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MODULATION OF SEIZURE ACTIVITY BY *ALCHORNEA LAXIFLORA* IN MICE

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ABSTRACT
The study investigated the effects of the extracts of *Alchornea laxiflora* in four models of experimental seizures, the strychnine-, pentylenetetrazole-, picrotoxin-, and the maximal electroshock-induced seizure tests. The rationale was to provide information on the acute toxicity and the anticonvulsant potential of the extracts, as well as the possible involvement of GABAergic and opioidergic pathways in mediating the anticonvulsant effects of the extracts. Mice of both sexes (n=6) weighing 18 – 22 g were used in the study, and were randomised into control and test groups, which summed up to seven (7) groups. The control group (1) received 10 % Tween 80 (vehicle), 0.1 ml/10 g mouse while the test groups (II, III, IV, V, VI) were administered graded doses (100, 200, 400, 800, 1600 mg/kg, p.o.) of the extracts. The standard group (VII) received standard drugs, Diazepam (2 mg/kg, i.p.) and Phenytoin sodium (25 mg/kg, i.p.). In another set of experiments in mice (n=6), a specific dose of the extracts (1600 mg/kg, p.o.) was used to evaluate the involvement of the GABAergic and the opioidergic pathways in the anticonvulsant effects of the plant extracts. Flumazenil (2 mg/kg, i.p.) and Naloxone (5 mg/kg, i.p.) were used in assessing the signalling pathway(s) mediating the anticonvulsant activity of *A. laxiflora*.The animals were observed for seizure threshold (latency), death threshold, duration of tonic hind limb extension and the seizure recovery time of the animals. They were individually scored and recorded after observation in the observation cage, 30 min post intra-peritoneal and 1 h post oral administrations of vehicle, extracts or drugs. The results showed that the LD_{50} for the aqueous and methanol extracts of *A. laxiflora* in the oral route was > 1600 mg/kg respectively, and found to be safe in animals. However, the LD_{50} (i.p.), was found to be 400 mg/kg for the methanol extract, which was relatively toxic, and > 1600 mg/kg for the aqueous extract. *A. laxiflora* demonstrated significant ($P < 0.05$) anticonvulsant activity, by the increase in seizure threshold and death threshold, decrease in the duration of tonic hind limb extension, and the decrease in seizure recovery time of the animals. The study concluded that the extracts of *A. laxiflora* have anticonvulsant activity in mice. The anticonvulsant effects were found to be mediated neither *via* the GABAergic nor the opioidergic pathway.

KEYWORDS: convulsion, recovery time, maximal electroshock, pentylenetetrazole, picrotoxin, strychnine.

BACKGROUND
Alchornea laxiflora (Bentham) Pax and Hoffman (Euphorbiaceae) is a deciduous shrub or a forest understorey (found between the forest canopy and the ground cover) tree of about 6m high growing in Nigeria. The leaves are thinly textured turning an attractive yellow or red in dry season, whiles the young leaves appear purple in colour (Hutchinson and Dalziel, 1937). It is found in the riverrine vegetation and mixed deciduous woodland, often on rocky outcrops in the Cameroons, and it is widespread in the Central and Southern tropical Africa. *A. laxiflora* is commonly known as lowveld beadstring, while the local names are Urievwu (Urhobo), Uwenuwen (Edo), Ububo (Igbo), Ijan or Pepe (Yoruba). The leaves of *A. laxiflora* are employed in ethnomedicine for the management of neurological and cardiovascular disorders *viz.* anxiety, insomnia, hypertension etc. The decoction of the leaves is used in the treatment of inflammatory and infectious diseases, as well as an important component of anti-malarial formulations (Adewole, 1993). The leaves are recorded as amongst those used to preserve the moisture of kolanuts in packing (Muanya, 2009). The stem (especially, the branchlets) is used in Nigeria as chewing sticks for teeth cleaning (Farnsworth et al., 1985). The plant enters the Yoruba incantation to make "bad

