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NEUROPHARMACOLOGICAL EVALUATION OF ETHANOLIC LEAF EXTRACT OF *ALTERNANTHERA BRASILIANA* (L.) KUNTZE (AMARANTHACEAE) IN MICE

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ABSTRACT: *Alternanthera brasiliana* is a medicinal plant used in the folkloric treatment of malaria, pains, infections and diabetes in South-western states of Nigeria. Recent reports indicate some central activities for the plant. This study evaluated ethanolic leaf extract of the plant for novelty-induced behaviour, anxiety, sedation and convulsion in mice. Dried powdered leaves of the plant were extracted with 70% ethanol, filtered and concentrated *in vacuo* to obtain ethanolic leaf extract (ELE) of *A. brasiliana*. The acute toxicity (LD_{50}) of the ELE was determined through oral (p.o.) and intraperitoneal (i.p.) routes. ELE (250, 500 and 1000 mg/kg, p.o.) was evaluated for novelty induced behaviours, anxiolytic, sedative and anticonvulsant activities. The LD_{50} values obtained for the ELE were ≥5000 mg/kg, p.o. and 3808 mg/kg, i.p. The ELE (500-1000 mg/kg) caused significant ($p<0.05$) increase in rearing [$p<0.05$, $p< 0.01$; $F_{(4, 25)}= 37.0$] and locomotor [$p<0.05$, $p< 0.01$; $F_{(4, 25)}= 36.0$] activities; caused increase in the number of head dips at 1000 mg/kg [$p<0.01$, $F_{(4, 25)} = 9.0$] and non-significantly increased time spent on the open arms of the elevated plus maze; caused significant increase in sleep latency [$p<0.01$, $F_{(4, 25)}=37.0$] but decrease non-significantly total sleeping time induced by ketamine. Finally, the ELE at all the doses used did not protect the animals against PTZ, strychnine or incidence of tonic hind limb extension on MES. This study concluded that ethanolic leaf extract of *A. brasiliana* on the central nervous system is stimulatory; possess moderate anxiolytic activity but lack anticonvulsant activity.

INTRODUCTION: In the recent times, there has been a renewed focus on medicinal plants research worldwide due to their widespread use in traditional settings particularly in developing countries [1]. This trend was partly associated with their relatively safe and cheap status and claims of efficacy against a wide variety of chronic or incurable diseases such as cancer, diabetes, arthritis etc. [2].

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The importance of medicinal plants has gained global acceptability culminating in the issuance of several international Guidelines and Acts by the World Health Organization (WHO) and European Union (EU) relating to rationale use of herbal medicines [3].

Alternanthera brasiliana (L.) O. Kuntze, (Amaranthaceae) is an herbaceous plant indigenous to tropical and sub-tropical regions of South America (Brazil) and Australia [4, 5]. *A. brasiliana* is described as perennial, prostrate and branchy presenting a circular to polygonal stem in transection, long internodes and swollen nodes, at which opposite leaves attach. The flowers are inconspicuous, white in clusters, composed of

hermaphrodite and monocyclic flowers [6]. The leaves are purple, simple, entire, and decussate presenting uniserate epidermis [7]. *A. brasiliana* is widely found in several other countries including Nigeria (South-Western states), where it is found freely growing in the wild, along the highways and in uncultivated land. *A. braziliana* otherwise known as *Oko Oru* among the Yoruba people of Nigeria is an important medicinal plant gaining new attention by the herbalists in areas where it is found.

In Brazil the plant is particularly popular as an herbal agent used in the treatment of inflammation, infections, cough, cancerous growth and pains [8, 9]. Globally, the ethnomedicinal uses of the plant include treatment of colds, grippes and headache [10]; as antipyretic [6]; treatment of diarrhoea [5]; night blindness, diarrhoea, dysentery and post-natal complaints [6]; as antipyretic and anti-diarrhoea agent [11].

FIG.1: *ALTERNANTHERA BRASILIANA* PLANT GROWING IN THE BUSH

In South-West Nigeria, *A. brasiliana* is an important medicinal agent used in the management of diabetes, pain and cough among other folkloric uses.

Pharmacological activities reported on the various extracts obtained from the plant include; wound healing [6, 12], antibacterial [4, 13], anti-inflammatory and analgesic [8]; anxiolytic and anticonvulsant activities [9]; anti-inflammatory, analgesic, anxiolytic and locomotor effect of the aqueous leaf infusions [14].

