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INVESTIGATION OF ANTI-DIABETIC POTENTIAL OF DIODIA SARMENTOSA IN ALLOXAN-INDUCED DIABETIC ALBINO RATS

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ABSTRACT

Purpose: Natural remedies from medicinal plants are considered to be effective and safe alternative treatments for diabetes mellitus. *Diodia sarmentosa* (Rubiaceae) commonly called tropical button weed, has been used locally in the treatment of diabetes. This study demonstrates the hypoglycaemic and anti-diabetic activity of the aqueous ethanol extract and ethylacetate fractions of *Diodia sarmentosa* in alloxan-induced diabetic rats. **Methods**: 1000g of the leaves (air dried at 25^0C for 7days) was subjected to extraction using 80% aqueous ethanol and then fractionated with ethylacetate. This gave a percentage yield of 13.299%w/w, 28.30%w/w, and 11.62%w/w for aqueous ethanol extract, ethylacetate soluble and insoluble fraction respectively. Three doses (1000mg/kg, 500mg/kg and 250mg/kg body weight) of the three samples were administered to the test groups while Glibenclamide at dose of 2.5mg/kg body weight was administered to the control group. Comparing the activity of the plant to the standard drug, the activity of all the doses of the aqueous ethanol extract and ethylacetate insoluble fraction was comparable with that of the standard drug (Glibenclamide). **Result**: As regard to the dose response, the highest activity resides in the highest dose of sample administered (i.e 1000mg/kg body weight) followed by the 500mg/kg and the 250mg/kg body weight (1000mg/kg>500mg/kg>250mg/kg). The aqueous ethanol and ethylacetate soluble fractions caused a significant ($p>0.05$) reduction in blood glucose of the diabetic rats. **Conclusion**: Thus, this present study validates the medicinal potential of *Diodia sarmentosa* in diabetes treatment.

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INTRODUCTION

Diabetes mellitus is a disorder of carbohydrate, fat and protein metabolism with elevations in fasting and postprandial blood glucose and a significant increased risk of various organ problems, atherosclerosis, nerve dysfunction and stroke [1]. It is the most common endocrine disorder affecting more than 300million people worldwide [2]. It is ranked among the leading causes of death especially when all its fatal complication are taken into account [3]. Diabetes mellitus is a major health problem with increasing prevalence all over the world. This is evident in the projection by the WHO that by 2030, diabetes mellitus will become the seventh leading cause of death in the world [4]. Worldwide prevalence of diabetes for all age groups was estimated to be 2.8% in 2000 and it is projected to be 5.4% in 2025 and has been associated with sedentary lifestyle [5]. The global prevalence of type 2 diabetes is estimated to reach 4.4% by 2030. Among the various type of diabetes mellitus, type 1 diabetes account for about 5-10% of diabetics diagnosis, while type 2 account for 90% [6].Total number of patients with type 2 diabetes will reach 366 million in 2030 [7, 8].

According to the 2004 estimates, the Diabetes Association of Nigeria (DAN) puts the diabetics' population in Nigeria at about 10 million and about half of this number is in Lagos state because of its very cosmopolitan nature. In a separate study by Akinkugbe et al, crude prevalence rate of 7.2% was reported for the Lagos mainland (one of the LGA's in the present survey) in a national non-communicable disease survey [9]. Blood glucose level is normally maintained in a very narrow range, usually 70 to 120 mg/dL [10]. Globally, herbal medicine has been used extensively in the treatment of diabetes and other diseases that constitute growing health problems among residents in Nigeria especially the less privileged ones who cannot afford orthodox medicine. This situation is further complicated by the fact that most antidiabetic drugs in use today present with serious side effects that have placed limitations to their usage. This study is thus designed to scientifically establish the antidiabetic activity of *D. sarmentosa and* to justify the use of the plant by traditional practitioners for the treatment of diabetes mellitus and also to determine the toxicity profile of the plant. Management of this disorder involves lifestyle changes, proper dieting, exercise and administration of insulin preparations or hypoglycaemic medications [11]. Although, oral hypoglycaemic agents and insulin are cornerstones in the treatment of various types of diabetes, their uses are limited by high cost, low efficacy, associated side effects, failure to prevent the long-term complications thus leading to non-compliance to therapy and ultimately treatment failure [12]. Hence, the management of the disease remains a major health care challenge in Nigeria and other developing countries. This has prompted researchers globally to explore and assess new methods and more appropriate ways to control the disease and its complications[12]. Treating diabetes with plant-derived compounds which are accessible and do not require laborious pharmaceutical synthesis seems highly attractive and potentially rewarding. The increasing public demand for natural products possessing anti-diabetic activity with fewer side effects has led the pharmaceutical industries and academic researches to pay more attention to medicinal plants [13]. Over the years, a number of successes have been recorded in the treatment of diabetes using herbs by local medical practitioners in Nigeria and globally. One example of such is metformin (biguanide) is traced to *Galega* officinalis [14, 15]. This study is therefore aimed at evaluating the antidiabetic activity of ethanol extract and ethylacetate fractions of the leaf of *Diodia sarmentosa* in alloxan-induced diabetic rat model.

