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Anxiolytic, Sedative and Hypothermic Effects of Aqueous Leaf Extract of *Vernonia amygdalina Del.* (Asteraceae) in Albino Mice

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Authors' contributions

This work was carried out in collaboration between all authors. This work emanated from the M. Phil. thesis of author IJO. Author IJO conceived and designed the study, performed and managed the literature search and statistical analysis, wrote the protocol and the first draft of the manuscript. Author IOR was the main supervisor while author OIA co-supervised the work. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study evaluated the anxiolytic, sedative and hypothermic effects of aqueous leaf extract of *Vernonia amygdalina* in Mice.
Study Design: One-factor two control groups experimental design.
Place and Duration of Study: Department of Pharmacology, Faculty of Pharmacy,

**Corresponding author: Email: tobiimoru@yahoo.com;*

Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria, between October 2012 and January 2013.
Methodology: Animal models of novelty induced behaviours (rearing and locomotion), anxiolysis (T-maze and hole-board), sedation (amylobarbitone induced hypnosis) and hypothermia (rectal temperature measurement) were utilized in this study. Five different groups of white albino mice of both sexes weighing 23 – 28g (n=5 or 6) were randomly selected. Group 1 was the control (normal saline, 10 ml/kg, i.p.), group 2 was the positive control (diazepam, 1mg/kg, i.p.), while group 3, 4 and 5 were treated with aqueous leaf extract at 50, 100 and 200mg/kg, i.p., respectively. All animals in each group were pre-treated for 30 minutes before assessment.
Results: *V. amygdalina* at 50 mg/kg showed anxiolytic activity by significantly (P<0.001) increasing the frequency of head-dip compared to control, and also a significant (P =.05) decrease and increase (P<0.001) in latencies to withdrawal from the closed and open arms of the elevated T-maze respectively. However, at 100-200mg/kg, *V. amygdalina* showed sedative activity by significantly (P<0.001) decreasing rearing, locomotion (P<0.001) and head-dip frequency (P<0.001) in mice. Furthermore, *V. amygdalina* (100-200mg/kg) caused significant (P<0.001) decrease in sleep latency and significantly (P<0.001) increased sleep duration in amylobarbitone-induced sleeping test indicating sedative activity. *V. amygdalina* (50-200mg/kg) also caused significant (at 30 min, 60 min, 90 min and 120 min; P=.05) reduction in rectal temperature in mice compared to normal saline and diazepam.
Conclusion: The aqueous leaf extract of *V. amygdalina* may possess anxiolytic, sedative and hypothermic effects, hence justifying its folkloric medicinal use.

Keywords: Vernonia amygdalina; anxiolysis; hypnosis; hypothermic; T-maze; novelty behaviours;

1. INTRODUCTION

Vernonia amygdalina Del. (synonym: *Gymnanthemum amygdalinum*) [1], commonly called bitter leaf is a perennial shrub or small tree of 2-5meter in height that grows throughout tropical Africa. It belongs to the family *Asteracea*, has a rough bark with dense back straits, and elliptic leaves that are about 6mm in length. The leaves are green and have a characteristic odour and bitter taste [2]. The macerated leaves of the plant are consumed as vegetables and condiments, and a source of green leafy vegetable for culinary application [3].

In the African traditional medicine, practitioners use the plant as an anti-helmintic, anti-malarial, and as a laxative [4]. It stems are used as a digestive tonic, appetizer and febrifuge as well as for the topical treatment of wounds [5]. The stems are use as chewing sticks for oral hygiene and for management of some dental problems [6]. It is also used by traditional birth attendants to aid the expulsion of the placenta after birth, aid post-partum uterine contraction, increase lactation and control post-partum haemorrhage [7,8]. Odugbemi and Akinsulire [9], summarized that *V. amygdalina* among other uses, is used in the treatment of nervous diseases.

The leaves of *V. amygdalina* have been shown to have certain appreciable quantities of ascorbic acid and caroteinoids [10]. Calcium, iron, potassium, phosphorus, magnesium, copper and cobalt have also been found in significant quantities in the leaves of the plant

[9,10]. It also contain significant quantities of lipids [10] and proteins with high essential amino acid score [11]. The chemical compositions of *V. amygdalina* include oxalates, phytates, tannins, stigmastane-type saponins such as vernoniosides [10,11]. A-series saponins, steroidal saponins, sesquiterpene lactones e.g. vernolide, vernodalol, vernolepin, vernodalin and hydroxyvernolide [12]. Other phytochemicals present in the leaves of *V. amygdalina* are flavonoids, terpenes, coumarins, phenolic acids, lignans, xanthones and anthraquinones [13].

Pharmacological evaluation of extracts of *V. amygdalina* has shown that it possessed antioxidant properties [14]. Antibiotic, antimicrobial and antimalarial properties [13,15]. Anticancer and anti-tumor properties [16]. Hypoglycaemic and antidiabetic properties [17]. Oxytocic property [18]. Hepato- and nephron- protective properties [19,20]. Serum lipid modulation property [1,21]. Pesticidal property [22].

Anxiety, reported to be affecting about one-eighth of the world, is an unpleasant effect characterized by a tense and physically exhaustive alertness focused on an impending and inevitable thought, not objectively apparent danger or emergency (doomsday syndrome), along with a painful awareness of being powerless to do anything about the situation [23]. Sedation, defined as the reduction of irritability or agitation by administration of a sedative agent [24] and hypothermia (reduced temperature), are thought to be closely linked or related, as previous studies have shown that agents with significant CNS depression exhibited these effects [25].

