

Effects of the Aqueous and Methanol Extracts of *Alchornea laxiflora* in Rodent Models of Experimental Psychosis

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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 mouse models of experimental psychosis, the apomorphine-induced climbing behavior and apomorphine-induced stereotypy tests. This was with a view to providing information on the acute toxicity and the antipsychotic potential of the plant extracts. 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 (7) groups. The control group (I) 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 a standard drug, Chlorpromazine (2 mg/kg, i.p.). The animals were observed for climbing and stereotypic behaviours in a wire mesh and observation cage respectively. They were individually scored after observation for 2 min at 10, 20, 30 and 45 min intervals post intra-peritoneal and oral administrations of vehicle, extracts or drugs. The LD₅₀ 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 LD₅₀ (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. The results showed that *A. laxiflora* produced significant ($P = 0.000$) attenuation of apomorphine-induced climbing and apomorphine-induced stereotypic behaviours. The study, therefore, concluded that *A. laxiflora* possesses antipsychotic activity in mice.

Keywords: *Alchornea laxiflora*, antipsychotic, climbing, stereotypy, apomorphine, chlorpromazine and mice

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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), Uwenuwun (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-mial 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 Sick cell anaemia (Muanya, 2009). The bioactive chemical constituents from *A. laxiflora* include flavonoids, which is the dominant 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. An *in vivo* study in mice has also, shown that the methanol extract of the leaves of *A. laxiflora* possesses sedative and anxiolytic activities (Nwonu, 2011). The present study was designed to evaluate the antipsychotic potential of the plant in view of the manifold and unpalatable adverse effects and draw backs associated with the existing antipsychotic medications.

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 °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 °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 °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 and 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. 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 LD₅₀.

Drugs

These drugs and chemical reagents were used in the study: Ethanol, Methanol (BDH Chemicals Ltd., Poole, England), Apomorphine, Chlorpromazine and Polyoxyethylene sorbitan monololate (Tween 80) (Sigma-Aldrich Inc., St. Louis, USA).

Experimental Designs

Acute Toxicity Tests

The acute toxicity and LD₅₀ 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 (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. LD₅₀ (the index of acute toxicity) was calculated within 24 h, as the geometric mean of the dose that caused 100 % mortality and that which produced no deaths (0 % mortality) in mice. 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.

Apomorphine-induced Stereotypic Behaviour in Mice

The method earlier described by Siquira *et al.* (1998) was adopted in the study. Mice were divided into seven groups, with a control as group one (I) (10 % Tween 80, 0.1 ml/10 g) and graded doses (100, 200, 400, 800, 1600 mg/kg, p.o.) of ALM, and thus served as groups two (II), three (III), four (IV), five (V) and six (VI) respectively, while the seventh (VII) group received the reference drug, Chlorpromazine 2 mg/kg, i.p. ALM was administered 1 h prior to apomorphine injection (35 mg/kg, i.p.), and animals were individually observed for 2 min in an observation cage (45 x 25 x 25 cm) at 10, 20, 30 and 45 min intervals post apomorphine administration. Stereotypic behaviors were scored as follows: Absence of stereotyped behavior (0); Presence of stereotyped movements of the head and intermittent sniffing (1); Sniffing and chewing (2); Chewing and intense licking (3).

Apomorphine-induced Climbing Behaviour in Mice

Adult mice were randomly divided into seven groups (n=5). The first (I) group received 10 % Tween 80 (0.1 ml/10 g), the vehicle. The second (II), third (III), fourth (IV), fifth (V) and sixth (VI) groups received ALM at doses of 100, 200, 400, 800 and 1600 mg/kg, p.o. respectively, while a seventh (VII) group received the reference drug, Chlorpromazine 2 mg/kg, i.p. 1 h post treatment. All the animals were treated with apomorphine (30 mg/kg, s.c.). Each mouse was placed singly in a wire mesh stick cage and the climbing behaviour was observed for 2 min at 10, 20 and 30 min interval post apomorphine administration and scored thus: 0 = four paws on the floor; 1 = fore feet holding the vertical bars; 2 = four feet holding the vertical bars (Protais *et al.*, 1976; Costall *et al.*, 1978).

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

Acute Toxicity Tests

The LD₅₀ was 400 mg/kg, i.p. and > 1600 mg/kg, p.o. for the methanol extract (MeOH EXT), and > 1600 mg/kg, i.p. and p.o. for the aqueous extract (AQ EXT).