medicine" rebound to sender (Burkill, 1994). A previous report demonstrated that extract from the leaves of *A. laxiflora* can reverse sickling phenomenon *in vitro*, and thus can be employed in themanagement of Sickle cell anaemia (Muanya, 2009). The bioactive chemical constituents from *A. laxiflora* include flavonoids, which is the dorminant constituent in the leaves of the plant but present in lesser quantities in the roots and stems, exhibited anti-microbial activity (Ogundipe *et al*., 2001), and this activity has been found to be significant against gram –ve and gram +ve organisms. This justifies the use of the plant as chewing stick in folkloric medicine. Farombi *et al*. (2003) demonstrated the anti-oxidant property of *A. laxiflora* leaf and root extracts, thus validating its use in the preservation of the moisture content of kolanuts during packing. A recent study on the phytochemical screening of *A. laxiflora* leaves revealed the presence of alkaloids, flavonoids, phenols, saponins and tannins (Osuntokun and Olajubu, 2015; Osabiya *et al*., 2017). Alkaloid, flavonoid and tannin-laden medicinal plants exert anxiolytic, sedative and hypnotic effects on laboratory animals. Plants with sedative activity have anticonvulsant properties (Nwonu, 2017). These phyto-chemicals (secondary metabolites) may be responsible for the central nervous system depressant effects of *A. laxiflora*. The present study, therefore, was designed to examine the modulatory potential of *A. laxiflora* in experimental seizures and the possible role of GABAergic and opioidergic pathways.

METHODS

Plant Collection

Alchornea laxiflora Bentham leaves were collected at the medicinal plant garden, Pharmacognosy plot II, Teaching and Research Farm located within the Obafemi Awolowo University campus. The plant was identified and authenticated in the Faculty herbarium by Mr. I. I. Ogunlowo, a field taxonomist with the Department of Pharmacognosy. A voucher specimen (Voucher number: Ife – 17592) of the leaves of *A. laxiflora* was deposited at the Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria.

Plant Extraction

The leaves of the plant were allowed to air-dry at laboratory room temperature (about 37 ^{o}C), and then pulverised, using a milling machine (Christy and Dorris Ltd., Model No. 7445). The powdered plant material (350 g) was subjected to cold extraction in a percolator (thrice) using 2.5 h of 100 % methanol (absolute methanol) for 72 h, with occasional stirring. The marc was re-extracted using another equal volume of methanol for 72 h. The filtrate generated was concentrated to dry residue in a rotary evaporator under reduced pressure at 40 ^{o}C. The extraction process yielded 90.0 g of sticky, black crude extract (25.7 %). The aqueous extraction process was carried out using hot extraction method. The pulverised plant (500 g) was extracted using boiling method under reflux. The extraction was made to simmer for 3 h. The decoction (menstrum) was concentrated to dryness *in vacuo* using the rotary evaporator at 40 ^{o}C. Little amount of methanol was added to the aqueous extract to facilitate easy concentration to dryness. The weight of the dry extracts was determined and the percentage yield calculated. The extraction process for the decoction yielded 38.6 g (7.7 %) of a sticky, dark brown crude extract.

Animals

Adult albino mice (Vom strain of the National Veterinary Research Institute, Vom, Jos, Nigeria) of both sexes (18 − 22 g) were used in the study. Animals were bred and housed in galvanised cages in a well-lit and aerated room of 12/12 h light/dark cycle in the animal facility, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife. Animals had unimpeded access to safe drinkable water and standard laboratory pellet diet (Guinea Feeds Brand, Bendel Feeds and Flour Mills, Ltd, Ewu, Edo State, Nigeria). The animal cages were regularly cleaned. All the animals were maintained on ideal environmental and nutritional state throughout the period of the study. Animals were allowed to acclimatise for 30 min before being used for experiment where they were moved from the animal facility to the laboratory.

Preparation of Extracts

The extracts of *A. laxiflora*were prepared fresh on each day of the experiment using 10 % Tween 80 as vehicle. The two extracts were administered to mice. The dosing of animals was based on the size of the experimental animals. The volume of the vehicle used was 0.1 ml/10 g mouse. Injection was administered slowly orally for the test doses, while both the oral and intra-peritoneal routes were used in the determination of acute toxicity and the LD_{50}.

Drugs and Chemicals

These drugs and chemical reagents were used in the study: Methanol, Ethanol (BDH Chemicals Ltd., Poole, England), Pentylenetetrazole, Picrotoxin, Strychnine, Phenytoin sodium, Polyoxyethylene sorbitan monotolate (Tween 80) (Sigma-Aldrich Inc., St. Louis, USA).

