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Full Length Research Paper

Neuropharmacological studies on ethyl acetate fraction of *Securinega virosa* root bark extract

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This study was conducted to evaluate the neuropharmacological activities of the ethyl acetate fraction of methanol root bark extract of *Securinega virosa* using *in vivo* models in laboratory animals. The fraction (125, 250 and 500 mg/kg) did not protect the animals against tonic hind limb extension induced by electroshock but produced a dose-dependently protection of animals against clonic spasm induced by pentylenetetrazole, with the highest protection of 66.67% produced by the highest dose tested. The fraction significantly ($P < 0.01$) and dose-dependently decreased the mean latency to sleep and increased mean sleep duration in mice treated with ketamine. However, it did not significantly increase the number of foot slips in the beam walking assay. These findings suggest that the ethyl acetate fraction of *Securinega virosa* root bark contains bioactive principle (s) that possesses sedative and anticonvulsant activities.

Key words: *Securinega virosa*, traditional medicine, epilepsy, sedative, electroshock, pentylenetetrazole, ketamine.

INTRODUCTION

Securinega virosa Roxb (Ex. Willd) Baill. family: Euphorbiaceae; is a commonly used medicinal plant which has enjoyed wide patronage among traditional practitioners in West Africa. It is a dense, low branching, many branched shrub, sometimes a small spreading tree up to about 6 m high, although, more commonly 2 to 3 m, evergreen or deciduous. It is widely distributed throughout tropical Africa (Dalziel, 1936). The local names of *S. virosa* in Nigeria include “Tsuwaawun karee, Gussu, Gwiiwar karee” (Hausa), “Iranje” (Yoruba), “Njisi nta” (Ibo), “Shim shim” (Kanuri), “kartfi-kartfi” (Shuwa arabs) and “Camal, cambe, came” (Fulani) (Neuwinger, 1996).

The root and leaf decoctions (separately) are drunk for fever in many parts of Africa including the south-western Nigeria. In Ivory Coast, the root is said to possess analgesic property and is used for labour and other pains (Neuwinger, 1996). In many parts of Africa including the north Eastern Nigeria, the root and leafy twig decoctions are used for the treatment of epilepsy. The root decoction is used as sedative in children to send them to sleep (Robert, 1961).

Previous studies in our laboratory showed that the crude methanol extract of *S. virosa* possesses anticonvulsant and sedative activities (Magaji et al., 2007, 2008). In an attempt to isolate and characterize the anticonvulsant and sedative principles of the root bark of the plant, the crude extract was successively partitioned

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into petroleum ether, chloroform, ethyl acetate and n-butanol. In this study, we report the anticonvulsant and sedative properties of the ethyl acetate fraction of *S. virosa* methanol root bark extract.

MATERIALS AND METHODS

Plant

The plant material was collected in February, 2009, in Basawa-Zaria, Sabon Gari local Government area of Kaduna State, Nigeria. The plant was identified by Messrs Umar Gallah and Musa Muhammad of the Herbarium section of the Department of Biological Sciences, Ahmadu Bello University Zaria-Nigeria, by comparing with existing voucher specimen (No 918).

Extraction and fractionation

The root bark of the plant was removed and dried under shade until constant weight was obtained. It was subsequently size-reduced to obtain the powdered root bark. The powdered root bark (1000 g) was extracted with 4 litres of methanol (70%) in a soxhlet apparatus for 72 h. The resultant extract was then concentrated *in vacuo* resulting into a brownish residue (9.5% yield), subsequently referred to as crude methanol root bark extract (CME). CME (50 g) was dissolved in water, filtered and the filtrate successively partitioned with petroleum ether, chloroform, ethyl acetate and n-butanol. The ethyl acetate fraction was concentrated *in vacuo* affording a light brownish residue (3.4% yields) subsequently referred to as ethyl acetate fraction (EAF).

Animals

Day old Rangers cockerels (34 ± 4 g) were obtained from the National Animal Production Research Institute (NAPRI), Shika, Kaduna state, Nigeria. Swiss albino mice of either sex (20 ± 2 g) were obtained from the Animal House Facility of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, Nigeria. Mice, housed in polypropylene cages at room temperature were maintained on standard rodent feed and water *ad libitum.* All experimental protocols were in accordance with the Ahmadu Bello University Research policy and ethic and regulations governing the care and use of experimental animals as contained in “Principles of laboratory animal care” (NIH Publication no. 85-23, revised 1985). The experiments were conducted in quiet laboratory between hours of 900 h to 1600 h.

Drugs/chemicals and treatment

EAF, normal saline, ketamine, diazepam, phenytoin and sodium valproate were administered via the intraperitoneal routes. Pentylenetetrazole was given subcutaneously. All administrations were at volumes equivalent to 10 ml/kg.

