Neuromuscular Inhibitory Effects of the Aqueous and Methanol Extracts of *Alchornea laxiflora* in Adult Albino Mice

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Abstract- The study investigated the median lethal dose and the effects of the aqueous and methanol extracts of *Alchornea laxiflora* in two neurobehavioural models of motor coordination, the traction (grip strength) and rotarod tests. This was with a view to providing information on the acute toxicity and the effects of the plant extracts on neuromuscular function. 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. Mice of both sexes (n=6) weighing 18 – 22 g were used for the study, which were randomized into control and test groups, which summed up to seven (VII) 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 Diazepam (4 and 10 mg/kg, i.p.). The animals were individually observed for fall off time from the revolving horizontal bar in the rotarod test and for the loss of muscle grip or grip strength in the traction test. They were appropriately scored after observation at intervals of 30, 60, 90, 120 and 150 min post intra-peritoneal and oral administrations of vehicle, extracts or drugs respectively. The results showed that *A. laxiflora* possesses significant ($P \leq 0.05$) inhibitory effect on neuromuscular function due to the increase in the time taken for the animals to grasp the horizontally stretched wire with the hind limbs in the traction test. However, the extracts did not demonstrate any significant ($P > 0.05$) effects on the fall off time in the rotarod performance test. The study concluded that *A. laxiflora* possesses skeletal muscle relaxant activity in mice.

Index Terms: *Alchornea laxiflora*, rotarod, traction, diazepam, motor coordination and mice

I INTRODUCTION

Alchornea laxiflora (Bentham) Pax and Hoffman (Euphorbiaceae) is a deciduous shrub or a forest understorey tree of about 6m high growing in Nigeria. The leaves are thinly textured turning an attractive yellow or red in dry season, while 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 has demonstrated that extract from the leaves of *A. laxiflora* can reverse sickling phenomenon *in vitro*, and thus can be employed in the management 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, exhibit 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. Another study has also, shown that the methanol extract of the leaves of *A. laxiflora* possesses sedative and anxiolytic activities in mice *in vivo* (Nwonu, 2011). This novel development necessitated further scientific enquiry into the neuromuscular inhibitory effects of the plant.

II MATERIALS AND METHODS:

Plant Collection

Alchornea laxiflora Bentham leaves were collected in the month of February, 2013 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. Ifeoluwa I. Ogunlowo, a 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 litres of 100 % methanol (absolute methanol) for 72 hours, with occasional stirring. The marc was re-extracted using another equal volume of methanol for 72 hours. 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 hours. 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 acclimatize for 30 min before being used for experiment where they were moved from the animal facility to the laboratory. The guidelines for the care and use of animals in neuroscience and behavioural research (NIH, 1991; NRC, 1996) were strictly adhered to.

Preparation and Dosing

A. laxiflora extracts were prepared fresh on each day of the experiment using 10 % Tween 80 as vehicle. All the extracts were administered to 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 intraperitoneal routes were used in the determination of acute toxicity and the LD_{50}.

Drugs

The following drugs and chemical reagents were used in the study: Diazepam (F. Hoffmann-La Roche, Basel, Switzerland), Ethanol and Methanol (BDH Chemicals Ltd., Poole, England), Tween 80 (Sigma-Aldrich Inc., St. Louis, USA).

EXPERIMENTAL DESIGNS

Acute Toxicity Tests

The acute toxicity and LD_{50} of the plant extracts were determined using the Lorke's Method (1983). The graded doses (100, 200, 400, 800, 1600 mg/kg, i.p. and p.o.) of *A. laxiflora* was used for toxicity testing. The number of death(s), behavioural changes (and 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 calculated within 24 h. Animals were observed hourly for the first 8 h, then 6 hourly for 24 h, and then daily for 14 days (Wafai and Mehta, 1986). The number of deaths were recorded on the day of experiment, and those that survived the acute toxicity were weighed daily for 14 days. Weight increase in each animal was regarded as having survived the acute toxicity, and thus the experiment was discontinued.

