Scholars Academic Journal of Pharmacy
Abbreviated Key Title: Sch Acad J Pharm
ISSN 2347-9531 (Print) | ISSN 2320-4206 (Online)
Journal homepage: http://saspublishers.com

OPEN ACCESS

Medicine

Antidiabetic Evaluation of *Zingiber zerumbet* Linn Rhizome (Zingibereceae) Collected from Agulu of Anambra State, Nigeria

Onwunyili Amaka Roseline[1*], Ezea Charity Chinasa[1], Ibe Chioma Ifeoma[1], Ifebi Hope Morris[1], Ikeh Obiageli Eucharia[1]

[1]Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, PMB 5025, Awka, Anambra State, Nigeria

DOI: 10.36347/sajp.2024.v13i04.001 | **Received:** 15.02.2024 | **Accepted:** 19.03.2024 | **Published:** 08.04.2024

***Corresponding author:** Onwunyili Amaka Roseline
Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, PMB 5025, Awka, Anambra State, Nigeria

Abstract **Original Research Article**

Background and objectives: In Nigeria, there are wide use of medicinal plants for different health conditions like diabetes. *Zingiber zerumbet* rhizome is one such plant used for health challenges. This study assessed the effectiveness of *Zingiber zerumbet* in a diabetic rat model. ***Methods***: Extraction was carried out with methanol, using cold maceration. Fractionation was carried out with n-hexane, ethyl acetate and butanol. The antidiabetic studies were done using Alloxan induced diabetic rat model. Haematological parameters and liver and kidney function enzyme levels were analyzed using standard protocols. ***Results***: The glucose levels were reduced significantly by 68.17, 67.68, 54.29, 66.81, 77.74, 64.00, 60.89, 69.12, 62.11 and 58.26 % for 100mg/kg of Glibenclamide, 100 mg/kg crude extract, 250 mg/kg crude extract, 500 mg/kg crude extract, 250 mg/kg n-hexane, 500 mg/kg n-hexane, 250 mg/kg Ethylacetate, 500 mg/kg Ethylacetate, 250 mg/kg butanol and 500 mg/kg butanol respectively. The liver and kidney function enzymes were brought to control at $p<0.05$ significance. The haematological parameters were also brought to control at $p<0.05$ significance**.** ***Conclusion***: The extract and fractions of *Zingiber zerumbet* rhizome exhibited potential antidiabetic effect in rat model. Further studies are required to isolate, purify, characterize and structurally elucidate the particular bioactive constituent(s) responsible for the observed antidiabetic effect.

Keywords: Antidiabetes, *Zingiber Zerumbet* Rhizome, Alloxan, Natural Product.

INTRODUCTION

Diabetes is a persistent metabolic ailment characterized by heightened blood glucose levels, resulting in gradual harm to vital organs such as the heart, blood vessels, eyes, kidneys, and nerves. Type 2 diabetes, predominantly affecting adults, emerges when the body develops insulin resistance or fails to produce adequate amounts of it. Over the last three decades, the incidence of type 2diabetes has surged significantly across nations of varying economic statuses. Conversely, type 1 diabetes, formerly termed juvenile or insulin-dependent diabetes, is a lasting condition marked by insufficient insulin production by the pancreas. The prevalence of diabetes and its incidence have steadily climbed in recent decades [1]. World Health Organization (WHO) estimates the prevalence of diabetes in Nigeria to be 4.3%, and the prevalence is largely attributed to the lifestyle changes caused by urbanization and its results; industries producing unhealthy diets, including sugar-sweetened drinks, lack of exercise, tobacco use, and harmful use of alcohol [2]. Even though there are orthodox medicines available for the management of diabetes, Nigeria still resorts highly to the use of herbs in the management of treatment of diseases. One of such diseases for which herbal medicine is utilized in Nigeria is Diabetes. The utilization of traditional or herbal medicine has persisted since ancient times, exhibiting a wide array of practices. Its application and significance primarily hinge on the community's cultural background engaging in herbal remedies. These remedies rely on the active phytochemicals present in plants and are thus integral to treatment methodologies in over 300 ethnic groups nationwide. However, the practice encounters a significant obstacle in terms of documentation and a need for clinical validation, particularly in rural areas. This deficiency poses a considerable challenge in establishing standardized herbal medicine protocols suitable for clinical application. Despite this, the country boasts an abundant

array of medicinal plants with reported efficacy supported by research [3-9].

The present study investigated the potential of the medicinal plant *Zingiber zerumbet* Linn Rhizome (Zingibereceae) as an antidiabetic agent. Previous studies documented that *Zingiber zerumbet* has been shown to possess several biological activities, including antimicrobial, anti-inflammatory, antipyretic, anti-cancer, and antioxidant properties [10]. *Z. zerumbet* rhizome has been traditionally used for treating colds, ulcers, nausea, menstrual discomfort, and headaches [11]. Irrespective of the continual use of the *Z. zerumbet* rhizome in southeastern Nigeria for different purposes, studies have yet to investigate its antidiabetic property in Nigeria.

