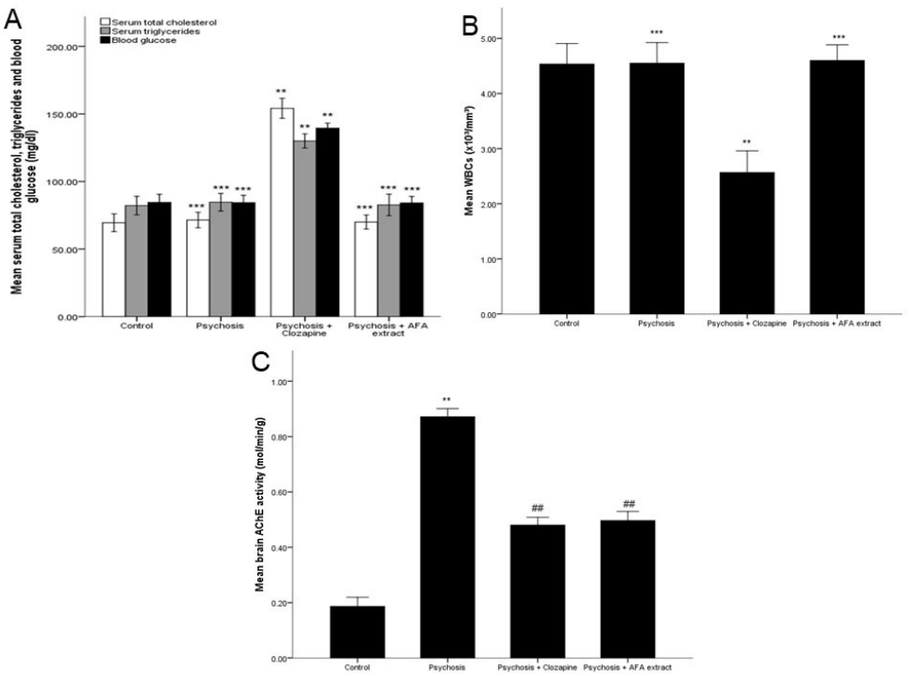

Fig. 3: (A) Mean serum total cholesterol, triglycerides and blood glucose levels (mg/dl), (B) Mean white blood cells count ($x10^3/mm^3$), (C) Mean brain AChE (mol/min/g). ** $P<0.001$ as compared with the control group, *** $P>0.05$ as compared with the control group, ## $P<0.001$ as compared with the psychosis group.

