Toxicity and Sedative Effect of *Voacanga Africana* Ethanolic Leaf Extract

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Received 10 September 2019; Revised 15 October 2019; Accepted 31 October 2019

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**Background:** Traditional plants have been good sources of alternatives for conventional drugs in recent years. The search for alternatives for conventional drugs becomes imperative most especially because of their high cost demand and side effects. The search for alternatives from traditional medicinal plants sources has been fruitful for many ailments and this has led to intense research on the potentials of many other plants that are used traditionally in different part of the world. This study assessed the sedative potentials of ethanol extract of *Voacanga africana* leaf (VAEE) as well as its toxicity.

**Methods:** Toxicity of the *Voacanga africana* leaf was examined using Lorké’s method while its sedative activity was evaluated using phenobarbitone and ketamine-induced sleeping time models in mice. The doses used in this study were 50, 100, 200, 400 and 800 mg/kg, administered intraperitoneal.

**Results:** Results revealed that the LD$_{50}$ of VAEE was greater than 5 g/kg. Using phenobarbitone-induced sedation, the sleep latency was found to be dose dependent and significantly decreased at higher dose when compared with normal saline while the total sleeping time was found to be significantly increased (p<0.05) as dosage increases.

**Conclusion:** The increase in total sleeping time using the two models showed that VAEE possesses marked sedative ability and can serve as a good alternative to conventional sedatives.

**Keywords:** *Voacanga africana*, Traditional medicine, Sedation, Phenobarbitone, Ketamine

1.0 INTRODUCTION

Traditional medicine using different plant parts have been one of the mainstays of disease treatment in Africa. It is the sole medical system for healthcare before the advent of conventional medicine [1]. Evidence based traditional medicine has shown that some of these herbs are potent in alleviating many bad health conditions [2]. Traditional medicine has received due attention in recent time due to its accessibility, economic viability, strong pharmacological potential and lesser side effects as compared to conventional ones [3]. Traditional medicine involves the extraction of different medicinal substances called phytochemicals found in different plant parts. These plant extracts usually contain spectrum of phytochemicals ranging from flavonoids to alkaloids, and terpenoids to anthraquinones [4]. In the development of new chemotherapeutic agents, medicinal plants are considered important in identifying potential useful chemical structures [5, 6]. Often times, the extracts are taken in the form of a concoction combining more than one plants, however, there are cases involving use of extract from single plant for the treatment of specific ailments. This aspect of traditional medicine has really aided conventional medicine as results of phytochemical screening of extracts found in concoctions that have formed the basis for the development of new drugs [7], thereby opening new area of research in pharmacology. Medicinal plants have long been exploited as sedatives [15]. Sedatives are substances used in treating sleeping disorders. These conventional sedating drugs used in addressing sleeping disorders are known with some side effects, tolerance and addiction issues and these have made imminent the search for alternatives [16].

One of the very good plant used in tropical Africa for traditional medicine is Voacanga Africana. This plant is a tropical rain forest shrub also found in the Guinea Savannah woodland belt [4]. It is cosmopolitan in all parts of West Africa [8, 9] and other countries such as Tanzania and Congo [10]. A mature V. africana crop is not more than 10 m tall. It has lowly branched stem with smooth grayish white bark. Leaves are simple, petiolate and decussately arranged. The flowers are pedicellate, mildly scented, corolla lobed with overlapping aestivation. The ovary is superior and bicarpellary. V. africana fruits are globose berry with brownish–white blotches having dark, bean–shaped seeds with denticulate ornamentation [11].

Various parts V. africana plant has been used for treatment of many diseases. Its bark is used in the treatment of a wide range of diseases [12]. Leaves and roots decoction are known for their antinociceptive activity [9], antioxidant and anti-inflammatory properties [13], antiulcer properties [10], usefulness in treatment of anaemia and other blood disorders [14] as well as in the treatment of malaria, diarrhea, infant convulsion, and heart arches [10]. According to Hussain et al [8], the medicinal properties of V. africana is attributed to more than 100 phytochemicals (indole alkaloids, ibogain, tabernanthin, ibogamine, vincanol, vincamone etc.) found in different parts of the plant. Considering the various reports on the medicinal use of V. Africana, coupled with the fact that the sedative and anxiolytic properties are not yet well documented, this research seeks to study the sedative activity of ethanolic extract of V. Africana leaf as well of its toxicity.

