



Mechanisms underpinning *Carpolobia lutea* G. Don ethanol extract's neurorestorative and antipsychotic-like activities in an NMDA receptor antagonist model of schizophrenia

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ABSTRACT

Ethnopharmacological relevance: Persistent ketamine insults to the central nervous system block NMDA receptors and disrupt putative neurotransmission, oxido-nitrosative, and inflammatory pathways, resulting in schizophrenia-like symptoms in animals. Previously, the ethnomedicinal benefits of *Carpolobia lutea* against insomnia, migraine headache, and insanity has been documented, but the mechanisms of action remain incomplete.

Aim of the study: Presently, we explored the neuro-therapeutic role of *Carpolobia lutea* ethanol extract (*C. lutea*) in ketamine-induced schizophrenia-like symptoms in mice.

Materials and methods: Sixty-four male Swiss (22 ± 2 g) mice were randomly assigned into eight groups (n = 8/group) and exposed to a reversal ketamine model of schizophrenia. For 14 days, either distilled water (10 mL/kg; p.o.) or ketamine (20 mg/kg; i.p.) was administered, following possible reversal treatments with *C. lutea* (100, 200, 400, and 800 mg/kg; p.o.), haloperidol (1 mg/kg, p.o.), or clozapine (5 mg/kg; p.o.) beginning on days 8–14. During the experiment, a battery of behavioral characterizations defining schizophrenia-like symptoms were obtained using ANY-maze software, followed by neurochemical, oxido-inflammatory and histological assessments in the mice brains.

Results: A 7-day reversal treatment with *C. lutea* reversed predictors of positive, negative and cognitive symptoms of schizophrenia. *C. lutea* also mitigated ketamine-induced neurochemical derangements as evidenced by modulations of dopamine, glutamate, norepinephrine and serotonin neurotransmission. Also, the increased acetylcholinesterase activity, malondialdehyde nitrite, interleukin-6 and tumor necrosis-factor-α concentrations were reversed by *C. lutea* accompanied with elevated levels of catalase, superoxide dismutase and reduced glutathione. Furthermore, *C. lutea* reversed ketamine-induced neuronal alterations in the prefrontal cortex, hippocampus and cerebellum sections of the brain.

Conclusion: These findings suggest that *C. lutea* reverses the cardinal symptoms of ketamine-induced schizophrenia in a dose-dependent fashion by modulating the oxido-inflammatory and neurotransmitter-related mechanisms.

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List of abbreviation

5-HT	Serotonin (5-hydroxytryptamine)	IL-6	Interleukin-6
AChE	Acetylcholinesterase	KET	Ketamine
AMP	Amphetamine	LPS	Lipopolysaccharide
APO	Apomorphine	MAO	Monoamine oxidase
BDNF	Brain derived neurotrophic factor	MDA	Malondialdehyde
CA	Cornu ammonis	MWM	Morris water maze
CERBL	Cerebellum	NE	Norepinephrine
CZP	Clozapine	DA	Dopamine
DG	Dentate gyrus	NMDA	N-methyl-D-aspartate
EAAT	Excitatory amino acids transporters	NMDAR	N-methyl-D-aspartate receptor
EECL	Ethanol extract of <i>Capolobia lutea</i> (<i>C. lutea</i>)	NO	Nitric oxide
FST	Force swimming test	NORT	Novel object recognition test
GABA	Gamma-aminobutyric acid	OFT	Open field test
Glu	Glutamate	PFC	Prefrontal cortex
GSH	Reduced glutathione	SCZ	Schizophrenia
HLP	Haloperidol	SOD	Superoxide dismutase
HC	Hippocampus	STR	Striatum
		TNF- α	Tumor necrosis factor-alpha

1. Introduction

Schizophrenia (SCZ) is the most complex spectrum of psychosis, an unpredictable and chronic disorder of mind-and-brain that strikes about 1–1.5% of the world's population (Howes and Murray, 2014). It is currently recognized as a multifactorial and neurodevelopmental syndrome affecting a wide range of cognitive and motor domains and neurobehaviors (Nestler et al., 2016). It is characterized by positive (e.g., hallucinations and delusions), negative (e.g., anhedonia and social withdrawal) and cognitive (e.g., executive and memory dysfunction) symptoms (Foussias and Remington, 2010; Ben-Azu et al., 2016a, 2016b). Although the aetiology of this SCZ remains unclear, some sheds of evidence suggest a strong interplay between genetics and environmental insults such as discrimination or economic hardship, adverse childhood events, adolescent drug abuse, maternal stress, infections, pregnancy complications, and nutritional deficiencies (Montrose, 2000; Li et al., 2010; Zwicker et al., 2018). These detrimental multi-factorial insults of SCZ have been categorized into several biopathophysiological hypotheses, the most important of which are neurotransmitter deregulation, redox disruption, neuroinflammation and mitochondrial dysfunction (Monler et al., 2015; Singh et al., 2020).

Homeostatic derangements in dopamine (DA), glutamate (Glu), gamma-aminobutyric acid (GABA), serotonin (5-hydroxytryptamine; 5-HT), norepinephrine (NE), and acetylcholine (ACh) neurotransmitters have all been linked to SCZ (Maldonado, 2013; Sobolewski et al., 2018; Ben-Azu et al., 2018a, 2018b). These neurotransmitter receptors are all distributed in diverse brain regions in varying proportions, and their physiological roles are critical in normal human responses to the environment. DA receptors, for example, regulate motor functioning, motivation, drive, and cognition (Ferrazzoli et al., 2018; Cools et al., 2019). Thus, any alteration in DA neurotransmission causes a wide range of behavioral dysfunctions. Most notably, the positive SCZ symptoms are mediated by hyperactivity in the mesolimbic DA pathway, whereas hypoactivity mediates the negative and cognitive SCZ symptoms in the mesocortical DA pathways (Marek et al., 2010; Ghosh et al., 2011). Furthermore, experimental evidence show that psychostimulants (e.g. amphetamine and cocaine) increase dopamine release and cause SCZ-like symptoms (Weidenauer et al., 2017; Ben-Azu et al., 2018c; Omeiza et al., 2022). Aside from the classical DA hypothesis of SCZ, glutamate (the most abundant excitatory neurotransmitter in the brain) and 5-HT (a key neurochemical responsible for modulating moods) are the other significant neurotransmitters implicated in the pathogenesis of SCZ. Previous research has shown that NMDAR hypofunction in the

cortex, limbic, and thalamic brain regions can lead to molecular, cellular, and behavioral changes associated with SCZ (Flores et al., 2016). This receptor has been extensively studied to mimic SCZ symptoms in animals following exposures to phencyclidine or ketamine, a non-selective NMDAR antagonist (Chatterjee et al., 2012; Monte et al., 2013; Ben-Azu et al., 2018a, 2018b, 2018c; Oshodi et al., 2021). In addition, the hyperactivity of 5-HT receptors such as 5-HT_{2A} in the cortex plays a crucial role in the pathophysiology of SCZ. 5-HT_{2A} agonists such as lysergic acid diethylamide (LSD) and psilocybin result in visual hallucinations and mystical delusions (Whelan and Johnson, 2018). Moreover, hyperactive serotonergic signaling and hypoactive glutamatergic pathways in the cortical and sub-cortical sub-fields of the brain have been characterized and strongly implicated in the misfiring DA system (Ben-Azu et al., 2018a).

Furthermore, almost all recent studies have found that SCZ is associated with increased levels of oxidative stress and inflammation, as evidenced by prooxidants overriding antioxidants and an imbalance between proinflammatory and anti-inflammatory cytokines favoring the former in animals and humans exposed to long-term NMDAR antagonists respectively (Maldonado, 2013; Ben-Azu et al., 2018d). The brain is known to be highly enriched with fats and consumes more basal oxygen, an increased rate of oxidative metabolism combined with weak antioxidant states, has been documented to result in oxidative cellular injury generally seen in the brains of SCZ patients (Smaga et al., 2015; Hardingham and Do, 2016; Howes et al., 2017). In addition, oxidative stress-induced brain damage is notably associated with neuroinflammation, characterized by increased release of proinflammatory cytokines and proteins (Arora and Chopra, 2013; Réus et al., 2015; Ben-Azu et al., 2019).

Current pharmacotherapy, which includes (a)typical antipsychotics that primarily target DA D₂ receptors, does not address the full spectrum of symptoms associated with the disorder, particularly the negative and cognitive domains (Iversen et al., 2018). Moreover, these therapeutic challenges are also associated with a wide range of undesirable side effects such as movement disorders, glucometabolic side effects, photo-sensitivity, agranulocytosis, and reproductive impairments (Gareri et al., 2003; Hudepohl and Nasrallah, 2012; Oyovwi et al., 2021). As a result, because SCZ is a condition with several hypotheses, antipsychotic medications with a broad spectrum of action or better pharmacological profiles from phytomedicine are continuously investigated! Indeed, earlier studies have revealed that phytotherapeutic research is a better source of lead compounds for complex neuropsychiatric disorders since plants contain bioactive secondary metabolites with

multi-pronged neuroprotective (Chatterjee et al., 2015; Ben-Azu et al., 2016a, 2016b, 2020; Omeiza et al., 2022) and chemopreventive (Hossain et al., 2021; Ağagündüz et al., 2022) functions.

Interestingly, *Carpolobia lutea* G. Don (also known as cattle stick, Oshunshun, Agba, or Angalagala in English, Yoruba, and Igbo languages) is a Polygalaceae family plant (listed at <http://www.theplantlist.org> or <http://mpns.kew.org>) that has been used ethnomedicinally to treat and/or manage a variety of systemic diseases, including migraine headache, insanity or mental illness, sleeplessness, and sterility, all of which are CNS-related ailments (Burkill, 1995; Mitaine-Offier et al., 2002; Yakubu and Jimoh, 2014; Beshel et al., 2019). Its popularity in traditional medicine in Nigeria may be attributed to its pharmacological safety and nutritional benefits, as previously reported (Nwidiu, and Nwafor, 2009; Manfo et al., 2011; Nwidiu et al., 2016; Omeiza et al., 2022). With relevance to this study, previous studies suggest that *C. lutea* demonstrates antipsychotic-like action against amphetamine, apomorphine, and ketamine behavioral mouse models of schizophrenia (Omeiza et al., 2022). Importantly, phytochemical fingerprinting of the ethanol leaves extract of *C. lutea* together with high-performance liquid chromatography from our earlier studies also confirms the existence of several bioactive or secondary metabolites having various pharmacological activities (Pandy and VijeePallam, 2017; Yadav et al., 2018a, 2018b; Omeiza et al., 2022). Many preclinical investigations have also revealed that *C. lutea* possesses antioxidant, antiinflammatory (Abiodun and Oshinloye, 2017), antinociceptive (Nwidiu et al., 2016), analgesic (Jackson et al., 2011), sedative (Beshel et al., 2019), cholinergic (Nwidiu et al., 2016), and cognitive-enhancing (Ajiwhien and Bisong, 2013) effects. In this study, we aimed to investigate further the plausible neurorestorative and antipsychotic-like mechanisms exerted by *C. lutea* in mitigating schizophrenia-like symptoms in a ketamine reversal murine model of schizophrenia.

2. Material and methods

Ethical statement

Sixty-four (64) male Swiss mice with an average weight of 23 g and a life of approximately 5 weeks were purchased from the University of Ibadan's Animal Breeding Unit. Mice were housed in an individually ventilated propylene cage (length 42, breadth 30 and height 27 cm) with a 12:12-h light/dark cycle, room temperature ($25 \pm 1^\circ\text{C}$), and unrestricted access to tap water, and a rodent pellet-like food. The experimental protocol and animal handling were carried out in line with the certification of the University of Ibadan's local ethical committee on preclinical biomedicine (UI-ACUREC/049-0521/26).

2.1. Plant material and extraction

Fresh *C. lutea* plants (leaves) were harvested, identified, and authenticated at the Taxonomy unit of the Forestry Research Institute of Nigeria (FRIN) in Ibadan with the herbarium number FHI 112961. The leaves of *C. lutea* were then extracted with 70% (v/v) ethanol using a standard process, as previously reported (Omeiza et al., 2022). Concentrations and appropriate doses of *C. lutea* ethanol extract (EECL) were diluted in distilled water before administration.

2.2. Drugs and extract

In this study, ketamine (KET 20 mg/kg, i.p.; Nirlife Aculife, India), haloperidol (HLP 1 mg/kg, p.o.; Sigma-Aldrich, St. Louis, USA), clozapine (CZP 5 mg/kg, p.o.; Sigma-Aldrich, St. Louis, USA), and *C. lutea* ethanol extract (100, 200, 400 and 800 mg/kg, p.o.) were used. The doses of ketamine (NMDA receptor blocker), haloperidol (typical antipsychotic), and clozapine (atypical antipsychotic) were chosen from

previous reports by Moreira and Guimarães (2005), Monte et al. (2013), and Ben-Azu et al. (2018a), respectively, whereas the doses of *C. lutea* ethanol extract (tested intervention) was chosen based on the LD₅₀ obtained from our earlier findings (Omeiza et al., 2022). The drugs and the extract were both dissolved or diluted in the vehicle (distilled water) and administered in a volume of 10 mL/kg per body weight animal either via intraperitoneal or oral routes.

