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Pretreatment with *Carpolobia lutea* ethanol extract prevents schizophrenia-like behavior in mice models of psychosis

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ABSTRACT

Ethnopharmacological relevance: Carpolobia lutea decoction is widely used as a phytotherapeutic against central nervous system-related disorders including insomnia, migraine headache, and mental illness in West and Central Tropical Africa.

Aim: This study was designed to investigate the antipsychotic activity of *Carpolobia lutea* (EECL) in mice models of psychosis.

Methods: Male Swiss mice (n = 5/group) were given EECL (100, 200, 400, and 800 mg/kg), haloperidol (1 mg/kg), clozapine (5 mg/kg) and vehicle (10 mL/kg) orally before amphetamine (5 mg/kg)-induced hyperlocomotion and stereotypy, apomorphine (2 mg/kg)-induced stereotypy, or ketamine (10, 30, and 100 mg/kg)-induced hyperlocomotion, enhancement of immobility and cognitive impairment.

Results: EECL (200, 400, and 800 mg/kg) prevented amphetamine- and apomorphine-induced stereotypies, as well as reduced hyperlocomotion induced by amphetamine and ketamine, all of which are predictors of positive symptoms. Regardless of the dose administered, EECL prevented the index of negative symptoms induced by ketamine. Furthermore, higher doses of EECL (400 and 800 mg/kg) also prevented ketamine-induced cognitive impairment, a behavioral phenotype of cognitive symptoms.

Conclusion: Pretreatment with EECL demonstrated antipsychotic activity in mice, preventing amphetamine-, apomorphine-, and ketamine-induced schizophrenia-like symptoms, with 800 mg/kg being the most effective dose.

1. Introduction

Schizophrenia is a neuropsychiatric disorder marked by positive symptoms (e.g., hallucination, delusion, and thought disorder), negative symptoms (e.g., asociality, anhedonia, and amotivation), and cognitive impairment (e.g., loss of learning and memory functions) (Foussias and Remington, 2010). It has affected approximately 21 million individuals globally, with a prevalence rate of 7.2 per 1000 people, and usually develops between the ages of 15 and 44, although with more prevalence in male (Haddad and Correll, 2018). Remarkably, schizophrenia has been named among one of the top eight leading causes of disability worldwide, making it the most burdensome and costly disease globally (Rössler et al., 2005).

Schizophrenia's etiology has been associated with numerous of factors, including genetic, biological, and environmental factors (Stilo and Murray, 2019; Zwicker et al., 2018; Howes et al., 2017). A growing body of evidence suggest that psychosocial stressors (Ben-Azu et al., 2020) or the misuse of psychotomimetic drugs including alcohol, tobacco, cannabis, cocaine, amphetamine, and ketamine are strongly linked to schizophrenia following alterations of neurochemical homeostasis (Murray et al., 2013; Bonomo and Proimos, 2005; Nordahl et al., 2003). Different neurochemical hypotheses such as dopamine, serotonin, and glutamate have long been known to play important roles in neurobehavioral activities like locomotion, cognition, motivation, arousal, and reward, among others (Uddin et al., 2017; Arain et al., 2013). Disruptions of these neurochemicals by psycho-stimulants are often used to screen drugs for potential antipsychotic effects (Ben-Azu et al., 2018a; 2018b; 2018c; 2018d). In rodents, amphetamines and

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apomorphines stimulate dopamine release and activate the dopamine receptor, thereby inducing schizophrenia-like positive symptoms. Notably, antipsychotic drugs are known to inhibit dopamine receptors (Rampino et al., 2019). Furthermore, ketamine, a non-competitive glutamate antagonist, mimics schizophrenia symptoms at varying doses and durations of induction (Chatterjee et al., 2015; Monte et al., 2013; Chatterjee et al., 2012a,b). Remarkably, ketamine also induces behavioral despair as well as cognitive impairment, both of which are predictors of negative and cognitive symptoms (Vijeepallam et al., 2016; Chatterjee et al., 2011; van den, 2010; Ellenbroek and Cools, 2002).

The current drugs used to treat this illness are classified as typical and atypical antipsychotics based on their mechanisms of action (Preskorn and Germann, 2019), but some of these pharmacotherapies are disadvantaged either due to undesirable side effects or because of the declining effectiveness after chronic clinical usage. Furthermore, these drugs particularly typical antipsychotics mostly ameliorate the positive symptoms; however, the negative and cognitive symptoms remain unabated in schizophrenic patients (Iversen et al., 2018; Barbato, 1998), therefore, necessitating the need for continuous search for better therapeutic agents with limited adverse effects.

Carpolobia lutea G. Don (*C. lutea;* family: Polygalaceae), also widely recognized as cattle stick, is an evergreen plant native to West and Central Tropical Africa (Nwidu et al., 2015). It is found in Guinea savannahs and rainforests of Nigeria, Cameroon, and Sierra Leone (Nwidu et al., 2015). Traditionally, *C. lutea* leaves, roots, and stem extracts have been identified as a multitasking herbal medicine used to treat various ailments including headache, sanity, sterility, malaria, rheumatoid pain, wounds (Yakubu and Jimoh, 2014; Nwidu et al., 2012a) as well as widely consumed as nutraceutical or food (Lykke and Padonou, 2019; N'guessan-Koffi et al., 2015). For instance, the dried bark and leaf of this plant are prepared as snuff and concoction by the Ibibio and Efik tribes in Nigeria to treat migraine headaches (Nwidu et al., 2015) and mental episodes (Mitaine-Offer et al., 2002; Burkill, 1995), respectively. Additionally, previous reports on the pharmacologic screening revealed that *C. lutea* possesses antinociceptive (Nwidu et al., 2016), antioxidant, anti-inflammatory (Abiodun and Oshinloye, 2017), sedative (Beshel et al., 2019), aphrodisiac (Yakubu and Jimoh, 2014), antidiarrheal (Nwidu et al., 2011), antimicrobial (Nwidu et al., 2012b), and neuroprotective properties (Ajiwhen and Bisong, 2013). Recently, Beshel et al. (2019) confirmed the sedative property of *C. lutea* in naïve Swiss white mice based on ethnomedicinal claims. However, until now, its sedative effect on behavioral hyperactivity and psychotic stereotypies which are obviously amplified in psychotic patients has not been investigated using schizophrenia disease models. Thus, the present study was designed to evaluate the antipsychotic-like activity of ethanol extract of *Carpolobia lutea* in animal models predictive of human psychosis.

2. Material and methods

2.1. Animals

Male Swiss mice (average weight: 20 ± 2 g) were obtained from the University of Ibadan's Central Research Animal Holding Facility. The mice were housed in polystyrene cages under standard housing conditions (light/dark cycle: 12 h, temperature: 25 ± 2 °C and relative humidity: 60 ± 5%), fed a standard rodent diet, and given fresh water *ad libitum*. They were acclimatized in the laboratory for 10 days prior to the commencement of experimental protocol. Behavioral assessments in the open field test (OFT), force swimming test (FST), and Y-maze test were used in this study to measure the parameters of positive, negative, and cognitive schizophrenia-like symptoms (Ben-Azu et al., 2016a, 2016b), respectively, between 8.00 a.m. and 12.00 p.m. in a quiet, temperature-controlled (25 ± 2 °C) room. All experiment protocols were carried out following the criteria outlined in the University of Ibadan's Ethical Research Committee in Ibadan, Oyo State, Nigeria with the reference code: UI-ACUREC/049–0521/26.

2.2. Plant material and extraction

Fresh *Carpolobia lutea* plants (leaves) were harvested, identified, and authenticated by Mr Egunjobi, Ayodele James at the Taxonomy unit of the Forestry Research Institute of Nigeria (FRIN) in Ibadan with the herbarium number: FHI 112961. The leaves were rinsed with tap water and air-dried for 21 days under the shed until they reached a constant weight of 3.95 kg. Following that, dried leaves were pulverized into a coarse powder (0.45 kg) using a miller, macerated in 4.5 L of 70% (v/v) ethanol for 72 h at room temperature (25 ± 2 °C), and the mixture was shaken intermittently. The mixture was filtered through absorbent cotton and No. 1 Whatman's paper, and the filtrate was concentrated to dryness in a rotary evaporator, yielding a dark green residue or crude ethanol extract of *Carpolobia lutea* (EECL; 45 g) at 40 °C temperature and low pressure. The dried or crude extract was then refrigerated (4 °C) until it was used. A portion of the EECL was used for phytochemical screening, while the remaining portion was used for an *in-vivo* study. EECL was dissolved in distilled water before administration.

2.3. Phytochemical analysis

2.3.1. Qualitative phytochemistry of EECL

The EECL was screened for secondary metabolites such as alkaloids, flavonoids, tannins, saponins, triterpenoids, cardiac glycosides, and steroids using the standard protocols as previously described (Lima et al., 2021; Gul et al., 2017).

2.3.2. Phytochemical fingerprinting of the high performance liquid chromatography (HPLC) analysis of EECL

HPLC analysis was performed using Shimadzu Ultra-Fast liquid chromatography (Shimadzu Corporation, Kyoto, Japan) to determine the preliminary phytochemical fingerprinting of EECL according to the previous procedure established in our laboratory using gallic acid, p-coumaric acid, and rutin as reference compounds to identify related compounds (Ariyo et al., 2020; Adeoluwa et al., 2019; Ajayi et al., 2017).

2.4. Acute toxicity test

To determine the mean lethal dose (LD_{50}) of EECL, a method previously described by Lorke (1983) was employed and the LD_{50} was computed as the geometric mean of the lowest dose that led to death and the highest dose that resulted in no death (Fig. 1).