Diseases and disorders of the nervous system among many include: Parkinson's disease, Alzheimer's disease, Multiple Sclerosis, Epilepsy, Anxiety, Insomnia, Depression e.t.c. [26,27]. Even though ethnomedicinal information on *V. amygdalina*, has indicated its use in the treatment of nervous diseases, there is however paucity of information on the neuropharmacological profile of this plant. Hence this study, in order to elucidate the neuropharmacological profile of *V. amygdalina*, was carried out to evaluate the effect of aqueous leaf extract of *V. amygdalina* on anxiety, sedation and rectal temperature in mice.

2. MATERIALS AND METHODS

2.1 Plant Material and Preparation of *V. amygdalina* Extract

Fresh leaves of *V. amygdalina* were collected from a private farm in Kosere Area of Ifewara in Ife-East Local Government Area of Osun State on the 15th of August, 2012 and were authenticated by Mr. G. Ibhanesebhor of the Department of Botany, Faculty of Science, Obafemi Awolowo University. The voucher specimen of the leaves of plant was prepared and deposited at the Herbarium Unit of the Department of Botany, Faculty of Science, Obafemi Awolowo University, Ile-Ife, with Voucher No: IFE 16901.

Fresh leaves of *V. amygdalina* were collected, air-dried and milled into powder with the aid of electric grinder. A powdered leaf of *V. amygdalina* (512g) was extracted cold in 7 litres of water with continuous shaking for 48 hours in a mechanical shaker. The mixture was filtered and the filtrate concentrated using a rotary evaporator at a maximum temperature of 45ºC to obtain the crude aqueous extract of the plant. Further drying of the extract was carried out using the freeze-dryer to obtain semi-solid extracts. The total dried aqueous extract obtained from the leaf of *V. amygdalina* was 51.87g given 10.13% w/w yield. The semi-solid paste of

the aqueous extract of the plant was then stored in the refrigerator at 4ºC until it was needed for use.

2.2 Experimental Animals

White albino mice of both sexes weighing between 23g and 28g were obtained from the Animal House, Department of Pharmacology, Faculty of Pharmacy, Obafemi Awolowo University (OAU), Ile-Ife, Osun State. The animals were kept under conducive laboratory conditions and fed with standard animal feed (Grower's mash), and water *ad libitum*. The "principle of laboratory animal care" (National Institute of Health-NIH publication No. 85-23) guidelines and procedures were followed in this study. The Ethical Committee of the Faculty Postgraduate Committee, Faculty of Pharmacy, Obafemi Awolowo University, approved the research work.

2.3 Drugs and Laboratory Materials

Diazepam (Valium® Swipha Nig. Ltd, Nigeria), amylobarbitone sodium (Eli Lilly and Company Limited, Basingstoke, England), observation cage, elevated T-maze apparatus, hole-board apparatus, stop watch, manual counter, syringes and needles, weighing balance and digital thermometer.

2.4 Administration of Extracts

The aqueous extracts were administered to mice through the intraperitoneal route. The volume of extracts administered intraperitoneally was 10mL per kg or 0.1mL per 10g of body weight of the animal in all cases. The intraperitoneal route was used in this study because it gives faster and more consistent results and test are readily reproducible. This route is preferred in CNS tests because of the possibility of interference of metabolic processes with the test agents given through the oral route [28].

2.5 Acute Toxicity

The acute toxicity (i.p. LD_{50}) of the aqueous leaf extract of *V. amygdalina* was estimated in 13 white albino mice using standard method of Lorke [29]. Briefly, this includes two phases: The 1st phase uses 3 animals for each dose level 10, 100 and 1000mg/kg. The mice are kept under the same laboratory conditions and observed for signs of toxicity which include but is not limited to paw-licking, stretching, respiratory distress and mortality for the first critical four hours and after 24 hours the number of death per group is recorded. The result obtained from this test is used as a basis for selecting the subsequent doses in the 2nd phase following a standard table. The 2nd phase involved administering four different doses to one mouse per group and the mice are observed for signs of toxicity for the first critical four hours and thereafter 24 hours for mortality. The intraperitoneal median lethal dose (LD_{50}) was calculated as the geometric mean of doses that caused 0 and 100% mortality respectively. The $LD_{50} = \sqrt{(A \times B)}$, where A = maximum dose that caused 0% mortality and B = minimum dose that caused 100% mortality. The working doses (i.e. treatment doses) used in this experimental work were arrived at by the formula $1/3 \times LD_{50}$. All treatment doses were below the third of the LD_{50}.

2.6 Assessment of the Effect of *V. amygdalina* Extract on Rearing and Locomotion in the Open Field Apparatus

The observation cage was used in this assessment. Thirty male mice were allotted into five groups of six mice each. The first group received 10mL normal saline per kg body weight i.p., the second group was 1.0mg diazepam per kg body weight i.p. while the third, fourth and fifth groups received 50, 100 and 200 mg extract per kg body weight i.p. respectively. Thirty minutes later, the animals were placed directly from their home cages into the observation cage. All animals were observed and scored singly in the cage. Rearing was scored for 30 minutes while line crossing was scored for the first 10 minutes of the 30 minutes duration. Rearing (vertical locomotion) is defined as lifting of the fore limbs off the floor completely [30], while line crossing was counted (with manual counter) when the animal crossed a line with all the limbs. Each animal was used only once and the floor of the cage cleaned with 100% methanol after each assessment to remove olfactory cue from one animal to the other [24]. The animals used were fasted overnight but allowed free access to water. All assessments were carried out between 09:00 and 17:00h [31].

