

## Research Article



## Studies on the Antidepressant Effect of Root Bark Extract of *Voacanga africana* in Murine Models

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### ABSTRACT

In Nigerian ethnomedicine, the root bark of *Voacanga africana* Stapf. (Apocynaceae) is used in the treatment of depression associated with mental and psychiatric disorders. According to World Health Organization (WHO) recent global estimates, over 300 million people are estimated to be suffering from depression. Therefore, this study was designed to evaluate the antidepressant effect of the root bark extract of *V. africana* using murine models, in order to scientifically verify the folkloric use. Forced swim test (FST) and phenobarbitone induced sleeping time test were the neuropharmacological models employed in the study. Results showed that the root bark of *Voacanga africana* extract (VAE) showed abolition of depressive-like behavior in the FST in a dose dependent fashion, with VAE (400 mg/kg) and amitriptyline (15 mg/kg), showing highest reduction of the immobility time to 70.40 and 49.50 seconds from 133 second of the control, respectively. The extract also significantly ( $p < 0.05$ ) and dose dependently, potentiated the onset of sleep and duration of sleep in phenobarbitone induced sleeping time in tested animals. At 400 mg/kg dose VAE almost doubled the duration of sleep while diazepam (3 mg/kg), a standard agent, tripled same parameter. However, the acute toxicity test showed that the extract gave an estimated LD<sub>50</sub> of 1250 mg/kg. In conclusion, the extract of *Voacanga africana* possesses antidepressant and sedative activities thus confirming its use in the treatment of psychiatric disorders in folk medicine.

**Keywords:** *Voacanga africana*, antidepressant, forced swim test, rats.

### INTRODUCTION

Depression is a common life threatening psychiatric disorder that negatively affects the mood, feelings and thoughts of an individual. It usually interferes with the quality of life of the individual and at same time causing feelings of sadness, loss of interest in activities once enjoyed, withdrawing from social life, dejected or feeling of worthlessness<sup>1</sup> However, according to World Health Organization (WHO) reports, depressive disorders and anxiety disorders are the two common mental disorders affecting the global population.<sup>2</sup> It has been estimated that over 300 million people suffer from depression globally which is about 4.4% of the world's population. Depression is often associated with decreased quality of life, disability, increased cost of healthcare, poverty and risk factor to other health disorders.<sup>3</sup> Many a times depression may lead to suicide and estimates have it that about one million lives are lost to suicide annually representing a total of about 3000 suicides per day.<sup>4</sup> Standard agents employed in the management of depressive disorders often have limited efficacy with attendant serious side effects, hence the need to discover new therapeutic agents from natural sources is of paramount importance. One of the medicinal plants used in Nigerian ethnomedicine for the treatment of depressive disorders is the root bark preparation of *Voacanga africana* Stapf. (Apocynaceae). *V. africana* has been reported to possess antiulcer<sup>[5]</sup>, antiamoebic and antispasmodic,<sup>6</sup> anti-addictive as well as psychedelic<sup>7</sup> activities. Therefore, this study was

designed to evaluate the antidepressant activities of *V. Africana* root bark extract using neuro-pharmacological models.

### MATERIALS AND METHODS

#### Animals

Adult albino mice (18-30 g) of either sex were used for the phenobarbitone induced sleep time test and acute toxicity test, while adult Sprague-Dawley rats (150-250 g) of either sex were used for forced swim test. The animals were maintained with a 12 hour light/dark cycle, allowed free access to food and water prior to behavioural experiment and were transported in their cages to the laboratory 30 minutes before the experiment to get acclimated. All animal experiments were conducted in compliance with the National Institute of Health Guide for Care and Use of Laboratory Animals (Pub No. 85-23, revised 1985) and in accordance with the University of Nigeria Ethics Committee on the use of laboratory animals, registered by the National Health Research Ethics Committee (NHREC) of Nigeria, with the number; NHREC/05/01/2008B.

#### Extraction of plant materials

Fresh root barks were collected in the month of November, from Nsukka, Enugu State, Nigeria; and were botanically identified by Mr. A. Ozioko of International Center for Ethnomedicine and Drug Development (InterCEDD), Nsukka, Nigeria. The fresh root bark was rinsed, air dried under the shade and milled to a coarse



powder. About 800 g of the powdered root bark were extracted with methanol by cold maceration for 48 hours.<sup>[8]</sup> The filtrate was concentrated using rotary evaporator which yielded the methanol extract of *Voacanga africana* (VAE) used for the experiment. The percentage yield of the extract was determined.

