British Journal of Pharmaceutical Research
12(6): 1-14, 2016, Article no.BJPR.28629
ISSN: 2231-2919, NLM ID: 101631759

SCIENCEDOMAIN *international*
www.sciencedomain.org

Ethanol Extracts of *Terminalia ivorensis* (Chev A.) Stem Bark Attenuates the Positive, Negative and Cognitive Symptoms of Psychosis in Experimental Animal Models

Benneth Ben-Azu[1*], Adegbuyi Oladele Aderibigbe[1], Olusegun Adebayo Adeoluwa[1,2] and Ezekiel Oluwagbenga Iwalewa[1]

[1]Department of Pharmacology and Therapeutics, College of Medicine, University of Ibadan, Ibadan, Oyo State, Nigeria.
[2]Department of Pharmacology and Therapeutics, College of Medicine and Health Sciences, Afe Babalola University, Ado Ekiti, Ekiti State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author BBA wrote the protocol, managed the experimental process, performed the statistical analysis of the study, managed the literature searches, wrote the first draft of the manuscript and final version of the manuscript. Author AOA designed the study, managed the experimental process and managed the analyses of the study, author OAA identified the species and extraction of the plant. Author EOI managed the analyses of the study and final version of the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJPR/2016/28629
Editor(s):
(1) Partha Krishnamurthy, Department of Pharmacology, Toxicology and Therapeutics, University of Kansas Medical Center, USA.
Reviewers:
(1) B. C. Ephraim-Emmanuel, Bayelsa State College of Health Technology, Bayelsa State, Nigeria.
(2) Fábio Tonissi Moroni, Universidade Federal de Uberlândia Faculdade de Medicina, Brazil.
Complete Peer review History: http://www.sciencedomain.org/review-history/15863

Original Research Article

Received 28th July 2016
Accepted 13th August 2016
Published 20th August 2016

ABSTRACT

Aims: This study was undertaken to evaluate the antipsychotic property of the ethanol extract of *T. ivorensis* (EETI) stem bark in mice
Study Design: The study used experimental animal models predictive of human psychosis in mice
Place and Duration of Study: Neuropharmacology Laboratory, Department of Pharmacology and Therapeutics, Faculty of Basic Medical Sciences, University of Ibadan, Oyo State, Nigeria,

**Corresponding author: E-mail: pharmben4ever@yahoo.com;*

between June 2013 and July 2014.
Methodology: Antipsychotic activity of EETI [125-1000 mg/kg, per os (p.o.)] was assessed based on the inhibition of stereotyped behavior induced by apomorphine (1 mg/kg, i.p.) or ketamine (10 mg/kg, i.p.) and ketamine-induced hyperactivity for positive symptoms in mice. Ketamine-enhanced immobility in forced swim test (FST) and reversal treatment of ketamine-induced cognitive dysfunction for negative and cognitive symptoms in mice respectively, and drug-induced ptosis and catalepsy in mice were also employed to further evaluate the antipsychotic property of EETI.
Results: EETI (125-1000 mg/kg, p.o.) significantly ($p<0.05$) inhibited apomorphine or ketamine-induced stereotypy, and ketamine-induced hyperactivity. Moreover, EETI significantly ($p<0.05$) attenuated the enhanced immobility and ameliorated the cognitive dysfunction by ketamine (30 and 20 mg/kg, i.p.), respectively in mice. EETI also dose dependently depleted the monoamine as indexed by the ptosis paradigm, however did not demonstrate cataleptic behavior as indexed on the catalepsy scale which suggest lack of expyramidal symptoms.
Conclusion: This study provides valuable evidence which suggests that *T. ivorensis* contain biologically active constituents that possess antipsychotic activity. Thus, justifying its ethnomedicinal claims in the management of psychotic disorders.

Keywords: Psychosis; schizophrenia; antipsychotics; Terminalia ivorensis.

1. INTRODUCTION

Psychosis (e.g. Schizophrenia) is a heterogeneous chronic neurological disease characterized by severe behavioral perturbations with distorted or non-existent sense of reality [1] that affects an average of approximately 1% of the World's population [2]. It commonly begins in late adolescence years [3] and often associated with polygenetic, environmental and neuro-developmental vulnerability factors [4]. It is represented by a group of complex symptoms characterized by positive (hallucinations, delusions, disorganized speech and thought), negative (flat expressions, anhedonia), and cognitive (deficits in learning, working memory) symptoms [5].

The dopamine dysregulation with hyperfunction of the mesolimbic dopamine system was the original tenet theory underlying the basis of schizophrenia [6] and the first animal models were developed on the basis of pharmacological manipulation in an attempt to mimic this feature [7], which respond to drugs that affect majorly the dopaminergic system, but does not replicate the negative or cognitive symptoms seen in schizophrenia [8]. In contrast, a widely used animal model of schizophrenia involves the acute or repeated administration of sub-anaesthetic doses of ketamine [9]. In rodents, *N*-methyl-D-aspartic acid receptor (NMDAR) blockade induces hyperactivity, stereotypy behavior, deficits in prepulse inhibition [10,11], social interaction and memory [12], which models the positive, negative and cognitive symptoms of schizophrenia, respectively [13].

Drugs prescribed for the treatment of psychosis can be categorized as typical and atypical. The typical class of antipsychotics is effective against the positive symptoms, and also possesses extrapyramidal side effects. Whereas the atypical class are effective in ameliorating the positive, negative as well as the cognitive symptoms and possess lesser extrapyramidal side effects however, accentuates greater risk of metabolic disorders including diabetes, agranuloctosis etc. Long term use of these drugs may even cause oxidative imbalance and thereby further enhance the progression of the disease [14,15]. In this contest, we need drugs with lesser side effects. Hence, natural products become the drug of choice and investigations have been extended for the search of novel and better tolerated molecules from plant sources. Moreover, Natural products structurally have characteristic high chemical diversity, biochemical specificity and other medicinal properties that make them favourable as lead structures for the remedies of a number of disorders including neuropharmacological activity [16].

Terminalia ivorensis is an indigenous plant from the family Combretaceae [17]. It is commonly called idigbo in Nigeria and some African countries [18]. Many bioactive studies carried out on *T. ivorensis* stem bark revealed that the plant showed great promise as antioxidant [19,20], antibacterial activity [21], anti-inflammatory and anti-arthritis [22] activities. In ethno-medicine, the pulverized leaves of the plant are used as poultice to treat burns and bruises [18]. The decoction of the bark plant is also used in Ivorian traditional medicine for sores and other

numerous diseases including cough, diarrhea, hypertension, diabetes and tooth decay [18]. The stem bark of *T. ivorensis* is reported to be used as tranquilizers in the treatment of insomnia, epilepsy, and psychotic disorders in south Western Nigeria [23].

