Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

European Journal of Experimental Biology, 2013, 3(1):22-27

ISSN: 2248 –9215
CODEN (USA): EJEBAU

Modulatory role of methanolic leaf extract of *Cissus cornifolia* on blood glucose levels of normoglycemic wistar rats

Jimoh A., Tanko Y. and Mohammed A

Department of Human Physiology, Ahmadu Bello University, Zaria Nigeria

---

ABSTRACT

An appropriate management approach to any disorders of glucose tolerance necessitates a strong understanding of the mechanism involved in the disease process. The preliminary phytochemical screening of methanolic extract of Cissus cornifolia revealed the presence of alkaloid, flavonoids, saponins, steroid, terperiods and tannins. Also, the LD_{50} of the extract is found to be above 5000mg/kg orally. Three doses of the extract (50, 100 and 200mg/kg), 0.2ml normal saline and 1mg/ml glibenclamide as a standard anti-diabetic drug were orally administered daily for two weeks. The fasting blood glucose levels were determined at intervals of 0, 1, 3, 5, 7, 9, 11 and 13 days. At day 0 of the extract administered, there were no significant difference in blood glucose level of the extracts treated and when compared to negative control (normal saline 0.2ml). However, after 5 days of extract administration, there was a significant ($p<0.05$) decrease in blood glucose levels in all the three doses administered when compared to negative control (normal saline). As regard to the positive control (glibenclamide) there was a significant ($p<0.05$) in blood glucose levels when compared to negative control (normal saline).The effects of methanolic extracts (50mg/kg) Cissus cornifolia on glucose tolerance tests was investigated. There was a significant ($p<0.05$) decrease in blood glucose level at 30min of initiating glucose tolerance test when compared to normal saline group. The hypoglycemic effect was pronounced from 30 to 120min after treatment. In conclusion, the three doses of the extract administered showed both significant ($p<0.05$) hypoglycemic effect in Wister rats.

Key words: Blood glucose, hypoglycemic, *Cissus cornifolia*, Diabetes mellitus, Oral Glucose Tolerance Test (OGTT)

INTRODUCTION

The public health burden of diabetes mellitus (DM) has been dramatically increased worldwide. Fasting hyperglycemia is caused by unrestrained basal hepatic glucose output, primarily a consequence of hepatic resistance to insulin action [1]. Diabetes that starts in childhood or adolescence (type I) is usually more severe than that beginning in middle or old age (type 2), but may also develop in younger people [2]. The worldwide survey reported that the DM is affecting nearly 10% of the world's population[3].The prevalence of DM for all age-groups worldwide is projected to rise from 171 million in 2000 to 366 million in 2030[4] and has been projected as the world's main disaster and killer in the next 25 years [5].

Side effect of oral hypoglycemic agents and insulin therapy necessitated the search for more effective and safer antidiabetic drugs. Recommendation by the World Health Organization for scientific search for hypoglycemic agents from medicinal plants has become even more important [6].

Cissus cornifolia (bark) is a species of the genera *Cissus* that belongs to the family *vitaceae* and has been described as a multipurpose plant which is used extensively both for its nutritional and medicinal properties. It is an annual, sub-erect herb with a height of about 1.3m from the permanent woody base. The plant is distributed in the rocky suburbs and bush savannah in China and Northern Nigeria. The plant is locally called 'Riigarbirri (rope of the monkey) in Hausa [7].

African traditional medicine amongst which is being used by the Fulani of Northern Nigeria as a remedy for gonorrhea when taken with native natron, while the leaf sap is used among the Tanganyika as a sedative in cases of mental derangement, the root-decotion is also used for malaria, septic tonsil and pharyngitis [7].

The present study was undertaken to evaluate the effects of methanolic extract of *Cissus cornifolia* on the blood glucose levels of normoglycemic wistar rats.

MATERIALS AND METHODS

Plant material

Fresh leaves of *Cissus cornifolia* plants were collected from Kufena village, Zaria, Kaduna State, Nigeria in the month of November, 2010. It was identified and authenticated at the herbarium unit of Biological Sciences, Department, A.B.U. Zaria by mallam A.U. Gallah. It was given a voucher specimen (No. 024) and deposited at the herbarium.

