Research Paper

Antipsychotic-like effect of ethyl acetate fraction of *Terminalia macroptera* leaf in mice

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ABSTRACT

Psychosis is a chronic neuropsychiatric disorder that affects millions of individuals worldwide and impairs the quality of life and productivity of the patients. *Terminalia macroptera* Guill & Perr. (Combrataceae) is a plant that is used in the management of anxiety related disorders. The present study investigates the antipsychotic effects of ethyl acetate fraction of *T. macroptera* (EFTM) leaf in ketamine-induced psychosis in mice. Acute toxicity of EFTM was determine using Lorke's method. Ketamine (25 mg/kg) was injected once daily for 7 consecutive days in Swiss albino mice to induce psychosis. The effect of the extracts (100, 200 and 400 mg/kg) was evaluated against psychotic-like behaviors induced by ketamine including locomotor activity and stereotypy in the open field test, immobility duration in the forced swim test, and memory impairment using the Y- maze test. The acute anti-psychotic effect of EFTM was evaluated on apomorphine climbing test, while woodblock test was performed to assess its extrapyramidal side effects. The LD_{50} was found to be 3807 mg/kg p.o. which is considered safe. EFTM (100, 200 and 400 mg/kg) exhibited significant antipsychotic effect by reducing ketamine-induced hyperactivity, immobility, and memory deficit in mice, EFTM also suppressed stereotypic climbing behavior due to apomorphine. Accordingly, the antipsychotic activity of EFTM was not associated with extrapyramidal side effects as evidenced by lack of catalepsy. The study revealed that EFTM ameliorated psychotic-like symptoms and is devoid of extrapyramidal side effects in mice, underscoring its antipsychotic-like effect.

1. Introduction

Schizophrenia is one of the crucial neuropsychiatric disorders characterized by a severe and chronic mental impairment (Calne, 1980). It is manifested by numerous symptoms which affect the emotion, thoughts, perception, and will and commonly known to impair the quality of life of the patients (Geyer and Vollenweider, 2008). It is often categorized into positive (e.g., behavioral hyperactivity and hallucination), negative (e. g. affect flattening) and cognitive (e.g., learning and memory impairment) symptoms (Coyle, 2006).

Deregulation of dopaminergic neurotransmission, hypofunction of N-Methyl-D-aspartic acid (NMDA) receptors and gamma-amino butyric acid (GABAergic) activity, neuro-inflammation, reduced cholinergic firing, and increased oxidative stress have been confirmed to play prominent roles in the pathophysiology of schizophrenia (Yadav et al., 2018). A number of experimental models have been proposed to portray some core features of schizophrenia, including sub-anesthetic doses of NMDA receptor antagonists, such as phency-clidine (PCP),) dizocilpine (MK-801) and ketamine to induce

behavioral responses that model positive, negative, and cognitive schizophrenia-like symptoms in healthy human volunteers (Javitt and Zukin, 1991) and experimental animals (Chatterjee et al., 2012). And to test the preclinical antipsychotic profile of medicine such as apomorphine induced climbing test (Costall et al., 1978). Also, (Seeman, 2002) showed that ketamine is known to present as indirect dopamine D2 receptor antagonist; which are the major molecular culprits implicated in the pathophysiology of schizophrenia and its treatment targets (Chindo et al., 2012).

Classical neuroleptics used in the management of schizophrenia, such as Chlorpromazine and haloperidol, are inefficient against the negative and cognitive symptoms of the disease. Furthermore, the use of these conventional antipsychotics are linked to severe adverse effects, markedly extrapyramidal symptoms (EPS) and tardive dyskinesia, (Martin, 1997). However, the atypical class are effective in ameliorating the positive, negative and the cognitive symptoms and have reduced extrapyramidal side effects, but highlights greater risk of metabolic disorders such as diabetes and agranulocytosis. Also, long-term use of these drugs have been reported to induce oxidative imbalance and

thereby further worsening the progression of the disease (Meyer, 2011). Thus, the search for more effective antipsychotic agents with better or tolerable adverse effect is highly imperative. Accordingly, studies are now focused on searching for new and safer molecules from natural resources to decrease the severity and the progression of mental disorders (Chindo et al., 2012).

Terminalia macroptera Guill. & Perr., belong to the family of Combretaceae, is a tree up to 20 m in height. It is widely distributed in West Africa, occurring from Senegal, to Cameroun occasionally as far as Sudan and Uganda (Arbonnier, 2004). Literature survey reveals that *T. macroptera* possess various pharmacological properties including antianxiety (Bum et al., 2012), antioxidant (Zou et al., 2014; Kone et al., 2012), Immunomodulatory (Zou et al., 2014) and antimicrobial (Pham et al., 2011; Silva et al., 2002) effects. From a methanol extract of the leaves of the medicinal tree *Terminalia macroptera, cis*-polyisoprene, chebulic acid trimethyl ester, methyl gallate, shikimic acid, corilagin, rutin, narcissin, chebulagic acid, and chebulinic acid, were isolated. The antioxidant and radical scavenging effects of some of the substances identified in this plant may to some extent explain the medicinal uses of this tree in West Africa (Pham et al., 2011). Accordingly, the leaves of *T. macroptera* have been used as a common remedy for the treatment of psychosis among traditional medicine practitioners (Ior et al., 2017). but its antipsychotic property has never been investigated. Sequel to some preliminary neuropharmacological investigations on the leaf extract of *T. macroptera*, we therefore hypothesized that *T. macroptera* leaf could reverse the behavioral abnormalities induced by ketamine or apomorphine in mice. Therefore the present study was designed to investigate the effects of the ethyl acetate fraction of *T. macroptera* (EFTM) in ketamine and apomorphine-induced schizophrenia-like behavior in mice.