T. ivorensis stem barks are known to be rich in several phytochemicals including butilinic acid, oleanolic acid, terminolic acid, ellagic acid, glycirrhetic acid, saponins (triterpenoid ivorenosides A, B, and C), quercetin, steroids, [22], polyphenols, flavonoids, saponins, steroids [19], punicalagin and punicalin [20], some of which have demonstrated different biological effects among such as sedative, antioxidant, anti-neuroinflammatory, and neuroprotective properties [24,25]. Recently, Adeoluwa et al. [26] reported the sedating and analgesic effect of the stem bark in mice. In the same contest, Ben-Azu et al. [27] also demonstrated the possible neuroprotective compensatory mechanism of action of stem bark extracts of *T. ivorensis* in the reversal treatment of ketamine-induced schizophrenia-like behavior and oxidative damage in mice.

To further investigate the medicinal claims and neuropharmacological effect of *T. ivorensis*, the present study hypothesized that administration of ethanol extract of *T. ivorensis* stem bark might ameliorate some of the behavioral symptoms of schizophrenia. Hence, this study was undertaken to evaluate the effect of the ethanol extract of *T. ivorensis* stem bark (EETI) on animal models predictive of human psychosis.

2. MATERIALS AND METHODS

2.1 Plant Materials

The stem bark samples of *T. ivorensis* were collected in March, 2014 at the Olupkele Forest Reserve, Ibadan, Oyo state, Nigeria. Taxonomical identification and authentication of the plant were done by Mr. O. S. Shasanya, at the herbarium section of the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria. A voucher specimen with identification number, FHI 109800, was deposited and compared with the reference specimen.

2.2 Extraction of Plant

The stem bark was air-dried for 6 to 7 weeks, and pulverized with an electric crusher. Pulverized stem bark (200 g) was macerated in 70% ethanol (2 L) for 48 h after which it was filtered on absorbent cotton and on Whatmann 3 mm paper under sterile conditions. The filtrate was concentrated using Rotary evaporator (BUCHI® Rotavapor Model R-215, Buchi Labortechnick AG, Flawil, Switzerland) at 40°C to give a semisolid residue. The dark brown paste obtained was dried to a constant weight (7.6 g) in a desiccator before storage in a sterilized glass vial for use.

2.3 Experimental Animals

Adult male Swiss albino mice weighing 20-25 g used for this study were obtained from the Central Animal House, College of Medicine, university of Ibadan. The animals were housed five per plastic cage (42 x 30 x 27 cm) in a controlled environment at room temperature (25±1°C) with a 12:12 h light/dark cycle. They were fed with standard rodent pellet food and water *ad libitum* throughout the experimental period. They were acclimatized for at least 1 week prior to commencement of the experiments. The experiments were performed after approval of the protocol by the Ethics Committee of the University of Ibadan according to the National institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985). Also, efforts were made to minimize the suffering of the animals.

2.4 Drugs

Apomorphine (Sigma-Aldrich, St. Louis, USA), ketamine hydrochloride (Sigma-Aldrich, St. Louis, USA), haloperidol (Sigma-Aldrich, St. Louis, USA) and risperidone (Sigma-Aldrich, St. Louis, USA). Haloperidol was used as the positive control for apomorphine-induced schizophrenia while risperidone was used as the positive control antipsychotic for ketamine models, because ketamine-induced model of schizophrenia has been demonstrated to be more responsive to atypical antipsychotic [12]. Different doses of ketamine were used in this study: 10 mg/kg, i.p. for hyperactivity (positive symptom) [11], 20 mg/kg, i.p. for cognitive deficit (cognitive symptom) [5], and 30 mg/kg, i.p. for enhanced-immobility (negative symptom) [30]; as ketamine have been found to induced different schizophrenia-like behavioral phenotypes.

2.5 Acute Toxicity Study

The method described by Lorke [28] was used to determine the LD_{50}, which is the index of acute

toxicity. Albino mice (20–25 g) of either sex were used. This method involved an initial dose finding procedure, in which the animals were divided into three groups of three animals. Doses of 10, 100 and 1000 mg/kg were administered p.o.), one dose for each group. The treated animals were monitored for 24 h mortality and general behavior. From the results of the above step, four different doses of the extract (2000, 3000, 4000 and 5000 mg/kg) were chosen and administered per oral (p.o), respectively to four groups of one mouse per group. The treated animals were monitored for 24 h. The LD_{50} was calculated as the geometric mean of the lowest dose showing death and the highest dose showing no death.

2.6 Experimental Paradigms

2.6.1 Apomorphine-induced stereotypy

The anti-psychotic effect of EETI was assessed using the Apomorphine-induced stereotyped behavioral paradigm in mice predictive of human psychosis as previously described by Bourin et. al. [29]. The mice were randomly divided into six treatment groups (n = 5/group). Group 1 received vehicle (10 mL/kg, p.o.), group 2-5 were pretreated with EETI (125, 250, 500 and 1000 mg/kg, p.o), while group 6 was pretreated with haloperidol (HLP) (1 mg/kg, p.o.) as positive control. Sixty minutes thereafter, each animal in group 1-6 received i.p. injection of apomorphine (APO) (1 mg/kg) and mouse were placed immediately in a transparent observation chamber (20 cm × 20 cm × 23 cm). Thereafter, stereotype behaviors were observed for 2 minutes at 10, 15, 30, 45 and 60 minutes after APO injection. Stereotype behaviors were scored as: 0 = absence of stereotype behavior; 1 = presence of stereotype movements of the head; 2 = intermittent sniffing; 3 = chewing; 4 = intense licking. After each mice session, the observation chamber was cleaned with 70% ethanol to remove residual odour.

2.6.2 Ketamine-induced stereotypy

Ketamine-induced stereotypy was also employed to screen for the antipsychotic effect of EETI according to the method described by Yamamoto et. al.[11]. The mice were randomly divided into six treatment groups (n = 5/group). Group 1 received vehicle [10 mL/kg, (p.o.), while group 2-5 were pretreated with EETI (125, 250, 500 and 1000 mg/kg, p.o), while group 6 was pretreated with risperidone (RIS) (0.5 mg/kg, p.o.) as positive control. Sixty minutes thereafter, each animal in group 1-6 received i.p. injection of sub-anaesthetic dose of ketamine (KET) (10 mg/kg), and mouse was placed immediately in a transparent observation chamber (20 cm × 20 cm × 23 cm) and stereotypy was observed for 2 minutes at 10, 15, 20, 30 and 45 minutes respectively. Stereotyped behaviors were scored as described above.

2.6.3 Ketamine-induced hyperlocomotion (Open field test)

Ketamine-induced hyperlocomotion was also used to screen for the antipsychotic effect of extract as previous described by Yamamoto et. al.[11] with brief modification, the open field apparatus consisted of a wooden box measuring 35 x 30 x 23 cm with visible lines drawn to divide the floor into 36 (20 cm × 20 cm) squares with a frontal glass wall and placed in a sound free room. The mice were randomly divided into six treatment groups (n = 5/group) and treated as described in the above ketamine model of stereotypy. Thereafter, the animals were placed in the rear left square and left to explore it. The duration of immobility(s) and number of line crossed were recorded for 5 minutes using a stopwatch. After each mice session, the observation chamber was cleaned with 70% ethanol to remove residual odor.

