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Research Article

Neurobehavioral and Anticonvulsant effects of ethanol extract of *Albizia adianthifolia* leaves in experimental animals

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Abstract

Albizia adianthifolia (Mimosoideae) is a medicinal plant used in the management of infections, and central nervous system disorders. We evaluated neurobehavioral and anticonvulsant properties of the ethanol extract of *Albizia adianthifolia* leaves (EEAAL). Effects of EEAAL (2.5 - 20 mg/kg, i.p.) on novelty-induced behaviors were determined in mice using open-field and hole board tests. Anticonvulsant property of EEAAL (20 - 140 mg/kg, i.p.) was evaluated using pentylenetetrazole, picrotoxin and strychnine-induced convulsions assays. The extract was administered intraperitoneally. The LD_{50} of EEAAL was 282 mg/kg, i.p. The EEAAL (2.5 - 20 mg/kg) significantly inhibited rearing (105.4±9.5, 94.4±5.9, 67.2±7.4, 32.6±3.8) and grooming (48.0±3.6, 33.8±2.9, 25.4±1.6, 7.6±0.8) as compared with controls (171.2±14.0; 83.8±4.4). The EEAAL (5 - 20 mg/kg) significantly inhibited locomotor activity (48.4±5.3, 37.8±1.8, 13.0±2.7) compared with control (65.4± 3.6). EEAAL (2.5 - 20 mg/kg) significantly decreased exploration on hole-board (24.2±1.7, 21.6±2.1, 17.2±1.2, 9.8±1.9) compared with control (43.2±3.3).In PTZ-induced seizure, EEAAL (80 and 140 mg/kg) offered protection in 50% and 33.3% of the animals respectively. In strychnine and picrotoxin-induced convulsion, EEAAL (80 and 140 mg/kg) significantly delayed onset and latency to death compared with control respectively. EEAAL has sedative and anticonvulsant effects thus justify its use in the management of mental illness and neurological disorders.

Keywords: convulsion, elevated plus maze, pentylenetetrazole, pentobarbitone, diazepam

INTRODUCTION

Albizia adianthifolia (Schumach) W. F. Wight (Mimosoideae), known as ayinreta or igbabo in Yoruba and kawo in Hausa is a big tree found in moist and tropical forest zones as well as areas that are transitional to woodland (Zabala, 1997). Geographically it is distributed from the northern parts of the Eastern Cape in South Africa throughout the tropical countries up into Senegal in the west, Ethiopia in the east of Africa, Madagascar (Krige, 2007). It also occurs in Nigeria. Various plant parts are used in traditional medicine. Bark sap is applied to the eye to treat river blindness and conjunctivitis, and internally against respiratory complaints, as an anodyne and to treat allergic reactions; it is also applied to sores and to allay toothache (Watt and Breyer-Brandwyk, 1962). Pounded bark is applied externally to boils and itching skin, and internally as a vermifuge. A twig-bark decoction is administered as a purgative and anodyne (Van-Wyk and Gerick, 2000). In traditional South African medicine the bark of *Albizia adianthifolia* is used to improve memory and to treat Alzheimer's disease. The leaves are used internally against diarrhea and gonorrhea, and externally to treat wounds and sore feet. A fruit extract is drunk to relieve stomach-ache (Lemmens, 2007). The maceration of stem bark and root is used as an antidote against poison or applied in pomade on inflamed eye; the decoction of stem bark is drunk in the treatment of abdominal pains, typhoid fever and infections of urinary and respiratory tracts (Tamokou *et al*., 2012). The plant is also used in Nigeria to treat epilepsy and psychosis (Lawal *et al*., 2010). Some biological activities exhibited by *A. adianthifolia* have been documented. Haddad *et al.,* (2013) demonstrated that the ethanol extract of the root has *in vitro* immunomodulatory activity on the Jurkart T cell line and haemolytic property against sheep erythrocytes. The root extract of *A. adianthifolia* has been shown to possess antibacterial, anti-inflammatory and anticholinesterase effects (Eldeen *et al*., 2005). Memory-enhancing activity of the aqueous leaf extract in the 6-hydroxydopamine-lesion rodent model of Parkinson's disease has also been documented (Beppe *et al*., 2014). Tamokou *et al.,* (2012) demonstrated the antioxidant and antimicrobial activities of ethyl acetate extract, fractions and compounds from stem bark. It has been shown to contain alkaloids, glycosides, saponins, steroids, tannins, and astringents (Burkill, 1985). Orwa *et al.,* (2009) reported that the plant contains three flavonoids: okanin, melanoxetin and dihydroflavonol. The present study aims at evaluating the neurobehavioural and anticonvulsant properties of ethanol extract of *A. adianthifolia* leaves (EEAAL) in mice..

MATERIAL AND METHODS

Collection of plant materials: The leaves of *A. adianthifolia* were collected in March 2014 at the Botanical Garden, University of Ibadan, Ibadan, Oyo state, Nigeria. The taxonomical identification and authentication of the plant was carried out at the herbarium section of the Forestry Research

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Institute of Nigeria (FRIN), Ibadan, Nigeria. A voucher specimen with identification number 109833 was deposited and compared with the reference specimen.

