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Neuropharmacological activities of the aqueous fraction of methanol extract of *Securinega virosa* (Roxb. ex. Willd) Baill. root bark in mice

Mohammed G. Magaji[1], Kabiru Abubakar[2*] and Faruk Faruk[3]

[1]*Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. Nigeria.*
[2]*Department of Pharmacology and Toxicology, Usmanu Danfodiyo University, Sokoto. Nigeria.*
[3]*Department of Human Physiology, Gombe State University, Gombe. Nigeria.*

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Abstract
Securinega virosa (Euphorbiaceae) is a commonly used medicinal plant in the management of epilepsy and mental illnesses. Previously, the anticonvulsant and antipsychotic activities of the methanol root bark extract have been reported. In an attempt to isolate the bioactive principle(s) responsible for these activities, this study was designed to evaluate the anticonvulsant and CNS depressant activities of the aqueous fraction of the root bark extract in mice. The fraction at the dose of 500 mg/kg protected 66.67% of the mice against pentylenetetrazole (PTZ)-induced seizure and prolonged the onset of seizure in unprotected animals. The fraction did not offer significant protection against strychnine and 4-aminopyridine-induced seizures. At all the doses tested (125-500 mg/kg), the fraction significantly ($p < 0.05$) reduced the number of head dips (in the hole board test), upward stairs climbed and rearings (in the stair case test). In the beam walking assay, the fraction significantly ($p < 0.05$) increased the number of foot slips. The findings of the study suggested that aqueous fraction of the methanol root bark extract of *Securinega virosa* possesses anti-PTZ and sedative activities; and further lend credence to the use of the root of the plant in the management of epilepsy and mental illness.

Keywords: *Securinega virosa*; Epilepsy; Sedatives; Pentylenetetrazole

INTRODUCTION

Epilepsy is a chronic disorder characterized by recurrent seizure. Approximately 1% of the world's population has epilepsy, the second most common neurologic disorder after stroke. Although standard therapy permit control of seizure in 80% of the patients, millions have uncontrolled epilepsy [1]. Most of the currently available anti-epileptic drugs (AEDs) are associated with serious side effects, drug interactions as most AEDs are consumed for life, and expensive making traditional practitioners and medical herbs an option for patients [2]. Antipsychotics, the cornerstone of treatment of psychosis (a chronic recurrent neuropsychiatric disorder that alters the quality of life of the sufferers) are associated with a number of shortcomings, including poor efficacy and high toxicity profile [3]. Most people of the developing world rely on traditional medicine for their health care needs which makes it necessary to intensify research on medicinal plants with

* Corresponding author. *E-mail*: kabirsultan2002@gmail.com *Tel*: +234 (0) 8035863780
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goals of developing phytomedicines and "lead" compounds in drug development process. *Securinega virosa* is one of the most commonly used medical plants in West Africa [4]. Previously, the anticonvulsant and antipsychotic activities of the methanol root bark extract have been reported [2, 5]. In an attempt to isolate the bioactive principle(s) responsible for these activities, this study was designed to evaluate the neuropharmacologic-al activities of the aqueous fraction of the root bark extract in mice.

EXPERIMENTAL

Collection and identification of plant material. The whole plant, *Securinega virosa* was collected from Basawa town, in Sabon-Gari Local Government Area of Kaduna State, Nigeria, in November, 2012. The plant was identified and authenticated by Malam Musa and Umar Galla of the Herbarium Section in the Department of Biological Sciences, Ahmadu Bello University, Zaria, by comparing with existing specimen (NO 918). A voucher specimen was deposited at the herbarium for future reference.

Preparation of the aqueous fraction. The root was cleaned and the bark removed. The root bark was air dried, and crushed into coarse powder. The powered root bark (1000 g) was extracted with 2 L methanol (absolute) for 72 hours using Soxhlet extraction apparatus. The solvent was evaporated on a water bath at 40ºC to give a dark brownish residue (Average yield: 9.82 % w/w). About 50 g of the crude methanol extract was dissolved in water and filtered. The filtrate was successively partitioned with petroleum ether, chloroform, ethyl acetate and n-butanol. The mother liquor was subsequently referred to as the aqueous fraction (RAF).