RESEARCH DESIGNS

Acute Toxicity Tests

The acute toxicity and LD_{50} of the plant extracts were determined using the Lorke's Method (Lorke, 1983) with minor modifications. The graded doses (100, 200, 400, 800, 1600 mg/kg, p.o.) of *A. laxiflora* (ALM) were used for toxicity testing. The number of death(s), behavioural changes (including the nature of death), time of death were recorded. One animal (n=1) was used for each dose level in phase I study, while four animals (n=4) of three dose levels were chosen in the phase II. The same procedure was employed in both the intra-peritoneal and the oral routes of toxicity testing.LD_{50} (the index of acute toxicity) was determined within 24 h. The LD_{50} was calculated as the geometric mean of the dose that caused 100 % mortality and that which produced no deaths (0 % mortality) in mice.

Assessment of Strychnine-induced Seizure

The protocol described by Lehmann *et al*. (1988) was adopted with minor modifications. Strychnine (STR) convulsions followed by death were induced in male mice by the administration of STR nitrate (2.5 mg/kg, i.p.). A protective effect of the ALM administered orally 1 h prior to STR was recorded and compared with the reference drug,Diazepam 2 mg/kg, i.p. and control (10 % Tween 80, 0.1 ml/10 g, p.o.). The number of test animals which survived 30 min post STR injection served as a criterion for protection.

Assessment of Pentylenetetrazole-induced Seizure

The method previously described by Schmutz *et al*. (1990) was adopted with minor modifications. Clonic seizures were induced in male mice by the administration of pentylenetetrazole (PTZ), 100 mg/kg, i.p., and the protective effect of ALM recorded. The latency to clonic seizures was recorded. The reference drug and the control experiments, as well as the observation time were as stated in the STR-induced seizure test.

Assessment of Picrotoxin-induced Seizure

The method of Lehmann *et al*. (1988) was adopted with minor modifications. Clonic seizures was induced in male mice with the injection of picrotoxin(PTX), 10 mg/kg, i.p. A protective effect of ALM against PTX-induced seizures was recorded. 10 % Tween 80 and Diazepam (2 mg/kg, i.p.) served as control and reference drug respectively.

Assessment of Maximal Electroshock-induced Seizure

The test was performed as described by Schmutz *et al*. (1990) with minor modifications. Tonic seizures of the hind extremities of mice were induced by passing alternating current (100 Hz, 18 mA, 1.0 s) from an electroconvulsiometer (Model No. 57800 – 001, Ugo Basile Biological Apparatus, Italy) through a pair of ear-clip electrodes immersed in Ringer's lactate solution. The current used was predetermined prior to the experiment and was the maximal current that caused hind limb extension in all mice in the experiment. The latency to clonic seizures, duration of tonic hind limb extension and post recovery time from tonic hind limb extension seizure was determined in the test groups of mice. An animal was considered to be protected, if the characteristic electroshock convulsive seizure pattern was absent. Phenytoin sodium 25 mg/kg, i.p. served as a reference drug.

Effect of Flumazenil on the Anticonvulsant Activity of *A. laxiflora* and Diazepam on PTZ-induced Seizure

Six (6) groups of mice (n=6) were used in the study. The control group (10 % Tween 80, 0.1 ml/10 g, p.o.) received PTZ (100 mg/kg, i.p.) while the second group received Flumazenil (2 mg/kg, i.p.) 5 min prior to the administration of PTZ. The third group of animalsreceived Diazepam (0.5 mg/kg, i.p.)15 min prior to PTZ. The fourth group was administered Flumazenil5 min prior to the administration of Diazepamand 15 min pre-administration of PTZ. The fifth group received ALM (1600 mg/kg, p.o.) 30 min prior to the administration of PTZ while the sixth group was injected Flumazenil 5 min before the administration ofALM and 30 min pre-injection of PTZ.

