

<https://doi.org/10.48047/AFJBS.6.14.2024.12428-12450>



African Journal of Biological Sciences

Journal homepage: <http://www.afjbs.com>



Research Paper

Open Access

Antidiabetic activity of methanol leaf extract of *Manihot esculenta* crantz (cassava leaf) in alloxan induced diabetic mice: A Potential Alternative for Diabetes Mellitus treatment

¹OMOIRRI MOSES AZIAKPONO, ¹OLOCHE JEREMIAH JOHN, ¹NKECHI JOAN OKONKWO-UZOR, ¹AMARACHUKWU UKAMAKA ONWUZULINGBO, ¹IBEH CLETUS NNODUM, ¹AJEGI INNOCENT FOUNTAIN, ¹OBI DORIS MESOMACHUKWU, ¹CHUKWUNONSO CHUKWUDIKE ONWULIGBO *²JOSEPH OYEPATA SIMEON, ²ADEGBUYI, ADEKUNLE THEOPHILUS, ²JOSEPH OPEYEMI TOSIN, ²ZUBAIRU SEBASTINE ALIYU

¹Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Federal University, Oye-Ekiti, Ekiti State, Nigeria.

¹Department of Pharmacology and Therapeutics, Federal University of Health Sciences, Otukpo, Benue State, Nigeria

¹Faculty of Pharmaceutical Sciences, Nnamdi Azikwe University, Enugu, Nigeria

¹Department of Pharmaceutical Sciences, Chukwuemeka Ojukwu University, Igbariam

¹Faculty of Basic and Clinical Sciences, Nnamdi Azikwe University, Akwa, Nnewi.

¹Department of Pharmaceutics, Chukwuemeka Odumegwu University, Igbariam

¹Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, Chukwuemeka Odumegwu Ojukwu University, Igbariam, Anambra State, Nigeria.

¹Faculty of Pharmaceutical Sciences of Pharmaceutics, Chukwuemeka Odumegwu University, Igbariam

²Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Federal University, Oye-Ekiti, Ekiti State, Nigeria.

²Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Federal University, Oye-Ekiti, Ekiti State, Nigeria. Email

²Department of Pharmacology, Faculty of Pharmacy, Lead City University, Ibadan, Nigeria. Email address: joseph.opeyemi@lcu.edu.ng

²Department of Pharmacology and Therapeutics, Faculty of Pharmacy, Gombe State University, Nigeria

***Corresponding author: Joseph Oyepata Simeon**

Email address: oyepata.joseph@fuoye.edu.ng

Phone number: +2348038248352

Volume 6, Issue 14, Sep 2024

Received: 15 July 2024

Accepted: 25 Aug 2024

Published: 05 Sep 2024

doi: [10.48047/AFJBS.6.14.2024.12428-12450](https://doi.org/10.48047/AFJBS.6.14.2024.12428-12450)

Abstract

Aim:Therefore, this study evaluated *Manihotesculenta* treatment in the control of diabetes damage and pancreatic complications using animal models.**Method:**Diabetes was induced in mice after overnight fast with 150 mg/kg alloxan intraperitoneally. After 72 h, those with plasma glucose levels >200 mg/dl were classified as diabetic. Five diabetic rats in each group were treated daily for 14 days orally with 100, 250, and 500 mg/kg of the extract, and metformin (100 mg/kg) while another group was untreated. **Result:** The two doses, (250 and 500 mg/kg) of the methanol leaf extract of *Manihot esculenta* Crantz as well as Metformin produced significant ($P<0.05$) reduction in the fasting blood glucose levels when compared with the untreated control from day 3 to 14th day of treatment. The fasting blood glucose reduction was dose dependent. 500mg/kg of the extract had the highest reduction in fasting glucose level on the 4th day (101.3 ± 4.91 , 77.91%) The reduction was consistent, as the extracts gradually reduce blood glucose level on daily bases. **Conclusion:** Analysis of the obtained data indicates the effect of *S. striata* in improving the complications of diabetes in rats and can be considered for therapeutic purposes.

keyword: *Manihot Esculenta*, antidiabetic, acute toxicity, antioxidant, mice

Abbreviation DM Diabetes mellitus,FBG Fasting blood glucose,ME *Manihot Esculenta*,MET Metformin,OGTT Oral glucose tolerance test,QE Quercetin,ROS Reactive oxygen species,SEM Standard error of the mean,WHO World Health Organization

Introduction

Diabetes, commonly known as diabetes, is a group of endocrine diseases caused by persistent blood sugar (Lartey et al., 2021). Diabetes occurs when the pancreas does not produce enough insulin or the body cells do not respond to the hormone. There are generally two types of DM; type I and type II. Type I diabetes is also known as insulin-independent diabetes, which means lack of insulin; Type II diabetes is also known as noninsulin-independent diabetes because insulin does not secrete or work on insulin. 1 Diabetes is the leading cause of disease and death worldwide; According to the World Health Organization, the prevalence of diabetes in adults was estimated at 346 mil

lion (6.1%) in 2011. ² This number is expected to increase to approximately 439 million (7.7%) by 2030 (Babaiedarzi et al., 2018). The World Health Organization predicts that deaths from diabetes will increase by two-thirds between 2008 and 2030.² In Diabetes Mellitus, changes in defense mechanisms that scavenge endogenous free radicals lead to negative inhibition of oxygen reactive species, causing oxidative damage and tissue damage. (Jonathan et al., 2018; Simeon et al., 2023). Research shows that streptozotocin may help treat diabetes because it has the ability to destroy pancreatic beta cells, possibly through a free radical process. At the same time, diabetic patients with poor glycemic control will develop microvascular and macrovascular complications such as nephropathy, retinopathy, neuropathy, and cardiovascular disease (Palanisamy et al., 2020). These problems increase the poverty of the patient, constitute a significant part of the costs of individuals with diabetes, and lead to economic poverty in the country (Spaeth 2017). Besides insulin, other treatments for type 1 diabetes include total pancreas and islet transplantation, but this depends on the individual's pancreatic disease, depending on its deficiency and effectiveness (Fujitani 2017). However, most of the drugs currently used to treat type 2 diabetes are effective and longlasting (Vaeli et al., 2021). The main goal of diabetes care is to reduce the risk of microvascular and macrovascular complications by normalizing blood pressure, lipid profile and glycemic status. The specific goal of glycemic control is to achieve a certain glycemic hemoglobin (HbA1c) level, as good glycemic control is important to reduce the risk of longterm microvascular complications in type 1 and type 2 diabetes (Tehrani et al., 2011). HbA1c can be measured at any time of the day, regardless of fasting time or the contents of the previous meal. Screening can also be done using portable devices and small blood samples (Meier 2008). Analysis of glycated hemoglobin (HbA1c) in the blood, called red blood cell (RBC) half-life, can provide a person's blood sugar level over the past 2-

3 months (Meier 2008).

