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Antipsychotic and anticonvulsant effects of crude aqueous leaf extract of *Vitex doniana* Sweet (Verbenaceae) In mice

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Abstract

Background: This work evaluated the antipsychotic and anticonvulsant effects of crude aqueous leaf extract of *Vitex doniana* in mice. This was to validate the use of *V. doniana* in the treatment of mental disorders and epilepsy in folkloric medicine.

Methods: Antipsychotic effect of *V. doniana* was evaluated using the animal models of psychosis: swimming-induced grooming, ketamine-induced hyperlocomotion and apomorphine-induced climbing tests. Anticonvulsant effect was evaluated using animal models of convulsion: maximal electroshock (MES), pentylenetetrazole (PTZ) and strychnine. Five groups of white albino mice (n = 6) were randomly selected. Group 1 was the control (normal saline, 10 ml/kg, i.p.), group 2, 3 and 4 were treated with aqueous leaf extract of *V. doniana* at 250, 500 and 1000 mg/kg p.o. for antipsychotic experiments and 100, 200 and 400 mg/kg, i.p. for the anticonvulsant experiments, while group 5 was the positive control (appropriate standard drugs).

Results: The doses of 500 and 1000 mg/kg of *V. doniana* showed significant antipsychotic, while at all the doses tested, it showed varying degrees of anticonvulsant activity.

Conclusions: It can be concluded, that *V. doniana* possesses significant antipsychotic and antiepileptic effects, thus providing pharmacological justification for some of it ethnomedicinal uses.

Keywords: Swimming-induced grooming; ketamine-induced hyperlocomotion; apomorphine-induced climbing; maximum electroshock; pentylenetetrazole; strychnine.

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Background

Vitex doniana (Synonyms: *Vitex cuneata Schumach & Thonn, Vitex cienkowskii kotschy & peyr., Vitex pachyphylla* Baker; Family: *Verbenaceae*) is a medium sized deciduous and semi-evergreen tree measuring 8 – 18 meters high, and with a heavily rounded crown and a clear pole up to 5 meters. It has a rough, pale brown or gray bark with fine fissures running down the trunk, and with bases of the old trees having oblong scale. The leaves are arranged like the fingers of a hand (palmate) and have five leathery leaflets, with the middle leaflet being the largest. The tips of the leaf are rounded or emarginate, while the bases are cuneate. The leaf is dark green above, pale greyish to green below, thickly leathery, and with a few or no scattered stellate hairs on the upper surface. The petals of the flower are white, with exception on the largest lobe, which is purple, and in dense opposite and axillary cymes. The flowers are small, blue or violet, and are 3 to 12 cm in diameter, with only a few open at a time. *Vitex doniana* fruit is oblong and about 3 cm long. The fruit is green when young but turns purplish-black on ripening and with a starchy black pulp. The fruit contains one hard conical seed. The seed is 1.5 to 2 cm long, and 1 to 1.2 cm wide [1,2].

Vitex doniana has copious ethnomedicinal uses. Leaf sap is used as an eye drop to treat conjunctivitis and other eye complaints. The leaf decoction is smeared externally as a galactagogue and against headache, stiffness, measles, rash, fever, chickenpox and hemiplegia, and internally as a tonic, anodyne and febrifuge, and to treat respiratory diseases. The pastes of pounded leaves and bark are applied to wounds and burns. A root decoction is administered orally to treat ankylostomiasis, rachitis, gastro-intestinal disorders and jaundice, and as an anodyne. The powdered bark added to water is taken to treat colic, and a bark extract is used to treat stomach complaints and kidney troubles. The bark is also used against leprosy and liver diseases, and to control bleeding after childbirth. Dried and fresh fruits are eaten against diarrhoea, and as a remedy against lack of vitamin A and B. The twigs are used as chewing sticks for teeth cleaning [1]. The hot aqueous extract of *V. doniana* leaves is used for the treatment of stomach and rheumatic pains, inflammatory disorders, diarrhoea and dysentery. The root has been used to treat epilepsy, nausea, and colic [3]. The stem bark extract of the tree is used for the control of hypertension, treatment of stomachache, pains, disorders, indigestion and sterility [4]. *Vitex doniana* like *Vitex rivularis* is said to have been used ethnomedically to cure madness, insanity and epilepsy [5–8].

The leaf extract of *V. doniana* has been found to have anti-inflammatory and analgesic effects [9]. The aqueous leaf extract of *V. doniana* has also shown antioxidant property [10]. The stem bark extract of *V. doniana* has been found to have a marked dose-dependent hypotensive activity in both normotensive and hypertensive rats. Extracts from the stem bark have also shown different level of in vitro trypanocidal activity against *Trypanosoma brucei* [4,11,12]. Other reported pharmacological activities of *V. doniana* include: hypolipidaemic effects [13], hypolipidaemic and antidiabetic effects [14].