Preparation of extract: The air-dried leaves (100 g) were pulverized and soaked in 50% ethanol (2 L) for 72 h. The filtrate was concentrated with a rotary evaporator to give a semisolid residue and evaporated to dryness to form solid residue (23 g). It was kept in the desiccator for further use. The dried extract was then subsequently reconstituted in distilled water at appropriate concentrations for the various experiments.

Animals: Albino mice weighing between 20 – 25 g used in this study were obtained from the Laboratory Animal Centre of the College of Medicine, University of Ibadan, Nigeria. The animals were kept in hygienic and well-ventilated compartments, maintained under standard environmental conditions and fed with standard rodent pellet (Livestock Feed PLC, Lagos, Nigeria) and water *ad libitum*. The experimental procedures adopted in this study were in accordance with the United States National Institutes of Health Guidelines for Care and Use of Laboratory Animals in Biomedical Research (NIH, 1985).

Drugs and Chemicals: Yohimbine (Sigma - Aldrich St. Louis, MO, USA), phenobarbital (Sigma, St. Louis, USA), picrotoxin (Sigma-Aldrich, St. Louis, USA), strychnine (Sigma, USA) and pentylenetetrazole (Sigma-Aldrich, St. Louis, USA), diazepam (Roche, Basel Switzerland), ciproheptadine, propanolol, haloperidol and atropine (Sigma-Aldrich, St. Louis, USA).

Acute toxicity test: The method described by Lorke (1983) was used to determine the LD_{50}, an index of acute toxicity. Albino mice (20 – 25 g) were used. This method involved an initial dose finding procedure, in which the animals were divided into three groups of three animals each. Doses of 10, 100 and 1000 mg/kg were administered intraperitoneally (i.p.), one dose for each group. The treated animals were monitored for 24 h for mortality and general behaviour. From the results obtained, four different doses of (800, 600, 400 and 200 mg/kg) were chosen and administered i.p. respectively to four groups of one mouse each. The treated animals were monitored for 24 hours. The LD_{50} was then calculated as the geometric mean of the highest dose showing no death and the lowest dose showing death.

Neurobehavioral assays

Effect of EEAAL on novelty-induced rearing (NIR) and grooming (NIG) in mice: The method described by Ajayi and Ukpomwan, (1994) and Onigbogi *et al.,* (2000) was used to assess the frequency of rearing and grooming. Animals were divided into six treatment groups of five mice each and their behavioural profiles under the influence of the extract were assessed individually in a white Plexiglas cage measuring (45 cm × 25 cm × 25 cm) with one transparent side for clear observation. The animal were divided into 6 groups (n = 5) and behavioral measurement was carried out after i.p. administration of vehicle (0.2 mL/20 g distilled water) to group 1 and different doses of the extract (2.5, 5, 10 and 20 mg/kg, i.p) to groups 2-5. Diazepam (2 mg/kg, i.p.), which was used as the reference drug, was administered to group 6. The animals were placed directly from home cage into an opaque Plexiglas observation cage with only one side transparent for observation. Each animal was used only once, with the observation cage cleaned with 70% ethanol and allowed to dry after each assessment to remove olfactory cue from previous animal to the other. The frequency of rearing and grooming episodes were separately quantified by using a manual counter and a stop watch. The total frequency was summed up for a period of 30 min. Rearing was taken as the number of times the mouse was standing on its hind limbs or with its forelimbs against the wall of the observation cage or in the free air while grooming was determined as the number of body cleaning with paws, picking of the body and pubis with mouth and face washing actions

Effect of EEAAL on spontaneous locomotor activity of mice in the open field: Motor activity was measured in an open field apparatus consisting a white Plexiglas box (45 cm × 25 cm × 25 cm) with a painted black grid dividing the floor into 16 (7 × 7 cm) equal squares. The mice were divided into six groups (n=5 per group).. Group 1 was given the vehicle (0.2 mL/20 g distilled water), while groups 2-5 received different doses of the extract of *A. adianthifolia*, (2.5, 5, 10 and 20 mg/kg, i.p) respectively and group 6 received diazepam (2 mg/kg). Thirty minutes after a single i.p. injection of extract and vehicle, the animals were placed singly in one of the corners of the box and the number of squares crossed with all four paws was counted for 5 min. The cages were cleaned with 70% ethanol at intervals when the animal is removed (Akanmu *et al*., 2011).

Effect of EEAAL on exploratory behaviour of mice on hole-board apparatus: The effect of the extract on the frequency of head dipping was determined in the hole-board with a number of holes (usually 16) distributed evenly on the floor through which the animal can poke. The board is elevated so that the mouse poking its nose into the hole does not see the bottom. . The animals were divided into six groups (n=5 per group)**.** . Group 1 was given distilled water (0.2 mL/20 g, i.p), while groups 2-5 received different doses of the extract of *A. adianthifolia*, (2.5, 5, 10 and 20 mg/kg, i.p) respectively and group 6 received diazepam (2 mg/kg). The animals were placed singly on the apparatus 30 minutes after administration of the extract at different doses. The number of times that each animal dipped its head into the holes was counted for the period of 5 min (Door *et al*., 1971).