Many African medicinal plants such as bitter melon, cyclops and periwinkle are effective against many diseases such as diabetes (2018). *Manihot esculenta*, commonly known as cassava, is a type of plant used to treat diabetes and other conditions such as menorrhagia, abdominal pain, and wound healing. Cassava is an important crop in tropical countries because its roots are rich in carbohydrates. As a summer crop, *Manihot esculenta* are grown as a staple food in many African countries and many southern provinces of China (Ravi and Aked., 1996 ; Christer et al. et al., 2009). In addition to the main nutrients in cassava leaves, recent research has focused on some bioactive compounds (e.g. flavonoids, carotenes, anthocyanins) that may help protect human health (Kubo et al., 2006; Almeida Siqueira et al., 2007; Byamukama et al., 2009). It has been reported that the leaves contain many flavonoids (such as quercetin, rutin, kaempferol, etc.) and most of the flavonoids act as antioxidants in the body (Kubo et al., 2006). As an important antioxidant bioactive compound, flavonoids can kill oxygen anions, hydroxyl radicals, and singlet oxygen (Hollman et al., 1999; Montoro et al., 2005).

Many studies have confirmed that *Manihot esculenta* have antibacterial, antifungal and antiviral properties. (Haruna et al., 2020). However, there is some information that *Manihot esculenta* are used to treat diabetes. Therefore, this study aimed to evaluate antidiabetic effects of methanol leaf extract of *Manihot esculenta* in alloxan-induced diabetic mice and its potential use in the treatment of diabetes mellitus.

Material and Method

This study was conducted at the Nnamdi Azikiwe University, Awka, Anambra State, Nigeria between August 11, 2023 and November 29, 2023. Awka, Nigeria is in the Nigerian National Urban Areas category with GPS coordinates of Latitude : 5.033

and Longitude 7.167. Awka has a summer season, a rainy season, and a hot, dry climate; Annual temperatures range from 69°F to 87°F¹³.

Plant collection

Fresh leaf of *Manihot esculenta* were acquired in a conservative medicinal garden in Awka, Nigeria, from a medicinal store. The plants were verified by the Department of Botany at the in Nnamdi Azikiwe University Anambra State, Nigeria. The voucher specimens were stored in the herbarium department (No:NAU/919).

Preparation of methanol leaf extract of *Manihot esculenta*

The method described by Trono et al, (2018) was adopted. The leaves were cleaned; air dried at room temperature for three (3) weeks and were pulverised using blender (Super Master Co., Ltd, Osaka, Japan). The methanol extract of *Manihot esculenta* was prepared using 279g of the powdered leaves mixed in 831 ml (1:3 w/v) of 80% methanol for 72 hours while it was stirred intermittently. The combined extract was filtered through a vacuum filter using Whatman No.1 filter paper. The resulting solution was evaporated using water bath heater at 55°C to yield 52.1g of the crude extract corresponding to a percentage yield of 18%. The extracts were stored at -4°C until used.

Animals

male mice (21-31 g) were obtained from the Animal House of Nnamdi Azikiwe University. They were fed standard animal pellets and given unlimited amounts of water. The Nnamdi Azikiwe University College of Health Sciences Animal Ethics Committee granted approval and consent were obtained for animal experiments (NAU/2023/124). Rats (n=6) were randomly assigned to different treatment groups. Animal experiments were carried out under the same conditions. The care and

handling of animals followed established public health guidelines in the Guide for the Care and Use of Laboratory Animals (2011).

Procedure for LD₅₀ determination of *Manihot esculenta* Extract

The median lethal dose (LD₅₀) for *Manihot esculenta* was determined using modified Lorke's method¹⁴. Sixteen (16) rats were used for the determination of the median lethal dose. The sixteen rats were randomized into seven groups; four groups of three rats each were used for the first phase and were given 10, 100, 200 and 400 ml/kg bodyweight respectively. Subsequently, four groups of one rat each were used for the second phase and given 500, 1000, 1500 and 2000 ml/kg bodyweight. The animals were monitored for changes in behaviour and mortality within 2 hrs, 24 hrs and 14 days after a single administration of the extracts. LD₅₀ values were calculated using the formular below:

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

Where: D₀ = Highest dose that gave no mortality

D₁₀₀ = Lowest dose that produced mortality

Phytochemical screening Procedure for *Manihot esculenta*

Qualitative phytochemical screening

Qualitative phytochemical tests were performed on *Manihot esculenta* to determine the presence of secondary metabolites such as alkaloids, flavonoids, saponins, cyanogenic glycosides, reducing sugars, phenolic substances, and triterpenes according to standard protocols. For each test, a small amount (1–2 g) of *Manihot esculenta* was reconstituted in distilled water/another solvent before use.

Quantitative phytochemical analysis

Total phenolic content

The total phenolic content of the *Manihot esculenta* was determined by the Folin-Ciocalteu method. A volume of 100 μl of 5% Folin-Ciocalteu reagent was added to 20 μl of 0–5 mg/ml of the *Manihot esculenta* followed by 80 μl of 7.5% Na_2CO_3 in a well plate and mixed. The mixture was then incubated at room temperature in the dark for 1 h and the absorbance measured at 765 nm using the Infinite M200Pro microtiter plate reader (Tecan, Austria). Gallic acid at a concentration range of 0–5 mg/ml was used to plot the standard calibration curve from which the concentration of phenol in each *Manihot esculenta* concentration was estimated. The total phenolic content of the extract was expressed in mg of Gallic acid equivalents (GAE)/g of *Manihot esculenta* for triplicate determinations.