2.7 Assessment of the Effect of *V. amygdalina* Extract on Head-Dipping on the Hole-Board Apparatus

The hole-board apparatus was used in this assessment. Thirty male mice were randomly allotted into five groups of six mice each. The first group received 10mL normal saline per kg body weight i.p., the second group was administered 1.0mg of diazepam per kg body weight i.p. while the third, fourth and fifth groups received 50, 100 and 200 mg extract per kg body weight i.p. respectively. Thirty minutes later, the animals were placed directly from their home cages to the hole-board apparatus and allowed to freely explore for 5 minutes. After each observation, hole-board apparatus was cleaned with 100% methanol to remove scent cue left from the preceding animal. The head-dip was scored using manual counter.

2.8 Assessment of the Effect of *V. amygdalina* Extract on Anxiety in the Elevated T-maze Apparatus

Twenty-five male albino mice were randomly allotted into five groups of five mice each. The first group received 10mL normal saline per kg body weight i.p., the second group was given 1.0 mg of diazepam per kg body weight i.p. while the third, fourth and fifth groups received 50, 100 and 200 mg extract per kg body weight i.p. respectively. Thirty minutes later, scoring each animal singly; for the inhibitory avoidance task (in the closed arm), the animal was placed at the end of the closed arm and the latency to withdraw from this arm with the four paws was recorded in three successive trials made at 30 seconds intervals. While for the one-way escape task which initiated 30 seconds after the completion of the avoidance tasks, the animal was placed at the end of one of the open arms and withdrawal latency from this arm was similarly recorded with stop watch [32].

2.9 Assessment of the *V. amygdalina* Extract on Amylobarbitone Sodium-Induced Sleeping Time in Mice

The method described for screening intravenous anaesthetics was used [33] with minor modification. In this study, the i.p. route was used instead of the i.v. (through tail) which was indicated because the i.p. route was faster and more reliable with mice. Two important parameters are normally evaluated. The first is the loss of righting reflex confirmed when the

animal, placed on its back fails to recover from this position within 60 seconds. The second parameter includes onset and duration of loss of righting reflex (in second or minutes). The onset (latency to sleep) is defined as the period from injection to the time of loss of righting reflex, while the duration (total sleeping time or total time of loss of righting reflex) is the period when the animal remained un-stimulated or does not respond to stimuli (shown by inability to move its head of body). The total sleeping time in this study is taken as the time of loss of consciousness (loss of righting reflex) and recovery of righting reflex. Recovery is considered to have occurred when the animal after spontaneous righting, would re-right itself within 15 seconds when placed on its back [33].

Thirty male mice were randomly allotted into five groups of six mice each. The first group was given 10ml/kg, i.p., the second group was given diazepam (1mg/kg, i.p.), whiles the third, fourth and fifth groups received 50, 100 and 200mg/kg, i.p. respectively. Thirty minutes later, animal were administered 20mg/kg of amylobarbitone sodium [34]. The latency period and total sleeping time were noted and recorded for each mouse as described above.

2.10 Assessment of the Effect of *V. amygdalina* Extract on Rectal Temperature in Mice

Five groups of albino mice of both sexes consisting of six animals each were randomly selected. Group one was administered with 10ml/kg, i.p. while group two received 1mg/kg, i.p. of diazepam. Group three to five were administered 50, 100 and 200mg/kg, i.p. of extract respectively. The rectal temperature of each mouse in all the groups were taken with digital thermometer (thermoprobe) by inserting the probe 2cm deep into the anus of the mice shortly before treatment time and at 30, 60, 90, 120 minutes after treatment. The test was done between 10:00 and 14:00h [35]. The mean ± SEM was then calculated for each group [36].

2.11 Statistical Analysis

Results are expressed as mean ± SEM. Statistical difference was determined by one-way analysis of variance (ANOVA) followed by a post hoc test (Student Newman-Keuls Test (SNK)). Difference was considered statistically significant with $p < 0.05$. Computer software Graph pad PRISM® version 3.00 was used for the analysis.

3. RESULTS

3.1 Acute Toxicity

For the first critical four hours during the acute toxicity studies, animals that received higher doses of the aqueous leaf extract of *V. amygdalina* were also noticed to be passive and were also seen stretching, while those that received lower doses of extract were seen active when placed back into their home cages. However no death was recorded during the hours. The aqueous leaf extract of *V. amygdalina* was found to have median lethal dose (LD_{50}) in mice of 894mg/kg i.p.

3.2 Effect of Aqueous Leaf Extract of *V. amygdalina* on Rearing and Locomotion in the Open Field Apparatus

3.2.1 Effect of aqueous leaf extract of *V. amygdalina* on rearing in the open field apparatus

Aqueous leaf extract of *V. amygdalina* (50-200mg/kg, i.p.) induced significant ($F_{4,20}$ = 154.3; p<0.001) inhibitory effect on rearing in mice compared to both control (normal saline) and diazepam groups. Diazepam (1mg/kg i.p.) also significantly (p<0.001) inhibited rearing in the animal in this group as compare to the control group (Fig. 1a).

3.2.2 Effect of aqueous leaf extract of *V. amygdalina* on locomotion of mice in the open field apparatus

Aqueous leaf extract of *V. amygdalina* at all the doses tested (50-200mg/kg, i.p.) caused a significant ($F_{4,20}$=39.02; p<0.001) decrease in the number of lines crossed within the observation period compared to both control (normal saline) and diazepam groups. While 1 mg/kg, i.p. diazepam also caused a significant ($F_{4,20}$=39.02; p<0.001) decrease in the number of lines crossed within the observation period (Fig. 1b).