There are lots of disorders and diseases of the nervous system some of which include: Psychoses, epilepsy, Parkinson's disease, Alzheimer's disease, multiple sclerosis, anxiety, insomnia, depression, etc. [15,16]. Psychoses, e.g., schizophrenic, schizoaffective, and affective illnesses, are common with a lifetime prevalence of 2–3% and account for a high percentage of the grave morbidity [17]. Schizophrenia is an overwhelming psychiatric disorder that disturbs cognition, emotion, language and thought, and it usually affects 0.5%–1.5% of the population [18]. Epilepsy, a disorder of the brain characterized by an enduring predisposition to generate epileptic seizures, and by neurobiological, cognitive, psychological, and social consequences, is one of the most common and serious neurological disorders of the brain [19,20]. Epidemiological studies have shown that 50 Million persons suffer from epilepsy worldwide, and that 20-30% of these persons have seizures that are resistant to treatment with the presently existing antiepileptic drugs [21].

The various side effects associated with current neuroleptics, such as: rebound insomnia, sedation, muscle relaxation, withdrawal and tolerance; antihistaminic, anticholinergic and sexual dysfunction, have limited their use in patients [22]. The search for novel drugs from medicinal plants has progressed significantly owing to their having less side effects and better tolerability [23].

Even though *Vitex doniana* is said to have been used ethnomedically to cure madness, insanity and epilepsy [8], there is still dearth of information on the neuropharmacological profile of this plant. Hence, this study will seek to substantiate, or otherwise, the folkloric medicinal claims of the use of *V. doniana* in the treatment of mental problems and epilepsy.

Methods

Plants collection and authentication

Fresh leaves of *V. doniana* were collected by the researcher and Mr. I. I. Ogunlowo of the Herbarium Unit of Department of Pharmacognosy, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria, from a farmland at Ondo Road, Ile-Ife Osun State. It was identified and authenticated by Mr. I. I. Ogunlowo of the Herbarium Unit of Department of Pharmacognosy, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria. The voucher specimen of the leaves was prepared and deposited at both the Herbarium Units of the Department of Botany, Faculty of Science, and Department of Pharmacognosy, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, as Voucher Nos.: IFE-17732 (Ife Herbarium) and FPI-2174 (Faculty of Pharmacy Herbarium).

Animals used

The animals used for this study were adult mice (18 – 25 g) of both sexes. All the animals were bred and housed in well-lit and aerated room in the Animal House, Faculty of Pharmacy, Obafemi Awolowo University, Ile - Ife. They were maintained under natural daylight/night condition. All animals had free access to water and standard commercial diet (Vital Feed® brand, produced by Grand Cereals Limited, a subsidiary of UAC Nigeria PLC). The experiments were carried out between 9.00 am and 3.00 pm. The animals were fasted overnight prior to the experiments. They were used in groups of six per dose level of the crude extract, positive and negative controls. Each mouse was used only once.

Laboratory materials used

Observation cage, cylindrical cage, swimming cylinder, Ugo Basile electroconvulsive machine (Model 57800, Ugo Basile Biological Research Apparatus, Italy), stopwatch, manual counter, syringes and needles and weighing balance.

Drugs used

Diazepam (Roche, Basel, Switzerland); haloperidol, pentylenetetrazole, strychnine, apomorphine, (Sigma chemicals Co, St. Louis, Missouri, U.S.A.), ketamine (Rotex medica), and normal

saline (Unique Pharmaceutical Limited, Lagos, Nigeria). Crude extract was dissolved in required volume of normal saline. The drugs and crude extract were freshly prepared on each day of the experiments.

Preparation of extracts

The fresh leaves of *V. doniana* were collected, air-dried and milled into powder with the aid of electric grinder. Powdered leaves of *V. doniana* (500 g) were extracted hot in 7 litres water respectively with continuous shaking for 48 hours in a mechanical shaker. The mixture of plant was filtered, and the filtrate concentrated using a rotary evaporator at a maximum temperature of 45^{O}C to obtain the crude aqueous extract of the plant. Further drying of the extracts was carried out using the freeze-dryer to obtain solid extract. The total dried aqueous extract obtained from 500 g of leaf of *V. doniana* was 69.90 g (13.98% w/w yield). The solid granules of the aqueous extracts of the plant was then stored in the refrigerator until it was needed for use.

Administration of extracts

The aqueous extracts were administered to mice through the oral route, except for the anticonvulsant assay which was through the intraperitoneal route. The volume of extracts and drugs administered orally was 10 mL per kg or 0.1 mL per 10 g of body weight of the animal, while the volume of extracts and drugs administered intraperitoneally was 5 mL per kg of body weight of the animal [24]. The intraperitoneal route was used in the anticonvulsant assay because it has been reported that even though clinically efficacious anticonvulsant drugs are usually administered orally, preclinical testing should be started with parenteral (usually intraperitoneally administration), to ensure that the gastrointestinal tract is not interfering with the results. That only in the case of activity by this route should the efficacy of the compound after oral administration be studied [25].

