

Short Communication

Anti-diabetic properties of *SECURINEGA VIROSA* (Euphorbiaceae) leaf extract

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This study was undertaken to evaluate the hypoglycemic effect of methanol extract of *SECURINEGA VIROSA* leaves on blood levels of streptozocin-induced diabetes rats. Three doses of the extract (100, 300 and 600 mg/kg) were administered intraperitoneally. After 2 h of extract administration there was no significant change in the blood glucose levels in all the three doses of the extract. Also after 4, 8 and 24 h of extract administration there was a significant ($p < 0.05 - 0.001$) decrease in the blood glucose levels in all the three doses of the extract. The preliminary phytochemical screening revealed the presence of reducing sugars, cardiac glycosides, resin, tannins, saponins, glycosides, flavonoids, glycerin carbohydrate, anthraquinone and steroids. The median lethal dose (LD₅₀) in rats was calculated to be 1264.9 mg/kg body weight.

Key words: *Securinega virosa*, hypoglycemic activity, streptozocin, diabetes mellitus.

INTRODUCTION

Diabetes is one of the oldest known diseases of the man whose devastating effect is increasing by the day and severity almost at epidemic level. It is a disease of disordered metabolism of carbohydrate, protein and fat which is caused by the complete or relative insufficiency of insulin secretion and /or insulin action (Balkau et al., 2000). The number of people suffering from the disease worldwide is increasing at an alarming rate with a projected 366 million peoples likely to be diabetic by the year 2030 as against 191 million estimated in 2000 (Wild et al., 2004). Developing countries are the most affected because of expensive and inadequate treatments (Djrolo et al., 1998), coupled with the side effect associated with these drugs. Thus the search for a new drug with low cost, more potential and without adverse effects becomes inevitable. A great number of medicinal plants have been used in the treatment of diabetes in different parts of the world, some of which are without scientific scrutiny al-

though World Health Organization (WHO) had encouraged and recommended the use of plants as an alternative therapy for diabetes (WHO, 1980). Evaluation of the antidiabetic potentials of these plants becomes necessary to provide scientific proof and justify their uses in ethnomedicine.

Securinega virosa is one of the great African medicinal plants described as a true "cure all", of which all parts are used as remedies, particularly the root (Neuwinger, 1996). It is widely distributed throughout tropical Africa, but can also be found in India, Malaya, china and Australia (Dalziel, 1936) The leaves are used in many parts of Africa in the treatment of fever, body pain, stomach-ache rheumatism, diarrhea, pneumonia and epilepsy (Neuwinger, 1996). The present study was designed to test the hypoglycemic effect of methanol extract of *S. virosa* leaves in streptozocin-induced diabetes.

MATERIAL AND METHODS

Plant material

The leaves of *S. virosa* were collected from Samaru town, in

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Sabon-gari Local Government Area of Kaduna State, Nigeria, in May 2006. The plant was identified and authenticated by Malam M. Musa of the Herbarium Section in the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria. A voucher specimen (NO 918) was deposited at the herbarium for future reference.

Extract preparation

The leaves were cleaned, air dried for 14 days and then crushed into coarse powder with a pestle and mortar. 100 g of the powdered leaves was extracted with 500 ml methanol for 72 h using Soxhlet extraction apparatus. The solvent was evaporated on a water bath to give an average yield of 12.63% (w/w). The extract was then stored in a desiccator. Solutions of the extract were prepared freshly for the study.

Chemicals used

All chemicals and drugs were obtained commercially and were of analytical grade.

Acute toxicity study

The lethal dose (LD₅₀) of the plant extract was determined by method of Lorke (1983) using 13 rats. In the first phase rats were divided into 3 groups of 3 rats each and were treated with the methanol extract of the leaves at doses of 10, 100 and 1000 mg/kg body weight intraperitoneally. They were observed for 24 h for signs of toxicity. In the second phase 4 rats were divided into 4 groups of 1 rat each and were also treated with the methanol leaves extract at doses of 600, 1000, 1600 and 2900 mg/kg bodyweight (ip). The median lethal dose (LD₅₀) was calculated using the second phase.

Phytochemical screening

The methanol extract of the leaves obtained was subjected to preliminary phytochemical screening, to identify the chemical constituents. The methods of analysis employed were those described by Trease and Evans (1989).

Animals and Induction of diabetes mellitus

Twenty five Wistar rats of both sexes weighing 120 - 180 g were used for the study of the effects of methanol extract of *S. virosa* leaves on the blood glucose levels of the animals. They were kept in standard cages at 25°C and 12 h light/dark condition in the animal room of the Department of Human Physiology, ABU, Zaria. The animals were fed on commercial feeds and were given water *ad libitum*. The animals were fasted from feeds for 12 h before the commencement of each experiment, but were allowed water *ad libitum*. The rats were injected with streptozocin dissolved in citrate buffer pH 4.5 in a dose of 60 mg/kg body weight intraperitoneally. Since Streptozocin is capable of producing fatal hypoglycemia as a result of massive pancreatic release of insulin, the rats were treated with 20% glucose solution intraperitoneally after 6 h (Stanley and Venugopal, 2001). They were kept for the next 24 h on 5% glucose solution to prevent hypoglycemia (Stanley and Venugopal, 1997). After a period of one week the rats with blood glucose levels greater than 200 mg/dl were considered diabetic and used for this research work.