MATERIALS AND METHODS

Collection and Identification of Plant Material

Samples of fresh, healthy rhizomes of *Z. zerumbet* were collected in September 2022 from the Agulu metropolis of Anambra state. The botanist at the International Centre for Ethnomedicine and Drug Development (INTERCEDD) Nsukka Enugu State, Nigeria, authenticated the plant sample. The voucher specimen was deposited in the herbarium at the Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Sciences, Agulu campus, Nnamdi Azikiwe University, Awka, with herbarium number PCG/ 474/Z/024.

Chemical: Methanol (JHD), Ethyl acetate (JHD), Butanol (JHD) and Hexane (JHD),

Equipment/Instruments/Apparatus

Pulveriser (Gx 160 Delmar 5.5 HP, Honda Motor CO., Ltd., Japan), Electronic Weighing balance (Ahaus, china), Rotary evaporator (Büchi Rotavapor R-200), Water bath (Searchtech instruments), Separating funnel, Vecstar furnace, Desiccators, Vacuum pump (CVC 2000 Vacuubrand), Refrigerator/Freezer, hotplate and stirrer (JENWAY, UK), Magnetic stirrer (Variomag Multipoint HP), Oven, Centrifuge Pico (Heraeus), Microhematocrit centrifuge (JENALAB medical, England), Glucose Monohydrate (JHD,China), Glucometer (Acu-answer, China) Alloxan (Labchem, India) e.t.c.

Extraction

About 3000 g of the pulverized rhizome powder was cold macerated in 5 litres of methanol for 72 hours with intermittent shaking. The resulting solution was filtered, and the filtrate was pre-concentrated in a rotary evaporator at 50 ºC; after that, the extract was dried to a constant weight in a water bath at the same 50 ºC to obtain the methanol extract.

Fractionation

The methanol crude extract (50 g) was dissolved in 200 ml of distilled water and subjected to liquid-liquid partition successively with 500 ml of n-hexane, ethylacetate and butanol using 1000 ml separating funnel to obtain n-hexane, ethylacetate and butanol soluble fractions, respectively. The fractions were pre-concentrated using a rotary evaporator at 50ºC and dried using a water bath at 50 ºC.

Experimental Animals

Adult Swiss albino mice (20 -30 g) used for the study were obtained from the animal house of the Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Agulu campus, Nnamdi Azikiwe University, Awka. They were housed in standard aluminium cages under uniform temperature and humidity laboratory conditions and allowed free access to standard pelletized feed (Vital feeds, Nigeria) and water *ad libitum* with 12 hr light/dark cycle.

They were acclimatized to the environment for seven days before the start of the study. All animal care, handling and experiments complied with the NIH guide for the care and use of laboratory animals. Animal ethics approval was obtained from the Animal Ethics Committee of the Nnamdi Azikiwe University, Awka, Nigeria.

Acute Toxicity Study of the Methanol Rhizome Extract of *Z. Zerumbet*

The acute toxicity test was carried out using Lorke's method as modified by Agyigra *et al*., 2017 [12]. The test was performed in two phases. In the first phase, three (3) groups of 3 mice in each group were orally given 10, 100 and 1000 mg/kg body weight respectively of the extract. The animals were observed for 24 hours post-administration for signs of toxicity and mortality. In the second phase, 4 mice were weighed, marked, and randomized into four groups, with one mouse in each group. They were orally administered 2000, 3000, 4000 and 5000 mg/kg body weight of the extract, respectively. The animals were again observed for signs of toxicity (behavioural responses, tremors, convulsion, salivation, diarrhoea, lethargy, sleep and coma) and mortality for 24 hours.

Antidiabetic Study

Alloxan was used for the induction of diabetes in Swiss albino mice. A 150 mg/kg of Alloxan with the post-oral treatment of 2000 mg/kg glucose monohydrate was administered intraperitoneally within 15 mins as described by Gbolade *et al*., (2008) [13], Tahara *et al*., (2008) [14]. Blood samples were drawn from the tail vein 48 hours post-induction for fasting blood glucose concentration determination using Accu answer Glucometer to confirm hyperglycemia. Mice with fasting blood glucose levels>150 mg/dl were considered diabetic.

Diabetic mice were randomly divided into five groups of five mice in each group; Group 1 received 10 ml/kg distilled water, Group 2 received 100 mg/kg

glibenclamide, while Groups 3-5 received 100, 250, and 500 mg/kg of the extract orally, respectively. Blood samples were collected from the tail vein after an overnight fast at intervals of 0hr, 30mins, 1hr, 2hrs, 4hrs, 6hrs, Day 3, and Day 7 to determine glucose concentrations using Accu answer Glucometer.

The most active doses (250 and 500 mg/kg) from the preliminary antidiabetic study of the doses of the crude extract were used for the antidiabetic evaluation of the derived fractions (n-hexane, ethyl acetate, and butanol, respectively) as well as positive and negative control.

Percentage reduction = (A-B)/A x 100.
Where A =Mean FBGL at Day 0, B =Mean FBGL at corresponding time and days

Biochemical Studies

Blood was collected in non-heparinised tubes. The blood samples were centrifuged at 4000 g for 15 mins, and the supernatant was separated and stored at 4 ºC to maintain enzyme activity.