Effect of Naloxone on the Anticonvulsant Activity of *A. laxiflora* on PTZ-induced Seizure

Four (4) groups (n=6) of animals were used in the study. The control group (10 % Tween 80, 0.1 ml/10 g, p.o.) received PTZ (100 mg/kg, i.p.) while the second group received Naloxone (5 mg/kg, i.p.) 5 min prior to the administration of PTZ. The third group of mice received ALM (1600 mg/kg, p.o.) 30 min prior to PTZ. The fourth group was administered Naloxone 5 min prior to the administration of ALM and 30 min pre-injection of PTZ.

Statistical Analysis

Results were expressed as Mean±S.E.M. Analysis of experimental data was done using one-way ANOVA and multiple comparison of treatment groups was performed by employing the Student-Newman-Keuls (post-hoc) test,using the primer of biostatistics (Version 3.01) (Glantz, 1992). Probability level of ≤ 0.05 (5 %) was considered statistically significant for all treatments relative to control (Ingelfinger *et al*., 1994; Bland, 2000).

RESULTS

The LD_{50} was 400 mg/kg, i.p. and > 1600 mg/kg, p.o. for the methanolextract (MeOH EXT), and > 1600 mg/kg, i.p. and p.o. for the aqueous extract (AQ EXT).

Table 1: Effect of Methanol Extract of *A. laxiflora* on STR-induced Seizure in Mice.

Treatment group (mg/kg, p.o.)	Latency (Min)	Death Latency (Min)	% Protection
CTR	1.87±0.03	2.62±0.08	—
100	2.03±0.11	2.87±0.25	—
200	2.25±0.17	3.11±0.36	—
400	2.50±0.16	3.96±1.02	—
800	2.80±0.25	3.82±0.32	—
1600	3.11±0.20	3.59±0.17	—
DZP (2 mg/kg, i.p.)	3.99±0.89*	6.30±1.35**	50.00

Values are expressed as Mean±SEM, n=6; One-way ANOVA followed by Student-Newman-Keuls (S-N-K) multiple comparison (post hoc) test.*P< 0.001, **P< 0.05compared to control. *Indicates a significant difference from control, 10 % Tween 80.

Table 2: Effect of Aqueous Extract of *A. laxiflora* on STR-induced Seizure in Mice.

Treatment group (mg/kg, p.o.)	Latency (Min)	Death Latency (Min)	% Protection
CTR	1.87±0.03	2.62±0.08	—
100	2.30±0.08	3.17±0.08	—
200	1.93±0.03	2.99±0.06	—
400	2.23±0.06	3.99±0.42	—
800	2.53±0.20	3.20±0.25	—
1600	2.14±0.12	2.89±0.20	—
DZP (2 mg/kg, i.p.)	3.99±0.89*	6.30±1.35**	50.00

Values are expressed as Mean±SEM, n=6; One-way ANOVA followed by S-N-K post hoc test.
*P< 0.001, **P< 0.05 compared to control.

Table 3: Effect of Methanol Extract of *A. laxiflora* on PTZ-induced Seizure in Mice.

Treatment group (mg/kg, p.o.)	Latency (Min)	Death Latency (Min)	% Protection
CTR	1.01±0.10	3.63±0.28	—
100	1.02±0.23	3.91±0.41	—
200	1.38±0.21	10.39±2.45**	33.33
400	1.17±0.15	5.15±0.35	16.70
800	0.85±0.06	4.83±0.25	—
1600	1.55±0.22	5.96±0.18	50.00
DZP (2 mg/kg, i.p.)	NS	ND	100.00

Values are expressed as Mean±SEM, n=6; One-way ANOVA followed by S-N-K post hoc test.
P> 0.05, **P< 0.05 relative to control.

Table 4: Effect of Aqueous Extract of *A. laxiflora* on PTZ-induced Seizure in Mice

Treatment group (mg/kg, p.o.)	Latency (Min)	Death Latency (Min)	% Protection
CTR	1.01±0.10	3.63±0.28	—
100	1.23±0.14	9.04±1.38**	83.33
200	1.93±0.19*	9.35±0.93**	66.67
400	1.02±0.05	4.38±0.08	—
800	2.57±0.08*	ND	100.00
1600	1.16±0.16	10.58±1.26**	—
DZP (2 mg/kg, i.p.)	NS	NS	100.00

Values are expressed as Mean±SEM, n=6; One-way ANOVA followed by S-N-K post hoc test.
*P< 0.001,**P< 0.001relative to control.