Pharmacological studies

Acute toxicity test

The method of Lorke (1983) was used. This involves using the 13-animal model for rapid determination of LD_{50}. The 1st phase uses 3 animals for each dose level 10, 100 and 1000 mg/kg. The mice were kept under the same laboratory conditions and observed for signs of toxicity which include but not limited to paw-licking, stretching, respiratory distress and mortality for the first critical four hours and after 24 hours the number of deaths per group is recorded. The result obtained from this test is used as a basis for selecting the subsequent doses in the 2nd phase following a standard table. The 2nd phase involved administering four different doses to one mouse per group and the mice are observed for signs of toxicity for the first critical four hours and thereafter 24 hours for mortality. The intraperitoneal median lethal dose (LD_{50}) was calculated as the geometric mean of doses that caused 0 and 100% mortality respectively.

The $LD_{50} = \sqrt{}$ (A x B), where A = maximum dose that caused 0% mortality and B = minimum dose that caused 100% mortality.

Neuropharmacological studies

The working doses (i.e. treatment doses) used in this experimental work were arrived at by the formula 1/2 x $LD_{50.}$ All treatment doses were below the half of the $LD_{50.}$

Antipsychotic tests of V. doniana in mice

Assessment of the effect of V. doniana extract on swimming induced grooming

The animals for this assay were pretreated with normal saline (10 ml/kg, p.o.), the crude extract (250, 500 and 1000 mg/kg, p.o.). One hour after treatments, mice were placed individually in swimming cylinder (8 x 8 x 18 cm high) filled with water (32^{O}C) for three min. The reference, haloperidol (dopamine receptor antagonist) treated positive control group received a dose (2 mg/kg, p.o.) 60 minutes before being placed individually to swim for three minutes. They were then removed and dried with towel for 30 seconds and placed immediately into single Perspex boxes. The number and the total duration of grooming episodes were recorded for 15 min [26]. Haloperidol (2 mg/kg, p.o.) served as standard reference.

Assessment of the effect of V. doniana extract on apomorphine-induced climbing test

Animals for this study were divided into 5 groups (n=6). The negative control group were treated orally with normal saline (10 ml/kg, p.o.), the treatment groups received the crude extract (250, 500 and 1000 mg/kg, p.o.). Fifty minutes after treatments the animals were placed into cylindrical cage, with walls of vertical metal bars 2 mm diameter 1 cm apart, surmounted by a smooth surface. After 10 minutes habituation the mice were treated with apomorphine (1.5 mg/kg, s.c.) [26]. The standard reference, haloperidol (dopamine D_2 receptor antagonist) treated positive control group received a dose (2 mg/kg, p.o.) 50 min before apomorphine injection. Ten minutes after apomorphine (APO) treatment, each animal was observed in the cylindrical cage for 15 minutes. Every minute the climbing behaviour was scored as follow: four paws on the floor (0), forepaws grasping the wall (1), four paws grasping the wall (2). Animals were also rated for repetitive sniffing as a measure of stereotypy according to the following scale: 0 = no sniffing, 1 = moderate sniffing, little snout contact with cage walls or floor, 2 = constant sniffing, persistent snout contact. Scores for both behaviours were summed for each individual and group means were calculated [26].

Assessment of the effect of V. doniana extract on ketamine-induced hyperlocomotion

The Open Field was used to evaluate the animals' exploratory activity [27,28]. Animals were treated orally with normal saline (10 ml/kg, p.o.), the crude extract (250, 500 and 1000 mg/kg, p.o.), One hour after treatments, each mouse received ketamine (10 mg/kg i.p.) and was immediately put in Open Field arena and observed for the number of squares crossed (with the four paws) during three minutes after one minute for acclimatization (locomotor activity). Ataxia (the frequency of staggered movements and number of falls) were also observed and recorded. The reference drug, haloperidol (dopamine D_2 receptor antagonist) treated positive control group received a dose (2 mg/kg, p.o) 30 min before ketamine (10 mg/kg, i.p.) injection. Haloperidol (2 mg/kg, p.o) served as standard reference.

Anti-convulsant evaluation of V. doniana in mice

Maximum electroshock-induced convulsion in mice

The method of Swinyard and Kufferberg [29] and Browning, [30] was employed. Thirty mice of both sexes were randomly allotted into five groups of six mice each. Group (1) was given normal saline (10 ml/kg, i.p.) which served as the vehicle (negative control), groups (2-4) were given the crude extracts (100, 200 and 400 mg/kg, i.p.), while group (5) was given Sodium valpoate (75 mg/kg, i.p.). Thirty minutes later, maximum electroshock was administered to induced seizure in the mice using Ugo Basile electroconvulsive machine (Model 57800) with an electrode clipped to each ear of the mice. The current, shock duration, frequency and pulse width were set and maintained at 18 mA, 1.0 s, 100 pulse per second and 0.5 ms respectively. Abolition of Hind Limb Tonic Extension (HLTE) was considered as protection from electroshock [31,32].

Pentylenetetrazole-induced convulsion in mice

The method of Swinyard *et al*. [33] was employed. Thirty mice of both sexes were randomly allotted into five groups of six mice each. Group (1) was given normal saline (10 ml/kg, i.p.) which served as the vehicle (negative control), groups (2-4) were given the crude extracts (100, 200 and 400 mg/kg, i.p.), while group (5) was given Diazepam (5 mg/kg, i.p.). Thirty minutes later, mice in all the groups received 85 mg/kg (i.p.) pentylenetetrazole. Mice were observed for over a period of 30 minutes. Absence of an episode of clonic spasm of at least 5 seconds duration indicated a compound's ability to abolish the effect of pentylenetetrazole on seizure threshold.

