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Cholinergic Mediated Effects of Alchornea Laxiflora in Mice Behaviours

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Introduction: *Alchornea laxiflora* is a member of the Euphorbiaceae family, which is used in folk medicine. The work examined the effects of the extracts of *A. laxiflora* on novelty-induced behaviours in mice. It also, evaluated the effects of the extracts on model systems of insomnia, and the signalling pathways mediating the inhibitory effects of the extracts. The purpose of the study was to determine the hypno-sedative effect and the neural pathways involved in the biological effects of the extracts. Methods: Male and female mice (n=6), weighing 18 – 24 g were used for the research, and were randomly distributed into control, test, and reference groups. The control group (1) received 5 mL/kg, p.o. of 10 % Tween 80 (vehicle), while the test groups (2, 3, 4, 5, and 6) were administered 100, 200, 400, 800, 1600 mg/kg per oral of the extracts. The reference group (7) received diazepam (1−2 mg/kg, i.p.) and pentobarbital sodium (40 mg/kg, i.p.). The animals were observed for rearing, grooming, spontaneous locomotion, head dip, loss and re-gain of righting reflexes. The scoring for neurobehavioural activity was performed in the open-field model system for each mouse, 30 and 60 min after intra-peritoneal and oral administrations respectively, for both the aqueous and the methanol extracts. Results: The results showed a significant ($P \leq 0.05$) decrease in rearing, grooming, spontaneous locomotion, and head dip. *A. laxiflora* also, produced a significant ($P \leq 0.05$) prolongation of sleeping time in both extracts. Conclusion: The study concluded that the extracts of *A. laxiflora* have hypno-sedative activity in mice. The signaling pathways mediating these effects were found to be through the stimulation of muscarinic-cholinergic and α_2-adrenergic receptors.

Keywords: assessment, model system, novelty-induced behaviour, hypno-sedative, signalling pathway

INTRODUCTION

Alchornea laxiflora is a deciduous, erect, and evergreen shrub of 7-10 m in height belonging to the Euphorbiaceae family. The flowers are unisexual and the fruits are even, dark green to brown, slightly hairy with about three seeds. The leaves, roots, and stems are used in traditional medicine for the treatment and management of nervous and cardiovascular disorders, such as anxiety, insomnia, and high blood pressure. The leaf extract of *A. laxiflora* demonstrated promise in reversing sickling process *in vitro*, hence its use in the management of sickle cell anaemia (Muanya,2009). The active constituents from *A. laxiflora* exhibit anti-microbial activity (Ogundipe et al.,2001), which is significant against disease-causing microorganisms. This is the basis for its use as chewing stick by many locals and city dwellers in the tropics. The leaves are also used for wrapping kola nuts during packaging and storage, so as to maintain the moisture content, and therefore, prevent their damage.

MATERIALS AND METHODS

Plant Collection

A. laxiflora was collected within the university community. The plant specimen was identified and authenticated in the Faculty of Pharmacy herbarium by Mr. Ifeoluwa Ogunlowo of the Department of Pharmacognosy, Obafemi Awolowo University, Ile-Ife.

Plant Extraction

The leaves of *A. laxiflora* were dried at laboratory room temperature, and ground using a mechanical device. The powdered material (350 g) was cold-extracted using 2.5 L of absolute methanol for 72 h. The filtrate generated was concentrated to dryness in a rotary evaporator at 40 ^{o}C under a reduced pressure. The aqueous extraction process used the hot extraction method. 500 g of the ground leaf material was extracted using boiling method

under reflux. The extraction lasted for 3 h. This was concentrated to a dry residue in a rotary evaporator at 40 ^{o}C. Both specimens were stored in the refrigerator until they were needed for experiment.

Animals

The Vom strain of albino mice (male and female, and weighing 18 − 24 g) was used in the study. They were housed in clean metal cages at room temperature. The animals were fed with standard laboratory pellet diet and had free drinkable water. They were maintained at a 12 h light/ dark cycle throughout the period of the experiment. They were allowed for 1 h to acclimatise to the laboratory environment before commencement of the study.

Ethics Statement

The study was conducted in consonance with the guidelines for animal care in research (The Norwegian National Committee for Research Ethics in Science and Technology (NENT,2018). Also, the guidelines for the care and use of animals in neuroscience and behavioural research (NIH,1991; NRC,1996) were strictly complied with.

Preparation of Extracts

Fresh preparation of the extracts was made on each day of the experiment using 10 % Tween 80 as vehicle. The two extracts were administered to mice. The dosing was based on the size of the experimental subjects. The volume of the vehicle used was 5 mL/kg or 0.1 mL/10 g mouse.

Chemicals and Drugs

Diazepam (F. Hoffmann-La Roche, Basel, Switzerland), flumazenil, naloxone, cyproheptadine, pentobarbital sodium, yohimbine (all from Sigma-Aldrich Inc., St. Louis, USA), atropine (Pauco Pharmaceutical Industries Ltd., Awka, Nigeria) and polyoxyethylene sorbitan monotolate (Tween 80) (Sigma-Aldrich Inc., St. Louis, USA) were used.

Acute Toxicity Tests

The acute toxicity (LD_{50}) of the extracts was determined using the Lorke's method (Lorke, 1983). The graded doses (100, 200, 400, 800, 1600 mg/kg, p.o.) of *A. laxiflora* (ALM) were used for toxicity testing.

Assessment of the Effects of *A. laxiflora* on Novelty-induced Behaviours

The procedure by Zapata-Sudo et al.(2010) was used in evaluating the effects of the aqueous (AQ) and methanol (MeOH) extracts on rearing, grooming and spontaneous locomotion in mice. Animals were placed individually from their home cages into an opaque plexiform observation cage (45 x 25 x 25 cm) with only one side transparent for observing the animals. Assessment of novelty-induced behaviours (NIBs) followed the administration of 10 % Tween 80 (vehicle) and graded doses of the extracts of *A. laxiflora* to different groups of mice. Seven groups of mice were used in testing the effects of the extracts, control (CTR), and the reference drug. Groups 2 − 5 (n=6) received 100, 200, 400, 800, 1600 mg/kg, p.o. of *A. laxiflora*, while the vehicle (10 % Tween 80, 5 mL/kg mouse, p.o.) and reference drug (diazepam 1 mg/kg, i.p.) were administered to groups 1 and 7 respectively.

Assessment of the Effects of *A. laxiflora* on Head Dip

The effect of *A. laxiflora* extracts on the frequency of exploratory head dips in mice was evaluated using the method described by Stanley et al.2005 and Kumar et al.2008. The animals were administered graded doses of 100, 200, 400, 800, 1600 mg/kg, p.o. of *A. laxiflora* extracts, 10 % Tween 80 as vehicle, 0.1 mL/10 g mouse, p.o., and diazepam 1mg/kg, i.p. Animals were then placed individually on a board (40 x 40 cm) with 16 holes symmetrically distributed in four rows 1 h or 30 min after administration of the extracts of *A. laxiflora*, vehicle, and diazepam respectively. The number of times that the animal's head dips into the hole using the pinnae as landmark during 5 min interval was recorded.