2. Materials and methods

The fresh leaves of *T. macroptera* were collected, from its natural habitation at Jengre, Bassa Local Government Area of Plateau State, Nigeria. The plants were first identified on the field using keys and description given in the Flora of West Tropical Africa. (Hutchinson and Dalziel, 1958). They were later authenticated in the herbarium section of the Federal College of Forestry, Jos, by Mr. J.J. Azila, a taxonomist. A voucher specimen (FHJ 227) was deposited at the herbarium for future reference. The dried and powdered leaves of *T. macroptera* was extracted by cold maceration for 72 h in 70% methanol, the dried extract was then subjected to liquid/ liquid fractionation using n-hexane, ethyl acetate, butanol and water, to yield n-hexane, ethyl acetate, n-butanol and aqueous fractions 1.9%, 8.3%, 4.7% and 5.4% respectively. After a pilot bioassay of all the fractions (result not shown), EFTM was chosen for this study.

2.1. Experimental animals

Male and female Swiss albino mice weighing between 25 and 30 g (7 weeks old) were used for the experiment. They were kept in a well-ventilated room in hygienic plastic cages with wood shavings as bedding in the animal facility of the Department of Pharmacology, Faculty of Pharmaceutical Sciences, University of Jos. Nigeria. The animals were kept under standard environmental conditions; temperature of 25 °C and a 12 h day/night cycle. They were given free access to food (standard animal feed) produced by the Animal house, University of Jos and clean water ad libitum under hygienic conditions. The animals were allowed to acclimatize in the laboratory environment prior to any experiment. Experimental animal groups used in the present study consisted of six mice in each group. The investigation conformed to the Guide for the Care and Use of Laboratory Animal Published by the US National Institute of Health (NIH No. 85–23, revised 1996). All protocols were also approved by the University of Jos Animal Care and Use in collaboration with the office

of Laboratory Animal Welfare (OLAW). With Reference: Assurance Approval- UJ/FPS/F17-00379.

2.2. Drugs and chemicals

All drugs and extract were prepared fresh on the days of the experiment, they were dissolved in normal saline. EFTM was first dissolved in dimethyl sulfoxide (DMSO), to improve solubility before diluting in normal saline. Vehicle (normal saline) was administered in a volume of 10 mL/kg/body weight. Drugs used include Normal saline (Dana Pharmaceuticals, Nigeria), haloperidol, apomorphine hydrochloride, risperidone, ketamine hydrochloride (Sigma Aldrich), and chemicals include methanol, ethyl acetate, n- butanol, n-hexane (Sigma-Aldrich, St. Louis, USA).

2.3. Acute studies

2.3.1. Preliminary study

An acute preliminary study was done to determine the optimum dose of EFTM that blocks the ketamine induced hyperlocomotor activity in mice. A graded dose of EFTM was administered in a dose range of 50–600 mg/kg p.o., 1 h prior to ketamine (25 mg/kg) administration, Mice were distributed into seven groups of 6 mice each, as follows: the control received saline only at 0 min and 60 min, the ketamine group received Ketamine (25 mg/kg, i.p.) and saline 1 h later, groups 3, 4, 5, 6 and 7 received graded doses of EFTM (50, 100, 200, 400 and 600 mg/kg) respectively and ketamine (25 mg/kg i.p.) 1 h later. The number of line crossing in the open field apparatus was used as an end-point assay to determine the optimum EFTM concentration that diminishes the ketamine-induced hyperlocomotion.

2.3.2. Acute oral toxicity study

The acute oral toxicity of EFTM was evaluated in Swiss albino mice (Lorke, 1983). The study involved two phases; the first phase was carried out as follows. Nine mice were grouped into three groups (n = 3). The mice were weighed and the doses were calculated according to their body weight. The crude extracts were suspended in normal saline. Each group received 10, 100 and 1000 mg/kg p.o. body weight of EFTM respectively. The animals were carefully observed for about 60 min for signs of toxicity or mortality, and subsequently the animals were observe every 8 h for a period of 24 h after administration of the fraction. When no mortality was observed in this phase, the second phase of the experiment was conducted, three mice were grouped into three groups of one mice each. The groups received 1500, 2900 and 5000 mg/kg per oral (p.o.) body weight of EFTM respectively. The mice were observed for signs of toxicity or mortality for another 24 h. The LD50 was calculated using the formula: $\sqrt{(D_0 \times D_{100})}$ where D_0 = Highest dose that produced no mortality in mice, D_{100} = Lowest dose that produced mortality.

2.4. Effect of EFTM on apomorphine induced stereotypic climbing

The method previously described by Costall et al. (1978) was adopted. This test examines the neuroleptic (anti-dopaminergic) potentials of EFTM. Thirty mice were randomly distributed into 5 groups of 6 mice each. Animals in the first group were administered 10 mL/kg normal saline p.o 1 h before 1.5 mg/kg apomorphine intraperitoneally (i.p.). Risperidone (an atypical antipsychotic drug) was used as positive control in this experiment. Risperidone was orally administered at 0.5 mg/kg dose 1 h before apomorphine 1.5 mg/kg i.p. EFTM was administered at doses 100, 200, and 400 mg/kg (p.o.) 1 h before the administration of apomorphine. After the administration of apomorphine, each mouse was placed in a cylindrical mesh cage and the frequency and duration of climbing was observed for 30 min at intervals of 10 min

2.5. Effect of EFTM on spontaneous motor activity

The open field was employed to determine the effect of EFTM on spontaneous motor activity (SMA) in mice. Mice (n = 6) were grouped into 5 treatment groups. Group 1 received saline 10 mL/kg. Animals in 3 groups were treated with EFTM (p.o.) 100, 200 and 400 mg/kg respectively. The atypical antipsychotic drug risperidone, used as positive control, was administered at 0.5 mg/kg p.o to animals in group 5. One hour later, each animal was kept in the center of an open field chamber (72 cm × 72 cm× 36 cm). The number of lines crossed and the duration of ambulation(s) for 5 min was recorded (Brown et al., 1999).