2.0 METHODOLOGY

2.1 Study Location

This study was conducted between October 2017 and April 2018 at the Department of Pharmacology, Faculty of Pharmacy, Obafemi Awolowo University (OAU), Ile-Ife, Osun State, South-West, Nigeria.

2.2 Plant Collection and Identification

Fresh leaves were collected in November 2017 from a wild V. africana tree growing near the University Health Centre, Obafemi Awolowo University (OAU), Ile-Ife, South-West, Nigeria. Leaf samples were identified and authenticated by an expert botanist, after which they were deposited at the Pharmacognosy herbarium (FPI 2134) of the same institution for research and reference purposes.

2.3 Extract Preparation

Fresh leaves of V. africana were oven-dried and coarsely powdered. 800g of the powdered leaf was used in the extraction process. Extraction was carried out in cycles in which 200 g of the powdered leaf was extracted using 200 ml of 50% v/v ethanol at a temperature of 70℃ in soxhlet apparatus, and each cycle lasted for 48 hours. The mixture obtained was pooled and filtered using Whatman Filter paper number 1 and the solvent from the filtrate was removed by air-evaporator under reduced pressure and low temperature. The V. africana ethanolic extract (VAEE) was then stored at 4℃ pending use.

2.4 Experimental Animals

Eighty-three Swiss albino male mice (Mus musculus), 3 – 4 weeks old weighing between 18g and 25g were obtained from the animal house, Department of Pharmacology,
Faculty of Pharmacy, Obafemi Awolowo University, Ile Ife. Animals were maintained under standard environmental conditions (28 ± 1°C, 70 – 80 % humidity, 12 hour light/dark cycle) at the Drug, Research and Production Unit laboratory, OAU, Ile Ife. The animals were fed with standard rodent diet (Livestock Feed Plc, Lagos, Nigeria) and water ad libitum. The mice were acclimatized for fourteen days before the commencement of the experiment. The cages were cleaned once a week with five mice (n=5) per cage. All procedures were approved by the committee on animal use and care of the Obafemi Awolowo University, Ile-Ife, Nigeria and conducted in accordance with internationally accepted principles for laboratory animal use and care [17].

2.5 Determination of LD50

The acute toxicity profile of the ethanol leaf extract of *V. africana* was determined using the method described by Lorke [18]. Three mice each were orally fed with 10, 100, 1000mg/kg of body weight of VAEE and a mice each was fed with 1600, 2900 and 5000mg/kg of body weight VAEE. Mortality was then recorded after 24 hours in each group.

2.6 Effects of the Extracts on Phenobarbitone-Induced Sleeping Time

Healthy albino mice (35) weighing between 18 and 25 g were fasted for 24 hours before the experiment. They were randomly assigned into seven groups (n=5) per group. Group I was administered with normal saline (NS) (10 ml/kg, i.p), group II was treated with standard drug diazepam (DZM) (1 mg/kg, i.p), group III, IV, V, VI and VII were treated the extract VAEE 50, 100, 200, 400 and 800 mg/kg, i.p respectively. After 30 minutes of administration of extracts, Phenobarbitone sodium (40 mg/kg, i.p) was administered 30 minutes after pretreatment. The sleep latency is defined as the time in minutes after treatment with phenobarbitone that the animal presents with loss of righting reflex whilst the time in minutes between loss and regain of righting reflexes was taken as a index of hypnosis or sleeping time [19].