Few phytochemical studies have been conducted to evaluate the chemical constituents of this plant. Phenols, flavonoids, phytosterols, tannins, alkaloids, saponins and carbohydrate have been reported to be present in the n-hexane fraction of the leaf extract [4]. Aqueous leaf extract of *A. brasiliana* was reported to contain flavonoids, mainly 3-O-robinobioside derivatives of kaempferol and quercetin [8].

From our literature search we found no comprehensive report on the central effect of the leaf ethanolic extract of *A. brasiliana* and considering the various biological activities that have been reported for this plant, it is imperative to evaluate it for some CNS effects as a first step in our quest to identifying plants with considerable central activities. Thus our aim in this study was to evaluate the ethanolic leaf extract of this plant for some neuropharmacological activities using standard animal models.

MATERIALS AND METHODS:

Plant collection and identification:

Alternanthera brasiliana leaves were collected within the campus of the Obafemi Awolowo University and identified by Mr. G. Ibhanesebhor, the taxonomist, Department of Botany, Faculty of Sciences, OAU, Ile-Ife and herbarium number Ife 16959 was obtained.

Preparation of plant materials:

The collected leaves were air dried for two weeks at room temperature, grounded into coarse powder and 550 g of the pulverized powder extracted with 70% ethanol for 72 h and thereafter filtered. The filtrate obtained was concentrated *in vacuo* at 40 ^{0}C using rotary evaporator to obtain 91.5 g (16.6% w/w) ethanolic leaf extract (ELE) of the plant and was refrigerated until use.

Drugs:

Diazepam (Valium ^{R} Roche, Switzerland), pentylenetetrazole (Sigma, USA), ketamine (Sigma, USA), strychnine (Sigma, Switzerland, MSDS), phenytoin sodium (Sigma, Poole, UK) and other reagents used were of analytical grade.

Experimental animal: Albino mice of both sexes (18-25 g) were obtained from the Animal House,

Department of Pharmacology, Faculty of Pharmacy, Obafemi Awolowo University, Ile- Ife, Nigeria. They were maintained under natural daylight/night condition. All animals had free access to drinking water and standard commercial diet (Guinea Feeds Brand, Bendel Feeds, Nigeria). The study was approved by the Faculty of Pharmacy Postgraduate Committee and all animal experiments were carried out in strict compliance with the National Institute of Health (NIH, 1985) as being implemented by the Obafemi Awolowo University Research Committee.

Methods:

Acute toxicity studies:

Acute toxicity effect of *A. brasiliana* was assessed in mice using the intraperitoneal route (i.p.) and oral route (p.o.) according to Lorke's method [15]. For each route, the procedure was divided into two phases. In the first phase, 3 animals per dose levels of 10, 100 and 1000 mg/kg were used and the animals were monitored for 24 h for mortality. There was no mortality at 1000 mg/kg in either route; hence in the 2nd phase one animal each was used per dose level at 1600, 2900 and 5000 mg/kg separately for the two routes and the animals were also monitored for 24 h for mortality and general behaviour. The LD_{50} value was calculated for each route separately and was estimated as the geometric mean of the lowest dose causing death and the highest dose causing no death according to the formula below:

$$LD_{50} = (A \times B)^{½}$$

Where A = maximum dose producing 0% death, and B = minimum dose producing 100% death Working Doses ≤ ½ (LD_{50})

Experimental design:

In all the subsequent tests, five groups of mice (n=6) were randomly selected and arranged as follows:

Group 1: Negative control treated with normal saline (10 ml/kg, p.o.)

Groups 2-4: Test groups treated with the ethanolic leaf extract (ELE) of *A. brasiliana* (250, 500 and 1000 mg/kg, p.o.)

Group 5: Positive control treated with the appropriate standard drug.

All animals were orally pretreated with normal saline or extract for 1 h prior to test, while positive drugs were administered ½ h intraperitoneally prior to test.

Behavioural and exploratory studies:

Novelty-induced behaviours (NIB):

Different groups of mice pretreated with normal saline, ELE (250-1000 mg/kg) or diazepam (1 mg/kg) as described above were evaluated for novelty-induced behavioural effects of rearing, grooming and locomotor [16]. Each mouse was placed inside plexiglas's cage and observed for rearing, grooming and locomotion for 30 min.

Anxiolytic studies:

Hole board test:

The hole- board is a flat space, (field) of 25 cm x 25 cm with holes (each 3 cm in diameter). Mice randomly grouped and treated as in previous section were tested on the hole board [17]. Diazepam (1 mg/kg) was used as positive control drug. Each treated mouse was dropped gently at the center of the hole- board and the number of head dips performed by the mouse was counted for a period of 5 minutes.