3.3 Effect of Aqueous Leaf Extract of *V. amygdalina* on Head-Dipping Frequency on the Hole-Board Apparatus

Aqueous leaf extract of *V. amygdalina* at 50 mg/kg, i.p. caused a significant ($F_{4,20}$ = 78.11; p<0.001) increase in the frequency of head-dip compared to the control, while at 100 and 200 mg/kg, *V. amygdalina* caused a significant ($F_{4,20}$ = 78.11; p<0.001) decrease in head-dip frequency compared to both control and diazepam groups. Diazepam (1mg/kg, i.p.) caused significant ($F_{4,20}$ = 78.11; p<0.001) increase in the frequency of head dips on the hole-board compared to the control group (Fig. 2).

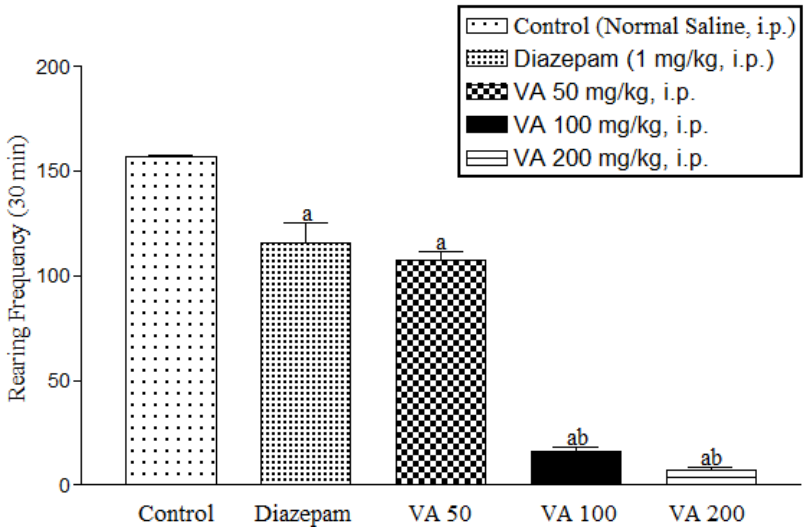

Fig. 1a. Effect of aqueous leaf extract of *V. amygdalina* (VA) on rearing frequency in mice

Each bar is expressed as Mean ± SEM of rearing frequency of mice in the open field; (n= 6 per group). a = p<0.001 compared to normal saline; b = p<0.001 compared to diazepam; (ANOVA; SNK)

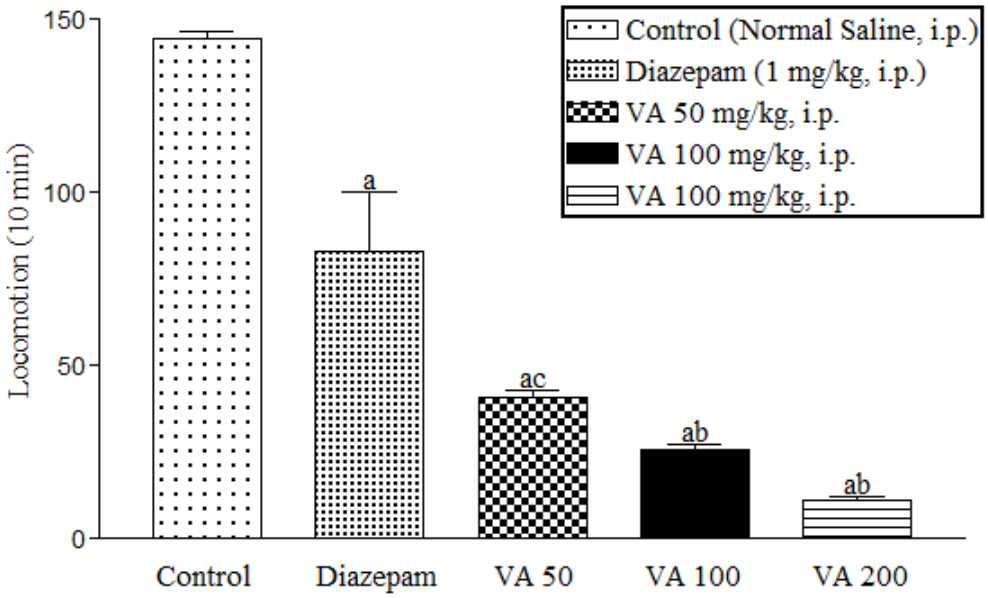

Fig. 1b. Effect of aqueous leaf extract of *V. amygdalina* (VA) on locomotion in mice
Each bar is expressed as Mean ± SEM of locomotion of mice in the open field; (n=6). a = p<0.001 compared to normal saline; b = p<0.001 and c = p<0.01 compared to diazepam; (ANOVA; SNK)

3.4 Effects of Aqueous Leaf Extract of *V. amygdalina* on Activities of Mice in the Elevated T-maze Apparatus

3.4.1 Effect of aqueous leaf extract of *V. amygdalina* on inhibitory avoidance task in mice

V. amygdalina at 50mg/kg (i.p.) and diazepam at 1mg/kg (i.p.) caused a significant ($F_{4,20}$ = 226.0; p<0.05) decrease in latency to withdrawal from the closed arm of the T-maze. Doses of 100 and 200mg/kg of *V. amygdalina* caused significant ($F_{4,20}$ = 226.0; p < 0.001) increase in latency to withdrawal from the closed arm of the T-maze compared to both the control (normal saline) and diazepam groups. However, there was no significant difference in the baseline latency, that is, the first time to withdraw from the closed arm (A_1) in all the treatment groups (including the diazepam group) compared to control (Fig. 3a).