2.6. Ketamine administration

Repeated ketamine administration induces behavioral deficits in rodents that are thought to recapitulate some cardinal features of schizophrenia including positive, negative, and cognitive symptoms (Chatterjee et al., 2015; Monte et al., 2013). The effect of EFTM was investigated on hyperlocomotion in the open field test, behavioral despair in the forced swim test and spatial memory in the Y-maze test induced by daily injection of ketamine in mice for 7 consecutive days. Ketamine (25 mg/kg) i.p. was administered daily. Risperidone, used as positive control, was administered at 0.5 mg/kg (p.o.) as above, 1 h after every dose of ketamine. The EFTM was prepared in saline and administered at doses of 100, 200, and 400 mg/kg (p.o.) respectively, 1 h after every dose of ketamine (Chatterjee et al., 2015).

2.7. Behavioral assessment of EFTM in Ketamine Induced Psychosis (KIP)

2.7.1. Open-field test
The open field test was used to evaluate the antipsychotic-like effect of EFTM on hyperlocomotion induced by the daily injection of ketamine (Ben-Azu et al., 2017). The experiments were carried out 24 h following the last dose of the 7 days administration of ketamine. The mice were placed individually in the center square of the open field chamber. The number of lines crossed were observed and recorded for a period of 5 min

2.7.2. Y-maze test
Memory function assessed by percentage alternation behavior using Y-maze test was evaluated as previously described (Casadesus et al., 2006). The apparatus consists of three identical arms (labeled A, B, C) at 120° to each other. Immediately after the test of locomotor activity (24 h following the last dose of the 7 days administration of ketamine), mouse were gently kept at the center of the Y-maze and allowed to freely explore all the three arms of the maze for a period of 5 min. The number and sequence of arm entries were observed and recorded, the apparatus was cleaned with 70% methanol following each test session to remove the residual odor from the preceding mice. The percentage alternation, which is a measure of spatial working memory (Monte et al., 2013), was calculated by dividing the total number of alternations by the total number of arm entries minus one and multiplied by 100. As shown in the formula: Percent alternations $= \frac{\text{Total number of alternations}}{\text{Total number of arm entries}-1}$ X100.

Alternation behavior was defined as consecutive entries into all three arms (i.e., ABC, CAB, or BCA but not BAB).

2.7.3. Forced swim test
The effect of EFTM on ketamine-enhanced immobility in forced swim test was evaluated using the method previously described by Chindo et al. (2012). Each mouse was placed in a standardized non opaque glass cylinder (height 44 cm, diameter 20 cm) filled with water to a depth of 30 cm at 25 °C and allowed to swim for 5 min (pretest session). Thereafter, mice received the treatment they had before for 7 days (2.6). One hour later, each animal was placed in the same transparent glass cylinder containing water to a depth of 30 cm at 25 °C and forced to swim

for 6 min, the immobility duration (the time the mouse glides in the water in an erect position and made only minor movements to avert sinking) was noted with a stopwatch and recorded. After each test, the mice were immediately removed from the glass cylinder, dried up with a towel and placed in an open space until entirely dried before returning to their home cages.

2.8. Effect of EFTM on catalepsy test

The propensity for EFTM to cause extrapyramidal symptoms was evaluated in the woodblock catalepsy test, according to the modified method previously described by (Costall and Naylor, 1974). Mice randomly distributed into 6 groups (n = 6), received once daily administration of one of the following treatments for 7 consecutive days: Normal saline, the typical antipsychotic haloperidol (1 mg/kg, p.o.), EFTM (100, 200, or 400 mg/kg, p.o.), or risperidone (0.5 mg/kg, p.o.). At 60 and 90 min after the last drug administration, mice were tested for catalepsy behavior. Each mouse was clasped gently on the shoulder and below the forepaws and carefully positioned on the upper edge of the wood block surface (L = 15 cm; W = 4 cm; H = 6) cm). The descent latency (DL) was taken as the time it took the mouse to descend from the wood block. A mousel was considered cataleptic if it remained on the block for more than 60 s (Ben-Azu et al., 2018).

2.9. Statistical analysis

The data were expressed as mean ± standard error of the mean (SEM). Statistical differences between control and treated were determined by analysis of variance (ANOVA) with Tukey post hoc test. Two way ANOVA was used in the apomorphine climbing tests and the catalepsy test considering the between factor treatment and the time course. Using Graph Pad Prism software version 6. A level of P < 0.05 was considered statistically significant for all tests.

3. Results

3.1. Preliminary acute test

The result of the acute preliminary test on graded doses of EFTM on locomotor activity showed a dose dependent decrease in the number of lines crossed. However, only 100–600 mg/kg groups were significantly (p < 0.0001) different from the ketamine only group, the decrease in line crossing caused by the 600 mg/kg EFTM group (mean - 42.3) was not appreciably different from the 400 mg/kg group (mean - 44.3) indicating that increase in dose beyond the 400 mg/kg was not necessary. From this findings EFTM 100–400 mg/kg groups produced the optimum activity against the ketamine induced hyperlocomotor activity, and were selected for further experiments. Fig. 1.

3.2. Acute toxicity test

Phase 1	Group	Dosage (mg/kg)	Number of death
	1.	10	0/3
	2.	100	0/3
	3.	1000	0/3
Phase 2	1.	1600	0/3
	2.	2900	0/3
	3.	5000	1/3

From the table $D_0 = 2900$ and $D_{100} = 5000$.

Therefore, the LD$_{50}$ of EFTM $=\sqrt{(D_0 \times D_{100})}=\sqrt{2900x5000}$ = 3807.89 mg /kg.