Assessment of the Effects of *A. laxiflora* on Motor Coordination in Mice

Rotarod Performance Test

Male mice (18-22 g) were pre-selected 72 h (with twice daily training) in advance to eliminate those animals which could not stay on the horizontal bar at a speed of 16 rpm for a period of 60 s. Animals were treated orally with vehicle (10 % Tween 80, 0.1 ml/10 g, p.o.) and graded doses of ALM (100, 200, 400, 800 and 1600 mg/kg, p.o.) or Diazepam (4 mg/kg, i.p.). The maximum period of time was 60 s, with three (3) reintroductions to the rota-rod (Tread Mill 7600; Model No. 09481, Ugo Basile Biological Apparatus, Comerio-(va)-Italy) were allowed (Rosiland *et al*., 1990).

Traction Test

The screening of animals was performed by placing the fore paws of male mice in a small twisted wire rigidly supported above a bench top. Normally, the mice grasp the wire with the fore paws and place at least one hind foot on the wire within 5 s, when allowed to hang freely. The test was conducted on five groups of animals (n=5), which were previously screened to exclude mice with limb dysfunction or other neuromuscular disorders, 1 h post administration of ALM (100, 200, 400, 800 and 1600 mg/kg, p.o.) or vehicle (10 % Tween 80, 0.1 ml/10 g, p.o.), while 30 min was allowed prior to Diazepam (10 mg/kg, i.p.) administration. The inability of animals to put at least one hind foot within 5 s was considered as failure in the traction (grip strength) test (Rudziket *et al*., 1973).

III RESULTS

Acute Toxicity Tests

The LD_{50} was 400 mg/kg, i.p. and > 3200 mg/kg, p.o. for the methanol extract, and > 1600 mg/kg, i.p. and p.o. for the aqueous extract.

Table 1. Effect of the Methanol Extract of *A. laxiflora* on Skeletal Muscle Relaxation: Rotarod Performance Teat

	Mean Time Spent on the Revolving Horizontal Bar at Different Time Intervals/ 60 s				
Treatment group (mg/kg, p.o.)	30 Min	60 Min	90 Min	120 Min	150 Min
CTR	60.00±0.00	60.00±0.00	60.00±0.00	60.00±0.00	60.00±0.00
100	60.00±0.00	60.00±0.00	60.00±0.00	60.00±0.00	60.00±0.00
200	60.00±0.00	60.00±0.00	59.78±0.22	60.00±0.00	59.90±0.10
400	60.00±0.00	60.00±0.00	60.00±0.00	60.00±0.00	60.00±0.00
800	60.00±0.00	57.40±2.60	58.50±1.50	60.00±0.00	60.00±0.00
1600	60.00±0.00	60.00±0.00	60.00±0.00	60.00±0.00	60.00±0.00
DZP (4mg/kg,i.p.)	2.80±0.92*	9.40±7.91*	18.60±11.04*	25.80±14.00*	55.80±4.20

One-way ANOVA revealed a significant difference between the treatments: F = 3865; F = 36.4; F = 13.63; F = 5.97; P = 0.000. The result shows no significant difference between the control and the test doses. However, a significant difference was observed between the control and the reference drug, diazepam. *Indicates a significant difference from control, 10 % Tween 80.

Table 2. Effect of Aqueous Extract of *A. laxiflora* on Skeletal Muscle Relaxation: Rotarod Performance Test

	Mean Time Spent on the Revolving Horizontal Bar at Different Time Intervals/ 60 s				
Treatment group (mg/kg, p.o.)	30 Min	60 Min	90 Min	120 Min	150 Min
CTR	60.00±0.00	60.00±0.00	60.00±0.00	60.00±0.00	60.00±0.00
100	60.00±0.00	60.00±0.00	60.00±0.00	60.00±0.00	60.00±0.00
200	60.00±0.00	60.00±0.00	60.00±0.00	60.00±0.00	60.00±0.00
400	60.00±0.00	60.00±0.00	60.00±0.00	60.00±0.00	60.00±0.00
800	60.00±0.00	60.00±0.00	60.00±0.00	60.00±0.00	60.00±0.00
1600	60.00±0.00	60.00±0.00	60.00±0.00	60.00±0.00	60.00±0.00
DZP (4mg/kg,i.p.)	2.80±0.92*	9.40±7.91*	18.60±11.04*	25.80±14.00*	55.80±4.20