Preliminary phytochemical screening. The crude extract and the fraction were subjected to preliminary phytochemical screening using standard protocols [6].

Animals. Swiss Albino mice of either sex (18 -22 g), were obtained from the Animal House Facility of the Department of Pharmacology and Therapeutics, Ahmadu Bello University Zaria, Nigeria. The animals were maintained in a well-ventilated room in the Animal house. They were fed on standard laboratory animal feed and water *ad libitum.* All experiments performed on laboratory animals in this study were in accordance with Ahmadu Bello University Research policy and followed the "principles of laboratory animal care" [7].

For each study, thirty (30) mice were randomly divided into five groups each consisting of six mice. The first group was treated with normal saline, the second, third and fourth groups were administered with the aqueous fraction (125-500 mg/kg) while the last group was treated with the standard drug. The fifth group was administered with sodium valproate 200 mg/kg (PTZ model), Phenobarbitone 30 mg/kg (for Strychnine and 4-Aminopyridine models) and diazepam 0.5 mg/kg (staircase test) 2mg/kg (hole board and beam walking assays). All drug treatments were by the intraperitoneal route (except where mentioned otherwise).

Acute toxicity study. The method previously described by Lorke [8] was adopted for the estimation of the median lethal doses (*po* and *i.p*) of the aqueous fraction in mice

Anticonvulsant studies

Pentylenetetrazole-induced seizure in mice. Thirty minutes post treatment with normal saline, fraction and standard drug, mice in all the groups received 85 mg pentylenetetrazole per kg, *s.c.* Mice were observed over a period of 30 minutes. Absence of an episode of clonic spasm of at least 5 seconds duration indicated a compound's ability to abolish the effect of pentylenetetrazole on seizure threshold [9].

Subcutaneous strychnine-induced seizure in mice. Thirty minutes post -treatment, mice in all the groups received 1.5 mg strychnine per

kg. The proportion of mice presenting convulsions as well as the onset of tonic convulsions was recorded. Abolition of tonic extensor jerks of the hind limbs within 30 minutes after strychnine administration was considered an indicator that the testing material could prevent strychnine-induced convulsions [10].

4-Aminopyridine-induced seizure in mice. The method adopted for this study was similar to those described previously [11, 12]. 30 minutes post treatment; 4-Aminopyridine was administered at a dose of 15 mg per kg body weight to each mouse. The mice were observed for 30 minutes for characteristic behavioural signs, such as hyperactivity, trembling, intermittent forelimb and hind limb clonus followed by hind limb extension, tonic seizures, opisthotonus and death. Ability of the extract to protect the mice from lethality within 30 minutes observation period was considered as an indication for anti-convulsant activity [12].

Behavioural Studies

Test for exploratory behaviour in mice. The method for the hole- board test in mice was similar to those described previously [13]. The apparatus used was a white painted wooden board (60 cm x 30 cm) with 16 evenly spaced holes (1cm diameter x 2 cm depth). Thirty (30) minutes post- treatment, each mouse was placed at a corner of the board and the number of head dips on the hole was counted using a tally counter during a 5 minute period [14]. A head dip was considered when the mouse dipped its head into the hole to the level of the eyes.

Staircase test in mice. The device used in this study consists of a wooden staircase similar to the one described by Simiand [15]. Thirty (30) minutes before placing each mouse individually on the floor of the Perspex box (with its back to the staircase), five groups each consisting of six mice were treated with normal saline, fractions or standard drug (diazepam 0.5 mg/kg). The behaviour of each mouse videotaped and number of upward steps climbed and rearings were recorded over a 5 minutes period. A step was considered climbed if a mouse placed all its four paws on it. Rearing was counted when a mouse rose on its hind limb both against the wall and on a step. The staircase was wiped with 70% ethyl alcohol and allowed to dry between tests to remove any olfactory cue.