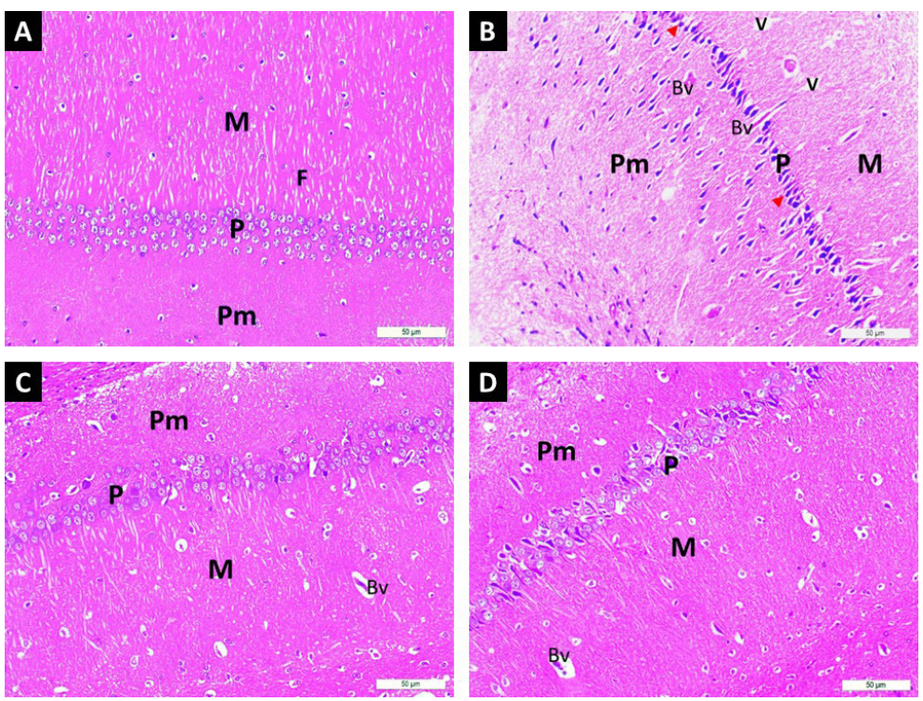

Fig. 4: H&E staining of brain sections at CA1 region of the rat hippocampus showing the polymorphic (Pm), pyramidal (P) and molecular (M) layers. (A) The control group showing normal pyramidal cells (P) and nerve fibers (F), (B) The psychosis group showing degenerated pyramidal cells (arrow head), vacuolations (v) and dilated congested blood vessels (Bv), (C, D) The psychosis group treated with clozapine and the psychosis group treated with AFA extract showing many normal pyramidal cells (P). (Scale bar = 50 µm).

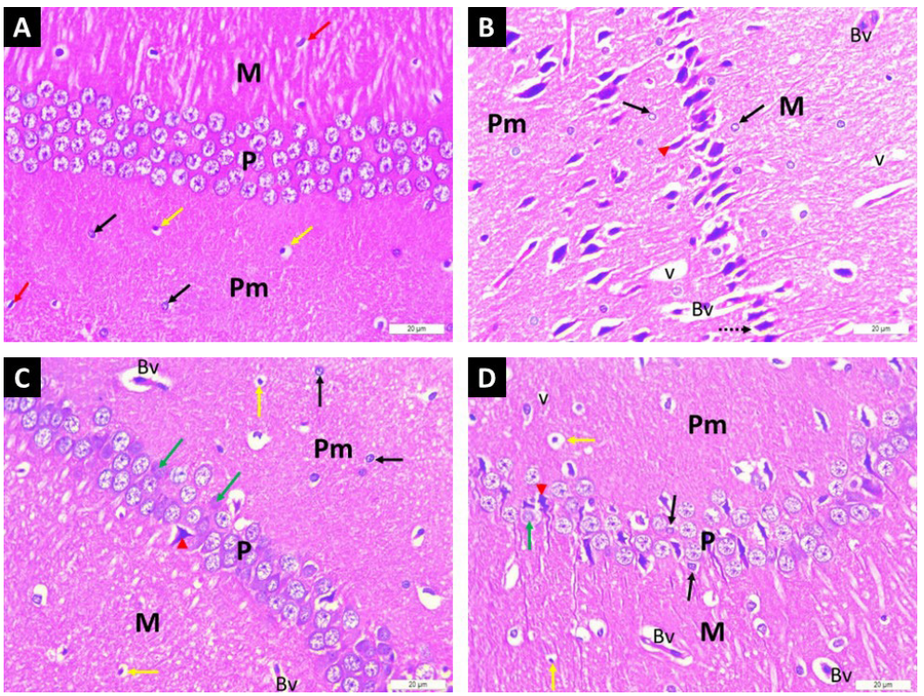

Fig. 5: H&E staining of brain sections at CA1 region of the rat hippocampus showing the polymorphic (Pm), pyramidal (P) and molecular (M) layers. Oligodendroglia (yellow arrow), microglia (red arrow) and astrocytes (black arrow) are embedded in the neuropil of both molecular and polymorphic layers. (A) The control group showing normal pyramidal cells (P), (B) The psychosis group showing degenerated pyramidal cells (arrow head) with perinuclear halos (dashed arrow), vacuolations (v) and dilated congested blood vessels (Bv), (C, D) The psychosis group treated with clozapine and the psychosis group treated with AFA extract showing many normal pyramidal cells (P), some degenerated pyramidal cells (arrow head) and swollen cells without nuclei (green arrow). (Scale bar = 20 µm).