One-way ANOVA revealed a significant difference between the treatments: F = 3865; F = 40.92; F = 14.06; F = 5.97; P = 0.000. The result shows no significant difference between the control and the test doses. However, a significant difference was observed between the control and the reference drug, diazepam. *Indicates a significant difference from control, 10 % Tween 80.

Table 3. Effect of the Methanol Extract of *A. laxiflora* on Muscle Relaxation: Traction Test

	Mean Time Hind Foot Grasp of an Elevated Horizontal Wire Mesh/ 5 s			
Treatment group (mg/kg, p.o.)	30 Min	60 Min	90 Min	120 Min
CTR	0.51±0.04	0.55±0.05	0.61±0.05	0.51±0.05
100	0.61±0.04	0.54±0.03	0.51±0.04	0.71±0.09
200	0.42±0.02	0.42±0.06	0.31±0.05	0.51±0.06
400	0.42±0.03	0.69±0.09	0.92±0.06	0.97±0.10
800	0.53±0.05	0.69±0.03	0.64±0.04	0.98±0.05
1600	0.67±0.04*	0.84±0.06*	0.69±0.03	0.62±0.03
DZP (10 mg/kg, p.o)	5.00±0.00*	5.00±0.00*	4.21±0.79*	4.20±0.80*

One-way ANOVA revealed a significant difference between the treatments: F = 2333.68; F = 984.57; F = 20.69; F = 18.57; P = 0.000. The result shows a significant difference between the control and 1600 mg/kg, p.o.

at 30 min and 60 min respectively. A significant difference was observed between the control and the reference drug, diazepam at different time intervals. *Indicates a significant difference from control, 10 % Tween 80.

Table 4. Effect of the Aqueous Extract of *A. laxiflora* on Muscle Relaxation: Traction Test

Mean Time Hind Foot Grasp of an bElevated Horizontal Wire mesh/ 5 s

Treatment group (mg/kg, p.o.)	30 Min	60 Min	90 Min	120 Min
CTR	0.51±0.04	0.55±0.05	0.61±0.05	0.51±0.05
100	1.24±0.24*	1.37±0.45	1.94±0.48	2.67±0.99*
200	0.97±0.07*	1.34±0.44	1.03±0.22	1.15±0.13
400	1.06±0.07*	1.15±0.13	1.15±0.21	1.86±0.29
800	0.99±0.04*	1.12±0.20	1.26±0.35	1.05±0.12
1600	0.75±0.10	0.81±0.05	0.75±0.14	0.82±0.04
DZP (10 mg/kg, p.o)	5.00±0.00*	5.00±0.00*	4.21±0.79*	4.20±0.80*

One-way ANOVA revealed a significant difference between the treatments: F = 211.26; F = 35.25; F = 9.90; F = 6.78; P = 0.000. The result shows a significant difference between the control, the test doses and the reference drug, diazepam at different time intervals. *Indicates a significant difference from control, 10 % Tween 80.

IV DISCUSSION

Motor coordination refers to a balanced combination of movements that involves more than one part of the body. It is an index of an individual's ability to utilize the muscles and the joints, as well as the nerves and other parts of the human or animal body to perform a given task. The cerebellum plays a pivotal role in the neural control of movement, and damage to this part of the brain or its connecting structures and pathways results in the impairment of coordination (motor deficit), known as ataxia. Drugs that relax the skeletal muscles are essential in surgical practice, allowing a low, safe level of anaesthesia to be adopted (Randall *et al*., 2009). Skeletal muscle relaxants are drugs that act peripherally at the neuromuscular junction and muscle fibres or centrally in the cerebrospinal axis to decrease muscle tone and/or cause paralysis (Tripathi, 2008). Myorelaxants relieve musculoskeletal pain or spasm and severe musculoskeletal spasticity. In the study, the fall off time of animals from the revolving rod in the rotarod coordination test and the loss of muscle grip or grip strength (i.e. the inability of animals to place at least a hind foot to the horizontally stretched wire mesh elevated above the laboratory bench top within five seconds) in the traction tests were regarded as indices of skeletal muscle relaxation.