Assessment of the Effect of *A. laxiflora* on Pentobarbital-induced Sleep

The sleep potentiating effect of *A. laxiflora* extracts were investigated in five groups (n=7) of mice. Five groups received graded does of the extracts 100, 200, 400, 800, 1600 mg/kg, p.o. 1 h prior to the administration of 40 mg/kg, i.p. of pentobarbital sodium (PBT). A sixth group received 10 % Tween 80, the vehicle (0.1 mL/10 g mouse, p.o.) in which pentobarbital sodium was administered 1 h after the vehicle. The seventh group received only pentobarbital sodium (40 mg/kg, i.p.), and thus served as reference drug. The eight and nineth groups received 1 and 2 mg/kg, i.p. of diazepam respectively. Following the administration of pentobarbital sodium intra-peritoneally, the loss and re-gain of righting reflex was considered to indicate latency and duration of sleep respectively. The time interval between the loss of righting reflex and the re-gain of righting reflex is referred to as the sleeping time, while sleep latency is the time taken to lose or the onset of loss of righting reflex (Rolland et al.,2006; Askari et a.,2016).

Assessment of the Signalling Pathway Mediating the NIR Inhibitory Action of *A. laxiflora*

The effect of neurotransmitter antagonists on NIR inhibition by the extracts of *A. laxiflora* were used to determine the possible signalling pathways mediating the hypno-sedative effect of the extracts in mice. The antagonists were injected into the peritoneal cavity 15 min prior to the administration of 800 mg/kg, p.o. of *A. laxiflora*. Atropine, flumazenil, naloxone, yohimbine, and cyproheptadine were administered at a dose of 0.5 mg/kg, 2 mg/kg, 1 mg/kg, 1 mg/kg, and 2 mg/kg, i.p. respectively.

Each treatment group (n=6) consisted of male and female mice (18 − 24 g). 0.9 % physiological saline, 0.1 mL/10 g mouse, p.o. served as vehicle/control, and each experiment session was observed for 30 min, while recording the frequency of NIR.

Statistical Analysis

Experimental results were expressed as mean±S.E.M. Analysis of data was done using one-way ANOVA and comparison of treatment groups was carried out using the Student-Newman-Keuls (post hoc) test. The statistical package used was the primer of biostatistics (version 3.01). Probability level of 5 % ($P \leq 0.05$) was considered statistically significant for all treatment relative to control.

RESULTS

Acute Toxicity Tests

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

Rearing

In the MeOH EXT, there was a significant decrease in rearing at all the test doses (100 – 1600 mg/kg, p.o.), but dose-dependent at 200, 400, and 800 mg/kg, p.o. The highest decrease in rearing was at a moderately high dose, 800 mg/kg, p.o. while the lowest activity was at 100 and 200 mg/kg, p.o. At 100 and 200 mg/kg, p.o., the MeOH EXT showed equal pharmacological activity. The central nervous system (CNS) depressant activity of *A. laxiflora* was greater at all the test doses relative to the reference drug, diazepam (DZP) (Fig. I).

The AQ EXT showed a significant decrease in rearing at all the test doses, with an incremental loss of central depressant activity at 100 – 1600 mg/kg, p.o. At high doses of 800 and 1600 mg/kg, p.o., the decrease in rearing activity was comparable to that of DZP (Fig. II).

Grooming

The MeOH EXT significantly and dose dependently (400 – 1600 mg/kg, p.o.) decreased grooming. The effect of the extract at moderate (800 mg/kg, p.o.) and high (1600 mg/kg, p.o.) doses are comparably equal (Fig. III).

There was a decrease in grooming at 100 – 800 mg/kg, p.o. significant at 800 mg/kg, p.o., which also demonstrated the highest decrease in grooming, but lower to DZP. At low to moderate doses (100, 200, and 400 mg/kg, p.o.) of the AQ EXT, the decrease in grooming was comparably equal and lower to DZP. At the highest test dose (1600 mg/kg, p.o.), the AQ EXT did not decrease grooming, as the effect was comparable to the control, 10 % Tween 80 (Fig. IV).

Locomotion

There was a significant decrease in spontaneous locomotion at all the test doses (100 – 1600 mg/kg, p.o.) in the MeOH EXT, dose-dependent at 200 – 1600 mg/kg, p.o. The extract produced comparable effect at low doses (100 – 200 mg/kg, p.o.). At high doses (800 – 1600 mg/kg, p.o.), the effect of *A. laxiflora* was comparable to DZP (Fig. V). In the AQ EXT, there was a significant and dose dependent decrease in spontaneous locomotion at low, median, and moderately high doses (100 – 800 mg/kg, p.o.). At the highest test dose (1600 mg/kg, p.o.), there was a loss of decrease and spike in spontaneous locomotion. The decrease in mice locomotor activity at moderately high dose (800 mg/kg, p.o.) was comparable to DZP (Fig. VI).

Head Dip

The MeOH EXT showed a significant and dose-dependent decrease (100 – 1600 mg/kg, p.o.) in head dip relative to control. The effect of the MeOH EXT on head dip as observed at moderate and high doses (800 – 1600 mg/kg, p.o.) was comparably equal, but higher than that of DZP. The MeOH EXT at the median dose (400 mg/kg, p.o.) produced an effect equal to DZP (Fig. VII).

In the AQ EXT, there was a significant decrease in head dip at all the test doses, but dose-dependent at 100 – 400 mg/kg, p.o. The AQ EXT at low doses was comparable to DZP. The highest decrease in head dip was observed at median, moderate, and high doses (400 – 1600 mg/kg, p.o.), and the activity was higher to that of DZP (Fig. VIII).

Sleep Latency

The sleep latency (onset) was between 2.74±0.18 and 5.02±0.48 min post intraperitoneal pentobarbital administration in the MeOH EXT. A decrease in sleep latency was observed at 100, 800, but significant at 1600 mg/kg, p.o. relative to CTR. The highest test dose (1600 mg/kg, p.o.) produced the greatest activity in sleep latency. At low dose (200 mg/kg, p.o.), there was a prolongation in sleep latency, while the effect of the MeOH EXT at the median dose (400 mg/kg, p.o.) was comparable to the CTR and PBT. The effect of *A. laxiflora* at the highest test dose was comparable to the combined effects of PBT (40 mg/kg, i.p.) and DZP (2 mg/kg, i.p.). The effect of PBT on sleep latency was comparable to the effect of the MeOH EXT at the lowest test dose (100 mg/kg, p.o.), and at a moderately high dose (800 mg/kg, p.o.). The sleep latency was shorter at 2 mg/kg, i.p. of DZP than at 1 mg/kg, i.p. of DZP when PBT was administered prior to DZP (Fig. IX). In the AQ EXT, the sleep latency was between 3.91±0.16 and 4.92±0.05 min. There was no significant decrease in the latency to sleep at all the test doses (100 – 1600 mg/kg, p.o.) relative to CTR. However, prior administration of PBT (40 mg/kg, i.p.) followed by DZP (1 – 2 mg/kg, i.p.) significantly decreased the latency to sleep in mice (Fig. XI).