Mouse beam walking assay in mice. The method previously described by Stanley [16] was adopted with some modification for this study. Mice were trained to walk from a start platform along a ruler (80 cm long, 3 cm wide) elevated 30 cm above the bench by metal support to a goal box. Three trials were performed for each mouse, and were designed such that the mouse tested would be aware that there was a goal box that could be reached. Thirty (30) minutes post- treatment, each mouse was placed on the beam at one end and allowed to walk to the goal box. Mice that fell were returned to the position they fell from, with a maximum time of 60 s allowed on beam. The number of foot slips (one or both hind limb slipping from the beam) was recorded with the aid of a tally counter. The time taken to complete the task was also recorded.

Statistical analysis. Data were presented as Mean ± SEM and analysed using One-Way ANOVA followed post hoc Dunnet t-test for multiple comparison. A p value less than 0.05 was considered significant.

RESULTS AND DISCUSSION

The present study investigated the neuropharmacological activity of the aqueous fraction. The fraction contained similar classes of phytochemicals found in the crude methanol root bark extract. These include alkaloids, tannins, saponins, flavonoids, terpenes among others. The intraperioneal median lethal dose of the fraction was found to be greater than 2,000 mg/kg while the oral was found to be greater than 5, 000 mg/kg

suggesting that it is practically nontoxic orally [8]. The aqueous fraction produced a dose-dependent protection with the highest dose (500 mg/kg) producing 66.67% protection (Table 1). RAF at the dose of 250 mg/kg significantly ($p < 0.05$) increased the mean onset of seizure. Pentylenetetrazole (PTZ) is a known convulsant and anticonvulsant effect against PTZ test identifies compounds that can raise seizure threshold in the brain. The PTZ-induced seizures are similar to the symptoms observed in the absence seizures and drugs useful in treatment of absence seizures suppress PTZ-induced seizures [17]. Moderate anti-convulsant activity of the fraction against PTZ-induced seizures suggests the presence of bioactive compounds effective in the therapy of absence or myoclonic seizures.

The fraction did not protect the mice against strychnine-induced seizure. There was also no significant increase in the mean onset of seizure compared with the normal saline treated group. The fraction also did not protect the mice against 4-Aminopyridine-induced seizure (Table 1). These suggest that the activity of the fraction may not involve interaction with the glycine receptors or the potassium channels.

The aqueous fraction significantly ($p < 0.05$) and dose-dependently decreased the number of head dips in the hole board test (Figure 1). The Hole-board test is a simple model for measuring exploratory activity in rodents [18]. The behavioral head dipping in hole-board assay is sensitive to changes in the emotional state of the animals and increase in head dipping behavior is a reflection of anxiolytic activity [19]; whereas a decrease in the parameter reveals a sedative behavior [20].The aqueous fraction (RAF) significantly ($p < 0.05$) and dose-dependently decreased the total number of upward stairs climbed. It also significantly ($p < 0.001$) decreased the total number of rearing (Figure 2). Compounds that reduce rearing activity are said to possess anxiolytic activity [21]. Non-benzodiazepine compounds, such as neuroleptics, tricyclic antidepressants, and buspirone have been found to suppress both rearing and climbing behaviour in the staircase test. It is therefore plausible to suggest that since the fraction decreased both parameters (rearing and step climbed), non-preferentially, it possesses central depressant activity.

The mouse beam walking assay is a more sensitive model than rotarod in predicting clinical sedation in humans caused by novel drugs [16], the fraction did not significantly increase the number of foot slips, an index of motor coordination deficit, thus suggesting that the depressant effect of the fraction may be centrally mediated and not due to peripheral muscular blockade [22].