### Forced swim test

This model of screening antidepressant effect is divided into two phases, the induction phase and the testing phase as previously described by Porsolt et al.<sup>[9]</sup> The equipment for this experiment is a cylindrical Perspex tank (50 cm height, 18 cm diameter), filled to a depth of 30 cm with water that was kept at constant temperature of  $27 \pm 1$  °C and the water depth was always adjusted according to the rat's size, so that it cannot touch the bottom of the container with its hind legs. Briefly, during the induction phase, the animals were placed in water cylinder for 15 minutes, towel dried and returned to their home cages. Those rats that were able to stay afloat during 15 minutes training were separated and used for the test stage. After 24 hours the animals were subjected to the test stage. In the test stage rats were divided into five (5) groups (n=5). Group I received the vehicle (negative control), Groups II, III and IV received VAE, 100, 200 and 400 mg/kg per oral, respectively, while group V received amitriptyline (standard agent) 15 mg/kg. Thirty minutes after drug or extract administration, each rat was placed in the water cylinder for 5 minutes and monitored for duration of immobility within the period.<sup>[10]</sup> The animal was then removed from the cylinder, dried and kept back to the cage. After testing each animal, the cage was washed and refilled with fresh water. The duration of immobile was whenever rat remained floating passively in the water in a slightly hunched but upright position with its head just above the water surface.

### Phenobarbitone induced sleeping time test

Albino mice were divided into 5 groups (n=5). Group I received the vehicle, groups II, III and IV received the VAE 100, 200 and 400 mg/kg, respectively, while group V received diazepam (3 mg/kg). The administration extracts and drugs were done orally and each was given 30 minutes prior to the administration of phenobarbitone (35 mg/kg). Afterwards each animal was monitored for the latency or onset of sleep and duration of sleep. The onset of sleep was recognized by the loss of righting reflex (sleep induction time in minutes) and the time required to recover righting reflex (sleeping time) as previously described.<sup>[11]</sup>

### Acute toxicity test (LD<sub>50</sub>)

Determination of the acute toxicity and lethality (LD<sub>50</sub>) of VAE was performed in albino mice using the method described by Lorke.<sup>[12]</sup> Briefly, twelve (13) adult albino mice (20-30 g) of either sex with free access to food and clean water were used for the test. The animals here were divided into three (3) groups of three (3) animals per group. The 3 groups received accordingly, oral doses

of 10, 100 and 1000 mg/kg VAE, after which the animals were allowed free access to food and water, and monitored for deaths over a period of 24 hours. The mortality rate was observed and recorded and the results obtained were used as a guide in the stage two of the experiment. The result of the first stage showed no deaths in all the administered doses. Hence, higher doses of the extract; 1600, 2900 and 5000 mg/kg of VAE were further administered to one rat each. The mice were allowed free access to food and water after which they were observed for death over a 24-hour period. The results were obtained and recorded.

### Statistical Analysis

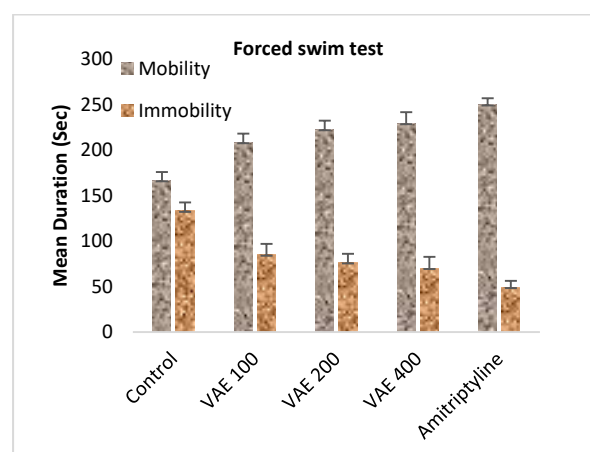
Data obtained were analyzed and expressed using one-way analysis of variance (ANOVA; SPSS Version 18) and expressed as Mean  $\pm$  SEM. Differences between mean were regarded significant at  $P \leq 0.05$ , using Dunnett's post hoc test.

### Acute toxicity test

The acute toxicity test showed that the LD<sub>50</sub> of VAE is estimated to be 1250 mg/kg per oral in mice. This is also an indication of its relative safety.

## RESULTS AND DISCUSSION

In this study we evaluated the antidepressant effect of *Voacanga africana* root bark in rodents. The extractive yield of the pulverized root bark was 54.5 g equivalent to 6.8% w/w of the starting material. In forced swim test, *Voacanga africana* extract (VAE) showed abolition of depressive-like behavior in the FST in a dose dependent fashion, with amitriptyline (15 mg/kg) and VAE (400 mg/kg) showing highest reduction of the immobility time to 49.50 and 70.40 seconds, respectively compared to 133 seconds of the control (Figure 1). However, as the duration of immobility decreases, the duration of mobility or struggling time consequently increases. Forced swim test (FST) is one of the approved animal neuropharmacological models to study the antidepressant effect of extract, bioactive compounds and pharmacological agents.

