The antidiabetic effects of ethnanol bulb extract of *Discorea bulbifera* in alloxan-induced diabetic Wistar rats

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Abstract

Objectives: Diabetes Mellitus, as one of the major diseases affecting human population all over the world has caused significant morbidity and mortality. The management of this condition has raised the demand for safe and cost effective remedial measures due to several side effects associated with the present use of modern medicines. Thus, it is crucial to explore other options for diabetes management such as the use of medicinal plants.

Materials and Method: Animals were divided into six groups of six rats each. All groups except the normal control group were administered a single intraperitoneal dose of 150 mg/kg alloxan monohydrate to induce diabetes. Following the induction of diabetes, rats were orally treated with 100 mg/kg, 200 mg/kg and 400 mg/kg in diabetic groups. Blood glucose level, haematologogical indices, liver function, kidney indices and lipid profile were all carried out.

Results: All treated rats responded positively to all treatment and hyperglycemia was reversed within seven days of treatments of continuous administration of the extracts. There was significant ($p<0.05$ and $p<0.01$) decrease in blood glucose as well as a significant increase in all haematological parameters when compared with diabetic control. The effect of the extracts on serum lipid profile, kidney and liver enzymes were also measured in diabetic and non-diabetic Wistar rats. *Dioscorea bulbifera* bulb extracts shows significant, reduction in total cholesterol, LDL, VLDL and improvement in HDL in diabetic rats after 21 days experiment.

Conclusion: The findings indicates that ethanol extracts of *D. bulbifera* bulb possesses hypoglycemic effects.

Keywords: *Dioscorea bulbifera*; Diabetes; Haematological parameters; Kidney indices; Liver indices; Rats

Introduction

There has been extensive research into herbal therapies and complementary medicine for the treatment of a variety of chronic and terminal diseases, including cancer, obesity, and diabetes for decades (1). Several allopathic drugs have been used to treat and control diabetes over the years, but non affordability due to increased prices, non-availability within communities, side effects of drugs reduce patient compliance (2). The need to mitigate side effects of orthodox and allopathic therapies, the high cost of drugs, and, to large degree, failure of some orthodox medications has prompted, revived scientifically oriented work in the field of ethnomedicine (2).

It is estimated that 25% of the world population is affected by diabetes mellitus. Despite considerable progress in the treatment of diabetes using oral hypoglycemic agents, search for newer drugs continues because of several limitations. The herbal drugs with antidiabetic activity are yet to be commercially formulated as modern medicines, though there are several therapeutic properties of medicinal plants. The adaptation of traditional medicine in different regions is done regardless of advanced international standards and methods of evaluation. Several countries do not have national policies to regulate practitioners on practices and traditional medicine remedies, most traditional medicines prepared by traditional practitioners develop microbial contamination at any stage of preparation process due to the lack of proper hygienic settings during preparation, handling and storage of herbal products (3, 4).

Since there is less stringent regulatory authority for medicinal plants products, there are need for research on medicinal plants to ensure quality, safety and authenticate the therapeutic claims made by traditional medicine healers or herbalists.

The therapeutic claims made by traditional healers range from prevention to cure of chronic diseases such as diabetes mellitus. Thus, it is crucial to explore other options for diabetes management like use of medicinal plants extracts. Medicinal plant based remedies are still the first choice in the developing countries because of their cost effectiveness, availability and minimum or no side effects (4). Diabetes is a metabolic disorder where the pancreas does not produce or body does not properly us insulin, a hormone that is required to convert sugar, starches, and other

food into energy. Diabetes mellitus is characterized by persistent increase in levels of blood glucose. Human body has to maintain the blood glucose levels at a very narrow range using hormone insulin and glucagon. The function of glucagon is causing the liver to release glucose from its cells into the blood for the production of energy while insulin transport glucose into tissue. Type 1 Diabetes leads to inability of pancreas to release insulin results in low rates of glucose uptake into muscles and adipose tissue while type 2 diabetes is due to tissue resistance to insulin (5).