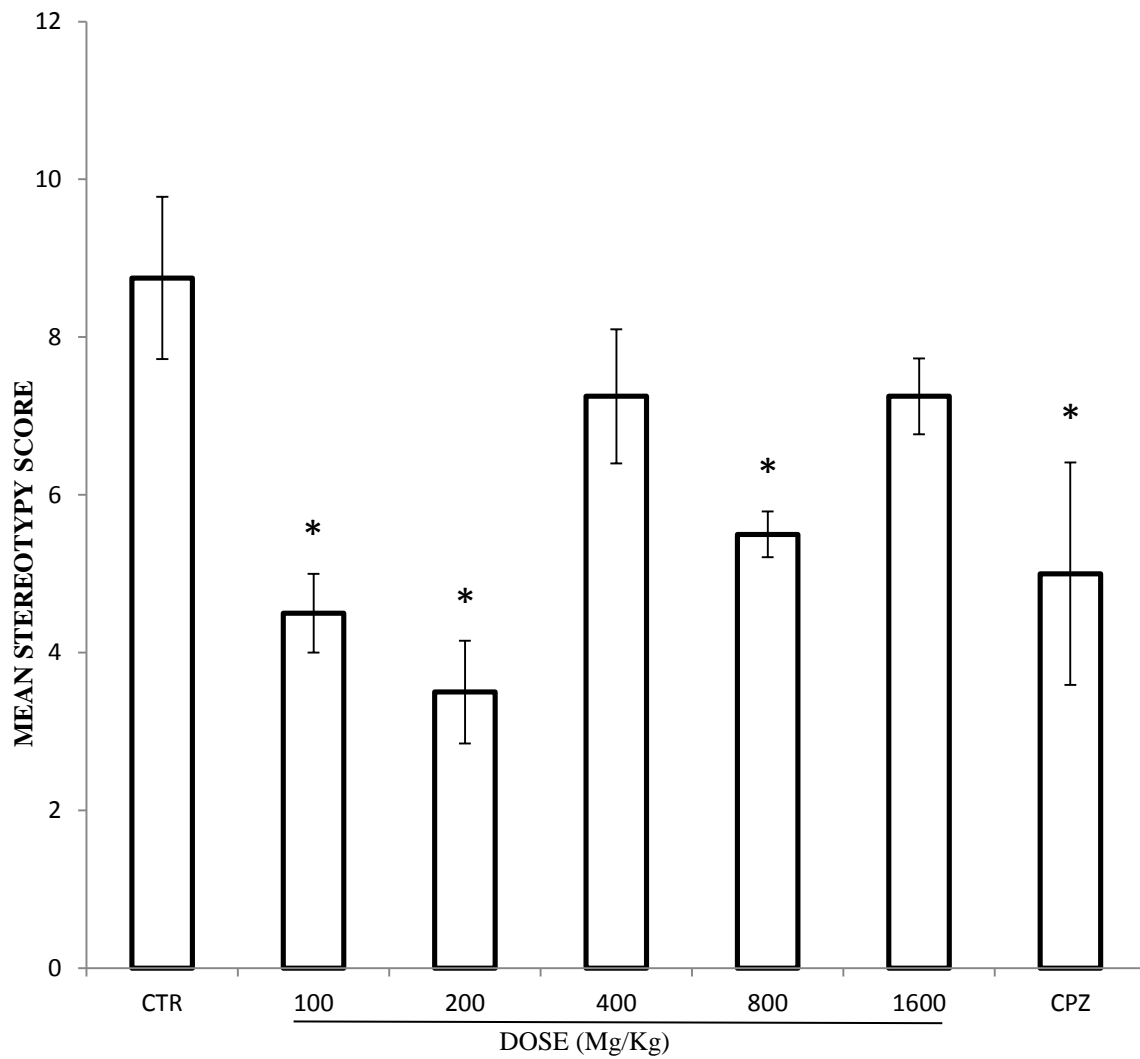


Fig. 1: Effect of the Methanol Extract of *A. laxiflora* on Apomorphine-induced Stereotypy
Each bar is expressed as Mean±SEM. One-way ANOVA revealed a significant ($F = 5.01$; $P = 0.000$) difference between the treatment groups. The result shows a significant decrease in the mean stereotypy score at 100, 200 and 800 mg/kg, p.o. compared to control. *Indicates a significant difference from control, 10 % Tween 80.