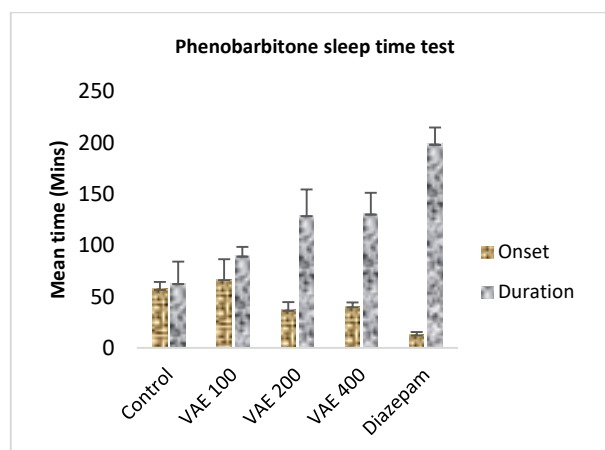


**Figure 1:** Duration of mobility and immobility of Forced swim test.

All values are significant at  $P < 0.05$ . VAE doses on mg/kg while amitriptyline is 15 mg/kg,  $n=5$ .

The FST is based on the assumption that immobility is a measure of behavioral despair in animals resembling a hopeless situation and inability to continue struggling.<sup>3</sup> *Voacanga africana* root bark extract (VAE) in this study corroborated with standard tricyclic antidepressant, amitriptyline, and reduced the immobility time in the FST. Reports have shown that similar herbal extracts used in folkloric medicine as well as standard agents used in the treatment of depression also reduced the immobility time in FST<sup>13, 14</sup> It is a pharmacological fact that most of the atypical antidepressants cause sedation among other established side effects. Hence in phenobarbitone induced sleep time test, the VAE significantly ( $p < 0.05$ ) and dose dependently, potentiated the onset of sleep and duration of sleep in tested animals. At 400 mg/kg dose VAE almost doubled the duration of sleep while diazepam (3 mg/kg), a standard agent, tripled same parameter (Figure 2). Therefore, the potentiation of sleeping time by the VAE may have been correlated with the mechanisms of central inhibition often associated with sedatives which calms and sedates the recipient, a desirable feature in controlling depression. In addition, standard antidepressant agents such as haloperidol might decrease dopaminergic transmission and prolongs barbiturate induced sleep by decreasing the activity of the nigrostriatal and mesolimbic dopaminergic pathway involved in critical activation and behavioural arousal as well as sensitizing the CNS to the depressant action of barbiturate.<sup>15</sup> Dopamine, serotonin and norepinephrine are neurochemicals that mediate the feeling of urge to go for reward and pleasurable tendencies and their depletion or lesion of neurons that secrete them might trigger pathological depression in humans.<sup>16</sup> Furthermore, the specific bioactive compound responsible for the antidepressant effect of VAE cannot be identified at this stage of the work, however reported phytochemical test on *V. africana* showed the presence of flavonoids, tannins, terpenoids, steroids and alkaloids.<sup>17</sup> Ibogaine, together with related alkaloids isolated from *V. africana*, has been shown to possess psychedelic, anti-addiction and stimulant activities<sup>7, 18</sup> and these compounds may be considered to be contributing to the overall antidepressant effects of *V. africana*. In our earlier study, terpenoids, flavonoids, glycosides, saponins and alkaloids have been reported to possess potent central nervous system effects.<sup>19</sup>

The mechanism through which the VAE exhibited the antidepressant effect might be related to the general mechanism of action of antidepressants which is inhibition of metabolism of neurochemicals such as serotonin and nor-epinephrine. The acute toxicity study showed that the  $LD_{50}$  of VAE was estimated to be 1250 mg/kg an indication of a relative safety and portrays the plant a potential candidate for further experimental research as proposed by Lorck.<sup>12</sup>



**Figure 2:** Mean time of onset and duration of sleep

VAE at 200 and 400 mg/kg and Diazepam showed significant value at  $P < 0.05$ ;  $n=5$ .

## CONCLUSION

Results of the study showed that *Voacanga africana* root bark extract exhibited antidepressant as well as sedative activities in rodents by reduction of the immobility time in forced swim test and potentiation of sleep in phenobarbitone induced sleep. Further research is required to isolate the possible bioactive compound responsible for the claimed activity.

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