D. bulbifera has been extensively used in the traditional medicine for the treatm`ent of a variety of ailments. Given its several ethnomedicinal uses, *D. bulbifera* has received more attention in past few decades. It is well known for its salty and bitter taste. Traditionally, *D. bulbifera* is used to treat cough, epistaxis, goiter, hemoptysis, pharyngitis, skin infections, piles, throat infections and to remove dandruff (6, 7).Tubers of *D. bulbifera* are roasted and cooked as vegetable and serves to cure cough, dysentery, piles, ulcers, diabetics, leprosy and syphilis (8). In Uganada, tubers of *D. bulbifera* are boiled and consumed by local people to treat HIV patients (9).

Materials and Methods

Collection of Plant and identification

Fresh *Discorea bulbifera* bulbs were collected from its growing habitat at Abi in Uzouwani local Government area of Enugu state of Nigeria in January 2021, and *Discorea bulbifera* bulb was transported to Pharmacology laboratory, Ebonyi State University, Abakaliki for extraction/process. Identification was done by Botanist at National Institute for pharmaceutical Research (NIPRD), Abuja

Bulbs preparation and extraction

Fresh bulbs of *D. bubifera* were washed with clean water chopped into small pieces and air-dried at room temperature for seven days in Pharmacology Department Laboratory, Ebonyi State University, Abakaliki. Thereafter, they were ground into coarse powder using mortar and pestle and passed through a 60 mesh sieve to obtain a fine powder 1700 g. In order to prepare the ethanol extract, 1500 g of the powder was macerated in 5 litres of 96 % ethanol for 48 hours while agitating

occasionally. The mixture was filtered using Whatman's No. 1 filtered paper. A yield of 35 g brownish extract was recovered after drying the filtrate on a water bath at reduced temperature of 40 °C. The recovered extract was stored in a sample container and stored in a refrigerator before the experiment.

Experimental animals

In this study, thirty-six (36) adult male Wistar rats weighing 180 - 200 g, were used. They were sourced from Animal House of the Department of Veterinary Medicine, University of Nigeria, Nsukka. The rats were kept in plastic cages and housed in Department of Pharmacology and Therapeutics, Faculty of Basic Clinical Sciences, Ebonyi State University, Abakaliki where experiment was carried out. The animals were allowed to acclimatize in laboratory environment for 14 days before commencement of the study, while their bedding were constantly cleaned every two days to prevent the animals from being infected. During the period of study, animals were fed with pellets (Vita Feeds Plc, Nigeria) and provided with clean water *ad libitum.* The National Institute of Health (10) Guide for the Care and Use of Laboratory Animals was utilized.

Acute Toxicity Test

The LD_{50} of *Discorea bulbifera* bulb was tested to determine its safety using Lorke (11) method. The studies were done in two phases. Mice used were fasted for 24 hours prior to the commencement of the experiment and randomly divided into 3 groups of 3 per cage in the first phase. Graded doses of the plant extract in ascending order (10 mg/kg, 100 mg/kg and 1000 mg/kg. They were orally administered with 10 mg/kg, 100 mg/kg and 1000 mg/kg of *D. bulbifera* bulb extracts in their different groups using orogastric cannula. The rats were observed for signs of toxicity such as hyper activity, salivation, paw-licking, writhing, muscle paralysis, respiratory distress and mortality within the first 4 hours and after 24 hours respectively. When no lethality was observed, phase 2 was then introduced. In the second phase, four groups of one rat in each cage were orally given the extract in geometrically increasing doses of 1600 mg/kg, 2900 mg/kg and 5000 mg /kg respectively. Thereafter, they were kept under similar conditions and observed

for signs of toxicity as stated above and mortality at first 4 hours, then 24 and 72 hours respectively for late toxicity.

Phytochemical Identification of Plant

The method as described by Mathews et al., (12) were adopted for the phytochemical analysis of the ethanol extracts of *D. bulbifera.* The metabolites assessed include tannins, saponins, alkaloids, flavonoids, terpenoids, steroids, anthraquinones, glycosides, phenols and reducing sugars.