2.5. Drug preparation and treatments

Amphetamine (AMP; 5 mg/kg), apomorphine (APO; 2 mg/kg), haloperidol (HLP; 1 mg/kg), clozapine (CZP; 5 mg/kg) (Sigma-Aldrich, St. Louis, USA) and ketamine (KET; 10, 30, and 100 mg/kg) (KET; Nirlife Aculife, India) used in this study were dissolved or diluted in distilled water (d.H_2O) and administered intragastically except AMP, APO and KET that were given intraperitoneally in a volume of 10 mL/kg per body weight animal. The ethanol extract of *C. lutea* (EECL) leaf was dissolved in d.H_2O and orally administered (100, 200, 400, and 800 mg/kg) based on the graded doses from LD_{50} result obtained and the previous study (Abiodun and Oshinloye, 2017). Meanwhile, the doses of AMP, APO and KET (negative control), HLP and CZP (standard control), and d.H_2O (vehicle control) were chosen based on earlier publications (Ben-Azu et al., 2016a, 2016b; Moreira and Guimarães, 2005).

2.6. Pharmacological treatment of animals

The experimental protocol for six different sets of animal experiments/models is shown in Fig. 1. Animal sampling approach described by Charan and Kantharia (2013) was employed in each experiment for animal grouping. Every model's performance was automatically

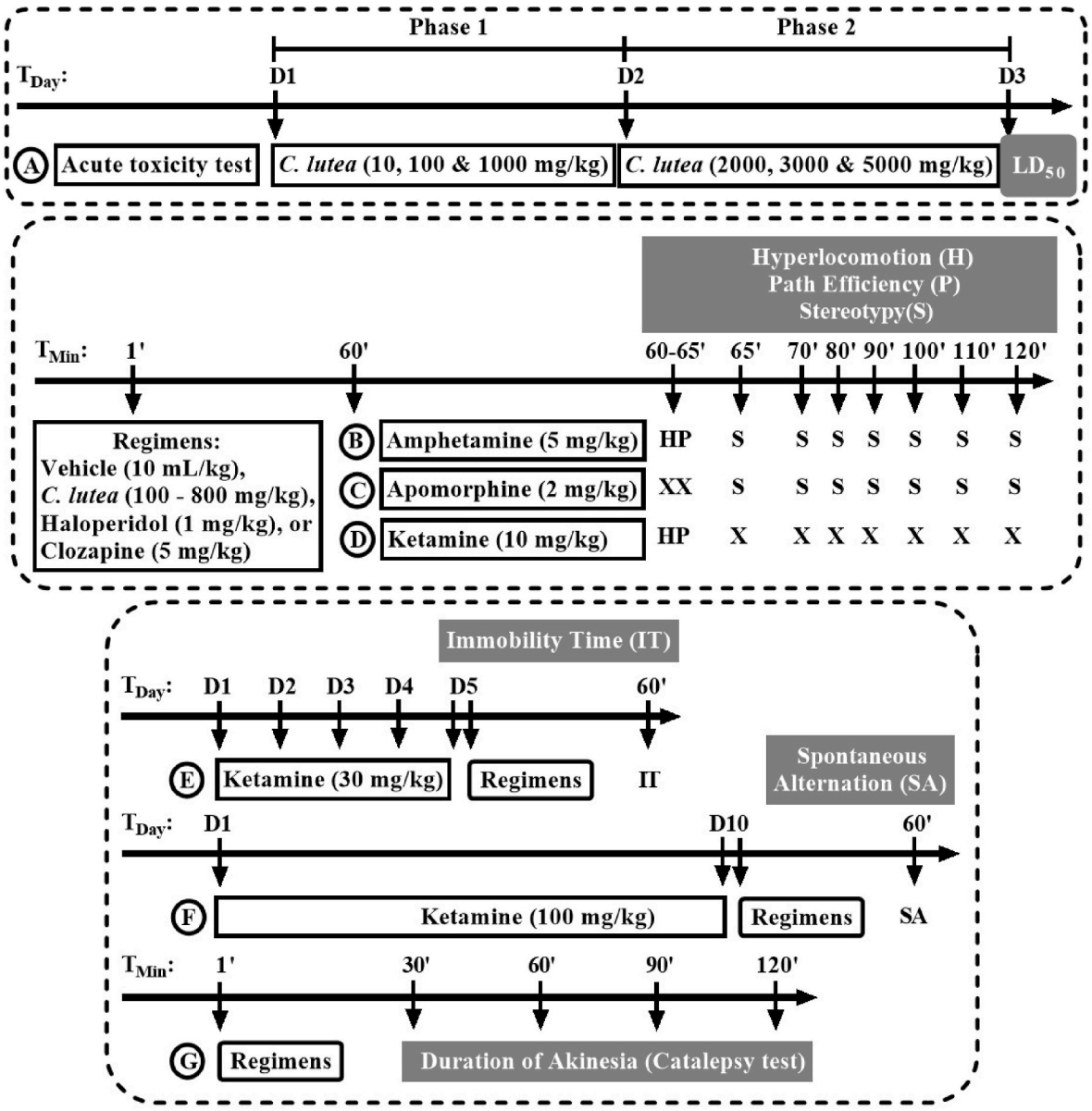


Fig. 1. Experimental timeline and design. A = Acute toxicity test; B = *Carpolobia lutea* (*C. lutea*) vs amphetamine-induced hyperlocomotion, path efficiency and stereotypy; C = *C. lutea* vs apomorphine-induced stereotypy in open field test; D = *C. lutea* vs ketamine-induced hyperlocomotion and path efficiency alteration in open field test; E = *C. lutea* vs ketamine-prolonged immobility time in force swimming test; F = *C. lutea* vs ketamine-induced spatial working memory deficits; G = Effect of *C. lutea* in catalepsy test. LD_{50} = Mean lethal dose.

recorded and saved on a computer by a video camera (Webcam) mounted above the apparatus (Omeiza et al., 2021).

2.6.1. Effect of EECL on amphetamine-induced hyperlocomotion and stereotypy

Using the method previously described by Macêdo et al. (2012), animals were challenged with amphetamine (5 mg/kg; i. p.) to assess the anti-psychotic effect of EECL, and parameters such as total distance travelled and path efficiency (which is a measure of how efficiently the mice move from the first to the last position) were first extracted as indicators of hyperlocomotion, followed by the determination of stereotyped behavior. Briefly, mice were distributed into eight treatment groups (n = 5) at random. Group 1 (vehicle control) and 2 (negative control) were pretreated with d.H_2O, (10 mL/kg, p. o.), groups 3 to 6 were given graded doses of EECL (100, 200, 400, and 800 mg/kg, p. o.), and groups 7 and 8 were given haloperidol (1 mg/kg, p. o.) and clozapine (5 mg/kg, p. o.) respectively. Sixty minutes later, each mouse was given a single dose of amphetamine (5 mg/kg, i. p) or d.H_2O, and then immediately transferred into a transparent open field observation arena (length × width × height: 20 cm × 20 cm × 23 cm), where the effects of EECL or drugs on the hyperlocomotion and path efficiency were determined using behavioral analysis system (ANY-maze version 6.3; Stoelting Co.; Wood Dale, IL) software within the first 5 min before stereotyped behavior was performed within 2 min at 65, 70, 80, 90, 100, 110, and 120 min. Path efficiency was calculated as the ratio of the straight line distance between the first and last positions to the total distance travelled by the mouse in the open field chamber during the exploration, and it ranged from −1 (imperfect) to +1 (perfect). Thereafter, stereotyped behavior was scored on a scale of 0–4, with 0 indicating no stereotyped behavior, 1, 2, 3, and 4 indicating the presence of stereotyped head movements, intermittent sniffing, chewing, and intense licking (Annafi et al., 2017). To estimate the overall effect of treatments, the area under the curve (AUC) known as the total stereotypy score was calculated as the sum of the stereotypy scores in 14 min

of seven trials. The observation chamber was thoroughly cleaned with 50% ethanol and allowed to dry before placing the next mouse on it; this eliminated residual odor after each test.

2.6.2. Effect of EECL on ketamine-induced hyperlocomotion and path efficiency alteration in open field test

In the second set of experiments, the antipsychotic effect of EECL on ketamine-induced hyperlocomotion and decreased path efficiency were examined according to the method earlier reported by Ben-Azu et al. (2016a). Following the 60 min pretreatment protocols described above, each mouse was given a single dose of ketamine (10 mg/kg; i. p.) or d. H_2O and placed immediately into a transparent open field observation cage (length × width × height: 20 cm × 20 cm × 23 cm). ANY-maze software (Version 6.3; Stoelting Co.; Wood Dale, IL) was used to examine behavioral characteristics such as hyperlocomotion and decreased path efficiency within a 5 min cut-off time. This software identifies the animal's position in the arena and estimates the distance travelled and path efficiency. The observation chamber was thoroughly cleaned with 50% ethanol and allowed to dry to reduce residual odor before placing the next mouse on it.

2.6.3. Effect of EECL on apomorphine-induced stereotypy in open field test

Apomorphine-induced stereotypy was also used to evaluate the antipsychotic effect of EECL, as previously reported by Ben-Azu et al. (2018d). Mice were randomly divided into seven treatment groups. Group 1 (negative control) was pretreated with d.H_2O, (10 mL/kg, p. o.), groups 2 to 5 were given EECL (100, 200, 400, and 800 mg/kg, p. o), and groups 6 and 7 received haloperidol (1 mg/kg, p. o.) and clozapine (5 mg/kg, p. o.), respectively. Sixty minutes after pretreatment with regimens, individual mice were given apomorphine or vehicle and immediately transferred into a transparent open field observation arena (length × width × height: 20 cm × 20 cm × 23 cm) as previously described with amphetamine-induced stereotypy (Annafi et al., 2017).