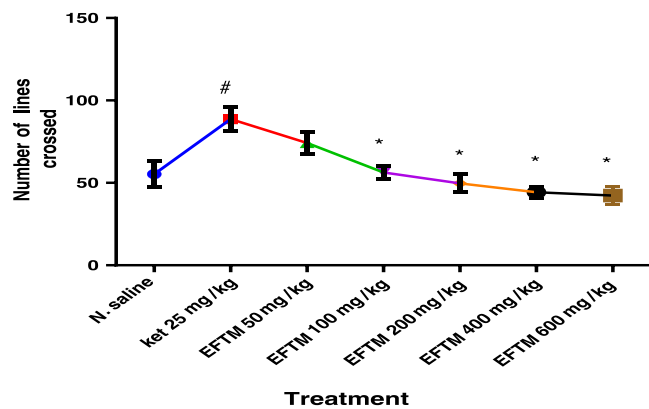The oral LD$_{50}$ of EFTM was found to be 3807 mg/kg.

Fig. 1. Acute effect of graded doses of EFTM on Ketamine-induced hyperlocomotion in open field test in mice. Values were expressed as mean ± S.E.M of 6 animals/group. # P < 0.05 compared to Ns group; * p < 0.05 compared to N saline + ketamine group (One way ANOVA followed by Tukey post-hoc test). Ket = Ketamine.

3.3. EFTM attenuated apomorphine-induced stereotypic climbing behavior

EFTM significantly attenuated apomorphine-induced stereotypic climbing behavior. As shown in Tables 1 and 2, intraperitoneal injection of apomorphine (1.5 mg/kg) resulted in marked stereotypic climbing behavior in mice characterized by initial rearing compared to vehicle control in mice at 10, 20, and 30 min. Pretreatment with EFTM at doses of 100, 200 and 400 mg/kg, p.o. significantly reduced the stereotyped climbing behavior (p < 0.05) and duration of stereotypic climbing (p < 0.05) at different time points ranging from 10 to 30 min. As expected, risperidone (0.5 mg/kg) also significantly decreased apomorphine-induced stereotyped climbing behavior at 10 and 20 min, to attain a total abolishment of the stereotypies at 30 min

3.4. EFTM mitigates spontaneous motor activity in open-field test

The effect of EFTM and risperidone on SMA in the open-field test is shown in Fig. 2. The extract or risperidone reduced SMA [F$_{(4, 25)}$ = 41.25 $P < 0.0001$]. Precisely, one-way ANOVA and Tukey post-hoc test showed that, EFTM at doses of 100, 200 and 400 mg/kg significantly mitigates SMA compared to vehicle-treated mice ($p < 0.05$); Risperidone (0.5 mg/kg, p.o.) significantly reduced SMA compared to saline group.

3.5. EFTM attenuates ketamine-induced hyperlocomotion in mice

The effect of EFTM and risperidone on ketamine-induced hyperlocomotion is shown in Fig. 3. One-way ANOVA and Tukey post-hoc test revealed that repeated treatment with ketamine (25 mg/kg) significantly induced hyperlocomotion when compared with the saline group

Table 1
Effect of Ethyl acetate Fraction of *T. macroptera* on Apomorphine (4 mg/kg) induced Stereotypic Climbing in Mice.

Treatment	Dose (mg/kg)	Number of Climbs at		
		10 min	20 min	30 min
Normal Saline	10 mL/kg	4.60 ± 0.03	4.58 ± 0.01	4.73 ± 0.04
EFTM	100	2.02 ± 0.05*	1.88 ± 0.04*	2.06 ± 0.05*
EFTM	200	2.03 ± 0.02*	1.91 ± 0.014*	2.06 ± 0.03*
EFTM	400	2.71 ± 0.03*	2.57 ± 0.05*	2.26 ± 0.05*
Risperidone	0.5	2.03 ± 0.18*	1.17 ± 0.38*	0.00 ± 0.00*

Values were expressed as mean ± S.E.M of (n = 6).
* $p < 0.05$ as compared to Normal saline group.

Table 2
Effect of Ethyl acetate Fraction of *T. macroptera* on the Duration of Apomorphine-induced Stereotypic Climbing in Mice.

Treatment	Dose (mg/kg)	Duration of Climbs at		
		0 − 10 min	10−20 min	20–30 min
Normal Saline	10 mL/kg	438.33 ± 7.62	411.67 ± 5.16	217.50 ± 3.68
EFTM	100	263.83 ± 4.73*	243.17 ± 4.16*	183.50 ± 2.32*
EFTM	200	256.83 ± 1.70*	231.17 ± 2.78*	142.50 ± 2.14*
EFTM	400	230.83 ± 2.80*	199.50 ± 18.28*	130.17 ± 2.09*
Risperidone	0.5	10.50 ± 1.26*	3.17 ± 1.28*	0.00 ± 0.00*

Values were expressed as mean ± S.E.M of (n = 6).
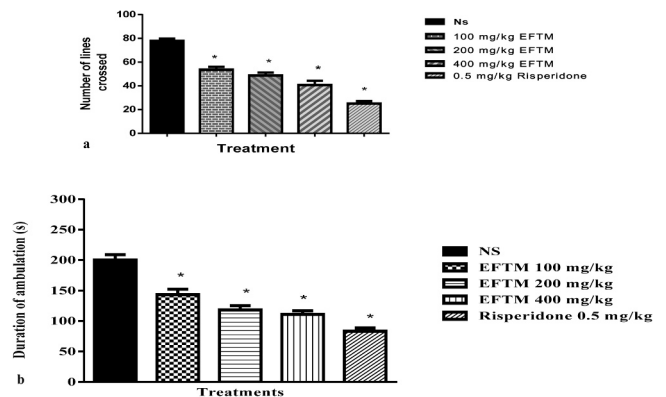* $p < 0.05$ as compared to Normal saline group.

