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Original Research Article

Antidiabetic Potentials of *Diodia sarmentosa* SW (Rubiaceae) Leaves on Alloxan-Induced Diabetic Rats

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Abstract

This study has investigated the antidiabetic potential of the extracts of *Diodia sarmentosa* (Rubiaceae) leaves on alloxan induced diabetic Wistar rats. The leaves are used to treat diabetes and various other disease conditions in traditional medicine. In this study, the leaves were air-dried and pulverized. A 1kg of the powdered leaves was extracted by maceration with n-hexane, chloroform and 70% methanol successively for three consecutive days, respectively. Phytochemical screening was conducted on the leaves using standard methods. Acute toxicity test was conducted on the rats by the Lorke's method. Diabetes was induced by the intraperitoneal injection of 120mg/kg body weight of alloxan monohydrate to the rats. Results of the phytochemical screening showed the presence of cardiac glycosides, saponins, tannins, triterpenoids and carbohydrates. The acute toxicity test indicated the plant to be safe since no fatality was recorded on the rats even at the dose of 5000mg/kg body weight. The n-hexane extract produced a peak significant ($p<0.05$) reduction of 75.3% in the blood glucose levels of the rats at day 7, comparable to 82.38% reduction by the control drug, glibenclamide. The chloroform and aqueous methanol extracts did not exhibit significant reductions in blood glucose levels. This study suggests that the n-hexane extract of *D. sarmentosa* leaves possesses significant ($p<0.05$) antidiabetic activity. This tends to justify its use in ethnomedicine for the management of diabetes and its related conditions.

Keywords: *Diodia sarmentosa*, (Rubiaceae), Antidiabetic, Alloxan-induced diabetic Wistar rats.

INTRODUCTION

Diabetes mellitus is a metabolic disorder of insulin characterized by hyperglycaemia and alterations in carbohydrate, fat and protein metabolism [1]. It causes significant morbidity and mortality due to microvascular and macrovascular complications [2]. In a review article by Jung, *et al.*, [3], it was reported that traditional medicines from readily available medicinal plants offer a great potential for the discovery of new antidiabetic drugs. And that most of the plant extracts exhibited hypoglycemic, hypolipidemic, and antioxidant effects in animals as well as in humans, which may be useful in treating diabetes and its complications. According to this report, the chemical constituents with the greatest effect on post-prandial hyperglycaemia include insulin, amylin analogues, and glucosidase inhibitors.

It has also been suggested that in hyperglycaemia associated with diabetes, the use of aldose reductase inhibitors is useful in the treatment of diabetic complications [4]. The identified plant-derived aldose reductase inhibitors include the isoquinoline alkaloids, berberine chloride and palmatine iodide. Aldose reductase catalyzes the reduction of glucose to sorbitol. Sorbitol does not readily diffuse across cell membranes, and when it accumulates it produces osmotic stresses on the cells by drawing water into them. The intracellular accumulation of sorbitol has been implicated in the chronic complications of diabetes, especially in the microvascular damage to the retina, kidney, and nerves [5-7]. Some plant extracts have been reported to exert antidiabetic effects by stimulating insulin release from the pancreatic beta cells. These plants include the aqueous leaf extract of *Aegle marmelos*, and the ethyl ether extract of *Allium sativum* [8]; the seeds of *Acacia arabica* [9]; and the aqueous extract of *Agrimony eupatoria* [10].

The leaves of *Diodia sarmentosa* have been reported to be used by traditional medicine practitioners in Southern Nigeria to manage diabetes mellitus. It is a straggling perennial evergreen plant with alternate leaf arrangement with climbing stems up to about 3m. The

leaves are opposite ovate to ovate lanceolate. The inflorescence consists of several white flowers clustered in the leaf axils. It can be found in gallery forest, grassy places, in the light shade of big trees, humus soil, old secondary swampy forest, on the roadside of secondary forest, in swampy open places and also in cultivated fields and bush fallow re-growths in the forest zones [11]. It is widespread in tropical Africa, Asia and America.

In Nigeria, *D. sarmentosa* leaves are also used for treating eczema; its juice is used to stop bleeding, and the plant is used as anti-abortifacient [12]. In addition, the ethanol extract of the whole plant was reported to have anti-ulcer activity [13]. Three iridoid glycosides, asperuloside, geniposidic acid and asperulosidic acid; a coumarin glycoside, scopolin; and 6 flavonoids, rutin, kaempferol-3-0-rutinoside, quercitrin, astragalin, isoquercitrin and quercetin were isolated from the methanolic extract of the whole plant of another species, *D. teres* [14]. The present study aims at investigating the *in vivo* antidiabetic potential of *Diodia sarmentosa* in alloxan-induced diabetic rats.