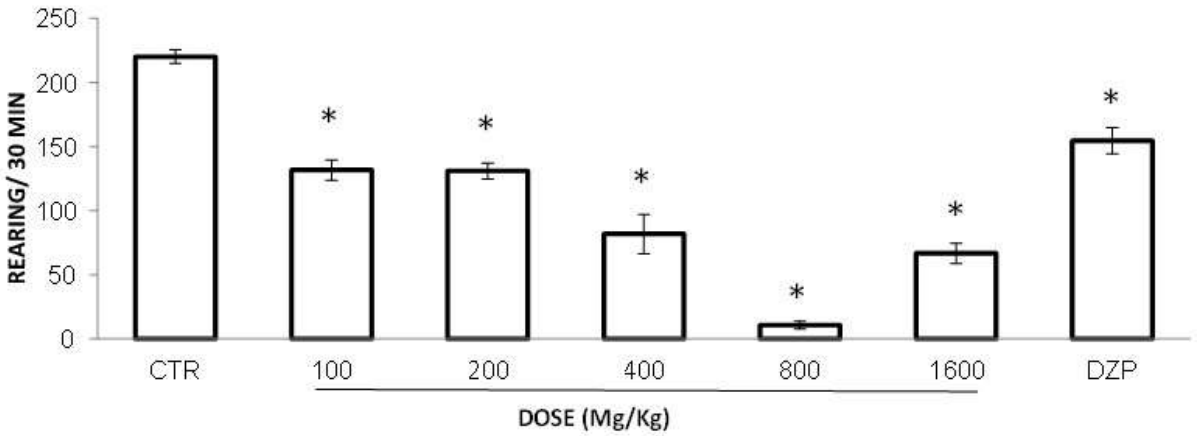

Fig. I. Effect of MeOH EXT on Rearing. Each bar is expressed as Mean±SEM. One-way ANOVA revealed a significant (F = 59.35; P = 0.000) difference between the treatment groups. *Indicates a significant difference from control, 10 % Tween 80.

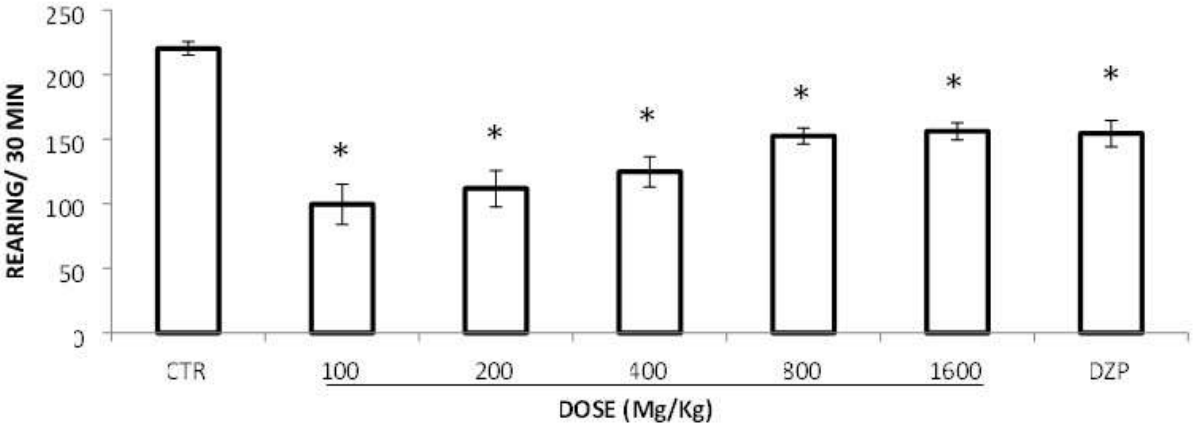

Fig. II: Effect of AQ EXT on Rearing. Each bar is expressed as Mean±SEM. One-way ANOVA revealed a significant (F = 13.89; P = 0.000) difference between the treatment groups. *Indicates a significant difference from control, 10 % Tween 80.

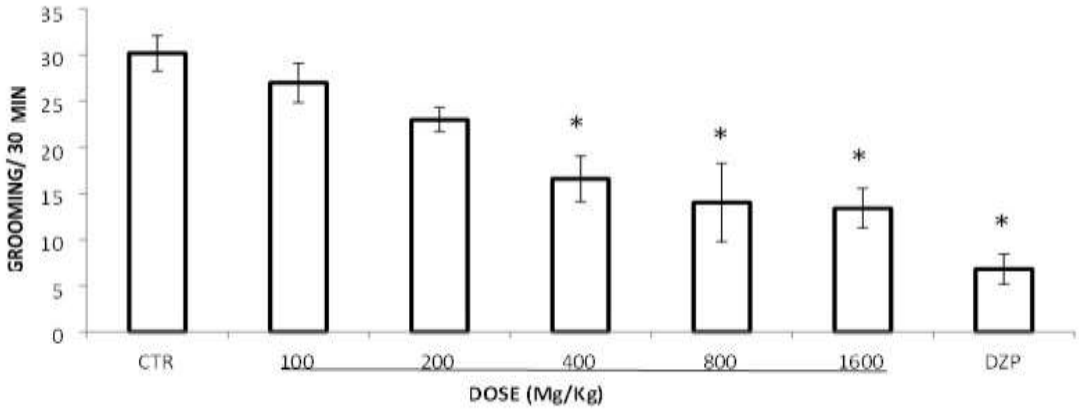

Fig. III: Effect of MeOH EXT on Grooming. Each bar is expressed as Mean±SEM. One-way ANOVA revealed a significant (F = 11.65; P = 0.000) difference between the treatment groups. *Indicates a significant difference from control, 10 % Tween 80.

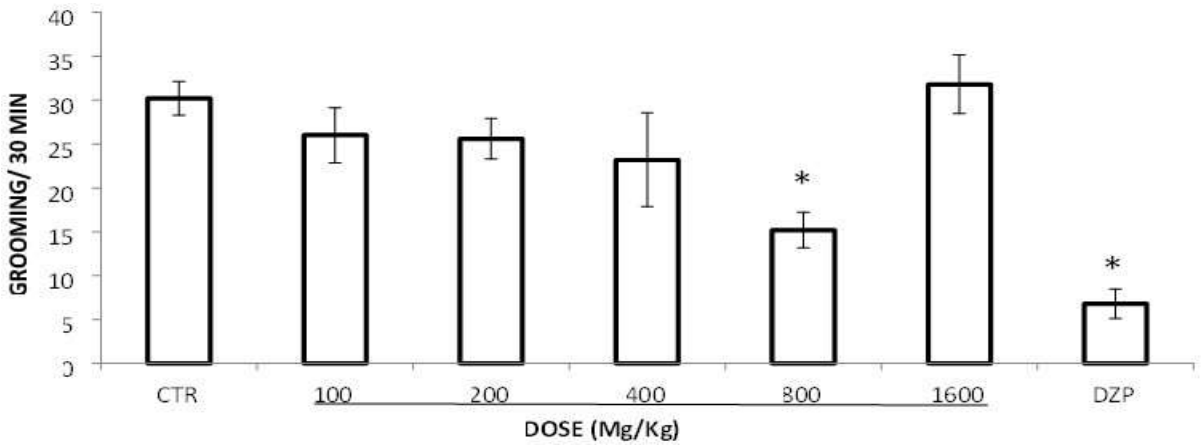

Fig. IV: Effect of AQ EXT on Grooming. Each bar is expressed as Mean±SEM. One-way ANOVA revealed a significant (F = 8.34; P = 0.000) difference between the treatment groups. *Indicates a significant difference from control, 10 % Tween 80.