2.6.4. Effect of EECL on ketamine-prolonged immobility time in force swimming test

To assess the antipsychotic effect of EECL on ketamine-prolonged immobility time, the protocol previously described by Chindo et al. (2012) modified by Ben-Azu et al. (2018d) was employed. Mice were randomly assigned to one of two groups: test (n = 35) or vehicle control (d.H_2O, n = 5). The test and the vehicle groups were exposed to ketamine (30 mg/kg, i. p.) and vehicle (10 mL/kg, p. o.) for 5 consecutive days. On day 5 and after treatments, individual mice were first allowed to habituate to the standard transparent glass cylinder (height × diameter: 46 cm × 20 cm) with 30 cm depth of water at 25 °C and afterwards forced to swim for 5 min (pretest session). Twenty-four hours later (day 6) and before the treatments, mice in the test groups were further divided into seven subgroups (n = 5/subgroup) and labeled as groups 2–8. Groups 1 (normal control) and 2 (negative control) received vehicle (d.H_2O, 10 mL/kg, p. o.), groups 3–6 received EECL (100, 200, 400, and 800 mg/kg, p. o.), and groups 7 and 8 received haloperidol (1 mg/kg, p. o) and clozapine (5 mg/kg, p. o.). Sixty minutes thereafter, each mouse was placed in a standard transparent glass cylinder containing water and forced to swim for 6 min (test session), which was automatically recorded by a camera set above the cylinders and saved on a computer. After disregarding the first 2 min animal tries to escape, the immobility time (passive swimming above the water) for the last 4 min was extracted using ANY-maze software.

2.6.5. Effect of EECL on ketamine-induced spatial working memory deficits

According to earlier report by Chatterjee et al. (2011) with a little modification, this experiment assessed the effect of EECL on ketamine-induced spatial working memory deficits. Mice were randomly sorted into two groups: test (n = 35) or normal control (vehicle or d.H_2O, n = 5). The test and normal control groups were given ketamine (100 mg/kg, i. p.) and vehicle (10 mL/kg, p. o.) for 10 days, respectively. On day 10, before the treatments, mice in the test group were divided into seven subgroups (n = 5/subgroup) and labeled as groups 2–8. As noted previously, Groups 1 and 2 received vehicle (d. H_2O, 10 mL/kg, p. o.), Groups 3–6 had EECL (100, 200, 400, and 800 mg/kg, p. o.), and groups 7 and 8 received haloperidol (1 mg/kg, p. o.) and clozapine (5 mg/kg, p. o.), respectively. Individual mice were then placed in a Y-maze apparatus comprised of three arms labeled A, B, and C that is, all the same length = 21 cm, breadth = 7 cm, and height = 15.5 cm, and each arm symmetrically separated from the other by 120°. Each mouse spent 8 min in the apparatus, and its exploration was automatically recorded and saved on a computer by a camera set above the apparatus. By allowing the mouse to explore all three arms of the labyrinth, spontaneous alternation (which is a predictor of spatial working memory) was measured using ANY-maze software and this parameter is driven by mice's intrinsic desire to explore previously unexplored arms. Based on the software settings, spontaneous alternation was calculated as the total number of correct alternations (ABC, BCA, or CAB but not ABA or BAB)/(total arm entries – 2) (Monte et al., 2013). To remove the bias caused by the previous mouse's odor cues, the apparatus was cleaned properly with 50% ethanol and allowed to dry.

2.6.6. Effect of EECL in catalepsy test

In accordance with the report described by Ben-Azu et al. (2018d), the experiment on the effect of EECL in catalepsy test was performed to determine whether EECL is free of extrapyramidal symptoms (EPS), a symptom caused by antipsychotic drugs that block the nigrostriatal dopamine pathway - particularly the typical antipsychotics. Mice were divided into seven groups (n = 5/group) and labeled 1–7. These groups were given a vehicle (10 mL/kg, p. o), EECL (100, 200, 400, and 800 mg/kg, p. o.), haloperidol (1 mg/kg, p. o), and clozapine (5 mg/kg, p. o). Sixty minutes thereafter, individual mice were trained to gently grasp a catalepsy woodblock apparatus (length × breadth × height: 16 cm × 4 cm × 6 cm) with their forepaws. The duration of akinesia, an index of EPS (catalepsy), in 60-s cut-off time at 30, 60, 90, and 120 min on the woodblock were measured using a stopwatch. To estimate the overall effect of treatments, the area under the curve (AUC) or total duration of akinesia/240 s was calculated as the sum of the duration of akinesia in 240 s of four trials. In this study, a mouse is classified as cataleptic if its akinesia time and AUC exceed 30 and 120 s, respectively. Before testing another mouse, the apparatus was cleaned with 50% ethanol to remove the bias caused by the previous mouse's odor cues.

2.7. Statistical analysis

The collected data were analyzed with the Graph Pad Prism 8.04 software (La Jolla, CA) and expressed as mean S.E.M. One-way or two-way analysis of variance (ANOVA) was used to compare treatment groups, followed by a Bonferroni or Dunnett post-hoc test. P-values less than 0.05 ($p < 0.05$) was considered statistically significant.

3. Results

3.1. Acute toxicity of ethanol extract of C. L leaf in swiss mice

No mortality was recorded in the two phases but mice orally treated with EECL (5000 mg/kg, p. o.) were calmed within the first 2 h of administration. There were no stereotypical symptoms related with toxicity in any group. Hence, the LD_{50} of EECL in this study was calculated to be more than the highest dose of 5000 mg/kg tested, suggesting it is relatively safe.

3.2. EECL reduced amphetamine-induced hyperlocomotion in OFT

The total distance travelled, and path efficiency were used to define the effect of EECL on amphetamine-induced hyperlocomotion, as shown in Fig. 2. Here, one-way ANOVA and Bonferroni post hoc analysis

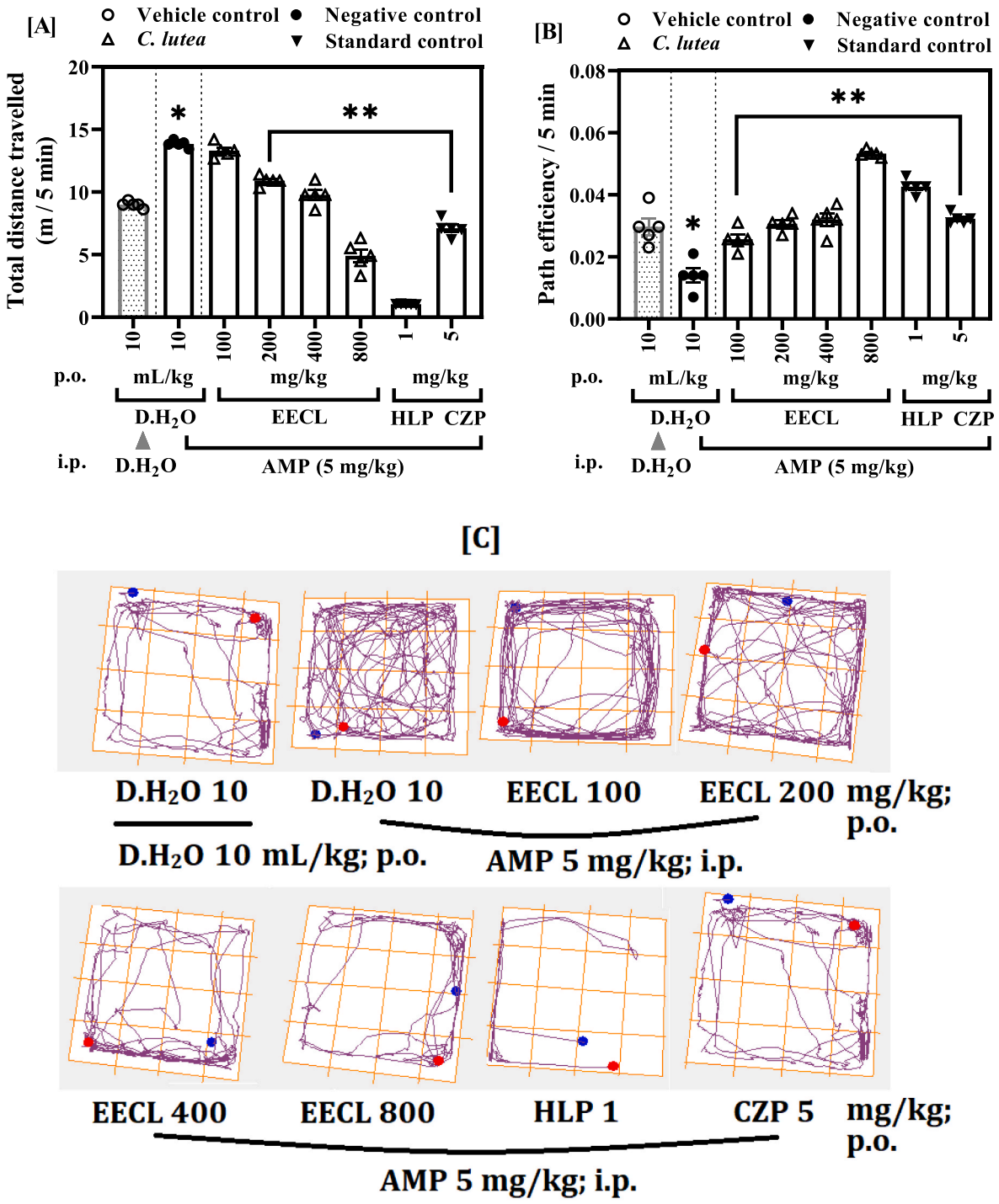


Fig. 2. EECL reduced amphetamine-induced hyperlocomotion in OFT. A) Total distance travelled, B) Path efficiency and C) ANY-maze trajectory plots of open field. Bar represents the mean ± S.E.M (n = 5/group); *P < 0.05 versus vehicle control; **P < 0.05 versus AMP-untreated mice (negative control); D. H_2O/VEH = Vehicle, AMP = Amphetamine; EECL = Ethanol extract of *Capolobia lutea*; HLP = Haloperidol; CZP = Clozapine; OFT = Open field test. Red and blue points in ANY-maze analysis = track start and track end. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