Strychnine-induced convulsion in mice

The method of Porter *et al*. [34] was employed. Thirty mice of both sexes were randomly allotted into five groups of six mice each. Group (1) was given normal saline (10 ml/kg, i.p.) which served as the vehicle (negative control), groups (2-4) were given the crude extracts (100, 200 and 400 mg/kg, i.p.), while group (5) was given Diazepam (5 mg/kg, i.p.). Thirty minutes later, mice in all the groups received 2.0 mg strychnine per kg, i.p. Abolition of tonic extensor jerks of the hind limbs was considered an indicator that the testing materials could prevent strychnine-induced convulsions.

Statistical analysis

Results are expressed as mean ± SEM. Statistical difference was determined by one-way analysis of variance (ANOVA) followed by a post hoc test (Student Newman-Keuls Test (SNK)). The results of convulsion experiments (percentage protection and mortality) were analyzed by non-parametric method (Chi square test). Difference was considered statistically significant with $P<0.05$ for all comparisons.

Results

Results of acute toxicity test (LD_{50})

The result of oral acute toxicity test (LD_{50}) of the aqueous leaf extract of *Vitex doniana* (AVD) was $\geq$ 5000 mg/kg. For the intraperitoneal acute toxicity test, the first critical four hours during the acute toxicity studies revealed that, animals that received higher doses of the aqueous leaf extract of *Vitex doniana* were noticed to be passive and were also seen stretching, while those that received lower doses of extract were seen active when placed back into their home cages. However, no death was recorded during the hours. The LD_{50} of AVD for the oral route of administration was > 5000 mg/kg. For the intraperitoneal route of administration, calculation of LD_{50} was done using the formula $LD_{50} = (A \times B)^{1/2}$, [35]; A is the maximum dose that cause 0% death i.e. 800 mg/kg, while B is minimum dose that caused 100% death i.e. 1,000 mg/kg. Therefore, the aqueous leaf extract of *Vitex doniana* was found to have median lethal dose (LD_{50}) in mice of 894 mg/kg i.p. (Table 1).

Antipsychotic activity of *V. doniana* in mice

Effects of *V. doniana* on swimming-induced grooming (SIG)

Effect of *V. doniana* on frequency of grooming in swimming-induced *grooming*

The sedative doses of *V. doniana* (500 and 1000 mg/kg; p.o.) in this study showed antipsychotic activity in that it significantly reduced [$F_{4,25} = 110.95$; $P<0.05$] the frequency of grooming compared to the vehicle in the swimming induced grooming model in mice. There was non-significant reduction in frequency of grooming in 250 mg/kg compared to the vehicle. Haloperidol (2 mg/kg; p.o.) group showed significant reduction ($P<0.05$) in frequency of grooming compared to the vehicle. The result is presented in figure 1a.

Effect of V. doniana on duration of grooming in swimming-induced grooming

V. doniana at 500 and 1000 mg/kg in this study showed antipsychotic activity in that it significantly reduced [$F_{4,25} = 820.99$; $P<0.05$] the duration of grooming compared to the vehicle in the swimming induced grooming model. *V. doniana* at 250 mg/kg showed no significant difference compared to the vehicle, while haloperidol (2 mg/kg p.o.) group showed significant reduction ($P<0.05$) in duration of grooming compared to the vehicle. the result is presented in Figure 1b.

Effects of V. doniana on Ketamine- induced hyperlocomotion

Effect of V. doniana on locomotion in Ketamine-induced hyperlocomotion

The results of this study showed that Ketamine (10 mg/kg; i.p.) increased the locomotive activity of mice in the ketamine-induced hyperlocomotion model compared to vehicle and the haloperidol groups. While there was no significant difference between 250 mg/kg of *V. doniana* compared to ketamine group, 500 mg/kg and 1000 mg/kg *V. doniana* significantly reduced [$F_{4,25} = 100.94$; $P<0.05$] locomotion compared to ketamine group. Although there was significant difference ($P<0.05$) between 250 mg/kg *V. doniana* compared to both 500 mg/kg and 1000 mg/kg *V. doniana*, there was no significant difference between 500 mg/kg and 1000 mg/kg dose levels. The result is presented in Figure 2a.

Effect of V. doniana on number of ataxias in ketamine-induced hyperlocomotion

Like it was observed in locomotion, the present result showed that Ketamine (10 mg/kg; i.p.) increased the number of ataxia of mice in the ketamine-induced hyperlocomotion model compared to vehicle

and the haloperidol groups. While there was no significant difference between 250 mg/kg of *V. doniana* compared to Ketamine group, 500 mg/kg and 1000 mg/kg *V. doniana* significantly reduced [$F_{4,25}$ = 46.88; P<0.05] number of ataxias compared to Ketamine group. Although there was significant difference (P<0.05) between 250 mg/kg *V. doniana* compared to both 500 mg/kg and 1000 mg/kg *V. doniana*, there was no significant difference between 500 mg/kg and 1000 mg/kg dose levels. The result is presented in Figure 2b.