Animal

A total of 40 adult Wistar rats weighing (150-250g) bred in the Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmaceutical Science, A.B.U. Zaria were used for the study. The animals were kept in well aerated laboratory cages in Human Physiology Department Animal House and were fed with grower and starter mash from Vital Feeds Company and water were provided during the stabilization period.

Plant preparation

The leaves were dried under the shade and ground into powder. The air-dried powdered plant (800g) material was extracted with 70% methanol and 30% aqueous using soxhlets apparatus; the solvent was removed in-vacuno and evaporated using rotatory evaporator to yield a residue of 150g of aqueous methanolic extract.

Phytochemical Screening of the Plant

Preliminary phytochemical screening of the crude extract of *Cissus cornifolia* was performed for the presence of its constituent using the following reagents and chemicals: Alkaloids – with Mayer's and Dragendorff's reagent[8,9]. Flavonoids with the use of mg and Hcl[10,11] tannins with 1% gelatin and 10% Nacl solutions and saponins with ability to produce- d[12].

Acute toxicity studies (LD_{50})

The median lethal doses (LD_{50}) of the plant extract was determined by method of [13] using 12 rats. In the first phase, rats were divided into three (3) groups of 3 rats each and were treated with the extract at doses of 10, 100 and 1000mg/kg body weight orally and observed for 24 hours for sign of toxicity. In the second phase, three rats were divided into three groups of 1 rat each and were treated with the extract at doses of 1600, 2900 and 5000mg/kg body weight orally. The LD_{50} values were determined by calculating the geometric mean of the doses for which 0/3 and 0/1 were found.

Experimental design of normoglycemic groups

Animal fasted overnight were randomly divided into 5 group of 5 rats (n = 5):
Group 1: As negative control and were treated with normal saline (0.2ml) orally.
Group 2: received 50mg/kg body weight of the *Cissus cornifolia* extract orally.
Group 3 : received 100mg/kg body weight of the *Cissus cornifolia* extract orally
Group 4: received 200mg/kg body weight of the *Cissus cornifolia* extract orally

Group 5: As a positive control group and were treated with glibenclamide 0.1mg/kg orally.

Experimental design for oral glucose tolerance test groups
Fasted rats were divided into three (3) groups of 5 animals (n=5) to each treatment after loading them with 2g of glucose.
Group I received normal saline 0.2ml orally.
Group 2 received 50mg/kg body weight of *Cissus cornifolia* orally
Group 3 received 1mg/ml of glibenclamide orally.

Determination of blood glucose level for normoglycemic group
The blood samples were collected by cutting the tail of the rats. Blood samples for blood glucose determination were collected from the tail at interval of 0, 1, 3, 5, 7, 9, 11 and 13 days respectively. Determination of the blood glucose level was divided by the glucose oxidase principle [14] using the one touch glulometer strips and reported as mg/dl.

Determination of blood glucose level for oral glucose tolerance test
Blood samples for blood glucose determination were collected from the tail at an interval of 0, 30, 60, 90, 120, 150 and 180min following the doses by the glucose oxidase principle[14] using the one touch glucometer strips and reported as mg/dl.

Statistical analysis
All the data were expressed as mean ± S.E.M. Statistical comparisons were performed by one way analysis of variance (ANOVA) using the Duncan's multiple range tests [15].A value of $p<0.05$ was considered statistically significant. The data were analysed using SPSS vision 17.0.

RESULTS

Preliminary phytochemical screening
The result from the preliminary phytochemical analysis of the leaf extract of *Cissus cornifolia* extract revealed the presence of carbohydrate, glycoside, flavonoids, saponins, steroids, terpenoids, tannins as shown in table I below.

Table I: Preliminary phytochemical constituents of *Cissus cornifolia*

Constituents/test	Inference
Carbohydrate	++
Cardiac glycoside	++
Alkaloid	+
Flavonoids	++
Saponins	+
Tannins	+
Stenoids/terpenoids	+

+ present

Acute toxicity studies
LD_{50} of methanolic leaf extract of *Cissus cornifolia* in wistar rats was found to be above 5000mg/kg body weight orally.