MATERIALS & METHODS

Fresh leaves of *Diodia sarmentosa* (Rubiaceae) were collected from farm areas in Ozoro, Isoko North LGA of Delta State Nigeria in April 2013. The fresh leaves were identified and authenticated by a botanist. Adult Wister albino rats (4-6 months old) of both sexes weighing 250-350g were obtained for this study. The animals were housed in metallic cages, acclamatized for two weeks under standard environmental conditions and maintained on a regular feed and water *ad libitum* before being employed for experiment. Fresh leaves of plant were collected and dried at room temperature (25±2^{o}C) for seven days. The dried powdered leaves of *Diodia sarmentosa* (1000 g) was extracted by maceration in 80% ethanol. The extracting vessel was shaken intermittently before being kept in a cool cupboard overnight. The solvent was filtered every day and replaced with fresh 80% ethanol. The extracts were first filtered with a white muslin cloth after which the filtrates were refiltered using a Whatman no 1 filter paper and suction pump (Buckner funnel). The resultant extract was weighed and stored in a clean sample bottle in a desiccator until required for analysis [16]. From the crude 80% aqueous

ethanol extract, 50 g was fractionated using ethyl acetate to obtain soluble and insoluble fractions of ethylacetate according with slight modification as done by other researchers [17–20]. The ethyl acetate fractions were concentrated in vacuum using a rotary evaporator to obtain a semi-solid mass. The concentrated ethylacetate fractions were then evaporated to dryness in a water bath at 40-45°C. The resultant extract was weighed and stored in a clean sample bottle in a desiccator until when required for analysis. The various extract yields were determined.

The animals were fasted overnight for 12 hours but allowed free access to water. Initial blood glucose level (zero time) was determined before assay using glucometer/strips (accuchek) as described by *Saleem et al* [21]. Rats in groups 1-3 were made hyperglycaemic by oral administration of 4g/kg glucose solution. Rats in group 4 were not glucose loaded or treated with extract. Extract of *D. sarmentosa* (1000mg/kg) was administered orally to animals in group 1 while glibenclamide (2.5mg/kg) was administered to group 2. Group 3 served as hyperglycaemic untreated control. Blood glucose was determined 30minutes after treatment and continued every 30minutes for three hours [16].

Alloxan-induced diabetic test

Induction of diabetes and grouping of diabetic rats

Animals were divided into 13 groups of 4 animals each. 12 groups were made to fast overnight and made diabetic by a single intraperitoneal injection of alloxan monohydrate (180mg/kg) dissolved in normal saline [22]. The animals turned diabetic within 7 days. Animals with blood glucose level ≥200mg/dl was considered to be diabetic and used for this study.

Statistical Analysis

Data from the study was statistically evaluated using one way Analysis of Variance (ANOVA) followed by Bonferroni *posthoc test* in statistical package for social sciences (SPSS version 20.0) to compare the equality of two or more independent groups of treated means with 5% level of significance. The test of significance may be taken as procedures that enable us to accept or reject hypothesis or to determine if observed samples differ significantly from expected results. Besides, the results are expressed as mean ± SD. $P<0.05$ was considered statistically significant.

RESULT

Phytochemical screening

The results of the preliminary phytochemical analysis of the leaf extract of *Diodia sarmentosa* revealed the presence of flavonoids, saponins, tannins, steroids and glycoside.