Table 5: Effect of Methanol Extract of *A. laxiflora* on PTX-induced Seizure in Mice.

Treatment group (mg/kg, p.o.)	Latency (Min)	Death Latency (Min)	% Protection
CTR	8.08±0.87	18.85±2.31	—
100	11.29±0.20*	16.54.±0.54	83.33
200	9.36±0.32	22.30±2.12	83.33
400	7.18±0.52	24.43±1.79	16.67
800	9.77±0.37	23.66±2.05	66.67
1600	8.06±0.40	16.10±0.58	83.33
DZP (2 mg/kg, i.p.)	12.80±0.68	ND	100.00

Values are expressed as Mean±SEM, n=6; One-way ANOVA followed by S-N-K post hoc test.
*P< 0.001, P> 0.05relative to control.

Table 6: Effect of Aqueous Extract of *A. laxiflora* on PTX-induced Seizure in Mice.

Treatment group (mg/kg, p.o.)	Latency (Min)	Death Latency (Min)	% Protection
CTR	8.08±0.87	18.85±2.31	—
100	10.26±0.19	18.88±3.13	66.67
200	8.37±0.82	16.35±0.92	66.67
400	9.62±0.86	17.32±1.10	50.00
800	11.99±1.11*	21.20±0.55	66.67
1600	10.15±0.44	18.19±1.88	16.67
DZP (2 mg/kg, i.p.)	12.80±0.68*	ND	100.00

Values are expressed as Mean±SEM, n=6; One-way ANOVA followed by S-N-K post hoc test.
*P< 0.05, P> 0.05 relative to control.

Table 7: Effect of Methanol Extract of *A. laxiflora* on MES-induced Seizure in Mice.

Treatment group (mg/kg, p.o.)	Latency (s)	D-THE (s) `	Recovery (s)
CTR	2.30±0.09	22.82±2.58	98.40±9.28
100	2.21±0.33	21.64±1.49	69.30±6.54***
200	2.23±0.21	15.62±1.41**	56.07±10.35***
400	2.33±0.25	15.90±0.49**	55.71±3.74***
800	2.63±0.20	15.71±0.65**	70.73±12.58***
1600	2.32±0.21	16.57±0.38**	48.22±5.53***
PHY (25 mg/kg, i.p.)	NS	NS	NS

Values are expressed as Mean±SEM, n=6; One-way ANOVA followed by S-N-K post hoc test.
P> 0.05, **P< 0.05, ***P< 0.05compared to control.

Table 8: Effect of Aqueous Extract of *A. laxiflora* on MES-induced Seizure in Mice.

Treatment group (mg/kg, p.o.)	Latency (s)	D-THE (s)	Recovery (s)
CTR	2.30±0.09	22.82±2.58	98.40±9.28
100	3.27±0.84	22.11±1.83	37.84±8.05*
200	2.06±0.21	21.23±1.00	63.50±12.41
400	2.22±0.16	20.12±1.68	83.00±8.38
800	1.51±0.12	17.09±0.28	76.80±9.32
1600	1.64±0.22	18.56±1.43	84.38±3.31
PHY (25 mg/kg, i.p.)	NS	NS	NS

Values are expressed as Mean±SEM, n=6; One-way ANOVA followed by S-N-K post hoc test.
P> 0.05, P> 0.05, ***P< 0.05relative to control.

Table 9: Effect of Flumazenil on the Anticonvulsant Activity of the Methanol Extract of *A. laxiflora* and Diazepam on Pentylenetetrazole-induced Seizures in Mice.

Treatment group	Latency (Min)	Death Latency (Min)
CTR	1.01±0.19	3.63±0.28
FLU	1.59±0.35	8.18±1.03**
DZP	2.80±0.58*	8.13±0.28**
FLU+DZP	1.36±0.26	5.88±1.13
ALM	1.55±0.22	5.96±0.18
FLU+ALM	0.79±0.03	4.42±0.66

Values are expressed as Mean±SEM, n=6; One-way ANOVA followed by S-N-K post hoc test.
*P< 0.05, **P< 0.001relative to control.

Table 10: Effect of Naloxone on the Anticonvulsant Activity of the Methanol Extract of *A. laxiflora* on Pentylenetetrazole-induced Seizures in Mice.