Elevated plus maze:

The elevated plus maze (EPM) was used to evaluate anxiety in laboratory animal [18]. The EPM for mice consists of two open arms 30 x 5 cm and two closed arms 30 x 5 x 15 cm with an open roof, arranged such that the two arms of each type were opposite each other. The maze was elevated to a height of 39 cm; the animal behaviour was recorded 2 m away in the same room.

Mice treated as in previous section were tested on the EPM. Diazepam (1 mg/kg) was used as positive control drug. Each mouse was observed for the number of entries into the open and closed arms, and the total time spent in the open and closed arms, for 5 minutes.

Sedative test:

Ketamine-induced hypnosis: The effect ELE on ketamine-induced hypnosis was evaluated as

described [19]. Ketamine (100 mg/kg, i.p.) was used to induce hypnosis. Pretreated mice with normal saline or extract as described earlier were injected with ketamine, while diazepam (1 mg/kg) served as positive control drug.

The time interval between the administration of ketamine until the loss of the righting reflex was recorded as onset of sleep (sleep latency), while the time from the loss to regaining of the righting reflex was recorded as the duration of sleep (total sleeping time).

Anticonvulsants experiments:

Pentylenetetrazole (PTZ)-induced convulsion in mice:

PTZ (85 mg/kg, i.p.) was used to induce tonic-clonic convulsions [20]. Different groups of mice were pre-treated with normal saline, the ELE (250, 500 or 1000 mg/kg) or diazepam (1 mg/kg, i.p.), prior to PTZ (85 mg/kg, i.p.). Each animal was observed for tonic-clonic convulsion. Animals that survived beyond 30 minutes were regarded as being protected. The onset of convulsion and time of death of each mouse was recorded.

Strychnine-induced convulsions:

Strychnine (2 mg/kg, i.p.) was used to induce tonic-clonic convulsions [21] instead of PTZ and the experiment repeated as in the previous section but diazepam (1 mg/kg, i.p.) served as positive control. Each animal was observed for tonic-clonic convulsion and animals that survived beyond 30 min were regarded as being protected. The onset of convulsion and time of death of each mouse was recorded.

Maximal electroshock seizure (MES):

Mice were randomly distributed into different groups (n=6) and pre-treated as described above with normal saline, ELE (250, 500 and 1000 mg/kg, p.o.) or phenytoin sodium (25 mg/kg, i.p.) prior to application of electrical shock [22]. Each mouse was subjected to maximal electroshock (18 mA, 1s, 100 pulses/second, and pulse width of 0.5 ms) delivered through the ear lobes by electrode clamp (trans-auricular ear clips), using an Electro-Convulsiometer, UGO BASILE ECT Unit (Italy). Protection against tonic hind limb extension (THLE) was regarded as positive effect.

Statistical analysis:

All data were presented as mean± SEM and the results were analyzed by ANOVA followed by Dunnett's test as post hoc using Graph Pad InStat® Biostatistics software (GraphPad Software, Inc., La Jolla, USA). The treated groups were compared to the negative control group and the level of significance set at $\leq p<0.05$.

RESULTS:

Acute toxicity:

There was no mortality up to 5 000 mg/kg in mice treated orally with the ELE of *A. brasiliana*, hence the LD_{50} was estimated to be ≥5000 mg/kg, p.o., however, in the intraperitoneal route, there was mortality at 2900 mg/kg but none at 5000 mg/kg, hence the LD_{50} was calculated to be 3808 mg/kg, i.p.

Effects of ELE of *A. brasiliana* on novelty-induced behaviours in mice:

The ELE caused increase in rearing at all the doses (250, 500 and 1000 mg/kg) but statistically significant at 500 and 1000 mg/kg [$p<0.05$, $p<0.01$; $F_{(4,25)} = 37.0$] when compared to the control group; however, diazepam (1 mg/kg) caused significant decrease ($p<0.01$) when compared to the control group. The ELE (250, 500 and 1000 mg/kg) caused increase in grooming but not statistically significant when compared to the control group; however, diazepam (1 mg/kg) caused significant decrease (*$p<0.05$) compared to the control group.

The extract at all the doses used caused increase in locomotion compared to the control group but statistically significant at 500 and 1000 mg/kg [$p<0.05$, $p<0.01$; $F_{(4,25)}=36.0$] however, diazepam (1 mg/kg) caused significant decrease ($p<0.01$) compared to the control group (**Fig.2 A-C**).