Aspartate Aminotransferase (AST)

The studies were carried out using standard AMP diagnostic kits (Stattogger Strasse 31b 8045 Graz, Australia)

About 0.1 ml of sample was mixed with Reagent 1 (phosphate buffer, 100 mmol/l, pH 7.4; L-aspartate, 100mmol/l; and α-ketoglutarate, 2 mmol/l), and the mixture was incubated for exactly 30 mins at 37 ºC. 0.5 ml of Reagent 2 (2,4-dinitrophenylhydrazine (2 mmol/l) was added to the reaction mixture and allowed to stand for exactly 20 mins at 20-25ºC. Then, 5 ml of sodium hydroxide (0.4 mol/l) was added and mixed. The absorbance of the sample against the reagent blank was read after 5 mins with a spectrophotometer at 546 nm, and the level of AST was calculated.

Alanine Aminotransferase (ALT)

The studies used standard AMP diagnostic kits (Stattogger Strasse 31b 8045 Graz, Australia). A 0.1 ml of diluted sample was mixed with 0.5 ml of Reagent 1 Phosphate buffer (100 mmol/L, pH 7.4), L alanine (100 mmol/L), and α-oxoglutarate (2 mmol/L), and the mixture incubated for exactly 30 minutes at 37ºC. 0.5 ml of 2, 4-dinitrphenylhydrazine (2 mmol/L) was added to the reaction mixture and allowed to stand for exactly 20 minutes at 20-25 ºC. Then, 5.0 ml of NaOH (0.4 mol/l) was mixed. The absorbance of the sample against the reagent blank was read after 5 minutes with a spectrophotometer at 546 nm, and the level of ALT was calculated.

Alkaline Phosphatase (ALP)

The studies used standard AMP diagnostic kits (Stattogger Strasse 31b 8045 Graz, Australia). One vial of substrate R1b (p-nitro-phenylphosphate) was reconstituted with the appropriate volume of R1a (diethanolamine buffer, $MgCl_2$) and was termed 'Reagent' 0.05 ml of the serum was mixed with 3.0 ml of the reagent and initial absorbance was read at 405 nm; a timer started simultaneously. Absorbance was reread at the same wavelength after 1, 2, and 3 minutes.

Urea Nitrogen (BUN) (Teco Kit)

The BUN enzyme reagent was reconstituted according to the instructions on the product leaflet. Fresh tubes were labelled blank, test, and standard. 1.5 ml of BUN enzyme reagent was pipetted into all and was allowed to equilibrate at room temperature for 5 mins. To the tube labelled "blank", 10 ul of deionized water was added. To the tubes labelled "Samples", 10 ul of each group's respective samples was added. To the tubes labelled "Standard", 10 ul of the standard reagent provided in the kit was added. They were appropriately mixed and were incubated for 5 mins at 37ºC. After the incubation, 1.5 ml BUN colour developer supplied in the kit was added to all the tubes and again incubated for 5 mins at 37ºC. The absorbance was read at 630 nm using a reagent blank to zero the spectrophotometer. The concentration of the Urea Nitrogen was calculated using the formula.

Absorbance of unknown (test))/(Absorbance of standard) X concentration of standard

Creatinine (Direct Endpoint Procedure)

Equal volumes of creatinine picric acid reagent and creatinine buffer reagent provided in the kit were well mixed; it was termed "working reagent". Test tubes were labelled "blank", "standard", and "test". 3.0 ml of the "working reagents" was pipette into all the respective tubes. To the tube labelled "blank", 100 ul of distilled water was added. To the tubes labelled "standard", 100 ul of standard reagent provided in the kit was added. To the tubes labelled with different samples according to groups, 100 ul of each sample was added respectively. It was mixed and heated in a water bath for 15 mins at 37ºC. It was cooled, and the absorbance was read at 520 nm using a reagent blank to zero the spectrophotometer. The creatinine value was calculated using the formula.

(Absorbance of unknown (test))/(Absorbance of Standard) x concentration of standard

Hematological Studies

Determination of Microhematocrit/ Packed Cell Volume

The blood sample may be obtained from a capillary puncture or a tube of venous blood with the anticoagulant EDTA added. The blood is drawn by capillary action into capillary tubes of minimal diameter sealed. To do this, the clean end of the tube is placed in sealing clay. This makes a tight seal and prevents contamination with blood. The tube is then put in a special microhematocrit centrifuge. The sealed ends of the tubes are placed against the rubber gasket, and the

open ends are towards the centre. After being centrifuged for the prescribed time (usually 3-5 minutes), the hematocrit (%) is read by placing the tube in a particular microhematocrit reader.

Estimation of Haemoglobin Content of the Blood (Sahli Method)

The dilution tube is filled approximately to mark 10, with fresh 0.1 N HCl. The blood pipette is pricked with the finger and sucked up about 20 mm^3 of blood, the blood on the outside is wiped off and dried, and the volume is adjusted exactly to the mark by tapping on the nail or touching the tip of the pipette lightly with cotton wool. Gently blow the blood into the acid and suck it up and down three times to mix well. Stand the mixture for 5 minutes to allow time for the formation of acid haematin. Add distilled water dropwise from a pipette, stirring with a glass rod after each addition. Continue to add water until the dilution tube's tint is darker than the standard's. Compare the tubes against bright, diffused light while holding a sheet of white paper behind them. Take the reading of the upper level of the fluid in the dilution tube. Continue diluting until the test is just appreciably paler than the standard. Take the average of this reading and the previous one as the correct reading. Repeat the experiment described above, but add diluting fluid dropwise until the blood-acid solution exactly matches the standard. Take a reading and compare it with the previous one.