2.3. Experimental design

After 10 days of acclimatization, experiment units were randomly assigned to one of two major groups: normal control (n = 8) or test (n = 56), with distilled water (10 mL/kg, p.o.) or ketamine (20 mg/kg, i.p.) administered for the first seven days. On day 8, the test group was further subdivided into seven subgroups (n = 8/subgroup) to establish the schizophrenic reversal protocol described by Monte et al. (2013). While the normal control group continued its regimen until day 14, subgroup (i) tagged as a negative control was administered ketamine and vehicle without intervention, and subgroups (ii–v) were exposed to co-administration of ketamine with gradually increasing doses of *C. lutea* (100, 200, 400 and 800 mg/kg, p.o., respectively) and subgroup (vi–vii designated as standard controls) received co-administration of ketamine with haloperidol (1 mg/kg, p.o.) and clozapine (5 mg/kg, p.o.) respectively. All treatments were administered once per day between 7:00 and 8:30 a.m., with a 45-min inter-treatment interval. Neuro-behavioral characterization in open field test, force swimming, Morris water maze and novel object recognition were performed between days 8–15 to reveal the extract's anti-antipsychotic-like effect on positive, negative, and cognitive symptoms, respectively (Fig. 1). Between 9:30 a.m. and 1:30 p.m., a video camera (Webcam) was positioned directly above each paradigm to capture behavioral activities of the mice in an unbiased pattern across the groups, and ANY-Maze video tracking system software from Stoelting Co. (Version 6.3; Wood Dale, IL) was used to track and analyzed behavioral data as previously described (Omeiza et al., 2021). On day 16, all mice were sacrificed by cervical dislocation, and those for biochemical assays (n = 5/group) were transcardially perfused with 20 mL of normal saline (0.9% w/v NaCl) and 40 mL of sodium phosphate buffer (0.1 M, pH 7.4), while those for histopathological examination (n = 3/group) were transcardially perfused with 60 mL of 10% formol saline (0.1% w/v formaldehyde + 0.9% w/v NaCl). The excised brain tissue of each animal was weighed, rinsed, and homogenized in sodium phosphate buffer (0.1 M, pH 7.4), centrifuged for 15 min at 4°C at 5400 g, and the supernatant was stored at -20°C for biochemical assays, while those for histopathological examination were individually fixed in 10% formol saline for 72 h before being sectioned into the prefrontal cortex (PFC), hippocampus (HC), and cerebellum (CERBL).

2.4. Behavioral tests

2.4.1. Assessment of hyperlocomotion using open field test (OFT)

The open field apparatus (length 20 cm, breadth 20 cm, and height 23 cm), made of wood was used to assess the antipsychotic effects of *C. lutea* on motor activity status, using total distance covered and average speed as predictors of hyperlocomotion of mice treated with ketamine as an index of one of the positive symptoms of SCZ (Maia-de-Oliveira et al., 2015; Ben-Azu et al., 2018c).

2.4.2. Assessment of behavioral despair using force swimming test (FST)

As previously documented by Porsolt et al. (1977), the FST was employed to assess the antipsychotic-like effects of *C. lutea* on ketamine-enhanced behavioral despair in mice. Briefly, each mouse was immersed vertically in an inescapable cylinder of water (diameter 10 cm, height 25 cm) at a room temperature ($25 \pm 1^\circ\text{C}$) for 6 min cut-off

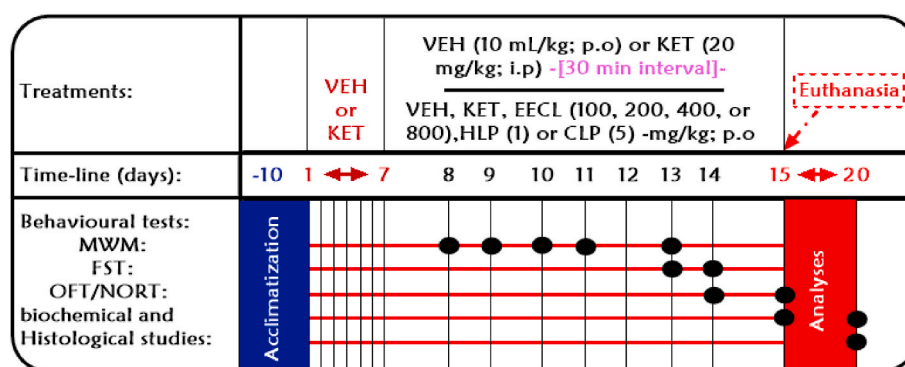


Fig. 1. The methodology employed to explore the plausible mechanisms of action of *C. lutea* against ketamine induced schizophrenia-like symptoms in mice. MWM = Morris water maze, FST = Force swimming test, OFT = Open field test, NORT = Novel object recognition test, VEH = Vehicle, KET = Ketamine, EECL = Ethanol extract of *Carpolobia lutea*, HLP = Haloperidol, CZP = Clozapine.

time at each stage of FST. The first stage is habituation, and the second stage is a test, with a 24-hr inter-stage interval, and the total duration of time each mouse stayed immobile during this stage was recorded as immobility time, which is an indicator of behavioral despairs or negative of SCZ.

2.4.3. Assessment of cognitive deficit using Morris water maze (MWM) and novel object recognition (NOR) tests

The MWM and NOR tests were used to assess *C. lutea*'s antipsychotic effects on ketamine-induced spatial and nonspatial learning and memory impairments, as discussed previously by Omeiza et al. (2021) and Ben-Azu et al. (2018c) with little modifications (Fig. 1).

2.5. Neurochemical assays

2.5.1. Assay of neurotransmitters

The ELISA analytical kits for the determination of glutamate, dopamine, norepinephrine, and serotonin were all obtained from Hangzhou Eastpharm Limited (Torrance U.S.A) and assays were performed according to the manufacturer's instructions and absorbent was read with a microplate reader (Spectramax M-5 (Molecular Devices, Sunnyvale, CA) equipped with Softmax Pro v 5.4 (SMP 5.4). Before use, all reagents, standard solutions, and samples were brought to room temperature, and their concentrations were expressed as $\mu\text{mol}/\text{mg}$ tissue or $\mu\text{g}/\text{mg}$ protein.

2.5.2. Determination of monoaminergic and cholinergic metabolizing enzymes activity

The activity of the brain monoamine oxidase (MAO), an index of monoaminergic system function, was measured using the method described earlier by Abolaji et al. (2018). An aliquot was mixed with sodium phosphate buffer (0.1 M, pH 7.4) and benzylamine hydrochloride (25 L), and the reactive mixture was incubated for 30 min at $25 \pm 1^\circ\text{C}$ before the reaction was terminated by adding perchinnic acid (10%) and centrifuging at 1500 g for 10 min at 4°C . The change in absorbance of the supernatant per min was then measured at 280 nm against a blank reagent using a microplate reader, and the MAO activity was reported as M of MAO/min/mg protein.

The activity of the brain acetylcholinesterase (AChE), a marker of cholinergic nervous system function, was assessed using the procedure reported earlier by Ellman et al., 1961). An aliquot was mixed with sodium phosphate buffer (0.1 M, pH 7.4), 5,5'-dithio-bis (2-nitrobenzoic acid) (DTNB), and acetylthiocholine iodide. The change in absorbance of the reactive mixture at 412 nm was measured using a UV spectrophotometer (752P UV-VIS spectrophotometer, Searchtech, UK) for 10 min at 2 min intervals against blanks, and the rate of AChE activity was directly proportional to color formation as a result of the reaction between thiocholine and DTNB. This activity was calculated and denoted

as μM of AChE/min/mg protein.

2.5.3. Assay of oxidative-nitrosative stress markers

The brain MDA concentration, a key indicator of tissue lipid peroxidation injury, was determined using the previously reported method (Ohkawa et al., 1979). An aliquot was incubated at 80°C for 45 min with Tris KCl (0.15M, pH 7.4), TCA (30%, pH 3.4), and TBA (0.75%, pH 3.2), then the reactive mixture was cooled to 25°C and centrifuged at 3000 g at 4°C for 15 min. The supernatant was then measured at an absorbance of 532 nm against a blank reagent using a microplate reader named above, and the concentration of MDA was denoted as nM of MDA/mg protein.

The brain SOD activity, a classic example of an enzymatic antioxidant, was evaluated using the Misra and Fridovich method (1972). Upon adding an aliquot to carbonate buffer (0.05 M, pH 10.2), the reaction was initiated by adding freshly prepared adrenaline (0.3 mM) to the mixture, and the absorbance was quickly read at 480 nm at 60 s intervals for 3 min as previously described (Misra and Fridovich, 1972). The brain GSH concentration, a typical example of a non-enzymatic antioxidant, was determined using the method earlier reported (Moron et al., 1979). An equal volume of aliquot and TCA (20%, pH 3.4) were mixed and centrifuged at 10,000 g for 20 min at 4°C with a yellow mixture formed by the addition of DTNB (0.6 mM) and sodium phosphate buffer (0.2 M, pH 8.0). Absorbance was measured at 412 nm against a blank reagent using a microplate reader (Spectramax M-5 (Molecular Devices, Sunnyvale, CA) equipped with Softmax Pro v 5.4 (SMP 5.4), and the GSH concentration was directly proportional to color formation, denoted as μM of GSH/mg protein.

The brain nitrite content, which is an excellent determinant of nitric oxide (NO) synthesis, was determined using the Griess reagent (Green et al., 1981). An equal volume of aliquot and Griess reagent (Green et al., 1981) were mixed, incubated and the absorbent was read at 540 nm UV spectrophotometer, and the nitrite concentration was determined from the standard curve obtained from sodium nitrite (0–100 μM) then denoted as μM of NO/mg protein.

2.5.4. Assay of inflammatory cytokines

The levels of interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF- α) in the brain were measured using an ELISA according to the manufacturer's instructions. Before use, all reagents, standard solutions, and samples were brought to room temperature, and their concentrations were expressed as pg/mg protein.

2.6. Histopathological evaluations

Following euthanasia, intracardiac perfusion with 10% formol saline, brain excision, and 72 h of brain fixation with 10% formol saline, each brain tissue was dissected, and isolates [prefrontal cortex,

cerebellum, and hippocampal zone (CA1, CA3 and DG)] were stained with hematoxylin and eosin (H and E) for microscopic assessments, as previously reported by Imam et al. (2016). The sections were examined under a light microscope (magnification: $\times 400$; Nikon, Tokyo, Japan) for histopathological alterations in each isolate, and the number of normal and aberrant cells per mm² in each group (n = 3/group) was quantified using Image J analyzer software (NIH Image, Maryland, US).

2.7. Statistical analysis

GraphPad Prism version 8.0.2 software (La Jolla, CA) was used to analyze the data, and the experimental results are presented as means standard error of mean (S.E.M.). One-way and two-way ANOVA were used to assess treatment effects across groups and between groups, followed by Tukey's post hoc test. Alpha (α) was set at 0.05 [(*) in comparison to vehicle control animals] and 0.05 [(#) in comparison to ketamine-untreated mice]. Unless otherwise indicated, the "n" value represents the number of animals used per group.

3. Results

3.1. Effect of *C. lutea* (EECL) on ketamine-induced schizophrenia-like symptoms

As shown in Fig. 2 A-C, the OFT trajectory plots of all groups exposed

to *C. lutea* and/or ketamine during the 5 min assessment showed varying dramatic differences (Fig. 2 A). Based on the analysis of the positive symptoms of schizophrenia in the OFT chamber (Fig. 2 B and C), the average distance travelled [F (7, 32) = 18.96, $P < 0.0001$, n = 5/group], a hyperactivity index, was elevated ($p < 0.05$) in ketamine-treated mice when compared to VEH-control mice, but this positive characterization induced by ketamine was reduced ($p < 0.05$) in mice that received *C. lutea* (100, 200, 400 and 800 mg/kg), haloperidol (1 mg/kg), and clozapine (5 mg/kg) (Fig. 2 B). Correspondingly, the average speed [F (7, 32) = 11.20, $P < 0.0001$, n = 5/group] of mice exposed to ketamine (20 mg/kg) alone increased ($P < 0.05$) in comparison to VEH-control mice, but this effect was found to be reduced ($p < 0.05$) in ketamine-treated mice with reversal agents like EECL (100, 200, 400, and 800 mg/kg), haloperidol (1 mg/kg), and clozapine (5 mg/kg) (Fig. 2 C).

As regards the negative symptoms in the FST (Fig. 2 D), total immobile time or immobility time [F (7, 32) = 4.125, $P = 0.0025$, n = 5/group], a measure of behavioral despair (or negative symptoms) was higher ($p < 0.05$) in the mice treated with ketamine when compared to VEH-control, but lowered ($P < 0.05$) in the mice treated with *C. lutea* (200, 400 and 800 mg/kg). Furthermore, total time immobile was also decreased ($P < 0.05$) in the clozapine-treated (5 mg/kg) mice with no significant differences at $p > 0.05$ in mice treated with haloperidol (1 mg/kg) and *C. lutea* (100 mg/kg) when compared to ketamine-treated mice.