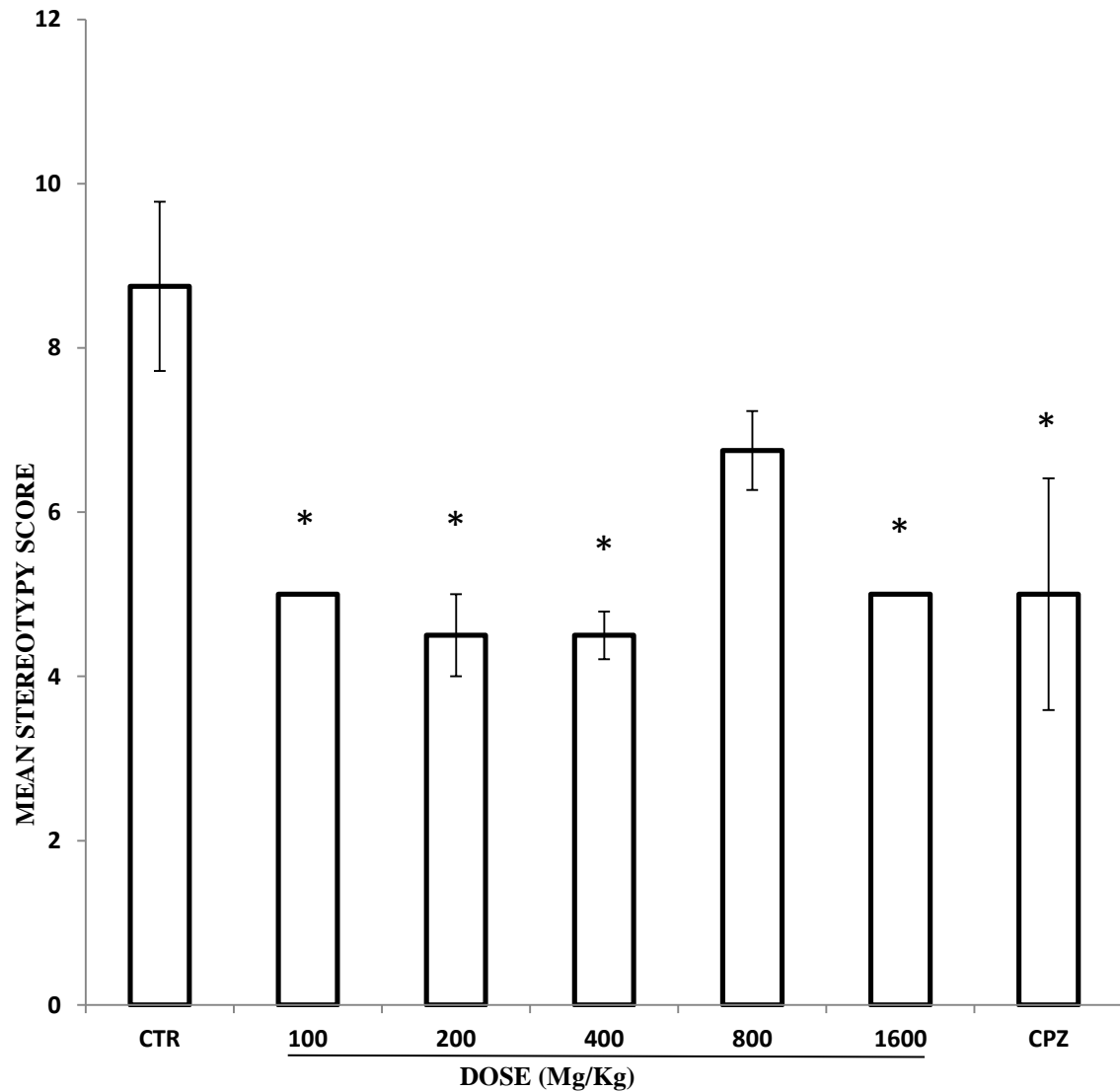


Fig. 2: Effect of the Aqueous Extract of *A. laxiflora* on Apomorphine-Induced Stereotypy. Each bar is expressed as Mean±SEM. One-way ANOVA revealed a significant ($F = 4.76$; $P = 0.000$) difference between the treatment groups. The result shows a significant decrease in the mean stereotypy score at 100, 200, 400 and 1600 mg/kg, p.o. The activity at the highest dose tested is comparable to that of the reference drug, chlorpromazine. *Indicates a significant difference from control, 10 % Tween 80.