Fig. 2. a Effect of EFTM on number of line crossing in open-field test in mice. Bars value were expressed as mean ± S.E.M of 6 animals/group. * P < 0.05 compared to Ns group (One way ANOVA followed by Tukey post-hoc test). Ns = Normal saline. b Effect of EFTM on number of line crosing in open-field test in mice. Bars value were expressed as mean ± S.E.M of 6 animals/group. * P < 0.05 compared to Ns group (One way ANOVA followed by Tukey post-hoc test). Ket + ketamine, Ns = Normal saline.
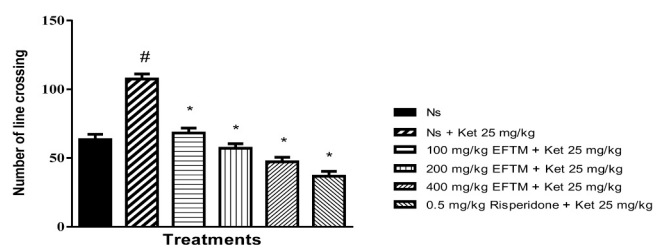

Fig. 3. Effect of EFTM on Ketamine-induced hyperlocomotion in open field test in mice. Bars value were expressed as mean ± S.E.M of 6 animals/group. #P < 0.05 compared to Ns group; * p < 0.05 compared to Ns + ketamine group (One way ANOVA followed by Tukey post-hoc test). Ket = Ketamine, Ns = Normal saline.

($p < 0.05$). Pretreatment with EFTM (100, 200 and 400 mg/kg, p.o.) significantly alleviates hyperlocomotion induced by ketamine [F$_{(5, 30)}$ = 221.9; $P < 0.0001$]. Similarly, pretreatment with risperidone (0.5 mg/kg, p.o.) significantly ($p < 0.05$) decreased hyperlocomotion in ketamine treated mice.

3.6. EFTM attenuates ketamine-induced cognitive impairment in mice

The effects of EFTM and risperidone on cognitive deficits induced by injection of ketamine in mice using Y maze test is shown in Fig. 4. Repeated injection of ketamine decreased the percent of alternations performance when compared with vehicle-treated mice ($p < 0.05$ following one way ANOVA analysis. Pretreatment with EFTM at (100,
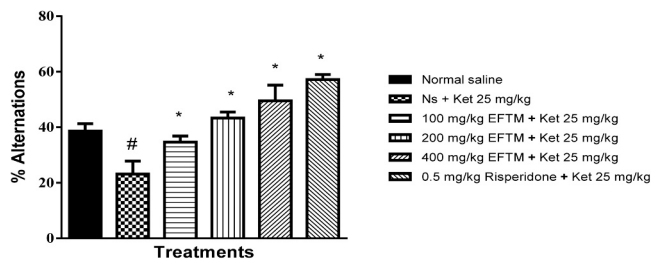
Fig. 4. Effect of EFTM on Ketamine-induced Cognitive Deficit Bars value were expressed as mean ± S.E.M of 6 animals/group. # $P < 0.05$ compared to Ns group, * $P < 0.05$ compared with ketamine + Ns group (One way ANOVA followed by Tukey post-hoc test). Ket = Ketamine, Ns = Normal saline.

200 and 400 mg/kg p.o.) significantly alleviates ketamine-induced cognitive deficit in a dose-dependent manner [$F_{(5, 30)} = 56.26$; $P < 0.0001$]. Similarly, risperidone (0.5 mg/kg, p.o.) significantly alleviated memory deficit due to ketamine treatment (p < 0.05).

3.7. Effect of EFTM on ketamine-enhanced immobility in forced swim test

The effect of EFTM and risperidone on ketamine-enhanced immobility time in forced swim test in mice is shown in Fig. 5. Repeated administration of ketamine produced a significant upsurge in the duration of immobility when compared with saline-treated mice ($p < 0.05$). However, treatment with both EFTM (100, 200 and 400 mg/kg, p.o.) and risperidone (0.5 mg/kg, p.o.) significantly alleviate [$F_{(5, 30)} = 169.6$; $P < 0.0001$] ketamine-induced increased duration of immobility in the forced swim test.

3.8. Effect of EFTM on catalepsy test in mice

The catalepsy effect of EFTM as observed on DL in the wood block test is shown in Fig. 6. Haloperidol (1 mg/kg, p.o.)-treated mice showed significant (p < 0.05) increase in DL on the wood block at 60 and 90 min compared to saline-treated mice. Administration of EFTM at 100, 200 and 400 mg/kg p.o. or risperidone (0.5 mg/kg) did not increase the DL of mice after 60 and 90 min post treatments relative to vehicle control groups.

4. Discussion

The result of this study provide evidence that, EFTM leaf has potential value in the management of psychotic like symptoms. The acute toxicity study revealed that EFTM at an LD_{50} of 3807 mg/kg caused no observable signs of toxicity or mortality in mice, signifying its obvious safety (Lorke, 1983). EFTM attenuated spontaneous motor activity, apomorphine stereotypic climbing behavior and ketamine-induced hyperlocomotion, cognitive deficit in the Y- maze test, and immobility
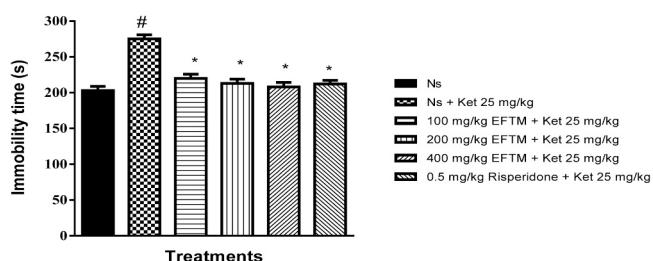