Anxiolytic test:

Effects of the ELE of *A. brasiliana* on head-dipping in mice:

The ELE at all the doses (250, 500 and 1000 mg/kg) increased head dips when compared to the control group but statistically significant ($p<0.01$) only at 1000 mg/kg ($F_{(4,25)}= 9.0$). Diazepam (1 mg/kg) also caused significant increase ($p<0.01$) in head dips when compared to the control group (**Fig.3**).

Effect of ELE of *A. brasiliana* on the EPM: The ELE at all the doses used did not cause significant change in the percentage number of entries into the open arms and increased time spent on the open arms of the EPM but non-significantly when compared to the control group. However, diazepam (1 mg/kg) caused significant ($p<0.01$) increase in the % of entries into and total time spent on the open arms of the EPM when compared to vehicle and test groups (results not presented).

Effect of ELE of *A. brasiliana* on ketamine-induced hypnosis: The ELE at all the doses significantly [$p<0.01$; $F_{(4,25)}=37.0$] increased sleep latency (SL) compared to vehicle but diazepam (1 mg/kg) caused significant ($p<0.01$) decrease in SL compared to vehicle. Also, the extract at all the doses used (250, 500 and 1000 mg/kg) caused non-significant but dose-dependent reduction in total sleeping time (TST) when compared to vehicle. However, diazepam caused significant ($p<0.01$) increase in TST compared to the other groups (**Fig.4**).