Effect of EEAAL on mice behaviour on Elevated plus maze test: The elevated plus maze test was carried out to assess the anxiety like behaviour effect of the extract. It is a modification of the apparatus validated for mice by Lister (1987). The EPM apparatus is made of wood consisting of two open arms (30 x 5 x 0.25 cm) which are essentially unprotected boards and two closed arms (30 x 5 x 15 cm) which are bordered by walls emanating from a common central platform (5 x 5 cm) and elevated to a height of 50 cm above floor level. The animals were divided into six (n=5 per group). Group 1 received distilled water (0.2 mL/ 20 g, i.p), groups 2-5 while groups 2-5 received different doses of the extract of *A. adianthifolia*, (2.5, 5, 10 and 20 mg/kg, i.p) respectively and group 6 received diazepam (1 mg/kg, i.p.) thirty minutes before observation. At the start of the session the mouse was placed at the edge of an open arm, with its head facing the center and

allowed to explore the maze for 5 min. Seventy percent (70%) ethanol was used to clean the plus maze and allowed to dry after each animal to prevent odor bias. During the test period, the following measurements were recorded: the total number of arm entries and the time spent in open and closed arms. An entry with all feet put into one arm is defined as an arm entry in this experiment. The percentage of entries into the open arms and closed arms based on the total arms entries were also calculated for each animal. The percentage of time spent in the open arms and the closed arms was calculated over the 5-minute test. The index of open arm avoidance (Trullas and Skolnick, 1993) was calculated as [100 – (% time on open arms + % entries into the open arms)/2].

Mechanism of action: In another set of experiments, mice were pre-treated 15 min prior with neurotransmitter blockers to evaluate the mode of actions of the *A. adianthifolia* on novelty-induced rearing and grooming, locomotor activity head dip in mice. The following receptor blockers were used: atropine (muscarinic antagonist, 0.5 mg/kg), haloperidol (dopamine D_2 antagonist, 0.2 mg/kg), ciproheptadine (5-HT antagonist, 0.5 mg/kg) and yohimbine (α_2-adrenergic antagonist, 1 mg/kg) (Aderibigbe *et al*., 2010).

Anticonvulsant assays

Pentelenetetrazole (PTZ), Strychnine and Picrotoxin-induced convulsions in mice: Female Swiss Albino mice (20 – 25 g) were divided into six groups (n = 6) in each group. Group 1 received distilled water (10 mL/kg). Groups 2 - 5 received extract (20, 40, 80 and 140 mg/kg, i.p.) respectively. Group 6 received phenobarbital (40 mg/kg). All the treatments were administered intraperitoneally 30 min prior to administration of PTZ (85 mg/kg), strychnine (2 mg/kg) and picrotoxin (7 mg/kg). The mice were then observed for onset of seizure, latency to death/recovery as well as the percentage of protection against mortality for 24 h (Hosseinzadeh and Parvardeh, 2004).

Statistical analysis

All data are presented as Mean ± SEM. The results were analyzed by One-way analysis of variance (ANOVA) and post hoc tests (Student's-Newman-Keuls) were carried out to determine the source of significant main effect using GraphPadInStat® Biostatistics software. The level of significance for all tests was set at $p < 0.05$.

RESULTS

Acute toxicity test

The LD_{50} of the crude extract of *A. adianthifolia* was found to be 282 mg/kg i.p. body weight in mice.

Effect of EEAAL on novelty-induced rearing and grooming behaviour in mice.

EEAAL (2.5, 5, 10, and 20 mg/kg) induced a dose dependent decrease in the novelty-induced rearing and (10 and 20 mg/kg) on grooming activity in mice compared with the control. A significant reduction in the frequency of rearing ($p < 0.05$) and grooming ($p< 0.05$) episodes was observed when compared with the control (Fig. 1a and Fig. 1b).

Effect of EEAAL on locomotor activity in open field

Intraperitoneal administration of the crude extract (2.5 mg/kg) showed no significant reduction ($p > 0.05$) in the number of line crossed while (5 - 20 mg/kg) significantly reduced the number of line crossed ($p < 0.05$) when compared to the control. Diazepam also significantly ($p<0.05$) reduced locomotor activity (Fig. 2).