Induction of Diabetes

The method of induction was adopted from Akuodor et al., (13) and Ossai *et al.* (14). The animals were fasted for 24 hours with free access to water prior to the induction of diabetes. Diabetes was induced in overnight fasted male Wistar rats by a single intraperitoneal injection (i.p.) of freshly prepared solution of 150 mg of alloxan dissolved in normal saline. All animals were allowed 5% glucose solution to overcome drug-induced hypoglyceamia (15), then plasma glucose was measured to confirm the diabetic state after 72 hours. The blood glucose measurement was obtained by pricking of the rat tail tip vein. A drop of blood was placed on a glucose test strip and the glucose concentrations were determined by using a compatible Glucometer (Accu Check ®Roche Pharmaceutical, Germany).Fasted alloxan-rats with blood glucose concentrations greater than or equal to 200 mg/dL were considered diabetic and recruited for the study.

Experimental Design and treatment

A total of 36 Wistar rats weighing between 180 g to 200 g were used for the study. The ethanol extract group was made up of 6 groups (n=6).

- Group 1: No induction of diabetes and no treatment (normal control).
- Group 2: Induction of diabetes with no treatment. (Diabetic control)
- Group 3, 4 and 5 were treated using 100 mg/kg, 200 mg/kg and 400 mg/kg of the ethanol extract of *D. bulbifera* bulb
- Groups 6: Induction of diabetes treated with glibeclamide (positive control)

The rats were sampled as follows; day zero for induction, day 3 for blood glucose level after alloxan induction, day 7, day 14 and day 21 for blood glucose measurement, haematological parameters and biochemical parameters ((liver enzymes and kidney parameters).

Determination of blood glucose level.

The method adopted was that of Preethi *et al* (16). The rats' blood glucose level was ascertained using an Accucheck Glucometer. To measure the glucose level using the glucometer, a blood sample collected via the tail vein of a conscious rat was put onto the glucose test strip. When a blood glucose level exceeded 200 mg/dl and deemed diabetic. Blood glucose levels were measured at 72 hours after induction. The blood glucose concentration was then measured 7, 14, and 21 days after the onset of diabetes.

Sacrificing the animals and Sample Collection

After 21 days of blood glucose estimation in rats, whole blood was collected from retro-orbital plexus under mild halothane anesthesia. An autoanalyzer (Chem 200, Germany) was used to determine the following indices; haematological, total cholesterol (TC), triglycerides (TG), and high density lipoprotein (HDL), whereas serum low density lipoprotein (LDL) and very low density lipoprotein (VLDL) were determined adopting Friedewald et al. (17) equations. Kidney indices (electrolytes, urea, creatinine and uric acid) and liver enzymes, AST, ALT and ALP were also assessed.

Determination of Body Weight and Organ Weight

Body weight of experimental animals was checked/determined at week 0 (before, ar induction / before administration), every seven days and last day of experiment before collection of blood. Percentage weight change was later calculated as follows.

Percentage weight change (%) =final-intialbodyweight (g)intialbodyweight (g)X1001

Statistical Analysis

Data obtained were analyzed using one-way analysis of variance (ANOVA) in Statistical Package for Social Sciences (SPSS version 26), and the results were expressed as mean ± standard error of the mean (SEM). Differences between the means of the treated and control groups were further subjected to Dunnett's post hoc test and considered significant at $P < 0.05$.

Results

Phytochemical analysis

The constituents present in the extract, including tannins, flavonoids, alkaloids, terpenoids, saponins, reducing sugar, glycosides and steroids

Acute Toxicity tests

The result of the acute toxicity study of the *D. bulbifera* bulb is displayed in table 2. It showed that the ethanol bulb extract of the plant was greater than 5000 mg/kg as no animal died or showed sign of toxicity during two phases of the study.

Effect of ethanol extract of *D. bulbifera* bulb on body weight of Wistar rats

There was a significant ($p < 0.05$ and $p < 0.01$) weight increase in all the rats given various doses of the extract and those treated with glibenclamide. However, there was a considerable weight loss in diabetic control rats (Table 1).

Effect of the ethanol leaf extract of *D. bulbifera bulb* on blood glucose levels in Wistar rats

Antihyperglycaemic impact of the leaf extract on blood glucose levels of the experimental rats is shown in Table2. Intraperitoneal alloxan administration into the rats significantly elevated blood glucose levels in Wistar rats compared with control (normal) rats. The blood glucose levels increased from 88.52 to 240.58 g/dL. Treatment with the ethanol leaf extract of *D. bulbifera* at doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg significantly ($p<0.05$ and $p < 0.01$) reduced the blood glucose levels in dose dependent fashion compared with diabetic untreated rats. However, 400 mg/kg of the extract with the highest activity in the extract-treated groups compared favourably well with the glibenclamide (10 mg/kg) treated group.