Effect of V. doniana on apomorphine- induced climbing test in mice

V. doniana at 500 and 1000 mg/kg caused a significant reduction [$F_{4,25}$ = 340.98; P<0.05] in number of climbing and sniffing compared to vehicle in apomorphine- induced climbing. 250 mg/kg of *V. doniana* did not cause any significant difference in number of climbing and sniffing compared to vehicle. Haloperidol (2 mg/kg, p.o.) caused a significant difference (P<0.05) in number of climbing and sniffing compared to vehicle. The result is presented in Figure 3.

Anticonvulsant assessment of V. doniana in mice

Effect of aqueous leaf extract of V. doniana on Maximum electroshock-induced convulsion in mice

In the control group, the entire animals exhibited hind limb tonic extension (HLTE) after electroshocks. Pretreatment with Sodium Valpoate (75 mg/kg, i.p.) gave a 50% protection against HLTE. All the doses of *V. doniana* tested gave a varying degree of protection against HLTE. 100 mg/kg like the reference drug (Sodium Valpoate) gave 50% protection against HLTE, and also significantly shortened [$F_{4,25}$ = 6.18; P<0.05] the recovery time from HLTE compared to the vehicle group. 200 mg/kg and 400 mg/kg gave 88.33% and 66.67% protection from HLTE respectively, and 200 mg/kg significantly reduced (P<0.05) recovery time from HLTE. The result is presented in Table 2.1.

Effect of aqueous leaf extract of V. doniana on Pentylenetetrazole (PTZ)- induced convulsion in mice

In the control group of animals, PTZ (85 mg/kg; i.p.) consistently induced tonic-clonic convulsions. There was 100% occurrence of tonic convulsion and mortality. While pretreatment with diazepam (5 mg/kg, i.p.) significantly suppressed both clonic and tonic PTZ convulsions, with 0% mortality, pretreatment with *V. doniana* at any of the tested doses did not block both the clonic and the tonic PTZ convulsions. However, *V. doniana* at all the doses tested significantly delayed [$F_{4,25}$ = 164.30; P<0.05] the time of death. The result is presented in Table 2.2.

Effect of aqueous leaf extract of V. doniana on Strychnine-induced convulsion in mice.

In the control group of animals, 2 mg/kg, i.p. of strychnine induced tonic-clonic convulsions in all the animals in the group and with 100% mortality. Neither 5 mg/kg of diazepam nor *V. doniana* at all the doses tested protected the animals against death. 100% mortality was recorded in all groups of animals. However, 5 mg/kg i.p of diazepam, and 200 mg/kg *V. doniana* significantly [$F_{4,25}$ = 15.69; *P* = .05] delayed the time of death caused by strychnine. The result is presented in Table 2.3.

Discussion

This study investigated the acute toxicity profile, antipsychotic and anticonvulsant effects of the aqueous leaf extract of *Vitex doniana* (AVD) in mice models.

The AVD was found not to yield any toxicity, nor did it produce any varied symptoms of deferred toxicity in terms of atypical behaviours in mice when administered orally up to 5000 mg/kg body weight according to Lorke's method of acute toxicity testing [35]. Intraperitoneally however, the AVD was found to have a median lethal dose (LD_{50}) of 894 mg/kg, i.p. in mice. Thus, it can be inferred that AVD, based on general categorizations of toxic compound, is safe in mice when administered through the oral route [36]. The AVD can be said to be moderately toxic to the experimental animal model (mice) used in this study when administered intraperitoneally. Lorke, [35], stated that substances toxic at less than 1 mg/kg are considered to be highly toxic; and considering that the LD_{50} estimate of the plant extract of AVD was far above this toxicity level thus, AVD can be said to be moderately toxic intraperitoneally. In addition, *Vernonia amygdalina* with a similar LD_{50} of 894 mg/kg i.p., has been adjudged to be moderately toxic [37].

The 500 mg/kg and 1000 mg/kg doses of AVD showed antipsychotic activity in this present study. Reports have shown that drugs or substances, that elicited reduction of grooming induced by the immersion of mice in water, possessed antipsychotic activity [38]. AVD at 500 mg/kg and 1000 mg/kg showed significant reduction in both frequency and duration of swimming- induced grooming thus elicited antipsychotic activity in this model. Also, in this study, 500 mg/kg and 1000 mg/kg of AVD reduced hyperlocomotion induced by ketamine. Ketamine is a competitive antagonist of NMDA receptors, and it does induce behavioural effects, both in healthy humans and experimental animals, that are similar to positive, negative and cognitive symptoms of schizophrenia [39]. However, haloperidol and risperidone- both neuroleptics, have been established to decrease locomotor activity. This decrease in locomotor activity is believed to be probably due to reduction in the excitability of the CNS or through sedative effects of these neuroleptics [39]. Thus, AVD, in the present study, and some other reported traditional medicinal agents [40], have shown antipsychotic activity by their ability to reduce hypermobility caused by ketamine. AVD also caused reduction in ataxia induced by ketamine, which also suggests its antipsychotic activity, and which may involve amelioration of glutaminergic neurotransmission; as dysfunction of glutaminergic neurotransmission mediated via the NMDA receptor has been implicated in the pathophysiology of psychotic illness such as schizophrenia [41]. This study also showed that AVD at 500 and 1000 mg/kg significantly reduced apomorphine-induced climbing and sniffing. Studies have shown that neuroleptic substances inhibit apomorphine- induced behaviour, and that the inhibitory action is mediated via the antagonism of D_1 and D_2 dopamine receptors [38]. Since AVD, in this present study, significantly reduced number of climbing and sniffing behaviour compared to vehicle in apomorphine- induced climbing; it is suggestive of its antipsychotic activity that is mediated through antagonism of D_1 and D_2 dopamine receptors system.