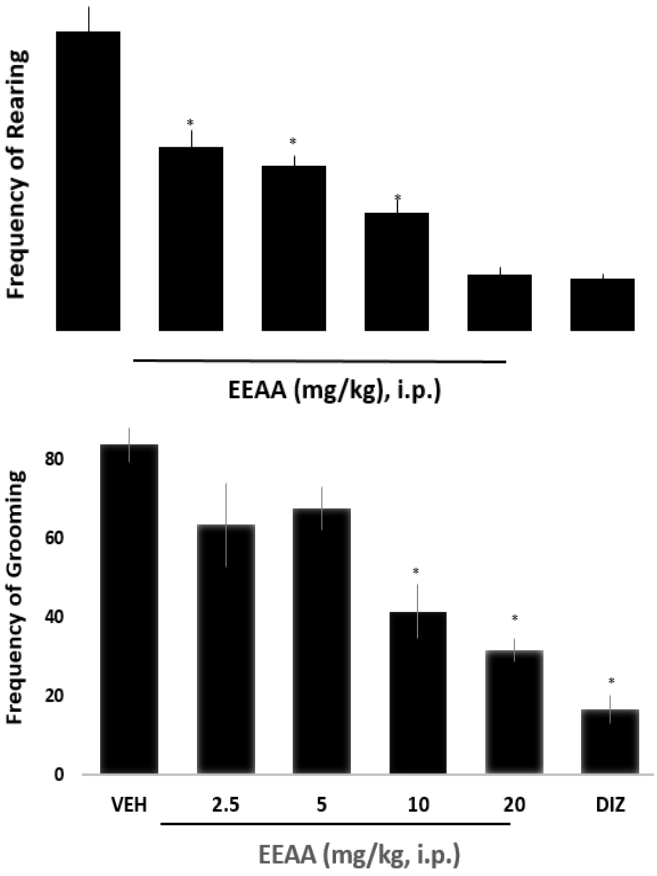

Fig. 1:
a- Effect of *A. adianthifolia* on rearing behavior in mice.
b-Effect of *A. adianthifolia* on grooming behavior in mice.
The results are expressed as Mean ± SEM (n= 5). One way ANOVA revealed that there is significant [$F (5, 24) = 42.16$, $p< 0.0001$] difference between various treatment groups.
* indicates significant difference from the control $P < 0.05$.
DIZ= Diazepam

Effect of *EEAAL* on the frequency of head dip on hole-board

Intraperitoneal administration of the crude extract (2.5 – 20 mg/kg) induced significant dose dependent reduction ($p < 0.05$) in the frequency of head-dip in mice compared to control, diazepam also caused a significant ($p < 0.05$) decrease in the frequency of head-dips (Fig. 3).

Effect of EEAAL on the elevated-plus maze (EPM) in mice

The extract (2.5 - 20 mg/kg, i.p.) showed no significant ($p > 0.05$) increase in the frequency of open arm entries ($p > 0.05$), and percentage (%) of open arm duration ($p > 0.05$) when, compared to the control. However, diazepam (1 mg/kg, i.p.) showed a significant ($p < 0.05$) increase in the frequency of open arm entries and % open arm duration compared to control (Table 1).

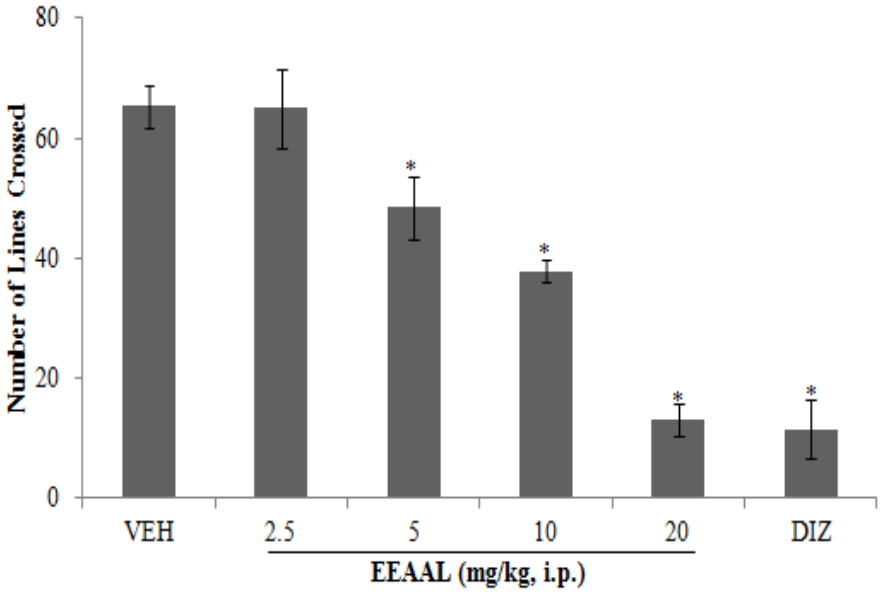

Fig. 2:
Effect of EEAAL on locomotor activity in open field The results are expressed as Mean ± SEM (n= 5). One way ANOVA revealed that there is significant [F (5, 24) = 35.30, p< 0.0001] difference between various treatment groups.
* indicates significant difference from the control p< 0.05. DIZ = Diazepam