Acute toxicity studies

The LD_{50} of ethanol extract of *Diodia sarmentosa* in Wistar rats of both sex was found to be above 5000mg/kg body weight orally.

Table 1: Percentage yield of extract and fractions

Extract	% yield (%w/w)
80% aqueous ethanol extract	13.3
Ethylacetate insoluble fraction	65.3
Ethylacetate soluble fraction	14.9

DISCUSSION

The present study investigated the phytochemical, pharmacological, and anti-diabetic activities of the leaves of *Diodia sarmentosa.* The aqueous ethanol leaf extracts of *Diodia sarmentosa* was obtained by cold maceration of 1000g of the starting material for 96 hours (4 days). The percentage yield of ethanol extract, ethylacetate insoluble and ethylacetate soluble fraction of the plant were calculated to be 13.29% w/w, 65.28% w/w and 14.9% w/w respectively. The phytochemicals of medicinal importance detected in the crude powder leaves of the plants, aqueous ethanol extract, ethyl acetate soluble fraction and insoluble fraction as presented in the results are saponins, flavonoids, steroids, glycosides and tannins, while alkaloids, phlobatannins, cyanogenetic glycosides and anthraquinones were not detected. Flavonoids and tannins are phenolic compounds. Plant phenolics have been found to be a major group of compounds that act primarily as antioxidants or free radical scavengers [23]. Evaluating the hypoglycaemic activity of the plant in alloxan-induced diabetic rats; the aqueous ethanol extract and ethyl acetate insoluble fractions of *D. sarmentosa* at various doses of 250, 500 & 1000 mg/kg body weight and also the standard drug (glibenclamide, 2.5 mg/kg b.w) caused a significant decrease ($p<0.05$) in the blood glucose level of the diabetic rats. During the oral glucose tolerance test, it was found that the the postpandrial blood glucose levels of the rats increased to the peak at 30 minutes after oral administration of glucose. Treatment with ethanol extract (1000 mg/kg) produced a significant ($P<0.05$)

hypoglycaemic effect (24.66%) in glucose loaded rats at 60minutes. The ethanol extract evoked a progressive decrease in blood glucose level up to 210 minutes. Result showed that glibenclamide also evolved a significant (P<0.05) suppression of blood glucose occurring from 1hour at 22.06%. The pronounced effect being recorded was at 210 minutes with 35.69% and 43.62% for aqueous ethanol extract and glibenclamide respectively.

From the results obtain, it could further be deduced that the anti-diabetic activity of the 80% aqueous ethanol extract and ethylacetate insoluble fraction at all doses were comparable with that of the standard drug, glibenclamide. Significant decrease was seen from day 3 in the group treated with ethanol and ethylacetate insoluble fraction and to almost normal by day 7 when compared to diabetic untreated control. In the ethylacetate soluble fraction, reduction of blood glucose in diabetic rat was not statistically significant (p<0.05) when compared to diabetic untreated values. Maximum activity was on day 6 fasting blood glucose at 80.04% for glibenclamide and 86.13% for 500 mg/kg aqueous ethanol extract group on day 8 of sacrifice (see Table1, 2, 3 and 4).

Tannins and saponins are also found to be effective antioxidants, antimicrobial, and anti-carcinogenic agents [24]. These activities are thought to be primarily due to the anti-oxidant activity of flavonoids. Furthermore, phytochemicals such as saponins, terpenoids, flavonoids and tannins are found to inhibit cancer cell proliferation, regulate inflammatory and immune response as well as protect against lipid peroxidation [23]. Some of these potential health benefits of polyphenolic substances have been related to the action of these compounds as antioxidants, free radical scavengers, quenchers of singlet and triplet oxygen and inhibitors of peroxidation [25]. This result suggests that the leaves of the plants relatively regulate inflammatory and immune response as well as protect against lipid peroxidation and complications [23].

Anti-diabetic Study

Table 2: Effect of fractions of *Diodia sarmentosa* and Glibenclamide on blood glucose of alloxan-induced diabetic rats in day 1 of treatment.