Determination of total flavonoids

The total flavonoid content of the *Manihot esculenta* was determined by the slightly modified aluminium chloride colourimetric method. Various concentrations of *Manihot esculenta* in distilled water were prepared by serial dilution; (10, 5, 2.5, 1.25, 0.625, 0.3125, 0.1563, 0.0781, 0.0391 mg/ml). Briefly, a volume (100 μl) of 2 % AlCl_3 was added to 100 μl of the *Manihot esculenta* (0.0391–5 mg/ml), followed by incubation in the dark for 10 minutes at room temperature. The absorbance of the solution was measured at a wavelength of 415 nm using the Infinite microtiter M200Pro plate reader. Quercetin (0–5 mg/ml) was used as a standard while distilled water was used as a blank. A calibration curve for quercetin was plotted from which the unknown concentration of flavonoids in each *Manihot esculenta* concentration was estimated. The total flavonoid content of *Manihot esculenta* was expressed in mg of quercetin equivalents (QE)/g of APE for triplicate determinations.

Estimation of Lipid Peroxidation

The method developed using the evaluation of lipid peroxidation by malondialdehyde (MDA) measurement⁷. Malondialdehyde (MDA) is the end product of lipid peroxidation and is a good marker of free radical-mediated damage and oxidative stress. The principle of this method is that MDA reacts with thiobarbituric acid (TBA) under acidic conditions and at high temperature (90–100 °C) to form a red MDA-(TBA)₂ complex, which can transmit light photometric measurement at 530 nm. In this procedure, 0.5 mL of 20% TCA was added to 0.5 mL of tissue

homogenate, followed by 1 mL of 0.67% TBA. The mixture was incubated in a 100°C water bath for 15 min, cooled, then 4 mL n-butanol was added and centrifuged at 3000 rpm for 15 min. The absorbance of the pure red supernatant is then read spectrophotometrically at 532 nm against a blank. MDA concentration is expressed in nmol/g tissue.

Determination of Reduced Glutathione (GSH)

Reduced Glutathione was determined using the DHHS method⁹. This test is based on the oxidation of GSH by 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB). DTNB reacts with glutathione (GSH) to form yellow 2-nitro-5-nitrobenzoic acid. . Thus, GSH concentration can be determined by measuring the absorbance at 412 nm. For this measurement, 50 µL of tissue homogenate is diluted in 10 mL of phosphate buffer (0.1 M, pH 8). Add 20 µL of DTNB (0.01 M) to 3 mL of the dilute mixture. The absorbance was read at 0.1412 nm based on the blank prepared during the same period.

Estimation of Catalase (CAT) Activity.

Catalase activity in plasma was determined using the modified method described by Atawodi¹². The method is briefly as follows: Blood (10 µL) is added to a test tube containing 2.80 mL of 50 mM potassium phosphate buffer (pH 7.0). The reaction was initiated by adding 0.1 mL of fresh 30 mM hydrogen peroxide, and the decomposition rate of hydrogen peroxide was measured on a spectrophotometer at 240 nm for 5 min. Catalase activity was calculated using a molar extinction coefficient of 0.041 mM⁻¹ cm⁻¹.

Induction of diabetic state in mice

The diabetic state was induced in normoglycaemic ICR mice by a single intraperitoneal injection of alloxan monohydrate (150 mg/kg). The alloxan was first weighed individually for each animal according to the body weight and then dissolved in 0.5 ml saline (0.9 % NaCl) just before administration to the animal. Two days after alloxan administration, the fasting blood glucose (FBG) of mice was determined, and those with FBG ≥ 200 mg/dl were classified as diabetic and, therefore, included in the study. Treatment with *Manihot esculenta* commenced 48 h after establishing the diabetic state of animals.

Treatment of animals

The alloxan-induced diabetic mice were divided into six (6) treatment groups of five (5) animals each. The first two groups were treated daily with normal saline (10 ml/kg) and metformin (100 mg/kg). the last three groups received *Manihot esculenta* of increasing dosage (100, 250 and 500 mg/kg). Five normoglycaemic littermates were put in a 6th group, which received an equal volume of water (0.5 ml) and represented the non-diabetic control. The animals were administered with/without *Manihot esculenta* or drug for four weeks by oral gavage. *Manihot esculenta* or standard drugs were reconstituted in sterilised distilled water before administration to the animals. The body weights of the animals in each group were determined weekly.

Blood sampling

At baseline, and the end of weeks 1, 2, 3 and 4, blood samples were collected by tail bleeding of the animals after an overnight fast (8 h) into serum separator tubes and centrifuged at 4500×g for 5 min to obtain serum The serum was transferred into fresh tubes and stored at −80 °C until use.

Serum glucose determination

Plasma glucose concentration was determined by the glucose oxidase method by following the manufacturer's protocol in assay kits (ELI Tech, Puteaux, France).), and measuring absorbance by the Biosystems A25 Chemistry Analyzer (Biosystems S.A, Barcelona, Spain).

.

Histological Studies

At the end of the experiment, the animals were sacrificed, and vital organs including the pancreas were harvested and fixed in 10% buffered formalin. The pancreatic tissue was processed using standard procedures as described by Grizzle et al. (2012). The tissue section was observed with a light microscope at a high magnification for histological changes and photomicrographs taken.

Statistical analysis

The data were expressed using the mean and standard error of the mean (SEM). Before Dunnett's post hoc test for multiple comparisons between the control and treated groups, one-way ANOVA was performed to statistically examine the data. $P \leq 0.05$ was considered significant.

Result

Acute Toxicity Test (LD₅₀)

No deaths were recorded after 24 hours of administration of the various doses (10, 100, 200 and 400 ml/kg body weight) of the ethanol extract of *Manihot esculenta*. In the second stage, four dose ranges were also used 500, 1000, 1500 and 2000 ml/kg body weight and there was no death after 24 hours. Therefore, the LD₅₀ is estimated at LD₅₀ ≥ 2000 ml/kg b.wt. in mice. It is therefore considered as safe (table 1).