revealed that amphetamine (5 mg/kg, i. p.) increased ($p < 0.05$) total distance travelled [$F(7, 32) = 240.2$, $P < 0.0001$], and decreased path efficiency [$F(7, 32) = 49.94$, $P < 0.0001$] in comparison to the vehicle control. However, a single dose of EECL (200, 400, and 800, not 100 mg/kg, p. o.) in a dose-dependent pattern reversed the effect induced by amphetamine on the total distance travelled. Moreover, the standard controls, HLP (1 mg/kg, p. o.) and CZP (5 mg/kg, p. o.) reduced the total distance travelled in amphetamine-treated mice (Fig. 2A). Regarding the path efficiency, pretreatment with both HLP (1 mg/kg; p. o.) and CZP (5 mg/kg, p. o.) as well as graded doses of EECL (100, 200, 400, and 800 mg/kg, p. o.) all increased ($p < 0.05$) path efficiency when compared to amphetamine-untreated mice (Fig. 2B). Overall, the trajectory plots of all groups in the amphetamine model exposed to vehicle, EECL, or standard drugs revealed varying dramatic differences during the 5 min exploration in the open field chamber (Fig. 2C).

3.3. EECL reversed ketamine-induced hyperlocomotion in OFT

One-way ANOVA and Bonferroni post hoc analysis revealed that ketamine (10 mg/kg; i. p.) increased ($p < 0.05$) total distance covered [$F(7, 32) = 50.18$, $P < 0.0001$] and decreased path efficiency [$F(7, 32) = 51.97$, $P < 0.0001$] in comparison to the vehicle control (10 mL/kg, p. o.) (Fig. 3). Notwithstanding, EECL (200, 400, and 800 not 100 mg/kg, p. o.) reversed the increased ($p < 0.05$) total distance travelled caused by ketamine. Similarly, HLP (1 mg/kg, p. o.), and CZP (5 mg/kg, p. o.) also lowered ($p < 0.05$) the total distance travelled in ketamine-untreated mice (Fig. 3A). Regarding the path efficiency, mice pretreated with graded doses (100, 200, 400, and 800 mg/kg, p. o.) of EECL showed an

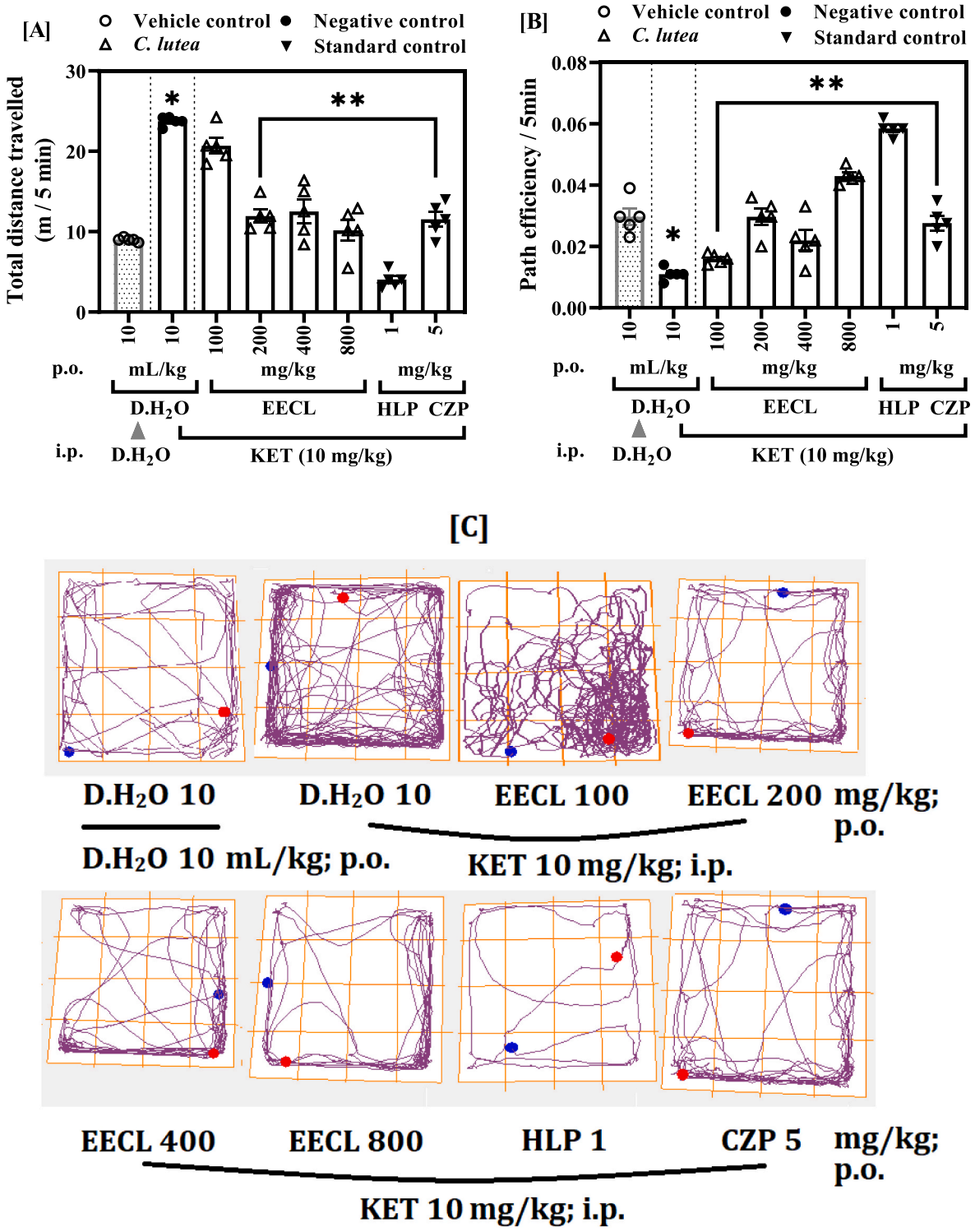


Fig. 3. EECL reduced ketamine-induced hyperlocomotion in OFT. A) Total distance travelled, B) Path efficiency and C) ANY-maze trajectory plots of open field. Bar represents the mean ± S.E.M (n = 5/group); *$P < 0.05$ versus vehicle control; **$P < 0.05$ versus KET-untreated mice (negative control); D. H_2O/VEH = Vehicle; KET = Ketamine; EECL = Ethanol extract of *Capolobia lutea*; HLP = Haloperidol; CZP = Clozapine; OFT = Open field test. Red and blue points in ANY-maze analysis = track start and track end. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

increase ($p < 0.05$) in path efficiency when compared to the ketamine-untreated group. Similarly, the reference antipsychotics, HLP (1 mg/kg, p. o.) and CZP (5 mg/kg, p. o.) overturned ($p < 0.05$) the effect of ketamine in mice (Fig. 3B). Altogether, during the 5-min exploration in the open field chamber, the trajectory plots of all groups in the amphetamine model exposed to vehicle, EECL, or reference drugs revealed varying dramatic differences (Fig. 3C).

3.4. EECL mitigates amphetamine-induced stereotypy in OFT

The effect of EECL on stereotypy is depicted in Fig. 4. In general, the stereotypy score in amphetamine-untreated mice remained high at all-time points, but this effect on the pretreated groups was variable at each time interval. Two-way repeated-measures ANOVA and Bonferroni post hoc analysis revealed a significant difference ($p < 0.05$) in stereotypy score with treatment [$F(6, 196) = 82.59$, $P < 0.0001$] and time [$F(6, 196) = 3.964$, $P = 0.0009$] effects, as well as the interaction between treatment and time [$F(36, 196) = 2.341$, $P = 0.0001$] in the experimental mice. Initially, there was a stable ($p < 0.05$) increase in stereotypy score between 65 and 80 min, and this effect was later amplified between 90 and 120 min in the amphetamine-untreated mice. However, pretreatment with EECL (400 and 800 mg/kg, p. o.), HLP (1 mg/kg, p. o.), and CZP (5 mg/kg, p. o.) reversed the amphetamine effect at 65, 70, 80, 90, 100, 110, and 120 min. Furthermore, there was no significant difference ($p > 0.05$) in stereotypy score between amphetamine and EECL at doses of 100 and 200 mg/kg, p. o. at 80 and 90 min, respectively (Fig. 4A).

Regarding the effect of EECL on total stereotypy score/14 min (AUC) (a parameter calculated as the sum of stereotypy scores observed in 2 min at 65, 70, 80, 90, 100, 110, and 120 min respectively in the open field chamber), one-way ANOVA and Dunnett post hoc analysis revealed that amphetamine-induced stereotypy [$F(6, 28) = 29.07$, $P < 0.0001$] was dose-dependently reduced by EECL (100, 200, 400, and 800 mg/kg, p. o) but relatively higher in amphetamine-untreated mice. Similarly, pretreatment with HLP (1 mg/kg, p. o.), and CZP (5 mg/kg, p. o.) also ($p < 0.05$) reduced total stereotypy in amphetamine-treated mice (Fig. 4B).

