



Original Research Article

Phytochemical Screening, Acute Toxicity study and Evaluation of Antidiabetic properties of the methanolic leaf extract of *Vernonia glaberrima* (Asteraceae)

M. I. Abdullahi^{1*}, A. Uba¹, A. Yaro², O. Maxwell³, A. J. Yusuf¹, S. Kabir¹, A. M. Alhasan¹, A. Umar¹, S. S. Bello¹, I. Nasir¹

¹Department of Pharmaceutical and Medicinal Chemistry, Usmanu Danfodiyo University, Sokoto, Nigeria

²Department of Pharmacology, Faculty of Medicine, Bayero University, Kano, Nigeria.

³Department of Pharmacology, Faculty of Medicine, Usmanu Danfodiyo University, Sokoto, Nigeria

*Corresponding Author: M.I. Abdulahi, Department of Pharmaceutical and Medicinal Chemistry, Usmanu Danfodiyo University, Sokoto, Nigeria

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ABSTRACT

Vernonia species, family *Asteraceae*, have been used for the treatment of diabetes mellitus and various other ailments in traditional medicine. *Vernonia glaberrima* is employed traditionally in Northern Nigeria for treating malaria, pain, dysmenorrhea, inflammation and microbial infections. The plant material was pulverized and macerated using methanol. Phytochemical screening was conducted on the crude methanol extract to test for the presence of secondary metabolites. Acute toxicity study of the crude extract was conducted (i.p.) using Swiss albino mice. The antidiabetic effect of the extract was evaluated by normoglycemic, glucose tolerance test and alloxan-induced diabetic models. The results of the phytochemical screening indicated the presence of flavonoids, saponins, tannins, steroids, glycosides, terpenes and alkaloids. The lethal dose (LD₅₀) was found to be 1265mg/kg suggesting the plant to be fairly toxic. The crude extract (300mg/kg) exhibited significant (p<0.05) hypoglycemic activity in alloxan-induced diabetic model when compared to the control but did not show significant change in the normoglycemic and oral glucose tests. These results suggest that the extract has a moderate hypoglycemic effect and may potentially be used for the treatment of diabetes mellitus without a major drawback of hypoglycemia.

Keyword: *Vernonia glaberrima* extract; Diabetes mellitus; blood glucose; phytochemical screening

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by a loss of glucose homeostasis with disturbances of carbohydrate, fat and

protein metabolism resulting from defects in insulin secretion, insulin action or both [1]. It is commonly accompanied with hyperglycemia,

hyperlipidemia, hyperaminoacidemia and hypoinsulinaemia as a result of a decrease in insulin secretion and insulin action [2]. It is frequently associated with the development of micro and macro vascular diseases such as neuropathy, nephropathy, cardiovascular and cerebrovascular diseases [3].

Global estimates indicates about 366 million people have diabetes mellitus, with type 2 making up to about 90% of the cases [4]. Diabetes mellitus occurs throughout the world but is more prevalent in the developing countries, thus, the disease is associated with reduced quality of life and increased risk factors for mortality and morbidity [5, 6].

Currently available therapies for diabetes include insulin and various oral antidiabetic agents such as sulfonylureas, biguanides, thiazolidinediones, α -glucosidase inhibitors and glinides [7]. Despite efforts to better manage patients with type 2 diabetes, attempts at maintaining near normal blood glucose levels in these patients remains unsatisfactory, as administration of most of these agents inadvertently lead to severe and at times, fatal hypoglycemia [8]. Some of these drugs are also bedeviled by varied contraindications and exhibit several adverse effects including hypoglycaemia, digestive and haematological manifestations [9, 10]. The above factors limit their utilization as medicines. Furthermore, these products could not easily be accessible in developing countries due to low income, necessitating research on safer antidiabetic agents.

An estimated 1000 plant species are being used in folk medicine for the management of diabetes [11]. The reduction in blood glucose levels are thought to be facilitated by the bioactive secondary metabolites including flavonoids, terpenoids, coumarins and other constituents present in these plants [12, 13].

Vernonia species, family *Asteraceae*, have been used for the treatment of diabetes mellitus and various other ailments in traditional medicine.