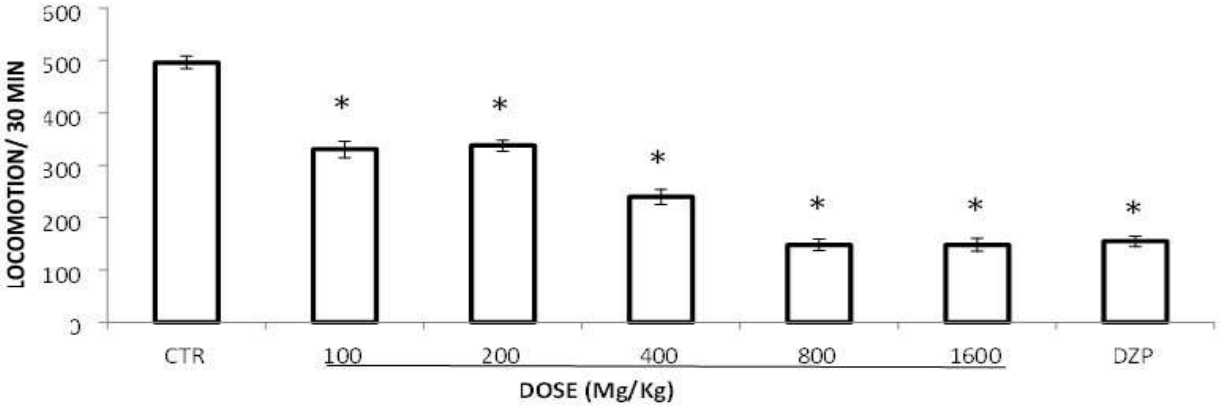

Fig. V: Effect of MeOH EXT on Spontaneous Locomotion. Each bar is expressed as Mean±SEM. One-way ANOVA revealed a significant (F = 110.80; P = 0.000) difference between the treatment groups. *Indicates a significant difference from control, 10 % Tween 80.

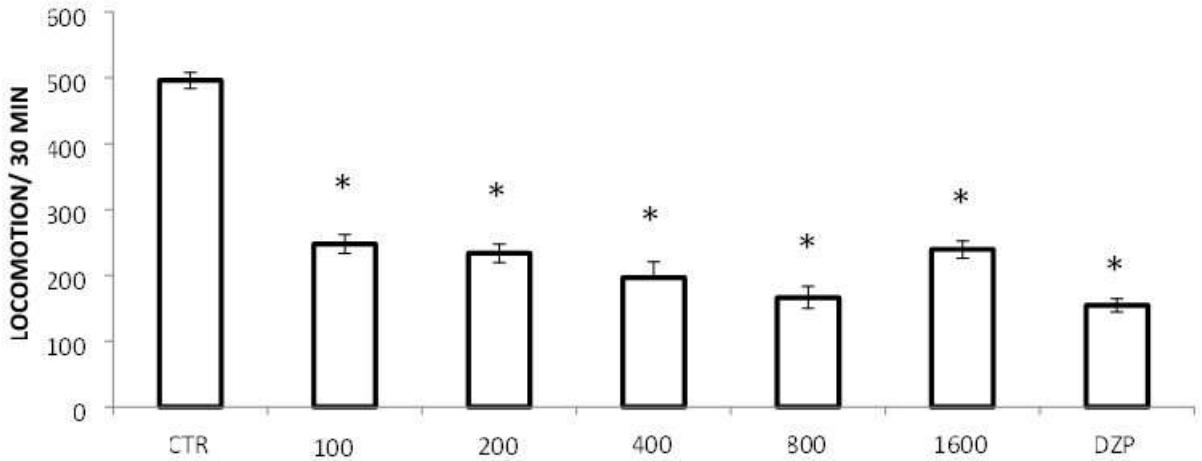

Fig. VI: Effect of AQ EXT on Spontaneous Locomotion. Each bar is expressed as Mean±SEM. One-way ANOVA revealed a significant (F = 73.79; P = 0.000) difference between the treatment groups. *Indicates a significant difference from control, 10 % Tween 80.

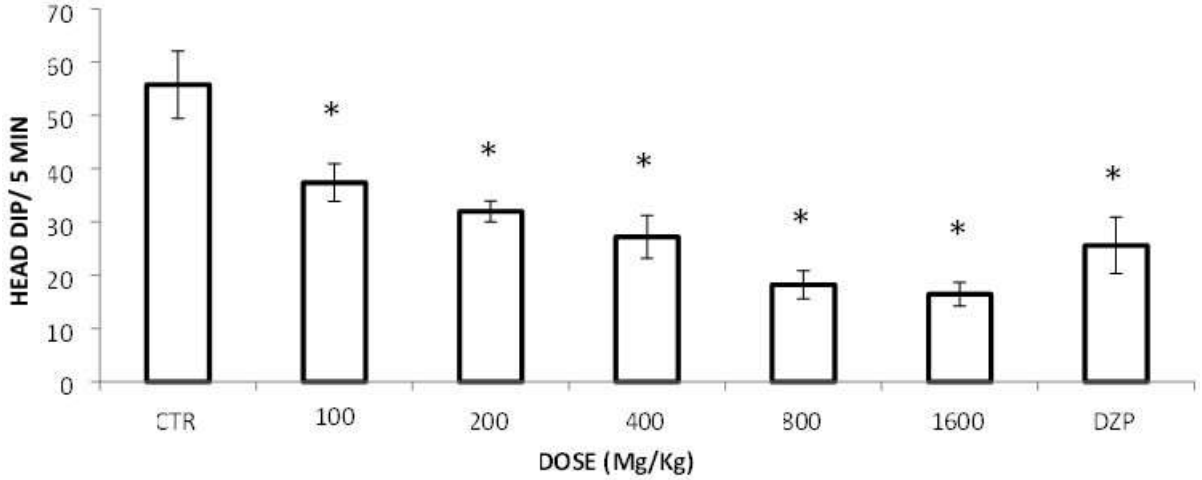

Fig. VII: Effect of MeOH EXT on Head Dip. Each bar is expressed as Mean±SEM. One-way ANOVA revealed a significant (F = 11.13; P = 0.000) difference between the treatment groups. *Indicates a significant difference from control, 10 % Tween 80.