2.7 Effects of the Extracts on Ketamine-Induced Sleeping Time

Thirty-five mice weighing between 18 and 25 g were randomly allotted into seven groups. Group I was administered with 10 ml/kg normal saline (i.p). Group II was injected with 1 mg/kg (i.p) of diazepam (standard reference drug). Groups III - VII were injected with various doses (50, 100, 200, 400 and 800 mg/kg, i.p respectively) of VAEE. Ketamine (100 mg/kg, i.p) was administered 30 minutes after the administration of test drugs. Sleep latency and total sleeping time was calculated as described for phenobarbitone-induced sedation.

2.8 Statistical Analysis

GraphPad prism, version 7.04 (UK) was used for the analysis. Data were analysed using one-way analysis of variance (ANOVA), followed by Dunnett’s multiple comparison test between the treated groups and controls. The results obtained were expressed as Mean ± SEM. The level of significance was set at 95% confidence interval for all treatment.

3.0 RESULTS

3.1 LD50 Determination

Results of acute toxicity study (Table 1) showed that ethanol extract of *V. africana* leaf via the oral route was not toxic. The value of the LD50 was ≥5000 mg/kg body weight. No mortality was recorded at the highest dose (5000 mg/kg body weight) tested.

Table 1: Summary of Acute Toxicity Test of Ethanolic Extract of *V. africana* Leaf in Mice.

<table>
<thead>
<tr>
<th>Dose (mg/kg, orally)</th>
<th>Mortality after 24</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0/3</td>
</tr>
<tr>
<td>100</td>
<td>0/3</td>
</tr>
<tr>
<td>1000</td>
<td>0/3</td>
</tr>
<tr>
<td>1600</td>
<td>0/1</td>
</tr>
<tr>
<td>2900</td>
<td>0/1</td>
</tr>
<tr>
<td>5000</td>
<td>0/1</td>
</tr>
</tbody>
</table>

3.2 Effect of Ethanol Extract of *Voacanga africana* Leaf on Phenobarbitone-induced Sedation

Results as shown in Table 2 revealed that diazepam and VAEE of 200, 400 and 800 mg/kg caused a dose-dependent significant reduction (p<0.05) in sleep latency (SL) induced by phenobarbitone (40 mg/kg) when compared with normal saline control (10 ml/kg) but was higher than the lower doses; 50 and 100 mg/kg body weight. Also, VAEE caused a significant increase (p<0.05) in the total sleeping time (TST) induced by phenobarbitone (40 mg/kg) when compared with normal saline (10 ml/kg) only at 200, 400 and 800 mg/kg.
3.3 Effects of Ethanol Extract of *Voacanga africana* leaf on ketamine-induced sedation

VAEE of 100, 200, 400 and 800 mg/kg caused a dose-dependent significant increase (p<0.05) in sleep latency induced by ketamine (100 mg/kg) compared to normal saline (10 ml/kg) . VAEE of 50, 100, 200, 400, and 800 mg/kg caused a dose-dependent significant increase (p<0.05) in the total sleeping time (TST) induced by ketamine (100 mg/kg) compared to normal saline (10 ml/kg), however, the TST for VAEE 50 and 100 mg/kg were significantly lower (p<0.05) when compared to diazepam while other tested doses were higher with only VAEE of 800 mg/kg body weight significantly higher (p<0.05) than the rest treatments (Table 3).

### Table 2: Effect of Ethanol Extract *Voacanga africana* of Leaf (VAEE) on Phenobarbitone-Induced Sedation.

<table>
<thead>
<tr>
<th>Treatment groups (n=5)</th>
<th>Dose (mg/kg)</th>
<th>Sleep Latency (Min)</th>
<th>Sleeping Time (Min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Saline (10 ml/kg)</td>
<td>10</td>
<td>20.8±0.33</td>
<td>132 ±1.72</td>
</tr>
<tr>
<td>Diazepam</td>
<td>50</td>
<td>21.6±0.82</td>
<td>103 ±3.73</td>
</tr>
<tr>
<td>Group III</td>
<td>200</td>
<td>13.2±0.77</td>
<td>403 ±6.81</td>
</tr>
<tr>
<td>Group IV</td>
<td>400</td>
<td>10.6 ±1.64</td>
<td>446.6±10.1*</td>
</tr>
<tr>
<td>Group VII</td>
<td>800</td>
<td>10.4 ±0.64</td>
<td>485.5±12.46*</td>
</tr>
</tbody>
</table>