Phytochemical screening

Crude methanol root bark extract and EAF were screened for the presence of alkaloids, tannins, saponins, flavonoids and cardiac glycosides using standard protocols previously described by Silva et al. (1998).

Acute toxicity study

Median lethal doses for crude methanol extract and EAF in mice were estimated using the intraperitoneal route. Briefly, the method was divided into two phases. In the initial phase, 3 groups of three mice each were treated with the fraction at doses of 10, 100 and 1000 mg/kg body weight *i.p*. and observed for signs of toxicity and death for 24 h. In the second phase, 3 groups each containing one mouse was treated with three more specific doses of the fraction (1600, 2900 and 5000 mg/kg) based on the outcome of the first phase. The lethal dose (LD_{50}) value was estimated by calculating the geometric mean of the lowest dose that caused death and the highest dose for which the animal survived (Lorke, 1983).

Maximum electroshock-induced seizure in chicks

Tonic hind limb extensions in chicks were induced by passing alternating electrical current (200 Hz, 90 mA, 0.8 s, 0.8 ms) through corneal electrode (Swinyard and Kufferberg, 1985; Sayyah et al., 2002). Thirty minutes before electroshock, five groups of chicks (n = 10) were treated with normal saline, EAF (125, 250 and 500 mg/kg) or phenytoin (20 mg/kg). Ability to prevent tonic hind-limb extension or to prolong its latency was considered as an indication of anticonvulsant activity (Swinyard, 1969; Sayyah et al., 2002).

Pentylenetetrazole-induced seizure in mice

Clonic seizures were induced in mice (n = 6) by treatment with 85 mg/kg (CD_{97}) pentylenetetrazole (Swinyard et al., 1989). Thirty minutes before treatment with the convulsant, mice were treated with normal saline, EAF (125, 250 and 500 mg/kg) or sodium valproate (200 mg/kg). Absence of an episode of clonic spasm of at least 5 s duration indicated a protective effect. The latency to the clonic spasm for each unprotected animal was also noted.

Ketamine induced sleep test in mice

The method previously described by Mimura et al. (1990) was adopted. Thirty minutes post-treatment with normal saline, EAF (125, 250 and 500 mg/kg) or diazepam (0.5 mg/kg), animals (n = 6) were administered with ketamine (100 mg/kg). The time interval between ketamine administration and loss of righting reflex was considered as latency to sleep while the time from the loss to regaining of righting reflex as the duration of sleep (Bastidas Ramirez et al., 1998; Rabbani et al., 2003).

Beam walking assay in mice

Mice previously trained to walk along a horizontal ruler (80 × 3 cm) from a start platform to a goal box (a hamster house) were used for the study. Thirty minutes post-treatment with the normal saline, EAF or the positive control (diazepam, 0.5 mg/kg), each mouse was placed on the beam (60 cm long and 8 mm in diameter) 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 (Stanley et al., 2005).

Statistical analysis

Results were expressed as mean ± standard error of mean.

Table 1. Phytochemical constituents present in the methanol root bark extract of *Securinega virosa* and its Ethyl acetate fractions.

Constituent	MRBE	EAF
Tannins	+	+
Flavonoids	+	+
Cardiac glycosides	+	+
Saponins	+	-
Alkaloids	+	-
Steroid/Terpenoids	+	-
Anthraquinone	-	-

CME: Methanol root bark extract of *Securinega virosa*; EAF: ethyl acetate fraction of methanol root bark extract of *Securinega virosa*; +: present; -: absent.

Statistical analysis was performed by analysis of variance (ANOVA); when a statistically significant result was obtained with ANOVA, a post hoc Dunnets t-test was performed for multiple comparisons. Values of $P < 0.05$ were considered significant.

RESULTS

Acute toxicity study

The intraperitoneal median lethal dose of EAF in mice was found to be 2154 mg/kg while that of the crude methanol root bark extract was found to be 774.6 mg/kg.

Preliminary phytochemical screening

Preliminary phytochemical screening revealed the presence of flavonoids, tannins and cardiac glycosides in EAF. However, it was negative for saponins and alkaloids found to be present in the crude methanol root bark extract (Table 1).

Maximal electroshock test in chick and pentylenetetrazole-induced seizure in mice

EAF did not protect the animals against tonic hind limb extension induced by electroshock; neither did it reduce the recovery time in the unprotected animals. Conversely, phenytoin (20 mg/kg) protected 100% of the animals against convulsion. EAF produced a dose-dependent protection of animals against clonic spasm induced by pentylenetetrazole, with the highest protection of 66.67% produced by the highest dose tested; 500 mg/kg. EAF also produced a significant ($P < 0.05$) reduction in the mean onset of seizure in the unprotected animals compared with normal saline treated control (Table 2).