2.6.4 Ketamine-enhanced immobility in forced swim test

The antipsychotic effect of EETI was also screened using ketamine-enhanced immobility in forced swim test paradigm, that is predictive of the negative symptoms of schizophrenia, which is reflected as a state of despair in mice as described by Chindo et. al.[30]. The reduction in the immobility time serves as a specific and selective index of antidepressant activity that can be used to alleviate the negative symptoms of schizophrenia. The mice were randomly divided into seven (7) treatment groups (n = 5/group). Group 1 was pretreated with vehicle (10 mL/kg, p.o.) once daily for 5 days while group 2-6 were pretreated with a sub-anaesthetic dose of KET (30 mg/kg, i.p.) once daily for 5 days. After which, each mice were placed in a standardized transparent glass cylinder (height 46 cm, diameter 20 cm) containing water at 25 ℃ to a depth of 30 cm and was forced to swim for 5 min (pretest session) 1 h after the last treatment (5^{th} day) with ketamine for habituation. Twenty four hours (24 h) after the last treatment (6^{th} day) with vehicle and KET respectively, group 2 received

vehicle (10 mL/kg, p.o.) as a negative control, group 3-6 were treated with EETI (125, 250, 500 and 1000 mg/kg, p.o), while group 7 was treated with RIS (0.5 mg/kg, p.o.) as positive control. Sixty minutes later, each animal was placed in the same transparent glass cylinder containing water at 25 ℃ to a depth of 30 cm and forced to for 6 minutes and the immobility time was recorded for a period of 5 minutes with a stopwatch (test session) after discarding activity in the first 1 minute, during which the animal tries to escape [31]. After each session, the mice was removed immediately from the cylinder, dried with a towel and kept in an open space until completely dried before returning the mice to their home cages.

2.6.5 Ketamine-induced cognitive dysfunction

The effect of the extract on spontaneous alternation performance was assessed using a Y-maze test (YMT), which allows the evaluation of cognitive searching behavior, as an index for the cognitive dysfunction of schizophrenia as described by Monte et. al.[5]. In this protocol, mice was divided into 6 groups (n=5/group). Group 1 was treated with vehicle (10 mL/kg, p.o.) once daily, while group 2-6 were treated with sub-anaesthetic dose of ketamine (20 mg/kg) once daily i.p. for 14 days. From the 8^{th} to 14^{th} day of treatment onwards, group 2 was additionally treated with vehicle (10 mL/kg, p.o.) once daily as negative control, while group 3-5 was treated additionally with extract (125, 250, 500 and 1000 mg/kg, p.o.) once daily, and group 6 also received in addition RIS (0.5 mg/kg, p.o.) once daily as a positive control, with a 30 min interval between treatments. Twenty four hours (24 h) after the last treatment (15^{th} day), each mouse was gently placed individually in the Y-maze apparatus, which consisted of three identical arms (33 × 11 × 12 cm each) in which the arms are symmetrically separated at 120° [32]. Specifically, each mouse was placed at the end of arm A, and allowed to explore all the three arms (labeled A, B, C) freely for 5 minutes, taking the following parameters: the number of arm visits and sequence (alternation) of arm visits visually. After each mice session, the observation chamber was cleaned with 70% ethanol to remove residual odor.

2.6.6 Ptosis induction

Drug-induced ptosis in psychotic paradigm is used as a specific index to show the level of monoamine depletion or hypofunction as described by Bourin et. al. [33]. Mice were divided into six groups (n=5/group). The group 1 was treated with vehicle (10 mL/kg, p.o.) while group 2-5 were treated with EETI (125, 250, 500 and 1000 mg/kg, p.o.), and the sixth group was treated with HLP (1 mg/kg, p.o.). All animals were kept in individual transparent glass ptosis observation chambers (20 cm above the bench top) immediately after treatment to allow for stable assessment of ptosis. The degree of ptosis of each animal was evaluated and recorded at 60, 90 and 120 minutes 60 minutes after treatment. The degree of ptosis was rated according to the following rating scale: 0, eyes open; 1, eyes one-quarter closed; 2, eyes half closed; 3, eyes three-quarter closed; and 4, completely closed. The results obtained were compared with control groups treated with vehicle.

2.6.7 Catalepsy test

In animals, a state of rigidity and immobility also known as catalepsy is a state in which the subject/experimental animal remain immobile in an unusual position. It is used as an index of extrapyramidal symptoms seen clinically from antipsychotic drugs [34]. The cataleptic effect of the EETI was investigated according to the modified version previously described by Costall and Naylor [35]. The animals was divided into six treatment groups (n = 5/group). The group 1 was treated with vehicle (10 mL/kg, p.o.) while group 2-5 were treated with EETI (125, 250, 500 and 1000 mg/kg, p.o.), and the sixth group was treated with HLP (1 mg/kg, p.o.) 60 minutes before testing for catalepsy. The test was done by gently placing the fore limbs of each animal on a horizontal plane wood surface (H = 6 cm; W = 4 cm; L = 16 cm) and the duration of akinesia (period of time the animal remained in one position, before initiating any active movement) in seconds was recorded. An animal is considered cataleptic if it remains on the block for 60 sec [13].

2.3 Statistical Analysis

The Data were expressed as Mean ± standard error of mean (S.E.M). The data were analyzed using one-way analysis of variance (ANOVA) followed by *post hoc* test (Newman-Keul) for multiple comparisons where appropriate using GraphPad InStat® Biostatistics software (GraphPad Software, version 4.0). A level of $P < 0.05$ was considered as statistically significant for all tests.

3. RESULTS

3.1 Acute Toxicity Test

The LD_{50} of the ethanol extract of *Terminalia ivorensis* stem bark was found to be 2236.06 mg/kg, p.o. body weight.

3.2 Effect of EETI on Apomorphine- and Ketamine-induced Stereotypy, and Ketamine-Induced Hyperlocomotion

Apomorphine (1 mg/kg, i.p.) and ketamine (10 mg/kg, i.p.) significantly ($P < 0.05$) induced marked stereotyped behaviors characterized by head movements, intermittent sniffing, chewing and intense licking in mice. In the same contest, ketamine (10 mg/kg, i.p.) also significantly ($P < 0.05$) induced-hyperlocomotion as indexed by an increase in the number of line crossings and decrease in duration of immobility(s) compared to the vehicle (10 mL/kg, p.o.) treated group, which are reflected in schizophrenic patient as a form of positive symptoms. Pretreatment with EETI (125, 250, 500 and 1000 mg/kg, p.o.) significantly ($P < 0.05$) prevented apomorphine-induced stereotyped (Fig. 1) or ketamine-induced stereotyped behaviors (Fig. 2), and ketamine-induced hyperlocomotion as indexed by a decrease in number of line crossings and duration of immobility(s) (Table 1) in a dose dependent manner compared to control. Similarly, effects were observed in animals treated with HLP (1 mg/kg, p.o.) or risperidone (0.5 mk/kg, p.o.), as they significantly ($P < 0.05$) prevented the manifestations of stereotyped behaviors induced by APO (1 mg/kg, i.p.) or ketamine (10 mg/kg, i.p.), and hyperlocomotion induced by ketamine (10 mg/kg, i.p.) in all paradigms, respectively.