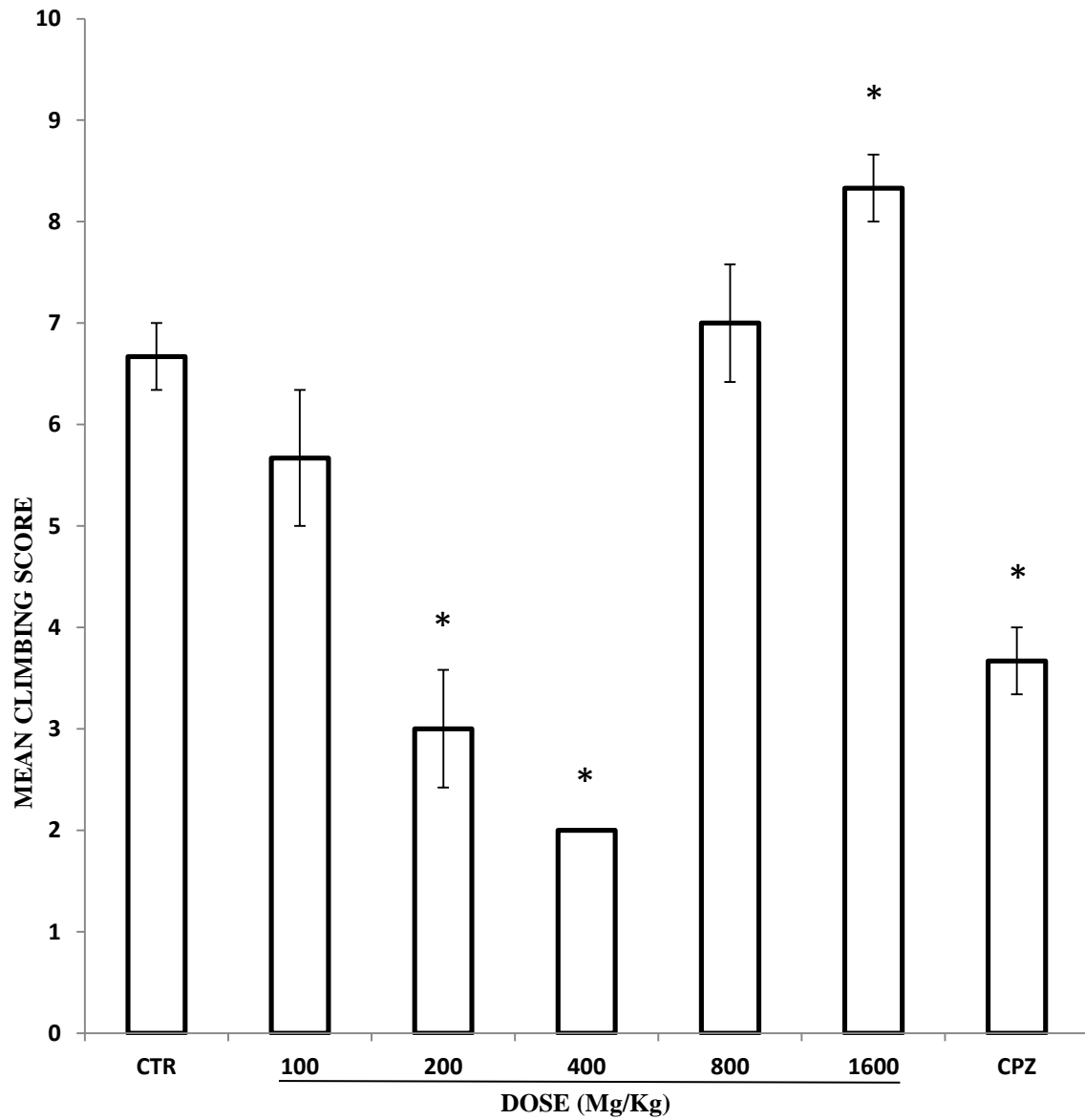


Fig. 3: Effect of the Methanol Extract of *A. laxiflora* in Apomorphine-induced Climbing
Each bar is expressed as Mean±SEM. One-way ANOVA revealed a significant ($F = 27.94$; $P = 0.000$) difference between the treatment groups. The result shows a significant decrease in the mean climbing score at 200 and 400 mg/kg, p.o. *Indicates a significant difference from control, 10 % Tween 80.