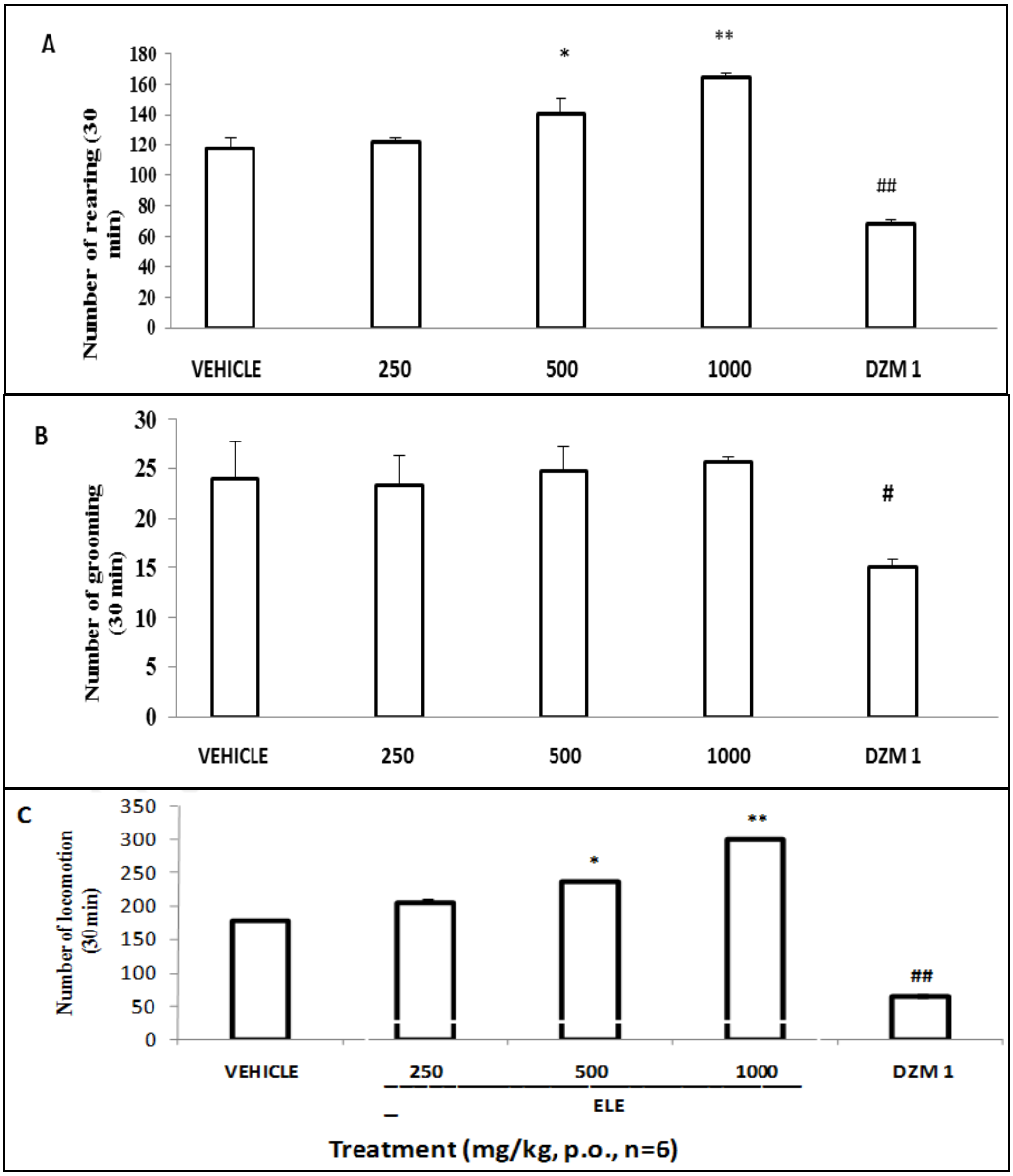

FIG.2: EFFECT OF ELE OF *A. BRASILIANA* ON NOVELTY-INDUCED REARING (PANEL A), GROOMING (PANEL B) AND LOCOMOTION (PANEL C) BEHAVIOUR IN MICE.

Each bar represent mean±SEM. VEHICLE, ELE and DZM represent normal saline, ethanolic leaf extract of *A. brasiliana* and diazepam (1 mg/kg, i.p.) respectively. N=6.

*p<0.05, **p<0.01; statistically higher than vehicle

##P<0.01, statistically lower than vehicle (ANOVA, Dunnett's)

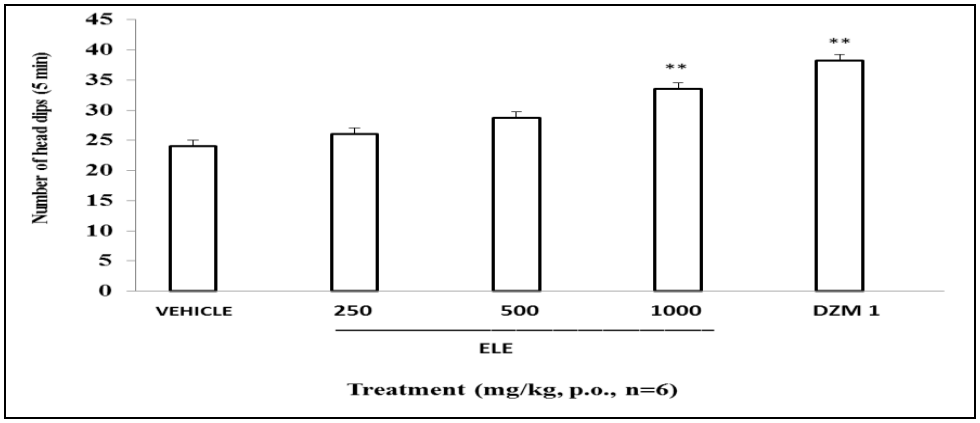

FIG.3: EFFECTS OF ELE OF *A. BRASILIANA* ON HEAD DIPS IN MICE

Each bar represents mean±SEM. VEHICLE, ELE and DZM represent normal saline, ethanolic leaf extract of *A. brasiliana* and diazepam (1 mg/kg, i.p.) respectively.