3.5. EECL reduces apomorphine-induced stereotypy in OFT

The effect of EECL on apomorphine-induced stereotypy is depicted in Fig. 5. Across all treatment groups, the stereotypy score decreased in a time-dependent pattern (from 10 to 60 min). Furthermore, two-way repeated-measures ANOVA (treatment and time) and Bonferroni post hoc analysis revealed that each treatment had a significant effect ($p < 0.05$) on stereotypy score. The effect of treatments [$F(6, 196) = 68.25$, $P < 0.0001$], time [$F(6, 196) = 37.88$, $P < 0.0001$], and the interaction of treatments and time [$F(36, 196) = 6.354$, $P < 0.0001$] on stereotypy score were all significant in the experimental mice. In the same trend, the stereotypy score also ($p < 0.05$) increased in apomorphine-untreated mice. However, pretreatment with EECL (400 and 800 mg/kg, p. o.), HLP (1 mg/kg, p. o.), and CZP (5 mg/kg, p. o.) ($p < 0.05$) reversed the apomorphine-induced effect at 65, 70, 80, 90, 100, 110, and 120 min respectively. However, there was no significant difference ($p > 0.05$) in stereotypy score between apomorphine- and EECL (100 and 200 mg/kg, p. o.)-exposed mice at 70, 80 and 90 min (Fig. 5A).

In the assessment of total stereotypy score/14 min (AUC), one-way ANOVA and Dunnett post hoc analysis showed that there was significant ($p < 0.05$) increase in total stereotypy score [$F(6, 28) = 103.1$, $P < 0.0001$] by apomorphine (2 mg/kg, i. p.). Nonetheless, in a dose-dependent manner, oral administration of EECL (100, 200, 400, and

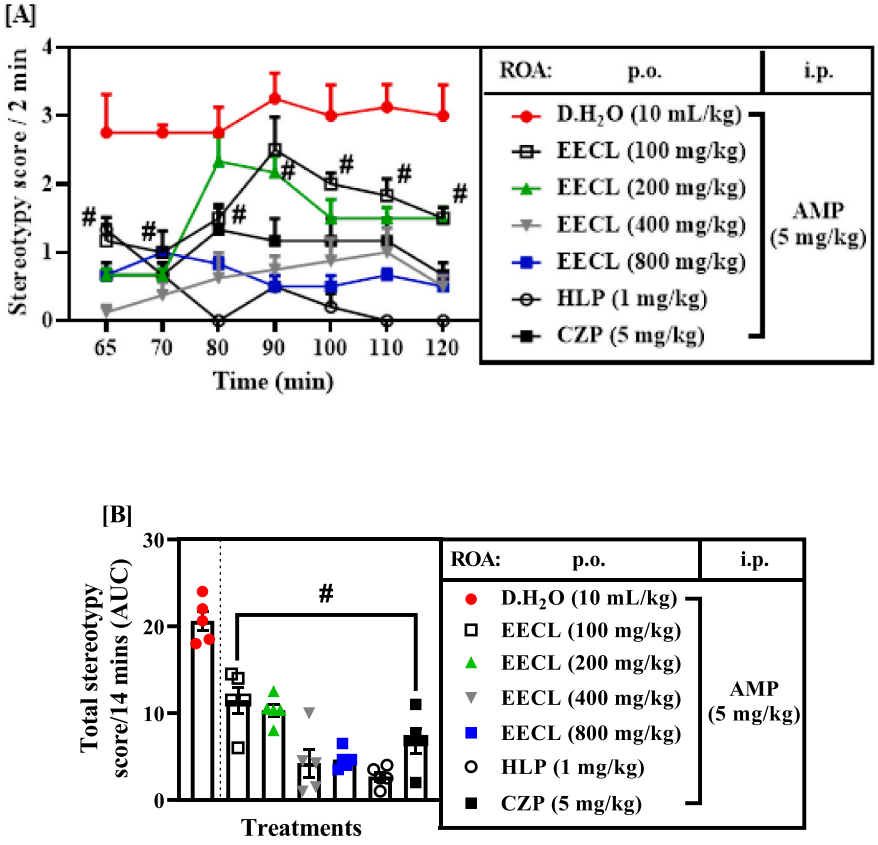


Fig. 4. EECL mitigates amphetamine-induced stereotypy in OFT. A) Stereotypy score and B) Total stereotypy score (AUC). Bar represents the mean ± S.E.M (n = 5/group). $^{\#}P < 0.05$ versus AMP-untreated mice ($D.H_2O$); D. H_2O/VEH = Vehicle; AMP = Amphetamine; EECL = Ethanol extract of *Capolobia lutea*; HLP = Haloperidol; CZP = Clozapine; AUC = Area under curve; OFT = Open field test.

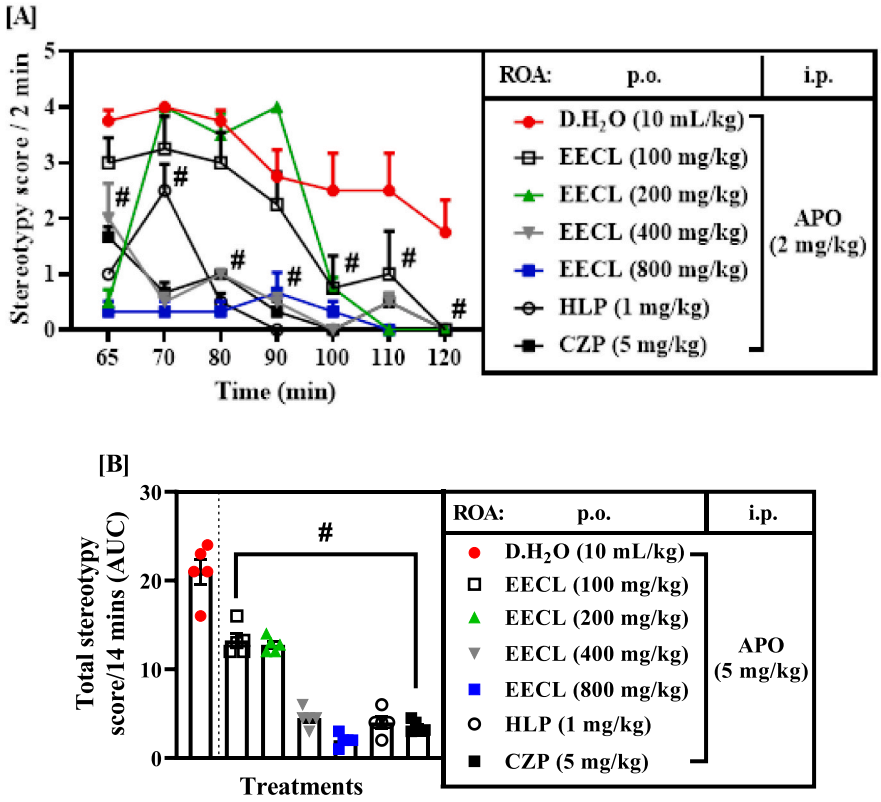


Fig. 5. EECL mitigates apomorphine-induced stereotypy in OFT. A) Stereotypy score and B) Total stereotypy score (AUC). Bar represents the mean ± S.E.M (n = 5/group). $^{\#}P < 0.05$ versus APO-untreated mice ($D.H_2O$); D. H_2O/VEH = Vehicle; APO = Apomorphine; EECL = Ethanol extract of *Capolobia lutea*; HLP = Haloperidol; CZP = Clozapine; AUC = Area under curve; OFT = Open field test.

800 mg/kg, p. o.) mitigated the effect induced in apomorphine-treated mice, and in a similar pattern, pretreatment with HLP (1 mg/kg, p. o.) and CZP (5 mg/kg, p. o.) also lowered total stereotypy score in apomorphine-treated mice (Fig. 5B).

3.6. EECL antagonizes ketamine-enhanced immobility time in FST

In the analysis of immobility time as an index of negative symptoms, one-way ANOVA and Bonferroni post hoc analysis showed that ketamine (30 mg/kg, i. p.) significantly ($p < 0.05$) enhanced immobility time [$F(7, 32) = 37.05$, $P < 0.0001$] when compared with vehicle control (10 mL/kg; p. o.). This effect of ketamine was ($p < 0.05$) reversed by the pretreatment with EECL (100, 200, 400 and 800 mg/kg, p. o.) and CZP (5 mg/kg, p. o.). Meanwhile, orally administered HLP (1 mg/kg, p. o.) did not ($p > 0.05$) produce any changes to immobility time (Fig. 6).

3.7. EECL ameliorates ketamine-induced spatial memory alteration in YMT

In the analysis of percentage alternation as a predictor of spatial memory deficits or cognitive symptoms, one-way ANOVA and Bonferroni post hoc analysis revealed that ketamine (30 mg/kg; i. p.) significantly ($p < 0.05$) decreased percentage alternation [$F(7, 32) = 13.42$, $P < 0.0001$] when compared to vehicle control (10 mL/kg, p. o.). However, when compared to ketamine-untreated mice, pretreated mice with EECL (400 and 800 mg/kg, p. o.) and CZP (5 mg/kg, p. o.) had higher percentage alternation. Moreover, the effect of HLP (1 mg/kg, p. o.) and EECL at 100 and 200 mg/kg, p. o. on percentage alternation was found to be unchanged ($p > 0.05$) in ketamine-untreated mice (Fig. 7).