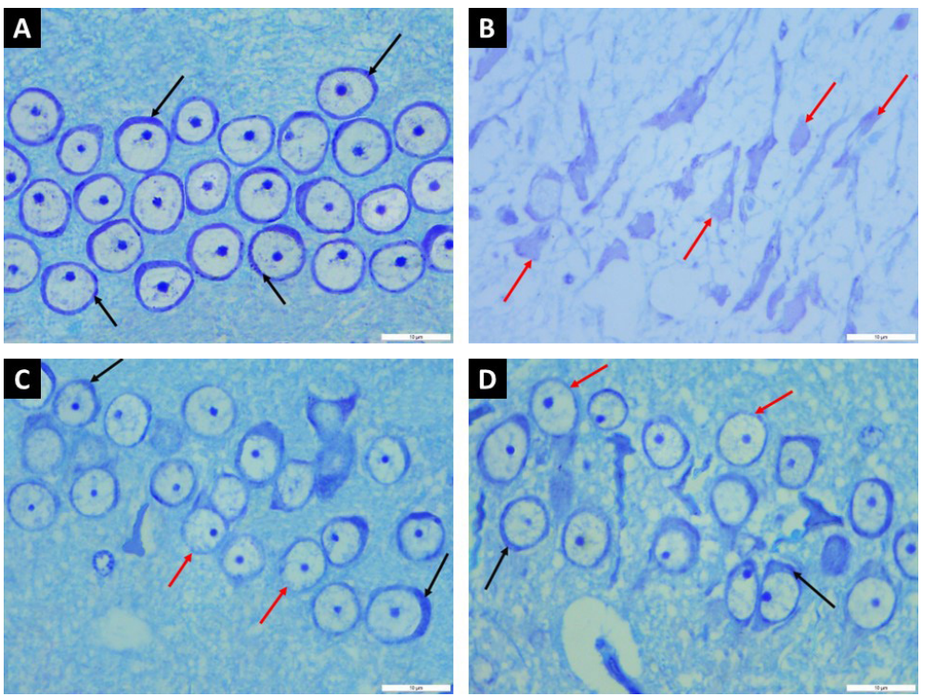

Fig. 6: Toluidine blue staining of brain sections at CA1 region of the rat hippocampus. (A) The control group showing multiple Nissl's granules (black arrow) in the cytoplasm of the pyramidal cells which appear dark blue in color, (B) The psychosis group showing apparent decrease of Nissl's granules in the cytoplasm of the pyramidal cells which appear faint blue in color, (C, D) The psychosis group treated with clozapine and the psychosis group treated with AFA extract showing apparent increase of Nissl's granules (black arrow) in the cytoplasm of many pyramidal cells which appear dark blue in color but few cells show faint blue Nissl's granules (red arrow). (Scale bar = 10 µm).

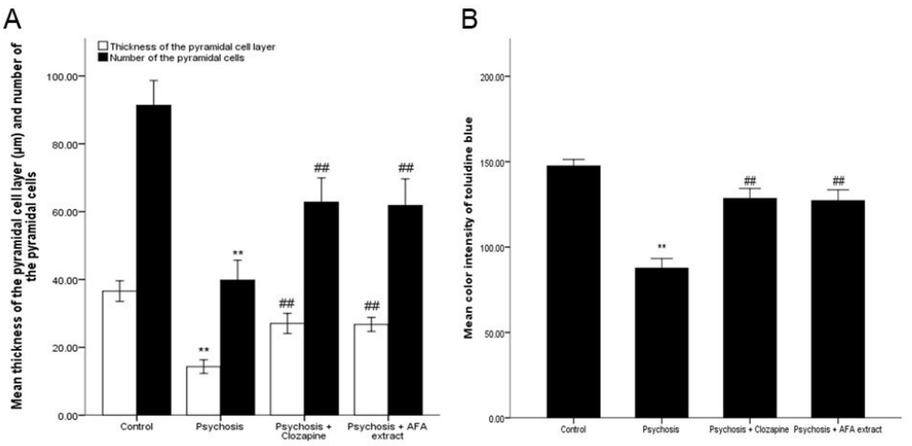

Fig. 7: (A) Mean thickness of the pyramidal cell layer (µm) and number of the pyramidal cells, (B) Mean color intensity of toluidine blue. ** P<0.001 as compared with the control group, ## P<0.001 as compared with the psychosis group.