**p<0.01; statistically higher than vehicle (ANOVA, Dunnett's)

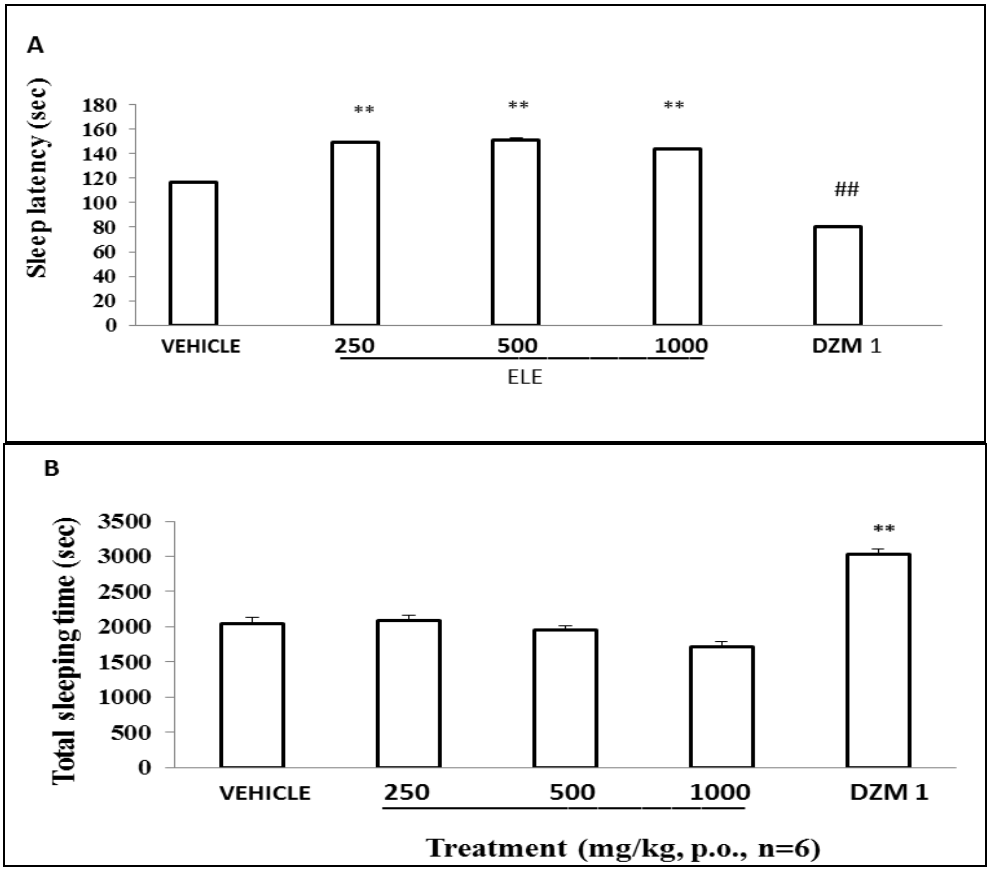

FIG.4: EFFECTS OF ELE OF *A. BRASILIANA* ON SLEEP LATENCY (PANEL A) AND THE TOTAL SLEEPING TIME (PANEL B) IN MICE.

Each bar represents mean±SEM. VEHICLE, ELE and DZM represent normal saline, ethanolic leaf extract and diazepam respectively.

**p<0.01; statistically higher than vehicle

##P<0.01, statistically lower than vehicle (ANOVA, Dunnett's)

Effect of ELE of *A. brasiliana* on PTZ-induced convulsion in mice: All the mice in the vehicle and ELE treated groups were not protected against PTZ-induced convulsion resulting in 0 % protection and 100 % mortality. However, diazepam (1 mg/kg) protected the animals against PTZ-induced convulsion with 100 % protection.

Effect of ELE of *A. brasiliana* on strychnine-induced convulsion in mice: Mice in the vehicle, extract (250, 500 and 1000 mg/kg) and diazepam (1 mg/kg) groups were not protected against strychnine-induced convulsion resulting in 0 % protection and 100 % mortality for all the groups.

Effect of ELE of *A. brasiliana* on MES: The vehicle and the extract at all dose levels (250, 500 and 1000 mg/kg) did not protect the animals against incidence of tonic hind limb extension (THLE) but phenytoin (25 mg/kg, i.p.) protected the animals against THLE. There was no mortality recorded in any group.

TABLE 1: EFFECT OF ELE OF *A. BRASILIANA* ON PTZ-INDUCED CONVULSION IN MICE

Treatment (mg/kg, p.o., n=6)	Onset of clonic convulsion (sec)	Time of death (sec)	Mortality (%)	Remark
VEHICLE	71.5 ± 2.6	402.3 ± 23.2	100	Not protected
ELE 250	72.8 ± 7.3	625.7 ± 118.5	100	Not protected
ELE 500	92.0 ± 11.1	520.7 ± 96.7	100	Not protected
ELE 1000	80.7 ± 2.4	518.5 ± 47.4	100	Not protected
DZM 1	1800.0 ± 0.0**	0.0 ± 0.0**	0	Protected

VEHICLE, ELE and DZM represent normal saline, ethanolic leaf extract and diazepam respectively.
**p<0.01; statistically different from all other groups (ANOVA, Dunnett

TABLE 2: EFFECT OF ELE OF *A. BRASILIANA* ON STRYCHNINE-INDUCED CONVULSION IN MICE

Treatment (mg/kg, p.o., n=6)	Onset of clonic convulsion (sec)	Time of death (sec)	% Mortality	% Protection
VEHICLE	285.7 ± 27.2	401.5± 80.9	100	0
ELE 250	245.5 ± 14.7	362.2 ± 75.7	100	0
ELE 500	282.7 ± 19.6	299.3 ± 24.3	100	0
ELE 1000	298.0 ± 36.8	579.0 ± 79.4	100	0
DZM 1	266.2 ± 43.1	335.0 ± 68.7	100	0

VEHICLE, ELE and DZM represent normal saline, ethanolic leaf extract and diazepam respectively.