Phytochemical screening of *Manihot esculenta* plant part (leaf extract)

The phytochemical screening of the *Manihot esculenta* showed the presence in different fractionated medium has listed in table 2. The result detected the presence of secondary metabolite such as flavonoids, phenols, tannins, terpenoids, steroids, carbohydrates and glycosides. (table 2).

Phytochemical screening of *Manihot esculenta*

Phytochemical analysis of *Manihot esculenta* revealed that flavanoid (2.58%) highest proportion in the extract. This is followed by alkaloid (1.14%) and saponin (6.20%) (table 3).

Effect of *Manihot esculenta* stem bark on activities of MDA, GSH, SOD and CAT

Changes in rat biochemical enzyme are shown in Table 4. Antioxidant enzyme status of rats in the control group and rats treated with zinc phosphide or combined with citrus juice is shown in Table 1. There was significant difference in the activity of CAT, SOD and GSH levels compared to rats administered *Manihot esculenta*. Control group. Zinc phosphide treatment alone caused a decrease in CAT, SOD and GSH activity levels compared to the control group ($p < 0.05$). Pretreatment with citrus juice reversed the decrease in play by increasing play to near control levels in rats pretreated with citrus juice. MDA activity only increased significantly in zinc phosphide-treated rats compared to the control group ($p < 0.05$). MDA activity in rats given L. citrus juice returned to normal compared to the control group ($p < 0.05$). (table 4)

Effect of *Manihot Esculenta* Crantz on fasting blood glucose

The two doses, (250 and 500 mg/kg) of the methanol leaf extract of *Manihot Esculenta* Crantz as well as Metformin produced significant ($P < 0.05$) reduction in the fasting blood glucose levels when compared with the untreated control from day 3 to 14th day of treatment (Fig 2,3 and Table 5). The fasting blood glucose reduction was dose dependent. 500mg/kg of the extract had the highest reduction in fasting glucose level on the 4th day (101.3 ± 4.91 , 77.91%) The reduction was consistent, as the extracts gradually reduce blood glucose level on daily bases (table 1 and figure 1 and 2).

Histopathology of pancreas

Histological observation of the pancreas at the termination of treatment of diabetic mice with *Manihot esculenta* and standard drugs showed the destruction of the beta cells, while the normoglycemic control group showed intact pancreatic beta cells. Treatment with metformin and *Manihot Esculenta* encouraged the restoration of the morphology of the beta cells of the pancreas in the diabetic mice (figure 3).

Table 1: Acute toxicity

PHASES	DOSE (ML/KG)	MORTALITY
PHASE 1	10	0/3
	100	0/3
	200	0/3
	400	0/3
PHASE 2	500	0/1
	1000	0/1
	1500	0/1
	2000	0/1

Table 2: Qualitative Phytochemical

Test	Observation	Inference
Alkaloid	Reddish brown	+
Saponin	Persistent frothing	+
Tannin	Blue black	+
Flavonoid	Yellow	+
Steroid	No blue or green	–
Terpenoid	Reddish brown	+
Cardiac glycosides	Reddish brown	+
Carbohydrate	Deep violet colour	+
Protein	White ppt	+
Reducing sugar	Yellow ppt	+

Phytoconstituent	Percentage
Flavonoid	2.58%
Alkaloid	1.14%
Saponin	6.20%

Table 3: Proximate analysis of *Manihot esculenta* Crantz leaves

Sample	Total ash (%)	Moisture content (%)	Sulfated ash (%)	Acid insoluble ash (%)	Water soluble ash (%)	Acid soluble extractive yield (%)	Water soluble extractive yield (%)

<i>Manihot esculenta</i> leaves	0.885	2.149	3.921	2.646	0.199	3.753	2.238
---------------------------------	-------	-------	-------	-------	-------	-------	-------

Table 4: Effect of *Manihot esculenta* leaf extract on activities of MDA, GSH, SOD and CAT

GROU P	TREATMENT	SOD (U/mg)	CAT (ug/mg)	MDA (µg/mg)	GSH (ug/mg)
1	Normal Control	7.45±0.45*	84.19±0.73*	41.31±0.42*	1.87 ± 0.22*
2	ZnP	26.20±0.04	115.94±0.34	21.27±0.27	0.34 ± 1.28
3	Vit.C 50 mg/kg	7.30±0.11*	83.08±0.11*	43.28±0.47*	1.68 ± 1.45*
4	<i>Manihot esculenta</i> 100 mg/kg	7.34±0.13*	88.03±0.70*	45.15±0.42*	1.55 ± 0.02*
5	<i>Manihot esculenta</i> 250 mg/kg	7.43±0.98*	87.32±0.19*	47.50±0.12*	1.90 ± 0.20*
6	<i>Manihot esculenta</i> 500 mg/kg	7.65±0.18*	81.12±0.27*	50.45±1.25*	1.54 ± 0.03*

All the values are expressed as mean ±SEM n=5. Data analyzed by ONE WAY ANOVA followed by Tukey's test). *P<0.05 is statistically significant

Table 5: Effect of *Manihot Esculenta* Crantz on fasting blood glucose

Group	Basal	Day 3	Day 7	Day 14
-------	-------	-------	-------	--------

Distilled water 10ml/kg (Diabetic induced group)	239.5 ± 9.50	293.50 ± 12.50 (-22.54%)	371.0 ± 7.77 (-54.90%)	373.7 ± 8.99 (-56.03%)
Metformin 100 mg/kg	367.7± 11.89	221.3 ± 10.91 (39.81%)	185.0 ± 8.14* (49.68%)	76.0 ± 5.68* (79.33%)
100mg/kg extract	585.7 ± 14.33	559.3 ± 5.36 (4.50%)	540.3± 8.37 (7.75%)	391.0 ± 9.29 (33.24%)
250mg/kg extract	467.3± 14.08	408.7 ± 11.26 (12.54%)	335.7 ± 4.48 (28.16%)	210.7 ± 8.41 (54.91%)
500mg/kg extract	458.7 ± 26.23	385.0 ± 18.77 (16.06%)	293.3 ± 10.48 (36.05%)	101.3 ± 4.91* (77.91%)
Distilled water 10 mg/kg (non- diabetic induced group)	77.0 ± 4.11	77.3 ± 4.76*	76.9.0 ± 7.55*	77.4 ± 4.35*

Values presented as Mean ± SEM, versus control (repeated measures ANOVA followed by post hoc turkey's test).

