ANTI-ANXIETY ACTIVITIES OF THE AQUEOUS AND METHANOL EXTRACTS OF *ALCHORNEA LAXIFLORA* IN ALBINO MICE

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Abstract- The study investigated the effects of the aqueous and methanol extracts of *Alchornea laxiflora* on two mouse models of anxiety, the elevated plus-maze and the staircase. This was with a view to providing information on the anxiolytic potential of *A. laxiflora*. Seven (7) groups of mice of both sexes (n=6) weighing 18 – 22 g were used for the study, which were randomized into control and the test groups. The control group (I) received 10 % Tween 80 (vehicle), 0.1 ml/10 g mouse, p.o. 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 reference group (VII) received standard drug, Diazepam (1 mg/kg, i.p.). The animals were observed for exploratory activity in the open and the close arms of the elevated plus-maze. The animals were also observed for rearing and steps-climbing of the staircase model. They were appropriately scored in the elevated plus-maze and the staircase paradigms individually 30 and 60 min post intra-peritoneal and oral administrations respectively for both the aqueous and the methanol extracts. The extracts demonstrated anxiolytic activity in the elevated plus-maze test, by the increase in the percentage entry into open arms, increase in the percentage time spent in the open arms, significant (P = 0.000) increase in the time spent in the open arms and a decrease in the index of open arm avoidance. In the staircase model, there was a significant (P = 0.000) decrease in rearing and a significant (P = 0.000) increase in the number of steps-climbing. The study concluded that the aqueous and the methanol extracts of *A. laxiflora* have anti-anxiety activities in mice.

Key words: *Alchornea laxiflora*, anxiety, elevated plus-maze, staircase and mice.

I. INTRODUCTION

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, 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).

II. MATERIALS AND METHODS

2.1 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. I. 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.

2.2 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.

2.3 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 and NRC, 1996) were strictly adhered to.

2.4 Preparation and Dosing of Animals

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 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}.

2.5 Drugs and Chemical Reagents

The drug and chemical reagents used in the study include: Diazepam (F. Hoffmann-La Roche, Basel, Switzerland), Polyoxyethylene sorbitan monotolate (Tween 80) (Sigma-Aldrich Inc., St. Louis, USA), methanol and ethanol (BDH Chemicals Ltd., Poole, England).

2.6 Research Designs

2.6.1 Acute Toxicity Tests

The acute toxicity and LD_{50} of the plant extracts were determined using the Lorke's Method (Lorke, 1983). The graded doses (200, 400, 800, 1600, 3200 mg/kg, i.p. and p.o.) of *A. laxiflora* (ALM) were 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. Increase in the weights of the animals was regarded as having survived the acute toxicity, and thus the experiment was discontinued.

2.6.2 Effects of the Extracts of *A. laxiflora* on Anxiety in the Elevated Plus-maze in Mice

The effect of the extracts of *A. laxiflora* on anxiety was investigated in mice using the elevated plus-maze model of anxiety (Montgomery, 1958; Handley and Mithani, 1984). The test is based on the natural aversion of rodents for heights and open spaces (Barnett, 1975).

The elevated plus-maze is a widely used behavioural assay for rodents and it has been validated to assess the anti-anxiety effects of pharmacological agents and steroid hormones, and to define brain regions (Gonzalez and File, 1997) and mechanisms underlying anxiety related behaviour (Pellow *et al*., 1985; Lister, 1987; Cortese and Phan, 2005). Elevated plus-maze consists of two open arms (30 x 5 x 0.25 cm) and two closed arms (30 x 5 x 15 cm) emanating from a common central platform (5 x 5 cm). The two pairs of identical arms are opposite to each other. The entire apparatus was elevated to a height of 50 cm above floor level. At the beginning of the experiment session, the mouse was placed at the centre of the maze, with its head facing the open arm and allowed to explore the maze for 5 min. To eliminate olfactory cues, 70 % ethanol was used to scrub the maze after each test session (Brown *et al*., 1999; Haque, 2001). The following parameters were scored; percentage of the time spent in the open arms and percentage of arm entries. 10 % Tween 80, 0.1 ml/10 g mouse, p.o. and diazepam 1 mg/kg, i.p. were used as vehicle and positive control (standard) respectively. The effects of 10 % Tween 80 and graded doses (100, 200, 400, 800 and 1600 mg/kg, p.o.) of the extracts of *A. laxiflora* were compared with the effect of the anxiolytic dose of diazepam, a standard anti-anxiety drug (Haefely, 1984). Doses of the extracts which did not affect motor coordination were used in the investigation of the anxiolytic potential of the extracts (Reddy and Kulkarni, 1997). Mice of both sexes (18 − 22 g) (n=6) were employed in the experiment and were all naive to the elevated plus-maze model. The index of open arm avoidance which is a measure of the level of anxiety (Trullas and Skolnick, 1993) was calculated thus:

[100 − (% time on open arm + % entries into open arm /2)

2.6.3 Effects of the Extracts of *A. laxiflora* on Anxiety using the Staircase Paradigm in Mice

The staircase test was carried out by the method described by Simiand *et al.* (1984). The staircase is made of wood and consists of five identical steps of 2.5 cm high, 10 cm wide and 7.5 cm deep surrounded by walls, the height of which (10 cm) was constant along the whole length of the staircase. A wooden box (15 x 10 x 10 cm) with one side open was placed facing the staircase. The mouse was gently placed on the floor of the box with its back directed to the staircase. During a 3 min period, the number of steps climbed and the number of rearings (or assisted rearings) performed were recorded. A step was considered climbed when all the four paws were placed on the step. Seven (7) groups of mice were administered with 10 % Tween 80, the vehicle (0.1 ml/10 g mouse, p.o.), Diazepam (1 mg./kg, i.p.) or ALM (100, 200, 400, 800 and 1600 mg/kg, p.o.) 30 min or 1 h prior to the experiment. The number of steps climbed and the rearing responses were recorded for each mouse. The apparatus was cleaned with 70 % ethanol between experiment sessions to remove faeces, odour and urine left by the previous animal, which can affect the behavioural response of the next animal. The experiment was carried out between 9.00 am and 5.00 pm.

2.6.4 Statistical Analysis

Results were expressed as Mean±S.E.M. Analysis of data was done using one-way ANOVA and multiple comparison of treatment groups was 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).

III. RESULTS

3.1 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.

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Treatment group (mg/kg, p.o.)	NOAE	NCAE	TSOA	% EOA	% TSOA	IOAA
CTR	4.60±0.81	8.20±1.85	89.60±8.83	35.94	29.87	67.10
100	8.00±0.89	3.00±1.48	144.00±13.93*	72.73	48.00	39.63
200	8.20±2.15	8.00±1.92	154.00±10.16*	50.62	51.33	49.02
400	7.40±0.40	10.00±0.71	118.00±2.70	42.53	39.33	59.07
800	5.00±0.63	2.80±0.66	147.60±16.43*	64.10	49.20	43.35
1600	7.00±1.76	2.40±0.68	173.20±6.02*	74.47	57.73	33.90
DZP (1mg/kg, i.p.)	8.40±0.98	7.40±2.01	118.00±12.34	53.16	39.33	53.75

Table 1: Elevated Plus-maze Test: Effect of the Methanol Extract of *A. laxiflora* on Anxiety
One-way ANOVA revealed no significant (F = 1.56; P = 0.195) difference between the treatments in the open arm entry. There was a significant (F = 6.52; P = 0.000) increase in the time spent in the open arms; an increase in the percentage entry and time spent in the open arms, and a decrease in the index of open arm avoidance at 100, 200, 800 and 1600 mg/kg, p.o. respectively relative to control. *Indicates a significant difference from control, 10 % Tween 80.