Effect of *D. bulbifera* bulb extract on haematological indices in rats

Table 3 shows a significant ($P < 0.05$) decrease in the levels of RBC, Hb, PCV, MCH, MCV, MCHC, RCDW as well as glibenclamide, after intraperitoneal administration of alloxan when compared with the normal control group. However, following intervention with the leaf extract

and glibenclamide at different doses applied, these haematological indices significantly ($p < 0.01$) increased compared with diabetic untreated group (Table 3).

Effect of *D. bulbifera* ethanol bulb extract on WBC differential in Wistar rats

There was significant ($p< 0.05$) observed reduction in serum WBC, neutrophils, lymphocyte, monocytes, eosinophils, basophils, and platelets following induction of hyperglycemia in rats with alloxan compared with normal control. The leaf extract significantly ($p< 0.01$) increased the levels of these parameters at both doses used when compared with the diabetic control group (Table 3). The best results were observed at 400 mg/kg of the extract and the standard drug (glibenclamide 10 mg/kg) (Table 4).

Effect of *D. bulbifera* bulb on lipid profile in Wistar rats

Table 5 presents the findings on *D. bulbifera's* impact on lipid profile parameters. The tables demonstrate that, in comparison to non-diabetic control rats, total cholesterol, triglycerides, and LDL cholesterol were considerably ($p < 0.05$) higher in diabetic control rats. When compared to the levels of diabetic control rats, the administration of *D.bulbifera* (100 mg/kg) considerably ($p < 0.05$) decreased the levels of total and LDL cholesterol in the diabetic rats and significantly ($p<0.01$) increased in VLDL

Effect of *D. bulbifera* bulb on Kidney Function in Wistar rats

There was a significant ($p < 0.05$) increase in urea concentration in group 2 (untreated group) compared to the normal control (group 1). There was a significant ($p < 0.01$) decrease in the level of Na+, K= Cl- urea and creatinine in extract treated and standard control group when compared to diabetic control group 1 (Table 6)

Effect of the Extract on Liver Function

The AST ALT, ALP and TP activities were significant at ($p <0.05$ and $p<0.01$) when compared to diabetic untreated group (Table 7)

Table 1: Effect of ethanol bulb extract of *Discorea bulbifera* on body weight of diabetic rats

Treatment	Day 1	Day 7	Day 14	Day 21
Normal control (10 mL/kg)	201.50±3.31	202.2±2.31	204.7±2.33	206.8±1.94
Diabetic control (10 mL/kg)	206.30±4.88	175.21±7.16[a]	162.2±3.54[a]	149.79±2.31[a]
D. bulbifera 100 mg/kg	205.65±2.40	190.45±2.33[a]	179.66±2.20[a]	174.45±1.45[a]
D. bulbifera 200 mg/kg	206.81±2.31	185.56±2.21[a]	181.18±2.14[a]	179.8±2.31[b]
D.bulbifera 400 mg/kg	205.72±2.33	193.02±2.36[a]	191.28±2.41[b]	189.21±1.48[b]
Glibenclamide 10 mg/kg	205.66±2.48	196.2±1.48[a]	192.2±1.23[a]	191.7±1.49

Values are expressed as mean ± SEM (n = 6); [a] $p < 0.05$ significant when compared to normal control; $p < 0.01$ significant when compared to diabetic control

able 2: Effect of ethanol bulb extract of *Discorea bulbifera* on blood glucose (mg/dL) in alloxan-induced diabetic rats.