3.3 Effect of EETI on Ketamine-enhanced Immobility in Forced Swim Test in Mice

Effect of EETI on ketamine-enhanced immobility in forced swim test, as measured by the duration of immobility time in forced swim test in mice is shown in Fig. 3. Ketamine (30 mg/kg, i.p.) significantly enhanced the immobility ($P < 0.05$) compared to the group treated with vehicle (10 mL/kg, p.o.) in the forced swim test in mice. EETI (125, 250, 500 and 1000 mg/kg, p.o.) significantly ($P < 0.05$) decreased immobility time in a dose-dependent manner compared to the group treated with ketamine (30 mg/kg, i.p.) alone (negative control). Similar effect was also observed in the group treated with RIS (0.5 mg/kg, p.o.), as it significantly ($P < 0.05$) decreased immobility time compared to the ketamine treated group (Fig. 3).

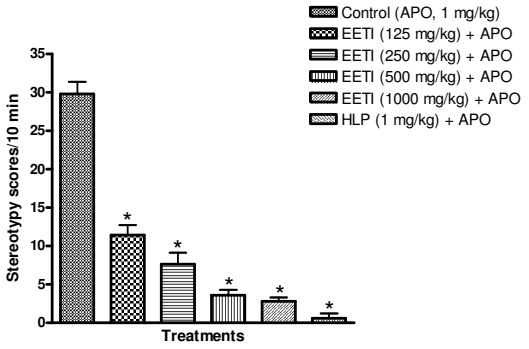

Fig. 1. Effects of EETI on apomorphine-induced stereotyped behavior

*Value represents the mean±S.E.M of 5 animals / group. One way ANOVA revealed that there is significant [F (5, 24) = 92.23, P < 0.0001] differences between various treatment groups. * Denotes P < 0.05 as compared with APO group, APO = Apomorphine, HLP = Haloperidol, EETI = Ethanol extract of T. ivorensis stem bark*

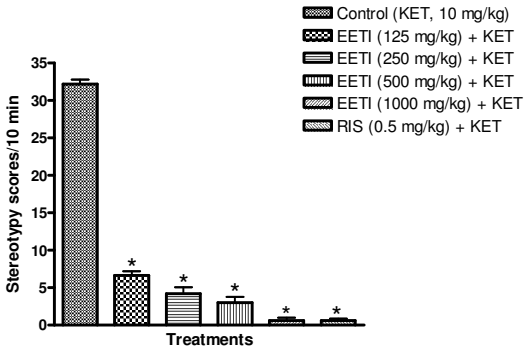

Fig. 2. Effects EETI on ketamine-induced stereotyped behavior

*Value represents the mean±S.E.M of 5 animals / group. One way ANOVA revealed that there is significant [F (5, 24) = 391.0, P < 0.0001] differences between various treatment groups. * Denotes P < 0.05 as compared with KET-treated group, KET = Ketamine, RIS = Risperidone, EETI = Ethanol extract of T. ivorensis stem bark*

3.4 Effect of EETI on Ketamine-induced Cognitive Dysfunction

The effect of ethanol extract on reversal treatment of ketamine-induced cognitive dysfunction was assessed by sequence of arm entry and number of arm entries in the Y-maze

Table 1. Effects of EETI on ketamine-induced hyperlocomotion

Treatments	Dose (mg/kg)	Number of line crossings	Duration of immobility
VEH	10 mL/kg	71.80±4.283	163.8±7.385
KET	10	232.8±8.628**	60.80±4.017**
EETI	125	70.80±7.664*	198.4 ±4.966*
EETI	250	59.00±7.635*	252.4±14.13*
EETI	500	32.00±2.720*	269.2±7.130*
EETI	1000	22.80±4.620*	283.6±4.622*
RIS	0.5	17.60±4.707*	289.0±7.906*

*Value represents the mean±S.E.M of 5 animals / group. One way ANOVA revealed that there is significant [F (6, 28) = 147.7, P < 0.0001] and [F (6, 28) = 111.8, P < 0.0001] differences between various treatment groups for number of line crossing(s) and immobility time, respectively. * Denotes P < 0.05 as compared with ketamine group, KET = Ketamine, RIS = Risperidone, EETI = Ethanol extract of T. ivorensis stem bark, VEH = Vehicle*

apparatus. Chronic ketamine (20 mg/kg, i.p.) treatment significantly (P < 0.05) induced cognitive dysfunction (memory impairment) compared to the group that received vehicle (10 mL/kg, p.o.) only following 14 days treatment. EETI (125, 250, 500 and 1000 mg/kg, p.o.) significantly reversed in a dose-dependent manner the cognitive dysfunction following treatment from the 8th to 14th day of treatment compared to the ketamine treated group. RIS (0.5 mg/kg, p.o.) compared to the ketamine treated group, significantly (P < 0.05) reversed the cognitive dsyfunction following treatment from the 8th to 14th day of treatment (Fig. 4).

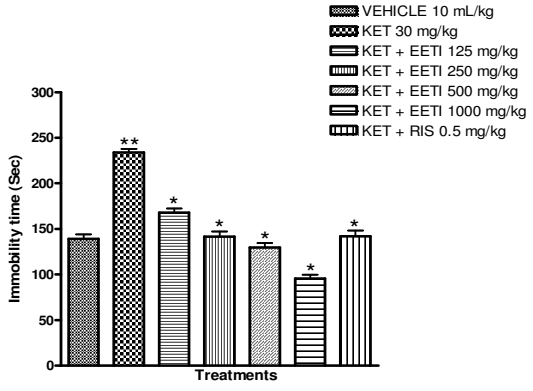

Fig. 3. Effect of EETI on ketamine-enhanced immobility in forced swim test in mice

*Value represents the mean±S.E.M of 5 animals / group. One way ANOVA revealed that there is significant [F (6, 26) = 72.64, P < 0.0001] difference between various treatment groups. ** Denotes P < 0.05 as compared to vehicle group. * Denotes P < 0.05 as compared with ketamine group. KET = Ketamine, RIS = Risperidone, EETI = Ethanol extract of T. ivorensis stem bark*

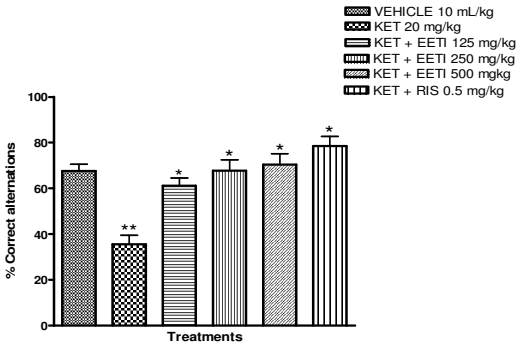

Fig. 4. Effect of EETI on reversal treatment of ketamine-induced cognitive dysfunction