Treatment group	Latency (Min)	Death Latency (Min)
CTR	1.01±0.10	3.63±0.28
NAL	0.80±0.04	4.35±0.31
ALM	1.55±0.22*	5.96±0.18*
NAL+ALM	1.14±0.09	5.06±0.28*

Values are expressed as Mean±SEM, n=6; One-way ANOVA followed by S-N-K post hoc test.

*P< 0.05, **P< 0.001relative to control.

Table 11: Effect of Flumazenil on the Anticonvulsant Activity of the Aqueous Extract of *A. laxiflora* and Diazepam on Pentylenetetrazole-induced Seizures in Mice.

Treatment group	Latency (Min)	Death Latency (Min)
CTR	1.01±0.10	3.63±0.28
FLU	1.59±0.35	8.18±1.03**
DZP	2.80±0.58*	8.13±0.28**
FLU+DZP	1.36±0.26	5.88 ±1.13
ALM	1.16±0.16	10.58±1.26**
FLU+ALM	0.97±0.04	2.77±0.31

Values are expressed as Mean±SEM, n=6; One-way ANOVA followed by S-N-K post hoc test.
*P< 0.05, **P< 0.001relative to control.

Table 12: Effect of Naloxone on the Anticonvulsant Activity of the Aqueous Extract of *A. laxiflora* on Pentylenetetrazole-induced Seizures in Mice.

Treatment group	Latency (Min)	Death Latency (Min)
CTR	1.01±0.10	3.63±0.28
NAL	0.80±0.04	4.35±0.31
ALM	1.16±0.16	10.58±1.26**
NAL+ALM	0.93±0.05	3.54±0.85

Values are expressed as Mean±SEM, n=6; One-way ANOVA followed by S-N-K post hoc test.
P> 0.05, **P< 0.001 relative to control.

DISCUSSION

Seizures are discrete, time-limited alterations in brain function associated with episodic high frequency discharge of impulses by a group of neurons (Fischer, 1998; Rang *et al*., 2019). Seizures start suddenly and usually stop on their own within one to three minutes. Epilepsy or seizure disorder is diagnosed after two or more episodes of unprovoked seizure. Epileptic seizure is characterised by a transient occurrence of signs and/or symptoms due to abnormal excessive or synchronous neuronal activity in the brain (ILAE, 2014). Clinical manifestations of a seizure reflect the area of the brain from which the seizure begins (seizure focus) and the spread of electrical activity. One out of every ten (1:10) people will have a single episode of seizure in their life time (Cincinnati Hospital, 2016). Clinical manifestations are numerous and varied, and includes: indescribable bodily sensations, pins and needles sensations, fear or depression, hallucinations, momentary jerks or head nods, staring with loss of awareness, convulsive movements lasting seconds to minutes. Convulsions on the other hand refer to specific type of seizures where the attack is primarily manifested by involuntary muscular contractions of the extremities, trunk, and head (Fischer, 1998; Randon House Dictionary, 2016). It has been observed that despite the many anti-seizure drugs (ASD) available for the treatment and management of seizure disorders (McNamara, 2006), asignificant percentage of the rural population in developing countries still depend on herbal remedies (Ojewole, 2008).

In the STR-induced seizure study, there was an increase in the threshold for seizures and a prolongation of death latency in both the methanol and the aqueous extracts at test doses, although this activity was not significant. This action was more prominent in the methanol extract, and showed a lack of anticonvulsant activity. However, diazepam (a standard drug) showed a significant increase in both the latency and death latency to seizures, and some degree of protection against seizures, indicating superiority in potency relative to the extracts. The absence of protection against STR-induced seizures, may suggest a lack of activity of *A. laxiflora* on post synaptic receptor inhibition mediated by glycine, an inhibitory CNS neurotransmitter.