Treatment group (mg/kg, p.o.)	NOAE	NCAE	TSOA	% EOA	% TSOA	IOAA
CRT	4.60±0.81	8.20±1.85	89.60±8.83	35.94	29.87	67.10
100	2.60±4.80	4.80±1.24	65.00±10.52	35.14	21.67	71.60
200	6.20±0.80	6.60±0.40	118.60±8.93	48.44	39.53	56.02
400	8.40±1.50	7.80±1.88	173.00±8.40*	51.85	57.67	45.26
800	8.60±0.87	8.00±1.18	94.60±2.29	51.81	31.53	58.33
1600	9.20±0.86	6.80±0.20	171.00±8.74*	57.50	57.00	42.75
DZP (1mg/kg,i.p.)	8.40±0.98	10.40±1.40	118.00±12.34	53.16	39.33	53.75

Table 2. Elevated Plus-maze Test: Effect of the Aqueous Extract of *A. laxiflora* on Anxiety
One-way ANOVA revealed no significant (F = 1.48; P = 0.220) difference between the treatments in the open arm entry. There was a significant (20.36; P = 0.000) increase in the time spent in the open arms; an increase in the percentage entry and time spent in the open arms, and a decrease in the index of open arm avoidance at 400 and 1600 mg/kg, p.o. relative to control. *Indicates a significant difference from control, 10 % Tween 80.

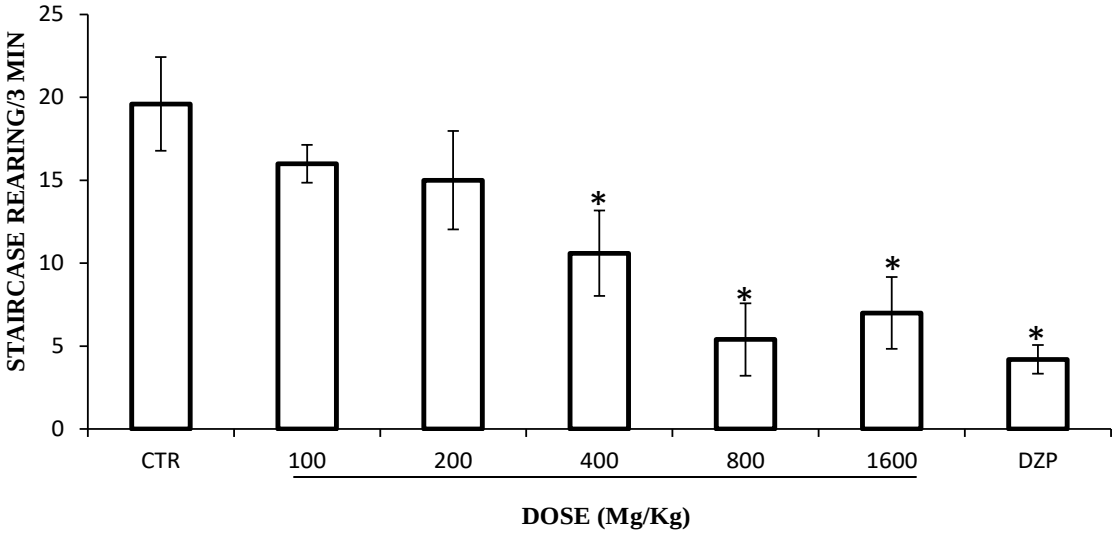

Fig. 1: Staircase Rearing Test: Effect of the Methanol Extract of *A. laxiflora* on Anxiety
Each bar is expressed as Mean±SEM. One-way ANOVA revealed a significant (F = 6.97; P = 0.000) difference between the treatment groups. The result shows a decrease in staircase rears at all the tested doses which was significant at 400 – 1600 Mg/Kg, p.o. However, the effect of reference drug, diazepam was higher compared to any of the tested doses. *Indicates a significant difference from control, 10 % Tween 80.