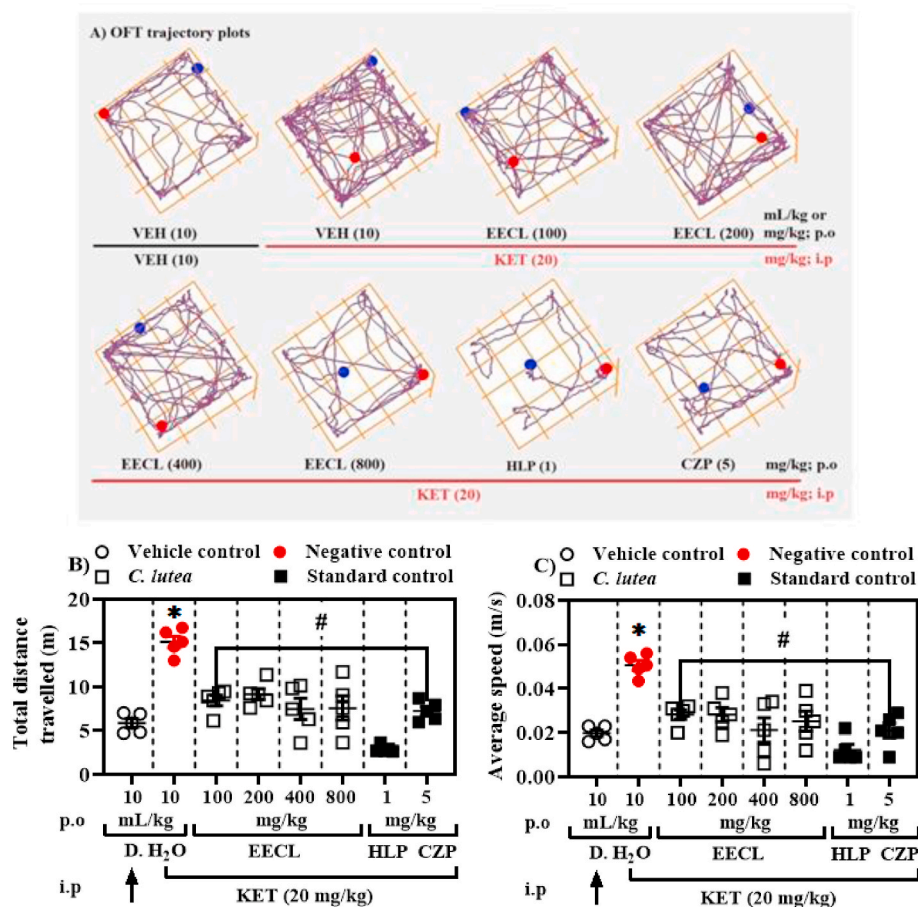


Fig. 2. Effect of *C. lutea* (EECL) on ketamine-induced schizophrenia-like symptoms. Positive symptoms: (A) Open Field Test (OFT) track diagram, (B and C) OFT data analysis; Negative symptoms: (D) Force Swimming Test (FST) data analysis; Cognitive symptoms: (E) Morris Water Maze (MWM) data analysis (learning days), (F) Path efficiency, (G) Cognitive score, (H) MWM track (I) Pathlength in target zone, (J) Average proximity to the target zone, (K) Annulus time, (L) MWM mean heat map and (M) Novel object recognition test (NORT) – Discriminating index. Data are expressed as mean $\pm$ S.E.M (n = 5/group). * $P < 0.05$ as compared VEH-control group; # $P < 0.05$ as compared ketamine-untreated group (One-way ANOVA followed by Tukey post-hoc test). VEH= Vehicle, KET = Ketamine; EECL = Ethanol extract of *Capolobia lutea* = *C. lutea*; HLP = Haloperidol; CZP = Clozapine, NO = Novel object, FO = Familiar object.

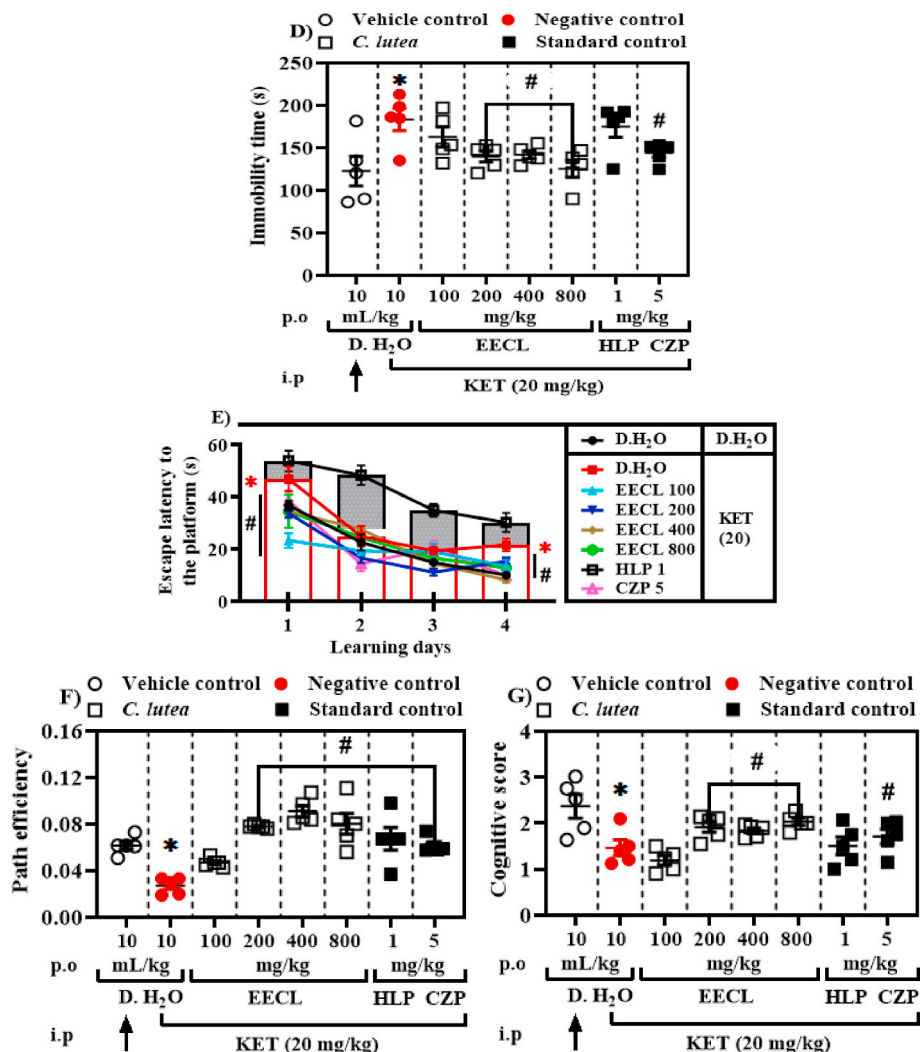


Fig. 2. (continued).

To evaluate the effect of *C. lutea* on cognitive symptoms (learning and memory deficits) induced by ketamine, mice were exposed to the MWM and NORT, as illustrated in Fig. 2 E-L. The time it took the experimental animal to locate the invisible platform during a 4-day learning trial in the MWM is known as escape latency, and it gradually decreased over time. Furthermore, a two-way ANOVA repeated measures with multiple comparisons: group [F (7, 160) = 31.40, $P < 0.0001$, $n = 6/\text{group}$], day [F (3, 160) = 103.5, $P < 0.0001$, $n = 6/\text{group}$] and the interaction (between-group vs. day) [F (21, 160) = 2.774, $P = 0.0002$, $n = 6/\text{group}$] revealed a significant ($p < 0.05$) effect of *C. lutea* and/or ketamine treatment on escape latency, particularly on day 4, where the haloperidol (1 mg/kg) reversal treatment and ketamine-untreated group had a higher ($P < 0.05$) escape latency than the VEH-control group (Fig. 2 E). On that same day, ketamine (20 mg/kg) reduced ($p < 0.05$) the path efficiency [F (7, 32) = 10.72, $P < 0.0001$, $n = 5/\text{group}$], a variable for measuring navigation efficiency to reach the invisible platform when compared to VEH-control group, but this learning and acquisition decline induced by ketamine was reversed ($p < 0.05$) in mice that were treated with 200, 400 and 800 mg/kg of *C. lutea*, and clozapine (5 mg/kg) but not haloperidol (1 mg/kg) (Fig. 2 F). Ketamine also decreased ($p < 0.05$) cognitive score [F (7, 36) = 5.357, $P = 0.0003$] in relation to VEH-control group. However, the administration of 5 mg/kg of clozapine, as well as 200, 400, 800 but not 100 mg/kg of *C. lutea* markedly reversed the effect (Fig. 2 G). This cognitive score was assessed using the mice's tracks on the last day of the

acquisition trial (Fig. 2 H).

Furthermore, a probe trial was conducted 24-h after the final acquisition day to further assess the cognitive processes like spatial reference, retrieval, and long-term memory. Three swimming indicators, pathlengths in the target zone, average proximity to the target zone, and annular time (that is, time spent in the annulus zone, which coincides with the target zone where the escape platform appears to be present), were used to examine the cognitive process status. One-way ANOVA analysis showed significant differences ($p < 0.05$) in the pathlengths in the target zone [F (7, 32) = 13.69, $P < 0.0001$, $n = 5/\text{group}$] of mice treated with ketamine (20 mg/kg) and VEH (10 mL/kg). Nonetheless, administration of *C. lutea* (200, 400 and 800 mg/kg) and clozapine (5 mg/kg) exhibited increased ($p < 0.05$) pathlengths in the target zone (Fig. 2 I). Regarding the average proximity to the target zone [F (7, 32) = 2.117, $P = 0.0702$, $n = 5/\text{group}$], there was an insignificant change when comparing ketamine-untreated mice to VEH-control mice. Similarly, neither haloperidol (1 mg/kg) nor clozapine (5 mg/kg) nor treatment with 100, 200, 400, or 800 mg/kg of *C. lutea* altered ($p > 0.05$) the average proximity to the target zone in comparison to ketamine-untreated (Fig. 2 J). When compared to VEH-control mice, ketamine treated mice had a shorter annulus duration [F (7, 36) = 19.16, $P < 0.0001$, $n = 5/\text{group}$]. Treatment with *C. lutea* (100, 200, 400, and 800 mg/kg), and clozapine (5 mg/kg) abrogated the long-term memory decline response induced by ketamine as annulus time was elevated at $p < 0.05$ and there were no other significant comparisons

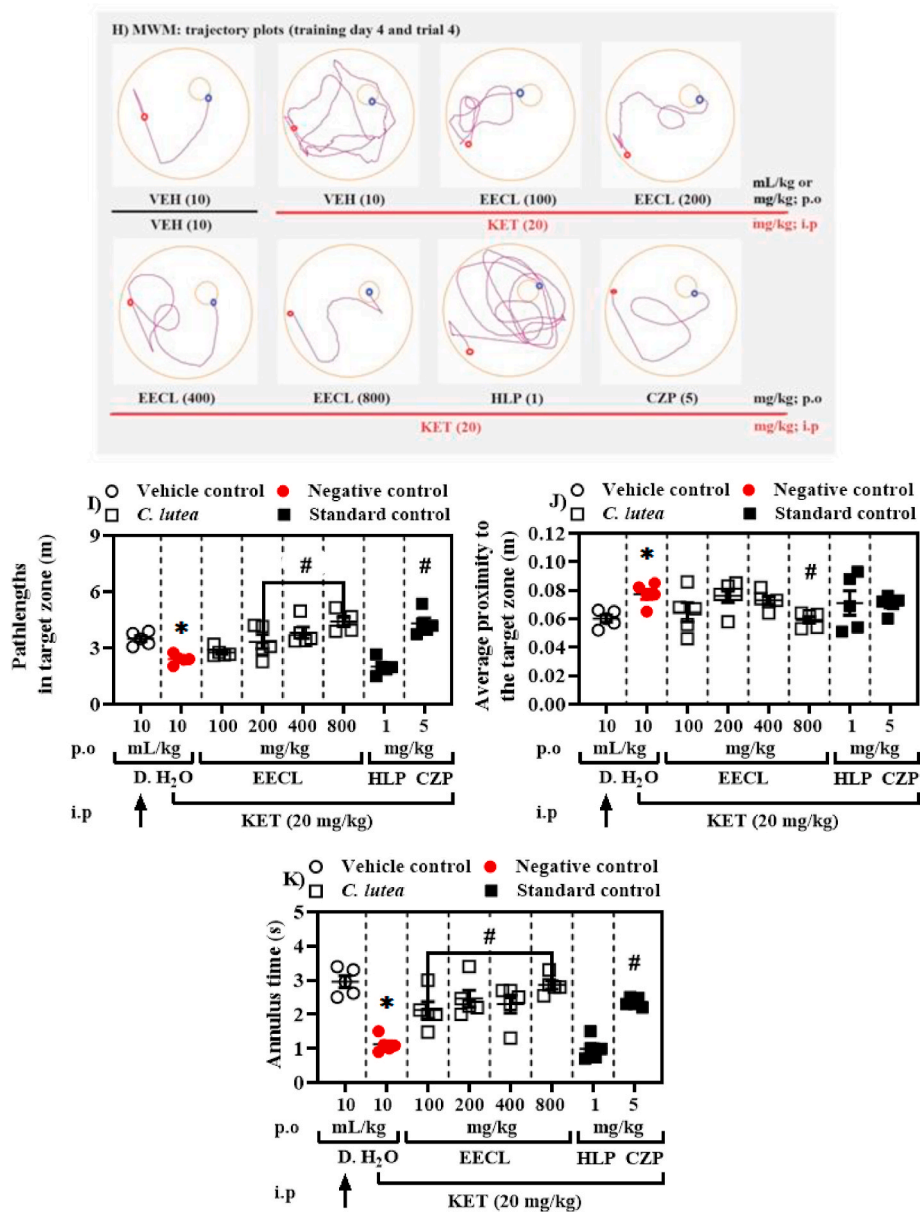


Fig. 2. (continued).

observed (Fig. 2 K). The mean heat map of the mice's searching strategy for an escape and an invisible platform that had been removed before the test is shown in Fig. 2L.

In NORT, as expected, the ketamine administration significantly ($p < 0.05$) resulted in impaired cognitive performance in mice, as evidenced by a lower discriminative index (DI) [$F(7, 37) = 6.509$, $P < 0.0001$, $n = 5/\text{group}$] when compared to VEH-control group, a non-spatial parameter similar to what was observed in the MWM's spatial learning and memory. However, the administration of oral *C. lutea* at higher doses of 200, 400, and 800 mg/kg, but not 100 mg/kg, reversed the Ketamine-induced effect. Similarly, the (DI) was found to be higher in the haloperidol (1 mg/kg)- and clozapine (5 mg/kg)-treated groups than in the ketamine treated group (Fig. 2 M).