In the PTZ-induced seizure, the methanol extract prolonged the onset of seizure at low and highest doses, while the death latency was prolonged at all the test doses, but significant at a moderately low dose. The methanol extract provided protection against seizures at moderately low doses and at the highest dose in the study, suggesting anti-seizure activity. The aqueous extract prolonged the latency to seizure at all the test doses, but significant at moderately low and high doses. It also, significantly prolonged death latency at low doses and at the highest test dose, while non-significantly prolonging death latency at a moderately low dose. The aqueous extract produced protection against death at low doses and total protection at a moderately high dose comparable to the reference drug, diazepam. The aqueous extract at moderately low and at the highest test doses did not provide protection against death similar to the control experiment, an indication of lack of anticonvulsant activity.

The methanol extract at the lowest test dose significantly prolonged the latency toseizures in the PTX-induced seizure model. There was a prolongation in the death latency to seizures at low to moderate test doses, which was not significant. The death latency to seizures in the methanol extract at the lowest and highest doses was

comparable to that of the control. The aqueous extract significantly prolonged the onset to seizures at a moderately high dose (an indication of anticonvulsant activity), with no significant increase in death latency to seizures. However, the aqueous extract provided protection against death of the experimental animals at all the test doses.

The latency (threshold) to seizures in the MES-induced seizures was not significantly prolonged in the methanol extract at all the test doses. There was a significant decrease in the duration of tonic hindlimb extension (D-THE) at low, moderate and high doses, an indication of the anti-seizure activity of the extract. The decrease in the recovery time of the test animals was significant at all the test doses, thus further lending credence that the methanol extract possesses anti-seizure property. In the aqueous extract, there was no significant prolongation in the latency to seizures at the lowest test dose. The decrease in the D-THE was not significant at all the test doses. There was, however, a decrease in the recovery time of the experimental animals, which was significant at the lowest test dose, and thus suggests an anticonvulsant activity. Phenytoin sodium, the reference drug, provided absolute protection of the test animals against MES-induced seizures. The absolute protection of the experimental animals against death was due to the low electric voltage and the short duration of the maximal electroshock, which was not experimentally programmed to electrocute the test animals. The total protection against MES-induced seizures by the reference drug, Phenytoin sodium, suggests an inhibitory action on the activation of Na^+ channels and possibly, the excitatory glutamatergic neurotransmission. The biochemical and physiological basis (neural mechanism) of MES-evoked seizures has been elucidated, and works through the activation of Na^+ channels and the excitatory glutamatergic neuronal transmission (Oraifo et al., 2012).

Flumazenil is a selective, competitive antagonist at the $GABA_A$-benzodiazepine receptor. It is an experimental tool, and is also employed clinically in the reversal of neurosedation arising from anoverdose or intoxication from the benzodiazepines. Flumazenil (FLU) was used in the study to determine the involvement of the GABAergic pathway in mediating the anticonvulsant effect of *A. laxiflora* in mice. FLU when administered prior to PTZ injection increased the latency to seizures relative to control, though not significantly while significantly increasing the death latency to seizures in the MeOH EXT. The MeOH EXT of *A. laxiflora* did not significantly increases the latency and death latency to seizures. DZP significantly increased the latency and death latency to seizures relative to control, an indication of anticonvulsant activity. Prior administration of FLU to DZP and PTZ produced no significant increase in latency and death latency to seizures. This was not unexpected due to the blockade of GABA-benzodiazepine receptors by FLU, and thus making prominent the convulsant effect of PTZ and decreasing the anti-seizure activity of DZP. This demonstrates that DZP works via the GABAergic pathway. The pre-injection of FLU to the MeOH EXT of ALM and PTZ did not decrease the latency to seizures significantly. The death latency to seizures was rather increased, although not in a significant manner implying that the anticonvulsant effect of *A. laxiflora* was probably not mediated through the GABAergic pathway. The AQ EXT of ALM on the other hand, increased the latency to seizures, not in a significant respect, but significantly increased the death latency to seizures. The administration of FLU prior to the AQ EXT of ALM and PTZ decreased both the latency and death latency to seizures non-significantly. This showed that GABAminergic receptors were probably not involved in mediating the anticonvulsant effect of *A. laxiflora* in mice.