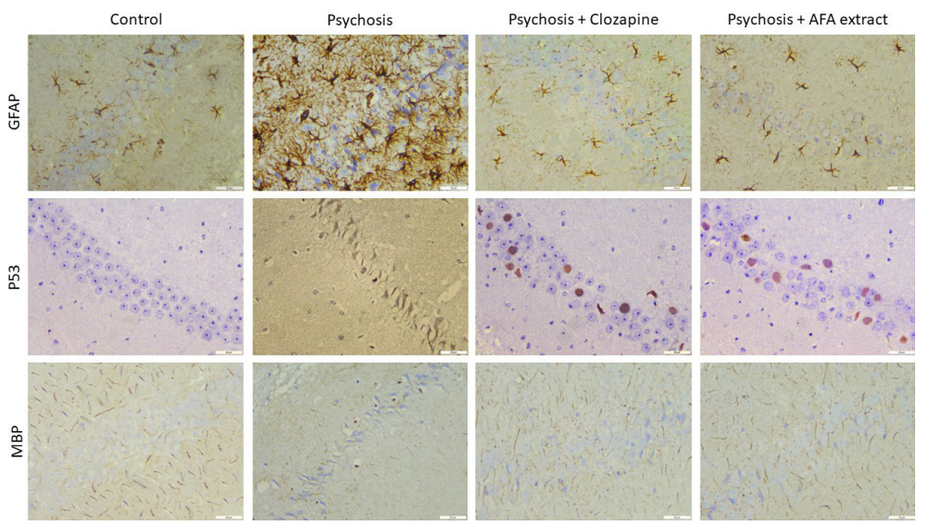

Fig. 8: Expression of GFAP, P53 and MBP in brain sections at CA1 region of the rat hippocampus. The psychosis group showed upregulation of GFAP, P53 and downregulation of MBP. The psychosis groups treated with clozapine and the psychosis group treated with AFA extract showed downregulation of GFAP, P53 and upregulation of MBP. (Scale bar = 20 µm).

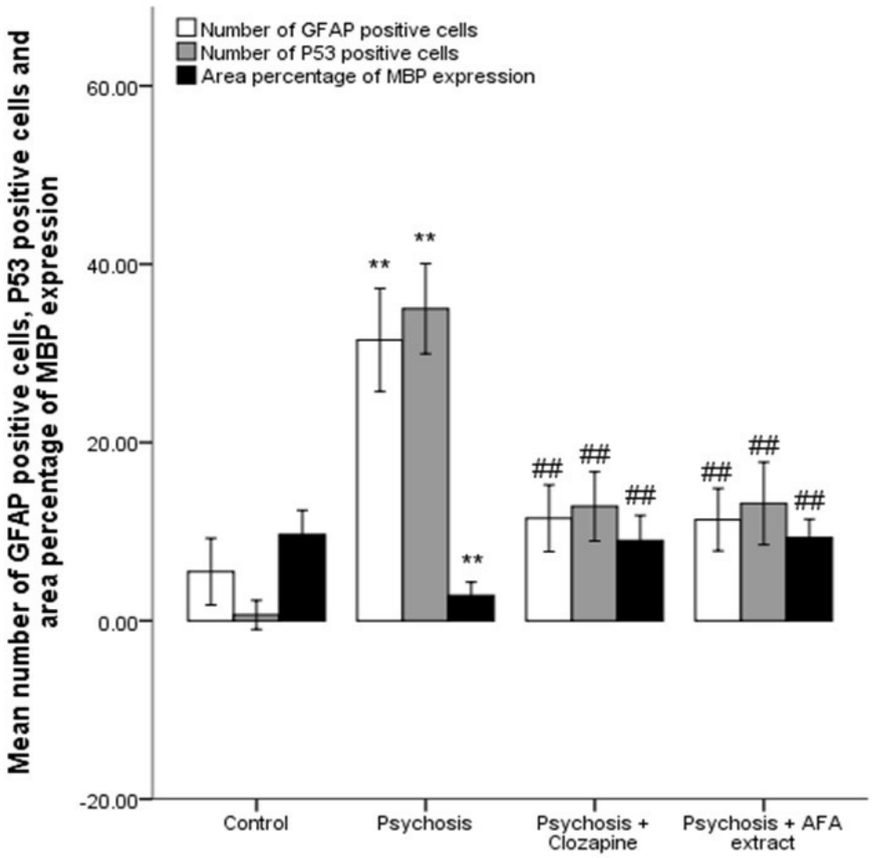

Fig. 9: Mean number of GFAP positive cells, P53 positive cells and area percentage of MBP expression. ** $P<0.001$ as compared with the control group, ## $P<0.001$ as compared with the psychosis group.