AVD at all the doses tested in this present study gave a varying degree of protection against maximum electroshock (MES) induced hind limb tonic extension (HLTE). The MES is a standard protocol that examines the ability of any test agents to protect against convulsions [42]. Protection against HLTE in the MES suggests anticonvulsant potential of antiepileptics that prevent the spread of the epileptic seizure from an epileptic focus during seizure

activity [43]. Antiepileptic drugs that are effective in the treatment of generalized tonic-clonic and partial seizures such as phenytoin, carbamazepine, oxcarbazepine and lamotrigine suppress HLTE in MES model [44]. AVD can also be said to possess antiepileptic activity since it protected from HLTE in the experimental animals used in this study. AVD at the doses tested in the study did not protect against PTZ's tonic - tonic convulsions. Anticonvulsant activity in PTZ model identifies compound that can raise the seizure threshold in the brain [45]. However, strychnine is a competitive antagonist of glycine in the spinal cord [46]. Agents that prolonged the onset of convulsion and death latency in strychnine- induced convulsion model are said to possess antiepileptic capability [47], AVD at the higher dose level (200 mg/kg, i.p.) used in this study, can be suggested to possess antiepileptic activity, because it caused prolongation of the onset of convulsion and death latency.

Phytochemicals such as flavonoids, alkaloids, terpenoids, phytosterols, phenols, tannins, amino and fatty acids have been reported to be responsible for many neuro- and psychopharmacology effects observed in different plant extracts [43]. Since the review of the phytoconstituents of *V. doniana* showed that the extract contains not a few of these chemicals, it is not impossible to attribute the various neuro- and psychopharmacology effects observed in *V. doniana*, in this present study, to these active principles, which may be centrally acting through the modulation of the glycine, GABA, dopamine and serotonin receptors systems. Further mechanistic work will be carried out to establish the mechanism of action of the extract.

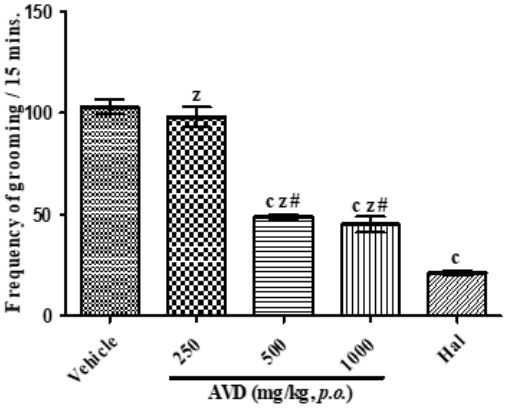

Figure 1a: Effect of aqueous leaf extract of *Vitex doniana* (AVD) on frequency of grooming in swimming induced grooming test in mice. Each bar is expressed as Mean ± SEM; (n = 6 per group). c = P<0.05 compared to vehicle (Normal saline; 10 ml/kg, p.o.). z = P<0.05 compared to positive control (Haloperidol (Hal); 1 mg/kg, i.p.) and # = P<0.05 compared 250 mg/kg to 500 mg/kg and 1000 mg/kg; (ANOVA; SNK).

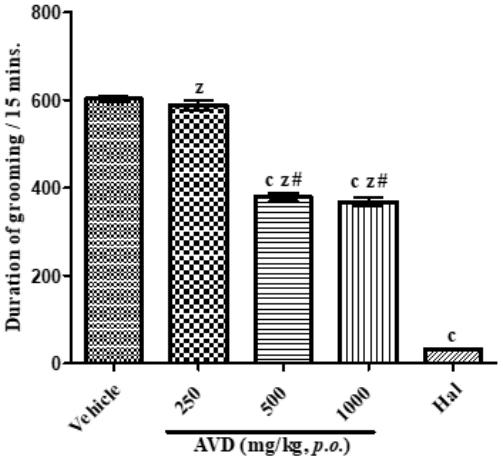

Figure 1b: Effect of aqueous leaf extract of *Vitex doniana* (AVD) on duration of grooming in swimming induced grooming test in mice. Each bar is expressed as Mean ± SEM; (n = 6 per group). c = P<0.05 compared to vehicle (Normal saline; 10 ml/kg, p.o.) z = P<0.05 compared to positive control (Haloperidol (Hal); 2 mg/kg, i.p.) and # = P<0.05 compared 250 mg/kg to 500 mg/kg and 1000 mg/kg; (ANOVA; SNK).