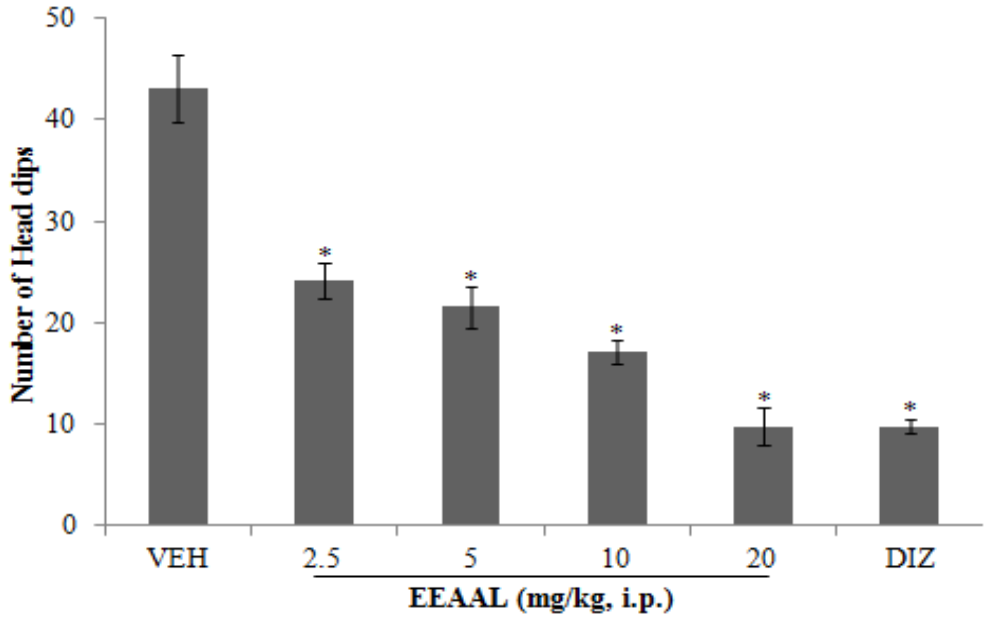

Fig. 3: Effect of EEAAL on frequency of Head dips
The results are expressed as Mean ± SEM (n= 5). One way ANOVA revealed that there is significant [F (5, 24) = 19.8, p< 0.0001] difference between various treatment groups.
* indicates significant difference from the control p< 0.05; DIZ = Diazepam

Effect of atropine, ciproheptadine, yohimbine and haloperidol on novelty induced rearing and grooming, head dip, and locomotor activity: Intraperitoneal administration of atropine, haloperidol, ciproheptadine, and yohimbine alone significantly (p< 0.05) reduced rearing, grooming, head dipping and locomotor activity; atropine had no significant (p> 0.05) effect on locomotor activity (Table 2).

Effect of pre-treatment with antagonists on the inhibitory effect of EEAAL on novelty induced rearing and grooming, head dip, and locomotor activity.: Pretreatment with atropine potentiated inhibitory effect of the extract on rearing, grooming and frequency head dip, but reversed the inhibitory effect on locomotor activity (p< 0.05); pretreatment with ciproheptadine reversed the inhibitory effect of the extract on rearing, grooming, frequency head dip and locomotor activity (p< 0.05); pretreatment with yohimbine potentiated inhibitory effect of the extract grooming and frequency head dip, reversed the inhibitory effect on locomotor activity (p< 0.05), but had no effect on rearing; pretreatment with haloperidol potentiated the inhibitory effect of the extract on rearing, grooming and frequency head dip but had no effect on locomotor activity (Table 3).

Table 1:
Effect of EEAAL on anxiety in mice using the Elevated-Plus Maze (EPM)

GROUPS	DOSE (mg/kg)	OPEN ARM ENTRY (Min)	OPEN ARM DURATION (Min)	%OPEN ARM ENTRY (Min)	%OPEN ARM DURATION (Min)	INDEX OF OPEN ARM AVOIDANCE
VEH	10mL/kg	3.00 ± 0.55	43.60 ± 9.09	27.36 ± 3.48	18.44 ± 4.078	77.12 ± 3.51
EEAAL	2.5	1.40 ± 0.40	24.40 ± 9.77	17.54 ± 4.18	10.76 ± 4.88	85.86 ± 4.49
	5	1.20 ± 0.58	17.60 ± 9.10	16.66 ± 6.97	8.82 ± 4.74	87.28 ± 5.59
	10	1.40 ± 0.40	11.00 ± 3.36	20.10 ± 5.51	4.48 ± 1.35	87.56 ± 3.40
	20	0.60 ± 0.40	4.60 ± 2.82	12.00 ± 8.00	1.66 ± 1.02	93.18 ± 4.46
DIZ	1	8.20 ±1.39*	249.4 ±3.74*	80.86 ±2.72*	86.82 ± 1.66*	16.18 ± 2.06*

*The results are expressed as Mean ± SEM (n= 5). One way ANOVA revealed that there is significant [F (5, 24) = 15.82, p< 0.001 (open arm entry); F (5, 24) = 182.2, p< 0.001 (open arm duration); F (5, 24) = 22.31, p< 0.001(%open arm entry); F (5, 24) = 91.75, p< 0.001 (%open arm duration); F (5, 24) = 50.94, p< 0.001 (index of open arm avoidance)] difference between treatment groups and control group. * indicates significant difference from the control p< 0.05; DIZ = Diazepam*

Table 2:
Effect of atropine, ciproheptadine, yohimbine, haloperidol, on novelty induced rearing and grooming, frequency of head dip, and locomotor activity