Ketamine induced sleep test in mice

EAF significantly ($P < 0.01$) and dose-dependently decreased the mean latency to sleep and increased mean sleep duration in mice treated with ketamine (Figure 1).

Beam walking assay in mice

EAF did not significantly affect the foot slips in mice. In contrast, diazepam (0.5 mg/kg) significantly increased the mean number of foot slips compared with normal saline treated control (Figure 2).

DISCUSSION

In the present study, we used maximal electroshock test and pentylenetetrazole induced seizure models to evaluate the anticonvulsant effects of ethyl acetate fraction of methanol root bark extract of *S. virosa*. Expectedly, the standard drugs used in both models; phenytoin and sodium valproate for maximal electroshock (MES) and pentylenetetrazole (PTZ)-induced seizure, respectively, offered 100% protection in the animals. However, the ethyl acetate fraction did not protect the animals in the MES model suggesting that its activities may not involve prevention of seizure spread from an epileptic foci; and it may not be beneficial in the management of generalized tonic-clonic seizure. The fraction protected 66.67% of the animals against PTZ and significantly increased the mean onset of seizure in unprotected animals. PTZ test identifies compounds that can raise seizure threshold in the brain (White et al., 1998) and drugs that reduce T-type Ca^{2+} currents, such as ethosuximide have been found to be protective against seizures induced by PTZ.

Previously, PTZ has been reported to interact with gamma aminobutyric acid (GABA) neurotransmitters and the GABA receptor complex (Loscher and Schmidt, 1988; De Deyn et al., 1992). Dopaminergic mechanism has also been implicated in PTZ-induced seizures. Drugs such as felbamate, which block glutamatergic excitation mediated by N-Methyl-D-aspartate (NMDA) receptors, have demonstrated activity against PTZ-induced seizures, suggesting the involvement of NMDA system in the initiation and propagation of PTZ-induced seizures (MacDonald and Kelly, 1993). It is therefore possible to suggest that the anti-PTZ activity of the fraction may involve one or more of these aforementioned mechanisms.

The ability of the fraction to reduce the latency to sleep and increase the total sleep time is an indication of its sleep inducing property. Similar influence in sleep indices were noticed with diazepam. However, greater reduction in the onset of sleep and increase in the total sleep time

Table 2. Effect of ethyl acetate fraction of methanol root bark extract of *Securinega virosa* against pentylenetetrazole-induced seizure and maximal electroshock tests.

Treatment (mg/kg)	MES		PTZ	
	Quantal protection against seizure	Mean recovery time (min)	Quantal protection against seizure	Mean onset of seizure (min)
N/Saline	0/10	5.86±1.58	0/6	3.51±0.43
EAF 125	0/10	7.22±0.88	2/6	8.99±2.85*
EAF 250	0/10	7.44±0.77	2/6	6.93±2.01*
EAF 500	0/10	8.00±0.85	4/6	7.12±1.87
PHT 20	10/10	-		
VPA 200			6/6	-

Protection against seizure expressed as quantal protection; Onset of seizure and recovery time were expressed as mean ± SEM; N/saline (normal saline); MES (maximal electroshock); PTZ (pentylenetetrazole); EAF (ethyl acetate fraction); VPA (sodium valproate); PHT (phenytoin); *P < 0.05; compared with N/Saline (10 ml/kg)-treated control.

