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Full Length Research Paper

Toxicity Assessment and Blood Glucose Regulation by *Cissampelos mucronata* Leaf Extracts in Wistar Rats with Streptozocin-Induced Diabetes

Tanko, Y. [1]*, Yaro, A. H.[2], Isa, A. I[3], Yerima, M.[4], Saleh, M. I. A.[1] and Mohammed, A.[1]

[1]Department of Human Physiology, Ahmadu Bello University, Zaria, Nigeria.
[2]Department of Pharmacology, Bayero University, Kano Nigeria.
[3]Department of Biology, College of Education P.M.B. 1021 Jalingo. Nigeria.
[4]Department of Pharmacology and Clinical Pharmacy Ahmad Bello, University Zaria, Nigeria.

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Preliminary phytochemical screening of the ethanol leaves of *Cissampelos mucronata* revealed the presence of reducing sugars, cardiac glycosides, resin, tannins, saponins, glycosides, flavonoids, glycerin and steroids. The median lethal dose (LD_{50}) in rats is greater than 5000 mg/kg body weight intaperitoneally. The hypoglycemic effect of ethanol extract of *C. mucronata* was also investigated in Streptozocin - induced diabetic rats. Single intraperitoneally administration of the extract at the doses of 200, 400 and 800 mg/kg. There was a significant decreased ($p<0.05$) in the blood glucose levels in all the doses administered. The dose of 200 mg/kg was more effective with the highest glycemic change of 67% after 24 h of extract administration than the other two doses of 400 and 800 mg/kg with glycemic change of 60%. The ethanol leaves extract of *C. mucronata* possess hypoglycemic activity in Streptozocin induced in diabetic rats.

Key words: *Cissampelos mucronata*, hypoglycemic activity, streptozocin, diabetes.

INTRODUCTION

Diabetes is a serious metabolic disorder with micro and macrovascular complications that results in significant morbidity and mortality (Rang et al., 1991). Chronic hyperglycemia during diabetes causes glycation of body protein that in turn leads to secondary complications affecting eyes, kidney, nerves and artery (Sharma, 1993).These may be delayed, lessened or prevented by maintaining blood glucose level close to normal. The increasing number of ageing population, consumption of calories rich diet, obesity and sedentary life style have led to a tremendous increase in number of diabetes world wide. According to WHO (1980) projection, the prevalence of diabetes is likely to increase by 35%. Currently there are over 150 millions diabetics world wide and this is likely to increase to 300 million or more by the year 2025. Statistical projection about India suggests that the number of diabetic will rise from 15 million in 1995 to 57 million in the year 2025 making it the country with the highest number of diabetics in the world (King, et al., 1998; Boyle et al., 2001)). Therefore it is necessary to look for a urgent solution to manage this problem.

The plant *Cissampelos mucronata* belongs to the family Menispermaceae is popular among traditional healers in Nigeria in treatment of anti diarrhoeal. The description of the plant morphology have been documented (Hutchinson and Dalziel, 1954). Traditionally the root bark is used to relieve dysmenorrhoea, to prevent abortion and also as a sedative (Ogwal et al., 1996; Oliver- Bever, 1997). Antispasmodic activity (Offiah et al., 1996) anti-ulcer activity (Akah and Nwafor 1999). In South African the root is

*Corresponding author. E-mail: yusuftanko@yahoo.com

used for schistosomiasis (Sparg et al., 2000). In India the root bark is used as an antivenin (Selvanayahtigram et al., 1994)

The present study was designed to test the hypoglycemic effect of petroleum ether fraction of *C. 2ucronate* in streptozocin- induced diabetes

MATERIALS AND METHODS

Chemical used

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

Plant material

Fresh leaves of *C. 2ucronate* were collected from Ahmadu Bello University Main campus Zaria in the month January 2007. The plant was identified by Mal. M. Musa at the herbarium unit of Biological Science Department A.B.U., Zaria where a voucher specimen (No. 6311) has been deposited.