*Value represents the mean±S.E.M of 5 animals / group. One way ANOVA revealed that there is significant [F (6, 28) = 77.33, P < 0.0001] difference between various treatment groups. ** Denotes P < 0.05 as compared to vehicle group. * Denotes P < 0.05 as compared with ketamine group. KET = Ketamine, RIS = Risperidone, EETI = Ethanol extract of T. ivorensis stem bark*

3.5 Effect of EETI on Ptosis Induction

Evaluation of EETI-induced ptosis at 60th, 90th, and 120th minutes post treatment, as evaluated on the ptosis scale is shown in Table 2. EETI (125, 250 mg/kg, p.o.) showed no significant (P > 0.05) induction of ptosis compared to the vehicle (10 mL/kg, p.o.). However, EETI (500 and 1000 mg/kg, p.o.), significantly (P < 0.05) induced ptosis when compared to the control (vehicle, 10 ml/kg, p.o.). HLP (1 mg/kg, p.o.) was shown to significantly (P < 0.05) induce ptosis compared to control (vehicle, 10 ml/kg, p.o.). However, there was a dose-dependent induction of ptosis with EETI (125, 250, 500 and 1000 mg/kg, p.o.), but significant (P < 0.05) induction of ptosis was only observed with doses of EETI (500, 1000 mg/kg,

Table 2. Effect of EETI on ptosis induction

Treatments	Dose (mg/kg)	60 min	90 min	120 min
VEH	10 mL/kg	0.00±0.00	0.00±0.00	0.20±0.20
EETI	125	0.60±0.24	0.40±0.24	0.60±0.24
EETI	250	0.20±0.20	0.40±0.24	0.80±0.20
EETI	500	1.60±0.40*	2.00±0.00*	2.00±0.00*
EETI	1000	2.80±0.20*	3.00±0.00*	3.00±0.00*
HLP	1	2.20±0.20*	2.40±0.24*	3.00±0.00*

The results are expressed as mean±SEM (n= 5). One way ANOVA revealed that there is significant [F (5, 24) = 22.99 P<0.0001 at 60 min; F (5, 24) = 52.49 P < 0.0001; F (5, 24) = 65.83 P<0.0001] at 60, 90 and 120 min respectively difference between various treatment groups.
HLP = Haloperidol, EETI = Ethanol extract of T. ivorensis stem bark, VEH = Vehicle

p.o.), when compared the control (vehicle 10 mL/kg, p.o.) (Table 2).

3.6 Effect of EETI on Cataleptic Behavior

The EETI (125, 250, 500 and 1000 mg/kg, p.o.) showed no significant ($P > 0.05$) prolongation in the duration of akinesia (cataleptic effect, an index of impaired extrapyramidal system), compared with the vehicle treated group (10 mL/kg, p.o.). However, HLP (1 mg/kg, p.o.) significantly ($P < 0.05$) prolonged the duration of akinesia above 60 seconds in comparison with the group treated with vehicle (Table 3).

Table 3. Effect of EETI on cataleptic behavior

Treatments	Dose (mg/kg)	Duration of akinesia / 60 secs
VEH	10 mL/kg	6.40±0.927
EETI	125	7.80±1.393
EETI	250	10.60±0.678
EETI	500	12.00±1.140
EETI	1000	23.80±1.497
HLP	1	62.00±2.915*

*Value represents the mean±S.E.M of 5 animals / group. * Denotes P < 0.05 compared to vehicle group (ANOVA followed by Newman Keuls post hoc test).*
HLP = Haloperidol, EETI = Ethanol extract of T. ivorensis stem bark, VEH = Vehicle

4. DISCUSSION

Herein we demonstrated that the acute toxicity test conducted in the study revealed that ethanol extract of *T. ivorensis* stem bark (EETI) was relatively safe for the animals, with an LD_{50} of 2236.06 mg/kg, p.o. body weight, as no sign of toxicity and death was observed at the chosen doses of 125-1000 mg/kg, p.o. for this experiment in mice. In this study, *T.ivorensis* was found to attenuate psychotic manifestations (positive, negative and cognitive symptoms) in mice.

Animal models used to study schizophrenia include both models of the full syndrome and models of specific signs of symptoms [36]. The behavioral studies using antagonism of stereotypy and hyperlocomotion induced by dopaminergic agonists (apomorphine) [37] and NMDA receptor antagonists (ketamine) [11] have traditionally been used to screen antipsychotic activity of novel antipsychotic agents. Stereotyped behaviors, which present itself as repetitive, ritualistic and functionless motor behavior [38], are one of the most prominent positive symptoms of psychosis [39]. Apomorphine activates post-synaptic dopamine D-2 receptors in the brain directly, and through this mechanism apomorphine accentuates locomotor activity leading to stereotypic behavior, resulting to repetitive and ritualistic functionless behavioral pattern [40,41]. Blockade of apomorphine-induced stereotyped behavior suggests neuroleptic activity [42,43]. The effect of the ethanol extract prepared from the stem bark of *T. ivorensis* (EETI) against apomorphine is therefore suggestive of possible interference with central dopaminergic neurotransmission and neuroleptic effect. This observation is consistent with earlier findings on the inhibition of apomorphine-induced stereotypy by the leaf extract of *Crassocephalum bauchiense* [41] and *Viscum album* [44]. In the same contest, Sonibare et al. [45] reported the inhibition of amphetamine-induced stereotypy by ethanol extract of *Lonchocarpus cyanescens.*

The finding that EETI significantly antagonized the stereotypy and hyperactivity (hyperlocomotion) induced by ketamine in a dose-dependent manner, also suggest that the

plant possess antipsychotic property. Increased motor activity is a validated animal paradigm employed in the evaluation of compounds suspected to have antipsychotic property [46]. Hyperactivity produced by ketamine at sub-anaesthetic doses is closely related to the psychotic agitation seen in patients with pychosis [47]. It is well known that dopaminergic mechanism play a central role in the mediation of stereotypic and locomotion activity, and ketamine may influence dopamine transmission and receptor activation via multiple mechanisms [48]. It is important to stress that biochemical data have shown that ketamine enhance dopamine release [49] and inhibit dopamine uptake [13] in the straitum and cortex respectively. Also, a possible mechanism by which ketamine might produce this adverse behavioral effects, at least partially, have been related to the blockade of NMDA receptors located on inhibitory GABAergic neurons in the limbic and subcortical brain regions [50]. This disinhibitory action has been reported to increase the neuronal activity and excessive dopamine release in the limbic striatal regions [51]. This hypothesis can explain some studies which have revealed a lower density of glutamatergic receptors in brain of schizophrenic patients [52]. The finding that the EETI significantly antagonized in a dose-dependent manner the hyperactivity (hyperlocomotion) and stereotypy-induced by ketamine, further suggest that the antipsychotic property of EETI might be due to its atypical mechanism of action via NMDA receptor thus, leading to the modulation of dopamine activity and hence the ability to show efficacy against positive symptoms. This observation is also consistent with earlier findings on the effect of ketamine on locomotion and stereotyped behavior, and inhibition of ketamine-induced stereotypy and hyperlocomotion by the root extract of *Panax quinquefolium* [13].