Fig. 5. Effect of EFTM on ketamine-enhanced immobility in forced swim test. Bars value were expressed as mean ± S.E.M of 6 animals/group. # $P < 0.05$ compared to vehicle group, *$P < 0.05$ compared with ketamine + Ns group. (One way ANOVA followed by Tukey post-hoc test). Ket = Ketamine, Ns = Normal saline.
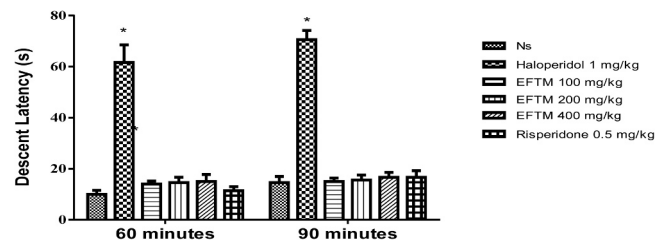

Fig. 6. Effect of EFTM on Catalepsy test in mice: Bars value were expressed as mean ± S.E.M of 6 animals/group. * P < 0.05 compared to Risperidone group. Data was considered significant above 60 s (two way ANOVA followed by Bonferroni post-hoc test). Ns = normal saline.

in the forced swim test. Accordingly, EFTM did not increase descent latency in the catalepsy test in contrast to haloperidol.

EFTM dose dependently decreased spontaneous motor activity, ketamine induced hyperlocomotion and apomorphine climbing behavior in a similar manner as risperidone. These effects suggest that EFTM possess antipsychotic-like activity against the positive symptoms of psychosis. Previous studies confirmed that atypical antipsychotic-like drugs mitigates psychotic-like symptoms such as; spontaneous motor activity, ketamine-induced hyperactivity and apomorphine-induced stereotypic climbing behavior respectively (Ben-Azu et al., 2018; Chatterjee et al., 2012; Costall et al., 1978; Pandy et al., 2012). The discovery that EFTM attenuated SMA, hyperactivity as well as apomorphine induced stereotypic climbing suggest a valuable role in psychosis. The ability of apomorphine to induce stereotypy has been well characterized and documented to be mediated through dopamine receptors stimulation (Pandy et al., 2012). Undeniably, dopamine hypothesis is a well-known theory on the pathophysiology of psychosis, the theory hinges on the blockade of dopamine receptors; a common mechanism of action of most antipsychotic agents (Meltzer, 1989). Consequently, the ability of EFTM to significantly attenuate the stereotyped behavior by apomorphine in mice may be related to anti-dopaminergic activity. Ketamine-induced hyperlocomotion and stereotypy; also describes an aspect of positive symptoms of schizophrenia (Vijeepallam et al., 2016), antipsychotic agents attenuate these symptoms. Furthermore, biochemical data have revealed that dopaminergic and glutamatergic neurotransmitters are implicated in central nervous system excitation (Yadav et al., 2018). Ketamine-induced hyperlocomotion and stereotypic behaviors were partly ascribed to the blockade of NMDA receptors situated on the inhibitory GABAergic neurons in the mesolimbic brain regions, leading to disinhibition and the consequential increase in neuronal excitations (Stahl, 2007). Also, ketamine may act as indirect dopamine agonist which explains the basis of ketamine-induced behavioral stimulation (Irifune et al., 1991). In this study, EFTM attenuated the increased in locomotor activity by ketamine, as evidenced by the decreased number of line crossing. Accordingly, these findings showed that EFTM may be a likely agent for the management of schizophrenia-like hyperactivity; resulting from NMDA receptors blockade on the inhibitory GABAergic system, that is often regarded as schizophrenia associated behavioral hyperactivity (Gottlieb et al., 2006).

EFTM also alleviated ketamine-induced cognitive deficit in Y-Maze test in a dose dependent manner. Risperidone was also effective as observed from earlier findings that report the amelioration of cognitive deficit in the Y-maze test by antipsychotic drugs (Ben-Azu et al., 2018; Monte et al., 2013). The propensity of EFTM to improve cognitive functions in psychotic disorders was evaluated in mice repeatedly treated with ketamine using Y-maze model. The Y-maze test is a well-documented animal paradigm used regularly for assessing agents with memory-enhancing property in rodents (Dawson et al., 1992). The Y-maze test is centered on the discovery that rodents tend to recall the sequence of arm entry, well known as spontaneous alternations. Rodents are known to always recall the last arm entered in order to alternate the

choice of arm, making the Y-maze test an appropriate model for short-term working memory (Casadesus et al., 2006). The finding that EFTM leaf significantly attenuate in a dose-dependent manner the cognitive dysfunction induced by ketamine implies that the plant may be valuable in the management of psychotic patients with memory deficit. Besides, cognitive dysfunction is particularly important because of its role in the quality of life of psychotic patients (Barch and Sheffield, 2014; Draper et al., 2009). Memory impairment triggered by ketamine is known to be connected to antagonism of glutamatergic neurotransmission, as well as stimulation of oxidative stress in the brain, acetylcholine, the neurotransmitter that performs critical role in memory, was seen to be reduced after ketamine injection (Chatterjee et al., 2012). Upsurge in the activity of acetylcholinesterase in the brain particularly in the hippocampal area also lends credence to ketamine-induced memory deficits (Ben-Azu et al., 2018; Chatterjee et al., 2012).

EFTM also attenuated the immobility duration in forced swim test, depicting its efficacy on the negative symptoms of schizophrenia. The negative symptoms of schizophrenia is a crucial feature of the disorder (Coyle, 2006). They are frequently present in the initial phase of the illness and endure throughout the entire progression of the disease, even during times of remission. The increase in immobility in forced swim test after repeated injection of ketamine, known as behavioral despair was previously described as a model of negative symptoms of schizophrenia, (Chatterjee et al., 2012; Chindo et al., 2012). Administration of EFTM decreased the period of immobility in mice in the forced swim test in a comparable fashion as risperidone. Risperidone and other antipsychotic drugs that are effective in the management of negative symptoms of schizophrenia act as antagonists of 5HT-2 receptors. Accordingly, this finding implies that EFTM possess atypical antipsychotic-like effect that warrant further assessment.