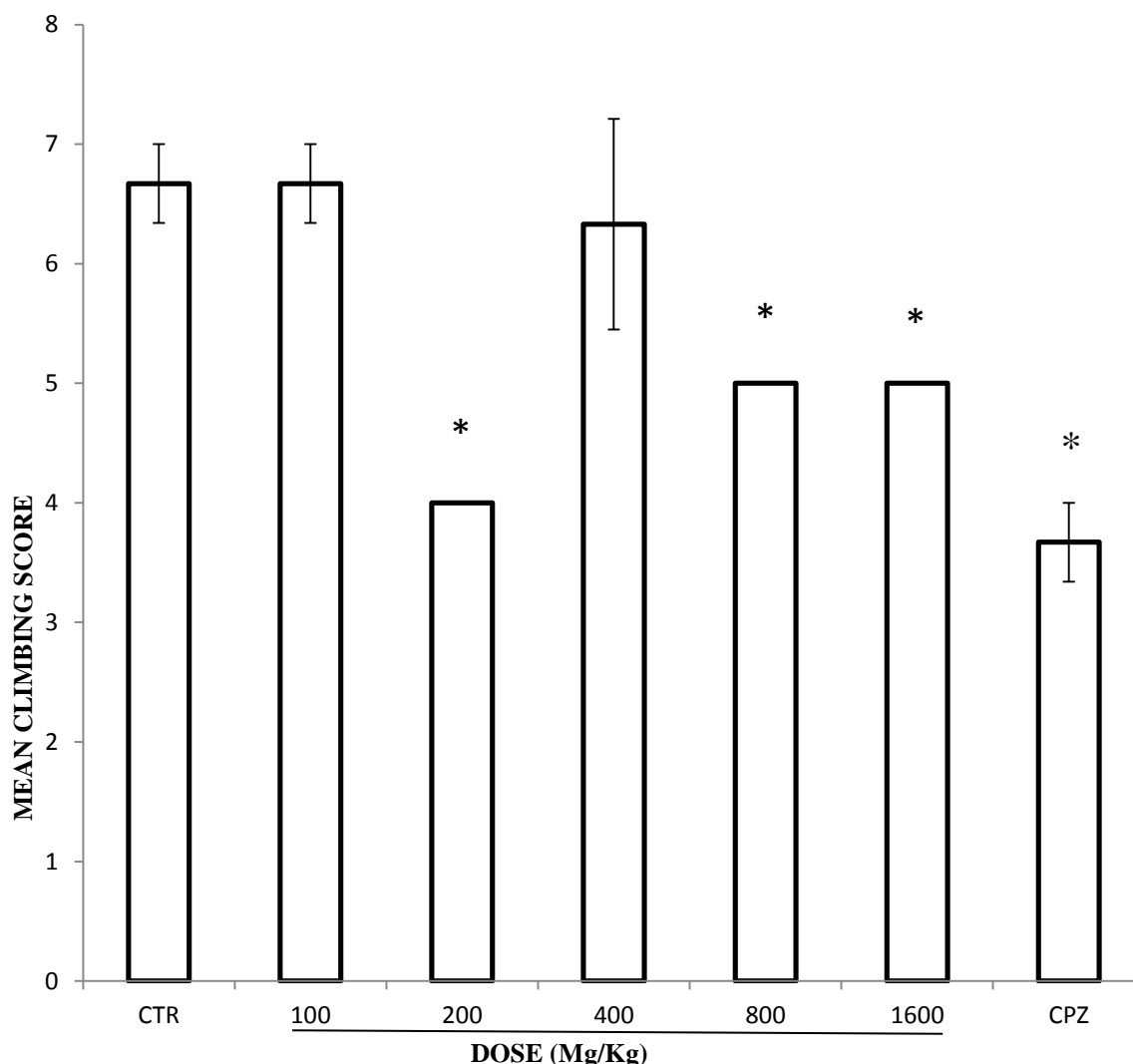


Fig. 4: Effect of the Aqueous Extract of *A. laxiflora* on Apomorphine-induced Climbing. Each bar is expressed as Mean±SEM. One-way ANOVA revealed a significant ($F = 9.89$; $P = 0.000$) difference between the treatment groups. The result shows a significant decrease in the mean climbing score at 200, 800 and 1600 mg/kg, p.o. compared to control. The mean climbing score at 200 mg/kg, p.o. has activity comparable to chlorpromazine, the reference drug. *Indicates a significant difference from control, 10 % Tween 80.