DISCUSSION

Ketamine model is considered one of the best pharmacological models of psychosis because it can produce positive, negative and cognitive symptoms[24]. To induce a rat model of psychosis, we used subanesthetic doses of ketamine which antagonizes N-methyl-D-aspartate receptors to induce psychomimetic symptoms[25].

In our study, we reported, for the first time to the best of our knowledge, a therapeutic role of AFA extract in psychosis. Clozapine and AFA extract reversed ketamine induced psychotic symptoms. This was demonstrated by a significant reduction in locomotor activity and elevation in sucrose preference and memory indices. Similar results were reported by George *et al*.[26] and Amiri *et al*.[27] who evaluated the therapeutic outcomes of clozapine in psychosis. Moreover, we observed a significant increase in sucrose preference and memory indices in the psychosis group treated with AFA extract as compared with the psychosis group treated with clozapine indicating better amelioration of negative and cognitive symptoms with AFA extract.

Since clozapine acts on multiple receptors in different brain regions, behavioral effects are unlikely to be attributed to a single neurotransmitter. However, positive symptoms improvement is mostly due to its antidopaminergic and 5-HT2A receptor modulation[28]. Hendouei *et al*.[29] stated that clozapine effect on the levels of glutamate, dopamine, GABA, serotonin and its antioxidant effect are thought to enhance negative symptoms.

The amelioration of the positive symptoms in the psychosis group treated with AFA extract may be due to the downregulation of dopamine signaling in the mesolimbic pathway by GABA content of AFA extract. This extract is also rich in phycocyanins, carotenoids, chlorophyll and polyunsaturated fatty acids which are responsible for its antioxidant anti-inflammatory properties that may encounter the oxidative stress hypothesis of psychosis[12]. Moreover, AFA extract phycocyanins have 200 times higher antioxidant power compared to other phycocyanins[30]. Zugno *et al*.[31] suggested the supplementation of omega-3 in psychotic patients as it enhances symptoms through increasing brain-derived neurotrophic factor besides preventing damage to lipids and proteins in the hippocampus, prefrontal cortex and striatum. This may explain the improvement in negative and cognitive symptoms of psychosis. In addition, vitamin B12, which is also present in AFA extract, was reported

to improve negative symptoms of psychosis. Furthermore, AFA extract contains tryptophan which increases the utilization of vitamin B complex, stabilizes emotions and has neuroprotective effects. Moreover, it is the precursor of serotonin which attenuates cognitive dysfunction[32].

AFA extract contains β-phenylethylamine (PEA) one of the trace amines which exerts its actions by binding trace amine associated receptor 1 (TAAR1)[12]. The role that may be played by PEA in psychosis is controversial and not well understood. Irsfeld *et al*.[33] stated that TAAR1 activation by PEA improves symptoms of psychosis and depression without causing the negative effects of dopamine receptor blockage. Also, Borah *et al*.[34] reported that PEA reduces striatal dopamine content through dopaminergic neurodegeneration. Recently, synthetic TAAR1 receptor agonists have been developed and introduced to trials as a new treatment of schizophrenia[35]. However, Ryu *et al*.[36] reported that acute PEA administration induced psychomotor behavior and activated dopamine receptors in the dorsal striatum. Importantly, endogenous trace amines like PEA are not suitable drug candidates as they are rapidly cleared in CNS[35]. Therefore, we referred the change of psychotic behavior with AFA extract to its antioxidants and neuroprotective vitamin content not to PEA. Further studies of the role of endogenous PEA content of AFA extract should be addressed.

We reported a significant elevation in percentage change of body weight in the psychosis group treated with clozapine. This is in line with Pillinger *et al*.[37] and Nikolić *et al*.[38] who referred the increase in body weight in male rats to increased serum corticosterone. Yuen *et al*.[39] stated that sympathetic hyperactivity and elevated plasma norepinephrine levels contribute to obesity with clozapine. Another explanation was reported by Abela *et al*.[40] who stated that clozapine increases appetite due to combined action at the serotonin 5-HT2C and histaminergic H1 receptors. High serum serotonin levels associated with clozapine inhibit glucose burning of brown fat and result in the development of obesity and diabetes[41].