Calculation

100 % on the Sahli scale in g Hb/100 ml is 14 g.
If X is Hb for 100 % reading and Y is the % reading obtained, then the Hb in the sample tested is = X(Y)

Red Blood Cell (RBC) Count

Several fluids may be used to dilute blood for an RBC count. The diluting fluid used for RBC count must be the isotonic solution. This prevents haemolysis or destruction of the red cells. Commonly used dilution fluids are Hayem's, Gower's and Ducie's.

A capillary sample of a well-mixed anti-coagulant blood sample is drawn into the RBC pipette to the 0.5 mark. Red cell diluting fluid is then drawn into the pipette to the 11 marks. The contents of the pipette are mixed thoroughly. The first 2 to 3 drops of blood are discarded. A cover glass is positioned on the hematocytometer. The tip of the pipette is touched to the edge of the cover glass, and one side of the chamber is allowed to fill by capillary action. The opposite side is filled in the same manner. If the fluid overflows into the depression around the platforms or if air bubbles occur, the chamber should be cleaned and refilled. After allowing the cells to settle for two to three minutes, the hematocytometer is placed carefully on the microscope stage. Locate the ruled areas using the low power (10 x) objective; the high power (40 x) objective is then rotated into place to perform the count. The RBC count is performed using the centre of the ruled area; within the centre square are twenty-five small squares. Of these twenty-five squares, the four corner squares and the centre square (marked a, b, c, d and e) are counted. Each of these five squares, in turn, contains four rows of squares that are counted using the left-to-right, right-to-left counting pattern. A hand counter tabulates the red cells in the five designated squares. The numbers of each of the five squares are recorded and totalled.

Statistical Analysis

Numerical data obtained from the study were expressed as the mean value ± standard error of the mean (SEM). Differences among means of control and tested groups were determined/analyzed using one-way analysis of variance (ANOVA). A probability level of less than 5 % ($p \leq 0.05$) was considered statistically significant. Graphical plots were done using Microsoft Excel 2007.

RESULT AND DISCUSSION

Extraction

The extraction of 3000 g of pulverized plant sample yielded about 216.73 g of a deep brown crude extract (CE). The percentage yield was 7.2 %. The yield of the natural extract strongly depends upon the solvent used for extraction due to solvent polarity [15].

Fractionation

The result of the Fractionation is presented in Table 1, showing the fractionation values of the solvent used for the fractionation of the extract. The crude extract was subjected to fractionation with solvents of increasing polarities to reduce the extract's complexities and to facilitate ease of compound isolation and a better understanding of the polarities of not only the solvent fractions but also that of the isolated compounds. It also facilitates understanding of the most bioactive fraction polarity. This experiment showed that the ethylacetate fraction was obtained in the highest yield (Table 1). This is an indication that the ethylacetate fraction may contain more components compared to the other fractions

Table1: Fractionation yield and appearance of the fractions

Fraction	Weight (g)	Color
n-hexane	11.47	Light yellow
Ethylacetate	41.65	Deep brown
Butanol	3.92	Brown
Water	2.11	Brown

Acute Toxicity

No toxicity was observed in the animals after the acute toxicity studies. The acute toxicity of methanol crude extract of *Zingiber zerumbet* rhizome was investigated to determine any adverse effect that may arise from a short-term (oral) administration of a single dose or multiple doses of the extracts (within 24-hour period). According to some expert recommendations, a chemical that has $LD_{50} \geq 5000$ mg/kg is practically non-toxic and may not likely cause any toxicity on short-term

exposure [16]. The oral acute toxicity and lethality test results did not exhibit any toxic symptoms or mortality, as shown in Table 10. The LD_{50} was established to be ≥ 5000 mg/kg, which suggests that this plant in high and low doses did not produce any significant changes, no gross, physical or behavioural changes such as hair erection, diarrhoea, sleepiness, coma, death and loss of appetite in the animal behaviour. This indicates that the administration of the plant crude extract showed no toxicity in the animal, and this low toxicity may have been responsible for its widespread use in different ethno-therapeutic interventions.

Antidiabetic Study

The result of the antidiabetic evaluations of the extract and fractions is presented in Table 2. The table shows the glucose levels of the groups of animals up to 7 days. The significance of the treatment was estimated after 7 days of treatment.

Fasting blood glucose estimation is an important diagnostic measure in diabetes [17]. The liver is pivotal in regulating glucose homeostasis during fed-fasting transition through gluconeogenesis and glycogenolysis. Deregulation of these important insulin-regulated mechanisms contributes to impaired fasting blood glucose, which is a risk factor for diabetes [18].

Alloxan-induced diabetes may be associated with impairment of basal insulin secretion. Alloxan-induced pancreatectomy may explain this, leading to abnormalities in beta cell function and Alloxan-associated hepatic oxidative effect affecting both hepatic insulin receptors and sensitivity [19]. Also, diabetes is a metabolic disorder characterized by hyperglycemia and can be treated with some medicinal plants found to have hypoglycemic effects. The most important is the *Zingiber zerumbet* rhizome [20].