Table 2: Percentage mortality of different doses of methanolic leaf extract of *Cissus cornifolia* administered orally during the first phase of the acute toxicity studies

Group (n=3)	Treatment (mg/kg)	Mortality	% mortality
1	10mg/kg extract	0/3	0
2	100mg/kg extract	0/3	0
3	1000mg/kg extract	0/3	0

Table 3: Percentage mortality of different doses of methanolic leaf extract of cissus cormifolia administered orally during the second phase of the acute toxicity studies

Group (n=1)	Treatment (mg/kg)	Mortality	% mortality
1	1600mg/kg extract	0/1	0
2	2900mg/kg extract	0/1	0
3	50000mg/kg extract	0/1	0

$LD_{50 \geq 5000}$

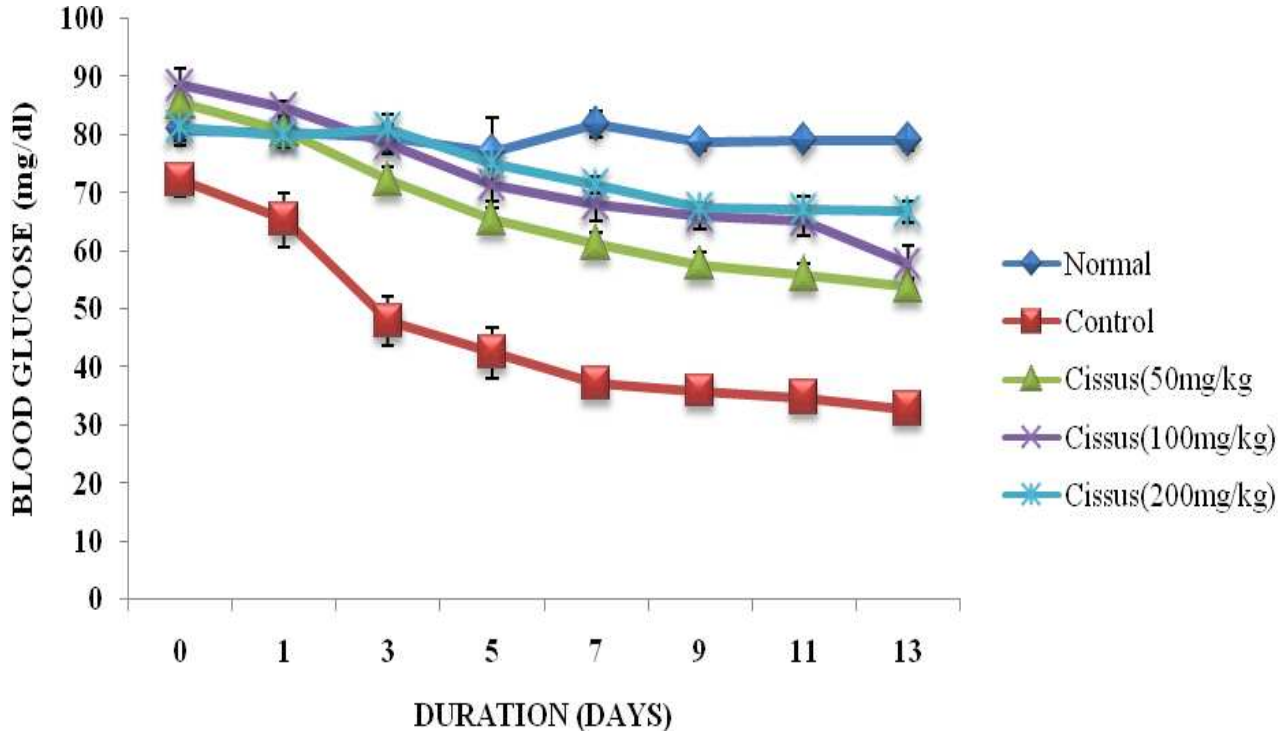

Fig.1: Effect of aqueous methanolic leaf extract of Cissus cornifolia on blood glucose levels of normoglycemic Wistar rats as compared with glibenclamide treated group and untreated control Values are expressed as mean ± SEM; n = 5

Value considered statistically when group. Compared with control group: a = $p<0.05$ significant and ns = not significant

The effect of the different doses (50, 100 and 200mg/kg) of the extract of *Cissus cornifolia a*nd the control groups (glibenclamide and normal saline groups) in normoglycemic wistar rat as shown in figure I above.