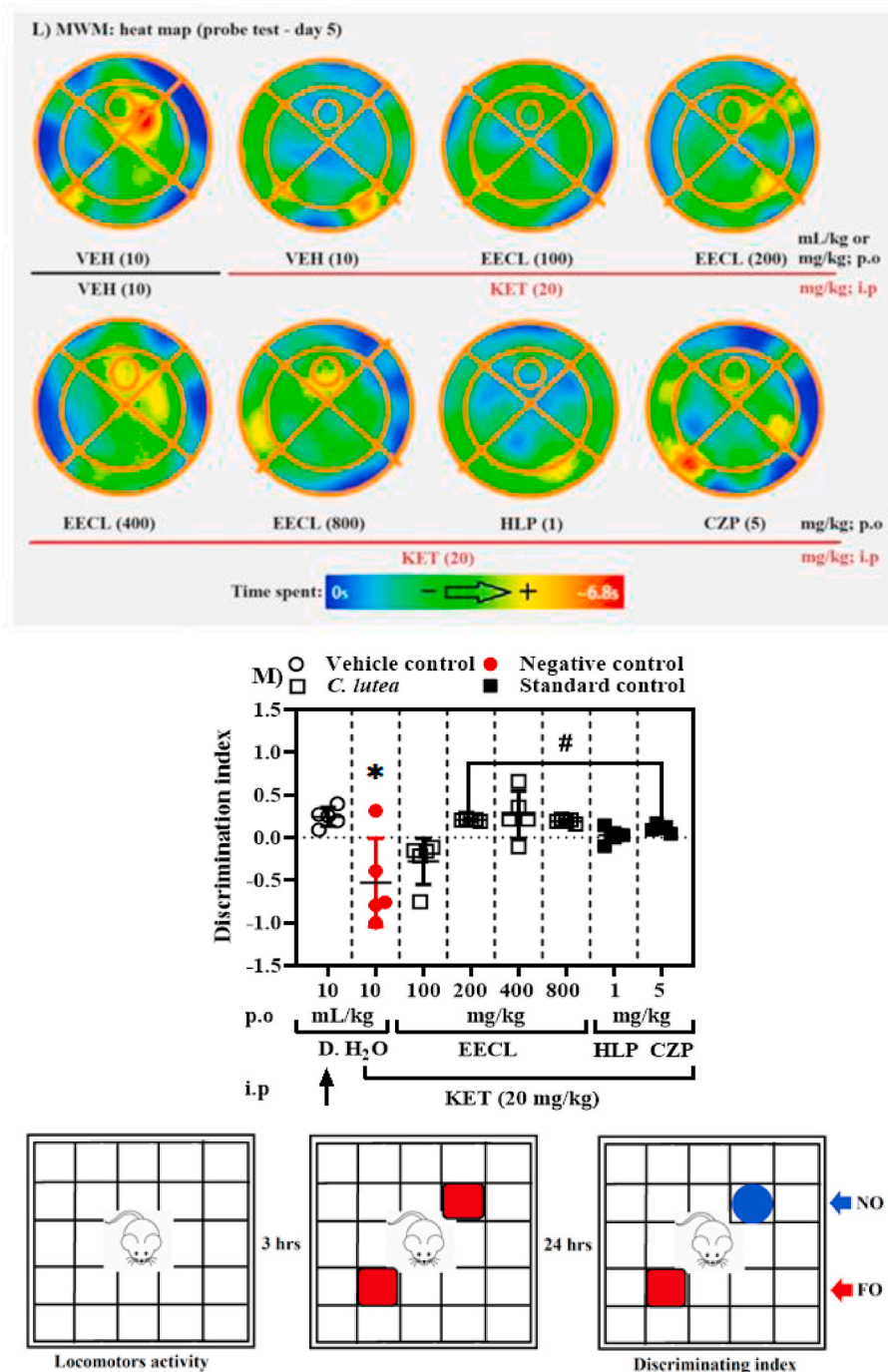
3.2. Effect of *C. lutea* on brain glutamate content in ketamine-induced schizophrenia

Glutamate (Glu) is a key neurotransmitter implicated in the pathogenesis of schizophrenia, and as shown in Fig. 3, ketamine-treated mice

had lower ($p < 0.05$) Glu levels [$F(7, 32) = 8.814$, $P < 0.0001$, $n = 5/\text{group}$] than the VEH-control group. However, when compared to ketamine-untreated mice, treatment with *C. lutea* at doses of 200, 400, and 800 mg/kg increased ($p < 0.05$) Glu level, but this effect was unaffected ($p > 0.05$) in mice treated with haloperidol (1 mg/kg), clozapine (5 mg/kg) and 100 mg/kg of *C. lutea* (Fig. 3).

3.3. Effect of *C. lutea* on brain monoamine contents and monoamine oxidase activity in ketamine-induced schizophrenia

To further understand the mechanism of action of *C. lutea*, the effect of *C. lutea* on ketamine-induced changes in monoamine (DA, 5-HT, and NE) contents and monoamine oxidase (MAO) metabolizing enzyme activity was investigated in mice brains. As shown in Fig. 4 A, the level of DA [$F(7, 32) = 24.46$, $P < 0.0001$, $n = 5/\text{group}$] was higher ($p < 0.05$) in the ketamine-treated mice compared to the VEH-control. However, treatment with *C. lutea* at 400, 800 mg/kg but not 100, and 200 mg/kg lowered ($p < 0.05$) the DA level which also annuls the effect of ketamine. Besides that, treatment with haloperidol (1 mg/kg) and clozapine



(5 mg/kg) reduced ($p < 0.05$) the effect of ketamine. The level of NE was also assessed and as depicted in Fig. 4 B, there was an insignificant increase ($p > 0.05$) in NE level [$F(7, 32) = 8.868$, $P < 0.0001$, $n = 5/\text{group}$] in ketamine-treated mice compared to VEH-control animals. Nevertheless, the administration of *C. lutea* at dose levels of 400 and 800 mg/kg, but not 100 and 200 mg/kg reduced ($p < 0.05$) the impact caused by ketamine. Furthermore, the involvement of the serotonergic (5-HT) system has also been linked to schizophrenia, although the result reveals that the level of 5-HT [$F(7, 32) = 3.680$, $P = 0.0050$, $n = 5/\text{group}$] in the ketamine-treated group was not affected ($p > 0.05$) when compared to the VEH-control group, nor did treatment with *C. lutea* (100, 200, 400, and 800 mg/kg), haloperidol (1 mg/kg), and clozapine (1 mg/kg) alter the 5-HT level when compared to ketamine-untreated

mice (Fig. 4 C). The MAO metabolizing enzyme activity [F (7, 32) = 6.969, P < 0.0001, n = 5/group] was insignificantly altered by ketamine exposure in relation to VEH-control treatment. However, when compared to ketamine-untreated mice, the administration of clozapine, rather than haloperidol or *C. lutea*, had a significant decrease (p < 0.05) (Fig. 4 D).

3.4. Effect of *C. lutea* on brain acetylcholinesterase activity in ketamine-induced schizophrenia

The effect of *C. lutea* and/or ketamine on the cholinergic system was investigated in mice brains to further validate the mechanism of action of *C. lutea*, as shown in Fig. 5. A significant ($p < 0.05$) increase in AChE

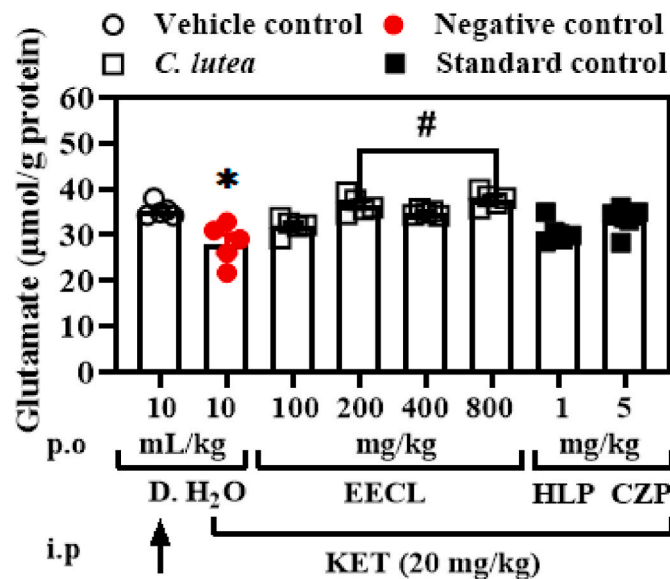


Fig. 3. Effects of *C. lutea* extract (EECL) on glutamate content in mice treated with ketamine-induced schizophrenia. Data are expressed as mean $\pm$ S.E.M ($n = 5$ /group). * $P < 0.05$ as compared VEH-control group; # $P < 0.05$ as compared ketamine-untreated group (One-way ANOVA followed by Tukey post-hoc test). D.H₂O/VEH = Vehicle, KET = Ketamine; EECL = Ethanol extract of *Capolobia lutea* = *C. lutea*; HLP = Haloperidol; CZP = Clozapine.

activity [$F(7, 32) = 10.4$, $P < 0.0001$, $n = 5$ /group] was observed in ketamine-treated mice compared to VEH-control mice, but *C. lutea* at doses of 200, 400, and 800 mg/kg, but not at 100 mg/kg, reversed the effect induced by ketamine. Correspondingly, clozapine (5 mg/kg) treatment significantly reduced ($p < 0.05$) AChE activity, but not haloperidol (1 mg/kg).

3.5. Effect of *C. lutea* on brain oxidative stress in ketamine-induced schizophrenia-like symptoms

To further validate *C. lutea*'s mechanism of action, the effect of *C. lutea* on ketamine-induced derangement in the oxidative status of mice was evaluated. Pro-oxidants (e.g., MDA) and antioxidants (e.g., SOD and GSH) were used as indexes of oxidative stress. As shown in

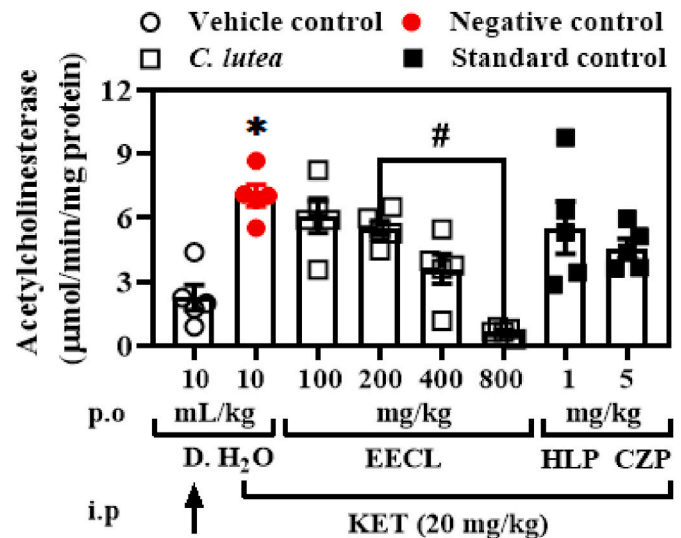


Fig. 5. Effects of *C. lutea* extract (EECL) on acetylcholinesterase activity in mice treated with ketamine-induced schizophrenia. Data are expressed as mean $\pm$ S.E.M ($n = 5$ /group). * $P < 0.05$ as compared VEH-control group; # $P < 0.05$ as compared ketamine-untreated group (One-way ANOVA followed by Tukey post-hoc test). D.H₂O/VEH = Vehicle, KET = Ketamine; EECL = Ethanol extract of *Capolobia lutea* = *C. lutea*; HLP = Haloperidol; CZP = Clozapine.

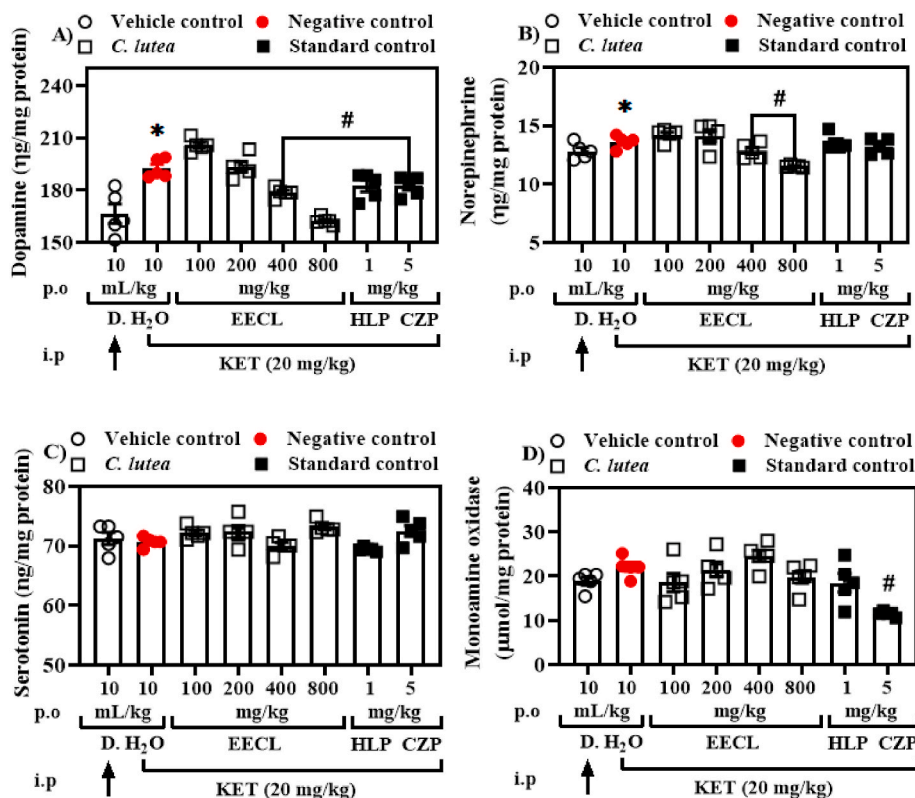


Fig. 4. Effects of *C. lutea* extract (EECL) on monoamine contents and monoamine oxidase activity in mice treated with ketamine-induced schizophrenia. (A) dopamine, (B) norepinephrine, (C) serotonin, and (D) monoamine oxidase. Data are expressed as mean $\pm$ S.E.M ($n = 5$ /group). * $P < 0.05$ as compared VEH-control group; # $P < 0.05$ as compared ketamine-untreated group (One-way ANOVA followed by Tukey post-hoc test). D.H₂O/VEH = Vehicle, KET = Ketamine; EECL = Ethanol extract of *Capolobia lutea* = *C. lutea*; HLP = Haloperidol; CZP = Clozapine.