In light of the results, continuous treatment of all induced diabetic mice with both glibenclamide and the methanol extract and fractions of *Zingiber zerumbet* rhizome at different doses significantly ($p < 0.05$) decreased the blood glucose level in diabetic mice. The percentage reduction in fasting blood glucose level showed a significant decrease with the standard drug (glibenclamide), crude extract and fractions of Z. zerumbet rhizome as compared with the diabetic control group and these reductions were done in a dose-dependent manner with the 250 mg/kg and 500 mg/kg doses bringing down the glucose concentration within seven days of administration which shows that this plant has an anti-hyperglycemic effect. The possible mechanisms responsible for this antidiabetic action of Zingiber zerumbet rhizome extract could be connected to their enhanced secretion of insulin from the beta cells of the pancreas or increased tissue uptake of glucose by enhancement of insulin sensitivity. It is also possible that the plant extract increased glucose removal from blood, decreased the release of glucagon or increased that of insulin, stimulated directly glycolysis in peripheral tissues or reduced glucose absorption from the gastrointestinal tract, ameliorated the oxidative stress attributed to the presence of a variety of phytoconstituents present in this plant [21]. It could also be connected to the inhibitory action of the extract on α-glucosidase, an enzyme found in the brush border of the intestine and responsible for converting polysaccharides into simple sugars. The inhibition of this enzyme slowed the elevation of sugar after a carbohydrate meal, which is one of the ways to decrease postprandial increases in blood glucose levels.

Table 2: Antidiabetic effects of the extract and the fractions

Group	Day 0	30 mins	1hr	2hrs	4hrs	6hrs	day3	day7
Control	108.0±6.93	107.3±2.67	109±4.31	106.1±14.11	107.2±8.94	105.0±10.11	90.0±6.42	92.4±4.55
10ml/kg distilled water	199.5±10.32	202.3±6.28	206±14.11	215.1.±10.32	228.1±6.2	226.3±4.62	226.0±7.26	272.14±10.56
100 mg/kg Glibenclamide	218±10.45	210.4±8.26	185±8.11	183.0±14.26	180±6.25	174.2±15.11	100.6±6.23	69.4±5.26*
100 mg/kg crude extract	211±16.06	209.6±14.11	205.0±11.26	197.6±6.42	200.0±9.26	210±6.44	105.7±4.57	68.2±3.47*
250 mg/kg crude extract	259.0±17.15	237.5±7.21	196.0±4.08	179. 0 ±8.23	170.4±8.23	171.4±11.21	223.5±7.08	118.4±4.26*
500 mg/kg crude extract	232.0±12.21	228.4±15.23	216±5.13	193.4±11.13	235.0±15.08	242.0±15.14	115.0±10.04	77.0±7.02*
250 mg/kg n-hexane	243.0±12.11	240.1±6.43	213±14.56	210.5±12.43	226.4±7.15	244.2±6.13	119.7±6.42	54.1±3.46*
500 mg/kg n-hexane	200±6.28	201.4±8.36	195.0±12.11	180.6±5.13	183.0±10.62	223.0±11.12	103.7±4.26	72.0±3.72*
250 mg/kg Ethylacetate	202.0±8.43	186.4±7.26	149.0±6.34	136.2±4.11	130.4±7.18	133.6±8.43	132.7±7.32	79.0±4.43*
500 mg/kg Ethylacetate	215.0±9.26	196.7±4.28	185.0±16.11	180.3±12.11	185.0±10.02	210.5±6.24	101.3±4.33	66.4±3.24*

Group	Day 0	30 mins	1hr	2hrs	4hrs	6hrs	day3	day7
250 mg/kg butanol	227.0±5.26	220.0±4..26	200.0±6.14	198.4±12.12	233.4±5.51	236.5±11.26	144.0±5.73	86.0±4.32*
500 mg/kg butanol	218.0±4.11	200.5±11.26	178.0±4.23	174.0±6.26	188.0±8.91	210.5±5.62	134.0±6.34	91.0±5.26*

Values are expressed as mean of 5 replicates ±SEM. Values with (*) are significantly comparable to the control values ($p<0.05$)

Percentage Reduction in Blood Glucose Level

The result of the calculation of the percentage reduction in blood glucose level is presented in Table 3. The table revealed more than 50 % reduction in blood glucose levels of all the treated groups.

Table 3: Percentage reduction in blood glucose levels of the treated animal groups

Group	30 mins	1hr	2hrs	4hrs	6hrs	day3	day7
10 ml/kg distilled water	0.00	0.00	0.00	0.00	0.00	0.00	0.00
100mg/kg Glibenclamide	3.67	15.14	16.06	17.43	20.18	53.85	68.17*
100 mg/kg crude extract	0.95	2.84	6.64	5.21	0.47	49.91	67.68*
250 mg/kg crude extract	8.30	24.32	30.89	34.21	33.82	13.71	54.29*
500 mg/kg crude extract	1.68	6.90	16.64	0.00	0.00	50.43	66.81*
250 mg/kg N-hexane	1.28	12.35	13.37	6.83	0.00	50.74	77.74*
500 mg/kg N-hexane	0.00	2.50	9.70	8.50	0.00	48.15	64.00*
250 mg/kg Ethylacetate	7.72	26.24	32.57	35.45	33.86	34.31	60.89*
500 mg/kg Ethylacetate	8.51	13.95	16.14	12.09	2.09	52.88	69.12*
250 mg/kg butanol	3.08	11.89	12.6	1.59	0.00	36.56	62.11*
500 mg/kg butanol	8.03	18.35	20.18	13.76	3.44	38.53	58.26*