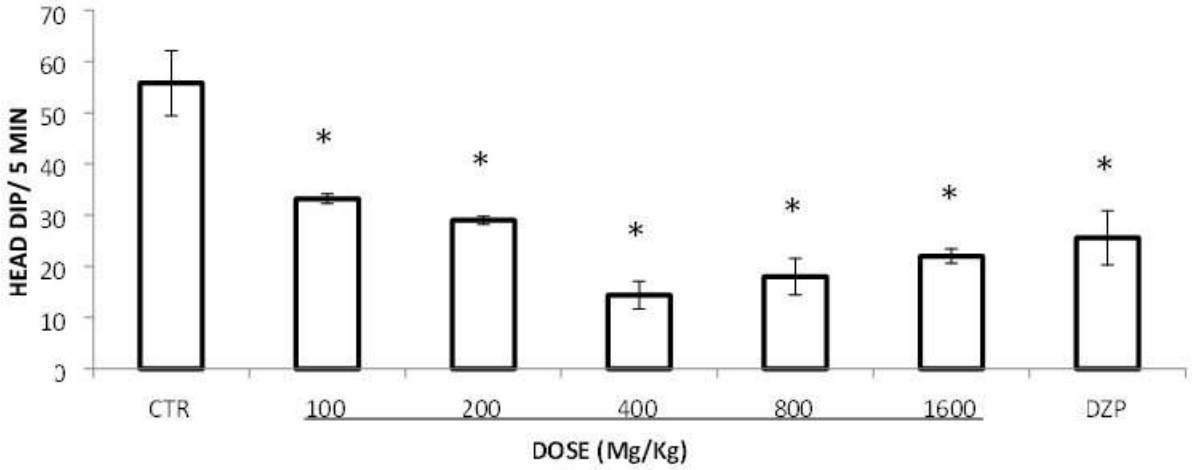

Fig. VIII: Effect of AQ EXT on Head Dip. Each bar is expressed as Mean±SEM. One-way ANOVA revealed a significant (F = 14.41; P = 0.000) difference between the treatment groups. *Indicates a significant difference from control, 10 % Tween 80.

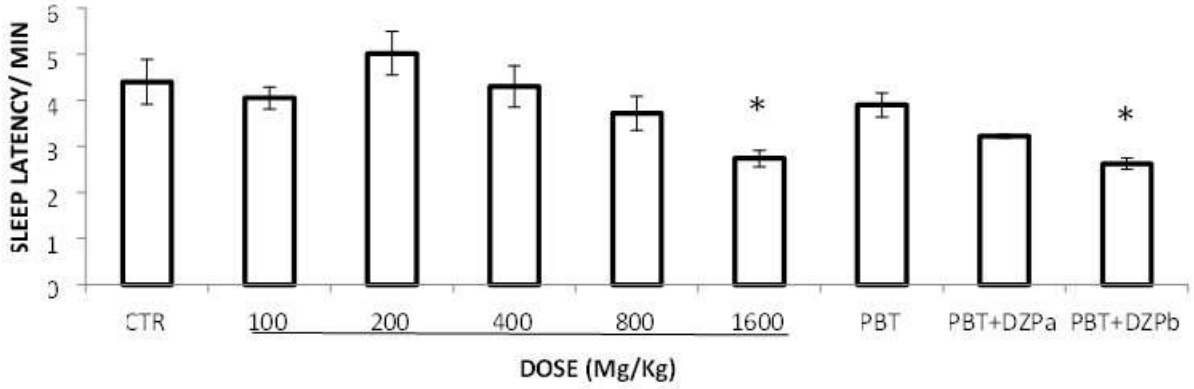

Fig. IX: Effect of MeOH EXT on Sleep Latency. Each bar is expressed as Mean±SEM. One-way ANOVA revealed a significant (F = 7.22; P = 0.000) difference between the treatment groups. *Indicates a significant difference from control, 10 % Tween 80.

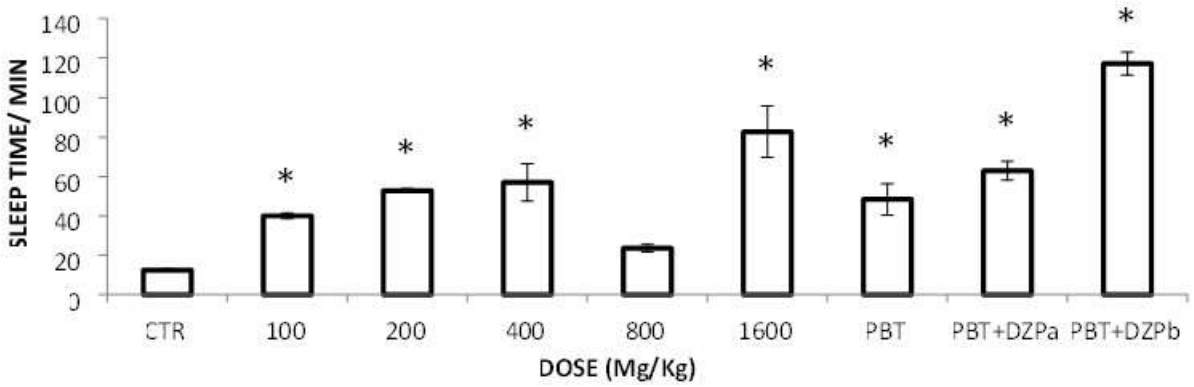

Fig. X: Effect of MeOH EXT on Sleep Time. Each bar is expressed as Mean±SEM. One-way ANOVA revealed a significant (F = 22.31; P = 0.000) difference between the treatment groups. *Indicates a significant difference from control, 10 % Tween 80.

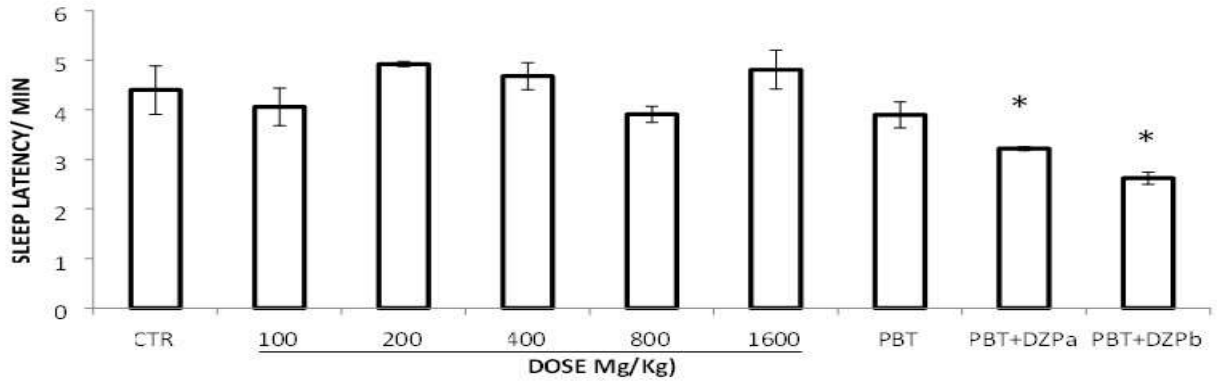

Fig. XI: Effect of AQ EXT on Sleep Latency. Each bar is expressed as Mean±SEM. One-way ANOVA revealed a significant (F = 7.22; P = 0.000) difference between the treatment groups*Indicates a significant difference from control, 10 % Tween 80.