Behavioral despair including flattening of affect and avolition are some of the major negative symptoms of schizophrenia, and forced swim test-induced immobility in rodent is an acceptable animal model of depression [53] that reflects a state of 'despair' in mice, and reduction in immobility time serves as a specific and selective index of antidepressant activity [30]. Therefore, the increase in immobility time in FST following repeated administration of a sub-anaesthetic dose of ketamine in the negative control group treated with ketamine alone in this study corroborates with previous studies [13,30]. In this contest, the antipsychotic property of the extract was further demonstrated by the reduction of ketamine-enhanced immobility in forced swim test. Although, the mechanisms by which negative symptoms are induced in ketamine psychosis are not fully understood, previous studies have reported the involvement of 5-hydroxytryptaminergic (5-TH) system in the negative symptoms of schizophrenia [13,54]. The findings by Chindo et. al. [30] suggests that ketamine-enhanced immobility in the FST might be mediated, at least in part, via 5-HT_{2A} receptors blockade, since 5-HT_{2A} receptor blockers such as clozapine, risperidone and paroxetine attenuated the ketamine-enhanced immobility time. Therefore, our finding that EETI demonstrated significant reduction of ketamine-enhanced immobility in FST in a dose-dependent manner with a comparable result to that of risperidone, further suggest that the extract may possess antipsychotic capability that may attenuate the negative symptoms of schizophrenia.

Cognitive impairments such as deficits in attention, executive function, working (short-term) memory, and long-term memory, are core symptoms in patients with schizophrenia [55]. Among these, learning and memory impairments are known to be particularly severe, and they are suggested to be major determinants of the amount of disability patients with schizophrenia experience in social and occupational functioning and in independent living [56]. The Y-maze test (YMT) paradigm has been used previously to evaluate the effects of antipsychotic drugs (e.g., risperidone) on learning and memory function in rodents [5]. Our findings also depict the memory impairing properties of ketamine as indexed by decreased percentage correct alternations on the YMT, which was reverted by EETI treatment and also by standard atypical drug, risperidone. Mechanistically, learning is associated with phosphorylation by calcium/calmodulin-dependent protein Kinase II (Ca^{2+}/CaMKII). Inhibition of NMDA receptor as a ligand-gated Ca^{2+} channel decreases this phosphorylation, which may explain the deficits in cognitive function induced by ketamine due to disruption of long-term potentiation (LTP) [57]. Moreover, ketamine also inhibits acetylcholine; a key player in the initial stages of memory formation [58] by antagonizing nicotinic acetylcholine receptor α-7nAchR [59]. Therefore, the learning and memory impairment by ketamine via NMDA receptor and reversal by EETI can also correlate its memory improving capability in this disorder.

Adversely, extrapyramidal symptoms (EPSs) are thought to result from decreased dopamine activity in the striatum, a preferential action of a novel agent against dopamine agonist-induced hyperactivity or stereotypy might serve as an indicator of a lower propensity to induced EPSs in patients [60]. This is because preferential blockade of D_2 receptors in the limbic system confers antipsychotic effects with little or no tendency to cause EPSs [43,60]. The EPSs or catalepsy test is a paradigm established in rodents to test the tendency of antipsychotics (e.g., haloperidol) to induce EPSs based on the prolongation of the duration of akinesia or immobility time upon an imposed posture [61]. However, the test for catalepsy demonstrated that the extract is devoid of catalepsy on the animals, as indexed by the duration of akinesia below 60 seconds compared to the vehicle-treated group. Also, the test for drug-induced ptosis, demonstrated that the extract elicited dose-dependent ptosis induction, as indexed by the dropping of the eye-lid of the animals, which is further suggestive of the suppression of the monoaminergic system [62] by EETI.

Taken together, these findings reveals that the ethanol extract of *T. ivorensis* stem bark contains biologically active substance(s) that might be acting centrally through the inhibition of dopaminergic pathway or the modulation of other pathway(s) including NMDA receptor of glutamatergic system linked to this dopaminergic transmission. The anti-psychotic potential of this extract need further investigation since drug used in the treatment of various psychosis abolished apomorphine- and ketamine-induced stereotyped behavior, ketamine-induced hyperactivity, ketamine-enhanced-immobility, and ketamine-induced cognitive deficit. It is known that this plant contains various phytochemical antioxidant constituents, many of which are flavonoid-based and have been shown to be ligands for the type A GABA ($GABA_A$) receptors where they exert their sedative and tranquilizing activities [63]. Other possible effects of these phytochemicals may include anti-neuroinflammatory, neuroprotective, chemoprevention, anti-oxidation actions [64,65]. Of note, a recent study demonstrated that ethanol extract of *T. ivorensis* treatment restores GSH levels and to a great extent reverses antioxidant defense alterations in the brain of mice treated with ketamine [27], hence may explain some of the beneficial behavioral effect (against the positive, negative and cognitive symptoms) demonstrated herein. Moreover, it is known that most psychotic patients are in a dangerous state of psychomotor excitement thus, requiring the use of drugs with tranquilizing or sedative properties [42,66].

5. CONCLUSION

This study provides valuable evidence that suggests that *T. ivorensis* contain biologically active constituents that possess antipsychotic activity. Thus, justifying its ethnomedicinal claims in the management of psychotic disorders.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The experiments were performed after approval of the protocol by the Ethics Committee of the University of Ibadan according to the National institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985). Also, efforts were made to minimize the suffering of the animals.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Kreyenbuhl J, Buchanan RW, Dickerson FB, Dixon L. The Schizophrenia patient outcomes research team (PORT): Updated treatment recommendations. Schizophrenia Bulletin. 2009;36:48-70.
2. Picchioni MM, Murray RM. "Schizophrenia". British Medical Journal. 2007;335:91-5.
3. Fanous AH, Kendler KS. Genetics of clinical features and subtypes of Schizophrenia: A review of the recent literature. Current Psychiatry Reports. 2008;10:164-170.
4. Lewis DA, Lieberman JA. Catching up on schizophrenia: Natural history and neurobiology. Neuron. 2000;28:325-34.
5. Monte AS, Greicy CS, Roger SM, Joanna KS, Júnia VS, Rafaela CC, Bruna MM, Ribeiro DF, Silvânia MM. Prevention and reversal of ketamine-induced schizophrenia related behavior by minocycline in mice: Possible involvement

of antioxidant and nitrergic pathway. Journal Psychopharmacology. 2013;11: 1032-1043.