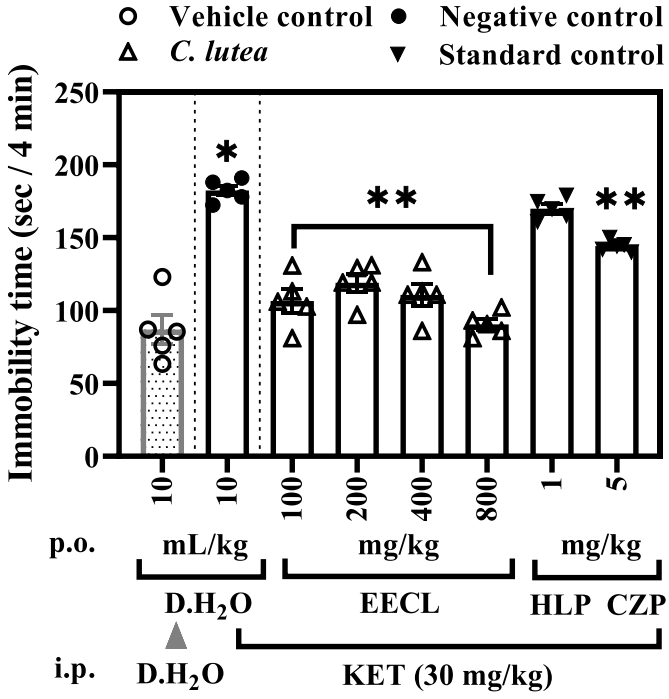


Fig. 6. EECL antagonizes ketamine-enhanced immobility time in FST. Bar represents the mean ± S.E.M (n = 5/group); *P < 0.05 versus Vehicle control; **P < 0.05 versus KET-untreated mice (negative control); D. H_2O/VEH = Vehicle, KET = ketamine; EECL = Ethanol extract of *Capolobia lutea*; HLP = Haloperidol; CZP = Clozapine; FST = Force swimming test.

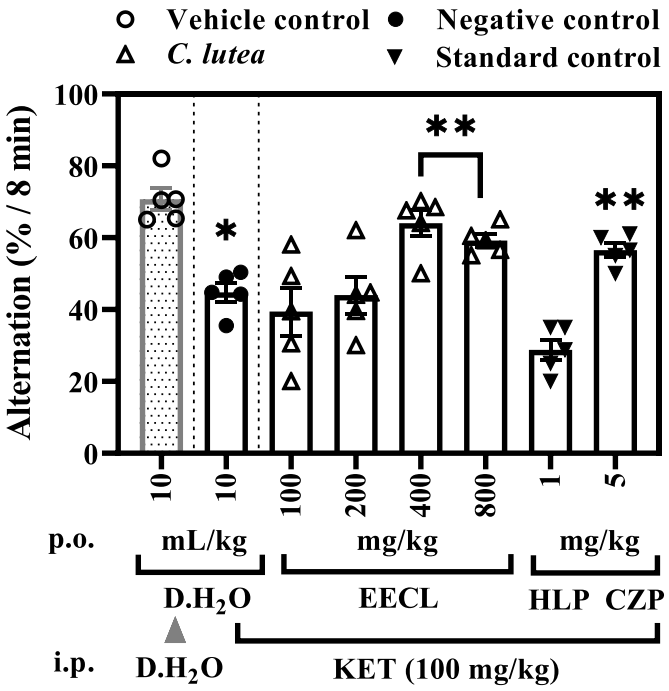


Fig. 7. EECL ameliorates ketamine-induced spatial memory alteration in Y-maze test using percentage alternation as an index. Bar represents the mean ± S.E.M (n = 5/group); *P < 0.05 versus vehicle control; **P < 0.05 versus KET-untreated mice (negative control); D. H_2O/VEH = Vehicle, KET = ketamine; EECL = Ethanol extract of *Capolobia lutea*; HLP = Haloperidol; CZP = Clozapine; YM = Y-Maze test.

3.8. Effect of EECL on catalepsy test

In the analysis of duration of akinesia (an index of cataleptic or extrapyramidal symptoms), a two-way repeated measure ANOVA and Bonferroni post hoc analysis revealed a significant difference ($p < 0.05$) in duration of akinesia with treatment [$F(6, 112) = 219.8$, $P < 0.0001$] and time [$F(3, 112) = 57.45$, $P < 0.0001$] effects, as well as the interaction between treatment and time [$F(18, 112) = 17.43$, $P < 0.0001$] effects. In comparison to vehicle control, the duration of akinesia in mice exposed to HLP but not EECL and CZP ($p < 0.05$) increased from 30 to 60 min, and this increase was sustained at 90 and 120 min (Fig. 8A).

Regarding the effect of treatments on the total duration of akinesia/240 s (AUC) (a parameter calculated as the sum of the duration of akineasia observed in 1 min at 30, 60, 90, and 120 min on the wood block), one-way ANOVA and Dunnett post hoc analysis revealed a significant ($p < 0.05$) increase in total duration of akinesia [$F(6, 28) = 130.3$, $P < 0.0001$] in mice treated with HLP (1 mg/kg, p. o.) but not EECL (100, 200, 400 and 800 mg/kg, p. o.) and CZP (5 mg/kg, p. o.) in relation to vehicle control (10 mL/kg, p. o.) (Fig. 8B). Data was considered significant above 30 and 120 s in Fig. 8A and **B** respectively.

3.9. Phytochemical analysis of ethanol extract of C. lutea leaf

The presence of alkaloids, Anthraquinones, flavonoids, phenolic, saponin, tannins, and terpenoids were found in the phytochemical screening of EECL (Table 1). A total of eight peaks detected at a typical UV absorption wavelength of 280 nm were resolved in further phytochemical analysis by HPLC fingerprinting of EECL (Fig. 9). Gallic acid, p-coumaric acid, and rutin had relative abundance of 3.98, 20.70, and 24.84, respectively, as measured by their peak areas (Fig. 9).

4. Discussion

Presently, dopamine (DA) enhancers (e.g., amphetamine and apomorphine) and non-competitive glutamate antagonist (e.g., ketamine) were found to insult the neural network, resulting in pathological features of schizophrenia-like behavior, as evidenced by hyperlocomotion, increased stereotypic behavior), behavioral despair, and spatial memory impairment. However, the single-dose administration of EECL pretreatment prevented these behavioral impairments related to schizophrenia-like behavior. Surprisingly, EECL's antipsychotic activity is comparable to that of the reference regimens especially the CZP. Furthermore, the phytochemical screening of EECL revealed some bioactive products, which suggests that EECL's antipsychotic activity could be attributed to the presence of the bioactive compounds including p-coumaric acid, gallic acid and rutin (Fig. 9), all of which have been largely reported to cross the blood brain barrier (BBB) to elicit neuroprotective and antipsychotic functions (Daroi et al., 2022; Daroi, 2021; Oshodi et al., 2021; Guven et al., 2015; Javed et al., 2012; Pandy et al., 2012).

Apart from neurochemical assessment of schizophrenia disease, behavioral phenotypes are crucial tool clinically used to diagnose schizophrenia in humans and measure behavioral phenotypes in rodents, as well as to evaluate the effects of antipsychotic compounds (Owens et al., 2016; Papaleo et al., 2016). Also, it is worth mentioning that no single animal model can perfectly mimic all of the symptoms of schizophrenia seen in humans. Hence, the current study used amphetamine, apomorphine, and ketamine models of schizophrenia to assess the neurotherapeutic impacts of EECL on drug-induced behavioral features linked to schizophrenia. Acute exposure to psychotomimetic substances like cocaine, amphetamine, heroin, and apomorphine have been shown to induce delusions and hallucinations, which are related to the excessive behavioral features, hyperactivity or stereotypy which are predictive of the positive symptoms of the disease (Leite et al., 2008). These drugs are well-known to inhibit dopamine transporter (DAT), thereby increasing dopaminergic neurotransmission, by blocking reuptake, increasing release from presynaptic terminals, preventing inhibitory interneurons from projecting to the dopaminergic system or acting as a dopaminergic agonist at D1 and D2 dopamine receptors in the mesolimbic neural system (Vijeepallam et al., 2016; Pandy et al., 2012). Furthermore, these stimulants also have local anaesthetic properties, most notably through the inhibition of fast sodium channels in peripheral nerve endings, but this activity is similar to that of a glutamate NMDA receptors (NMDARs) antagonists (e.g., ketamine and phencyclidine), which has been shown to induce a wide range of schizophrenia-like symptoms at various sub-anaesthetic dose levels (Ben-Azu et al., 2018a, 2018b; Georgiadou et al., 2014). For instance, ketamine, a non-competitive antagonist of NMDARs, is a modulator of dopamine and opioid transmission, and a serotonin transporter agonist (SERT) (Dare et al., 2015; Vollenweider, 2001). Overall, it acts by blocking NMDARs, resulting in NMDARs hypofunction, which has been strongly hypothesized in schizophrenia pathology (Kantrowitz and Javitt, 2010; Krystal et al., 1994).

In the present study, total distance travelled, path efficiency and total stereotypy score were used as predictive variables of the positive symptoms of schizophrenia. Acute amphetamine and apomorphine exposure were found to increase total stereotypy score while amphetamine and ketamine exposure increased total distance travelled. These effects of psychotomimetic substances were mitigated by EECL (Figs. 2–5). The possible mechanism of action of EECL may be that it inhibits increased cortical glutamatergic projection neuron firing, resulting in hyperactivation of the dopamine mesolimbic pathway (Marek et al., 2010). Similarly, previous reports have shown that dietary supplements (e.g., stigmasterol, rutin, gallic acid, scopoletin, and morin) (Yadav et al., 2018a,b; Pandy and VijeePallam, 2017) or plant sources like *Panax quinquefolium*, *Bacopa monniera*, *Synedrella nodiflora* and *Terminalia ivorensis* (Mishra et al., 2018; Amoateng et al., 2017; Ben-Azu et al., 2016; Chatterjee et al., 2012a,b), which contains bioactive metabolites, alleviate psychotic symptoms in acute and chronic animal models of schizophrenia.