Sleep Time

The total sleep time for the MeOH EXT was between 23.61±2.03 to 82.59±13.06 min. The MeOH EXT significantly and dose dependently (100 – 400 mg/kg, p.o.) increased the sleep time. The most prominent significant increase in sleep time was at the highest test dose (1600 mg/kg, p.o.). At a moderately high dose (800 mg/kg, p.o.), there was no significant increase in sleep time. The administration of PBT (40 mg/kg, i.p.) either singly or in combination with DZP (1 – 2 mg/kg, i.p.) significantly prolonged sleep time in mice. DZP in combination with PBT caused a deeper and longer sleep time at incremental doses (Fig. X). In the AQ EXT, sleep time was between 27.51±1.72 to 53.32±1.40. The AQ EXT significantly prolonged sleep time at all the test doses (100 – 1600 mg/kg, p.o.). The prolongation in sleep was most prominent at low (200 mg/kg, p.o.) and highest (1600 mg/kg, p.o.) test doses respectively. The potentiation in sleep time was followed by the activity observed at the lowest (100 mg/kg, p.o.) and the median (400 mg/kg, p.o.) doses. The least increase in sleep time was at a moderately high (800 mg/kg, p.o.) dose (Fig. XII).

Signalling Pathways Mediating the Effect of MeOH EXT

Five different neurotransmitter antagonists were used to study the signalling pathways mediating the inhibitory effect of the MeOH EXT (800 mg/kg, p.o.) on novelty-induced rearing (NIR) in mice. All the treatment groups produced a significant decrease in NIR relative to control (10 % Tween 80). The neurotransmitter antagonists significantly decreased rearing in comparison to the CTR, while significantly increased rearing in comparison to the MeOH EXT. Prior administration of atropine (ATR) significantly blocked the inhibitory action of the MeOH EXT, and increased rearing. Cyproheptadine (CYP) significantly facilitated the inhibitory action of the MeOH EXT, and decreased rearing. Flumazenil (FLU) significantly blocked the inhibitory action of the MeOH EXT, thus marginally increased rearing relative to the MeOH EXT.

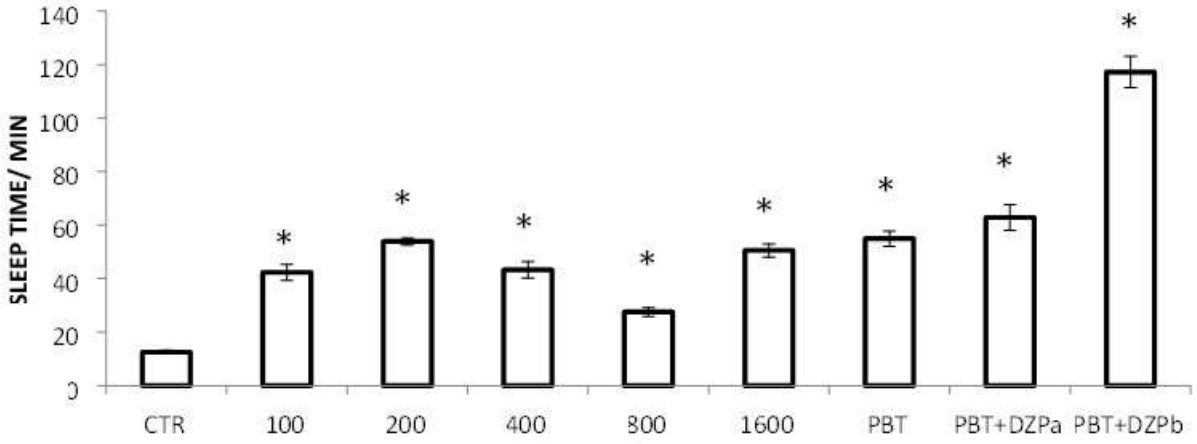

Fig. XII: Effect of AQ EXT on Sleep Time. Each bar is expressed as Mean±SEM. One-way ANOVA revealed a significant (F = 78.74; P = 0.000) difference between the treatment groups. *Indicates a significant difference from control, 10 % Tween 80.

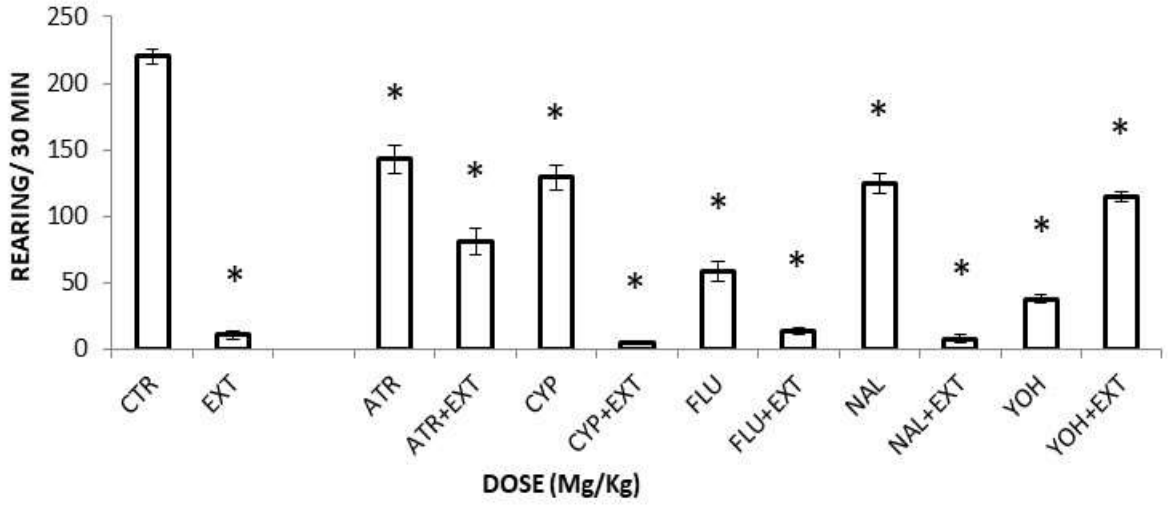

Fig. XIII: Signalling Pathway Mediating the Effect of MeOH EXT of *A. laxiflora*. Each bar is expressed as Mean±SEM. One-way ANOVA revealed a significant (F = 114.70; P = 0.000) difference between the treatment groups. *Indicates a significant difference from control, 10 % Tween 80.