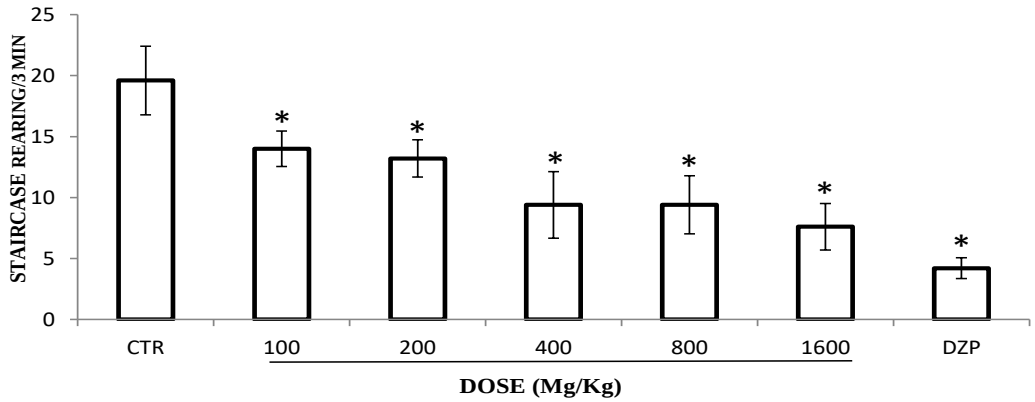

Fig. 2: Staircase Rearing Test: Effect of the Aqueous Extract of *A. laxiflora* on Anxiety
Each bar is expressed as Mean±SEM. One-way ANOVA revealed a significant (F = 5.62; P = 0.000) difference between the treatment groups. The result shows a significant dose dependent decrease in staircase rears at 100, 200, 400, 800 and 1600 mg/kg, p.o. The effect of the reference drug, diazepam on the staircase rears was higher compared to all the tested doses of the extract. *Indicates a significant difference from control, 10 % Tween 80.

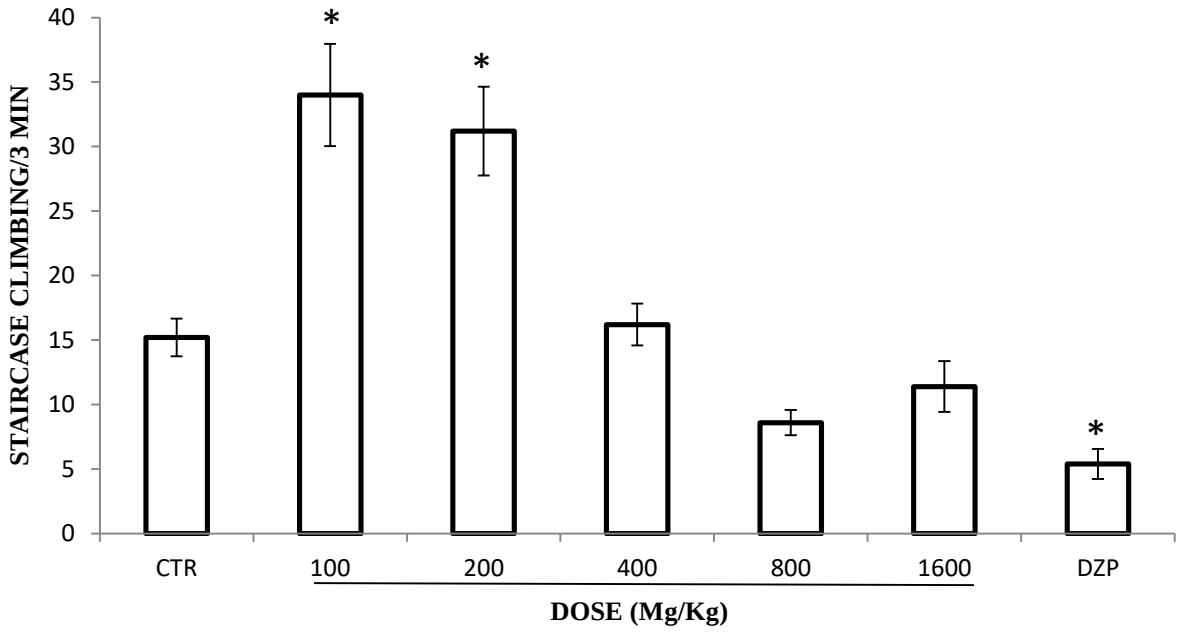

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Fig. 3: Staircase Step-Climbing Test: Effect of Methanol Extract of *A. laxiflora* on Anxiety
Each bar is expressed as Mean±SEM. One-way ANOVA revealed a significant (F = 16.42; P = 0.000) difference between the treatment groups. The result shows a significant increase in staircase step-climbing at 100 and 200 mg/kg, p.o. *Indicates a significant difference from control, 10 % Tween 80.