Fig. 6. ketamine-treated group demonstrated a significant increase ($p < 0.05$) in MDA [$F(7, 32) = 9.623$, $P < 0.0001$, $n = 5/\text{group}$] and a decrease in SOD levels when compared with VEH-control group, confirming oxidative stress. This effect was mitigated ($p < 0.05$) in mice treated with 200, 400, 800, but not 100 mg/kg of *C. lutea*, as MDA was diminished and SOD was up-regulated, indicating antioxidant capacity. The administration of haloperidol (1 mg/kg) and clozapine (5 mg/kg) showed no antioxidant effect in mice exposed to ketamine (**Fig. 6 A and B**). Despite this, GSH level [$F(7, 32) = 1.628$, $P = 0.1634$, $n = 5/\text{group}$] in ketamine-treated group was unaltered ($p > 0.05$) as compared to VEH-control group. Likewise, no significant ($p > 0.05$) difference in GSH level was noted in groups treated with *C. lutea* (100, 200, 400 and 800 mg/kg), haloperidol (1 mg/kg) and clozapine (5 mg/kg) when compared to ketamine-treated group (**Fig. 6 C**). In addition, **Fig. 6 D** illustrates the effect of *C. lutea* on ketamine-induced changes in nitrite level as a biomarker for nitric stress. In comparison to VEH-control mice, the level of nitrite [$F(7, 32) = 2.799$, $P = 0.0217$, $n = 5/\text{group}$] was insignificantly raised ($p > 0.05$) in ketamine-treated mice. Conversely, there was a significant reduction ($p < 0.05$) in nitrite levels in mice exposed to reversal treatment with *C. lutea* (200, 400, and 800, but not 100 mg/kg). Neither haloperidol (1 mg/kg) nor clozapine (5 mg/kg) had any effect ($p > 0.05$) on the nitrite level.

3.6. Effects of *C. lutea* on ketamine-induced neuroinflammation-mediated schizophrenia

To further validate *C. lutea*'s effect on ketamine-induced schizophrenia-like symptoms, pro-inflammatory cytokines such as IL-6 and TNF- α levels were measured in mice brains. Ketamine exposure resulted in a significant increase ($p < 0.05$) in IL-6 [$F(7, 32) = 3.733$, $P = 0.0046$, $n = 5/\text{group}$] when compared to VEH-control group. Ketamine-induced effect was however ameliorated ($p < 0.05$) by 400 and 800 mg/kg of *C. lutea* but not by 100 or 200 mg/kg of *C. lutea*. Neither the haloperidol (1 mg/kg) nor the clozapine (5 mg/kg) reversal treatments altered ($p > 0.05$) the IL-6 level in relation to ketamine-untreated mice (**Fig. 7 A**).

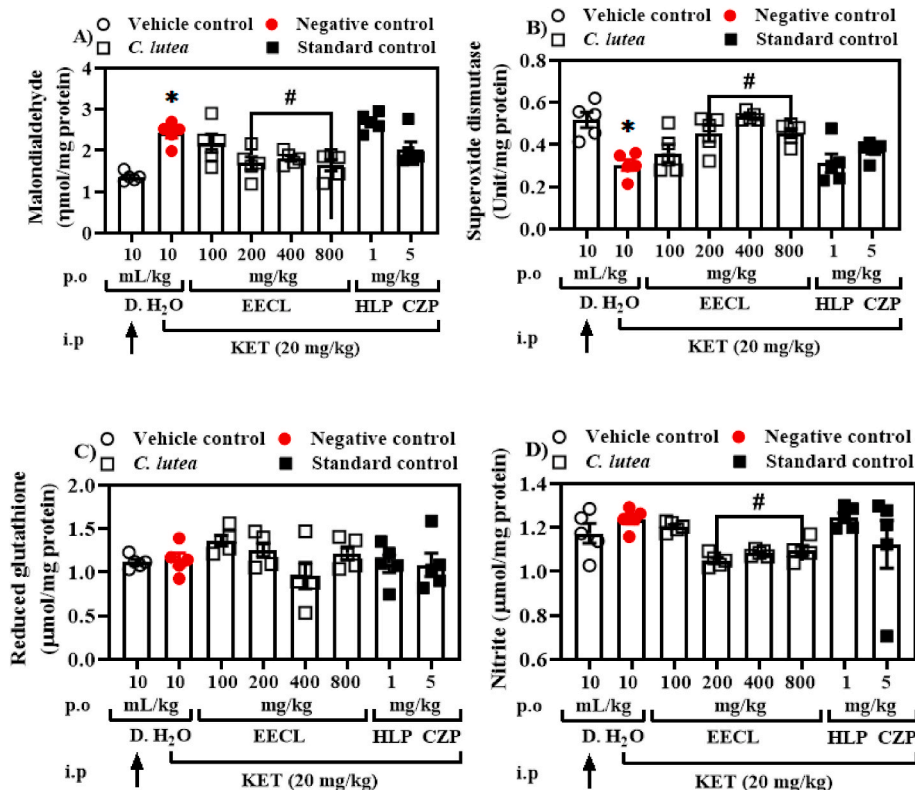


Fig. 6. Effects of *C. lutea* extract (EECL) on oxidative stress markers in mice treated with ketamine-induced schizophrenia. (A) lipid peroxidation, (B) superoxide oxidase, (C) glutathione, and (D) nitric oxide. Data are expressed as mean $\pm$ S.E.M ($n = 5/\text{group}$). * $P < 0.05$ as compared VEH-control group; # $P < 0.05$ as compared ketamine-untreated group (One-way ANOVA followed by Tukey post-hoc test). D.H₂O/VEH = Vehicle, KET = Ketamine; EECL = Ethanol extract of *Capolobia lutea* = *C. lutea*; HLP = Haloperidol; CZP = Clozapine.

Ketamine intoxication however resulted in an insignificant increase ($p > 0.05$) level of TNF- α [$F(7, 32) = 0.7488$, $P = 0.6331$, $n = 5/\text{group}$] when compared to VEH-control mice. However, none of the reversal treatments with *C. lutea*, haloperidol (1 mg/kg), or clozapine (5 mg/kg) had any effect ($p > 0.05$) on TNF- α level (**Fig. 7 B**).

3.7. *C. lutea* abates histological alterations of the prefrontal cortex in ketamine-induced schizophrenia

As shown in **Fig. 8**, the prefrontal cortex histoarchitecture showed more abnormalities in ketamine-untreated mice compared to VEH-control mice, with minimal or no alteration in neuronal settings evidenced by intact granular cells at the cortical layer (black arrow) and little vacuolization (red arrow). In comparison to the ketamine-untreated mice, mice treated with *C. lutea* (400 and 800 mg/kg), haloperidol (1 mg/kg), and clozapine (5 mg/kg) had less neuronal damage (**Fig. 8 A-H**). Correspondingly, the semi-quantitative analysis indicated that the ketamine-treated group had a higher ($p < 0.05$) % of damaged neuronal cells per field [$F(7, 16) = 22.00$, $P < 0.0001$, $n = 3/\text{group}$] than the VEH-control group. However, the distortion caused by ketamine intoxication was reversed ($p < 0.05$) in mice given 400, and 800 mg/kg of *C. lutea*, as well as haloperidol (1 mg/kg) and clozapine (5 mg/kg) (**Fig. 8 I**).

3.8. Effect of *C. lutea* on histological alterations of the CA1 hippocampal subfield in ketamine-induced schizophrenia

As depicted in **Fig. 9**, the histoarchitecture of the CA1 hippocampal region of the brain revealed no distinct differences between the groups exposed to ketamine without treatment and VEH-control, as they all have little or no disfigurement in their outer polymorphic, middle pyramidal, and inner molecular layers (black arrow). The pyramidal neurons are densely packed across the groups, with little vacuolization (red arrow). Likewise, there was no significant difference in neuronal settings between groups treated *C. lutea* (100, 200, 400, and 800 mg/

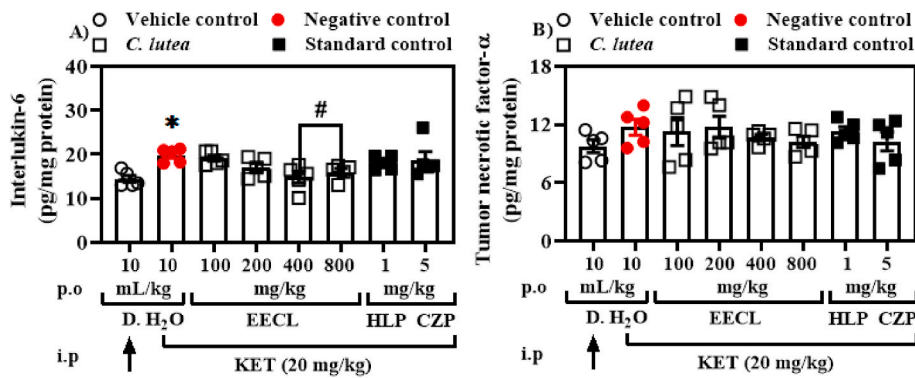


Fig. 7. Effects of *C. lutea* extract (EECL) on inflammatory cytokines in mice treated with ketamine-induced schizophrenia. (A) interleukin 6, and (B) tumor necrotic factor-α. Data are expressed as mean $\pm$ S.E.M (n = 5/group). *P < 0.05 as compared VEH-control group; #P < 0.05 as compared ketamine-untreated group (One-way ANOVA followed by Tukey post-hoc test). D.H₂O/VEH= Vehicle, KET = Ketamine; EECL = Ethanol extract of *Capolobia lutea* = *C. lutea*; HLP = Haloperidol; CZP = Clozapine.

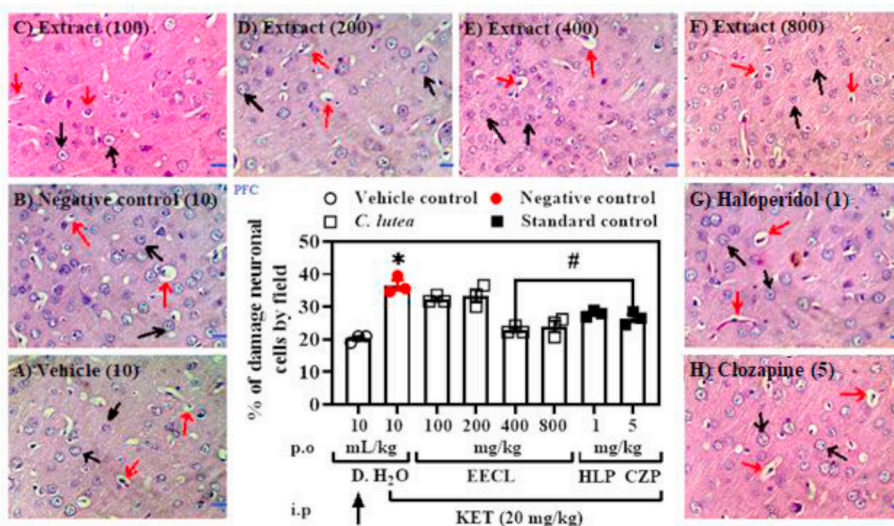


Fig. 8. Effects of *C. lutea* on ketamine-induced neuropathological changes (A–H) and neuronal degeneration (I) in the prefrontal cortex (PFC). Black arrow indicates normal neurons and red arrow indicates degenerative neurons. The slides were observed by light microscopy (H and E staining $\times$ 400, reference bar = 50 μ m). Bar represents the mean $\pm$ S.E.M (n = 3/group). *P < 0.05 as compared VEH-control group; #P < 0.05 as compared ketamine-untreated group (One-way ANOVA followed by Tukey post-hoc test). D.H₂O/VEH= Vehicle, KET = Ketamine; EECL = Ethanol extract of *Capolobia lutea* = *C. lutea*; HLP = Haloperidol; CZP = Clozapine. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

kg), as well as haloperidol (1 mg/kg) and clozapine (5 mg/kg) (Fig. 9 A–H). Regarding the semi-quantitative analysis, there was no change ($p > 0.05$) in the % of damaged neuronal (pyramidal) cells by field [F (7, 16) = 0.7533, $P = 0.6327$, $n = 3$ /group] between the ketamine-untreated and VEH-control groups. Also, the administration of 100, 200, 400 and 800 mg/kg *C. lutea*, as well as haloperidol (1 mg/kg) and clozapine (5 mg/kg), did not affect ($p > 0.05$) the % of damaged neuronal (pyramidal) cells by field when compared to ketamine-untreated group (Fig. 9 I).