TABLE 3: EFFECT OF ELE OF *A. BRASILIANA* ON MAXIMUM ELECTROSHOCK (MES)

Treatment (mg/kg, p.o., n=6)	Incidence of tonic hind limb extension (THLE)	%Mortality	Remark
VEHICLE	+	0	Not protected
ELE 250	+	0	Not protected
ELE 500	+	0	Not protected
ELE 1000	+	0	Not protected
PHS 25	-	0	Protected

VEHICLE, ELE and PHS represent normal saline, ethanolic leaf extract and phenytoin sodium respectively.

DISCUSSION:

This study investigated the acute toxicity profile of the ethanolic dried leaf extract of *A. brasiliana* orally and intraperitoneally in mice; while the extract was evaluated for novelty induced behaviours (rearing, grooming and locomotion), anxiolytic (elevated plus maze and hole board), sedative (ketamine-induced hypnosis) and anticonvulsant tests using pentylenetetrazole (PTZ)-, strychnine- and maximal electroshock (MES)-induced convulsion models. The results obtained showed that the extract was non-toxic orally but moderately toxic intraperitoneally; and also demonstrated significant increase in CNS activities which signifies central excitatory effect.

The LD_{50} values obtained were ≥5000 and 3808 mg/kg for oral and intraperitoneal routes respectively. From the values of the LD_{50} obtained here, the extract could be said to be relatively safer through the oral route when compared to the intraperitoneal route. This could be due to pharmacokinetic factors such as metabolism in the GIT, biodegradation, absorption rate, effect of food substance in the gut, enzymatic activity etc. [23, 24]. Accordingly, the ethanolic extract of this plant is assumed to be non-toxic and slightly toxic through the oral and intraperitoneal routes respectively [15, 25]. This acute toxicity results obtained here could partly explain the widespread use of the plant as a safe herbal agent in folkloric medicine and could be used to justify its continuous use in traditional settings.

The extract caused significant ($p<0.05$) increase in rearing, grooming and locomotion at all the doses used when compared with the negative control group (**Fig.2**) signifying central nervous system stimulant effect [26, 27]. The results obtained here were similar to those reported by Formagio et al. [14] and Barua et al. [12] in which both reported increase

in rearing and or locomotor behaviour of mice treated with the methanolic and aqueous extracts of the plant respectively. Drugs that stimulate the CNS normally increase rearing and locomotion [27, 28], while those that depress the CNS reduce rearing behaviour or locomotion [29]. Increase in rearing and locomotor behaviours is mainly modulated by enhanced dopamine neurotransmission [30].

Excessive grooming behaviours in animals induced naturally or experimentally are considered to be animal models of obsessive-compulsive disorder (OCD) [31]. In rodents the purpose of grooming include thermoregulation, stimulation of pheromone release, self-stimulation, increasing or decreasing arousal, self-cleansing and inhibiting irritation [32, 33]. Drugs that have depressant effect are known to suppress grooming in experimental animals, while those that have stimulatory effects increase grooming behaviour [34]. In this study, the extract at all the doses used increased grooming though non-significantly, suggesting that it possibly disrupted arousal state of the animal. The novelty-induced rearing and grooming behavioural responses are thought to be regulated by different neurotransmitters such as GABA, ACh, noradrenaline, serotonin, glutamate and dopamine [35], however, it cannot be predicted here the specific mechanism(s) of action of this extract in the mediation of the observed central excitatory activity.

The hole board and EPM are among the common models used in the evaluation of anxiolytic or anxiogenic properties of new drugs in rodents [36]. Hole board test is based on assumption that head-dipping of animals is inversely proportional to their anxiety state in moderately aversive environment [37]. In this study, the head dips increased at all the doses used (**Fig. 3**) but significantly ($p<0.05$) only at the highest dose (1000 mg/kg), which implies that the extract possess anxiolytic activity [36].