Extract preparation

The fresh whole plant was collected and dried under the shade and ground into powder. The powder (100 g) was macerated with ethanol for 48 h with occasional shaking. The extract was concentrated under reduced pressure to yield a dark green mass which weight 15 g and kept in dessicator until use.

Animals

Wistar strain albino rats of both sexes weighed between 120 – 150 g, which were bred in the Department of Pharmacology A. B.U Zaria. The animals were housed in standard environmental conditions of temperature (21 ± 2^{o}C), humidity (55 ± 10%) and a 12 h light- dark cycle. The animals were divided into extract treated groups and the control groups. All the animals were fasted for 12 h, but were allowed free access to water, before commencement of the experiments.

Phytochemical screening

The ethanol leaves extract of *C. 2ucronate* were subjected to preliminary phytochemical screening, to identify the chemical constituents. The methods of analysis employed were those described by Brain and Turner (1975).

Acute toxicity study

The lethal doses (LD_{50}) 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 ethanol extract of the plant at doses of 10, 100 and 1000 mg/kg body weight intraperitoneal. 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 aqueous extract at doses of 1000, 1600, 2900 and 5000 mg/kg bodyweight *(i.p).* The median lethal dose (LD_{50}) was calculated using the second phase.

Induction of diabetes

Diabetes mellitus was induced by single intraperitoneal dose of 60 mg/kg of streptozocin dissolved in 0.1 m fresh cold citrate buffer pH 4.5 into 12 h-fasted rats. On the third day of STZ- injection the rats were fasted for 6 h and blood was taken from tail artery of the rats (Burceline et al., 1995) . Rats with diabetes having hyperglycemia (that is, with blood glucose of 180 – 460 mg/dl) were taken for the experiment. The diabetic rats were then randomly selectively in different groups.

Experimental design

In the experiment, a total of 25 rats were used which were divided into 5 groups of 5 rats in each. The first group served as control, group 2 was given 6.iu./kg isophane insulin *i.p* (Stanley and Venugopau, 2001) while group 3, 4 and 5 received the extract at the doses of 200, 400 and 800 mg/kg (*i.p)* respectively.

Determination of blood glucose levels

All blood samples were collected from the tail artery of the rats at intervals of 0, 2, 4, 8 and 24 h. Determination of the blood levels was done by the glucose-oxidase principle (Beach and Turner, 1958) using the one touch basic (lifescan, mulpital CA instrument) and the result were expressed as mg/dl (Rhieney 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

Acute toxicity study (LD_{50})

The sign of toxicity were first noticed after 4 – 8 h of extract administration. There was decreased locomotor activity and decreased in sensitivity to touch. Also there was decreased feed intake, and prostration after 16 h of extract administration. There is no any mortality rate after administered 5000 mg/kg.

The median lethal dose (LD_{50}) in rats was calculated to be greater than 5000 mg/kg body weight.

Table 2 showed the results of the effects of three doses (200, 400 and 800 mg/Kg) of the extract of *C. 2ucronate* and insulin 6.i.u/kg on streptozocin-induced diabetes Wistar rats. All the three dose of extract showed a significant decreased in the blood glucose level ($p<0.05$) when compared to control normal saline at all the time intervals. The highest activity resides at the lowest dose of 200 mg/kg with percentage glycemic change of 67 % after 24 h of extract administration.

$$\%\ \textbf{Glycaemic change} = \frac{\text{Glucose concentration (2, 4, 8 or 24) – fasting blood glucose x 100}}{\text{Fasting blood glucose}}$$

Figure in parenthesis represent percentage glycaemic change.

Table 2. Effect of ethanol leaves extract of *Cissampelos mucronata* on streptozocin-induced diabetic wistar rats.