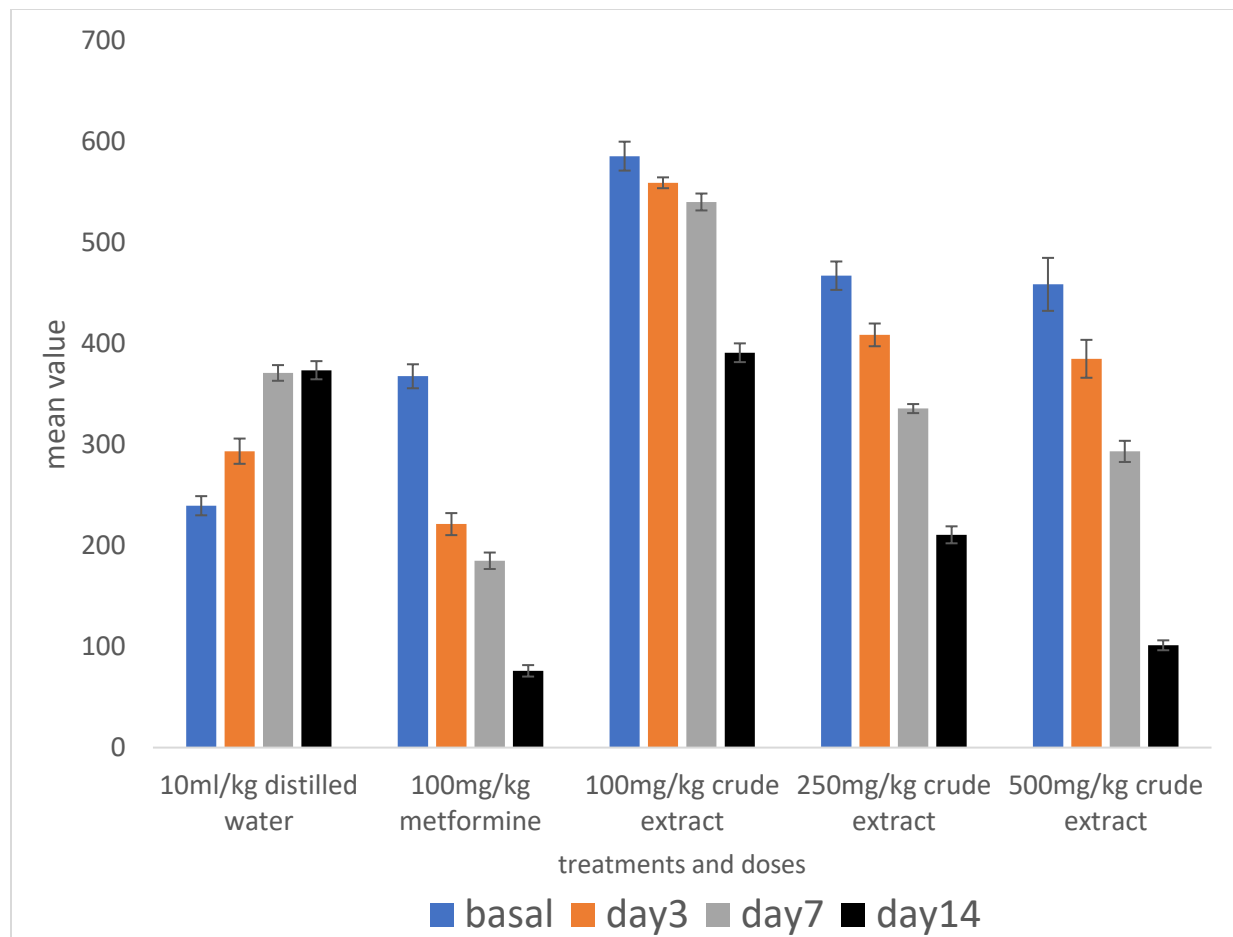


Fig 1: A barchart of mean FBGL against doses

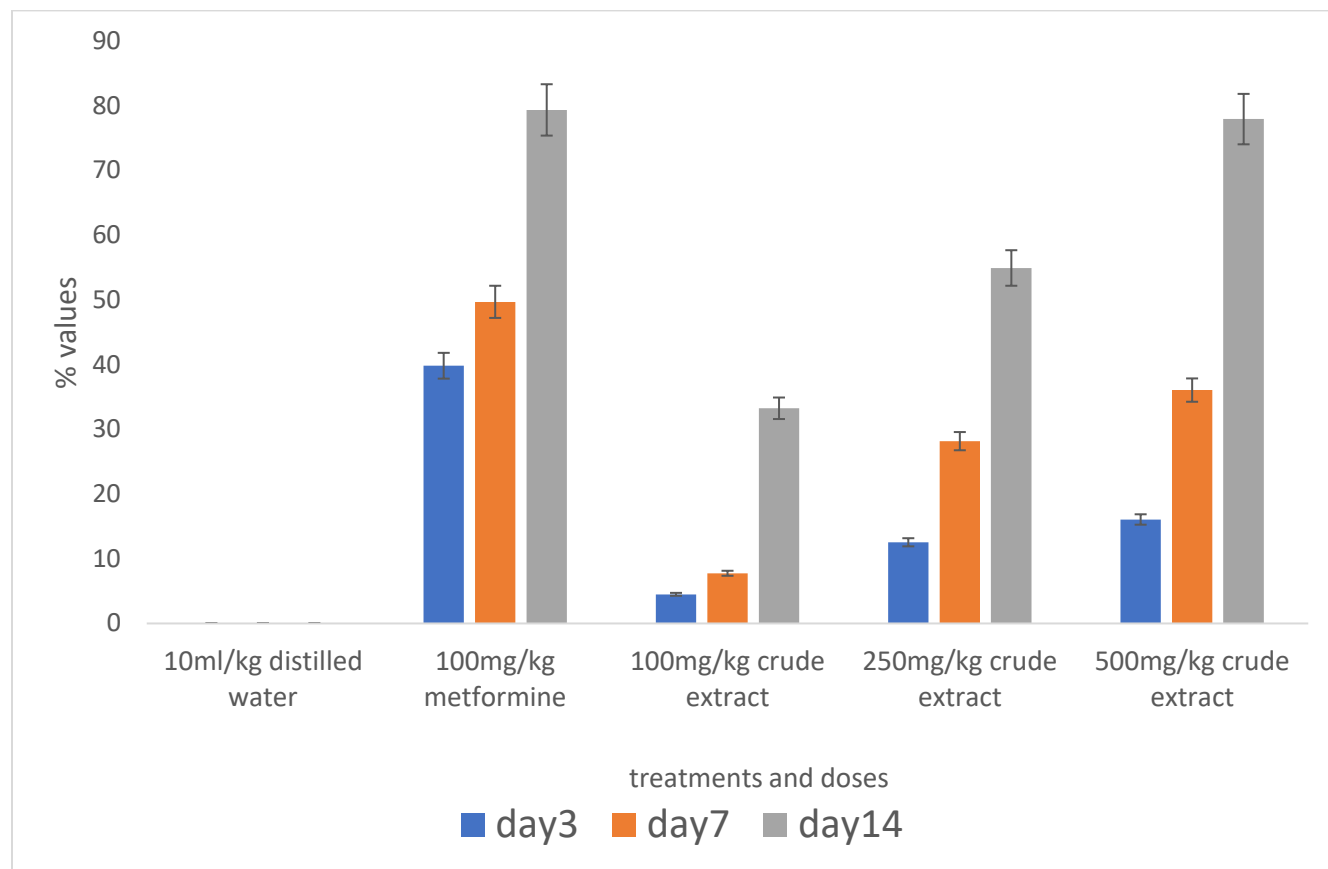


Fig 2: A bar chart of % glucose reduction against doses

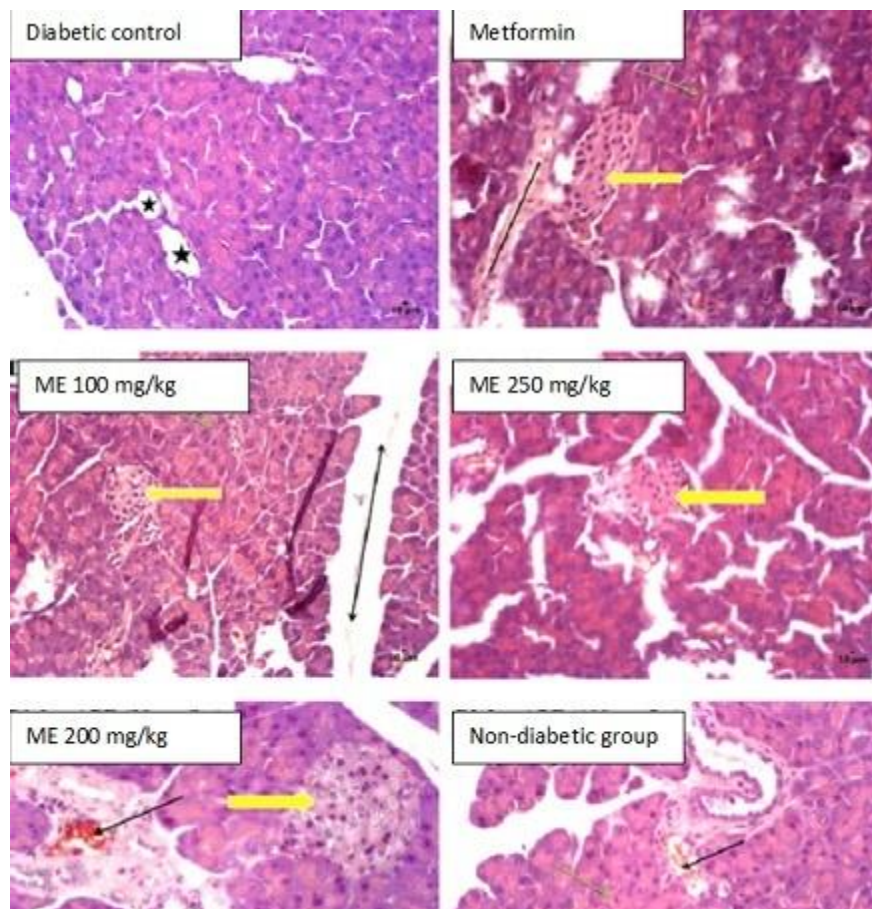


Figure 3: Powder microscopy showing the medullary rays taken at X100 magnification with the light Celestron Labs CB2000C Compound

Discussion

The LD₅₀ of *Manihotesculenta* is more than 2000 ml/kg. No animal deaths were recorded within 24 hours. This indicates that the plant is safe. For pharmacological studies of new drugs, important information about drug composition is often provided by phytochemical analysis of plant extracts. In this study, many bioactive molecules such as phenols and alkaloids were found in the qualitative analysis of different *Vitex*. This study aims to evaluate the anti-diabetic effect of methanol extract of cassava leaf extract on alloxan-induced diabetic rats and evaluate its application in human diabetes (DM). Alloxan-induced hyperglycemia is considered to be a suitable experimental model to study the hypoglycemic effects of antidiabetic agents in type II diabetes. In alloxan-induced diabetes, free radicals produced by alloxan can damage β cells, causing pancreatic malfunction and decreased insulin secretion, resulting in hyperglycemia (Joseph et al., 2024). In this study, male ICR mice were developed diabetes via intraperitoneal injection of alloxan (15

0 mg/kg body weight) and then treated with cassava and standard metformin for 4 days on the weekend. Throughout this study, the extract was used to treat alloxan-induced diabetes and lower blood sugar levels in rats. The fact that blood sugar is close to normoglycemia at the end of treatment shows that the extract has hypoglycemic activity. The hypoglycemic activity of this extract was compared to the highest concentration of metformin. Metformin works by increasing blood sugar in peripheral tissues. The hypoglycemic effect of potato may be due to the improvement of pancreatic β -

cell morphology, thereby improving pancreatic function in diabetic rats (Figure 3). This improvement is dose dependent. The presence of alkaloids and phenolic compounds (especially flavonoids) in the extract may be responsible for the observed hypoglycemic effects of the extract (Risbud and Bhonde 2002; Wazis et al. 2020). Blood sugar tests also showed that potatoes improved the ability of diabetic mice to control oral intake, similar to metformin.