MATERIALS & METHODS

Plant material: The fresh leaves of *Diodia sarmentosa* SW (Rubiaceae) were collected in Choba, Port Harcourt and authenticated by a taxonomist, Dr. M. Bassey in the Department of Botany, University of Uyo, Uyo, with a Herbarium number, UUH032/15.

Experimental animals: Healthy male and female Wistar albino rats weighing between 150-200g were used for this study. The rats were fed with standard feed and water *ad libitum*, throughout the study [15].

Phytochemical screening: Phytochemical screening of the leaves of the plant was carried out using standard procedures [16, 17].

Extraction of plant material: A 1kg of the dried and pulverized leaves was extracted successively with n-hexane, chloroform and 70% aqueous methanol. Each of the filtrates was evaporated to dryness *in vacuo*, in a rotary evaporator to obtain the respective extracts. Each of these extracts was then used for the biological assay.

Acute toxicity test: 18 animals of both sexes were used in the determination of the acute toxicity of the extract as previously reported [18].

Induction of diabetes mellitus: Hyperglycaemia was achieved by intraperitoneal administration of 120mg/kg body weight dose of alloxan monohydrate [19]. After 4 days, animals showing blood glucose levels above 200mg/dl were considered diabetic, and selected for the study.

Treatment schedule: The rats were divided into 6 groups of five rats each. Group 1 was normal control rats (non-diabetic, untreated); Groups 2-6 were diabetic rats. Group 2 were treated with glibenclamide, 2.5mg/kg body weight; Group 3 were diabetic untreated; Groups 4-6 were each treated with one of the three extracts at 1000mg/kg b.w., respectively

Day 1: Basal blood glucose levels at zero time were determined (with glucometer) before the oral treatment. The drug and extracts were administered immediately to rats in the appropriate cages. Animals in groups 1 and 3 were not treated. Blood glucose levels were determined at 60 minute intervals for the next 180 minutes as previously described [19]. Days 2-7: The blood glucose levels were taken once daily, 2 hours after the administration of drug and extracts.

STATISTICAL ANALYSIS

All data obtained from the study were expressed as mean ± SEM. Percentage reduction in blood glucose levels were calculated. Statistical analysis was done by ANOVA using SPSS package version 2.0.The significant difference ($p<0.05$) between the treated and untreated control animals was established by student's t-test.

RESULTS

Phytochemical screening: The results in Table 1 show the presence of cardiac glycosides, tannins, saponins, steroidal and triterpenoid nuclei. Alkaloids, anthraquinones, flavonoids and cyanogenic glycosides are absent.

Table-1: Results for the phytochemical screening of the dried leaf extract of *Diodia sarmentosa*

S/N	TESTS FOR METABOLITES	INFERENCE
1	Tannins (Ferric chloride test)	+
2	Phlobatannins	-
3	Alkaloids: a. Dragendorff's reagent b. Mayer's reagent c. Hager's reagent	- - -
4	Anthraquinones (Borntrager's test)	-
5	Carbohydrate (Molisch's test) Reducing Sugar test (Fehling's reagents)	+ -
6.	Saponins: a. Frothing test b. Emulsion test c. Haemolysis test	+ + +
7.	Steroids (Salkowski test) Triterpenoids (Liebermann-Burchard test)	+ +
8.	Cardiac glycosides: a. Deoxy sugars (Keller-Kiliani test) b. Cardenolides (Kedde's test)	+ -
9.	Cyanogenic glycosides	-
10.	Flavonoids: a. Sodium hydroxide test b. Shinoda test	- -

Key: + = positive (present); - = negative (absent).

Table-2: Results of the acute toxicity tests on the crude aqueous methanol extract of *Diodia sarmentosa*

DAY	DOSE, mg/kg body wt.	SURVIVAL	DEATHS
1	10 100 1000	3/3 3/3 3/3	0/3 0/3 0/3
2	1600 2900 5000	3/3 3/3 3/3	0/3 0/3 0/3

Acute toxicity test

As shown in Table 2, no death was recorded among the animals even at the highest dose of 5000mg/kg body weight, on Day 2.

