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Full Length Research Paper

Behavioural studies on the methanol leaf extract of *Securinega virosa* (Euphorbiaceae) in mice

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Securinega virosa **is used traditionally in the West African region as sedative in children and in mental illnesses. In this study, the behavioural effects of the methanol leaf extract of *S. virosa* were investigated in mice at doses of 25, 50 and 100 mg/kg, using diazepam-induced sleeping time, hole board and beam walking assay, all in mice. The results revealed that the extract at the highest dose tested (100 mg/kg) significantly ($p \leq 0.001$) prolonged the duration of diazepam-induced sleep without any effect on the latency to sleep at all the doses tested. The extract did not have any effect on the exploratory behaviour of mice in the hole board test; it also had no effect on the motor coordination of the mice in the beam walking assay. The intraperitoneal LD_{50} was found to be 1265 mg/kg while the preliminary phytochemical screening of the extract revealed the presence of alkaloids, tannins, saponins and flavonoids. These results suggest that the methanol leaf extract of *S. virosa* contains biologically active principles that are sedative in nature.**

Key words: Beam walking, exploratory behaviour, *Securinega virosa,* sleep.

INTRODUCTION

World Health Organization (WHO) estimated that about 450 million people world wide suffer from a mental or behavioral disorder (WHO, 2001). Majority of such patients in the developing countries still rely on traditional healing practices and medicinal plants for treatment of these conditions (Magaji et al., 2009). The inclusion of herbal medicines of proven safety and efficacy in the healthcare programs of developing countries is encouraged by the WHO because of the great potential they possess in combating various diseases (Amos et al., 2001). One of such medicinal plant which has enjoyed wide patronage among the people of tropical Africa is *S. virosa* (Neuwinger, 1996). *S. virosa* is a low branching, dioecious shrub, or a small tree, distributed throughout Tropical Africa (Dalziel, 1936).

The decoction of the leaves and roots is used for abdominal pain in Tanzania while the leaf decoction is drunk for fever by the Yorubas of South Western Nigeria. The decoction of the leaves with other herbs is used in Northern Nigeria for treatment of painful swellings (Neuwinger, 1996). A leaf sap of the plant is administered in cases of epilepsy and with other plants, as tranquilizers in mental illnesses in Tanzania (Burkill, 1994). A leaf decoction is used by traditional practitioners in northern Nigeria in the management of insanity (personal communication with Mr Ado Ibrahim) .The methanolic root bark extract have been reported to possess CNS depressant effect in laboratory animals (Magaji et al., 2008).

To our knowledge, there is no report on the behavioural effects of leaf extract of *S. virosa* in literature. This study, therefore reports the behavioural effects of methanol leaf extract of *S. virosa* using diazepam-induced sleep, hole-board test and beam walking assay in mice.

MATERIALS AND METHODS

Plant material

The whole plant was collected in Basawa town, Sabon Gari Local Government Area of Kaduna State, Nigeria in December, 2008.

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The leaves were authenticated by staff of the herbarium section, Department of Biological Sciences, Ahmadu Bello University (ABU), Zaria, Kaduna State, Nigeria, by comparing it with existing specimen (Voucher specimen number 918).

Preparation of extract

The leaves were air dried under shade until crispy and then size-reduced into powder with pestle and mortar. About 100 g of the powdered leaves was macerated with 500 mL methanol for 72 h with occasional shaking. The extract was concentrated *in vacuo* affording a yield of 13.2% w/w subsequently referred to as methanol leaves extract of *S. virosa*. Solution of extract was prepared freshly for each study.

Phytochemical screening

The screening was carried out in accordance with the standard protocol as described by Trease and Evans (1983).

Experimental animals

The pharmacological experiments were conducted using adult Swiss Albino mice of either sexes (weighing 18 to 30 g) obtained from Animal House Facility of Department of Pharmacology and Therapeutics, ABU Zaria-Nigeria. The mice were maintained on standard laboratory animal feed and water *ad libitum*. They were housed in polypropylene cages at room temperature with a 12 h light/dark cycle (6 am to 6 pm) and allowed to acclimatize with the laboratory environment for at least five days prior to the commencement of the study.

Drugs

Diazepam (Roche, Pakistan) (10 mg/2 ml). The drug was freshly prepared to the desired concentration with appropriate solvent just before use.