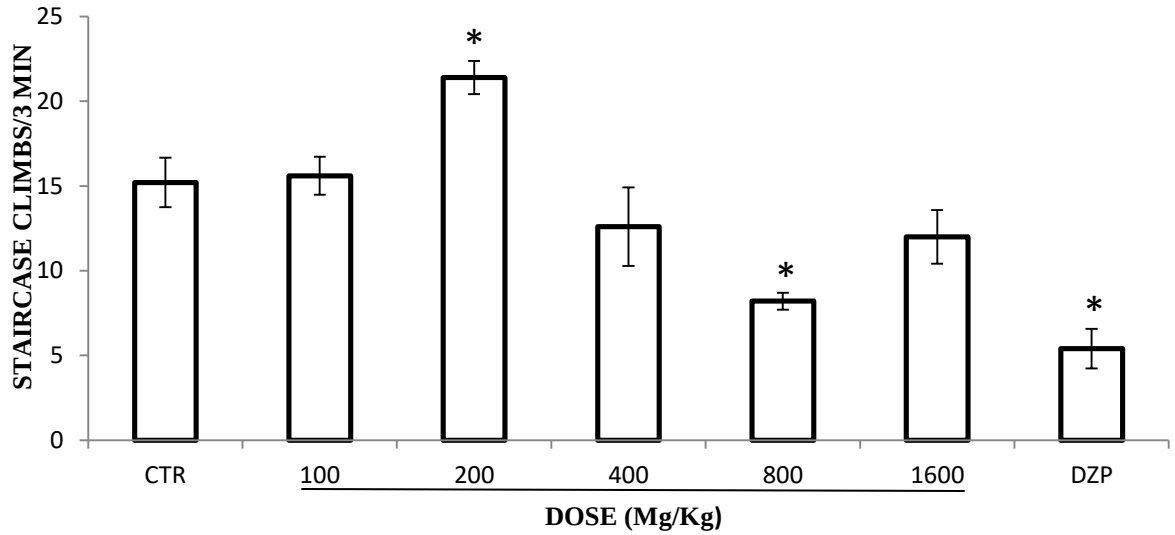

Figure 4: Staircase Climbing Test: Effect of Aqueous Extract of *A. laxiflora* on Anxiety
Each bar is expressed as Mean±SEM. One-way ANOVA revealed a significant (F = 13.84; P = 0.000) difference between the treatment groups. The result shows a significant increase in staircase step-climbing at 200 mg/kg, p.o. and a significant decrease in staircase step-climbing at 800 mg/kg, p.o. compared to control. *Indicates a significant difference from control, 10 % Tween 80.

IV. DISCUSSION

Anxiety is an adaptive response which makes an individual ready to confront the challenges of everyday life. It is characterised by psychological symptoms (e.g. tension, fear, apprehension, lack of concentration etc.) as well as sympathetic and physical or somatic symptoms (e.g. tachycardia, tremors, sweating, palpitations, fatigue, dizziness, headache, muscle aches, insomnia, gastrointestinal disturbances etc.) (Barar, 2006). Anxiety disorders are common, although females are more affected than the males. It is the most frequent mental illness in our communities. The current global prevalence of anxiety in adults is 7.3 % (4.8 – 10.9 %), and ranges

from 5.3 % (3.5 – 8.1 %) in the Africa region to 10.4 % (7.0 – 15.5 %) in Europe and other Anglophone regions (Baxter *et al.,* 2013). Anxiety may occur occasionally in the general population as part of normal life. Stressful life experiences such as bereavements, jilted love, rumours or threats of an impending terrorist attack, separations from loved ones or failure to pass examinations may precipitate in concerned individuals situations of prolonged anxiety. Persistent anxiety causes distress and emotional disturbances that interfere with social functioning and performance of daily tasks (Njau, 2002), and often leads to visceral organ dysfunction and neurological problems. It is increasingly becoming a public health problem. *Alchornea laxiflora* is used ethnomedically in the treatment of anxiety, insomnia, hypertension and other systemic pathological manifestations, hence this study.