3.4.2 Effect of aqueous leaf extract of *V. amygdalina* on one-way escape task in mice

V. amygdalina at all doses tested caused a significant ($F_{4,20}$ = 23.19; p < 0.001) increase in latency to withdrawal from the open arm of the T-maze. This increase is comparable to both control and diazepam groups. The dose of 1mg/kg, i.p diazepam also caused an increase in the latency to withdrawal from the open arm, but this increase was not significant. There was also no significant difference among all the treatment groups in baseline latency (E_1) to withdrawal from the open arm of the T-maze (Fig. 3b).

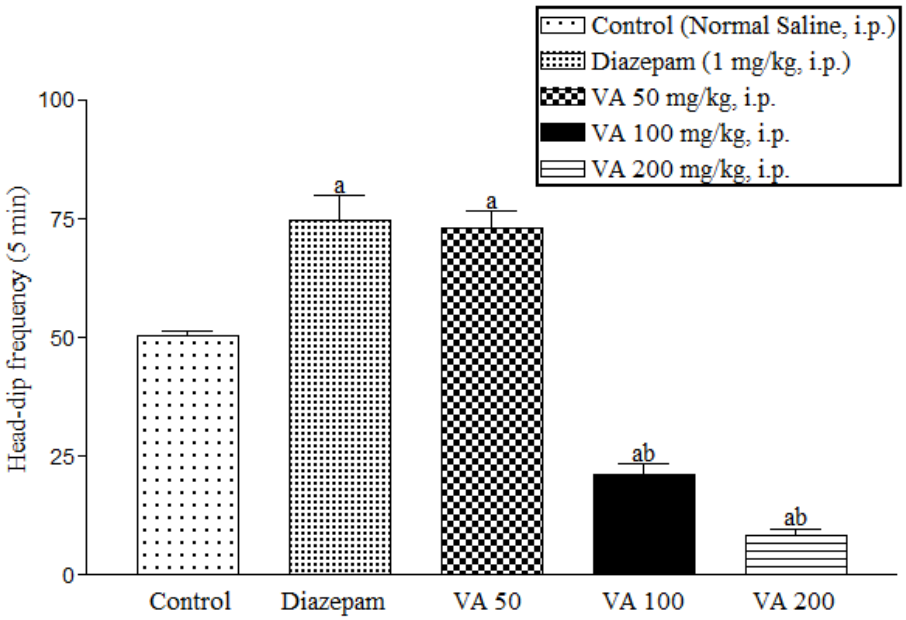

Fig. 2. Effect of aqueous leaf extract of *V. amygdalina* (VA) on head-dip frequency in mice

Each bar is expressed as Mean ± SEM of head-dip frequency of mice on the hole-board apparatus; (n=6 per group). a = p<0.001 compared to normal saline; b = p<0.001 compared to diazepam; (ANOVA; SNK)

3.5 Effect of Aqueous Leaf Extract of *V. amygdalina* on Rectal Temperature in Mice

There were no significant variations in the rectal temperature in the control group of animals. Pretreatment with *V. amygdalina* at the dose of 200mg/kg significantly produced a fall of body temperature at 30 min interval ($F_{4,25}$ = 19.45; p<0.001), 60 min ($F_{4,25}$ = 13.93; p<0.001), 90 min ($F_{4,25}$ = 15.76; p<0.001) and 120 min ($F_{4,25}$ = 8.31; p < 0.001). Pretreatment with *V. amygdalina* at the dose of 100mg/kg significantly produced a fall in rectal temperature at both 30 ($F_{4,25}$ = 19.45; p<0.05) and 60 min ($F_{4,25}$ = 13.93; p<0.01). While significant ($F_{4,25}$ = 13.93; p<0.01) hypothermia was observed in pretreatment with 50 mg/kg of *V. amygdalina* only at 60 min interval. The hypothermic effect of *V. amygdalina* on rectal temperature in this study showed a dose dependent relationship. Pretreatment with 1mg/kg showed significant (p<0.05) hypothermic effect at 30 and 60 min interval (Fig. 4).

3.5 Effect of Aqueous Leaf Extract of *V. amygdalina* on Amylobarbitone Sodium-induced Sleeping Time in Mice

V. amygdalina at 50mg/kg, i.p. caused a reduction in sleep latency with an increase in sleep duration, but these effects were not significant. However, *V. amygdalina* at higher doses of 100 and 200 mg/kg, i.p. caused significant ($F_{4,25}$ = 27.83; p<0.001) reduction in sleep latency with significant ($F_{4,25}$ = 18.11; p<0.001) increase in sleep duration compared to the control group. Diazepam (1 mg/kg, i.p.) caused a significant ($F_{4,25}$ = 27.83; p<0.001) reduction in sleep latency with a significant ($F_{4,25}$ = 18.11; p<0.001) increase in sleep duration compared

to control group of animals. Diazepam significantly ($F_{4,25}$ = 27.83; p<0.001) reduced sleep latency and significantly ($F_{4,25}$ = 18.11; p < 0.01) increase sleep duration compared to 50mg/kg of *V. amygdalina*. While 200 mg/kg of *V. amygdalina* significantly ($F_{4,25}$ = 18.11; p<0.05) increase the sleep duration compared to 1mg/kg, i.p. of diazepam (Figs. 5a and 5b).