*p* = 0.05. Results for sleep latency and sleeping time are expressed in means±S.E.M. (n=5)

### Table 3: Effects Of Ethanol Extract of *Voacanga africana* (VAEE) Leaf on Ketamine-Induced Sedation.

<table>
<thead>
<tr>
<th>Treatment groups (n=5)</th>
<th>Dose (mg/kg)</th>
<th>Sleep Latency (Min)</th>
<th>Sleeping Time (Min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Saline (ml/kg)</td>
<td>10</td>
<td>4.0 ± 0.4</td>
<td>56.4±2.58</td>
</tr>
<tr>
<td>Diazepam</td>
<td>1</td>
<td>4.4 ± 0.2</td>
<td>125.8±0.77</td>
</tr>
<tr>
<td>Group III</td>
<td>50</td>
<td>1.7±0.52*</td>
<td>77.4±1.37*</td>
</tr>
<tr>
<td>Group IV</td>
<td>100</td>
<td>5.8± 0.34*</td>
<td>96.0±1.06*</td>
</tr>
<tr>
<td>Group V</td>
<td>200</td>
<td>6.2 ±0.52*</td>
<td>127.0±1.20*</td>
</tr>
<tr>
<td>Group VI</td>
<td>400</td>
<td>6.3 ± 0.22*</td>
<td>129±2.21*</td>
</tr>
<tr>
<td>Group VII</td>
<td>800</td>
<td>5.4 ± 0.22*</td>
<td>142±0.40*</td>
</tr>
</tbody>
</table>

*p* = 0.05. Results for sleep latency and sleeping time are expressed in means±S.E.M. (n=5)

4.0 DISCUSSION

Folklore medicine has evidence of effectiveness of herbs in treating various sleeping disorders [20]. Conventionally, these disorders are also managed by modern medicinal drugs. Sleeping disorder is one of the common disorders and it is conventionally managed by drugs containing sedative substances. The use of these conventional sedating drugs is accompanied with negative feedbacks ranging from addiction to other side effects of the drugs. With growing concerns about the side effects of conventional sedative drugs, assessment of sedative potential of various extracts from different herbs become imminent. In a bid to finding alternatives to current conventional drugs, this study assessed the toxic impact and sedative potentials of ethanol extract of V. africana leaf using ketamine- and phenobarbitone-induced sedation models. According to Lorke's model [18], the LD50 is an index of acute toxicity and it is considered to be of little or no practical importance at values above 5.0g/kg. The result from the toxicity study shows that no mortality was recorded at 5000 mg/kg, therefore, the LD50 values of VAEE is >5 g/kg. Hence, as described in Lorke’s classification, the ethanol extract of V. africana leaf is non-toxic. The non-toxic nature of VAEE may account for the widespread and continuous use of the plant leaf as a safe herbal agent in traditional medicine in Africa.

The sedative-hypnotic action of phenobarbitone and other barbiturates is due to their interaction on the GABAA receptors which enhances GABAA transmission [21]. The binding site is distinct from that of benzodiazepines. Barbiturates potentiate GABA action on chloride entry into the neuron by prolonging the duration of the chloride-channel openings [22]. In addition, barbiturates can block excitatory glutamate receptors. VAEE was observed to shorten the onset of sleep in phenobarbitone-induced sedation at doses of 100, 200, 400 and 800 mg/kg except at 50 mg/kg, which might be due to its low dose compared to the other doses. At all the doses, VAEE also prolong the duration of sleep when using phenobarbitone-induced sedation model. This was similar to the findings of Fujimori [23] and Garige et al. [24] who proposed that the enhancement of the barbital hypnosis is a good index of central nervous system (CNS) depressant activity. It is known that substances possessing CNS depressant activity either decrease the time for the onset of sleep or prolong the duration of sleep or both. The result of phenobarbitone-induced sedation for VAEE revealed that VAEE possesses strong...
sedative ability as it decreased onset of sleep in mice and would allow users sleep easily as well as prolong the duration of the sleep.