Treatment	Dose (mg/kg b.w)	Daily blood glucose level			
		0HR	1HR	2HR	3HR
Diabetic treated with ethylacetate Insoluble fraction	1000	356.80±64.95 (11.11)*	295.00±85.68 (35.02)*	439.40±35.32 (11.69)	437.20±33.51 (17.02)
	500	401.40±53.92 (20.25)	454.00±92.25(10.41)	393.40±67.50(31.07)*	373.60±33.38(30.45)*
	250	336.60±40.18(33.12) *	586.00±9.62(15.64)	486.00±11.60(14.84)	493.60±74.34(8.10)
Diabetic treated with Ethylacetate soluble fraction	1000	355.40±54.13(19.02)	270.60±59.58(0.00)	297.00±46.09(4.21)	292.40±23.37(19.74)
	500	265.60±43.56 (11.05)	201.00±57.43 (25.72)	250.00±29.03 (12.28)	236.00±20.62 (3.36
	250	207.00±8.46 (30.68)	184.60±62.04 (31.78)	190.20±51.32 (33.26)	179.40±49.32 (26.54)
Diabetic treated with Glibenclamide	2.5 ml/kg	596.60±5.27 (18.54)	536.80±54.13 (5.93)	469.60±77.79 (17.72)	447.00±74.98 (16.78)
Diabetic untreated administered DMSO	3 ml/kg	503.30±18.99	506.75±10.09	570.70±21.69	537.13±97.42
Diabetic Untreated administered olive oil control	3 ml/kg	298.60±170.96	270.60±130.87	285.00±157.68	244.20±40.42
Normal		77.20±0.84	84.00±1.58	70.20±6.06	83.60±2.70

Values are mean ±SD; n= 5; figures in parenthesis indicate % decrease in blood glucose. Superscripted items (*) indicate significant different from control at P< 0.05.

Table 3: Hypoglycaemic effects of fractions of *Diodia sarmentosa* on post-treatment blood glucose(mg/dl) of diabetic rats from days 2-7 of treatment.

Treatment	**Dose (mg/kg)**	Daily Post- **Daily blood glucose level** blood glucose					
		DAY 2	**DAY 3**	**DAY 4**	**DAY 5**	**DAY 6**	**DAY 7**
Diabetic treated with ethylacetate Insoluble fraction fraction	1000	490.40±81 (14.17)	403.40±12 (31.14) *	497.60±69.31 (2.39)	340.20±20.54 (40.65) *	253.20±41.4 (57.33) *	227.60±26. (61.20) *
	500	544.60±53. (4.68)	518.40±33 (11.51)	488.00±29.83 (4.28)	356.80±55.26 (38.04) *	303.20±20.9 (48.90) *	247.80±54. (57.76) *
	250	494.80±74. (13.40)	496.40±74 (15.26)	262.80±1.30 (48.45) *	399.20±23.40 (30.68)	270.75±23.3 (54.37) *	255.50±41. (56.45) *
Diabetic treated with ethylacetate soluble fraction	1000	515.40±56. (7.97)	494.20±37. (2.75)	515.20±3.35 (35.44)	511.20±48.19 (14.37)	466.80±8.90 (33.6)	463.60±33. (19.95)
	500	440.00±64. (21.43)	446.20±29 (12.20)	466.80±3.96 (22.71)	352.40±74.61 (40.97)*	272.40±57.6 (22.04)	335.20±51. (42.13)*
	250	577.20±44. (3.07)	502.40±57. (1.14)	497.40±63.97 (30.76)	445.40±60.83 (25.39)	467.40±81.6 (33.77)	427.60±69. (26.17)
Diabetic untreated administered DMSO	3ml/kg	571.33±19.	585.80±11.	509.80±44.25	575.87±19.53	593.40±4.16	575.87±19.
Diabetic Untreated with oil control	3ml/kg	560.00±38.	508.20±60.	380.50±216.6	597.00±2.45	349.67±215.	597.00±2.4

Values are mean ±SD; n= 5; figures in parenthesis indicate % decrease in blood glucose. Superscripted items (*) indicate significant different from control at P< 0.05.

Table 4: Effect of fractions of *D. sarmentosa* and glibenclamide on fasting blood glucose(mg/dl) of alloxan-induced diabetic rats.