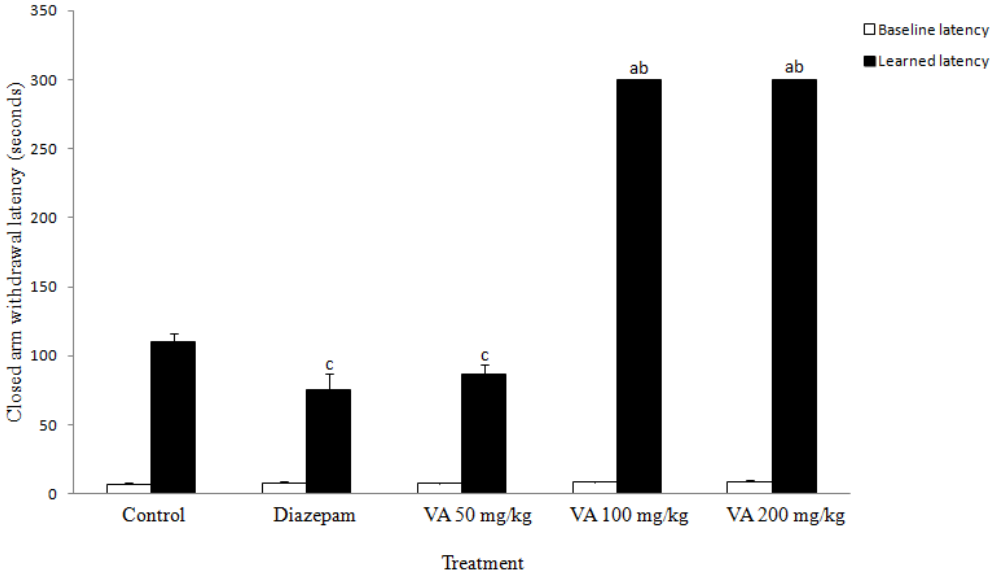

Fig. 3a. Effect of intraperitoneal injection of aqueous leaf extract of *V. amygdalina* on inhibitory avoidance task (latency of withdrawal from the closed arm) of mice on T-maze apparatus

Each bar is expressed as Mean ± SEM, (n=5 per group). a = p<0.001and c = p<0.05 compared to normal saline; b = p<0.001 compared to diazepam; (ANOVA; SNK)

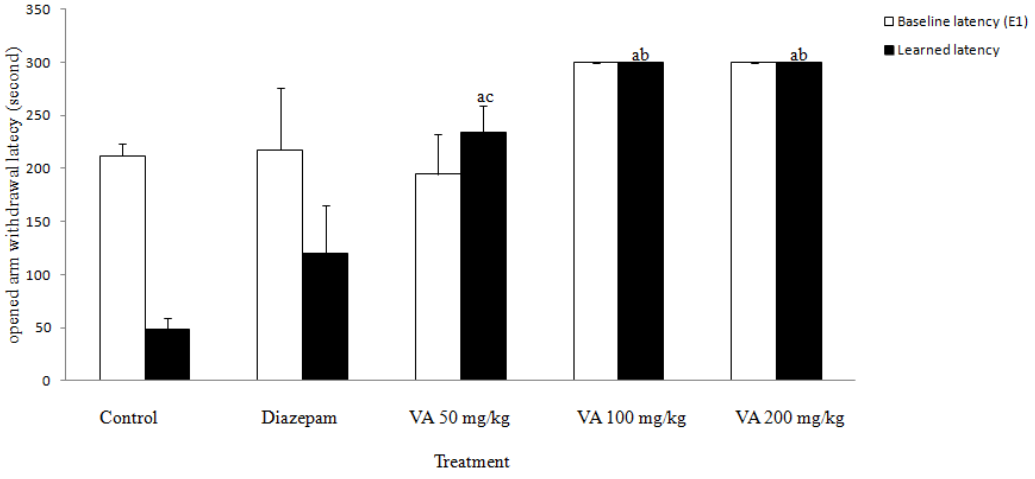

Fig. 3b. Effect of intraperitoneal injection of aqueous leaf extract of *V. amygdalina* (VA) on one-way escape task (latency of withdrawal from the opened arm) of mice on T-maze apparatus

Each bar is expressed as Mean ± SEM, (n=5 per group). a = p<0.001 compared to normal saline; b = p<0.001, and c = p<0.01 compared to diazepam; (ANOVA; SNK)