Naloxone (NAL) significantly facilitated the inhibitory action of the MeOH EXT, and decreased rearing. Yohimbine (YOH) significantly blocked the inhibitory action of the MeOH EXT, and increased rearing in mice (Fig. XIII).

Signalling Pathways Mediating the Effect of AQ EXT

Five different neurotransmitter blockers were also used to study the signalling pathways mediating the inhibitory effect of the AQ EXT (800 mg/kg, p.o.) on NIR in mice. All the treatment groups produced a significant decrease in NIR relative to control (10 % Tween 80). The neurotransmitter antagonists significantly decreased rearing in comparison to the CTR. FLU and YOH caused a significant decrease in rearing relative to the AQ EXT. However, ATR, CYP, and NAL did not significantly decrease rearing relative to the AQ EXT, rather the *trio* drugs produced a comparable effect on NIR to the AQ EXT. Prior administration of ATR significantly facilitated the inhibitory action of the AQ EXT, and decreased rearing. CYP significantly facilitated the inhibitory action of the AQ EXT, hence the decreased rearing. FLU significantly enhanced the inhibitory action of the AQ EXT, thus decreased rearing relative to the AQ EXT. NAL significantly facilitated the inhibitory action of the AQ EXT, and decreased rearing. YOH significantly enhanced the inhibitory action of the AQ EXT, hence the decreased rearing in mice (Fig. XIV).

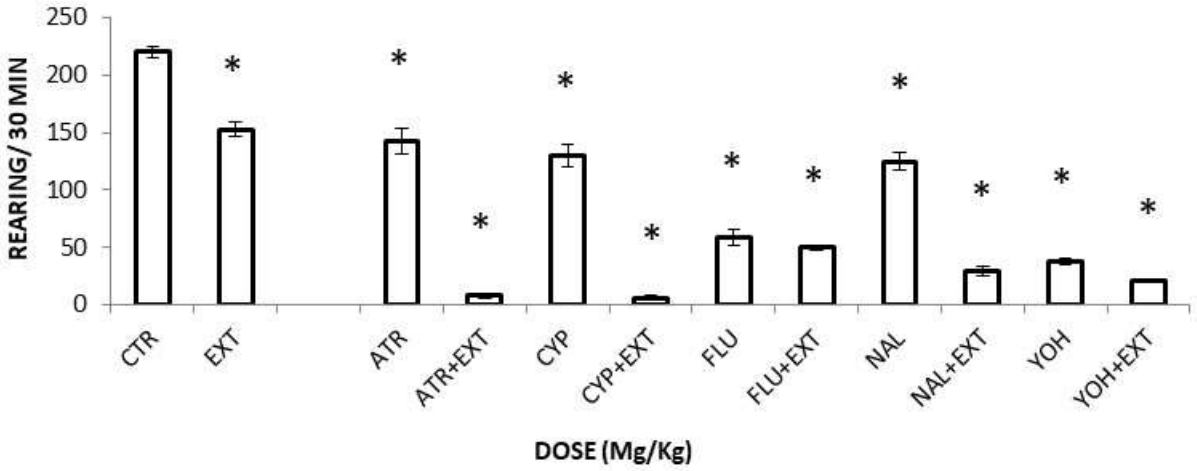

Fig. XIV: Signalling Pathway Mediating the Effect of AQ EXT of *A. laxiflora* Each bar is expressed as Mean±SEM. One-way ANOVA revealed a significant (F = 135.49; P = 0.000) difference between the treatment groups. *Indicates significant difference from control, 10 % Tween 80.

DISCUSSION

The study investigated the hypno-sedative effect and signalling pathways mediating the inhibitory effects of the MeOH and AQ EXTs of *A. laxiflora* in mice. The median lethal dose (LD_{50}) was determined in 24 h using the Lorke's method. The LD_{50} for the MeOH and AQ EXTs of *A. laxiflora* in the oral route was >1600 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 MeOH EXT and >1600 mg/kg for the AQ EXT. The LD_{50} (i.p.) was relatively toxic in the MeOH EXT, but safe orally. In the AQ EXT, however, the LD_{50} (i.p. and p.o.) was found to be safe. The result of the study demonstrated that the extracts of *A. laxiflora* have very low toxicity, as reflected in the high LD_{50} values for the intraperitoneal and the oral routes, and the absence of toxic systemic signs during the acute toxicity testing. Novelty-induced behaviours are actions (or activity) of animals, especially rodents in a new or unfamiliar environment. NIBs have been used by rodents as one of the survival strategies, especially for searching the environment for food, protection, and escape from predators. This behaviour has been utilised in the evaluation of candidates for both sedative activity and CNS excitation (Vogel,2002). Rearing is a vertically-directed, spontaneous locomotor activity (Onigbogi et al.,2000). It is a CNS-modulated excitatory activity, and evaluates the level of exploration of the animal in the open-field model system. Drugs that stimulate the CNS facilitate rearing, while those agents that depress the CNS inhibit rearing activity in animals. In the study, the MeOH EXT of *A. laxiflora* produced a significant and dose-dependent decrease on rearing, demonstrating a central depressant activity (Fig. I). Also, the aqueous extract produced a significant decrease in rearing (Fig. II), but the effect of the MeOH EXT was more pronounced at relatively higher doses (Fig. I), while the effect of the AQ EXT was more at lower doses (Fig. II). Grooming is a displacement response exhibited by rodents in an unfamiliar environment. It is an important behavioural characteristic associated with CNS de-activation (i.e., loss of normal brain activity), especially that of the reticular activating system of the medulla, and it shows how excited the animal is. Drugs and/or candidates with CNS depressant activity inhibit grooming in rodents. Both the AQ and the MeOH EXTs of *A. laxiflora* significantly and dose-dependently decreased grooming in mice (Fig. III and Fig. IV). The number of squares crossed generally correspond with the distance covered, and this activity evaluates the horizontal locomotion of animals. Square crossings measure exploration; it also measures anxiety[3]. The number of central square entries and the time spent in the central square by a mouse in the open-field model system has been described as a measure of exploration and anxiety. A high frequency of these neurobehaviours shows increased locomotion and exploration, and/or low level of anxiety (Brown et al.,1999). Centrally-acting depressant drugs have inhibitory effect on spontaneous locomotor activity (Haque et al.,2001) A reduction in spontaneous locomotion in mice is suggestive of CNS depressant activity (Cooper et al.,1996). This was demonstrated in the study, as both MeOH and AQ EXTs demonstrated a significant and dose-dependent decrease in spontaneous locomotion, an indication of CNS depressant activity (Fig. V and Fig. VI). Head dip measures the capacity of mice for exploration in a strange environment, and any agents that decreases this activity demonstrates sedative activity. The MeOH and AQ EXTs significantly and dose-dependently decreased the number of head dips in the experimental animals, thus