The result showed a significant decrease ($p<0.05$) in the blood glucose level with 50mg/kg after 0 day of the extract treatment when compared with the normal saline treated group, while 100mg/kg and 200mg/kg groups shows a significant decrease ($p<0.05$) in the blood glucose level after 1 day and 3 days of the extract treatment when compared with the normal saline treated group. The reference drug (glibenclamide) shows a significant decrease ($p<0.05$) in the blood glucose levels after 0 day of the extracts administered when compared with the normal saline group.

Also the effect of 50mg/kg of extract of *Cissus cornifolia* and the control group (glibenclamide and normal saline treated groups) in normoglycemic wistar rats treated with 50mg/kg body weight of glucose as shown in figure 2 above.

At 30min of initiating glucose tolerance fast, serum glucose concentration was higher than at zero time but decrease significantly from 30min to 150min, then increased at 180min after treatment (Fig. 2) methanolic extracts of *Cissus cornifolia* significantly decrease the level of serum glucose at a dose of 50mg/kg and glibenclamide at a dose of 1mg/ml from 30min to 120min but reinforced it at 180min following treatment. Similar results have been reported by Ghosh and Suryawanshi using some plant extracts but in diabetic rats [14].

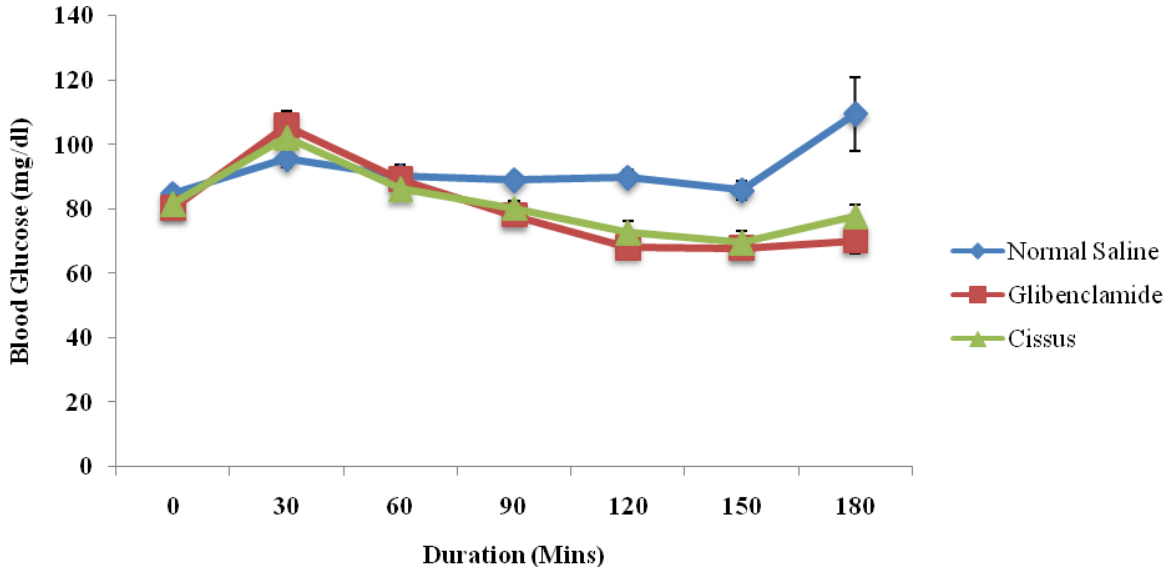

Figure 2: Oral glucose tolerance test showing the glycemic profile of aqueous-methanolic extract of *Cissus cornifolia* extrat as compared with glibenclamide treated group and untreated control group

Values are expressed as mean ±SEM; n = 5 Value considered statistically when compared with control group: a = p<0.05 significant and ns = not significant.

DISCUSSION AND CONCLUSION

In the present study, the hypoglycemic activity of methanolic leaf extract of *Cissus cornifolia* was assessed in normal rats. At 0 day of administration, there were no significant change in blood glucose level of all the experimental groups, whereas the three doses of the extracts (50, 100 and 200mg/kg) showed a significant hypoglycemic effect throughout the study period after 3 days of the administration.

The dose of glibenclamide (1mg/ml) as a positive control and 50mg/kg methanolic extract showed a significant decrease at 1 day of administration while 100mg/kg and 200mg/kg of the extract showed no significant decrease in blood glucose level when compared to negative control (normal saline). Similar results have been reported by [15] using some plant extracts but in diabetic rats.