3.9. *C. lutea* reverses neurodegeneration of the CA3 hippocampal subfield in ketamine-induced schizophrenia

As shown in Fig. 10 A, the neurons in the CA3 hippocampal area of the brain were severely condensed, shrunken, and had an irregular nuclear outline in the pyramidal layer of the ketamine-untreated group. However, the following exposures revealed little or no cellular damage or alterations: VEH-control, clozapine (5 mg/kg), and higher doses of *C. lutea* (400 and 800 mg/kg). Their pyramidal neurons were arranged in an orderly manner, with clear profiles and distinct nuclei and nucleoli, but mice treated with lower doses of *C. lutea* (100 and 200 mg/kg) and haloperidol (1 mg/kg) showed no difference, as there were also histoarchitectural distortions, neuronal vacuolization, and degeneration comparable to the ketamine-untreated group (Fig. 10 A–H). Correspondingly, the densitometric analysis revealed that ketamine-untreated mice had a higher ($p < 0.05$) % of damaged neuronal (pyramidal) cells per field [F (7, 16) = 10.73, $P < 0.0001$, $n = 3$ /group] than VEH-control mice. This degenerative effect shown in ketamine-treated mice was

reversed ($p < 0.05$) in mice administered with 400 and 800 mg/kg *C. lutea*. Clozapine (5 mg/kg) but not haloperidol (1 mg/kg), has a reversal effect ($p < 0.05$) like the higher doses of *C. lutea* (Fig. 10 I).

3.10. *C. lutea* improves histological alterations of the dentate gyrus in ketamine-induced schizophrenia

As shown in Fig. 11, the suprapyramidal blade of the dentate gyrus (DG) of the VEH-control mice had well-organized molecular, granular, and polymorphic layers, and the mature granular cells were tightly packed with little or no oedema fluid infiltration or vacuolization (black arrow) (Fig. 11 A). Meanwhile, mice treated with ketamine without reversal interventions show histoarchitectural disruption, as evidenced by the presence of pyknotic nuclei, shrinking cytoplasm, and hypocellularity of granular layer cells (red arrow) (Fig. 9 B). Ketamine-induced neuronal distortion, on the other hand, was reduced in mice given the highest doses of *C. lutea* (800 mg/kg) and clozapine (5 mg/kg) (Fig. 11 F and H). Treatment with lower doses of *C. lutea* (100, 200, and 400 mg/kg) and haloperidol (1 mg/kg) resulted in similar histoarchitectural disruption as the ketamine-treated group (Fig. 11 C–E and G). Furthermore, the morphometric analysis revealed an insignificant increase ($p > 0.05$) in the % of damaged neuronal (granular) cells per field [F (7, 16) = 0.9624, $P = 0.4895$, $n = 3$ /group] when comparing the ketamine-untreated and VEH-control groups. Also, when compared to the KET-untreated group, *C. lutea* (100, 200, 400, and 800 mg/kg), haloperidol (1 mg/kg), and clozapine (5 mg/kg) exposure showed no difference ($p > 0.05$) (Fig. 11 I).

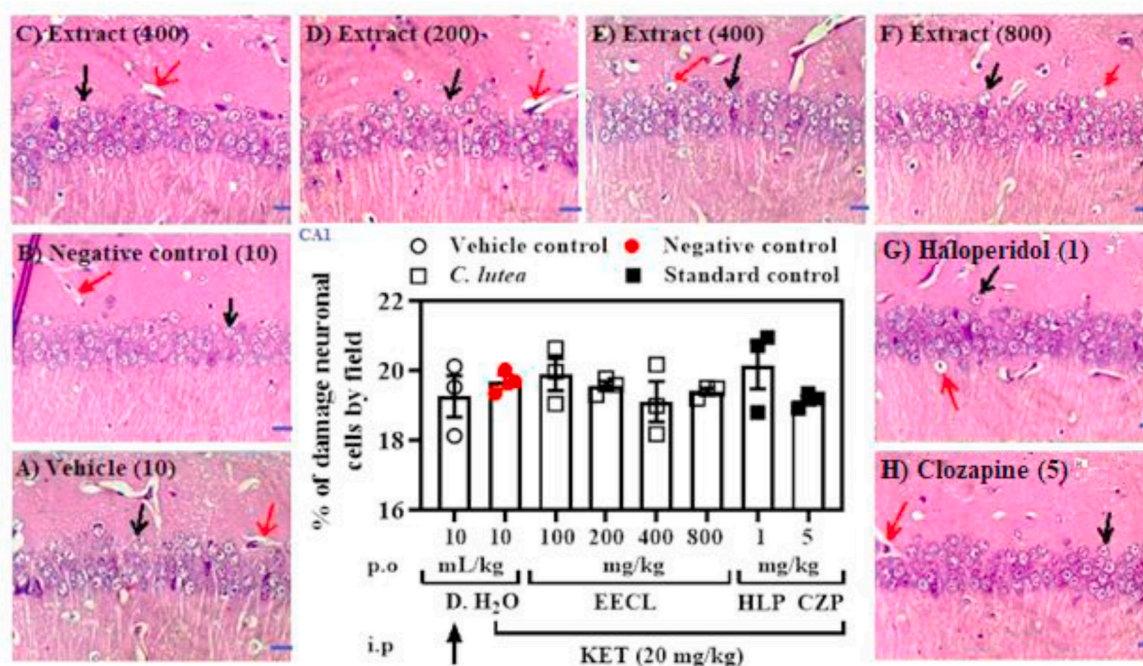


Fig. 9. Effects of *C. lutea* on ketamine-induced neuropathological changes (A–H) and neuronal degeneration (I) in the CA1 hippocampal region. Black arrow indicates normal neurons, and red arrow indicates degenerative neurons. The slides were observed by light microscopy (H and E staining $\times 400$, reference bar = 50 μ m). Bar represents the mean $\pm$ S.E.M (n = 3/group). *P < 0.05 as compared VEH-control group; #P < 0.05 as compared ketamine-treated group (One-way ANOVA followed by Tukey post-hoc test). D.H₂O/VEH = Vehicle, KET = Ketamine; EECL = Ethanol extract of *Capolobia lutea* = *C. lutea*; HLP = Haloperidol; CZP = Clozapine. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

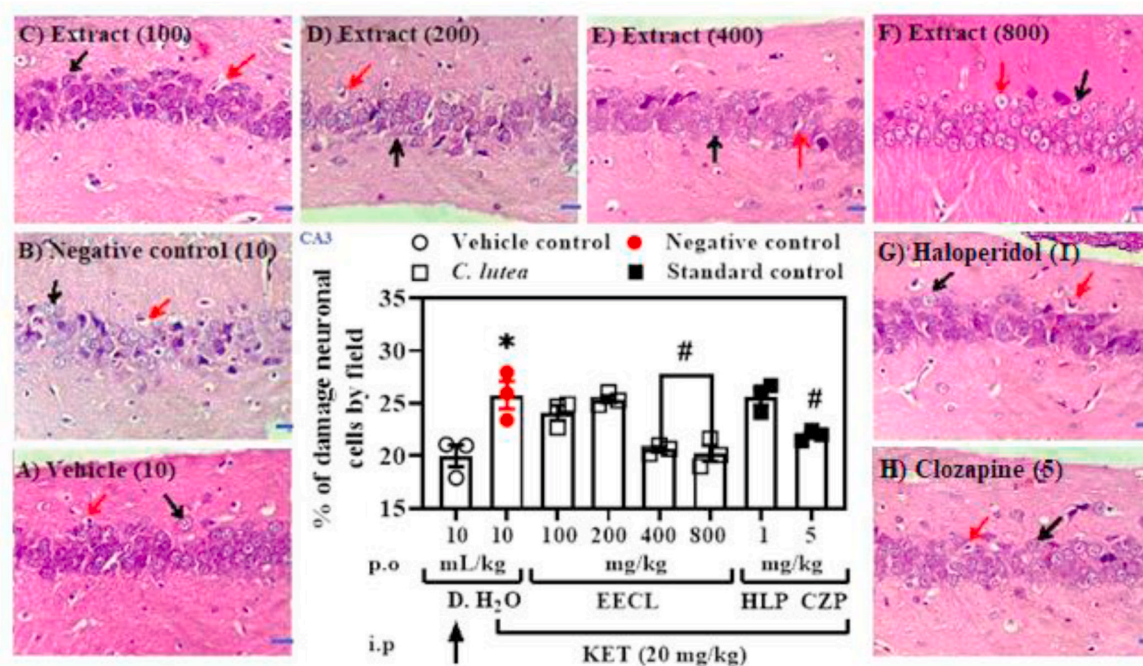


Fig. 10. Effects of *C. lutea* on ketamine-induced neuropathological changes (A–H) and neuronal degeneration (I) in the CA3 hippocampal region. Black arrow indicates normal neurons, and red arrow indicates degenerative neurons. The slides were observed by light microscopy (H and E staining $\times 400$, reference bar = 50 μ m). Bar represents the mean $\pm$ S.E.M (n = 3/group). *P < 0.05 as compared VEH-control group; #P < 0.05 as compared ketamine-untreated group (One-way ANOVA followed by Tukey post-hoc test). D.H₂O/VEH = Vehicle, KET = Ketamine; EECL = Ethanol extract of *Capolobia lutea* = *C. lutea*; HLP = Haloperidol; CZP = Clozapine. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

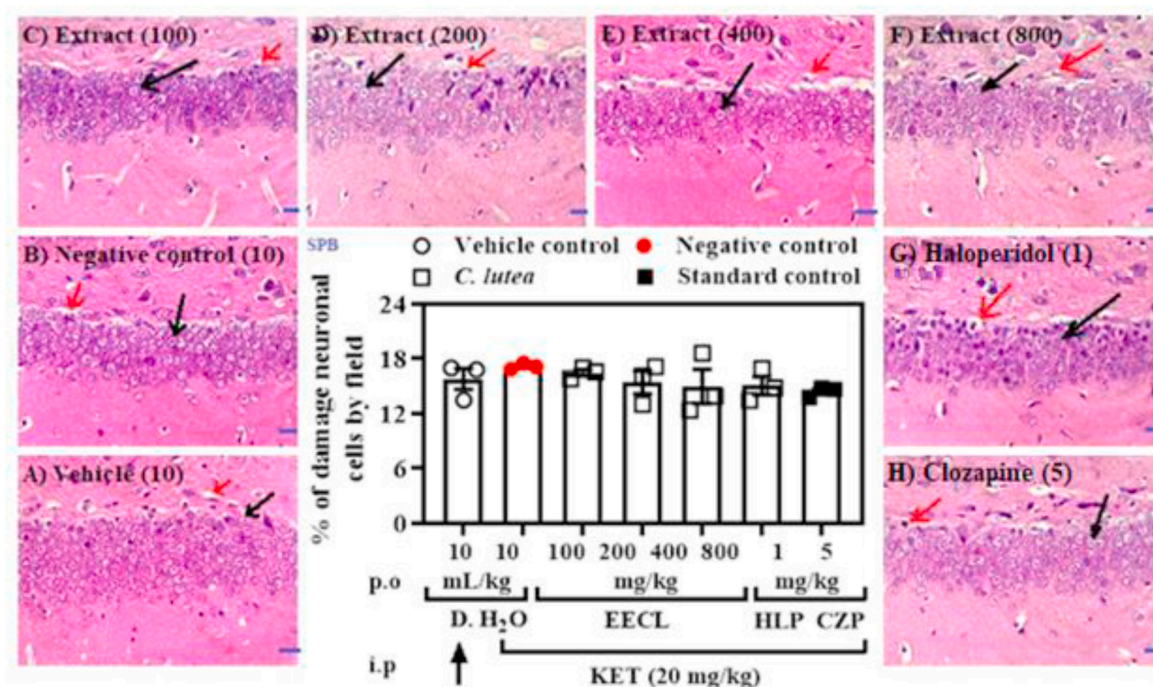


Fig. 11. Effects of *C. lutea* on ketamine-induced neuropathological changes (A–H) and neuronal degeneration (I) in the suprapyramidal blade of dentate gyrus (DG). Black arrow indicates normal neurons, and red arrow indicates degenerative neurons. The slides were observed by light microscopy (H and E staining $\times 400$, reference bar = 50 μ m). Bar represents the mean $\pm$ S.E.M (n = 3/group). *P < 0.05 as compared VEH-control group; #P < 0.05 as compared ketamine-untreated group (One-way ANOVA followed by Tukey post-hoc test). D.H₂O/VEH = Vehicle, KET = Ketamine; EECL = Ethanol extract of *Capolobia lutea* = *C. lutea*; HLP = Haloperidol; CZP = Clozapine. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