demonstrating the sedative activity of these crude drugs (Fig. VII and Fig. VIII). Pentobarbital-induced sleep has been used to elucidate CNS active properties of drugs (Adongo et al.,2014). Hypno-sedatives and antidepressants at high doses are known to prolong sleep time (Vogel and Vogel,1997). Antipsychotics also, prolong sleep time, while neuropsychostimulants shorten sleep time (Vogel and Vogel,1997). The prolongation of induced sleep is a proof of sedation, while induction of anaesthesia is a test for hypnosis (Trevor and Way,1997). Increased GABAaminegic transmission produces profound sedation in test animals (Gottesman,2002), which is an indication of depressant activity on the CNS. In the study, the highest decrease in the latency to sleep in the MeOH EXT was observed at very high doses, indicating that the sedation was heavy at very high doses, while at lower doses, the onset of sleep was comparable to that of the control or vehicle (Fig. IX). There was a decrease in sleep latency at the lowest test dose and at a moderately high dose (Fig. IX). An increase in sleep latency was also observed at a low dose in the MeOH EXT (Fig. IX). The highest increase in the duration of sleep was at the highest test dose, showing that the sedative efficacy was achieved at very high doses in the MeOH EXT (Fig. IX). The least duration of sleep was observed at a moderately high dose. An increase in the sleep latency relative to the control was observed at a low dose in the MeOH EXT (Fig. IX). The MeOH EXT significantly potentiated pentobarbital-induced sleep in a dose-dependent manner (Fig. X). In the AQ EXT, the highest decrease in the onset of sleep was at a moderately high dose, indicating that sedative activity was potent at a moderately high dose, while the highest increase in the duration of sleep was at low and highest test doses. *A. laxiflora* demonstrated sedative potency at low and very high doses in the AQ EXT (Fig. XI and Fig. XII). Previous phytochemistry reports 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 plant extracts exert anxiolysis and sedation in laboratory animals, and this was demonstrated in the study. These phyto-chemicals and secondary metabolites may be responsible for the CNS depressant activity of *A. laxiflora*. The investigation of the signalling pathways was carried out to determine the neurotransmitter systems involved in mediating the sedative activity of the MeOH and AQ EXTs. The neurotransmitter antagonists used in the work included atropine, cyproheptadine, flumazenil, naloxone, and yohimbine, which antagonise the following neurotransmitter systems *viz*. cholinergic, serotonergic, GABAergic, opioidergic, and the noradrenergic systems respectively. Novelty-induced behaviours are coordinated by the CNS, and are regulated through a host of neurotransmitter systems. These neurotransmitter pathways, include the dopaminergic, GABAergic, chlolinergic, serotonergic, opioidergic, glutamatergic, and the noradrenergic systems. The pretreatment of mice with atropine, a muscarinic prejunctional (presynaptic) receptor antagonist significantly blocked the inhibitory action of the MeOH EXT (Fig. XIII), suggesting that the inhibition of NIR by the MeOH EXT was possibly through the stimulation of muscarinic receptors, since atropine is a non-selective, reversible, competitive antagonist at muscarinic-cholinergic receptors. On the other hand, the AQ EXT, rather significantly facilitated the inhibitory action on NIR (Fig. XIV), which suggests that the inhibitory action of the AQ EXT on NIR was not mediated through the stimulation of muscarinic-cholinergic receptors. Differential polarities and absorptive capacities of the two solvents (methanol and water) used for the extraction process may be responsible for the variation in the actions of both extracts in the screening for possible cholinergic involvement. Cyproheptadine is a serotonin (5-HT) receptor antagonist with histamine H_1-receptor blocking activity. The administration of cyproheptadine to mice before the extract significantly facilitated the inhibitory action of the MeOH and AQ EXTs on NIR (Fig. XIII and Fig. XIV), which suggests that the inhibitory action of the MeOH and AQ EXTs on NIR was not mediated through the stimulation of 5-HT receptors. Flumazenil is a competitive antagonist at the GABA-benzodiazepine receptor. Prior treatment of mice with flumazenil marginally, but significantly blocked the inhibitory action of the MeOH EXT on NIR (Fig. XIII), which suggests that the inhibitory action of the MeOH EXT on NIR may be mediated through some degree by the stimulation of GABAminergic receptors. However, the AQ EXT did not show any stimulatory effects on the GABAminergic system, as prior administration of flumazenil significantly enhanced the inhibitory action of the AQ EXT on NIR (Fig. XIV). Naloxone is a non-selective, competitive opioid receptor antagonist. Its pretreatment in mice significantly facilitated the inhibitory action of the MeOH and AQ EXTs on NIR (Fig. XIII and XIV), thus suggesting that the inhibitory action of the MeOH and AQ EXTs on NIR was probably not mediated through the stimulation of opioid receptors. Yohimbine is a selective, pre-synaptic α_2-adrenergic receptor blocker. Administration of yohimbine before the extract significantly antagonised the inhibitory action of the MeOH EXT on NIR (Fig. XIII), and this suggests that the inhibitory action of the MeOH EXT on NIR was probably mediated through the stimulation of noradrenergic receptors. However, an enhancement in the inhibitory action of the AQ EXT on NIR was observed (Fig. XIV), which suggests that the inhibitory action of the AQ EXT on NIR was probably not mediated through the stimulation of noradrenergic receptors. The discrepancies observed in the signalling pathways mediating the biological activity of the MeOH and AQ EXTs of *A. laxiflora* was probably due to the differential extractive capacites of the solvents used in the extraction process. Methanol is a polar solvent, but it has the capacity to extract both polar and non-polar compounds. It is able to

penetrate the intracellular and the extracellular compartments of the plant cells, thus extracting all the bioactive constituents of the research plant. Water on the other hand, even though a polar solvent lacks the capacity to extract these bioactive compounds from the plant cells of *A. laxiflora* in the same proportion as methanol. Water can only extract polar molecules or compounds. Therefore, the differential solvent extraction capacities of methanol and water were probably responsible for the observed differences in the signalling pathways mediating the hypno-sedative effect in both extracts. The use of specific antagonists of receptor systems revealed that while some of these receptors were involved in the modulation of the inhibitory action of *A. laxiflora* on NIR, other receptors seem not to be involved in mediating the inhibitory action of the extracts in mice.

CONCLUSIONS

The study concluded that the extracts from *A. laxiflora* have hypno-sedative activity in mice. The signalling pathways mediating the effects of the extracts were found to be through the stimulation of muscarinic-cholinergic and α_2-adrenergic receptors.

Supplementary materials

The supplementary material / supporting for this article can be found online and downloaded at: https://www.isisn.org/article/

Author contributions

Conceptualisation and supervision, O.R.I.; Collection of data/ bench work, and data analysis, C.N.N.; Co-supervision and streamlining of the research, J.M.A. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement

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Informed Consent Statement

Not applicable.

Data Availability Statement

All of the data is included in the article/supplementary material.

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Conflict of interest

The authors declared that the study was performed in the absence of any conflicts of interest.

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