The genus *Vernonia* is known for having several species with food, medicinal and industrial uses [14]. *Vernonia* species commonly used in ethnomedicine include *Vernonia amygdalina*, *V. condensata*, *V. cineria*, *V. Guineensis*, *V. conferta* and *V. amygdalina* is the most studied member of the *Vernonia* genus and is reported to be the most studied plant in Africa [15]. It is the most traditionally used antidiabetic herb in Nigeria [16]. Other biological activities reported for the vernonia species include, anti-microbial [17, 18], anti-malarial [19, 20], analgesics and anti-inflammatory [21], cytotoxic activity [22-24], anti-oxidant [25] and hypolipidemic effect [26]. Sesquiterpene lactones and flavonoids are some of the major bioactive constituents isolated from the *Vernonia* plant extracts [17, 18].

Vernonia glaberrima Welw. Ex O. Hoffm (family *Asteraceae*), Shiwaákár-ján-gágári (Hausa language - N. Nigeria) is an erect shrub, 2 meters high, found on hillside grassland in Guinea to Northern Nigeria, Western Cameroon and Central Africa to Angola. It is reported to be used against malaria, migraine, psoric and dysmenorrhoea [14]. It is also employed traditionally in Nassarawa State, Northern Nigeria for treating pain, inflammation, vertigo and microbial infections (Personal Communication).

In this work, we conducted a preliminary screening of the phytochemical constituents present and assessed the acute toxicity and antidiabetic property of the plant, *V. glaberrima*.

MATERIAL AND METHODS

Collection and Identification of the Plant Sample

The plant sample of *V. glaberrima* was collected in Nasarawa State, Northern-Nigeria in June 2012 during rainy season. It was authenticated by U.S Gallah of the Herbarium unit, Department of Biological Sciences, Ahmadu Bello University, Zaria. A voucher specimen (No.

899) was deposited at the herbarium for future reference.

Preparation of extract

The leaves were removed, shade dried, pulverized, labelled and stored at room temperature in an air-tight container prior to extraction. The Powdered leaves (2500g) were extracted with 70% methanol using maceration method for 10 days with occasional shaking. The extract was evaporated in-vacuo using rotary evaporator at 40°C to obtain a gummy greenish product (400g) subsequently referred to as the crude methanol leaf extract VGLE.

Preliminary Phytochemical Screening

Portion (0.5g) of the crude methanol extract was subjected to preliminary phytochemical screening for the presence of secondary metabolites in accordance with standard procedures [27].

Experimental Animals

Swiss albino mice and adult Wister rats of either sexes weighing (15-38g) and (121-180g) respectively obtained from the Animal House Facility of the Department of Pharmacology and Toxicology, Ahmadu Bello University, Zaria, Nigeria, were used for the study. They were fed with commercial feeds and water *ad libitum* and maintained under standard conditions (12hr light and 12hr dark cycle) in propylene cages at 25°C room temperature. All experimental procedures were approved by the Animal right Ethic Committee of the university.

Acute Toxicity study

The method described by Lorke [28] was employed. The route of administration was intra-peritoneal. In the first phase, nine mice of either sex were divided into three groups containing three mice each. The first, second and third groups received 10mg/kg, 100mg/kg and 1000mg/kg respectively. The rats were observed continuously for behavioral, neurological, autonomic and any lethality in the

first 24 hours. From the result of the first phase, three mice were used for the second phase. They were given different doses 1600mg/kg, 2900mg/kg and 5000mg/kg of the extract, and were observed for any sign of toxicity and possibly death during the 24 hours.

The median lethal dose was calculated using the following formula;

$$LD_{50} = \frac{\sqrt{\text{minimal lethal dose} \times \text{maximal survival dose}}}{2}$$

Animals and induction of diabetes mellitus

Seventy-five Wistar rats of both sexes weighing 121-180 g were used for the study of the effects of extract of *V. glaberrima* on the blood glucose levels of the animals. They were kept in standard cages and maintained under standard conditions (12hr light and 12hr dark cycle) at 25°C room temperature in the animal room of the Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto. They were fed on commercial feeds and water *ad libitum*. The rats were fasted from feeds for 18-24 h before the commencement of each experiment, but were allowed water *ad libitum*. The rats were then randomly divided into five groups of five animals each for the three sets of experiments viz. Normoglycemic blood glucose level, Glucose Tolerance test and Alloxan-induced Diabetes mellitus tests.

Normoglycemic Blood Glucose Determination

Group I received propylene water (normal control), Group II received Glibenclimide (5 mg/kg *i.p.*) (standard control) while groups III, IV and V received the extract at a dose of 75mg/kg, 150mg/kg, 300mg/kg orally as propylene glycol water suspension, respectively.