Ketamine-induced sedation is one of the models used extensively in screening of sedatives and anaesthetics [25]. The model exploits both the uncompetitive antagonistic ability of ketamine to N-methyl-D-aspartate (NMDA) type of excitatory receptor system [26, 27] and the sedative effect of ketamine by gamma-amino butyric acid-A (GABAA) receptor potentiation [21]. In this study, 50, 100, 200, 400 and 800 mg/kg of VAEE was used to potentiate ketamine-induced sedation in mice in a dose-dependent manner. Onset of sleep (sleep latency) was significantly (p<0.05) decreased at 50 mg/kg and increases at higher doses of 100, 200, 400 and 800 mg/kg, this suggested that VAEE may have an excitatory effect at higher doses. This effect may be explained with an understanding of the underlying principle through which VAEE potentiate ketamine-induced sleep, as it may be due to the blockade of the NMDA receptor or rather the modulation of the GABAA receptor. This calls for concern as this may subject the plant to abuse and undermine its role as a sedative in some patients. Hence, the plant should be administered with caution at bedtime as it can interfere with the sleep pattern of the patient. Edi

However, VAEE increased the time of sleep significantly at all tested doses when compared with normal saline but it effects when compared with the standard drugs used in this study (Diazepam) was significantly lower at 50 and 100 mg/kg and significantly higher at 800 mg/kg. At 200 and 400 mg/kg, the impact of VAEE on sleeping time was in same range as that of Diazepam. Therefore, the effectiveness of VAEE in increasing sleeping time at higher doses when compared with the standard drugs established it sedative potentials. The sleep latency and duration of sleep in ketamine-induced sedation was observed to be shorter than that of phenobarbitone-induced sedation. This could be due to the fact that phenobarbitone is one of the longest-acting barbiturates (half-life of two to seven days) unlike ketamine whose duration of action is 30 minutes to 2 hours [28].

The increase in the incidence of addiction and other unwanted side effects such as drowsiness, drug “hangover” and confusion when conventional sedatives such as benzodiazepines and barbiturates are administered to patients calls for the development of better alternatives. A good source of these alternatives can be found in traditional medicinal plants such as V. africana. They possess very strong prospects of being the most probable short term sustainable solution to sleep disorders if they are fully exploited as sources of new sedative agents.

In conclusion, the present study indicated that ethanolic extract of Voacanga africana (VAEE) is non-toxic as no mortality was recorded as at 5g/kg. Using the Phenobarbitone-induced model, VAEE decreased onset of sleep and increased the sleeping time, thereby suggesting that the plant possesses sedative potentials. In slight contrast to the activities of VAEE using the Phenobarbitone-induced model, VAEE increased both onset of sleep and sleeping time when assessed using the ketamine-induced model. While elongation of sleeping time in both models suggests the plant possesses sedative potentials, the contrast in the onset of sleep in both models needs to be further research on. Also, further studies are needed to characterise the active compound (s)/metabolites responsible for its pharmacological activities and also examine the underlying mechanisms for its sedative effects.

Acknowledgements

The authors acknowledge the support of Mr I. I Ogunlowo for the identification of leaf samples and Miss Saheedat Tobiloba Adetayo for her assistance in reviewing the manuscript.

Conflict of interest

The authors declare that they have no conflicts of interest.

Authors Contribution

OIA conceived and designed the study, performed analysis, drafted and revised the manuscript. OAQ conceived and designed the study. Performed data collection and contributed to data analysis tools. HS contributed to study design and data analysis tools. OM contributed to data collection and writing of the manuscript. FM contributed to data collection and data analysis tools. All authors approved the final manuscript.

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