Acute toxicity study

The intraperitoneal (*i.p.*) LD_{50} of the extract in mice was estimated according to the method of Lorke (1983). The study was divided into two phases. In the initial phase, 3 groups of three mice each were treated with the methanol leaves extract of the plant at dosed 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, 4 groups each containing one mouse was injected with four more specific doses of the extract based on the result of the first phase. The LD_{50} value was determined by calculating the geometric mean of the lowest dose that caused death and the highest dose for which the animal survived (0/1 and 1/1).

Diazepam-induced sleep in mice

The method previously described by Rakotonirina et al. (2001) was adopted. Sleep potentiating effect of the plant extract was studied in group of mice that received diazepam *i.p.* at a dose of 20 mg/kg body weight thirty minutes after *i.p.* administration of extract (100 mg/kg, 50 mg/kg and 25 mg/kg) or normal saline 10 mL/kg with six mice in each group. The mice were placed individually in cages and observed. The onset and duration of sleep were determined for each animal. Onset of sleep was denoted by loss of rightening reflex (Ramirez et al., 1998), and the duration of sleep estimated as the time interval between the loss and recovery of the rightening reflex (Fujimori, 1965).

Hole-board test for exploratory behaviour in mice

The study was conducted using a wooden board measuring 20 cm by 40 cm with sixteen evenly spaced holes (Perez et al., 1998). The animals were randomly grouped into five groups each containing six mice. Group one served as the control group and was treated with normal saline 10 mL/kg *i.p.* Groups two, three and four were treated with the extract *i.p.* at doses of 100 mg/kg, 50 mg/kg and 25 mg/kg respectively; while those in group five received diazepam 0.25 mg/kg *i.p.* Thirty minutes after treatment, the mice were placed singly on the board and the number of times the mice dipped their head into the holes at the level of their eyes during a five minute trial period was counted using a tally counter.

Beam walking assay (motor co-ordination) in mice

The method previously described by Stanley et al. (2005) was adopted. The 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 supports to a goal box. Three trials were performed for each mouse to ensure that the mice learnt properly. The mice that successfully walked along the ruler were randomly divided into five groups of six mice each. The first group served as the control group and was treated with normal saline (10 mL/kg i.p.), second, third and fourth groups were treated with the extract at doses of 100 mg/kg, 50 mg/kg and 25 mg/kg respectively; while those in the fifth group received diazepam at a dose of 0.25 mg/kg. All treatments were by intraperitoneal route. Thirty minutes after treatment, each mouse was placed on the beam made of wood (8 mm in diameter and 60 cm long elevated 30 cm above the bench by metal supports) and allowed to walk to the goal box. Mice that fell were returned to the position they fell from with a maximum time of sixty seconds allowed on the beam. The number of foot slips was recorded with the aid of a tally counter.

Statistical analysis

The results were presented as mean ± SEM (standard error of mean) and analysed using one way ANOVA followed by Dunnett post hoc test for multiple comparisons. A difference was considered significant at $p< 0.05$.

RESULTS

Phytochemical screening

The preliminary phytochemical screening of the methanol leaf extract of *S. virosa* revealed the presence of alkaloids, tannins, saponins, flavonoids, cardiac glycol-sides, cyanogenic glycosides, resins, steroids/terpenoids and carbohydrates.

Acute toxicity study

The median lethal dose (LD_{50}) value of the methanol leaf extract of *S. virosa* in mice was found to be 1265 mg/kg body weight, intraperitoneally.

Table 1. Effect of methanol leaf extract of *S. virosa* on diazepam-induced sleep in mice.

Treatment/dosage	Onset of sleep (min)	Duration of sleep (min)
N/Saline 10 mL/kg	3.33 ± 0.21	85.33 ± 11.49
Extract 25 mg/kg	4.50 ± 0.50	120.17 ± 17.90
Extract 50 mg/kg	3.50 ± 0.43	129.17 ± 17.90
Extract 100 mg/kg	3.16 ± 0.40	188.83 ± 5.08*
One way ANOVA	df 3, 20 f = 2.246 p = 0.114	df 3,20 f = 5.993 p = 0.004

Values are mean ± SEM; n = 6 in each group, * significantly different from control at p < 0.001 (Dunnett post hoc test).

Table 2. Effect of methanol leaf extract of *S. virosa* on exploratory behavior (Head dip test) in mice.