Phytochemical studies on cassava have shown that it contains substances such as flavonoids, cinnamic acid, phenylpropanoids, flavonoids and glycosides (Hasim et al., 2016). Flavonoids are a group of secondary plant phenolics with powerful antioxidant properties. Independent studies have shown that flavonoids have anti-diabetic properties (Haruna et al., 2020). Therefore, the anti-diabetic and antioxidant properties of cassava may be related to flavonoids, a group of similar compounds. These results indicate that cassava methanol extract can induce insulin secretion in rats. The hypoglycemic effect of this extract may be due to the presence of insulin-like substances in the plant that stimulate B cells to produce more insulin, the high fiber content in the plant inhibits carbohydrate absorption, or the plants' ability to affect reproduction. pancreatic cells (Harborne 2008). Hypoglycemic agents are theorized to work through multiple mechanisms, including altering insulin secretion, increasing glucose-dependent insulin secretion, and promoting islet regeneration in the STZ pancreas of diabetic rats. Additionally, many scientific studies have reported the role of antioxidants in the prevention and treatment of diabetes. For example, treatment of STZ-severe diabetic animals with N-acetylcysteine (NAC), a known antioxidant, suppressed hyperglycemia and restored β -cell function by reducing oxidative stress (Tonchaiyaphum et al. 2013). On the other hand, STZ has been shown to damage pancreatic β cells (Baiubon et al., 2016). Accordingly, β -cell insulin content decreased in untreated diabetic rats, but pancreatic insulin content increased in treated rats.

The antioxidant effects of flavonoids may be the reason why they prevent glycation. Studies have shown that flavonoids are good scavengers of most oxidizing molecules (e.g. singlets), oxygen and many free radicals³⁶ and are associated with many diseases (Campbell et al., 2016 Haruna et al. et al., 2020). Antioxidant studies show that the antioxidant properties of the extract will affect the elimination of free radicals (Bahram et al., 2020), thus protecting cells from free radical damage and harmful substances. The antioxidant activity of the product can also be measured by its reduction in donating hydrogen atoms that help break free radical chains and therefore destroy the m.

Conclusion

Result from this study indicates that *Manihot esculenta* methanol leaf extract exhibits significant antidiabetic activity due to its hypoglycemic and antioxidant effects. This antidiabetic effect may be due to its ability to stimulate the reproduction and/or reactivation of pancreatic beta cells and therefore improve pancreatic function. The extract has significant antioxidant activity that neutralizes ROS and helps overcome some late complications of DM. The presence of alkaloids and phenolics (mainly flavonoids) may be responsible for the hypoglycemic activity and antioxidant effect of *Manihot esculenta*. These findings support the use of traditional herbs and antibiotics in the treatment of diabetes.

Funding

Nil

Ethics approval

The Nnamdi Azikiwe University College of Health Sciences Animal Ethics Committee granted approval and consent were obtained for animal experiments (NAU/2023/124).

Availability of data and materials

Available on request

Authorship contribution statement

All authors were involved in supervision, design of protocol, investigation, interpretation of result, Writing, Data curation, Visualization and editing

Declaration of competing interest

All authors of this paper declare that we have no competing financial interests or personal relationships that could have appeared to influence the work reported this paper.

Acknowledgments

Author will like to thank everyone who has supported this work.

Reference

Almeida, S. E. M., Arruda, S. F., Vargas, R. M., Souza, E. M. T. (2007). β -Carotene from cassava (*Manihot esculenta* Crantz) leaves improves vitamin A status in mice. *Comp Biochem Physiol C*, 146: 235-240.

Babaiedarzi A, Ghanbari S, Mehrad SM. (2022). Antidiabetic effects of *Scrophularia striata* ethanolic extract via suppression of *Pdx1* and *Ins1* expression in pancreatic tissues of diabetic rats. *Sci Rep* 12, 9813 (2022).

Bahrami G. *et al.* (2020). Anti-diabetic effect of a novel oligosaccharide isolated from *Rosa canina* via modulation of DNA methylation in Streptozotocin-diabetic rats. *DARU J. Pharm. Sci.* <https://doi.org/10.1007/s40199-020-00363-8> (2020).

Baiubon P, Kunanusorn P, Khonsung P, Chiranthanut N, Panthong A, Rujjanawate C. (2016). Gastroprotective activity of the rhizome ethanol extract of *Zingiber simaoense* YY Qian in rats. *J. Ethnopharmacol.*, 194 (2016), pp. 571-576

Byamukama, R., Namukobe, J., Kiremire, B. (2009). Anthocyanins from leaf stalks of cassava (*Manihot esculenta* Crantz). *Afri J Pure and Appl Chem.*, 3: 20-25

Campbell-TM, Rodriguez-Calvo, T. & Battaglia M. (2015). Abnormalities of the exocrine pancreas in type 1 diabetes. *Curr. Diab.Rep.* 15(10), 79.

Christer, J., Anna, W., Jiaming, Z., Xinwen, H., Chuanxin, S. (2009). Cassava, a potential biofuel crop in (the) People's Republic of China. *Applied Energy.*, 86: S95-S99.

Fujitani Y. (2017). Transcriptional regulation of pancreas development and β -cell function. *Endocrine J.* E17-0098 (2017).

Harborne AJ. (1998). *Phytochemical Methods a Guide to Modern. Techniques of Plant Analysis*. Springer Science & Business Media.

Haruna WC, Builders M, Simeon JO, Tosin JO(2020). Toxicological Study of the Effect of Ethanol Leaf Extract of *Pterocarpus santalinus* Extract on Liver of Wister Rats. *Nigeria biomedical Science Journal*. Page 17-29.

Haruna WC, Simeon JO, Builders M, Tosin JO(2020). Effect of ethanol leaf extract of *cassia angustifolia* extract on heart and lipid profile of wister rats. *African Journal of Pharmaceutical Research & Development*. Vol. 12 No.1. Page 1-8.