In the oral glucose tolerance test, the *Cissus cornifolia* extract showed significant reduction of serum glucose levels with 50mg/kg extract administered. The main mechanism by which the extract brings hypoglycemic effects most probably involves stimulation of peripheral glucose consumption. Furthermore, the glycemic profile observed in the glibenclamide group indicates that the extract of *Cissus cornifolia* acts on the liver or on peripheral glucose consumption. Similar results were earlier reported by [16]

LD_{50} of the extract was found to be above 5000mg/kg body weight in rats. The preliminary phytochemical screening of the extract revealed the presence of alkaloids, steroids, terpenoids, tannins, saponin and flavonoids effect of the flavonoids quercetin and ferulic acid on pancreatic β-cells leading to their proliferation and secretion of more insulin have been proposed by [17,18] as the mechanism by which they reduced hyperglycemia.

The flavonoids present may also be acting similarly thereby decreasing the blood glucose levels in normoglycemic Wistar rats. The extract might possess glibenclamide like effect on the liver or on peripheral glucose consumption.[19]

It is concluded that the methanolic leaf extract of *Cissus cornifolia* reduced serum glucose level in normal rats, however; the effect of 50mg/kg methanol extracts were more pronounced in normoglycemic rats. Furthermore, due to the presence of hypoglycemic phytochemicals (flavonoid etc], the leaf of *Cissus cornifolia* have a potential to provide raw materials for pharmaceutical industries.

Therefore, additional studies are needed for isolation and separation of bioactive compounds from the leaf of this important medicinal plant and also to carry out the OGGT on diabetic rats to see if the extract have insulin-like effect.

Acknowledgement
The author acknowledges the support provided to the study by Mallam Ya'u bello casual staff, Mallam bala and the animal house staff Department of Human Physiology, Ahmadu Bello University, Zaria, Nigeria.

REFERENCES

[1] Daily O, *Clin. ther*. **2004**, 6, 889-901.
[2] Bell GI, *Diabetes*, **1991**, 5, 413-417.
[3] Siddharth NS, *J. diabetol* **2001**, 3,11.
[4] Amos AF, McCarty DJ, and Zimmet P, *Diab Med*, **1997**, 14, 1-85.
[5] Edwin E, Sheeja E, Gupta VB, and Jain DC, *Pharma review*, **2006**, 1, 41-2.
[6] World Health Organization, *Technical report series*, **1980**, 646, 66
[7] Burkhill HM, The useful plants of West Tropical Africa. *2nd edn.*, Royal Botanical Garden, Kew, **2000**, pp 293-294.
[8] Farnsworth RN, *J. Pharm, Sci*, **1966**, 55, 225-276.
[9] Harborne JB, *Phytochemical methods*: *A guide to modern techniques of plant analysis*; 3rd Edition. Chapman and Hall, London, **1966**, 235.
[10] Silva LG, Lee IS, Kinghorn DA, Inc., In: *methods in Biotechnology Natural product isolation*. Cannell JPR (ed) Humana, Press Inc., Totowa, new Jersey, USA, **1993**, 4, 329-363
[11] Houghton PJ, and Ranan A, Laboratory handbook for functioning of natural extracts. Chapman and Hall, London, **1998**, 199.
[12] Lorke D, *Arch Toxico*, **1983**, 54, 275-287.
[13] Beach EF, and Turner JJ, (**1958**). An enzymatic method for glucose determination in the body fluids. *Clin. Chem*, **1958**, 4, 462-465.
[14] Duncan RC, Knapp RG, and Miller MC, Test of hypothesis in population means. In: Introductory Biostatistics for the health sciences, John Wiley and Sons Inc, **1977**, NY, 71-96.
[15] Ghosh S, and Suryawanshi SA, *J. exp. Biol* **2001**, 8, 746-759.
[16] Tanko Y, Jimoh A, Goji ADT, Muhammad A, and Musa KY, *J. Nat. prod. Plant Reson*, **2011**, 1(2), 1-7.
[17] Muhammad G, Ghulam Q, Fatima N, and Zahida P, *Diabetologia croatia* **2007**, 36-3.
[18] Ahesh T, and Menon PV, *Phytotherapy Research*, **2004**, 18, 123-127.
[19] Sri Balasubashim M, Rukkumami R, Viswanathan P, and Menon PV, *Phytotherapy Research*, **2004**, 18, 310-314.