Treatment/dose	Mean number of head dips in 5 min
N/Saline 10 mL/kg	4.33 ± 1.12
Extract 25 mg/kg	3.67 ± 0.76
Extract 50 mg/kg	3.33 ± 0.61
Extract 100 mg/kg	3.00 ± 1.15
DZP 0.25 mg/kg	9.83 ± 1.22*
One Way ANOVA	df 4, 25 f = 8.011 p = 0.00

Values are mean ± SEM; n = 6 in each group, * significantly different from control at p < 0.05 (Dunnett post hoc test).

Effects on diazepam induced sleep in mice

The methanol leaf extract of *S. virosa* did not affect the onset of sleep at the doses tested but significantly ($p < 0.001$) prolonged the duration of the diazepam induced sleep at the doses tested. The sleep time increased from 85.33 ± 11.49 min in the control group to about 120.17 ± 17.90 min to 129.17 ± 27.51 min and 188.83 ± 5.08 min at doses of 25 mg/kg, 50 mg/kg and 100 mg/kg body weight *i.p.*, respectively (Table 1).

Effects on exploratory behavioural patterns in mice

The extract at the doses tested showed a slight decrease in the number of head dips in the hole-board test compared with the control group, though the differences observed were not statistically significant. The standard drug, diazepam 0.25 mg/kg caused a significant increase in the exploratory behaviour ($p < 0.05$) (Table 2).

Effects on motor coordination (beam walking assay)

The extract showed no significant difference in the number of foot slips compared to the control group however, diazepam at 0.25 mg/kg significantly ($p < 0.05$) impaired motor coordination. It should be noted that the extract exhibited a non significant reduction in the number of foot slips in a non dose dependent manner relative to the diazepam treated group (Table 3).

DISCUSSION

This study reports some behavioural activities of the methanol leaf extract of *S. virosa.* The extract at the highest dose tested (100 mg/kg) significantly ($p < 0.001$) prolonged the duration of the diazepam induced sleep, which is in agreement with an earlier studies (Musa et al., 2006; Musa et al.,2008). By potentiating the Diazepam induced sleeping time, the extract seems to possess sleep inducing properties (Rakotonirina et al., 2001).

The hole board experiment is a measure of exploratory behaviour in animals (File and Wardil, 1975) and is an accepted parameter for evaluating anxiety conditions in animals (Crawley, 1985). The extract produced a non significant decrease in exploratory behaviour as shown by the decrease in the number of head dips in the hole-board test. A decrease in number of head dips reveals sedative behaviour (File and Pellow, 1985) and is thus a measure of CNS depressant activity (Adzu et al., 2002; Viswanatha et al., 2006).

The number of foot slips made by mice in the motor coordination test has been found to be a sensitive measure at determining benzodiazepine induced motor

Table 3. Effect of methanol leaf extract of *S. virosa* on motor coordination (beam walking assay) in mice.

Treatment/dosage	Duration of beam walk (s)	Number of foot slips
N/Saline 10 mL/kg	7.87 ± 1.88	0.50 ± 0.34
Extract 25 mg/kg	11.10 ± 0.94	0.33 ± 0.33
Extract 50 mg/kg	13.90 ± 2.09	0.33 ± 0.21
Extract 100 mg/kg	9.12 ± 1.29	0.83 ± 0.31
DZP 0.25 mg/kg	10.33 ± 1.86	3.80 ± 0.73*
One way ANOVA	df 4, 24 f = 1.955 p = 0.134	df 4,24 f = 12.678 p = 0.00

Values are mean ± SEM; n = 6 in each group, * significantly different from control at $p < 0.05$ (Dunnett post hoc test).

coordination deficits and is a good predicator of doses producing clinical sedation (Stanley et al., 2005). The extract had no observable effect on motor coordination when compared with the negative control suggesting that the inhibition effect observed in the other tests might be elicited centrally and not due to a peripheral neuromuscular blockade (Perez et al., 1998). It is therefore, possible that the sedative action of the extract was produced centrally.

The phytochemical screening of the methanol leaf extract of *S. virosa*, revealed the presence of alkaloids, tannins, saponins, flavonoids, cardiac glycosides, cyanogenic glycosides, resins, steroids/terpenoids and carbohydrates. The saponins and flavonoids have been reported by several researchers (Amos et al., 2001; Musa et al., 2006; 2008; Viswanatha et al., 2006; Won et al., 1980; Dubios et al., 1986) to be responsible for sedative and likewise to inhibit spontaneous motor activity in mice. The overall results revealed that the methanol leaf extract of *S. virosa* possess contains biologically active principles that are sedative in nature. Further studies are required to isolate the useful active components and elucidate their possible modes of action.

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