Values are expressed as mean of 5 replicates ±SEM. Values with (*) are significantly comparable to the control values ($p<0.05$)

Effects of the Treatments on the Liver and Kidney Function Enzymes of the Diabetic-Treated Animal Groups

The results of how the treatment affected the ALT, AST, ALP, urea and creatinine levels of the treated diabetic animal groups are presented in Table 4. There was a significant improvement in the blood parameters compared to the normal control group. This study's levels of AST, ALT ALP, urea and creatinine significantly increased in diabetic-induced mice [22]. The increase in the activities of AST, ALT, ALP, urea and creatinine may be due to the leakage of these enzymes from the liver cytosol into the bloodstream, which gives an indication of the hepatotoxic effect of diabetogenic agents (alloxan) in diabetic mice [23]. An increase in serum levels of AST shows hepatic injuries similar to viral hepatitis, muscle infarction and muscle damage. ALT, which mediates the conversion of alanine to pyruvate and glutamate, is specific for the liver and is a suitable indicator of hepatic injuries. In addition, ALP is membrane-bound, and its alteration is likely to affect the membrane permeability and produce derangement in the transport of metabolites. Urea and creatinine are synthesized in the liver, pancreas, and kidneys, and they are good indicators of the normal functioning of the kidney. An increase in the serum urea and creatinine levels are indicators of kidney dysfunction, that is, renal failure.

Impairment of urea and creatinine levels due to an increase in blood glucose level due to alloxan induction reduced kidney function in diabetic mice [24]. Significant return of these enzymes to basal serum value ranges following treatment with *Zingiber zerumbet rhizome* extract and fractions as compared with the negative control group may be connected to the prevention of intracellular and tissue enzyme leakage resulting from cell membrane stability or cellular regeneration, showing that this plant can protect the liver and kidney, particularly on membrane permeability due to their target oxidant and consequently can function as free radical scavengers [25]. The extracts can also inhibit lipid peroxidation, preventing alloxan-induced oxidative stress and beta cells. They can lead to increased secretion of insulin and decreased glucose levels fast as glibenclamide [26], though ALP slightly increased with treatment, which may be as a result of large bile duct obstruction, intrahepatic cholestasis, and infiltrative diseases of the liver or increased osteoblastic activity.

Table 4: Effects of the treatment on the liver and kidney functions of the animal groups

Group	ALT(U/I)	AST(U/I)	ALP(U/I)	UREA(mg/dl	CREATININE(mg/dl)
Normal control	32.0±2.32	43.4±6.12	62.4±3.21	41.5±3.85	0.62±0.04
10 ml/kg distilled water	73.4±3.47	83.5±2.16	58.9±9.63	62.5±4.11	1.64±0.06
100 mg/kg Glibenclamide	43.6±2.56*	47.3±1.62*	65.8±4.26*	43.6±2.11*	0.73±0.03*
100 mg/kg crude extract	51.2±3.13*	56.4±2.84*	60.1±8.11*	45.8±3.52*	0.81±0.04*
250 mg/kg crude extract	46.1±2.41*	51.2±3.04*	57.3±4.16*	40.1±5.26*	0.75±0.02*

Group	ALT(U/I)	AST(U/I)	ALP(U/I)	UREA(mg/dl	CREATININE(mg/dl)
500 mg/kg crude extract	45.6±4.10*	48.9±6.11*	63.5±7.21*	44.9±4.27*	0.79±0.04*
250 mg/kg N-hexane	48.1±6.40*	59.8±4.12*	60.5±6.14*	43.8±1.96*	0.83±0.06*
500 mg/kg N-hexane	46.3±8.00*	56.1±2.34*	59.3±8.12*	46.1±3.96*	0.81±0.03*
250 mg/kg Ethylacetate	42.4±2.81*	46.9±8.12*	61.4±4.11*	48.1±2.23*	0.76±0.02*
500 mg/kg Ethylacetate	45.1±3.01*	49.2±3.26*	59.5±6.13*	45.6±1.93*	0.69±0.06*
250 mg/kg butanol	51.6±0.59*	54.1±8.32*	61.4±5.13*	47.8±2.12*	0.72±0.05*
500 mg/kg butanol	46±0.43*	53.4±2.84*	57.9±9.14	45.9±8.26*	0..76±0.04*

Values are expressed as mean of 5 replicates ±SEM. Values with (*) are significantly comparable to the control values ($p<0.05$)

Effects of the Treatments of Blood Parameters of the Treated Animal Groups

The results of how the treatment affected the RBC, HB and PCV levels of the treated diabetic animal groups are presented in Table 5. There was a significant improvement in the blood parameters compared to the normal control group. The examination of blood has been described as a good way of assessing the health status of animals because it plays an important role in animals' physiological, nutritional and pathological status [27]. Haematological parameters provide information regarding the status of bone marrow activity and hemolysis [28]. It has been revealed in this study that haematological parameters in pre-treated groups showed abnormalities which correlate with the results of (Mansi KMS, 2006) [29]. This might be due to the destruction of matured red blood cells leading to low haemoglobin (Hb) count (because of the reaction of excess glucose with the haemoglobin to give rise to glycosylated haemoglobin) with a decrease in red blood cells (RBC) (an indication of imbalance between its synthesis and destruction) and packed cell volume (PCV) normally being affected by LADA-induced diabetes, an indication of anaemia [30]. Treatment with the extract and fractions significantly restored PCV, Hb and RBC to basal ranges when compared to the diabetic group.