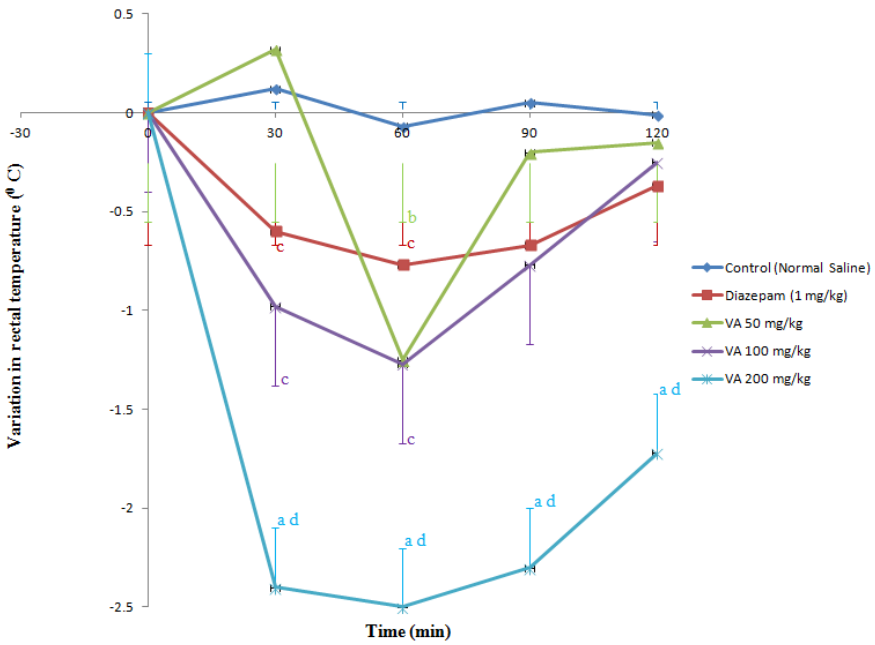

Fig. 4. Effect of intraperitoneal injection of *V. amygdalina* (VA) on rectal temperature in mice

Each line is expressed as Mean ± SEM; (n=6 per group). a = p<0.001, b = p<0.01, and c = p<0.05 compared to normal saline; d = p < 0.001 compared to diazepam; (ANOVA; SNK)

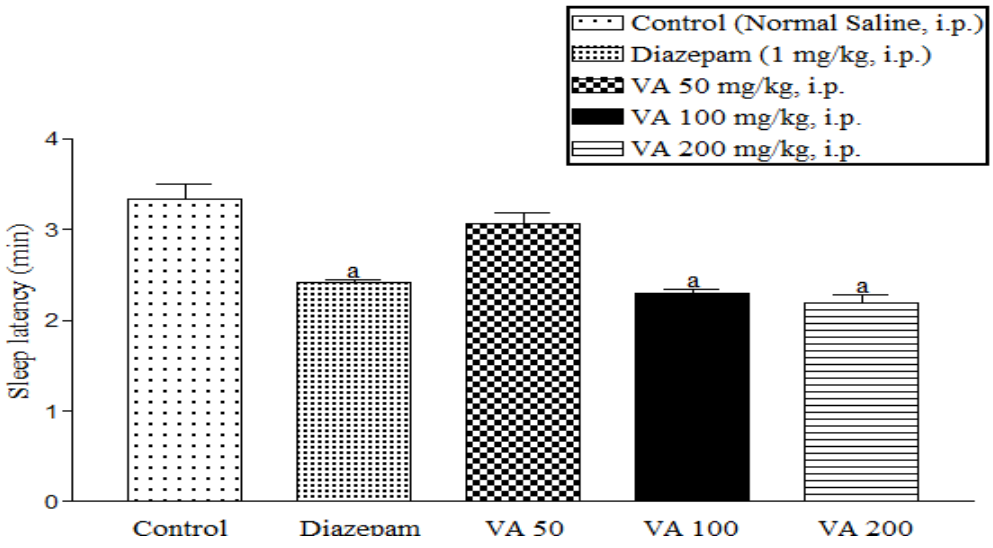

Fig. 5a. Effect of aqueous leaf extract of *V. amygdalina* (VA) on sleep latency in mice

Each bar is expressed as Mean ± SEM; (n = 6 per group). a = p < 0.001 compared to normal saline; (ANOVA; SNK)

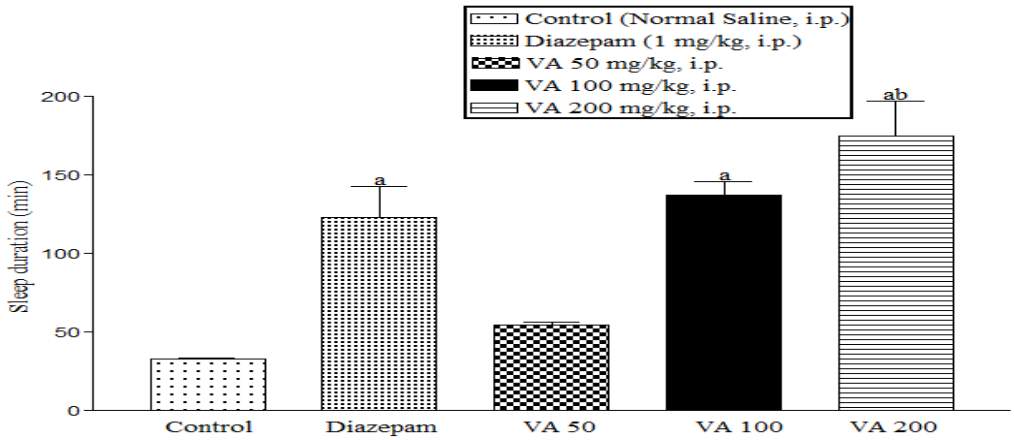

Fig. 5b. Effect of aqueous leaf extract of *V. amygdalina* (VA) on sleep duration in mice

Each bar is expressed as Mean ± SEM; (n = 6 per group). a = p<0.001 compared to normal saline, and = p<0.05 compared to diazepam; (ANOVA; SNK)

4. DISCUSSION

This study investigated the acute toxicity profile of aqueous leaf extract of *Vernonia amygdalina* (VA), and central nervous system effect of aqueous leaf extract of *V. amygdalina* in mice.