Experimental design

The Streptozocin-induced diabetic Wistar rats were randomly assigned into five groups (1 - 5) of five rats (n = 5) each as follows; Group 1 received normal saline i.p; Group 2 received biphasic isophane insulin 6 i.u/kg i.p (Stanley and Venugopal, 2001); Group 3 received 100 mg/kg body weight of the methanol extract of *S. virosa* leaves i.p; Group 4 received 300 mg/kg body weight of the methanol extract of the leaves i.p; and Group 5 received 600 mg/kg body weight of the methanol extract of the leaves i.p.

Determination of blood glucose levels

All blood samples were collected by cutting the tail-tip of the rats. Blood samples for blood glucose determination were collected from the tail at intervals of 0, 2, 4, 8 and 24 h. Determination of the blood glucose level was done by the glucose-oxidase principle (Beach and Turner, 1958) using the ONE TOUCH Basic (Lifescan, Milpitas, CA) instrument and results were reported as mg/dl (Rheney and Kirk, 2000).

Statistical analysis

Blood glucose levels were expressed in mg/dl as mean ± SEM. The data were statistically analyzed using ANOVA with multiple comparisons versus control group. The values of p < 0.05 were considered as significant (Duncan et al., 1977).

RESULTS AND DISCUSSION

Phytochemical analysis

Many secondary metabolites participate in a variety of anti-diabetic functions *in vivo* (Kako et al., 1997). Freshly prepared extracts were subjected to preliminary phytochemical screening test for various constituents. This revealed the presence of reducing sugars, cardiac glycosides, resin, tannins, saponins, glycosides, flavonoids, glycerin carbohydrate anthraquinone and steroids.

Acute toxicity study (LD₅₀)

The sign of toxicity were first noticed after 2 – 4 h of extract administration. There was decreased locomotor activity and decreased in sensitivity to touch. Also there was decreased feed intake and prostration after 4 h of extract administration. The median lethal dose (LD₅₀) in rats was calculated to be 1264.9 mg/kg body weight.

Anti-diabetic study

Streptozocin-induced hyperglycaemia has been described as a useful experimental model to study the activity of hypoglycaemic agents (Szkudelski, 2001). Streptozocin selectively destroyed the pancreatic insulin secreting β-cells, leaving less active cell and resulting in a diabetic state (Kamchoung et al., 1998; Szkudelski, 2001). Table

Table 1. Effect of methanol extract of *S. virosa* leaves on streptozocin-induced diabetic Wistar rats.

Treatment	Blood glucose level (mg/dl)				
	0 h	2 h	4 h	8 h	24 h
Group 1 Control (Saline)	277.0 ± 434.9	318.2 ± 27.4	330.6 ± 30.7	322.4 ± 28.9	328.2 ± 29.9
Group 2 (Insulin 6.i.u/kg)	272.8 ± 43.1 ^{ns}	239.4 ± 36.6 ^{ns}	188.4 ± 23.0 ^c	149.4 ± 16.6 ^c	137.0 ± 12.1 ^c
Group 3 (100 mg/kg)	273.2 ± 36.2 ^{ns}	238.2 ± 25.5 ^{ns}	205.4 ± 15.5 ^c	185.6 ± 9.35 ^c	165.4 ± 16.5 ^c
Group 4 (300 mg/kg)	277.4 ± 21.5 ^{ns}	256.2 ± 15.87 ^{ns}	219.8 ± 14.8 ^b	196.2 ± 16.4 ^c	174.4 ± 16.9 ^c
Group 5 (600 mg/kg)	271.0 ± 29.2 ^{ns}	264.4 ± 26.7 ^{ns}	252.0 ± 21.2 ^b	238.2 ± 22.4 ^b	199.0 ± 22.0 ^b

Vales are given as mean ± SD for 5 rats in each group.

Experimental groups were compared with diabetic control. Values are statistically significant at ^a = P < 0.05; ^b = P < 0.01; and ^c = P < 0.001.

^{ns} = Not significant.

1 shows the results of the effects of three doses (100, 300 and 600 mg/Kg) of the methanol extract of *S. virosa* leaves, insulin and control groups in streptozocin-induced diabetic Wistar rats. The insulin and the three doses of the extract did not show any significant change in the blood glucose levels when compared to untreated control after 2 h. However, after 4, 8 and 24 h of treatments the extract showed a significant ($p < 0.05 - 0.001$) decrease in the blood glucose levels when compared to untreated control. The 100 mg/kg dose which is the lowest dose was found to be more effective in the glycaemic change than the other two doses of 300 and 600 mg/kg body weight.

Conclusion

The study indicates that the methanol extract of *S. virosa* leaves possess anti-diabetic properties which suggest the presence of biologically active components. The extract might be promoting glucose uptake and metabolism or inhibiting hepatic gluconeogenesis. Result from the phytochemical analysis of *S. virosa* revealed the presence of flavonoids, which has also been isolated from the other plant and found to stimulate secretion or possess an insulin-like effect (Marles and Farnsworth 1995).

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