GROUPS	DOSE (mg/kg)	REARING	GROOMING	HEAD DIP	LOCOMOTOR ACTIVITY
VEH	10 mL/kg	171.20 ±13.99	83.80 ± 4.39	43.20 ± 3.34	65.40 ± 3.56
Atropine	0.5	122.20 ±3.09*	52.40 ± 2.84*	31.00 ± 2.24*	58.60 ± 2.29
Ciproheptadine	0.5	94.60 ± 7.45*	25.40 ± 3.12*	12.00 ± 1.23*	44.20 ± 2.22*
Yohimbine	1	126.60 ±7.17*	42.80 ± 2.25*	11.20 ± 0.97*	39.40 ± 4.09*
Haloperidol	0.2	100.60 ±8.72*	76.80 ± 4.08	12.40 ± 0.93*	19.80 ± 1.72*

*The results are expressed as Mean ± SEM (n= 5). One way ANOVA revealed that there is significant [F (5, 24) = 12.84, p< 0.001 (Rearing); F (5, 24) = 45.78, p< 0.001 (Grooming); F (5, 24) = 35.64, p< 0.001 (Head dip); F (5, 24) = 42.79, p< 0.001 (Locomotor activity)] difference between treatment groups and control group. * indicates significant difference from the control p< 0.05*

Table 3:
Effect of pre-treatment with antagonists on the inhibitory effect of the extract on novelty induced rearing and grooming, frequency of head dip and locomotor activity in mice.

GROUPS	DOSE (mg/kg)	REARING	GROOMING	HEAD DIP	LOCOMOTOR ACTIVITY
VEH	10 mL/kg	171.20 ±13.99	83.80 ± 4.39	43.20 ± 3.34	65.40 ± 3.56
EEAA	20	32.60 ± 3.83*	23.34 ± 2.98*	9.80 ± 1.86*	13.00 ± 2.70*
Atropine + EEAA	0.5	13.00 ± 2.51*	12.20 ± 2.48*	4.60 ± 0.81*	43.00 ± 3.08**
Ciproheptadine + EEAA	0.5	52.60 ± 7.11**	48.80 ± 8.48**	21.00 ± 2.30**	52.60 ± 5.53**
Yohimbine + EEAA	1	21.20 ± 3.48	13.60 ± 1.81*	5.40 ± 0.51*	54.60 ± 4.61**
Haloperidol + EEAA	0.2	10.80 ± 1.20*	8.80 ± 1.01*	3.40 ± 0.51*	5.80 ± 1.39

*The results are expressed as Mean ± SEM (n= 5). One way ANOVA revealed that there is significant [F (5, 24) = 20.84, p< 0.001 (rearing); F (5, 24) = 19.22, p< 0.001 (grooming); F (5, 24) = 29.95, p< 0.001 (haed-dip); F (5, 24) = 36.85, p< 0.001 (locomotor activity)] difference between treatment groups and control group. * indicates significant difference (further depression) from the EEAAL P< 0.05. ** indicates significant difference (reversal) from the EEAAL P< 0.05*

Effect of EEAAL on pentylenetetrazole-induced convulsion

Pentelenetetrazole (85 mg/kg) produced tonic seizures in all the animals. The extract at 20, 40 and 80 mg/kg i.p. did not alter onset seizures while at 140 mg/kg, there was significant prolongation of seizure onset. At 40, 80 and 140 mg/kg there was a significant prolongation of latency to death. The extract at 80 mg/kg protected 50% of animal from death and 33.3% at 140 mg/kg. The standard drug, phenobarbital (40 mg/kg) offered full protection without seizure in the animals (Table 4).

Effect of EEAAL on strychnine-induced convulsion

Strychnine produced tonic seizures in all the animals. The extract at 20 and 40 mg/kg i.p. did not affect the onset of seizures and latency to death while 80 and 140 mg/kg significantly (p<0.05) prolonged both the onset of convulsions and latency to death in the animals. The standard antiepileptic drug, phenobarbital (40 mg/kg i.p.) significantly (p< 0.05) prolonged both the onset of convulsions and latency to death in the animals (Table 5).

TABLE 4:
Effect of *A. adianthifolia* on PTZ -Induced Convulsion

Group	Dose (mg/kg)	Seizure Onset (min)	Latency to Death (min)	Number of survivals	Percentage Survival (%)
VEH	10 mL/kg	1.12 ± 0.05	4.77 ± 0.22	0/6	0
EEAAL	20	1.22 ± 0.06	11.11 ± 0.56	0/6	0
	40	1.36 ± 0.03	21.40 ± 5.66*	0/6	0
	80	1.71 ± 0.31	23. 03 ± 3.61*	3/6	50
	140	2.34± 0.46*	22.26 ± 5.01*	2/6	33.3
Phenobarbital	40	NC	NC	6/6	100

The results are expressed as Mean ± SEM (n= 6). One way ANOVA revealed that there is significant [F (4, 25) = 3.949, p< 0.001 (Onset); F (4, 20) = 5.022, p = 0.006 (Latency)] difference between various treatment groups.
* indicates significant difference from the control p< 0.05.; **NC**: No convulsion