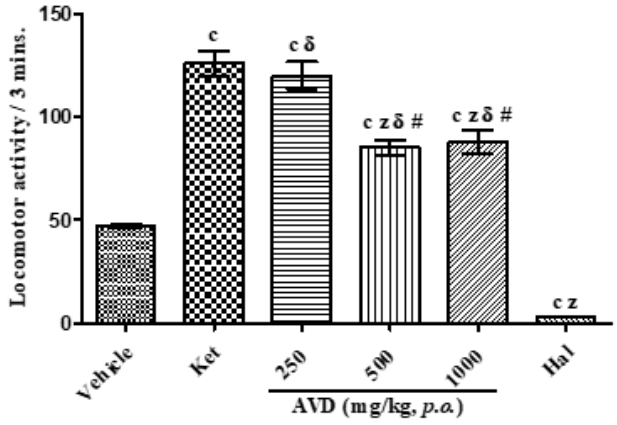

Figure 2a: Effect of aqueous leaf extract of *Vitex doniana* (AVD) on locomotor activity in Ketamine (Ket) induced hypermobility test in mice. Each bar is expressed as Mean ± SEM; (n = 6 per group). c = *P*<0.05 compared to Vehicle (Normal saline; 10 ml/kg, p.o.). z = *P*<0.05 compared to Ketamine (10 mg/kg, i.p.), and δ = P<0.05 compared to positive control (Haloperidol (Hal); 2 mg/kg, p.o.) and # = P<0.05 compared 250 mg/kg to 500 mg/kg and 1000 mg/kg; (ANOVA; SNK).

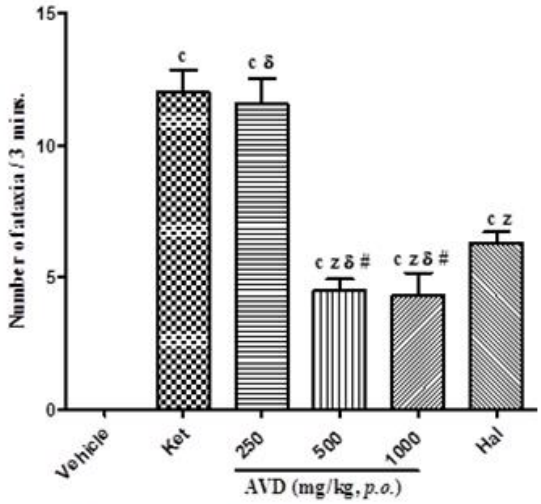

Figure 2b: Effect of aqueous leaf extract of *Vitex doniana* (AVD) on ataxia in Ketamine (Ket) induced hypermobility test in mice. Each bar is expressed as Mean ± SEM; (n = 6 per group). c = P<0.05 compared to Vehicle (Normal saline; 10 ml/kg, p.o.) z = P<0.05 compared to Ketamine (10 mg/kg, i.p.), and δ = P<0.05 compared to positive control (Haloperidol (Hal); 2 mg/kg, p.o.) and # = P<0.05 compared 250 mg/kg to 500 mg/kg and 1000 mg/kg; (ANOVA; SNK).

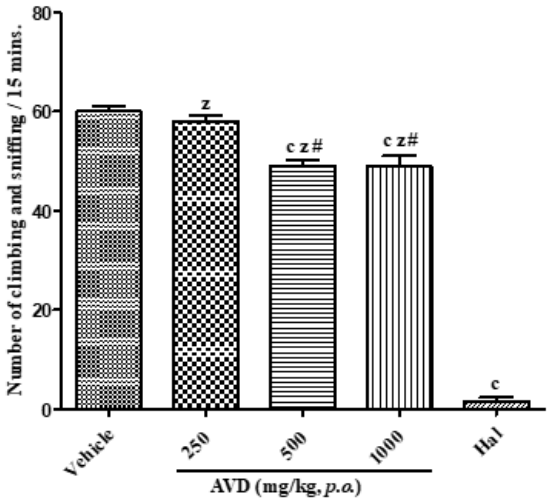

Figure 3: Effect of aqueous leaf extract of *Vitex doniana* (AVD) on numbber of climbing and sniffing in apomorphine (1.5 mg/kg; s.c.) induced climbing test in mice. Each bar is expressed as Mean ± SEM; (n = 6 per group). c = P<0.05 compared to vehicle (Normal saline; 10 ml/kg, p.o.), z = P<0.05 compared to positive control (Haloperidol (Hal); 2 mg/kg, p.o.), and # = P<0.05 compared 250 mg/kg to 500 mg/kg and 1000 mg/kg; (ANOVA; SNK).