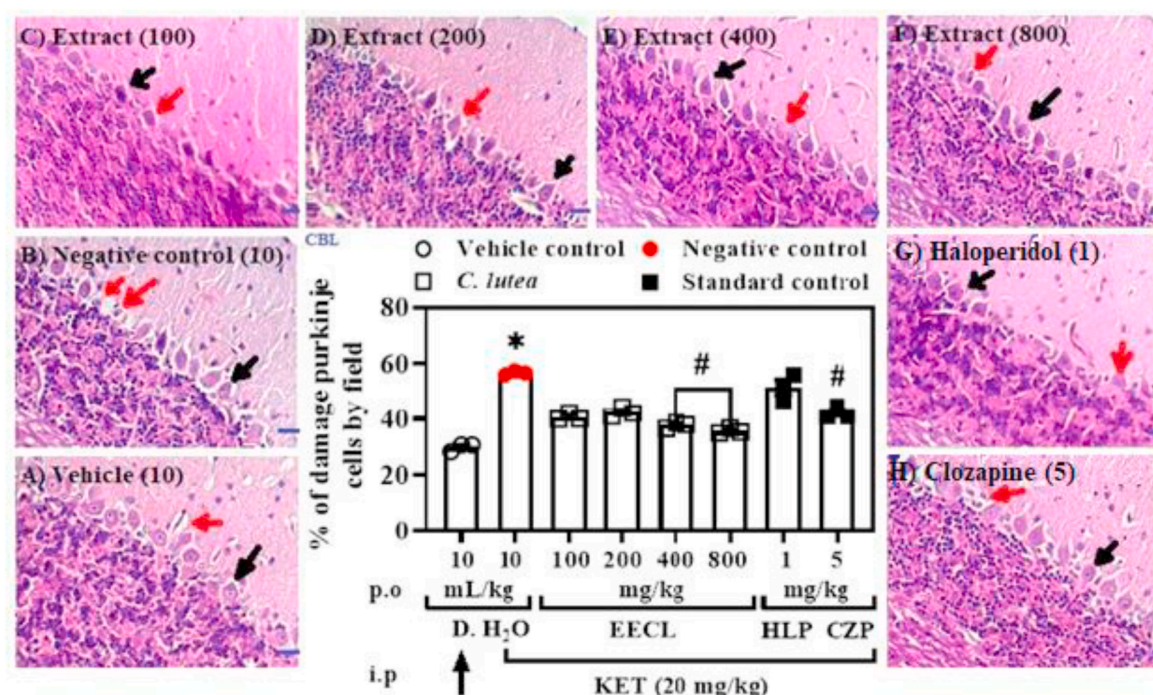


Fig. 12. Effects of *C. lutea* on ketamine-induced pathological changes (A–H) and neuronal degeneration (I) in the cerebellum. Black arrow indicates normal neurons, and red arrow indicates degenerative neurons. The slides were observed by light microscopy (H and E staining $\times 400$, reference bar = 50 μ m). Bar represents the mean $\pm$ S.E.M (n = 3/group). *P < 0.05 as compared VEH-control group; #P < 0.05 as compared ketamine-untreated group (One-way ANOVA followed by Tukey post-hoc test). D.H₂O/VEH = Vehicle, KET = Ketamine; EECL = Ethanol extract of *Capolobia lutea* = *C. lutea*; HLP = Haloperidol; CZP = Clozapine. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3.11. *C. lutea* attenuates histological alterations of the cerebellum in ketamine-induced schizophrenia

Fig. 12 shows areas of neurodegeneration in the cerebellum tissue of KET-treated mice, as demonstrated by Purkinje cells showing apoptosis with pyknotic nuclei, irregular dark cytoplasm, and wide interstitial spaces around the cells (red arrow). Furthermore, the overall histo-architectural settings of molecular, Purkinje, and granular layers are not well delineated (Fig. 12 B). In contrast, VEH-control mice and mice exposed to reversal treatment with graded doses of *C. lutea* (400 and 800 mg/kg), clozapine (5 mg/kg), but not haloperidol (1 mg/kg), showed little or no signs of degeneration (black arrow). Although, the degree of wide interstitial spaces around the cells observed in clozapine-treated mice was comparable to that observed in ketamine-untreated mice (Fig. 12 A and C–H). Regarding the morphometric analysis, the % of damaged neuronal (Purkinje) cells per field [$F(7, 16) = 48.59$, $P < 0.0001$, $n = 3/\text{group}$] was significantly higher in ketamine-untreated group than in VEH-control group, but this effect was reversed in groups treated with higher doses of *C. lutea* (400, 800 mg/kg), clozapine (5 mg/kg), but not haloperidol (1 mg/kg) (Fig. 12 I).

4. Discussion

The current study employed ketamine model of schizophrenia to investigate *C. lutea*'s neuroprotective potential and antipsychotic mechanisms of action, as preliminary evidence of its antipsychotic activity has previously been reported (Omeiza et al., 2022). In conformity with previous studies, mice intoxicated with subanesthetic repeated doses of ketamine for 14 days developed schizophrenia-like symptoms, derangement in neurochemicals, as well as disruption in the histological architecture, and neuronal settings of the brain regions (Monte et al., 2013; Vasconcelos et al., 2015; Oshodi et al., 2021). *C. lutea*, on the other hand, reverses ketamine-evoked positive, negative, and cognitive symptoms of schizophrenia with corrected neurotransmitters (Glu, DA, and NE, neglecting 5-HT) and metabolizing enzymes (MAO and AChE) well known to be implicated in the pathogenesis of schizophrenia. *C. lutea* also attenuates ketamine-induced oxido-inflammatory deregulations and restores morphological and morphometric alterations in the prefrontal cortex, hippocampal proper, dentate gyrus, and cerebellum. Above all, this study provides background data that confirms the ethnomedicinal significance of *C. lutea* as a phytotherapeutic agent against schizophrenia-like symptoms in animal models, vis-à-vis, the possible mechanisms of action based on our previous study (Omeiza et al., 2022).

The link between NMDA receptor (NMDAR) inhibition and schizophrenia pathobiology is well established (Jorratt et al., 2021). Ketamine is an NMDA receptor blocker that has been shown in rodents to cause behavioral phenotypes (Chatterjee et al., 2012; Monte et al., 2013; Ben-Azu et al., 2018a) similar to schizophrenia-like symptoms in humans (Krystal et al., 1994). Ketamine inhibits glutamatergic neurotransmission by non-competitively binding to NMDA receptor components of the glutamatergic synapse (Coyle, 2004). This hypofunction of the NMDA receptor in various parts of the brain (e.g. ventral tegmentum, striatum, prefrontal cortex, and hippocampus) indirectly causes upregulation and downregulation of dopamine release in the mesolimbic and mesocortical circuits, respectively, to induce positive, negative, and cognitive symptoms of SCZ (Chatterjee et al., 2012; Monte et al., 2013; Ben-Azu et al., 2018a, 2018b, 2018c; Oshodi et al., 2021). Thus, repeated ketamine administration was used in this study to investigate *C. lutea*'s antipsychotic-like activity on not only behavioral phenotypes but also neuropathological sequelae in schizophrenia. Furthermore, male Swiss mice were chosen as experimental units in this study to eliminate potential confounding factors, as cyclical changes in female sex hormones easily alter rodent neurobehaviors (Gioiosa et al., 2007), putting aside the fact that the incidence of schizophrenia is 1.4 times higher in males than females (McGrath et al., 2008).

According to previous reports (Chatterjee et al., 2012; Monte et al., 2013), our current findings demonstrated that ketamine exposure resulted in multiple SCZ-like symptoms in mice, as the Any-maze® tracking system revealed an increase in motor performance and behavioral despair, which predict the positive and negative symptoms of the disease in the open field and force swimming paradigms, respectively. This enhanced motor performance induced by ketamine is attributable to NMDAR hypofunction in the glutamatergic pathway, which in turn activates dopamine D2 receptors in the striatal regions of the mouse brain, resulting in hyperactivity or hyperlocomotion (Nakao et al., 2019). Similarly, increased behavioral despair has also been linked to an alteration in the serotonergic system in which ketamine has been found to interact, and thus induce the negative and cognitive symptoms specifically via upregulation of serotonin (5-HT_{2A}) receptors and elevated levels of serotonin in the cortex and hippocampal regions of the brain (Becker et al., 2003; Chindo et al., 2012; Ben-Azu et al., 2018a). Furthermore, as observed in this study, ketamine impairs cognitive function in the MWM, as mice exposed to ketamine without reversal treatment as well as haloperidol-treated animals learned less in the course of the acquisition trial. Correspondingly, the path length efficiency to the escape platform and cognitive score on the last day of the MWM acquisition trial of these two groups declined, indicating a learning deficit. During the MWM probe trial, the path length in the target zone, average proximity to the target zone, and annulus time were altered from normalcy in the ketamine-untreated and haloperidol-treated groups. These parameters were used to assess the impairment of spatial working memory and retentive (explicit) memory (Omeiza et al., 2021). Ketamine repeated exposure also reduced the discriminative index in NORT, a non-spatial learning and memory parameter that defines mice's ability to recognize novel objects (Rajagopal et al., 2014). Ketamine and haloperidol, when combined, altered the brain system's consolidation, potentiation, and recall processes. These findings support previous research that found NMDA receptors to be pertinent in cognition processes (Chatterjee et al., 2011; Lopes-Aguiar et al., 2020). As a result, any discrepancy in the physiology of NMDAR, which is ubiquitous in the hippocampus and prefrontal cortex, may result in schizophrenia-like cognitive symptoms. However, the administration of *C. lutea* reversed the effect of ketamine on the neurobehavioral alternations as indicated by a significant reduction in locomotor activity and immobility, as well as improvement in cognitive performance. These data are consistent with preliminary evidence from our previous research on the acute effect of *C. lutea*, as well as published preclinical reports on the use of medicinal plants as a source of biomolecules with phytotherapeutic value against various behavioral patterns associated with experimentally-induced schizophrenia in animals (Yadav et al., 2018a). It is also worthy of note that our findings on the inability of haloperidol to reverse ketamine-induced learning and memory impairments in the MWM and NORT are consistent with previous studies (Antonio, et al., 2016; Ben-Azu et al., 2018a, 2018b).

Worthy to note in this study is that the reversal effect of *C. lutea* on the positive symptoms of schizophrenia (Fajemiroye et al., 2016; Omeiza et al., 2022) are comparable to the available antipsychotic reference drugs haloperidol and clozapine in a dose-dependent manner. In addition, *C. lutea* treatment may alleviate negative symptoms in a way similar to clozapine but not haloperidol. The negative symptoms have been identified as a core feature of schizophrenia, with a distinct psychopathological dimension from other schizophrenia symptoms (Pelizza et al., 2020). Although most antipsychotics primarily target DA D2 receptors to reverse the positive symptoms, clozapine, but not haloperidol in part, was able to restore ketamine-enhanced negative symptoms because it has the affinity to block the 5-HT_{2A} receptors, which is well-known to be implicated in the molecular basis of the pathobiology of the negative symptoms of schizophrenia (Steward et al., 2004). Regarding the cognitive symptoms, higher doses of *C. lutea* (400 and 800 mg/kg) and clozapine but not haloperidol mitigate the cognitive decline induced by ketamine exposure. These results are in line with

observations by Ben-azu et al., (2018a, 2018b, 2018c, 2018d) and Omeiza et al. (2021), who reported that morin and melatonin supplementations reverse cognitive impairment in ketamine and lead models of hippocampus-dependent spatial and non-spatial learning studies, respectively.

To confirm the probable mechanism(s) of *C. lutea*'s anti-antipsychotic-like activity, we explored the cellular and molecular biomarkers well-studied in SCZ *in-vivo* and *in-vitro* models. Generally, repeated exposure to ketamine modulates the physiology of the various neurotransmitters and their neuronal networks by inhibiting glutamatergic NMDAR in GABAergic interneurons (Carlsson et al., 2001; Cohen et al., 2015). The simultaneous effect of NMDAR inhibition on all of the different neurotransmitter networks is notably directly associated with the illness's complexity and myriads of schizophrenia symptoms (Ben-Azu et al., 2018a, 2018b). In the current study, repeated ketamine exposure reduced glutamate levels in the whole brain, which could be attributed to several cascade pathways such as: decreased glutamate uptake at postsynaptic neuronal membranes, altered astrocytic glutamate transporter system-excitatory amino acids transporters (EAATs), insensitivity or underexpression of glutamate receptor molecules, and enhanced glutamate conversion to alpha (α)-ketoglutarate via glutamate dehydrogenase activity (Marmioli and Cavaletti, 2012). Ketamine-induced schizophrenia is linked to decreased glutamate levels in the brain, thereby resulting in hypostimulation of glutamate receptor subunits (particularly NMDAR), as well as depletion of GABAergic ensembles in the limbic and subcortical brain regions (Lewis and González-Burgos, 2008; Lisek et al., 2017; Ben-Azu et al., 2018b). This observation confirms the clinical studies showing that the extent of glutamate uptake by astrocytes or disruption of cortical and subcortical glutamatergic neurotransmissions determine the severity of schizophrenia (Khalaf et al., 2012; McCutcheon et al., 2020). Indeed, it has been suggested that an increased glutamate uptake by astrocytes may considerably contribute to its purported glutamatergic hypofunction (Herrmann et al., 2012; Lisek et al., 2017). Meanwhile, one important finding from this study is that *C. lutea* but not the reference antipsychotics (haloperidol and clozapine) increased cellular glutamate availability and thus alleviated ketamine-induced schizophrenia-like symptoms in mice via upregulation of the glutamatergic pathway. Although the abnormal secretion of glutamate does not always translate to improved glutamatergic neurotransmission, an excessive amount of this neurotransmitter in the synaptic cleft could be efficiently taken up by EAATs, which is an important clearance mechanism of glutamate from the synapse (Lisek et al., 2017). Previous studies have reported that ketamine-induced psychosis is thus associated with elevated expression of mesolimbic EAAT, suggesting its ability to induce NMDA receptor hypofunctionality (Lisek et al., 2017). However, in this study, the ability of *C. lutea* to improve ketamine-induced schizophrenia-like behavior could suggest a conformational configuration of glutamatergic receptors subunits including NMDA receptors. Of note, more studies on receptor expression are encouraged to elucidate the mechanism by which *C. lutea* modulate glutamatergic neurotransmission.