Treatment	Day 1	Day 7	Day 14	Day 21
Normal control 10 mL/kg	98.27 ± 1.35	100.48 ± 2.70	102.37 ± 2.65	102.52 ± 3.41
Diabetic control 10 mL/kg	255.50±0.51	302.60±0.30a	340.20±0.54a	379.62±0.40a
D.bulbifera 100 mg/kg	226.25±3.47	221.50±2.25[a]	191.50±3.38[b]	183.50±5.12[b]
	250.75±14.74	219.50±1.44[b]	189.25±2.95[b]	159.25±5.30[b]

D.bulbifera 200 mg/kg				
D.bulbifera 400 mg/kg	250.25±9.20	201.50±1.04[b]	187.00±2.86[b]	152.75±4.11[b]
Glibenclamide 10 mg/kg	216.75±8.20	195.75±7.09[b]	170.75±6.18[b]	124.25±4.01[b]

Values are expressed as mean ± SEM (n = 6); [a] $p < 0.05$ significant when compared with normal control; $p < 0.01$ significant when compared with diabetic control

Table 3: Effect of ethanol bulb extract of *Discorea bulbifera* on Red blood cell indices in alloxan-induced diabetic rats.

Treatment	RBC x10^{12}/L	Hb(g/dL)	PCV (%)	MCV(fL)	MCH (pg)	MCHC(g/dL)
Normal Control 10 mL/kg	8.20±0.18	16.22±0.25	48.40±0.23	56.29±0.10	18.6±0.12	37.22±0.13
Diabetic control 10 mL/kg	6.50±0.03	13.04±1.05	37.20±2.01	49.01±3.02	14.08±0.26	29.90±0.51
D. bulbifera 100 mg/kg	8.26±0.16	14.05±0.97[a]	42.80±3.54[a]	53.04±0.62[a]	16.50±0.13[a]	33.40±0.43[a]
D. bulbifera 200 mg/kg	7.76±0.30	14.26±0.21[a]	43.73±0.14[a]	53.11±0.13[a]	17.82±0.11[a]	34.40±0.34[a]
D. bulbifera 400 mg/kg	8.61±0.16	14.70±1.03[b]	45.4 ± 0.86[b]	54.03±0.12[b]	18.30±0.14[b]	35.59±0.21[b]
Glibenclamide 10 mg/kg	8.00±0.64	15.20±0.30[b]	46.61±1.10[b]	55.12±0.45[b]	18.60±0.12[b]	36.45±1.21[b]

Values are expressed as mean ± SEM (n = 6); [a] $p < 0.05$ significant when compared with normal control; $p < 0.01$ significant when compared with diabetic control

Table 4: Effect of ethanol bulb extract of *Discorea bulbifera* on White blood cell indices in alloxan-induced diabetic rats.

Treatment	WBC ($x10^9$/L)	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophil (%)	Basophil (%)	Platelets (10^9/L)
Normal control 10 mL/kg	8.24±0.50	38.20±0.33	56.54±0.54	13.93±0.27	0.44±0.11	0.31±0.07	216.66±6.92
Diabetic control 10 mL/kg	15.80±0.54	36.42±1.14[a]	48.69±1.08[a]	10.71±0.14[a]	2.31±0.12[a]	1.29±0.12[a]	804.22±68.04[a]
D. bulbifera 100 mg/kg	10.28±0.38	34.90±0.62[a]	51.48±1.22[a]	13.97±0.20[a]	0.09±0.02[b]	0.08±0.01[b]	544.00±31.51[b]
D. bulbifera 200 mg/kg	9.35±0.31	37.73±1.48[a]	47.40±0.94[a]	12.50±0.26[a]	0.26±0.07[b]	0.19±0.04[b]	501.64±28.17[b]
D. bulbifera 400 mg/kg	7.35±0.32	36.79±0.54[a]	45.14±1.29[b]	13.80±0.68[b]	0.62±0.53[b]	0.57±0.07[b]	465.52±19.24[b]
Glibenclamide 10 mg/kg	9.51±0.53	37.76±1.23[a]	51.29±0.87[a]	10.45±0.55[a]	0.19±0.04[b]	0.29±0.03[b]	555.78±20.67[b]

Values are expressed as mean ± SEM (n = 6); [a] $p < 0.05$ significant when compared with normal control; $p < 0.01$ significant when compared with diabetic control

Table 5: Effect of ethanol bulb extract of *D. bulbifera* on lipid profile (mg/dL) in alloxan-induced diabetic rats.