Treatment	Blood glucose levels(Mg/dl)				
	0 h	2 h	4 h	8 h	24 h
Control (N/Saline)	266±41.1	321±49.2	341±38.4	346±30.8	354±25.3
Insulin	267±19.3 ^{ns}	213±23.8 ^{a} (34%)	187±13.0 ^{a} (45 %)	156.4±14.8 ^{a} (55%)	102±2.93^{a} (71%)
200mg/kg	266±24.6 ^{ns}	206±18.2 ^{a} (39%)	165±11.6 ^{a} (52%)	147.4± 7.05 ^{a} (57%)	117±6.76 ^{a}(67%)
400mg/kg	265±40.1 ^{ns}	207±19.5 ^{a} (36%)	180±18.3 ^{a} (47%)	148±22.3^{a} (57%)	139±15.2 ^{a} (60%)
800mg/kg	268±48.5 ^{ns}	240±27.2 ^{a} (25%)	191±27.9 ^{a} (44%)	168±18.4^{a} (51%)	143±16.6 ^{a} (60%)

a = P<0.05 =Significant, ns=not significant n = 5.
Vales are given as mean ± SD for 5 rats in each group; experimental groups are compared with diabetic control. Values are statistically significant at a = P<0.05 ns = not significant.

Table1. Phytochemical constituent of the crude ethanol leaves extract of *Cissampelos mucronata.*

Constituents	Inference
Reducing sugar	+
Cardiac glycosides	+
Resin	+
Tannins	+
Saponins	+
Flavonoids	+
Glycerin	+
Steroid	+
Alkaloids	-

+ = Present, - = Absent

DISCUSSION

Medicinal plants are widely used by the populations of underdeveloped countries as alternative therapy. In Africa, hundreds of plants are used traditionally for the management and/or control of diabetes mellitus. Unfortunately only a few of such African medicinal plants have received scientific scrutiny.

Many secondary metabolites participate in a variety of anti-diabetic functions *in vivo* (Kako et al., 1997) . The glycemic change in blood glucose level of diabetic rat at different time intervals after intraperitoneal administration of the leaves extract of *C. mucronata* at the doses of 200, 400, and 800 mg/kg is shown in Table 2.

In relation to the diabetes rats that received 200,400 and 800 mg/kg bodyweight of the extract of *C. mucronata* there was a significant change in the blood glucose levels (p<0.05) when compared to the control group after 2, 4, 8 and 24 h of extract administration. In regard to the dose of 200 mg/kg of *C. mucronata* it significantly (P<0.05) lowered the blood glucose level when compared to control with percentage glycemic change of 67%. Also in relation to the doses of 400 and 800 mg/kg it also significantly lowered the blood glucose levels with percentage glycemic of change of 60% after 24 h of extract administration .The highest activity resides at the lowest dose of 200 mg/kg which was found to be more effective with percentage glycaemic change of 67% after 24 h of extract administration.

Phytochemical analysis of the ethanolic extract of *C. mucronata* leaves extract revealed the presence of flavornoids, saponins, tannins, cardiac glycosides resin, reducing sugar and steroids as shown in Table 1.

Polysaccharides, coumarins, flavonoids, terpenoids and a host of other secondary plant metabolites, include-ing arginine and glutamic acid, posses hypoglycaemic effects in various experimental animal models (Akah and Okafor, 1992; Marles and Farnworth, 1995; Ross, 2001; Ojewole, 2002). Tannin containing drugs have also been shown to demonstrate anti-diabetic activity (Iwu, 1980; 1983).

Effect of the flavonoids quercetin and ferulic acid on pancreatic -cells leading to their proliferation and secretion of more insulin have been proposed by Mahesh and Menon (2004) and Sri-Balasubashini et al., (2004) as the mechanism by which they reduced hypergly-caemia caused by Streptozocin in diabetic rats. The presence of flavonoids in the ethanol crude extract of *C. mucronata* may also be acting similarly thereby decreas-ing the high blood glucose levels of STZ-diabetic rats.

In conclusion, the present study suggest that ethanol leaves extracts of *C. mucronata* possess antidiabetic properties which suggest the presence of biologically active components which may be worth further investigation and elucidation.

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