6. Murray RM, Lappin J, Di Forti M. Schizophrenia: From developmental deviance to dopamine dysregulation. Eur Neuropsychopharmacol. 2008;18:129-134.
7. Jones PB, Barnes TR, Davies L, Dun G, Lloyd H, Hayhurst KP, Murray RM, Markwick A, Lewis SW. Randomized controlled trial of the effect on quality of life of second- vs first-generation antipsychotic drugs in schizophrenia: Cost Utility of the Latest Antipsychotic Drugs in Schizophrenia Study (CUtLASS 1). Arch Gen Psychiatry. 2006;63:1079-1087.
8. Featherstone RE, Rizos Z, Kapur S, letcher PJ. A sensitizing regimen of amphetamine that disrupts attentional set-shifting does not disrupt working or long-term memory. Behav Brain Res. 2008;189:170-9.
9. Bubenikova-Valesova V, Horacek J, Vrajova M, Hoschl C. Models of schizophrenia in humans and animals based on inhibition of NMDA receptors. Neurosci Biobehav Rev. 2008;32:1014-1023.
10. Jentsch JD, Roth RH. The neuropsychopharmacology of phency-clidine: From NMDA receptor hypofunction to the dopamine hypothesis of schizophrenia. Neuropsychopharmaco-logy. 1999;20:201-225.
11. Yamamoto M, Mizuki Y, Suetsugi M, Ozawa Y, Ooyama M, Suzuki M. Effects of dopamine antagonists on changes in spontaneous EEG and locomotor activity in ketamine treated rats. Pharmacology Biochemistry and Behavior. 1997;57:361-365.
12. Becker A, Grecksch G. Ketamine-induced changes in rat behavior: A possible animal model of schizophrenia. Test of predictive validity. Prog Neuropsycho-pharmacol Biol Psychiatry. 2004;28:1267-1277.
13. Chatterjee M, Seema S, Reena K, Verma AK, Gautam P. Evaluation of the antipsychotic potential of *Panax quinquefolium* in ketamine induced experimental psychosis model in mice Neurochemical Research. 2012;37(4):759-70.
14. Meltzer H. Antipsychotic agents & Lithium. In: Basic & clinical pharmacology, 12th eds. Katzung BG, Masters SB, Trevor A J. McGraw-hill companies; 2010.
15. Meyer JM. Pharmacotherapy of Psychosis and Mania. In: Goodman & gilman's the pharmacological basis of therapeutics, 10th eds. Brunton L, Chabner B, Knollman B. McGraw-Hill; 2011.
16. Zhang ZJ. Therapeutic effects of herbal extracts and constituents in animal models of psychiatric disorders. Life Sci. 2004;75:1659-1699.
17. Foli EG. *Terminalia ivorensis* A. Chev. In: Lemmens RHMJ, Louppe D, Oteng Amoako, AA. (Editors). Prota 7(2): Timbers/Bois d'œuvre 2. [CD-Rom]. PROTA, Wageningen, Netherlands; 2009.
18. Coulibaly K, Zirihi GN, N'Guessan K, Djaman AJ. Botanical study and evaluation of antifungal activity of *Terminalia ivorensis* and *Terminalia superba* (Combretaceae) on the growth *in vitro* of A*spergillus fumigatus*. Int J Ph Sci. 2010;2:904-912.
19. Ponou BK, Teponno RB, Ricciutelli M, Quassinti L, Bramucci M, Lupidi G, Barboni L, Tapondjou LA. Dimeric antioxidant and cytotoxic triterpenoid saponins from *Terminalia ivorensis* A. Chev. Phytochemistry. 2010;71:2108-2115.
20. Adiko VA, Barthelemy KA, Félix ZT, Kouamé MA, Coulibali S, Léon AD. Separation and characterization of phenolic compounds from *Terminalia ivoiriensis* using liquid chromatography-positive electrospray ionization tandem mass spectroscopy. Afr J Biotechnol. 2013;12:4393-4398.
21. Malcom SA, Sofowora EA. Antimicrobial activity of selected Nigerian folk remedies and their constituent plants. Lloydia. 1969; 32:512-517.
22. Iwu MM, Anyawu BN. Anti-inflammatory and anti-arthritic properties of *Terminalia ivorensis*. Fitoterapia. 1982;52:25-34.
23. Lawal IO, Uzokwe NE, Igboanugo AB, Adio AF, Awosan EA, Nwogwugwu JO. Ethno-medicinal information on collation and identification of some medicinal plants in Research Institutes of South-west Nigeria. Afr J Pharm Pharmacol. 2010;4:001-007.
24. Zijia Z, Liping L, Jeffrey M, Tao W, Zhengtao W. Antioxidant phenolic

compounds from walnut kernels (*Juglans regia* L). Food Chem. 2009;113:160-165.

25. Wang Y, Zhang H, Yuan Q. Purification antioxidant activity and protein-precipitating capacity of punicalin from Pomegranate husk. Food Chem. 2013; 138:437-443.
26. Adeoluwa OA, Aderibigbe AO, Agu GO, Adewole FA, Eduviere AT. Neurobehavioral and analgesic properties of Ethanol bark exctract of *Terminalia ivorensis* A. Chev. (Combrataceae) in mice. Drug Res. 2015;65(10):545-51.
27. Ben-Azu B, Aderibigbe AO, Ajayi AM, Iwalewa EO. Neuroprotective effects of the ethanol stem bark extracts of *Terminalia ivorensis* in ketamine-induced schizophrenia-like behaviors and oxidative damage in mice. Pharma. Biol.

    Available:http://dx.doi.org/10.1080/13880209.2016.1190382 [PubMed: 27250524]
28. Lorke D. A new approach to practical acute toxicity testing. Archieves Toxicology. 1983;54:275–287.
29. Bourin M, Poisson L, Larousse C. Piracetam interaction with neuroleptics in psychopharmacological tests. Neuro-psychobiology. 1986;19:93-6.
30. Chindo AB, Adzu B, Yahaya TA, Karniyus SG. Ketamine-enhanced immobility in forced swim test: A possible animal model for the negative symptoms of schizophrenia. Progress in Neuro-Psychopharmacology & Biological Psychiatry. 2012;38:310-316.
31. Kroczka B, Branski P, Palucha A, Pilc A, Nowak G. Antidepressant-like properties of zinc in rodent forced swim test. Brain Research Bulletin. 2001;55:297-300.
32. Dall'igna OP, Fett P, Gomes MW. Caffeine and adenosine A(2a) receptor antagonists prevent beta-amyloid (25–35)-induced cognitive deficits in mice. Exp Neurol. 2007;203:241-245.
33. Bourin M, Poncelet M, Chermat R, Simon P. The value of the reserpine test in psychopharmacology. Arznei-Forschung. 1983;33:1173-1176.
34. Leucht S, Corves C, Arbter D, Engel RR, Li C, Davis JM**.** Second-generation versus first-generation antipsychotic drugs for schizophrenia: A meta-analysis. Lancet. 2009;373:31-41.
35. Costall B, Naylor RJ. On catalepsy and catatonia and the predictability of the cataleptic test for neuroleptics activity. Psychopharmacology (Berlin). 1974; 34:233-41.
36. Geyer MA, Moghaddam B. Animal models relevant to schizophrenia disorders. Neuropsychopharmacology. 2002;50:690-701.
37. Omogbiya IA, Solomon U, Aderibigbe AO, Adewale GB. Jobelyn pretreatment ameliorates symptoms of psychosis in experimental models. J Basic Clin Physiol Pharmacol. 2013;24:331-336.
38. Morrens M, Hulstijn W, Lewi PJ, De Hert M, Sabbe BG. Stereotypy in schizophrenia. Schizophrenia Research. 2006;84:397-404.
39. American Psychiatric Association (APA). Schizophrenia and other psychotic disorders. In: Statistical manual of mental disorders. Washington, America Psychiatric Association. 2002:4:297-319.
40. Stolk JM, Rech RH. Antagonism of O-amphetamine by methylp-tyrosin. Behavioual evidence for participation of cathelcolamine stores and synthesis in the amphetamine stimulant response. Neuropharmacology. 1970;9:249-263.
41. Taïwe GS, Elisabeth NB, Emmanuel T, Amadou D, Fleur COM, Gwladys TZ et al. Antipsychot ic and sedative effects of the leaf extract *of Crassocephalum bauchiense* (Hutch.) Milne-Redh (Asteraceae) in rodents. Journal of Ethnopharmacology. 2012;143:213-220.
42. Davis KL, Kahn RS, Ko G, Davidson M. Dopamine in schizophrenia: A review and reconceptualization. American Journal of Psychiatry. 1991;148:1474-86.
43. Leite JV, Guimara˜es FS, Moreira FA. Aripiprazole, an atypical antipsychotic, prevents the motor hyperactivity induced by psychotomimetics and psychostimulants in mice. European Journal of Pharmacology. 2008;578;222-227.
44. Gupta G, Imran K, Muhammad A, Mahfoozur R, Shakir S, Shamim A, Mohammad JK, Khalid N, Sayeed A, Mohd M, Zubair A, Firoz A. Sedative, antiepileptic and antipsychotic effects of *Viscum album* L. (Loranthaceae) in mice and rats. Journal of Ethnopharmacology. 2012;141:810-816.