The catalepsy test indicated that EFTM in the same manner as risperidone did not cause catalepsy in mice. The stimulation of catalepsy in animals by typical antipsychotic agents like haloperidol is due to blocked of dopaminergic neurotransmission (Hoffman and Donovan, 1995). Therefore, the catalepsy test, as a model for assessing the propensity of antipsychotics to induce extra-pyramidal side effects based on the protraction of the period of akinesia, is normally carried out in rodents to differentiate between typical and atypical antipsychotic agents.

5. Conclusion

The study revealed that EFTM possess an atypical antipsychotic-like profile with modest efficacy against positive, negative and cognitive symptoms of schizophrenia, devoid of extrapyramidal adverse effects. This suggests that EFTM is safe and may be beneficial in the treatment of psychotic – like symptoms.

Compliance with Ethical Standards

Acknowledgment

Conflict of Interest

The authors declare no conflict of interest.

References

Arbonnier, M., 2004. Trees, shrubs and lianas of West Africa Dry Zones. Margraf Publishers, Weikersheim.

Barch, D.M., Sheffield, J.M., 2014. Cognitive impairments in psychotic disorders: common mechanisms and measurement. World Psychiatry 13, 224–232 http://doi:10.1002/wps.20145.

Ben-Azu, B., Aderibigbe, A.O., Omogbiya, I.A., Ajayi, A.M., Owoeye, O., Olonode, T., Iwalewa, E.O., 2018. Probable mechanisms involved in the antipsychotic-like activity of morin in mice. Biomed. Pharmacother. 105, 1079–1090. https://doi.org/10.1016/j.biopha.2018.06.057.

Ben-Azu, B.A., Omogbiya, I.A., Ajayi, A.M., Iwalewa, E.O., 2017. Morin pretreatment attenuates schizophrenia-like behaviors in experimental animal models. Drug Res. 67, 1–9. https://doi.org/10.1055/s-0043-119127.

Brown, R.,E., Corey, S.C., Mooren, A.K., 1999. Differences in measures of exploration and fear in MHC congenic C578/61 and B6-H-2K mice. Behav. Genet. 26, 263–271. https://doi.org/10.1023/a:1021694307672.

Bum, E.N., Neteydji, S., Djafsia, G., Njifutie, N., Taiwe, G.S., Rakotonirina, S.V., Rakotonirina, A., 2012. The aqueous extract of *Terminalia macroptera* possess anxiolytic and antipyretic activities in mice. Asian J. Pharm. Health Sci. 2 (4), 555–561.

Calne, D.B., 1980. Therapeutics in Neurology, Second ed. Blackwell Scientific Publications, Oxford, England.

Casadesus, G., Webber, K.M., Atwood, C.S., Pappolla, M.A., Perry, G., Bowen, R.L., Smith, M.A., 2006. Luteinizing hormone modulates cognition and amyloid-beta deposition in Alzheimer APP transgenic mice. Biochim. Biophys. Acta 1762, 447–452. https://doi.org/10.1016/j.bbadis.2006.01.008.

Chatterjee, M., Singh, S., Kumari, R., Verma, K.A., Palit, G., 2012. Evaluation of the antipsychotic potential of *Panax quinquefolium* in ketamine induced experimental psychosis model in mice. Neurochem. Res. 37, 759–770. https://doi.org/10.1007/s11064-011-0670-4. Epub 2011 Dec 22.

Chatterjee, M., Verma, M., Kumari, R., Singh, S., Verma, A.K., Dwivedi, A.K., Palit, G., 2015. Antipsychotic activity of standardized Bacopa extract against ketamine-induced experimental psychosis in mice: evidence for the involvement of dopaminergic, serotonergic, and cholinergic systems (Early Online:). Pharm. Biol. 53, 1850–1860. https://doi.org/10.3109/13880209.2014.976350.

Chindo, B.A., Bulus, A., Tijani, A.Y., Gamaniel, K.S., 2012. Ketamine-enhanced immobility in forced swim test: a possible animal model for the negative symptoms of schizophrenia. Prog. Neuropsychopharm. Biol. Psychiatry 38, 310–316. https://doi.org/10.1016/j.pnpbp.2012.04.018.

Costall, B., Naylor, R.J., 1974. On Catalepsy and Catatonia and the Predictability of the Catalepsy Test for Neuroleptic. Psychopharmacol (Berl) 34, 233–241. https://doi.org/10.1007/BF00421964.

Costall, B., Naylor, R.J., Nohria, V., 1978. Climbing behaviour induced by apomorphine in mice: a potent model for the detection of neuroleotic activity. Eur. J. Pharmacol. 50, 39–50. https://doi.org/10.1016/0014-2999(78)90251-0.

Coyle, J.T., 2006. Glutamate and schizophrenia: beyond the dopamine hypothesis. Cell. Mol. Neurobiol. 26, 365–384. https://doi.org/10.1007/s10571-006-9062-8.

Dawson, G.R., Heyes, C.M., Iversen, S.D., 1992. Pharmacological mechanisms and animal models of cognition. Behav. Pharmacol. 3, 286–297.

Draper, M.L., Stutes, D.S., Maples, N.J., Velligan, D.L., 2009. Cognitive adaptation training for outpatients with schizophrenia. J. Clin. Psychol. 8 (65), 842–853. https://doi.org/10.1002/jclp.20612.

Geyer, M.A., Vollenweider, F.X., 2008. Serotonin research: contributions to understanding psychoses. Trends Pharmacol. Sci. 29, 445–453. https://doi.org/10.1016/j.tips.2008.06.006.