Table 5: Effect of *A. adianthifolia* on Strychnine-Induced Convulsion

Group	Dose (mg/kg)	Onset of Seizure(min)	Latency to Death (min)
VEH	10 mL/kg	1.27 ± 0.05	0.17 ± 0.02
EEAAL	20	1.29 ± 0.03	0.13 ± 0.01
	40	1.46 ± 0.02	0.20 ± 0.02
	80	2.47 ± 0.26*	5.49 ± 0.80*
	140	3.04 ± 0.10*	4.03 ± 0.75*
Phenobarbital	40	4.08 ± 1.17*	5.16±0.80*

*The results are expressed as Mean ± SEM (n= 6). One way ANOVA revealed that there is significant [F (5, 30) = 48.71, p< 0.001 (Onset); F (5, 30) = 22.49, p<0.001 (Latency)] difference between various treatment groups. * indicates significant difference from the control p< 0.05.*
***NC**: No convulsion*

Table 6: Effect of *A. adianthifolia* on Picrotoxin-induced Convulsions

Group	Dose (mg/kg)	Onset of Seizure (min)	Latency to Death (min)
VEH	10 mL/kg	6.35 ± 0.18	5.15± 0.30
EEAAL	20	6.37 ± 0.19	5.44 ± 0.28
	40	6.21 ± 1.09	10.51± 2.27
	80	8.47 ± 1.03*	14.19±2.47*
	140	8.33± 0.47*	10.60±0.84
Phenobarbital	40	NC	NC

The results are expressed as Mean ± SEM (n= 6). One way ANOVA revealed that there is significant [F (4, 25) = 9.237, p = 0.004 (Onset); F (4, 25) = 5.193, p = 0.019 (Latency)] difference between various treatment groups.
** indicates significant difference from the control p< 0.05.*
NC = No convulsion

Effect of EEAAL on picrotoxin-induced convulsion
Picrotoxin produced tonic seizures in all the animals. The extract at 20 and 40 mg/kg i.p. did not affect the onset of seizures and latency to death, 80 and 140 mg/kg significantly (p<0.05) prolonged the onset of convulsions while 80 mg/kg alone significantly prolonged latency to death in the animals. The standard drug, phenobarbital (40 mg/kg) offered full protection without seizure in the animals (Table 6)

DISCUSSION

In this study, the effect of *A. adianthifolia* was examined for behavioral effect (rearing, grooming and locomotion activity) in a novel environment using the open field test (OFT) and anxiety on hole-board and elevated plus maze.
Animals exposed to a novel environment will display novelty induced rearing (NIR). This behavior is employed by rodents as one of the survival strategies in assessing the environment for food, protection and possible escape and used to classify substances as either stimulant or sedative (Blanchard *et al*., 2001). Rearing which involves an animal standing on its hind legs and raising its front paw on air or the walls of the maze is a measure of exploratory behaviour and considered to be a central excitatory behavior (Ajayi and Ukponmwan, 1994; Brown *et al*., 1997). The extract produced a dose dependent reduction in the frequency of rearing in the animals. Therefore the extract by inhibiting rearing is suggests a sedative property.

Similarly, EEAAL produced a reduction in novelty induced grooming (NIG) in the animals. Grooming is an important behavioral component in animals and is associated with de-arousal state of the central nervous system (CNS) that is absence of stimulation (Aderibigbe *et al*., 2010). Grooming described as face or head cleaning with paws or picking of the body and pubis with mouth (Ukponmwan *et al*., 1985) is a displacement response that is elicited by animals either by exposure to a novel environment, including holding and transportation to an observation room (Colbern *et al*., 1981; Jolles *et al*., 1979). It serves a variety of adaptive functions (Spruit *et al*., 1992) and plays a deactivating role in restoring homeostasis under stressful condition (Gispen *et al*., 1981). Inhibiting grooming therefore suggests the depressant effect of the extract on the CNS.

The novelty-induced rearing and grooming behavior response is regulated by multiple neurotransmitter system such as ᵞ-aminobutyric acid (GABA), cholinergic, adrenergic, opioid, serotonin, glutamate and dopamine receptors (Walting, 1998). The extract by inhibiting rearing and grooming behaviors suggests that it may be acting by blockade of dopamine, potentiation of GABA, inhibition of serotonin, inhibition of cholinergic neurotransmission and inhibition of

the stimulation of the excitatory neurotransmitter in the CNS (Jones *et al*., 1981; Strange *et al*., 1993).

Locomotion in animals like rearing is indicative of their exploratory and is also considered to be a central excitatory behavior (Ajayi and Ukponmwan, 1994). Neurotransmitter interaction between dopaminergic, adrenergic, glutaminergic and cholinergic systems are involved in locomotion in mice (Svensson *et al*., 1995) although, it is mediated mainly through dopaminergic pathway (Rang *et al*., 2005) and has been reported that drugs that enhance dopaminergic transmission increases locomotor activity (Akanmu *et al*., 2007). The EEAAL produced significant reduction in locomotor activity measured by the number of lines crossed further confirming its depressant effect as a decrease in locomotor activity in rodents is suggestive of a possible CNS depressant activity (Cooper *et al*., 1996).

Anxiety which is a state of excessive fear and characterized by motor tension, sympathetic hyperactivity, apprehension and vigilance syndromes (Sadock and Sadock, 2003) may interfere with intelligence, psychomotor function and memory (Pine *et al*., 1999). Among the models of anxiety disorders that are used in determining anxiolytic or anxiogenic properties of substance in rodents are hole-board and elevated plus maze (Akanmu *et al*., 2011).