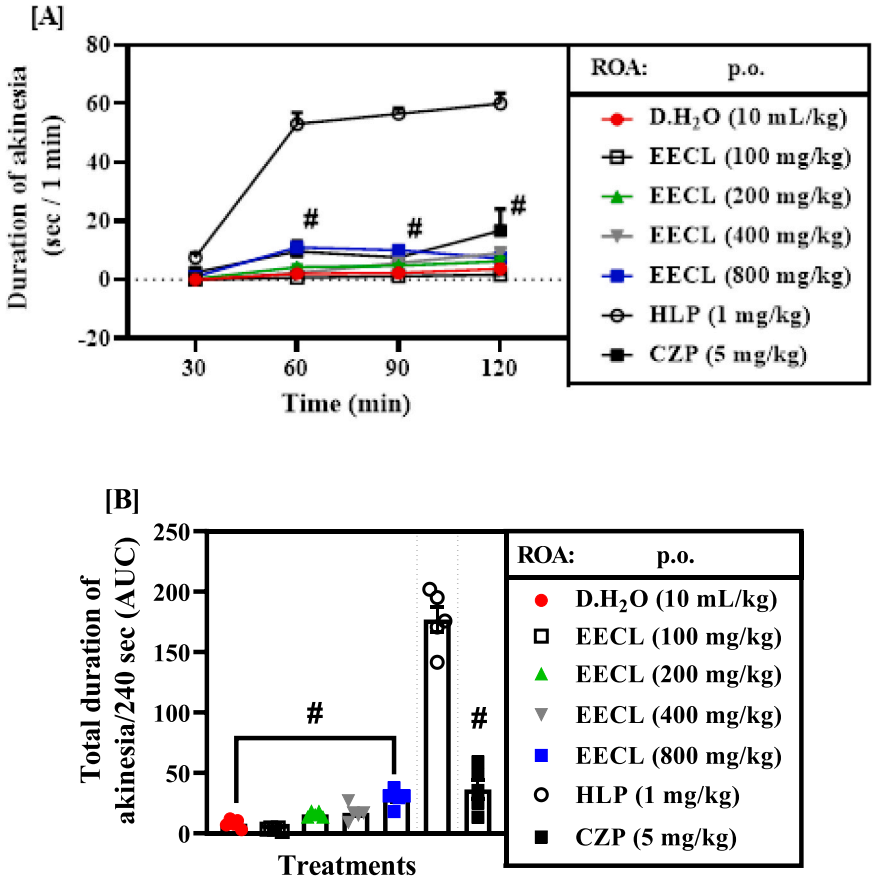


Fig. 8. Effect of EECL on catalepsy test. A) Duration of akinesia and B) Total duration of akinesia/240 s (AUC). Bar represents the mean ± S.E.M (n = 5/group). $^{\#}P <$ 0.05 versus HLP-treated mice; D. H_2O/VEH = Vehicle; EECL = Ethanol extract of *Capolobia lutea*; HLP = Haloperidol; CZP = Clozapine; AUC = Area under curve.

Table 1
Table showing the phytochemicals analysis of *C. lutea* leaf.

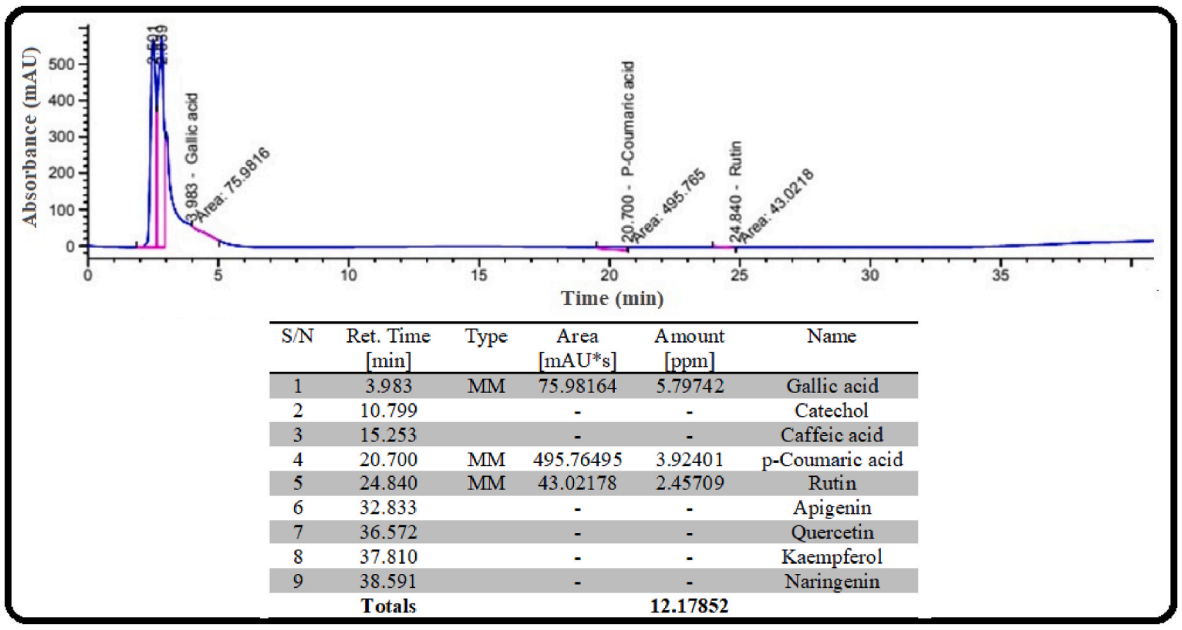
S/No	Constituents	Qualitative
1	Alkaloids	+
2	Anthocyanin	–
3	Anthraquinones	+
4	Carotenoids	–
5	Flavonoids	+
6	Phenolic	+
7	Saponins	+
8	Steroids	+
9	Tannins	+
10	Terpenoids	+

+ = present and - = absent.

Furthermore, in schizophrenia, alteration in motor functions include both hyperkinetic and hypokinetic movement disorders (Walther and Mittal, 2017; Tse et al., 2004). This is because many brain areas involved in motor coordination, planning, fine-tuning, and learning motion sequences, such as the motor and premotor cortices, basal ganglia, brainstem, cerebellum, and the white matter tracts connecting these components, have been implicated (Walther and Strik, 2012). The severity of damage in one area of the brain determines the kind of motor disorder that emanates. In this study, apart from the use of hyperlocomotion and stereotyped movement as indicators of positive symptoms, another notable finding was the decreased path efficiency, a parameter that measures the exploratory efficiency with which mice travel from the first to the last position in an open field in amphetamine and ketamine models. However, this effect was also reversed by graded doses of EECL (Figs. 2B and 3B), indicating its reversibility on the altered cerebro-cerebellar and nigrostriatal motor pathways (Gonzalez, 2004).

Negative symptoms of schizophrenia have proven to be significant challenges for biomedical scientists, as available (a)typical antipsychotics as such D2 receptor antagonists are only beneficial against positive symptoms but with little or no effect on the negative symptoms of the disease (Vardigan et al., 2010; Keshavan et al., 2005). While neither DA enhancers nor $5HT_{2A}$ receptor agonists induce negative symptoms, a subanesthetic dose of NMDARs antagonist, such as ketamine, has been shown to cause deficits in social interaction (Thiebes et al., 2017; Chatterjee et al., 2011) or behavioral despair (Ben-Azu et al., 2018d; Chindo et al., 2012). Ketamine is widely used to screen test compounds for probable antipsychotic-like activity in terms of their effect against negative symptoms of schizophrenia. It has been further reported that ketamine induces cellular disruptions in GABAergic–glutamatergic balance, as well as alterations in dopaminergic and serotoninergic signaling to induce the negative-like symptoms of schizophrenia (Ben-Azu et al., 2018a, 2018b; Muguruza et al., 2016; Chindo et al., 2012). This claim is supported by the fact that atypical antipsychotics, such as clozapine or risperidone, which are potent serotonin (5-HT) receptor antagonists, alleviate negative symptoms in schizophrenia patients. Meanwhile, increased 5-HTergic and dopaminergic activity is accompanied by decreased NMDA function, and vice versa. This intricate interaction of these neurotransmitter pathways may result in negative symptoms. In this study, ketamine administration caused behavioral despair in the mice. However, EECL reverses this effect as indexed by reduced immobility time, in the FST, indicating the antipsychotic-like property of EECL. This data is consistent with the previous report by Ben-Azu et al. (2018b), which also revealed morin's antipsychotic effect on negative symptoms in FST.

Aside from the well-known positive and negative symptoms induced



S/N	Ret. Time [min]	Type	Area [mAU*s]	Amount [ppm]	Name
1	3.983	MM	75.98164	5.79742	Gallic acid
2	10.799		-	-	Catechol
3	15.253		-	-	Caffeic acid
4	20.700	MM	495.76495	3.92401	p-Coumaric acid
5	24.840	MM	43.02178	2.45709	Rutin
6	32.833		-	-	Apigenin
7	36.572		-	-	Quercetin
8	37.810		-	-	Kaempferol
9	38.591		-	-	Naringenin
	Totals			**12.17852**	

Fig. 9. Representative result showing the HPLC chromatogram of EECL monitored at wavelength: 280 nm.

by subanesthetic dosing levels of ketamine, it is also known that ketamine interferes with cognitive performance such as attention, learning, and memory functions (Morgan et al., 2004). The NMDARs are abundantly expressed in many regions of the brain but are most densely concentrated in the hippocampus and cerebral cortex, which are crucial areas for learning and memory. Thus, the hypofunctionality of this receptor in these brain regions have been strongly linked to the pathogenesis of the cognitive dysfunction as well as disrupted neural dynamics in schizophrenia, and current antipsychotics have not been shown to efficiently alleviate these symptoms in schizophrenic patients (Gilmour et al., 2012). Moreover, haloperidol, a first generation antipsychotic drugs, has repeatedly been shown to worsen the learning and memory impairment in schizophrenia by producing so-called secondary deficit symptoms (Geyer and Moghaddam, 2002) and degeneration of cortical pyramidal neurons (Ben-Azu et al., 2019). However, there are conflicting evidence that the newer generation of antipsychotics (e.g., clozapine and olanzapine) that affect multiple dopamine and serotonin receptor subtypes may efficiently improve cognitive function (Adem et al., 2019; Meltzer et al., 2003). This is because the pathogenesis of cognitive symptoms is complex, involving many putative neurotransmitters of disconnected signal transduction pathways, such as dopamine, glutamate, GABA, serotonin, acetylcholine, and norepinephrine in the striatum, hippocampus, and cerebral cortex of the brain (Chatterjee et al., 2015, 2012; Zugno et al., 2014). In the current study, EECL (400 and 800 mg/kg, p. o.) assuaged the spatial working memory impairment induced by a 10-day ketamine intoxication, as evidenced by increased spontaneous alternation in the Y maze paradigm as an index of cognitive function, and this effect of EECL suggests its possible antipsychotic potential, similar to the result of the reference drug clozapine but not haloperidol. Although specific mechanistic studies are needed to clarify the precise mechanisms of action underlying this beneficial effect, the mechanism of action of EECL could be through its modulation of neurochemical pathways involved in the pathogenesis of schizophrenia. In agreement with previous study by Chatterjee et al. (2012a), *Panax quinquefolium* is well-known to contain bioactive compounds that reverse ketamine-induced cognitive impairment in mice.