The rotarod performance test was essentially employed to evaluate neurological (motor) deficits in mice. The loss of motor coordination is pathognomonic of many neurological disorders (e.g. transient ischaemic attack (mini stroke), cerebrovascular disease (stroke), traumatic brain injury, etc.). This clinico-pharmacological effect can be easily detected in cases of drug intoxication (Massaquoi and Hallet, 1998). In motor coordination deficits, there is abrupt loss of connectivity between the central nervous system (brain and spinal cord) and the muscles or direct abnormal functioning (dysfunction) of the muscles of the body. The loss of neural

connectivity can be between the central nervous system (CNS) and the motor end plate (the terminal portion where the axon of a motor neurone makes synaptic contact with a muscle fibre). It, therefore, means that motor deficits can occur anywhere along the neural stretch between the CNS and the motor end plate. Any drugs or candidate drugs that has the potential to induce skeletal muscle relaxation through the interference with the animal's ability to remain on a revolving (or rotating) rod at a specific speed is referred to as a skeletal muscle relaxant. The methanol and aqueous extracts of *A. laxiflora* did not affect rotarod performance in the animals, as all the animals stayed on revolving rod for 60 s without falling off the rod. It, therefore, means that the leaf extracts of *A. laxiflora* produced little or no effect on physical performance, endurance and inhibition of neuromuscular function, since there was no significant decrease in the fall off time of the animals from the rotating rotarod. Therefore, the leaf extracts *A. laxiflora* had no effects on motor coordination on the rotarod model.

The traction test was used to study neuromuscular function, and is often combined with the rotarod motor coordination test, a standard validated behavioural paradigm used to screen drugs and candidates. The methanol extract at the highest tested dose used in this study significantly inhibited neuromuscular function in the fore limbs of the animals stretched horizontally to a stretched wire at 30 and 60 min time intervals respectively. The time taken for the animals to put at least one of the hind limbs to the horizontally stretched wire was prolonged relative to control. Diazepam, the reference drug significantly prolonged the time taken for the animals to place a hind limb to the horizontally stretched wire at the different time intervals in the methanol extract. In the aqueous extract, there was a significant prolongation in the time taken for the animals to put at least one hind limbs to the horizontally stretched wire. This activity was demonstrated at 30 and 120 min time intervals in low to moderate and high doses, and at the lowest tested dose respectively. At 60 and 90 min time intervals, an increase in the time required for the animals to place either one of the hind limbs to the horizontally stretched wire was observed, but this was not significant. This was an indication of neuromuscular inhibition, and shows that *A. laxiflora* has the potential to induce neurological deficits, and thus modulate motor coordination. The loss of skeletal muscle grip in the animals was due to decreased neuromuscular excitability of the CNS induced by the plant extracts. However, diazepam, the standard drug and a benzodiazepine neurospasmolytic agent, produced a prominent effect on skeletal muscle relaxation relative to control by significantly decreasing motor coordination in the experimental animals at different time intervals.

V CONCLUSION:

The study concluded that *Alchornea laxiflora* possesses skeletal muscle relaxant activity in the traction test. However, the plant extracts did not demonstrate any motor coordination deficits in the rotarod performance test.

Statistical Analysis

All data were expressed as Mean±S.E.M. Analysis of data was done using one-way ANOVA and multiple comparison of treatment groups performed by employing the Student-Newman-Keuls 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 (Steel and Torrie, 1960).

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Conflicts of interest

The authors declare that there is no competing interests.