The aqueous leaf extract VA was found to have median lethal dose (LD_{50}) 894mg/kg, i.p. in mice, showing that this plant extract was moderately toxic to the experimental animal model (mice) used in this particular study. Ibrahim et al. [37] had reported a LD_{50} of 288.50mg/kg, i.p. of ethanolic extract of VA in mice, claiming a relative toxicity to the animal model used in their study. Lorke [29], stated that substances toxic at less than 1 mg/kg are considered highly toxic; and considering that the LD_{50} estimate of this plant extract was far above this toxicity level thus, this plant extract was moderately toxic to the experimental animal model (mice) used in this particular study.

This study also examined some of the neuropharmacological profile of *Vernonia amygdalina* (VA) including: anxiolytic, sedative and hypothermic activities in mice. VA aqueous leaf extract showed significant anxiolytic and sedative activities. At lower dose of 50mg/kg, VA showed significant increase in head-dips frequency on the hole-board apparatus, significant decrease in latency to withdrawal from the closed arm and significant increase in latency to withdrawal from the open arm on the elevated T-maze apparatus indicating its anxiolytic activity at this particular dose. VA showed a dose dependent sedative activity at higher doses of 100 and 200mg/kg as indicated by significant reduction in rearing and locomotion in the open field test, reduction in head-dips frequency on the hole-board apparatus and significant increase in both inhibitory avoidance and one-way escape tasks on the elevated T-maze apparatus, dose dependent increase in amylobarbitone-induced sleep duration and decrease in sleep latency, and also significant reduction in rectal temperature.

The anxiolytic effect of VA at 50mg/kg on the activity of mice as indicated by significant increase in head-dip frequency on the hole board apparatus corroborates the report of

Casarrubea et al. [38] who reported that the hole-board is an exploration-based assay widely used to asses features of anxiety-related behaviours in rodent and that an increase in head-dip frequency was suggestive of an anxiolytic activity. The anxiolytic effect of VA at 50mg/kg is further buttressed by the result from the elevated T-maze which also corroborates the report of Teixeira et al. [39] who reported that reduction in latency to withdrawal from the closed arm and increase in latency to withdrawal from the open arm are features of anxiolytic activities. Furthermore, diazepam a benzodiazepine anxiolytic caused significant reduction in latency to withdrawal from the closed arm, but showed no significant changes in one-way escape task. This also corroborates the report of Graeff et al. [40], which stated that the escape task is insensitive to doses of benzodiazepines that showed anxiolytic effect (decrease of withdrawal latency) in the avoidance task.

Rearing and locomotion are central excitatory locomotor behaviour associated with motivational state and arousal level and are regarded as 'arousal' or 'stress' phenomenon [41] and decrease in these index of alertness indicates a sedative effect [42]. In this study, it was observed that VA produced a decrease in rearing and locomotion in the open field test indicating a CNS inhibitory activity. This effect is comparable to that of diazepam (a sedative drug), which also reduced rearing and locomotion significantly compared to normal saline (control).

In the Hole-board model, a significant decrease in the exploratory head-dip frequency was observed after treatment with VA extract (100 and 200mg/kg), thus reinforcing the hypothesis that it has inhibitory activity at these doses. The effect of VA (100 and 200mg/kg) on the activity of mice on the T-maze showed that these doses of VA are rather sedative and not anxiolytic. VA at these doses caused increased in latencies to withdrawal from both the closed and open arms. This corroborates the report of Teixeira et al. [39], which stated that whenever the latencies are similarly increased or decreased, there is need for an independent assessment of motor drug effects which may affect the activity of the animals. Increase in both latencies revealed reduced motor activity, since the animals preferred to remain in both ends of the maze. In view of the fact that CNS depressants prolong barbiturate sleeping time [43], VA can be said to display sedative activity most especially at 100 and 200mg/kg. Sedation, muscle relaxation and hypothermia are thought to be closely linked or related as previous studies have shown that agents with significant CNS depression exhibited these effects [25]. VA at all doses tested in this study showed hypothermia in mice at different intervals thereby reinforcing its CNS inhibitory effect.

The T-maze for the screening for anxiolytic effect has proved valuable in identifying both the anxiolytic potentials of benzodiazepine/$GABA_A$ receptor-related agents and for detecting anti-anxiety effect of other agents with unrelated mechanisms e.g. 5-HT_{1A} [40]. In this context, the effectiveness of VA at 50mg/kg in producing anxiolytic effect in this model suggests a possible positive modulation of both the $GABA_A$ – chloride channel receptors complex and 5-HT_{1A} receptors complex. However, further studies will be needed using different receptors antagonists to ascertain whether or not the anxiolytic effect of VA at 50 mg/kg will be inhibited by these antagonists. Phytochemicals such as flavonoids, alkaloids and terpenoids have been reported to be responsible for anxiolytic and sedative effects observed in different plant extracts [44]. VA extract have been reported to be rich in these plant chemicals [45] and thus might be exerting its anxiolytic and sedative activities due to the presence of these phytochemicals. However, further study would be needed to isolate and characterize the active constituents in VA for possible CNS drug development.

Diseases and disorders of the nervous system among many include: Parkinson's disease, Alzheimer's disease, multiple sclerosis, epilepsy, anxiety, insomnia, depression e.t.c. [26,27] and VA has been touted to be useful in the treatment of nervous diseases in folk medicine [9]. Hence, this study justifies the use of VA in folk medicine, in that it showed inhibitory (on the CNS), anxiolytic and sedative activities. However, further work could be done to screen VA for muscle relaxant, analgesic and nootropic activities.

5. CONCLUSION

It can therefore be concluded, that *Vernonia amygdalina* may possess significant anxiolytic, sedative and hypothermic effects, thus providing pharmacological justification for some of its ethnomedicinal uses.

CONSENT

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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