45. Sonibare M A, Solomon U, Esther TS. Antipsychotic property of aqueous and ethanolic extracts of *Lonchocarpus cyanescens* (Schumach and Thonn.) Benth. (Fabaceae) in rodents. Journal of Natural Medicine. 2012;66:127-132.

46. Arnt J, Skarsfeldt T. Do novel antipsychotics have similar pharmacological characteristics? A review of the evidence. Neuropsychopharmacology. 1998;18:63-101.

47. Swerdlow NR, Braff DL, Taaid N, Geyer MA. Assessing the validity of an animal model of deficient sensorimotor gating in schizophrenic patients. Archives of General Psychiatry. 1994;51:139-54.

48. Benturquia N, Courtin C, Noble F, Marie-Claire C. Involvement of D1 dopamine receptor in MDMA-induced locomotor activity and striatal gene expression in mice. Brain Research. 2008;1211:1-5.

49. Irifune M, Shimizu T, Nomoto M. Ketamine-induced hyperlocomotion associated with alteration of presynaptic component of dopamine neurons in the nucleus accumbens of mice. Pharmacological Biochemical Behavior. 1991;40:399-407.

50. Duncan GE, Moy SS, Knapp DJ, Mueller RA, Breese GR. Metabolic mapping of the rat brain after subanaesthetic doses of ketamine: Potential relevance to schizophrenia. Brain Research. 1998;787:181-190.

51. Moghaddam B, Adams B, Verma A, Daly D. Activation of glutamatergic neurotransmission by ketamine: A novel step in the pathway from NMDA receptor blockade to dopaminergic and cognitive disruptions associated with the prefrontal cortex. J Neurosci. 1997;17: 2921-2927.

52. Kim JS, Kornhuber HH, Schmid-Burgk W, Holzmüller B. Low cerebrospinal fluid glutamate in schizophrenic patients and a new hypothesis on schizophrenia. Neuroscienc Letters. 1980;20:379-382.

53. Porsolt RD. Rodent models of depression: Forced swimming and tail suspension behavioral despair tests in rats and mice. Current Protocol in Neuroscience. 2001;8:1-10.

54. Oka M, Noda Y, Ochi Y, Furukawa K, Une T, Kurumiya S, et al. Pharmacological profile of AD-5423, a novel antipsychotic with both potent dopamine-D2 and serotonin- 5HT2 antagonist properties. Journal of Pharmacology and Experiment Therapy. 1993;264:158–65.

55. Harvey PD, Green MF, Keefe RS, Velligan DI. Cognitive functioning in schizophrenia: a consensus statement on its role in the definition and evaluation of effective treatments for the illness. J Clin Psychiatry. 2004;65:361-372.

56. Green MF, Kern RS, Braff DL, Mintz J. Neurocognitive deficits and functional outcome in schizophrenia: Are we measuring the "right stuff"? Schizophr Bull. 2000;26:119-136.

57. Mouri A, Yukihiro N, Akihiro N, Tomonobu N, Taknaobu T, Yoshimitsu Y, et al. Involvement of a Dysfunctional Dopamine-D1/*N*-Methyl-Daspartate- NR1 and Ca2+/Calmodulin-Dependent Protein Kinase II Pathway in the Impairment of Latent Learning in a Model of Schizophrenia Induced by Phencyclidine. Molecular Pharmacology. 2007;71:1598-1609.

58. Hasselmo ME. The role of acetylcholine in learning and memory. Current Opinions Neurobiology. 2006;16:710-715.

59. Coates KM, Flood P. Ketamine and its preservative, benzethonium chloride, both inhibit human recombinant alpha7 and alpha4beta2 neuronal nicotinic acetylcholine receptors in *Xenopus oocytes.* British Journal of Pharmacological. 2001;134:871-879.

60. Porsolt RD, Moser PG, Castagné V. Behavioral indices in antipsychotic drug discovery. Journal of Pharmacology and Experimental Therapy. 2010;333:632-8.

61. Geyer MA, Ellenbroek B. Animal behavior models of the mechanisms underlying antipsychotic atypicality. Prog Neuropsychopharmacol Biological Psychiatry. 2003;27:1071-9.

62. Bourin M, Poncelet M, Chermat R, Simon P. The value of the reserpine test in psychopharmacology. Arznei-Forschung. 1983;33:1173-1176.

63. Marder M, Paladini AC. GABA-areceptor ligands of flavonoid structure. Current Topics in Medicinal Chemistry. 2002;2(8): 853-867.

64. Zijia Z, Liping L, Jeffrey M, Tao W, Zhengtao W. Antioxidant phenolic compounds from walnut kernels

(Juglansregia L). Food Chem. 2009;113: 160-165.

65. Aguirre-Hernandez E, Gonzalez-Trujano ME, Martinez AL, Moreno J, Kite G, Terrazas T, et al. HPLC/MS and anxiolytic-like effect of quercetin and kaemferol flavonoids from *Tilia americana var. maxicana.* J Ethnopharmacol. 2010;127: 91-97.

66. Baldessarini RJ. Drugs and treatments of psychiatry and mania. In: Hardman JG, Limbird IE (eds) Goodman and Gilman's pharmacological basis of therapeutics, 10th edn. The McGraw Hill; 2001.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/15863