Oral Glucose Tolerance Test (OGTT)

OGTT for non-diabetic rats were performed according to the modified method [29]. Group I received propylene water (normal control),

Group II received Glibenclimide (5 mg/kg *i.p.*) (standard control) while groups III, IV and V received the extract at a dose of 75mg/kg, 150mg/kg, 300mg/kg orally as propylene glycol water suspension, respectively. After 30 min of drug/extract administration, the rat in each of the groups was orally administered with 200 mg/kg of glucose in form of solution.

Alloxan Induced Diabetic Rats

Alloxan monohydrate was used to induce diabetes by injecting the rats at a dose of 150 mg/kg body weight intraperitoneally. After 1 hour of alloxan administration, the animals were given feed and water *ad libitum*. The animals were kept under observation and after 72 hours, blood glucose was measured using the glucometer. The diabetic rats (glucose level 150-400 mg/dl) were selected and divided into five different groups for the experiment. Group I: control induced diabetic rats received propylene water, 1ml per oral. Group II: induced diabetic rats received Glibenclamide 5mg/kg body weight orally. Group III-V: induced diabetic animals received the extract at dose of 75mg/kg, 150mg/kg and 300mg/kg orally as a fine propylene glycol water suspension, respectively.

Determination of blood glucose levels

All blood samples were collected by cutting the tail-tip of the rats. Serum glucose of the blood samples from the tail vein was estimated at 0, 30min, 60min, 120min, 180min and 240min interval. The determination of the blood glucose level was done using the accu-chek glucometer and recorded in mg/dl [30].

STATISTICAL ANALYSIS

Data were statistically evaluated by use of one-way ANOVA, followed by student's t-test using version 18 of SPSS software and Microsoft Office Excel (2003). The values were considered significant at $p < 0.05$.

RESULTS

The result of preliminary phytochemical screening of the extract is shown in (Table 1).

Table 1: Preliminary Phytochemical Screening of *V. glaberrima*

Constituents	Test	Inferences
Alkaloids	a. Mayer's test	+
	b. Dragendorff's test	+
	c. Wagners reagent	+
Cardiac glycoside	a. Molisch's test	+
	b. Fehling solution	+
Flavonoids	a. Ferric chloride test	+
	b. NaOH test	+
	c. Shinoda test	+
Anthraquinones	a. Borntrager's test	-
Saponins	a. Frothing test	+
Steroids & Terpenes	a. Lieberman-Buchard	+
	b. Salkowski test	+
Tannins	a. Lead Sub-acetate	+
	b. Ferric chloride test	+

Key: + presence of constituent - absence of constituent

The hypoglycemic effects of *V. glaberrima* evaluated in the fasting normoglycemic animals by the oral administration of graded doses (75, 150 and 300mg/kg) of the methanol leaf extract produced insignificant hypoglycemic effect as shown in (Table 2).

The result of blood glucose level of oral glucose loaded rats as presented in Table 3 showed the graded doses of the extract produced insignificant transient decrease in blood glucose concentration (Table 3). The administration of graded doses of *V. glaberrima* leaf extract showed significant hypoglycemic activity in

alloxan-induced diabetic rats. There was significant ($P < 0.05$) decrease in blood glucose concentration (BGC) in the rats at higher dose (300mg/kg) when compared to the control hyperglycemic rats as indicated in (Table 4). The

standard drug, Glibenclamide, reduced the blood glucose levels in the normoglycemic model (Table 2) and the oral glucose loaded rats as observed in (Table 3).

Table 2: Effect of the methanolic extract of *Vernonia glaberrima* on blood glucose level of normoglycemic rats:

Group	Treatment	Blood glucose levels with time (mg/dl)					
		0 Min	30 Min	60 Min	120 Min	180 Min	240 Min
I	Control (Solvent)	107±4.16	106±4.44	104±4.63	92±5.77	100±6.04	88±4.37
II	Glibenclamide	115±4.38	109±4.42	90±10.55	63±6.77	73±6.14	64±5.71
III	75mg/kg	119±8.81	118±8.22	108±6.22	96±7.33	110±5.57	99±4.64
IV	150mg/kg	118±9.08	106±11.8	101±13.42	94±13.4	101±15.46	97.±4.64
V	300mg/kg	105±5.72	102±2.66	101±2.69	95±1.91	96±2.93	87±3.65