Gottlieb, M., Rocı'o, L., Marı'a, R.C., 2006. Neuroprotection by two polyphenols following excitotoxicity and experimental ischemia. Neurobiol. Dis. 23, 374–386. https://doi.org/10.1016/j.nbd.2006.03.017.

Hoffman, D.C., Donovan, H., 1995. Catalepsy as a rodent model for detecting antipsychotic drugs with extrapyramidal side effect liability. Psychopharmacol. (Berl) 120 (2), 128–133. https://doi.org/10.1007/BF02246184.

Hutchinson, J., Dalziel, J.M., 1958. Flora of West Tropical Africa, Second ed. Crown agents for Oversea Governments and Adminstrations, London.

Ior, L.D., Otimenyin, S.O., Okwori, V.A., Umar, D.M., Azila, J.J., 2017. Ethnobotanical survey of plants used in the management of mental illnesses in some selected local government areas of Plateau State. Nigeria. J. Pharmacog. Phytother. 9, 146–156. https://doi.org/10.5897/JPP2017.0464.

Irifune, M., Shimizu, T., Nomoto, M., 1991. Ketamine-induced hyperlocomotion associated with alteration of presynaptic component of dopamine neurons in the nucleus accumbens of mice. Pharmacol. Biochem. Behav. 40, 399–407. https://doi.org/10.1016/0091-3057(91)90571-i.

Javitt, D.C., Zukin, S.R., 1991. Recent advances in the phencyclidine model of schizophrenia. Am. J. Psychiatry 10 (148), 1301–1308. https://doi.org/10.1176/ajp.148.10.1301.

Kone, D., Diop, B., Diallo, D.D., 2012. Identification Quantitative Determination, and Antioxidant Properties of Polyphenols of Some Malian Medicinal Plant Parts Used in

Folk Medicine. In. J. Uddin, Macro To Nano Spectroscopy 132–142. http://doi.org/10.5772/38345.

Lorke, D., 1983. A new approach to practical acute toxicity testing. Arch. Toxicol. 53, 275–287. https://doi.org/10.1007/bf01234480.

Martin, A.R., 1997. Antipsychotic Agents. In: Wolf, M.E. (Ed.), Burger's Medicinal Chemistry, Fifth ed. Wiley-Interscience, p. 195. https://doi.org/10.1002/ddr.430350207.

Meltzer, H.Y., 1989. Clinical studies on the mechanism of action of clozapine: the dopamine-serotonin hypothesis of schizophrenia. Psychopharmacol 99, 518–527. https://doi.org/10.1007/bf00442554.

Meyer, J.M., 2011. Pharmacotherapy of Psychosis and Mania. In: Brunton, L., Chabner, B., Knollman, B. (Eds.), Goodman & gilman's the pharmacological basis of therapeutics, Tenth ed. McGraw Hill. https://doi.org/10.1036/0071422803.

Monte, A.S., de Souza, G.C., McIntyre, R.S., Soczynska, J.K., dos Santos, J.V., Macêdo, D.S., 2013. Prevention and reversal of ketamine-induced schizophrenia related behavior by minocycline in mice: possible involvement of antioxidant and nitrergic pathway. J. Psychopharmacol. 27, 1032–1043. https://doi.org/10.1177/0269881113503506.

Pandy, V., Narasingam, M., Mohamed, Z., 2012. Antipsychotic-like activity of noni (Morinda citrifolia Linn.) in mice. BMC Complement Altern Med. 12 (12), 186. https://doi.org/10.1186/1472-6882-12-186. PMID: 23082808; PMCID: PMC3487797".

Pham, A.T., Malterud, K.E., Paulsen, B.S., Diallo, D., Wangensteen, H., 2011. DPPH radical scavenging and xanthine oxidase inhibitory activity of *Terminalia macroptera*

leaves. Nat. Prod. Commun. 6, 1125–1128. https://doi.org/10.1016/j.jep.2011.08.029.

Seeman, P., 2002. Atypical antipsychotics: Mechanism of action. Can. J. Psychiatry 47, 27–38. https://doi.org/10.1007/BF02246184.

Silva, O., Ferreira, E., Pato, M.V., Canic, M., Gomes, E.T., 2002. In vitro anti-Neisseria gonorrhoeae activity of *Terminalia macroptera* leaves. FEMS Microbiol. Letters. 217, 271–274. https://doi.org/10.1111/j.1574-6968.2002.tb11487.x.

Stahl, S.M., 2007. Beyond the dopamine hypothesis to the NMDA glutamate receptor hypofunction hypothesis of schizophrenia. CNS Spectr. 12, 265–268. https://doi.org/10.1017/S1092852900021015.

Vijeepallam, K., Pandy, V., Kunasegaran, T., Murugan, D.D., Naidu, M., 2016. Mitragyna speciosa leaf extract exhibits antipsychotic-like effect with the potential to alleviate positive and negative symptoms of psychosis in mice. Front Pharmacol. 7 (7), 464. https://doi.org/10.3389/fphar.2016.00464. PMID: 27999544; PMCID: PMC5138496.

Yadav, M., Parle, M., Sharma, N., Jindala, D.K., Bhidhasra, A., Dhingra, M.S., Dhingra, S., 2018. Protective effects of *Spinacia oleracea* seeds extract in an experimental model of schizophrenia: possible behavior, biochemical, neurochemical and cellular alterations. Biomed. Pharmacother. 105, 1015–1025. https://doi.org/10.1007/s10787-017-0366-8.

Zou, Y., Giang, T.T.K., Nhat, H.,T.L., Kari, T.I., Hilde, B., Drissa, D., Terje, E.M., 2014. Enzyme inhibition,antioxidant and immunomodulatory activities, and brineshrimp toxicity of extracts from the root bark,stembark and leaves of *Terminalia macroptera*. J. Ethnopharmacol. 155, 1219–1226. https://doi.org/10.1016/j.jep.2014.07.004.