Table-3: Effect of the various extracts of the leaf of *Diodia sarmentosa* on blood glucose levels (mg/dl) of alloxan-induced diabetic rats (Day 1)

TREATMENT	DOSE (mg/kg body wt.)	DAILY BLOOD GLUCOSE LEVELS (mg/dl)			
		0 min.	60 min	120 min.	180 min.
n-Hexane extract	1000	530.0 ±47.51	373.67±18.28 (28.1)	385.57± 2.74	424.3± 16.67 (19.58)
Chloroform extract	1000	389.0± 7.23	421.33± 6.0	522.67±17.68	543.67±26.56
Aq. Methanol extract	1000	513.33± 2.58	516.00 ±2.79 (0.83)	460.67±19.46	490.33±29.17 (7.08)
Glibenclamide	2.5	255.00 ±9.76	217.33 ±8.36 (58.23)*	150.33±12.28 (38.5)*	186.0 ±16.13 (64.7)*
Diabetic untreated rats	-	531.00 ±5.50	520.33 ±3.78	370.0 ±15.20	527.67±17.07
Normal rats	-	118.00 ±3.79	118.00 ±3.79	128.00 ±4.26	119.00 ±6.11

Each value is represented as mean ± S.E.M, where n=5; * represent the values that are significantly ($p<0.05$) different compared to the diabetic untreated. The figures in parenthesis represent the % decrease in blood glucose levels.

Table-4: Results of the effects of the various extracts of *Diodia sarmentosa* on fasting blood glucose levels of alloxan-induced diabetic rats (Day2-7)

TREATMENT (mg/kg b.w)	DAILY BLOOD GLUCOSE LEVELS (mg/dl)					
	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7
n-Hexane (1000)	345.67± 7.68 (33.1)*	353.0± 29.19 (30.73)*	309.0± 19.29 (43.1)*	194.0± 3.15 (63.35)*	141.33± 8.52 (73.67)*	124.67± 1.48 (75.23)*
Chloroform (1000)	556.67± 9.67	597.33± 7.21	548.6± 27.79	564.33± 21.8	572.67± 22.0	534.37± 24.9
Aq. Methanol (1000)	399.00± 32.0 (22.87)	402.00± 42.7 (21.11)	434.00± 22.4 (20.22)	422.67± 22.2 (20.15)	311.33± 9.36 (41.9)*	406.67± 3.88 (19.2)
Glibenclamide (2.5)	158.3± 8.68 (69.39)*	124.6± 1.39 (75.52)*	113.67± 8.91 (79.10)*	105.0± 1.62 (80.16)*	107.3± 8.45 (80.0)*	88.67± 6.67 (82.38)*
Diabetic untreated	517.3± 19.1	509.5± 9.97	544.0± 17.95	529.3± 10.3	536.6± 5.54	503.3± 9.61
Normal rats	109.0± 3.6	100.3± 3.33	114.0± 7.55	113.0± 7.42	118.0± 7.42	120.6± 7.33

Each value is represented as mean ± S.E.M, where n=5; * represent the values that are significantly ($p<0.05$) different compared to the diabetic untreated. The figures in parenthesis represent the % decrease in blood glucose levels.

DISCUSSION

Table 2 shows that even at the highest dose of 5000mg/kg body weight there was no mortality of the tested rats. This implies that the extract is safe [18], and that its use in ethnobotany as an oral medication for treating dysentery is not deleterious to health at these tested dose ranges [20]. Table 3 shows that none of the extracts demonstrated significant reduction in blood glucose levels after 180 minutes on Day 1 of the study. However, the standard drug, glibenclamide showed a significant ($p<0.05$) 64.7% reduction at 180 minutes. This suggests that the extracts may not be effective in providing an immediate action against hyperglycaemia. In Table 4, the n-hexane extract produced a peak significant ($p<0.05$) reduction of 75.3% in blood glucose at day 7, when compared to the diabetic untreated group of rats. This is comparable to the peak reduction of 82.3% exhibited by the standard drug, glibenclamide. However, the chloroform and aqueous methanol extracts did not produce significant reductions. There are more than 400 plants with anti-diabetic properties and most of these plants contain flavonoids, carotenoids, terpenoids and alkaloids which have frequently been implicated in having this activity [10, 21, 22, 23,]. The phytochemical compounds found to be present in the leaf of *D. sarmentosa* include cardiac glycosides, saponins, tannins, triterpenoids and carbohydrates (Table 1). These constituents may be responsible in part for the observed significant activity of the n-hexane extract of the leaf of this plant, either singly, or in synergy with one another [24].

CONCLUSION

The results of this study show that the n-hexane leaf extract of *D. sarmentosa* possesses antidiabetic properties. This tends to justify its use in ethnomedicine for the management of diabetes mellitus.

Further work

Further work is on-going to isolate and characterize the active compounds responsible for this reported activity.

No conflict of interest

The authors do hereby affirm that there are no conflicts of interest on this work.

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