IV. Discussion

Advances in science and technology have contributed immensely to the improvement in the quality of life of humans. Notwithstanding, environmental and modern life stresses, associated trials and tribulations are responsible for the upsurge in the incidences of a manifold of mental illnesses (Abid *et al.*, 2006). These illnesses alter an individual's ability to think clearly, make good judgments, respond emotionally, communicate effectively, understand reality, and behave in an appropriate manner. The major use of antipsychotic drugs is in the treatment of mental and behavioural emergencies, e.g., mania, toxic delirium, etc. Antipsychotic drugs are also employed in the treatment of deviant antisocial behaviour, motor tics and intractable hiccup. The clinical efficacy of these medications in enabling patients with psychiatric illness lead more normal lives has been demonstrated in many controlled trials. However, these drugs are associated with many side effects and shortcomings. It has been documented that only 70 % of schizophrenic patients are controlled with the conventional (orthodox) antipsychotic drugs, while 30 % of these patients are pharmaco-unresponsive, and thus present a major therapeutic challenge to clinicians (Rang *et al.*, 2012).

Apomorphine is a non-selective dopamine receptor agonist which activates D_2 and to a lesser extent D_1 receptors (Guardia *et al.*, 2002). Apomorphine increases the intensity and duration of stereotypic behaviours by acting on post synaptic dopamine D_2 receptors (Stolk and Reck, 1970; Kwanashie and Nwinyi, 2009). Inhibition

of apomorphine attenuates and/or reverses the hyperactivity and stereotypy, suggesting interference with central dopaminergic neurotransmission (Moore and Axton, 1988; Wanibuchi and Usuda, 1990; Potter and Hollister, 2004). In the study, *A. laxiflora* significantly reduced apomorphine-induced climbing behavior and apomorphine-induced stereotypy, thus suggesting antidopaminergic activity. Apomorphine and amphetamine-induced stereotypic behaviours are mediated by the hyperactivity of dopaminergic mechanisms of the nigrostriatal and mesolimbic systems (Le Moal, 1985). Apomorphine is known to decrease motor activity in lower doses, while causing a stimulatory effect at higher doses (Strombom, 1976; Costal *et al.*, 1981; File *et al.*, 1989). The ability of a drug to antagonise amphetamine or apomorphine-induced climbing or stereotypic behaviour has been correlated with antipsychotic activity (Protais *et al.*, 1976; Costal *et al.*, 1978; Yaro *et al.*, 2007). Inhibition of apomorphine-induced climbing is suggestive of post synaptic striatal dopamine D₂ receptor blockade (Moore and Axton, 1988; Wanibuchi and Usuda, 1990; Potter and Hollister, 2004). This was demonstrated in the study.

LD₅₀ is an index of acute toxicity in laboratory animals. The LD₅₀ (i.p.) of the MeOH EXT was relatively toxic, but safe orally. In the AQ EXT, the LD₅₀ was found to be safe, both orally and intraperitoneally. *A. laxiflora* demonstrated antipsychotic activity by significantly attenuating apomorphine-induced climbing and apomorphine-induced stereotypic behaviours in the two plant extracts. The MeOH EXT produced a significant decrease in the mean stereotypic score, most prominent at low doses and at a moderately low dose. This effect showed that ALM possesses antipsychotic property. The decrease in the mean stereotypic score at low doses was higher relative to Chlorpromazine (CPZ), a standard antipsychotic drug. At moderate and high doses, the decrease in the mean stereotypic score was not significant. In the AQ EXT, there was a significant decrease in the mean stereotypic score at low, moderate and at the highest test doses. These effects are comparable to the effects produced by CPZ. However, no significant decrease in the mean stereotypic score was observed at a moderately high dose.

There was a significant decrease in the mean climbing score at low and high doses in the MeOH EXT, while at the lowest and moderate test doses, no significant decrease in the mean climbing score was observed. The significant decrease in the mean climbing score at low doses was comparable to the effect of CPZ. The AQ EXT significantly decreased apomorphine-induced climbing behaviour at low and moderate doses, an indication of antipsychotic activity. This effect was higher relative to the effect of the reference drug, CPZ. At the lowest and high doses of the AQ EXT, no significant decrease in the mean stereotypic score was observed. The ability of drugs to attenuate apomorphine-induced climbing and stereotypic behaviours correlates with antipsychotic activity, and thus lends pharmacological credence to the hypotheses of central activity, which might be related to anti-dopaminergic actions on the limbic system as proposed by Anca *et al.* (1993) and Morrais *et al.* (1998).

V. Conclusion

The study concluded that *A. laxiflora* possesses significant antipsychotic activity by the attenuation of apomorphine-induced climbing and stereotypic behaviours in mice.

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