Table 1: Effect of aqueous fraction of methanol root bark extract of *Securinega virosa* against some chemo-convulsants in mice

Treatment (mg/kg)	Chemo-convulsants					
	PTZ		Strychnine		4-AP	
	Quantal protection	Mean onset of seizure	Quantal protection	Mean onset of seizure	Quantal protection	Mean onset of seizure
N/Saline	0/6	3.5 ± 0.43	0/6	6.50± 0.43	0/6	14.40 ± 3.31
RAF 125	1/6	6.00 ± 1.00	0/6	7.83 ± 1.01	0/6	12.67 ± 1.26
RAF 250	2/6	7.25 ± 1.60*	0/6	6.83 ± 0.40	0/6	14.67 ± 2.03
RAF 500	4/6	4.0 ± 0.00	0/6	7.83 ± 0.83	0/6	13.83 ± 1.08
VPA 200	6/6	-	n/a	n/a	n/a	n/a
PBT 30	n/a	n/a	6/6	-	5/6	22.33

Data presented as Quantal protection and Mean ± SEM; * $p < 0.05$ (Dunnet post hoc test; compared with the control); n=6; RAF (Aqueous fraction); VPA (Sodium valproate); PBT (Phenobarbitone); n/a= not applicable

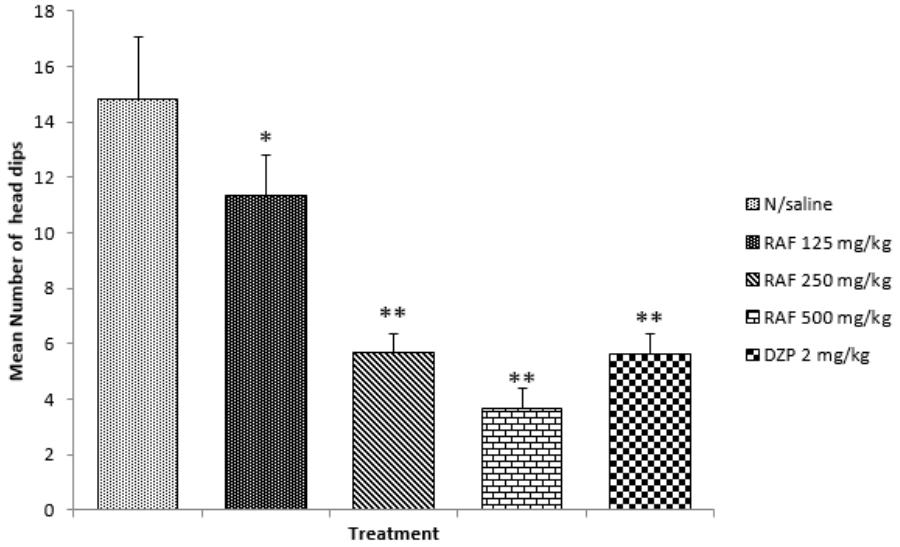

Figure 1: Effect of aqueous fraction of methanolic extract of *Securinega virosa* root bark on exploratory activity of mice in hole board test; RAF (aqueous fraction); DZP (Diazepam); number of heap dips presented as mean ± SEM; *P<0.01; **P< 0.001; n=6