Hasim H, Falah S, Dewi LK. (2016). Effect of boiled cassava leaves (*Manihot esculenta* Crantz) on total phenolic, flavonoid and its antioxidant activity. *Curr. Biochem.*, 3 (3), pp. 116-127

Jonathan EE, Esther OA, Ibrahim AO. (2018). Antidiabetic Effects of the Ethanolic Root Extract of *Uvaria chamae* P. Beauv (Annonaceae) in Alloxan-Induced Diabetic Rats: A Potential Alternative Treatment for Diabetes Mellitus. *Advances in Pharmacological and Pharmaceutical Sciences*, vol. 2018, Article ID 1314941, 13 . <https://doi.org/10.1155/2018/1314941>.

Joseph Opeyemi Tosin, Joseph Oyepata Simeon. (2024). Hematological, biochemical and histological evaluation of sub-chronic administration of ethanol leaf extract of *Canscora decussate* on Kidney and Stomach of wistar rats. *JOPAT Vol 23(1) 1361– 1375, Jan – June. 2024 Edition*. <https://dx.doi.org/10.4314/jopat.v23i1.11>

Joseph Oyepata Simeon, Joseph Opeyemi Tosin. (2024). Evaluation of synergistic cytotoxic potential of *Zingiber officinale* and *Allium sativum* on Wistar rats Testes *JOPAT Vol 23(1), 1208 - 1219, January - June. 2024*. <https://dx.doi.org/10.4314/jopat.v23i1.4>

Joseph, O. S., Joseph, O. T., El-Gazzar, A. M., Mahmoud, M. H., Omoirri, M. A., & Gaber, E. S. B. (2024). Toxicological evaluation of ethanol leaf extract of *Pterocarpus santalinus* on lungs, stomach, brain and hematological parameters of Wistar rats. *Cogent Food & Agriculture*, 10(1), 2303828. Link: <https://www.tandfonline.com/doi/full/10.1080/23311932.2024.2303828>

Joseph, O. S., Omoirri, M. A., & Joseph, O. T. (2024). Evaluation of the phytochemical Constituent, acute toxicity and antiulcer activity of *Duranta erecta* fruit extracts. *Journal of Herbs, Spices & Medicinal Plants*, 1–12. <https://doi.org/10.1080/10496475.2024.2359430>

Kubo, I., Masuoka, N., Nihei, K., Brigitta, B. (2006). Maniçoba, a quercetin-rich Amazonian dish. *J Food Com Anal.*, 19: 579-588.

Lartey NL, Asare-Anane H, Ofor EK, Antwi S, Asiedu-Larb SJ, Ayertey F, Okine LKN. (2021). Antidiabetic activity of aqueous stem bark extract of *Annickia polycarpa* in alloxan-induced diabetic mice. *Journal of Traditional and Complementary Medicine*, Volume 11, Issue 2, 2021, Pages 109-116.

Meier JJ. (2008) “Beta cell mass in diabetes: a realistic therapeutic target?” *Diabetologia*, vol. 51, no. 5, pp. 703–713.

Oyepata JS and Adekunle AT. Haematological, Renal and Gastric Effects of Ethanol Leaf Extract of *Bauhinia variegata* Linn. *Trop J Phytochem Pharm. Sci.* 2024; 3(2):174-178. <http://www.doi.org/10.26538/tjpps/v3i2.3>

Oyepata JS and Opeyemi JT. (2024). Evaluation of Proliferative Potential of *Musa Paradisiaca* Stem Juice on the Pancreatic Cells of Wistar Rats. *Trop J Nat Prod Res.* 2024; 8(1):6035-6038. <http://www.doi.org/10.26538/tjnpr/v8i1.46>

Palanisamy M, Kandhasamy L, Govindraj V, Jagadeesh P, Sengottayan SN, Muthugoundar SS. (2020). Effect of *Manihot esculenta* (Crantz) leaf extracts on antioxidant and immune system of *Spodoptera litura* (Lepidoptera: Noctuidae). *Biocatalysis and Agricultural Biotechnology*, Volume 23, 2020, 101476, ISSN 1878-8181, <https://doi.org/10.1016/j.bcab.2019.101476>.

Ravi, V., Ake, J. (1996). Review on tropical root and tuber crops. II. Physiological disorders in freshly stored roots and tubers. *Cri Rev Food Sci Nutr.*, 36: 711-731.

Risbud MV and Bhonde. RR. (2002) “Models of pancreatic regeneration in diabetes,” *Diabetes Research and Clinical Practice*, vol. 58, no. 3, pp. 155–165, 2002

Simeon, JO., Tosin JO. 2024. Toxicological Evaluation of Sub-Chronic Administration of Ethanol Leaf Extract of *Ocimum canum* on Lipid Parameters, Heart Histology and Feed

Consumption of Wistar Rats. Caliphate Journal of Science and Technology Journal/Caliphate Journal of Science and Technology/Vol. 6 No. 1. Pp. 70-76.

Spaeth JM. (2017). Defining a novel role for the Pdx1 transcription factor in islet β -cell maturation and proliferation during weaning. *Diabetes* 66(11), 2830–2839.

Tahrani AA, Bailey CJ, Del Prato S, and Barnett AH. (2011) “Management of type 2 diabetes: new and future developments in treatment,” *The Lancet*, vol. 378, no. 9786, pp. 182–197.

Tonchaiyaphum P, Arpornchayanon W, Khonsung P, Chiranthanut N, Pitchakarn P, & Kunanusorn P. (2021). Gastroprotective activities of ethanol extract of black rice bran (*Oryza sativa* L.) in rats. *Molecules*, 26(13): 3812.

Vaeli N, Honarvar B, Esfandiari N & Aboosadi ZA. (2021). A laboratory study on extracting active ingredients from *scrophularia striata* boiss using ultrasound-assisted supercritical fluid extraction technique. *S. Afr. J. Chem. Eng.* 35, 111–117 (2021).

Wazis CH, Joseph OS, Modupe B, Joseph OP (2020). Effect of Ethanol Leaf Extract of *Pterocarpus santalinus* Extract on Kidney of Wister Rats. *Nigerian Biomedical Science Journal* Vol. 17 No 1. Page 35-47.