Table 5: Effects of the treatment on the blood parameters of the animal groups

Group	RBC ($x10^9$)	HB (g/dl)	PCV (%)
Normal control	6.7±1.41	13.3±1.21	40.0±1.26
10 ml/kg distilled water	5.0±1.36	10.0±1.04	30.1±1.43
100 mg/kg Glibenclamide	6.2±1.39*	12.0±1.32*	36.8±2.11*
100 mg/kg crude extract	5.9±1.21*	11.7±0.09*	35.6±1.04*
250 mg/kg crude extract	6.2±1.59*	12.2±1.02*	37.8±1.43*
500 mg/kg crude extract	6.0±1.93*	12.1±1.42*	36.4±1.26*
250 mg/kg N-hexane	5.7±0.89*	11±0.09*	34.5±1.06*
500 mg/kg N-hexane	6.1±1.34*	12.1±1.26*	37.6±1.59*
250 mg/kg Ethylacetate	6.1±0.92*	12±1.43*	36.4±1.41*
500 mg/kg Ethylacetate	5.8±1. 11*	11.2±1.27*	35.2±1.28*
250 mg/kg butanol	6.2±0.89*	12±121*	37.6±1.11*
500 mg/kg butanol	6.1±1.42*	11.9±0.06*	36.4±1.28*

Values are expressed as mean of 5 replicates ±SEM. Values with (*) are significantly comparable to the control values ($p<0.05$)

CONCLUSION

This study provided evidence indicating that the methanol extract and the fractions of *Zingiber zerumbet* rhizome significantly reduced glucose levels in diabetic mice. In addition, treatment with the extract and fractions caused the recovery of certain altered biochemical and haematological parameters of diabetic animals. Further studies are required to isolate, purify, characterize and structurally elucidate the particular bioactive constituent(s) responsible for the observed antidiabetic effect, which may result in the development of a potent antidiabetic agent with low toxicity and better therapeutic index.

Acknowledgements

The authors sincerely thank the Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, for providing the opportunity to conduct the research. The authors would also like to express their gratitude to the teaching and non-teaching staff of the Department of Pharmacognosy and Traditional Medicine and The Department of Pharmacology and Toxicology for their assistance.

Conflict of Interest: There are no competing interests.

REFERENCES

1. World Health Organization, 2024. Diabetes. *https://www.who.int/health-topics/diabetes#tab=tab_1* (accessed on February 21th, 2024)
2. World Health Organization, 2022. Stakeholders call for increased access to diabetes education. *https://www.afro.who.int/countries/nigeria/news/stakeholders-call-increased-access-diabetes-education* (accessed on February 21th, 2024)
3. Ejiofor, I. I., Zaman, K., & Das, A. (2017). Antidiabetic evaluations of different parts of