REFERENCES

[1] Adewole, A.A. Personal communication with local traditional medical practitioners in Ibadan, Nigeria, 1993.

[2] Burkill, H.M. The useful plants of West Tropical Africa, Edition 2, Vol. 2, Families E-I, Royal Botanic Gardens, Kew, 1994: pp. 144 − 150.

[3] Farnsworth, N.R., Akerele, O., Bingel, A.S., Soejarto, D.D. and Guo, Z.G. Medicinal plants in therapy, Bull. WHO 63, 1985: 965 – 981.

[4] Farombi, E.O., Ogundipe, O. O. Uhunwangho, E., Adeyanju, M.A. and Olarenwaju, M.O. Anti-oxidant properties of extracts from *Alchornea laxiflora* (Benth.) Pax and Hoffman. *Phytotherapy Research* 17 (7), 2003: 713 − 716.

[5] Glantz, A.S. The Primer of Biostatistics (Version 3.01), McGraw–Hills Inc., 1992.

[6] Gupta, M., Mazumder, U.K. and Chakrabatis, S. CNS activities of the methanolic extract of *Morringa oleifera* root in mice. *Fitoterapia* 70, 1999: 244 − 250.

[7] Hutchinson, J. and Dalziel, J.M. Flora of West Tropical Africa. Crown Agents for Overseas Government and Administration, London, Vol. 1 (2), 1937: 600 – 605.

[8] Lorke, D. A new approach to practical acute toxicity testing. *Archives of Toxicol*ogy 54, 1983: 275 − 287.

[9] Massaquoi, S.G. and Hallet, M. Ataxia and other cerebellar syndromes. In: Parkinson's disease and other movement disorders. Jankovic, J. and Tolosa, E. (eds.), Baltimore: Williams and Wilkins, 1998, pp. 623 – 686.

[10] Muanya, C. Herbal medication shows promise in the management of Sickle cell anaemia. The Guardian, February 19, 2009, pp. 35 − 36.

[11] NIH Guidelines for the care and use of animals in neuroscience and behavioural Research, 1991. National Institutes of Health.

[12] NRC Guidelines for the care and use of animals in neuroscience and behavioural Research, 1996. National Research Council. Academic Press: Washington, DC. 12.

[13] Nwonu, C.N. Neuropharmacological effects of the methanolic leaf extract of *Alchornea laxiflora* Benth. (Euphorbiaceae) in mice, 2011. M.Sc. Thesis, Obafemi Awolowo University, Ile-Ife, Nigeria.

[14] Ogundipe, O.O., Moody, J.O., Houghton, P.J. and Odelola, H.A. Bioactive chemical constituents from *Alchornea laxiflora* (Benth.) Pax and Hoffman. *Journal of Ethnopharmacology* 74 (3), 2001: 275 − 280.

[15] Randall, M., Kendall, D. and Alexander, S. 2009 FASTtrack Pharmacology, Pharmaceutical Press, London, 2009, pp. 183.

[16] Rosiland, J.H., Hunskaar, S. and Hole, K. Diazepam attenuates morphine antinociceptive test dependently in mice. *Pharmacology and Toxicology* 66 (5), 1990: 382 – 386.

[17] Rudziket, A.D., Hester, J.B., Tang, A.H., Staw, R.N. and Friis, W. The Benzodiazepines. Raven Press, New York, 1973, pp. 285 – 297.

[18] Steel, R. G. D. and Torrie, J. H. Principles and Procedures of Statistics, McGraw-Hills Publishing Company Inc., London, 1960, pp. 13 – 26.

[19] Tripathi, K.D. Skeletal Muscle Relaxants. Essentials of Medical Pharmacology, 6th ed., Jaypee Brothers Medical Publishers (P) Ltd., New Delhi, 2008, pp. 339.

[20] Wafai, Z.A. and Mehta, V.L. Some neuropharmacological actions of 3-methyl-5-phenyl-(4-methyl)–s. *Indian Journal of Pharmacology* 18, 1986: 89 − 94.