Treatment	**TC (mg/dl)**	**TG (mg/dl)**	**HDL (mg/dl)**	**LDL (mg/dl)**	**VLDL (mg/dl)**
Normal Control 10ml/kg	64.20±3.25	63.70±0.49	27.40±1.44	22.30±2.80	10.28 ±
Diabetic control 10ml/kg	130.60±4.06	115.20±4.12[a]	48.80±1.73[a]	114.30±3.54[a]	32.10 ± 0.93[a]
D.bulbifera 100 mg/kg	73.80±4.58[a]	76.90±2.42[a]	65.70±1.83[a]	20.60 ±2.41[b]	13.50 ± 0.96[b]
D.bulbifera 200 mg/kg	110.80±2.34[a]	102.30±2.34[b]	35.90±2.91[a]	49.70±1.54[a]	20.33 ± 1.09[b]
D.bulbifera 400 mg/kg	98.70±3.43[b]	93.50±4.70[b]	43.40±2.94[b]	35.10±2.73[b]	17 .17 ± 1.35[b]
Glibenclamide (10 mg/kg)	89.60±3.55[a]	81. 40±3.60[a]	46.55±2.70[a]	35.80±2.45[a]	16.65±2.10[a]

Values are expressed as mean ± SEM (n = 6); [a] $p < 0.05$ significant when compared with normal control; $p < 0.01$ significant when compared with diabetic control

Table 6. Effect of ethanol bulb extract of *D. bulbifera* on kidney indices in alloxan induced rats

Treatment	Na^+(mmol/L)	K^+ (mmol/L)	Cl^- (mmol/L)	Urea(mg/dl)	Creatinine (mg/dl)
Control 10 mL/kg	136.67±1.22	4.67±0.58	102.60±3.71	24.47±6.14	0.70.11
Diabetic control 10 mL/kg	137.80±2.93	4.85±0.62[a]	102.80±2.51[a]	42.20±13.39[a]	1.46±0.46[a]
D. bulbifera 100 mg/kg	136.42±1.22[a]	4.42±0.16[a]	101.62±3.01[a]	33.6±23.45[b]	0.95±0.74[b]
D. bulbifera 200 mg/kg	136.70±0.45[a]	3.86±0.74[b]	100.40±9.89[a]	28.80±7.16[b]	0.78±0.27[b]
D. bulbifera 400 mg/kg	135.48±2.88[a]	3.60±0.47[b]	99.80±0.45[b]	28.48±12.26[b]	0.75±0.41a[b]
Glibenclamide 10 (mg/kg)	136.41 ± 2.38	3.52 ± 0.51[b]	96.62 ± 1.85[b]	26.29±2.61[b]	0.71±0.18[b]

Values are expressed as mean ± SEM (n = 6); [a] $p < 0.05$ significant when compared with normal control; $p < 0.01$ significant when compared with diabetic control

Table 7: Effect of ethanol bulb extract of *Discorea bulbifera* on liver enzymes in alloxan induced diabetic rats

Treatment	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	TP (g/dl
Normal Control 10 mL/kg	84.20±10.45	50.4011.61	150.60±13.68	5.50±0.05
Diabetic control 10 mL/kg	150±20.97[a]	85.70±13.24[b]	144.40±9.92[a]	5.20±8.12[a]
D. bulbifera 100 mg/kg	120.60±5.51[a]	68.55±6.73[a]	116.40±6.68[b]	5.70±0.22[a]
D. bulbifera 200 mg/kg	58.40±4.50[b]	55.40±1.63[a]	105.60±7.31[b]	5.42±0.19[a]
D. bulbifera 400 mg/kg	44.80±3.66[b]	50.32±3.16[a]	92.60±4.62[b]	5.40±0.13[a]
Glibenclamide 10 mg/kg	44.33±0.88[b]	48.60±3.58[a]	90.33±2.40[b]	4.37±0.62[b]

Values are expressed as mean ± SEM (n = 6); [a] $p < 0.05$ significant when compared with normal control; $p < 0.01$ significant when compared with diabetic control

Discussion

Diabetes is a chronic metabolic condition recognized worldwide as an important cause of premature death and disability, especially in the developing world. According to the WHO, the number of adult people suffering from diabetes has almost quadrupled since 1980 mainly due to the increase number of people living with diabetes mellitus type 2 and related factors driving it including obesity and overweight. Additional 2.2 million of deaths are associated with the increase risk of cardiovascular diseases due to the high blood glucose concentrations. The cost of managing diabetes can be catastrophic in poor population. There is an urge to seek other possibilities of managing diabetes in order to reduce the high rate of mortality. In fact, about 80% of the population relies on herbal medicines especially in developing countries due to the low cost and availability of these medicines. The WHO has requested several governments to include herbal medicines with proven efficacy and safety in their healthcare programs (18, 19).