Treatment	**Dose (mg/kg)**	**Fasting blood glucose**		
		DAY 4	**DAY 6**	**DAY 8**
Diabetic treated with ethylacetate Insoluble fraction	1000	70.80±7.46 (38.44)*	53.80±2.49 (27.79)*	50.40±0.89 (20.09)*
	500	88.60±8.08 (81.27)*	87.40±22.61 (85.55)*	69.80±14.52 (82.41)*
	250	107.80±23.82 (60.48)*	184.40±35.75 (65.34)*	167.40±16.35 (78.60)*
Diabetic treated with Ethylacetate soluble fraction	1000	241.20±47.92 (49.42)	139.20±15.34 (3.69)*	275.60±88.83 (21.17)*
	500	188.80±54.64 (38.30)*	293.40±68.10 (6.61)	270.40±28.91 (1.73)*
	250	307.20±67.07 (14.89)	248.00±49.05 (8.50)	288.40±47.13 (9.88)*
Diabetic treated with Glibenclamide	2.5mg/kg	162.80±3.90 (67.69)	82.80±40.12 (82.26)*	96.40±22.68 (80.04)*
Diabetic untreated administered DMSO		571.33±19.61	585.80±11.10	509.80±44.25
Diabetic Untreated administered olive oil control	3ml/kg	306.0±157.7	275.25±103.15	265.75±123.62
Normal		74.20±0.84	81.00±1.58	80.20±6.06

Values are mean ±SD; n= 5; figures in parenthesis indicate % decrease in blood glucose. Superscripted items (*) indicate significant different from control at P< 0.05.

Oral glucose tolerance test

Table 5: Effects of aqueous ethanol extract (1000mg/kg) of *Diodia sarmentosa* and glibenclamide on blood glucose (mg/dl) level of glucose loaded rats.

	Blood Glucose level (mg/dl)								
	0 hr	**30min**	**60min**	**90min**	**120min**	**150min**	**180min**	**210min**	**240min**
Hyperglycaemic rats (treated with extract 1g/kg)	98.20 ±16.04 (0.89)	123.80 ±17.85 (16.54)	110.00 ±13.69 (24.66)*	102.40 ±15.69 (26.51)*	97.40 ±15.90 (28.91)	91.80 ±19.64 (29.75)*	83.00 ±13.27 (27.41)*	74.60 ±6.43 (35.69)*	69.60 ±11.28 (31.09)
Normal rats(mg/dL)	74.80 ±15.47	77.20 ±7.98	74.80 ±12.34	72.60 ±6.80	75.40 ±13.52	72.40 ±8.08	68.40 ±4.16	66.40 ±3.44	67.40 ±3.97
Hyperglycaemic rats (treated with glibenclamide 2.5mg/ kg)	92.00 ±6.04 (5.48)	140.80 ±23.30 (5.08)	113.80 ±16.35 (22.06)*	111.00 ±7.78 (20.34)*	119.20 ±13.65 (12.99)	87.20 ±3.96 (33.27)*	78.40 ±6.88 (31.43)*	65.40 ±7.92 (43.62)*	68.80 ±4.76 (31.88)
Hyperglycaemic (untreated, control)	97.33 ±4.73	148.33 ±9.02	146.00 ±6.56	139.33 ±15.95	137.00 ±10.39	130.67 ±9.45	114.33 ±21.36	116.00 ±7.00	101.0 ±4.00

Values are mean ±SD; n= 5; figures in parenthesis indicate % decrease in blood glucose. Superscripted items (*) indicate significant different from control at P< 0.05

CONCLUSION

This works assess the efficacy and usefulness of *Didia sarmentosa* as anti-diabetic agent, which if explored further would be highly beneficial in the pharmaceutical world as its extracts have a promising outcome in reducing plasma glucose. Its investigates the anti-diabetic property of the leaves of *Diodia sarmentosa*. With the successful induction of insulin dependent diabetes in albino rats, the leaves of the plants were found to reduce significantly both fasting and post-treatment blood glucose levels and mechanism of reduction in blood glucose maybe due to its ability to restore damaged beta cells of islet. The antidiabetic activity of the plant was resident in the 80% aqueous ethanol extract and ethylacetate insoluble fraction. Comparing the activity of the plant to the standard drug, the plant was about the same efficacy as the standard drug when used under the same conditions.

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Nil

CONFLICT OF INTEREST

The authors declare no conflict of interest

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