In addition, our study reported that clozapine significantly increased serum total cholesterol, triglycerides and blood glucose levels. This is in line with Pillinger *et al*.[37] and George *et al*.[17] who declared that clozapine enhances cholesterol and triglycerides synthesis via activating sterol regulatory element-binding proteins which initiate lipogenic gene expression. Hyperglycemia was referred to disruption of glucose homeostasis, enhanced glucagon secretion, and stimulated glycogenolysis.

Furthermore, we confirmed a significant reduction in WBCs count in the psychosis group treated with clozapine highlighting agranulocytosis which is one of the most serious side effects of clozapine. This is in line with Islam *et al*.[42] and Mijovic & MacCabe.[43] who reported that clozapine is metabolized by myeloperoxidase to a nitrenium ion which triggers apoptosis of granulocytes.

Unlike clozapine, AFA extract did not induce pathological changes in body weight and biochemical parameters. This is in line with Sanaei *et al*.[44] who reported that increased glycated hemoglobin (HbA1c) is due to low hemoglobin levels. AFA extract, which is a rich source of iron, increases hemoglobin which reduces HbA1C level decreasing blood glucose. They also reported that decreased blood glucose in diabetic rats by AFA extract may be due to stimulation of β-cells of islets of Langerhans to increase insulin production. Ku *et al*.[45] added that AFA extract has the ability to lower serum total cholesterol and triglycerides through modulating absorption of intestinal cholesterol and expression of hepatic lipogenic gene. The maintaining action of AFA extract on white blood cells may be attributed to increased bone marrow-derived stem cells (BM-SCs) which produce WBCs[46].

Cognitive deficits are usually linked to cholinergic dysfunction[47]. We confirmed a significant increase of brain AChE activity in the psychosis group. The cognitive improvement in the psychotic rats treated either with clozapine or AFA extract may be due to modulation of acetylcholine indicated by the significant decrease of brain AChE activity. This is supported by Andrabi *et al*.[48] who declared that clozapine ameliorated cognitive symptoms in schizophrenic mice through decreasing AChE activity.

We also reported a significant reduction in brain weight in the psychosis group which may be attributed to the cytoarchitectural alterations and increased turnover as explained by Takahashi *et al*.[49]. There was a significant increase in brain weight in the psychosis groups treated either with clozapine or AFA extract.

In agreement with the clinical improvement, the psychosis groups treated either with clozapine or AFA extract showed histological and immunohistochemical recovery. Many pyramidal cells appeared normal but some cells were degenerated. Few vacuolations and few congested blood vessels were found in the neuropil of both molecular and polymorphic layers. There was a significant increase in the pyramidal cell layer thickness, the number of the pyramidal cells and Nissl's granules in toluidine blue staining of the cytoplasm of the pyramidal cells. In addition, there was a significant reduction in astrocytes number confirmed by GFAP immunostaining and a significant reduction in the expression of P53 protein. GFAP is the primary intermediate filament of astrocytes and its increase is an indication of gliosis following injury of the central nervous system[50]. Gao *et al*.[51] stated that P53 is associated with psychosis and might be one of its susceptibility genes by regulating apoptosis.

The amelioration findings of clozapine are in line with Kusumi *et al*.[52], Chang *et al*.[53], George *et al*.[17] and Gammon *et al*.[54] who referred these effects to neurogenesis besides the expression of neurotrophic factors such as brain-derived neurotrophic factor.

The amelioration changes of AFA extract may be referred to its neuroprotective effect due to vitamin B12

content and the antioxidant anti-inflammatory components that inhibit neuronal toxicity and protect against neurodegeneration[14]. Furthermore, AFA extract enhances mobilization of BM-SCs which can migrate to site of tissue damage, differentiate into neurons and promote tissue regeneration[46,55]. The deficits in inhibitory interneurons besides connectivity between hippocampus and prefrontal cortex in psychosis proposed stem cell derived interneuron transplants as a new therapeutic strategy. However, this strategy is not feasible and carry several risks[56]. Therefore, AFA extract can provide an endogenous source of stem cells without the risks of transplants.