The benefits of plants and herbs in human health cannot be held in disdain. The bioactive constituents of *D. bulbifera bulb* can be evaluated by carrying out phytochemical analysis on the bulbs and other parts of the plants. The phytochemical analysis of *D. bulbifera* in this study revealed the presence of flavonoid, tannins, steroids, reducing sugars, alkaloids and glycosides (Table 4.1) in both ethanol and n-hexane extracts of *D. bulbifera* bulb. These identified metabolites have been identified in the bioactivity and effectiveness of extracts of herbs and plants (20). The ethanol extracts of the bulb contained higher quantity of flavonoids, steroids and glycosides. Although, saponins was not found in n-hexane fraction of the extract, while phenol and anthraquinon were totally absent in the ethanol and hexane fraction of *D. bulbifera* bulb. The present study showed that ethanol was a better solvent as it had higher quantities of the metabolites than the n-hexane extract. Flavonoids are known for their antioxidant, anti-inflammatory and anti-carcinogenic properties hence its attribution to the efficacy of most natural plants.

The study was designed to determine the effects of *Dioscorea bulbifera* bulb ethanol and n-hexane fraction extracts in alloxan-induced diabetes in Wistar rats and to compare the results with anti-diabetic potentials activity of reference drug, glibenclamide. In this study, the animals were made diabetic and treated with varying doses of the extracts for 21 days. The blood glucose level in

animals were estimated at 1st, 7th, 14th, and 21st day of the study to determine the potency of the extract of *D. bulbifera* bulb in controling blood glucose level in rats induced with diabetes.

Therapeutic effects of medicinal plant extracts are due to their phytochemical constituents with varieties of biological activities (21). In this study, phytochemical screening of the ethanol bulb extract of *D. bulbifera* revealed the presence of alkaloids, cardiac glycosides, steroids, carbohydrate, flavonoids, saponin, tannins and terpenoids. *D. bulbifera* bulb extract was found to reduce the glucose level in alloxan-induced hyperglycemic animals. A therapeutic approach to maintain normal blood glucose levels is suppression of the production and/or absorption of glucose by inhibiting either the α-amylase or α-glucosidase enzymes (22). Alpha amylase breaks down starch into oligosaccharides and disaccharides, which are further hydrolysed by α glucosidase to produced glucose and other monosaccharides, which are then absorbed in the small intestine (14). In this study, the bulb extract of the plant brought about 3-fold decrease in the plasma blood glucose. This observation, coupled with the reduction in glucose levels strongly suggests that the plant possesses antihyperglycemic effect.

The potentials of the extract to lower the diabetic complications by assessing the lipid profile, and liver function tests were also studied. For the evaluation of lipid profile, concentrations of total serum cholesterol (TC), and triglycerides (TG), high density lipoprotein (HDL), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) were estimated. The liver enzymes, alanine transferase, aspartate transferase and alkaline phosphate were also estimated to study the hepatic function. The diabetes mellitus is a chronic metabolic disorder and it is also associated with several secondary complications such as hyperlipidemia, atherosclerosis, hypertension, diabetic nephropathy, diabetic neuropathy and diabetic keto acidosis. Hyperlipidemia is one of such common complication of diabetes which is characterized by increase in serum total

cholesterol (TC), triglycerides (TG), LDL and VLDL. The azotemia is condition which is due to the accumulation of nitrogenous waste products like urea and creatinine in blood and usually found during diabetic nephropathy. Along with other risk factors such as hypertension, smoking, obesity etc., increasing importance has been given to secondary hyperlipidaemias is the causation of accelerated atherosclerosis ((23). Hyperlipidaemia as a metabolic abnormality is frequently

associated with diabetes mellitus. The most characteristic lipid abnormality in diabetics is hypertriglyceridaemia, with or without associated increase in plasma cholesterol (24).