Table 1. LD_{50} Determination of *Vitex doniana* aqueous leaf extract in mice

Oral			
First phase			
Dose of aqueous leaf extract of *Vitex doniana* (mg/kg)	No of mice used	No of animals that died	Mortality rate
10	3	0	0/3
100	3	0	0/3
1000	3	0	0/3
Second phase			
1,600	1	0	0/1
2,900	1	0	0/1
5,000	1	0	0/1
Intraperitoneal			
First phase			
10	3	0	0/3
100	3	0	0/3
1000	3	1	1/3
Second phase			
800	1	0	0/1
1000	1	1	1/1
1,600	1	1	1/1
2,900	1	1	1/1

Table 2.1. Effect of intraperitoneal administration of aqueous leaf extract of *Vitex doniana* (AVD) on hind limbs tonic extension (HLTE) in maximal electro-shock (MES) induced convulsion in mice

Treatments (mg/kg)	MEAN ± S.E.M (SEC)				
	Onset of HLTE	Duration of HLTE	Recovery time	Quantal Protection	% Protection
Vehicle (ml/kg) 10	3.50 ± 0.25	15.00 ± 0.83	94.00 ± 6.30	0/6	0.00
AVD 100	1.90 ± 0.12	19.00 ± 2.00*#	49.00 ± 11.00*#	3/6	50.00*
AVD 200	2.20 ± 0.00	23.10 ± 0.00*#@	44.00 ± 0.00*#	5/6	88.33*#@
AVD 400	3.00 ± 0.74	23.00 ± 0.05*#@	81.00 ± 2.50@$	4/6	66.67*#@$
VAL 75	3.40 ± 0.37	13.00 ± 0.20	86.00 ± 11.00	3/6	50.00*

Data are expressed in mean ± SEM of latency to hind limb tonic extension (HLTE), duration of HLTE, recovery time from HLTE, quantal protection and percentage of protection from HLTE.* = P<0.05 compared to vehicle (Normal saline), # = P<0.05 compared to positive control (Sodium valpoate (VAL)), @ = P<0.05 compared 100 mg/kg to 200 mg/kg and 400 mg/kg, and $ P<0.05 compared 200 mg/kg to 400 mg/kg. (ANOVA; SNK).

Table 2.2. Effect of intraperitoneal administration of aqueous leaf extract of *Vitex doniana* (AVD) on clonic and tonic seizure following Pentylenetetrazole (PTZ) (85 mg/kg, i.p.) induction in mice

Treatments (mg/kg)	MEAN ± S.E.M (SEC)		
	Onset of clonus	onset of tonus	Time of death
Vehicle (ml/kg) 10	84.78 ± 15.04	156.00 ± 29.00	171.80 ± 29.17
AVD 100	87.87 ± 8.21#	255.00 ± 47.00#	334.70 ± 87.46*#
AVD 200	88.68 ± 8.86#	307.00 ± 49.00#	395.20 ± 57.25*#
AVD 400	67.35 ± 5.78#	237.00 ± 59.00#	388.00 ± 48.79*#
DZM 5	1800.00 ± 0.00*	1800.00 ± 0.00*	1800.00 ± 0.00*

Data are expressed in mean ± SEM of latencies to clonus, tonus, and time of death. * = P<0.05 compared to vehicle (Normal saline), and # = P<0.05 compared to positive; control (Diazepam (DZM)). (ANOVA; SNK).

Table 2.3. Effects of intraperitoneal administration of aqueous leaf extract of *Vitex doniana* (AVD) on clonic and tonic seizure following Strychnine (2 mg/kg, i.p.) induction in mice

Treatments (mg/kg)	MEAN ± S.E.M (SEC)		
	Onset of clonus	Onset of tonus	Time of death
Vehicle (ml/kg) 10	177.20 ± 16.87	199.20 ± 18.98	214.80 ± 17.90
AVD 100	83.02 ± 8.02*#	96.93 ± 12.55*#	123.20 ± 13.36#
AVD 200	195.00 ± 12.94@	265.50 ± 28.20@	468.40 ± 86.03*#@
AVD 400	129.20 ± 30.41	136.30 ± 29.32#$	258.20 ± 56.43#$
DZM 5	205.00 ± 28.20*	309.90 ± 40.98*	673.90 ± 73.49*

Data are expressed in mean ± SEM of latencies to clonus, tonus, and time of death. * = *P* <0.05 compared to vehicle (Normal saline), # = *P* <0.05 compared to positive control Diazepam (DZM)), @ = *P* =.05 compared 100 mg/kg to 200 mg/kg and 400 mg/kg, and $ = P<0.05 compared 200 mg/kg to 400 mg/kg. (ANOVA; SNK).

Conclusions

From this study, the following deductions can be drawn on the antipsychotic and anticonvulsant effects of *Vitex doniana* in mice:

- Aqueous leaf extract of *V. doniana* is safe in oral administration, but moderately toxic in intraperitoneal administration in mice.
- It can be concluded, that *V. doniana* possesses significant antipsychotic and antiepileptic effects, thus providing pharmacological justification for some of its ethnomedicinal uses.

Authors' Contribution

This work was carried out in collaboration between all authors. This work emanated from the Ph.D. research works of author JOI. Author JOI conceived and designed the study, performed and managed the literature search, statistical analysis wrote the protocol, carried out the laboratory works and wrote the first draft of the manuscript. Author AMA was the main supervisor, and provided some materials used in the work, while author IAO co-supervised the work, provided some materials used in the work and also assisted in some of the laboratory works. All authors read and approved the final manuscript.

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Conflict of interest

The authors declare that they have no competing interests.

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