In our study, the psychosis group showed a significant reduction in the expression of MBP affecting myelinization of nerves in the central nervous system. This indicates a possible decreased myelination in the pathophysiology of psychosis. This is in agreement with Akosman *et al*.[6], Raabe *et al*.[57] and Valdés-Tovar *et al*.[58] who stated that myelination maintains connectivity between different brain structures to support integration processes such as memory, perception or cognition. A significant rise in the expression of MBP in the psychotic rats treated either with clozapine or AFA extract was observed. This is in line with Xu *et al*.[59] and Templeton *et al*.[60] who declared that clozapine enhances remyelination by decreasing inflammatory cytokines and supporting maturation of oligodendrocytes which are capable of remyelinating axons. Moreover, high levels of dopamine are reported to act as endogenous neurotoxins and damage oligodendrocytes by increasing free radicals. Therefore, the antidopaminergic action of clozapine can reverse white matter damage.

The effect of AFA extract on MBP may be due to vitamin B12 which promotes myelin formation and reduces Wallerian degeneration. Vitamin B12 enhances generation of MBP and prevents homocysteine accumulation preventing oxidative damage[61].

CONCLUSION

Subanesthetic ketamine administration induces psychosis with degenerative changes in the hippocampus due to cholinergic dysfunction, upregulation of P53 protein and decreased myelination. Clozapine administration improves behavioral and degenerative changes of psychosis but induces major side effects including metabolic syndrome and agranulocytosis. AFA extract improves psychotic behavior, ameliorates degenerative changes in the hippocampus, decreases brain acetylcholinesterase activity and enhances remyelination without causing the major side effects of clozapine.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

الدور العلاجي المحتمل لمستخلص أفانيزومينون المائي مقارنا بالكلوزابين على نموذج الكيتامين للذهان في الجرذان

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المقدمة: تصيب الاضطرابات الذهانية ما يقرب من ٠,٧٥ ٪ من السكان في جميع أنحاء العالم مع وجود مخاطر كبيرة من المضاعفات والوفيات، ويتسبب الكلوزابين والعقاقير المضادة للذهان الحالية في مضاعفات خطيرة. مستخلص أفانيزومينون المائي له خواص مضادة للأكسدة والالتهابات وواقية للأعصاب.

الهدف: الهدف من العمل هو تقييم الآثار العلاجية المحتملة لمستخلص أفانيزومينون المائي في الذهان بناء على دراسات سلوكية وكيميائية حيوية ونسيجية مرضية في قرن آمون.

مواد وطرق البحث: تم تقسيم ستين من ذكور الجرذان البيضاء البالغين إلى خمس مجموعات: المجموعة الضابطة، مجموعة مستخلص أفانيزومينون المائي (٢٠٠ مجم/ كجم عن طريق الفم)، مجموعة الذهان (٢٥ مجم كيتامين/ كجم بالحقن داخل البريتون)، مجموعة الذهان المعالجة بالكلوزابين (٥ مجم/ كجم بالحقن داخل البريتون)، مجموعة الذهان المعالجة بمستخلص أفانيزومينون المائي. تم حقن الكيتامين من اليوم الأول حتى اليوم الرابع عشر بينما تم إعطاء الكلوزابين ومستخلص أفانيزومينون المائي من اليوم الثامن حتى اليوم الحادي والعشرين، تم إجراء اختبارات سلوكية هي فحص المجال المفتوح وفحص تفضيل السكروز وفحص التعرف على الأجسام الجديدة كما تم إجراء تحاليل كيميائية حيوية وبعد ذبح الفئران تعرض المخ لدراسات نسيجية وهستوكيميائية مناعية.

النتائج: تسبب الكيتامين في ظهور الأعراض الإيجابية والسلبية والمعرفية للذهان وزيادة نشاط إنزيم أستيل كولين إستراز بالمخ كما أدى إلى حدوث تغيرات تنكسية في نسيج قرن آمون مع زيادة الخلايا الإيجابية لصبغتي GFAP, P٥٣ ونقص في صبغة MBP؛ وقد أدى الكلوزابين ومستخلص أفانيزومينون المائي إلى عكس التغيرات العرضية والتنكسية في قرن آمون، وتقليل نشاط إنزيم أستيل كولين إستراز بالمخ، وتقليل الخلايا الإيجابية لصبغتي GFAP, P٥٣ وزيادة في صبغة MBP ولكن أدى الكلوزابين إلى زيادة الوزن وتغير مستويات الدهون وارتفاع الجلوكوز في الدم ونقص كرات الدم البيضاء.

الاستنتاج: مستخلص أفانيزومينون المائي له آثار علاجية على الذهان المستحث بالكيتامين دون التسبب في الآثار الجانبية للكلوزابين.