The study examined the anti-anxiety activities of *A. laxiflora* in mice. The median lethal dose (LD_{50}) was determined in 24 h using the Lorke's method (Lorke, 1983). The LD_{50} for the aqueous and methanol extracts of *A. laxiflora* in the oral route was > 3200 and > 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 and > 1600 mg/kg for the aqueous extract. The LD_{50} (i.p.) was relatively toxic in the methanol extract, but safe orally. In the aqueous extract, however, the LD_{50} (i.p. and p.o.) was found to be safe. This was not unexpected as absorption of drugs is faster via the intra-peritoneal route than the oral route due to very large surface area for absorption in the peritoneal cavity relative to the alimentary tract. It is also known that absorption and bioavailability via the intra-peritoneal route is about 100 %, while in the case of the oral route, inactivation of the extract in the hepatocytes of the liver and to a lesser extent in the lungs and the stomach (first-pass effect) will reduce the fraction of the extract that gets to the systemic circulation, and thus influence the biological effect of the extract. The LD_{50} value depends on the route of administration. The LD_{50} values are found to increase with the following sequences of routes: intravenous > intra-peritoneal > subcutaneous > oral.

The elevated plus-maze (EPM) is a simple and highly validated behavioural paradigm for anxiety in rodents (Pellow *et al.,* 1985; Dutt *et al.,* 2011). It is conceivably the most common neuropharmacological model used to study anxiety in rodents. The EPM is a standard model for investigating anxiolytic and anxiogenic properties of drug substances and candidate drugs (Lister, 1987). It is a widely used behavioural model to search for new benzodiazepine-like anxiolytic agents, and to define brain regions (Gonzalez and File, 1997) and mechanisms (Handley and Mithani, 1984; Pellow *et al.,* 1985; Cortese and Phan, 2005). Anxiolytic agents reduce animal's natural aversion to the open arms, and promote the exploration thereof. On the other hand, the animal's forced or voluntary passages into the open arms are associated with endocrine and behavioural changes, indicative of increased anxiety (Felipe *et al.,* 2008).
In the study, the methanol extract increased the percent entry and percent time spent in the open arms, and significantly increased the time spent in the open arms at lower and higher doses. The methanol extract decreased the index of open arm avoidance (IOAA) in almost all the doses tested, an indication of anxiolysis. The aqueous extract also, demonstrated anti-anxiety activity by increasing the percent entry and percent time spent in the open arms. It significantly increased the duration of mice exploration in the open arms at a moderately low dose and at the highest tested dose, while decreasing the IOAA, which indicates anxiolytic activity.

The staircase paradigm was also, used in evaluating anxiolytic activity by purporting step-climbing to reflect exploratory or locomotor activity, while rearing is an index of anxiety (Vogel and Vogel, 1997). A decrease in rearing and an increase in staircase step-climbing correlates with anxiolytic activity, while increased rearing and decrease in staircase step-climbing are indicative of anxiety and sedation respectively (Vogel and Vogel, 1997; Ago *et al.,* 2007; Dutt *et al.,* 2011). This was demonstrated in the study, since both the methanol and aqueous extracts indicated anti-anxiety activity at all the tested doses in the staircase rearing, and at lower doses in the staircase step-climbing tests. The methanol extract significantly decreased staircase rearing at moderate and high doses, indicating anxiolytic activity. The methanol extract significantly demonstrated antianxiety activity at low doses by increasing the staircase step-climbing behaviour in the animals. ALM produced sedation at a moderately high dose in the methanol extract by decreasing staircase step-climbing. In the aqueous extract, however, ALM significantly decreased staircase rearing at all the experimental doses and significantly increased staircase step-climbing at a low dose, an indication of anxiolytic activity. It also, significantly decreased staircase step-climbing at a moderately high dose, thus demonstrating sedative activity.

V. CONCLUSION:

The study concluded that the aqueous and the methanol extracts of *A. laxiflora* possesses anti-anxiety activities in mice *in vivo.*

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