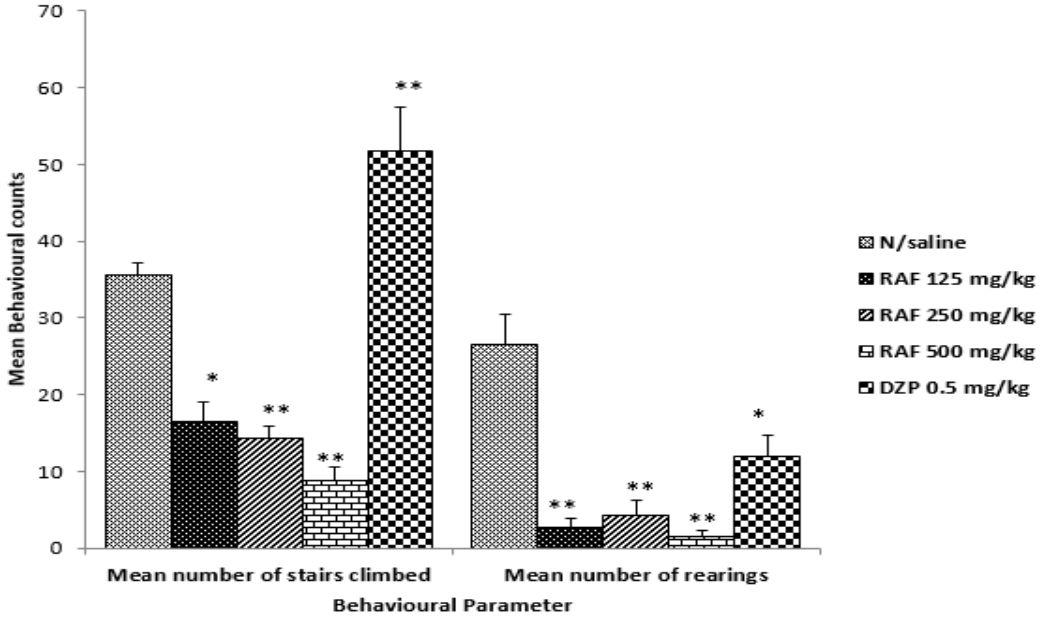

Figure 2: The Effect of aqueous fraction of methanolic root bark extract of *Securinega virosa* on the behaviour of mice in staircase test. N/saline (10 ml/kg); RAF (aqueous fraction, 125, 250 and 500 mg/kg); DZP (diazepam). Data presented as mean ± SEM; n=6; *P<0.01, **P<0.001

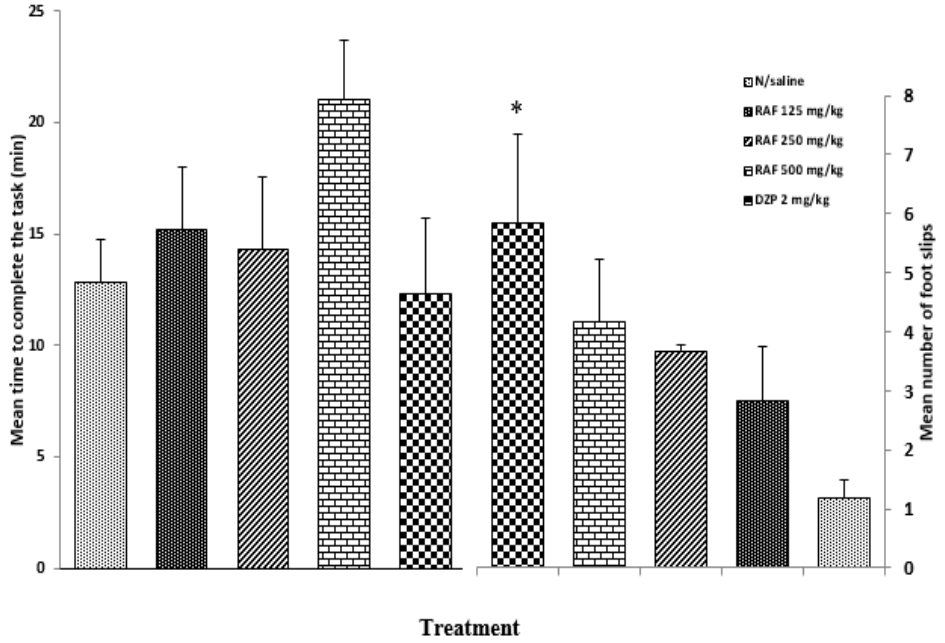

Figure 3: Effect of aqueous fraction (RAF) of methanolic root bark extract of *Securinega virosa* on motor coordination using beam walking assay in mice; DZP (diazepam); Data presented as mean ± SEM; *P<0.01; n=6

Alkaloids, flavonoids, saponins and steroids found to be present in the fraction have been previously reported to possess anticonvulsant and CNS depressant activities and may be responsible for the observed activity of the aqueous fraction.

The findings of the present study suggest that aqueous fraction possesses anticonvulsant and CNS depressant activities and further lend credence to the ethnomedicinal use of the root of *Secuinega virosa* in the management of epilepsy and mental illness.

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