Other than the deregulated glutamatergic/GABAergic pathways caused by ketamine's inhibition of NMDAR via a cascade of downstream changes in neuronal activity, calcium entry, and epigenetic machinery, it also includes catecholamine (DA and NE), serotonin, and cholinergic dysfunctions (Carlsson et al., 2001; Coyle, 2004; Ghosh et al., 2011). In particular, NMDAR inhibition has been linked to increased sensitivity and density of dopamine D2 receptors, as evidenced by an increase in DA levels in the limbic striatal regions (Iasevoli et al., 2014; Chatterjee et al., 2012). Consequently, increased DA levels lead to increased norepinephrine biosynthesis as a precursor (Fryar-Williams and Strobel, 2015), and excessive levels of NE have been strongly associated with stress-induced hyperactivity or positive symptoms of schizophrenia via inhibition of the striatal α 1-adrenergic receptors (Maletic et al., 2017). Repeated ketamine exposure also had the effect of sparing monoamine oxidase (Chatterjee et al., 2015), which is a major catecholamine

catabolizing enzyme (Edmondson and Binda, 2018) in the nervous system. This is consistent with our current findings, which show that ketamine increases DA and NE levels without main effect on MAO activity. These findings partly explain the positive symptoms but are also linked to the negative and cognitive symptoms of schizophrenia (Chatterjee et al., 2012; Venkataramaiah et al., 2021). Notwithstanding, *C. lutea* administration reversed the catecholamine hyperfunction observed in ketamine-exposed mice, as evidenced by lower DA and NE concentrations in the brain. This reversal effect of *C. lutea* is similar to that of the reference antipsychotics (haloperidol and clozapine), implying that *C. lutea* may elicit an hypocatecholaminergic effect by manipulating the DA D2 receptor, which is still the same receptor that available antipsychotics block to alleviate the positive symptoms of schizophrenia. So, is it possible that *C. lutea* could also exert its antipsychotic-like activity via adrenergic receptors in the brain, although more studies are needed to clarify this assertion?

Regarding the involvement of serotonin in schizophrenia, it was previously explored that glutamatergic neurons in the cerebral cortex had hyperfunctioning of 5-HT2A receptors, resulting in an excess of serotonin or 5-HT2A receptors overexpression. This has been identified as the underpinning mechanism associated with the negative and cognitive symptoms of schizophrenia (Chatterjee et al., 2012). In this study, neither ketamine nor the reversal treatments influenced serotonin concentrations in the brain, implying that the antipsychotic-like effects of *C. lutea* and clozapine on negative systems may be mediated by mechanisms other than the serotonergic pathway. Now, the question is whether ketamine induces negative symptoms through another pathway, and if so, which pathway does *C. lutea* utilize to reverse ketamine-induced immobility time, which is an index of negative symptoms. Indeed, our finding contradicts the outcome of Chatterjee et al. (2012), which showed that after repeated ketamine treatment, serotonin levels in the hippocampus remained unchanged while increasing in the cortex and striatum. This disparity could be explained by the fact that Chatterjee et al. (2012) measured serotonin contents in different regions of the brain while our present study focused on the entire brain as a pharmacological compartment. This serves as one of the limitations of our study given that the concentrations and functionality of these neurotransmitters vary in different regions of the brain (Ben-Azu et al., 2018a). Furthermore, suppression in acetylcholine levels in the cortex and the hippocampus have also been linked to cognitive symptoms of schizophrenia (Zugno et al., 2015). In this study, repeated ketamine intoxication notably increased the activity of AChE, an enzyme that hydrolyzes acetylcholine. This finding is consistent with previous studies which showed that repeated intraperitoneal injection of ketamine causes elevation of whole-brain AChE activity (Ben-Azu et al., 2018c). The cholinergic system derangement caused by repeated ketamine exposure, on the other hand, was reversed by higher doses of *C. lutea* with a greater effect than the atypical reference antipsychotic clozapine, implying that *C. lutea* and clozapine may mediate their neuroprotective and antipsychotic-like actions against cognitive symptoms via regulation of the cholinergic pathway in the brain (Chatterjee et al., 2011; Ben-Azu et al., 2018a, 2018c). These findings suggest the possibility of new pharmacological interventions for the treatment of schizophrenia's cognitive symptoms.

Aside from neurotransmitter homeostatic dysfunction in the brain, persistent oxidative-nitrosative stress and inflammation have also been implicated in the etio-pathomechanism of schizophrenia (Monte et al., 2013; Ben-Azu et al., 2018a, 2019; Rossetti et al., 2020). Oxidative stress is a metabolic condition in which the body releases more free radicals than it has anti-oxidant status, thereby resulting in a state of redox imbalance (Olayaki et al., 2018). This deleterious condition impairs normal cellular metabolism, particularly in lipid-rich tissues or organs that also demand more oxygen, especially the brain (Abdulrahim et al., 2021). Thus, the brain's vulnerability to oxidative insult has resulted in several neurological illnesses, including schizophrenia (Monte et al., 2013). In the current study, repeated ketamine intoxication elevated

lipid peroxidation and reduced superoxide dismutase however without affecting the level of reduced glutathione, indicating the presence of oxidative stress. This is consistent with earlier research that supports oxidative damage in ketamine-induced schizophrenia-like symptoms (Ben-Azu et al., 2016; Chaves et al., 2020). Moreover, one of the principal causes of cell damage in aerobic organisms exposed to oxidative stress has been identified as lipid peroxidation of biological membranes (Birben et al., 2012). It also causes alteration in membrane fluidity, which can lead to the inactivation of membrane-bound receptors or enzymes (Ayala et al., 2014). Consequently, elevated lipid peroxidation and a reduction in superoxide dismutase, a detoxifying non-enzymatic antioxidant agent on the other hand might disrupt neurotransmission, which has been shown in this study to contribute to schizophrenia-like behavior. Also, an insignificant increase in nitrite was observed in ketamine-untreated mice. Indeed, it has been previously reported that ketamine-induced oxidative stress has been associated with fluctuations in nitrite levels, a marker for nitroergic stress, which has been implicated in the abnormal synaptic plasticity largely reported in schizophrenic brains (Sampaio et al., 2018). Another major culprit in this mental ailment is inflammation, which is a complex response to brain cellular attacks that can be triggered by the mere existence of oxidative–nitrosative stress and vice versa (Arora and Chopra, 2013). To bring about repair, inflammation plays a crucial role in the CNS's innate immunity (Ransohoff and Brown, 2012). But, in chronic neurological states, inflammation becomes persistent, resulting in neuronal cell damage, and this phenomenon has also been linked to schizophrenia, as evidenced by previous studies on ketamine's capabilities to potentiate LPS-induced inflammation (Ben-Azu et al., 2019; Réus et al., 2017). In this study, the increased involvement of IL-6 is believed to negatively impact the survival of brain neurons, and neurotransmitter homeostasis is commonly implicated in the pathogenesis of schizophrenia (Gilmore et al., 2004). Conversely, reversal treatment with *C. lutea* rather than reference antipsychotics (haloperidol and clozapine) negated ketamine's effect on the redox and inflammatory systems, indicating that *C. lutea* has antioxidative and anti-inflammatory activity. This study supports the findings of Abiodun and Oshinloye (2017), who found that *C. lutea* prevented the depletion of colonic antioxidants and anti-inflammatory biomarkers in murine models. Relevantly, these thus strengthen the previous research that revealed that *C. lutea* contains bioactive compounds used ethnomedicinally to ameliorate oxido-inflammatory pathologies (Nwidi et al., 2016).

The histopathological findings in this study complement the behavioral and biochemical findings because the data revealed distortions in brain regions that have previously been associated with schizophrenia (Ben-Azu et al., 2018c). In lines with the previous reports, repeated ketamine intoxication disrupted histoarchitectural settings of the brain regions, as increased damage was found in the neurons of the granular layer of the PFC and DG, as well as the pyramidal layer of the hippocampal CA3 area (Zou et al., 2009; Ben-Azu et al., 2018c; Yadav et al., 2018a,b; Ben-Azu et al., 2019). By implication, the schizophrenia-like core symptoms earlier reported may be associated with the compromises in the structural and functional integrity of the prefrontal cortex and hippocampus (Ben-Azu et al., 2018a, 2018b, 2018c). It is known that the prefrontal cortex and hippocampus play a critical role in the cognitive and other behavioral brain functions, and numerous observations suggest that any alteration of these regions could trigger the progression of schizophrenia (He et al., 2009). Aside from the fact that the NMDARs are more densely expressed in these brain regions, the prefrontal cortex and hippocampus (comprising of Cornu Ammonis—CA1 through CA4, dentate gyrus, subiculum, and the entorhinal cortex) are highly susceptible to oxidative invasion (Arnsten, 2009; Xu et al., 2019; Omeiza et al., 2021). Accordingly, repeated ketamine brain

insult has heretofore been noted in this study to induce lipid peroxidation and elevated cytokine production (Yadav et al., 2017; Ben-Azu et al., 2019; Murray et al., 2021). Furthermore, because the hippocampus has extensive connections with the cerebral cortex, hypothalamus, and amygdala, among other regions, damage to the hippocampus has been found to alter the function of other regions of the brain (Anand and Dhikav, 2012). Thus, the complexity of negative and cognitive symptoms of SCZ may be tied to the vulnerability of the hippocampal brain regions (Meyer-Lindenberg et al., 2005; Tregellas et al., 2014; Ghoshal and Conn, 2015). Also, repeated ketamine administration is known to cause neurodegeneration in the cerebellum (Onaolapo et al., 2019). This is as demonstrated by Purkinje cells presenting apoptosis with pyknotic nuclei and irregular dark cytoplasm, as well as an increase in the percentage of damaged neuronal cells by field. However, *C. lutea*'s ability to overturn ketamine-induced neuronal degeneration in this study indicates that it has therapeutic potential on cerebellum motor and cognitive functions, which is consistent with that of Khalaf et al. (2012) and Shuvaev et al. (2021). Of note, the histological findings of this study show that reversal treatment with *C. lutea* reduced ketamine-induced neuronal degeneration, especially in animals given higher doses. *C. lutea*'s neuroprotective activity adds to previous evidence that plants with bioactive compounds like flavonoids and phenols, which *C. lutea* also has, may have neuroprotective properties against ketamine-induced neurotoxicity (Jakkala and Ali, 2015; Ben-Azu et al., 2016). Interestingly, we found that the effectiveness of *C. lutea* as an intervention against ketamine-induced morphological changes was greater than that of the reference antipsychotics, particularly haloperidol. Previously, haloperidol has been reported to exacerbate the neurodegenerative effect following ketamine administration on the CA3 and DG subregions of the hippocampus (Valvassori et al., 2021). So, the existence of several bioactive compounds in plants, each of which has been shown to elicit pharmacological effects (Pandy and VijeePallam, 2017; Yadav et al., 2018a; 2018b; Omeiza et al., 2022), may be at the core of *C. lutea*'s multiple actions in the reported brain regions and against schizophrenia-like symptoms.

Of note, some limitations of this study include the lack of histological investigation of the striatal brain region, BDNF immunoassays, and the use of molecular techniques such as Western blot which could help in the receptors elucidation and further antipsychotic mechanisms of action of *C. lutea*. However, as a build up to our previous study (Omeiza et al., 2022), the present findings further strengthen the antipsychotic potential of *C. lutea*. Also, the current findings further support the earlier reports of ketamine-induced schizophrenia involving blockade of NMDAR, thereby causing dysregulation of multiple neurotransmitters (e.g., DA, Glu, 5-HT, NE, and ACh) and oxido-inflammatory systems in the brain, as well as disrupting histological architecture in experiment rodents. Our findings however showed that *C. lutea* reverses the cardinal symptoms of schizophrenia in ketamine-treated mice through rescuing these implicated systems. Notably, the *C. lutea* extract previously reported to contain phytonutrients in this study elicits multitarget effects comparable to atypical antipsychotics with a wider spectrum of effects (Fig. 13). Moreover, in the context of safety profile, we believe that *C. lutea* is known to be relatively safe based toxicological investigations (Manfo et al., 2011; Nwidi et al., 2016) unlike some currently used antipsychotic agent like haloperidol (Ben-Azu et al., 2018d, 2019). Given this background, *C. lutea* could be a source of promising molecules or lead compounds against the treatment of schizophrenia-like features, particularly negative and cognitive symptoms. More basic as well as clinical investigations into the specific bioactive constituents of the extract with antipsychotic-like effect is therefore recommended for further studies.

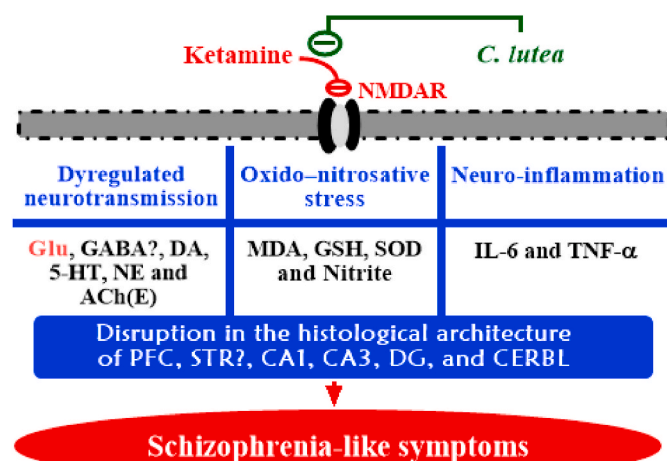


Fig. 13. Graphical abstract illustrating the mechanistic basis by which *C. lutea* wages war against ketamine-induced schizophrenia-like symptoms.

Consent for publication

This is not applicable.

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Noah A. Omeiza: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Resources, Software, Visualization, Writing – original draft, Writing – review & editing. **Adewale Bakre:** Supervision. **Benneth Ben-Azu:** Formal analysis, Methodology, Writing – review & editing. **Abimbola A. Sowunmi:** Resources, Data curation. **Halimat A. Abdulrahim:** Resources, Data curation, Formal analysis. **Joseph Chimezie:** Resources. **Sodiq O. Lawal:** Resources. **Olusegun G. Adebayo:** Resources, Formal analysis. **Abdullateef I. Alagbonsi:** Resources, Writing – review & editing. **Olugbenga Akinola:** Writing – review & editing. **Amos O. Abolaji:** Supervision, Data curation. **Adegbuyi O. Aderibigbe:** Supervision, Conceptualization, Methodology, Writing – review & editing.

Declaration of competing interest

The authors declare no conflict of interest.

Data availability

Data will be made available on request.

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