Naloxone is a pure, non-selective opioid receptor antagonist. It is a research tool, and is used to investigate drugs and/or candidates that mediate their actions and effects via the opioidergic pathway. It is also used in emergency clinical medicine to reverse opioid intoxication, diagnose opioid dependence, reverse endogenous opioid-induced hypotension in stressful states, as well as in the reversal of neonatal asphyxia triggered by opioids (e.g. morphine, pethidine or meperidine, demerol, nubain, fentanyl, stadol etc.) during childbirth. Naloxone administration prior to PTZ decreased the latency to seizures but increased the death latency to seizures in a non-significant manner. The MeOH EXT of *A. laxiflora* significantly increased both the latency and the death latency to seizures, which showed anticonvulsant activity. Pre-injection of NAL to ALM and PTZ increased the threshold for the development of seizures (non-significantly) and significantly increased the mortality threshold in Swiss albino mice, and thus demonstrates that *A. laxiflora* did not mediate its anticonvulsant effect through the opioidergic pathway. To implicate the opioidergic system, a pharmacodynamic blockade of the opioid receptors by NAL would rather significantly decrease the threshold and the mortality threshold to seizures respectively. The AQ EXT did not significantly increase the latency to seizures. However, there was a significant increase in the death latency to seizures, which indicated anticonvulsant activity. Pre-treatment of animals with NAL, and then ALM followed by PTZ decreased the latency and the death latency to seizures, although not in a significant manner. This showed that the anticonvulsant properties of the AQ EXT were perhaps not mediated via the opioidergic pathway. The findings from the study is in tandem with a previous study (Nwonu, 2017) on the signalling mechanisms/pathways mediating the effects of the extracts of *A.laxiflora*, which did not implicate the GABAminegic pathway, neither was the opioidergic pathway involved in mediating the central nervous system depressant effect of the extracts.

The protection against death of the experimental animals in the PTZ-induced seizure models suggests that *A.*

laxiflora may exert its effect on neuronal transmission probably mediated by other pathways, other than through the $GABA_A$ and/or k-opioid receptors (K-OPr). It has been established that activation of $GABA_A$ and/or k-opioid receptors (K-OPr) open Cl^- channel and creates a state of cellular negativity leading to hyperpolarization, and ultimately decreases neuronal cell firing (Nassiri-Asi *et al*., 2007). The anticonvulsant activity of the K-OPr agonists has been elucidated (Nassiri-Asi *et al*., 2007). K-OPr agonists have been shown to be effective against bicuculline, maximal-electroshock (MES) and excitatory amino acid-induced seizures (Nassiri *et al*., 2007). These K-OPr agonists attenuate the kindling of seizures evoked by repeated PTZ administration (Yajima *et al*., 2000; Manocha *et al*., 2003). The endogenous opioid peptide (e.g. dynorphins) binds to K-OPr, and has demonstrated anti-seizure activity in some studies (Kaminski *et al*., 2007). *A. laxiflora* probably had no agonist action on opioid receptors (mu, kappa, delta or nociceptin/orphanin receptors) to cause neuronal hyperpolarisation, neither did it induce the attenuation of seizures evoked by PTZ as demonstrated in the study. K-OPr agonists have modulatory actions on $GABA_A$ receptor-induced seizures (Yajima *et al.*, 2000). $GABA_A$ receptors are directly coupled to Cl^- channels, and with the effect of *A. laxiflora* on PTZ-induced seizures, *A. laxiflora* extracts did not seem to have caused the blockade of experimentally-inducedseizures through the $GABA_A$ receptor-linked chloride ion channel. The signalling pathways mediating the anticonvulsant effect of *A. laxiflora*were probably via other pathways.

CONCLUSION

The study concluded that *A. laxiflora* possesses anticonvulsant activity. The signalling pathways mediating the anticonvulsant effects of the extracts were found not to be through the GABAergic and the opioidergic pathways.

Ethical Considerations

Compliance with ethical guidelines

The study was approved by the University Research Committee through the Postgraduate College Board of the Obafemi Awolowo University, Ile-Ife, Nigeria (Ethical Approval No. PHP11/12/H/2768). The guidelines for the care and use of animals in neuroscience and behavioural research (NIH, 1991; NRC, 1996) were strictly adhered to.

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Competing Interests

The authors declare that there was no conflicts of interest in the work.

Contribution of Authors

CN wrote the proposal for the study, designed the experiments, and carried out the bench work. OI muted the idea for the study, and in conjunction with JA streamlined and supervised the research. All the authors read the work, revised and approved the final manuscript for submission.

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