Phytochemicals are secondary plant metabolites that have been shown to have chemopreventive and therapeutic effects on a wide range of diseases (Singh et al., 2019; Saxena et al., 2013; Dillard and German, 2000). It is worth noting that herbal plants contain complex mixtures of chemical compounds that work independently or synergistically to maintain health (Gurib-Fakim, 2006). In the present study, phytochemicals such as alkaloids, anthraquinones, flavonoids, phenolic, saponin, tannins, and terpenoids were found in EECL which are in agreement with previous findings (Olayinka et al., 2019; Nwidu et al., 2016). *In-vivo* and *in-vitro* studies have shown that the physiological properties of these phytochemicals in EECL can help prevent a variety of diseases including neurological related disorders (Nwidu et al., 2017; Dare et al., 2015; Ajiwhen and Bisong, 2013). Apart from the fact that *C. lutea* has been used in Nigerian traditional medicine to treat migraine headaches and psychotic episodes; it also prevents reproductive dysfunction in males, as evidenced by its aphrodisiac action (Yakubu and Jimoh, 2015).

This aphrodisiac effect also demonstrated that EECL or its secondary metabolites can cross the blood-brain barrier, as it is well-known that higher centres in the brain control the physiology of the reproductive system. In addition, HPLC fingerprinting of EECL revealed the presence of gallic acid, p-coumaric acid, and rutin. Meanwhile, gallic acid and rutin, for example, have been shown by Yadav et al. (2018a) and Pandy and VijeePallam (2017) to have psychopharmacological activities, as they demonstrated a significant decrease in positive, negative and cognitive symptoms in ketamine mouse models of schizophrenia. This suggests that the EECL could have a wide range of medicinal applications because the phytochemicals have been shown in previous studies to have significant antioxidant, anti-inflammatory, anticholinesterase, anxiolytic, antinociceptive, and aphrodisiac effects (Abiodun and Oshinloye, 2017; Nwidu et al., 2017; Jackson et al., 2011) and currently antipsychotic-like activity.

The neuroprotective functions of EECL's metabolites have been largely elucidated. Notably, p-coumaric acid has been reported to attenuate brain intraluminal monofilament occlusion-induced embolic cerebral ischemia (Guven et al., 2015), as well as brain senescence induced by neurotoxicants such as d-galactose- (Daroi, 2021) and lipopolysaccharide (Daroi et al., 2022)-induced neuroinflammation and apoptosis. More importantly, rutin which is a 3,3′,4′5,7-pentahydroxyflavone-3rhamnoglucoside widely distributed in fruits and vegetables, is a neuroactive flavonoid derivative (Oshodi et al., 2021; Kreft et al., 1997). It possesses strong antioxidant and anti-inflammatory effects and demonstrates neuroprotective functions in neurological diseases characterized of neurochemical derangement, oxidative stress, and

neuroinflammation (Oshodi et al., 2021; Javed et al., 2012). Also, it elicits central nervous system-depressant activity (Fernandez et al., 2006), reverses haloperidol-induced tardive dyskinesia (Bishnoi et al., 2007), and exhibits antipsychotic-like effect (Pandy et al., 2012). Its antipsychotic mechanisms involve enhancement of $GABA_A$- and augmentation of cortical and subcortical glutamic acid decarboxylase-67-mediated GABAergic inhibitory activity, up-regulation of central cholinergic function, and down-regulation of nicotinamide adenine pathways (Oshodi et al., 2021) in mice brains. Additionally, gallic acid, which is a natural polyphenolic agent with the ability to cross the blood brain barrier (Mansouri et al., 2013) is known to possess protective effect against psychotic activities. The findings of Yadav et al. (2018) also revealed that the antipsychotic activity of gallic activity in mice brains involves inhibition of ketamine-induced oxidative stress, neuroinflammatory surge, reduction of dopamine and AChE levels but with increased concentration of GABA concentration. These findings suggest the antipsychotic-like activity of EECL in this study might in turn be due to the presence of rutin, gallic acid, and p-coumaric acid. Although it is well-known that the EECL contains several other bioactive phytoconstituents (Table 1) that are beneficial in therapy, the EECL's safety level was still examined, and the mean lethal dose (LD_{50}) of EECL was found to be greater than 5000 mg/kg. This finding is in close range to the findings of Nwidu and Nwafor (2009), who found the LD_{50} of EECL to be 3850.0 mg/kg; p. o. suggesting its relative safety. The disparity between the LD_{50} of both studies might be attributed to an environmental influence on the *C. lutea* plant or the pharmacological strategy used for the toxicity profile. Regarding the extrapyramidal side effects, 800 mg/kg; p. o. single oral dosage of EECL was shown to be devoid of extrapyramidal effect as evidenced by a reduced duration of dyskinesia in the woodblock catalepsy test, which is consistent with the findings of Amoateng et al. (2017).

In conclusion, the study showed that EECL demonstrates antipsychotic-like effect in experimental models of schizophrenia for the first time. We showed that acute EECL supplementation prevents the neurobehavioral impairments linked to the positive, negative, and cognitive symptoms seen in schizophrenia similarly to clozapine (Fig. 10). Notably, the bioactive metabolites of EECL may be responsible for the antipsychotic-like effect demonstrated in this study. We also showed that EECL has a high safety profile and is devoid of extrapyramidal symptoms. Thus, these findings suggest that EECL might be an excellent candidate for further investigation of possible underlying mechanisms of action.

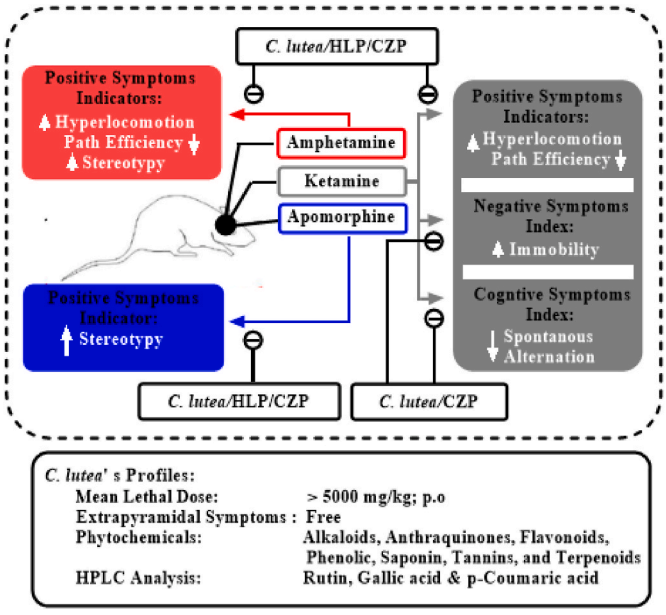


Fig. 10. Acute antipsychotic-like activity of EECL vs schizophrenia-like symptoms in experimental mice models of psychosis.

Consent for publication

Not applicable.

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CRediT authorship contribution statement

Noah A. Omeiza: conceived and designed the experiments, carried out the experiments, analyzed and interpreted the data, Formal analysis, Data curation, wrote the manuscript, Writing – original draft. **Adewale G. Bakre:** conceived and designed the experiments, supervised the work, Supervision. **Halimat A. Abdulrahim:** carried out the experiments. **Happy Isibor:** Data curation, carried out the experiments. **Precious U. Ezurike:** Data curation, carried out the experiments. **Abimbola A. Sowunmi:** analyzed and interpreted the data, Formal analysis, Data curation. **Benneth Ben-Azu:** analyzed and interpreted the data, Formal analysis, Data curation, critically revised the manuscript. **Adegbuyi O. Aderibigbe:** conceived and designed the experiments, critically revised the manuscript, supervised the work, Supervision, All authors reviewed, edited, and approved the manuscript, Writing – review & editing.

Declaration of competing interest

The authors declare no conflict of interest, financial or otherwise.

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List of Abbreviation

5-HT	5-hydroxytryptamine; serotonin
AMP	Amphetamine
APO	Apomorphine
AUC	Area under curve
CZP	Clozapine
DA	Dopamine
DAT	Dopamine transporter
EECL	Ethanol extract *Carpolobia lutea*
EPS	Extrapyramidal symptoms
FST	Force swimming test
FRIN	Forestry Research Institute of Nigeria
GABA	Gamma-aminobutyric acid
HLP	Haloperidol
KET	Ketamine
LD_{50}	Mean lethal dose
NMDA	N -methyl-D-aspartate
NMDARs	N -methyl-D-aspartate receptor
OFT	Open field test
SERT	Serotonin transporter
YMT	Y-maze test

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