In this study, alloxan-induced diabetes in control animals caused elevation of serum cholesterol, triglycerides, and urea as a consequence of secondary complications of diabetes. Animals of therapeutic groups treated with *D. bulbifera* bulb (100mg/kg, 200mg/kg and 400 mg/kg) have shown significant reduction in above serum parameters. The ethanol and n-hexane extracts of *D. bulbifera* prevented the loss in body weight in alloxan-induced diabetic rats and from this it is evident that the extracts possesses significant beneficial effects on body weight. This may be due

to the protein sparing effect of *D. bulbifera* which prevent protein degradation of those results in improvement of body weight. Lack of insulin in diabetes makes the cell to starve for glucose, so the cell uses proteins as an alternative energy source which ultimately results in weight reduction. Numerous studies have shown an association between hyperglycemia and decreased body weight of diabetic animals (25). It was observed that alloxan induced diabetes is associated with the reduction in the weight of animals and the relative weight of kidney, liver and spleen in rats (26). The reduction of glucose might be brought by the stimulation of pancreatic β- cells of Langerhans to produce insulin or it prevent the absorption of glucose from the intestine or it may enhance the glucose uptake in peripheral tissues (27, 28).

In this study, the effects of the ethanol and hexane extracts on haematological paramete also explore. The results showed that diabetic control rats had abnormalities of most of the

measured haematological parameters compared with non-diabetic treated rats. The observed results are in support of Bunza and Dallatu who reported haematological abnormalities in diabetic rats (29). The reduction in the number of platelets is of paramount important because they are known for their essential role blood clotting. Platelets are also involve in the repair of walls of blood vessels. They as well play an important role in acute phase reaction to inflammation. Treatment of rats with the ethanol extract of *D. bulbifera* bulb prevented the decrease in platelets in a dose-dependent manner (30). Though, the ethanol extract had upper hand in the observe

reduction. The findings suggest that *D. bulbifera* bulb extract may exhibit some beneficial effects on haematological disorders.

The effect of flavonoids on pancreatic βcells leading to their proliferation and secretion of more insulin has been proposed by Ghorbani et al. (31) as the mechanism by which they reduce hyperglycemia caused by alloxan in treated rats. The study concludes that whole tuber extract of *D. bulbifera* contains high phytochemical contents that exhibited potent and appreciable antioxidant and antihyperglycemic activity in alloxan-treated rats thus preventing the occurrence of hyperglycemic disorders. It will be rewarding to isolate the active principle(s) responsible for these activities in subsequent studies.

Conclusion

The data obtained from this present investigation provide evidence that *D. bulbifera* bulb has hypoglycaemic effects on blood glucose concentrations of diabetic rats and may be effective in cases of glucose tolerance impairment. However, it can be speculated that the antidiabetic activity of *D. bulbifera* may be due to non-specific mechanism and not due to the stimulation of insulin release from pancreatic beta cells since alloxan used in this study is well known to work by depleting the pancreatic beta cells thus reducing the release of insulin from these cells. Also, the synergistic effect of different bioactive chemicals may have a crucial contribution to the potential hypoglycaemic action of the plant species.

The LD_{50} of the plant revealed that the plant is safe and/ or non-toxic in mice when doses up to 5000 mg/kg of body weight of animals. All the above data obtained from *D. bulbifera* studies indicate that the plant species has antidiabetic activities which support or justify the reported folkloric use of *Discorea bulbifera* bulb in the treatment and/ or management of diabetes in South Eastern Nigeria

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Compliance with ethical guidelines

The ethical approval of the experiments in this study was obtained from the Institutional Animal Ethics Committee of Ebonyi State University, Nigeria, with the reference number (EBSU/RIC/UREC/VOL.07/057).

Conflict of interest statement

We declare that there is no conflict of interests.

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Authors' contributions

Study design: Akuodor GC, Eke DO and Ofor CC; The literature search and editing of the manuscript: Enendu AC, Akpan JL and Ofornakara U; Statistical analysis: Nweke HD, Anele DO and Nwodo ES; Experiments and final approval: All authors;

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