However, diazepam (1mg/kg) a standard anxiolytic agent increased head dips significantly ($p<0.01$) compare to the negative control. The EPM has been used extensively for screening for anxiolytic effects of new agents and has been very effective in identifying the anxiolytic potential of benzodiazepine-like or GABA receptor related agents but may not be reliable in detecting anti-anxiety effects through unrelated mechanisms e.g. 5-HT partial agonists like buspirone [38]. Generally, anxiolytic drugs increase the time spent on the open arms and increase the number of entries into open arm entries during the test [39], with increase in open arm entry parameter being the most representative indices of anxiolytic activity [36]. The extract at all doses did not increase the percentage time spent in open arms suggesting that it may be devoid of anxiolytic activity on the EPM model. Although the extract caused a non-significant increase in the percentage of time spent on the open arms, the result cannot be disregarded, since it was earlier noted that clinical effects appear only after chronic treatment [40]. The resu

lts obtained here for the EPM are conflicting with the results of previous studies in which methanolic leaf extract of the same species from another region were reported to demonstrate positive effect at doses of 100-600 mg/kg, p.o., on the EPM [9], but confirm that of Formagio et al. [14] who reported lack of anxiolytic effect of its aqueous extract (200-400 mg/kg) on the EPM. The reason for the differences in the results obtained could be due to variation in their phytochemical constituents, experimental procedure factor and genetic differences of the animal species used in the studies [41, 42, 43].

The result obtained here is not unusual for non-sedative agent for example, extract of *Nepeta persica* with proven excitatory effect have been reported to exert appreciable anxiolytic activity [44]. Ketamine-induced hypnosis is an extensively used model to screen for sedative and anaesthetic agents [45]. The extract caused significant increase in SL induced by ketamine but decreased TST non-significantly when compared to the control group (Figure 4A-B). Substances that cause significant decrease in SL and prolong TST are regarded as sedatives [44] and those that prolong SL and shorten TST are regarded as stimulant [46], hence, it could be inferred from the results obtained here that this extract possess stimulatory or central excitatory effect.

PTZ induces seizure by glutamatergic excitation mediated by NMDA receptors or blocking GABA–

BZD receptor mediated neurotransmission [47]. It has also been proposed that PTZ induces seizure by increasing the central noradrenergic activity [48]. The extract at all the doses tested did not protect the animals against PTZ-induced convulsion (Table 1) suggesting lack of significant anticonvulsant activity. These anticonvulsant results contradicted a previous report in which methanolic extract of the plant was shown to possess significant anticonvulsant activity against PTZ--induced convulsion model [9]. The reason for the discrepancies in these assays could not be ascertained but aforementioned factors relating to chemical composition and genetic factors may be considered.

Strychnine-induced convulsions are closely linked with glycine inhibition in the spinal cord. The extract at all the doses used was ineffective against the strychnine-induced convulsion (**Table 2**) indicating it is devoid of any component that might interact or modulate glycine receptor pathway. Diazepam (1 mg/kg, i.p.), a standard anticonvulsant agent failed to protect the mice against strychnine-induced convulsion which was consistent with previous studies which show that diazepam at low dose was ineffective against strychnine-induced convulsion [49].

The maximal electroshock test (MES), in which tonic hind limb extension are induced by bilateral corneal or trans-auricular electrical stimulation, is thought to be predictive of anticonvulsant drug efficacy against generalized tonic-clonic seizures [50]. MES causes several changes at the cellular level including facilitation of Ca^{2+} entry into the cells in large amounts and thus, prolonging the duration of convulsions or facilitate the entry of other positive ions like Na^{+} and its blockade can prevent the MES-induced tonic hind limb extension [51, 52]. The extract did not protect the animals against incidence of tonic hind limb extension (THLE) but phenytoin (standard drug) protected the animals against THLE (**Table 3**).

The result of the MES obtained here are in agreement with previous report of Barua et al. [9], who reported lack of activity for the leaf methanolic extract (200-400 mg/kg, p.o.) against the MES.

It is not possible to pin-point the specific compound(s) that could be responsible for the observed central activities in this study. However, the various phyto-constituents mentioned earlier including secondary metabolites such as phenols, flavonoids, phytosterols, tannins, alkaloids and saponins or their constituents may contribute to the overall activities of the extract. Several flavonoids have been isolated from this plant and have been shown to be responsible for some of the biological activities reported in previous studies. Therefore, it will be vital in subsequent studies to isolate the compounds present in this plant and evaluate them for central effect in furtherance of the search for novel bioactive drugs to mitigate the ever-increasing or re-emerging debilitating diseases affecting mankind.

CONCLUSION: The result obtained in this study suggested that the ethanolic leaf extract of *Alternanthera brasiliana* on the central nervous system is stimulatory; possess moderate anxiolytic activity but lack anticonvulsant activity.

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CONFLICT OF INTEREST: The authors declare that there are no conflicts of interest.

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