Each value represents Mean ± SEM. Values are statistically significant at * $P < 0.05$ compared with control

Table 3: Effect of the methanolic extract of *Vernonia glaberrima* on blood glucose level of oral Glucose loaded rats

Group	Treatment	Blood glucose levels with time (mg/dl)					
		0 Min	30 Min	60 Min	120 Min	180 Min	240 Min
I	Control (Solvent)	99±5.46	120±4.85	113±5.59	102±3.65	103±3.18	97.4±2.98
II	Glibenclamide	101±6.47	*99±6.1	*78±4.63	*60±4.43	*64±3.59	*70±8.48
III	75mg/kg	118±4.41	119±2.6	108±4.42	108±1.66	108±3.89	102±3.85
IV	150mg/kg	109±4.2	113±2.94	106±3.8	103±5.25	105±2.85	100±4.49
V	300mg/kg	99±5.17	116±4.88	100±4.48	97±4.5	101±6.22	93±3.04

Each value represents Mean ± SEM. Values are statistically significant at * $P < 0.05$ compared with control

Table 4: Effect of the methanolic extract of *Vernonia glaberrima* on blood glucose level of alloxan induced diabetic rats

Group	Treatment	Blood glucose levels with time (mg/dl)					
		0 Min	30 Min	60 Min	120 Min	180 Min	240 Min
I	Control (Solvent)	360±43.83	352±68.48	312±89.52	300±76.45	296±74.34	272±77.77
II	Glibenclimide	373±130.64	318±89.18	261±94.94	243±94.92	244±102.73	211±92.73
III	75mg/kg	471±56.73	423±63.12	378±64.16	344±68.77	326±61.19	319±53.86
IV	150mg/kg	307±83.85	290±83.7	290±93.70	294±103.2	268±94.38	269±94.08
V	300mg/kg	*331±73.4	*288±78.61	*268±65.8	*255±67.66	*234±66.84	*201±61.93

Each value represents Mean ± SEM. Values are statistically significant at * P<0.05 compared with control

DISCUSSION

Preliminary phytochemical screening of methanolic leaf extract of *V. glaberrima* revealed the presence of various chemical constituents including alkaloids, tannins, flavonoids, saponins, glycosides, steroids and terpenoids (Table 1). These are secondary metabolites with definite biological activities [31].

The intraperitoneal LD₅₀ of the crude methanol extract was found to be 1265mg/kg indicating that the extract is fairly toxic [28].

The extract exhibited antihyperglycemic activity in alloxan-induced rat model at the three graded doses. It was significant (P<0.05) at the highest administered dose of 300mg/kg when compared to control. The extract did not significantly decrease the blood glucose levels in both the normoglycemic and glucose tolerance test models. This suggests that the extract may exert its antihyperglycemic effect without causing hypoglycemia commonly observed in most of the antidiabetic drugs.

Glibenclimide, the standard drug produces hypoglycemia in normal animals by stimulating the pancreatic beta-cells to produce more insulin and by increasing the deposition of glycogen in the liver, which may not be

effective in alloxan-induced diabetic animals because alloxan treatment causes a permanent destruction of the beta cells [32]. The extract might possess insulin-like effect on peripheral tissues either by promoting glucose uptake and metabolism and/or inhibiting hepatic gluconeogenesis [33].

The reduction of BGC levels in the alloxan-induced rats may however be attributable to other possible mechanisms. *V. amygdalina* was also reported to control blood glucose levels without inducing severe hypoglycemic effect [34].

Secondary constituents might have contributed to the observed antihyperglycemic activity of the extract. It was earlier proposed that the hypoglycemic effect of *V. amygdalina* could be due to the insulin production, stimulation and release from the beta-cells possibly by the sesquiterpene lactones and other bitter constituents present in the plant [35]. Moreover, saponins [36, 37], flavonoids [38-40], alkaloids [41, 42], steroids and terpenes [43, 44], and glycosides [45, 46] have been reported to have good antidiabetic, hypolipidemic and antihyperglycemic activities [47-50].

CONFLICT OF INTEREST STATEMENT

None Declared

CONCLUSION

The findings of this research suggest that the methanolic leaf extract of *Vernonia glaberrima* may contain bioactive constituent(s) with antidiabetic activity which can be potentially used for the treatment of diabetes mellitus without a major drawback of hypoglycemia.

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