Vernonia amygdalina. *IOSR Journal of Pharmacy and Biological Sciences*, *12*(4), 23-28.
4. IfedibaluChukwu, E. I., Aparoop, D., & Kamaruz, Z. (2020). Antidiabetic, anthelmintic and antioxidation properties of novel and new phytocompounds isolated from the methanolic stem-bark of Vernonia amygdalina Delile (Asteraceae). *Scientific African*, *10*, e00578.
5. Ii, E., Zaman, K., & Das, A. (2017). Effect of Extracts of Vernonia Amygdalina in Helminthiasis-A Tropical Neglected Disease. *Open Access Journal of Pharmaceutical Research*, *1*(8).
6. Ololade, A. M., Anowi, F. C., Anwuchaepe, A. A., & IfedibaluChukwu, E. I. (2023). Pharmacognostic Study and Hepatoprotective Activity of the Methanolic Extract and Fractions of Leaves of Picralima nitida Apocyanaceae. *Sciences of Phytochemistry*, *2*(1), 114-127.
7. Okoben, F. C., Ejiofor, I. I., Mbagwu, I. S., Ajaghaku, D. L., & Anowi, F. C. (2024). Effects of Methanolic Leaf Extract and Fractions of Irvingia Gabonensis on Hematological Parameters in Wistar Rats with Splenomegaly. *Sciences of Pharmacy*, *3*(1), 9-17.
8. Perpetual, E. A., Ifedibaluchukwu, E. I., Lotanna, A. D., Obodike, E. C., & Ifeoma, I. C. (2024). In-Vivo Antidiarrhoea Evaluations of Methanol Leaf Extract and Fractions of Chrysophyllum albidum. *Sch Acad J Pharm*, *1*, 23-29.
9. Okoye, K. F., Anowi, F. C., Ejiofor, I. I., & Ololade, O. M. (2023). Pharmacognostic study and sedative activity of Bryophyllum pinnatum stem methanol extract and fractions. *Sciences of Phytochemistry*, *2*(2), 127-138.
10. Yob, N. J., Jofrry, S. M., Affandi, M. M. R., Teh, L. K., Salleh, M. Z., & Zakaria, Z. A. (2011). Zingiber zerumbet (L.) Smith: a review of its ethnomedicinal, chemical, and pharmacological uses. *Evidence-Based Complementary and Alternative Medicine*, *2011*.
11. Hong, T. Y., Tzeng, T. F., Liou, S. S., & Liu, I. M. (2016). The ethanol extract of Zingiber zerumbet rhizomes mitigates vascular lesions in the diabetic retina. *Vascular Pharmacology*, *76*, 18-27.
12. Agyigra, I. A., Ejiofor, J. I., & Magaji, M. G. (2017). Acute and subchronic toxicity evaluation of methanol stem-bark extract of Ximenia americana Linn (Olacaceae) in Wistar rats. *Bulletin of Faculty of Pharmacy, Cairo University*, *55*(2), 263-267.
13. Gbolade, A. A., & Adeyemi, A. A. (2008). Anthelmintic activities of three medicinal plants from Nigeria. *Fitoterapia*, *79*(3), 223-225.
14. Tahara, A., Matsuyama-Yokono, A., Nakano, R., Someya, Y., & Shibasaki, M. (2008). Effects of antidiabetic drugs on glucose tolerance in streptozotocin-nicotinamide-induced mildly diabetic and streptozotocin-induced severely diabetic mice. *Hormone and metabolic research*, 880-886.
15. Romdhane, M., & Gourdon, C. (2002). Investigation in solid–liquid extraction: influence of ultrasound. *Chemical engineering journal*, *87*(1), 11-19.
16. Mbagwu, I. S., Akah, P. A., & Ajaghaku, D. L. (2020). Newbouldia laevis improved glucose and fat homeostasis in a TYPE-2 diabesity mice model. *Journal of ethnopharmacology*, *251*, 112555.
17. American Diabetes Association. (2010). Diagnosis and classification of diabetes mellitus. *Diabetes Care, 33*(Suppl. 1), S62– S69.
18. Al-Goblan, A. S., Al-Alfi, M. A., & Khan, M. Z. (2014). Mechanism linking diabetes mellitus and obesity. *Diabetes, metabolic syndrome and obesity: targets and therapy*, 587-591.
19. Rosa, C. V. D. D., Campos, J. M. D., Sá Nakanishi, A. B. D., Comar, J. F., Martins, I. P., Mathias, P. C. D. F., ... & Natali, M. R. M. (2018). Food restriction promotes damage reduction in rat models of type 2 diabetes mellitus. *PloS one*, *13*(6), e0199479.
20. Kar, A., Choudhary, B. K., & Bandyopadhyay, N. G. (2002). Comparative evaluation of hypoglycaemic activity of some Indian medicinal plants in alloxan diabetic rats. *Journal of Ethnopharmacology*, *84*(1), 105–108.
21. Malik, N. H., Ghumman, S. A., Irfan, H. M., & Akram, M. (2012). Hypoglycemic and hematological effects of aqueous extract of Thymus serpyllum Linn. in alloxan-induced diabetic rabbits. *African Journal of Pharmacy and pharmacology*, *6*(40), 2845-2850.
22. Nwanjo, H. U. (2007). Free radicals scavenging potential of the aqueous extract of Viscum album (Mistletoe) leaves in diabetic wistar rats hepatocytes. *The Internet Journal of Nutrition and Wellness*, *3*(2), 1-11.
23. Saggu, S., Sakeran, M. I., Zidan, N., Tousson, E., Mohan, A., & Rehman, H. (2014). Ameliorating effect of chicory (Chichorium intybus L.) fruit extract against 4-tert-octylphenol induced liver injury and oxidative stress in male rats. *Food and chemical toxicology*, *72*, 138-146.
24. Kamal, A. (2014). Impact of diabetes on renal function parameters. *Ind J Fund Appl Life Sci*, *4*(3), 411-6.
25. Sharifudin, S. A., Fakurazi, S., Hidayat, M. T., Hairuszah, I., Aris Mohd Moklas, M., & Arulselvan, P. (2013). Therapeutic potential of Moringa oleifera extracts against acetaminophen-induced hepatotoxicity in rats. *Pharmaceutical biology*, *51*(3), 279-288.
26. McCarty, M. F. (2005). Nutraceutical resources for diabetes prevention–an update. *Medical hypotheses*, *64*(1), 151-158.
27. Muhammad, N. O., Adeyina, A. O., & Peters, O. M. (2000). Nutritional evaluation of fungi treated cocoa bean shell. *Nigerian J. Pure Appl. Sci*, *5*, 1059-64.
28. Iniaghe, O. M., Egharevba, O., & Oyewo, E. B. (2013). Effect of aqueous leaf extract of Acalypha

wilkesiana on hematological parameters in male wistar albino rats. *British journal of pharmaceutical research*, *3*(3), 465.

29. Mansi, K. M. S. (2006). Effects of administration of alpha-melanocyte stimulating hormone (?-MSH) on some hematological values of alloxan-induced diabetic rats. *Am J Pharmacol Toxicol*, *1*, 5-10.
30. Muhammad, N. O., & Oloyede, O. B. (2009). Haematological parameters of broiler chicks fed Aspergillus niger - fermented Terminalia catappa seed meal-based diet. *Global J Biotechnol Biochem*, *4*, 179–18.