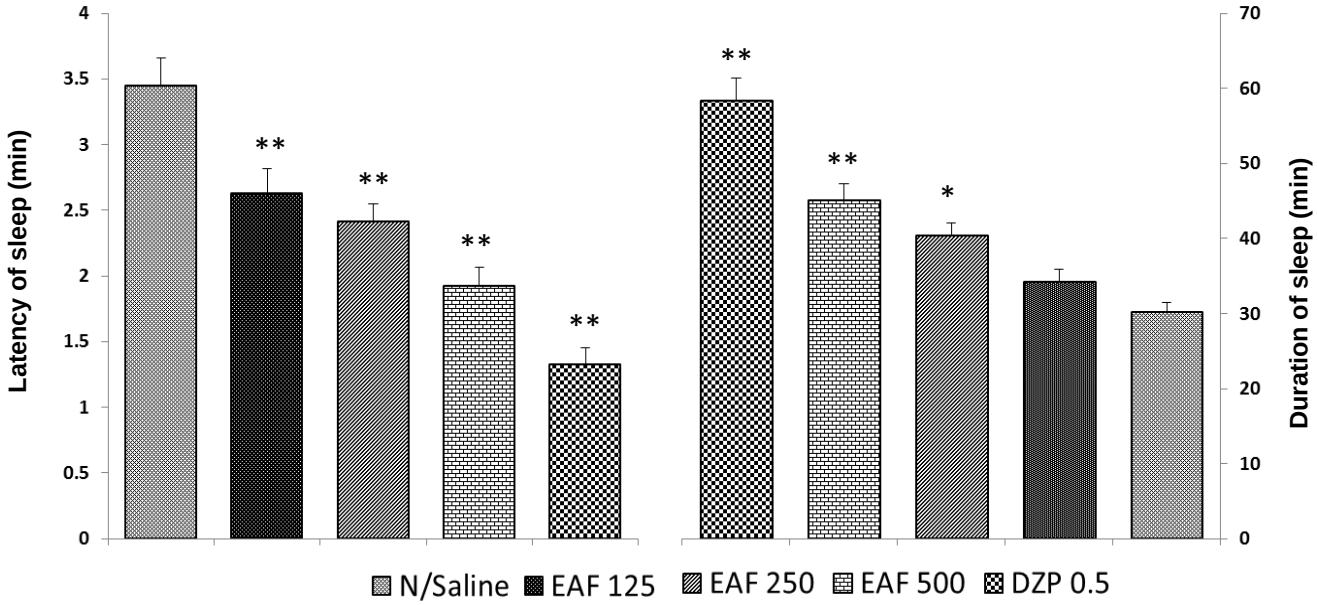

Figure 1. Effects of ethyl acetate fraction (EAF) and diazepam (DZP) on ketamine-induced sleep in mice. Latency to sleep and duration of sleep were expressed as mean ± SEM; *P < 0.01; **P < 0.001; compared with N/Saline (10 ml/kg)-treated control.

produced by the standard agent (diazepam) is indicative of lesser sedative potential of the fraction.

The fraction did not significantly increase the number of slips in the beam walking assay for motor coordination, an indication that its effect may be centrally mediated and not due to peripheral muscular blockade (Perez et al., 1998). Diazepam significantly increased the number of slips. This result is consistent with previous data which indicated that benzodiazepines induce motor coordination deficit in the beam walking assay (Stanley et al., 2005; Danjuma et al., 2009).

Previous works have reported the anticonvulsant and sedative properties of flavonoids (Johnston, 2005; Yao et al., 2010; Hanrahan et al., 2011). Flavonoids, found to be present in the ethyl acetate fraction of methanol root bark extract of *S. virosa*, may therefore be responsible for the observed anticonvulsant and sleep promoting activities. Further purification and pharmacological investigations are needed to identify the active principle(s) responsible for the anticonvulsant and sedative properties of the ethyl acetate fraction of the *S. virosa* methanol root bark extract.

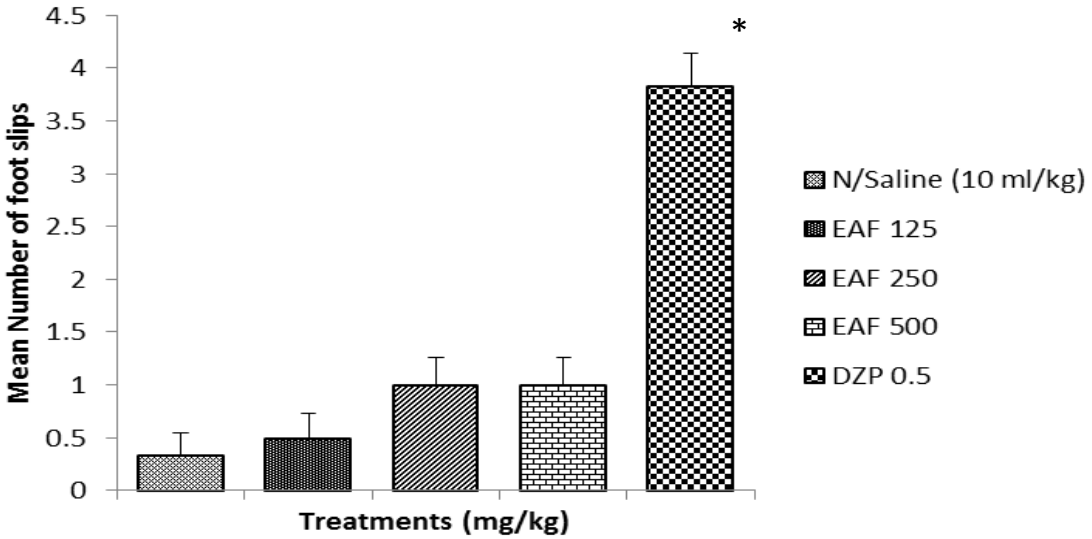

Figure 2. Effects of ethyl acetate fraction (EAF) and diazepam (DZP) on motor coordination in mice. Number of foot slips expressed as mean ± SEM; *P < 0.05, compared with normal saline treated group.

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