The EEAAL produced significant reduction in the number of head dips in the hole-board test. The hole-board provides a simple method for measuring the response of rodents to unfamiliar environment and it's sensitive to changes in emotional state of animals (Nolan and Parkes, 1993; Takeda *et al*., 1998). This test is based on assumption that head-dipping is inversely proportional to their anxiety state of animals in moderately aversive environment (Bilkei-Gorzo and Gyertyan, 1996) and an increase in head dip into holes indicates reduced anxiety (Akanmu *et al*., 2011). It is also used as a measure of exploratory effect and reveals sedative property of substances. Reduction in number of head dip by the extract further suggests the sedative property of the extract.

Similarly, EEAA at all doses did not increase open arm entry and duration in the elevated plus maze (EPM). EPM test is an anxiety based test based on the natural aversion of rodents to heights and open spaces and rests on the conflict between the innate tendencies of the rodent to explore novel environments and avoid open and brightly lit areas (Akanmu *et al*., 2011). Mice and rats prefer the closed arms but ventures out into the open arm if they are less anxious under the influence of anxiolytic drugs. The extract also caused a decrease in percentage open arm entry and duration. The index of open arm avoidance shows that the extract has no anxiolytic effect.

The neural mechanism of action of EEAAL was also investigated in this study by interaction of the extract (20 mg/kg) with antagonists of different systems involved in the regulation of neurobehaviors in animals. Administration of the antagonists; atropine and haloperidol potentiated the inhibitory effect of EEAAL, ciproheptadine and propanolol reversed the reduction while yohimbine did not reverse nor potentiate the inhibitory effect of EEAAL on novelty induced rearing (NIR). This suggests that β-adrenergic and serotonergic systems are involved in the inhibition of NIR of EEAAL. In the same vein, atropine, yohimbine and haloperidol potentiated the inhibitory effect of EEAAL on novelty induced grooming (NIG) excluding muscarinic, α_2-adrenergic and dopaminergic system involvement while ciproheptadine reversed the effect thus implicating serotonergic system in the inhibition of NIG of EEAAL.

The inhibitory effect of EEAAL on frequency of head dip was potentiated by atropine, yohimbine and haloperidol while ciproheptadine reversed it. This also suggests the involvement of β-adrenergic and serotonergic system in the inhibitory effect of *Albizia adianthifolia*. Alsothe inhibitory effect of EEAAL on locomotion was reversed by atropine, ciproheptadine and yohimbine while haloperidol showed no effect. This suggests the involvement of muscarinic, serotonergic and α_2-adrenergic systems in the inhibitory effect of the extract and precludes the dopaminergic system.

Pentylenentetrazole (PTZ) is a non-competitive antagonist of GABA that has been widely used as animal model of chemical induced seizure because it is highly sensitive for comparing different substances under standardized conditions (Shafaroodi *et al*., 2004; Samini *et al*., 2005). Pentylenentetrazole causes convulsion by inhibiting chloride ion channels associated with GABA at $GABA_A$, receptors. Meldrum (1982) noted that the enhancement and inhibition of GABA neurotransmission attenuates and enhances convulsion respectively. The extract delayed seizure onset and latency to death. The extract at 80 and 140 mg/kg prevented death in 50% and 33.3% of animals respectively, suggesting the interaction of the extract with GABA-ergic transmission. PTZ test is assumed to identify anticonvulsant drugs effective against generalized clonic seizures (Loscher and Schmidt, 1988; De Deyn *et al*., 1992), thus the extract may therefore be effective for this type of seizure. Phenobarbitone used as the positive control antagonized PTZ as there was no convulsion in the animals.

Strychnine has been demonstrated to have a well-defined mechanism of convulsant action which involves direct antagonism of inhibitory spinal cord and brain reflexes of glycine thus increasing spinal reflexes (Amole *et al*., 2009) Under normal conditions, binding of glycine to the glycine chloride channel causes inward increase of flow of chloride thus hyperpolarizing the cell and inhibiting its ability to propagate nerve signals but with strychnine there is increased nerve signal transmission. The extract delayed both onset of seizure and latency to death implying that it might be acting through glycine receptor in the spinal cord and brain stem.

The effect of the extract on picrotoxin induced convulsion was also investigated in thus study. The extract though did not protect against death but significantly delayed the onset of seizure and latency to death. The standard drug phenobarbital offered protection against death. Picrotoxin is known to elicit seizure by antagonizing the effect of GABA through blockade of the chloride channels linked to $GABA_A$ receptors (Rang *et al*., 2005). The result therefore suggests that the extract may be producing its anticonvulsant property by opening of chloride channels associated with $GABA_A$ receptors.

In conclusion the extract of the leaves of *A. adianthiofolia* has both central inhibitory and anticonvulsant effects. Its CNS inhibitory effect may be due to involvement of serotonergic, adrenergic and muscarinic systems, while its anticonvulsant property may be due to GABA and glycine enhancement in the brain

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