

ORIGINAL ARTICLE

Effect of Aqueous Extract of *Cymbopogon Citratus* on Blood Glucose Level, Lipid Profile and Histology of Diabetic Rats

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ABSTRACT

Background: Diabetes is considered as the foremost cause of morbidity and mortality. This study determined the effect of aqueous extract of *Cymbopogon Citratus* on blood glucose level, lipid profile and histology of diabetic rats.

Methods: One gram of *C. citratus* dissolved in 10 mL of distilled water was used to prepare 100 mgmL⁻¹ stock of the extract; while intra-gastric tube (attached to a syringe) was utilized to administer the extract for the 21 days treatment. Alloxan was used to induce diabetes in rats. Twenty Wistar rats were equally and randomly assigned to three groups. Group 1 was treated with 400 mg/kg of *C. citratus* extract, group 2 with 800 mg/kg of *C. citratus*, group 3 with 500 µg/kg of glibenclamide, and control group (negative control) that was left untreated. For all animals, blood glucose level, lipid profile, and histopathology examination were undertaken.

Results: Treatment with 400 mgkg⁻¹ of *C. citratus* extract showed 58.84% decrease in blood glucose level, while the blood glucose level of diabetic rats treated with glibenclamide (500 µg/kg) decreased to 61.19%. A significant decrease (89.5%) was observed in low density lipoprotein-cholesterol (LDL-C) level after treatment with 800 mgkg⁻¹ of *C. citratus* and glibenclamide, while treatment with 400mgkg⁻¹ of *C. citratus* demonstrated a significant increase (35.8%) in high density lipoprotein-cholesterol (LDL-C) level. There was a mild fatty infiltration in glomerulus of rats treated with *C. citratus* (400 mgkg⁻¹) and glibenclamide (500 µg/kg).

Conclusion: *C. citratus* extract illustrated anti-hyperglycemic and anti-lipidemic potentials in diabetic rats with healing effects in kidney, liver and pancreas.

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Introduction

Diabetes mellitus (DM) is still a metabolic disorder in developing countries. Blood and urine have been used to diagnose diabetes; however,

DM is usually characterized by prolonged high glucose level due to inability of the body to secrete enough insulin and/or use insulin maximally that result in hyperglycemia (1). The World Health

Organization reported a prevalence rate of 2% for diabetes in Nigeria (2). An elevated blood sugar level (Hyperglycemia) and increased fatty acid, triglyceride, and low-density lipoprotein concentrations (Dyslipidemia) can be noticed in this metabolic syndrome (3). The global occurrence of DM has been shown to be related to a sedentary lifestyle and obesity (4). Elevated intakes of sugar and saturated fats, as well as poor consumption of poly-unsaturated fatty acids (PUFAs) have been reported to contribute the progression of DM (5, 6). DM by triggering hyperglycemia can lead to atherosclerosis, retinopathy, neuropathy, hypertension, hyperlipidemia and nephropathy (7, 8). The increased risk of DM relies on elements such as environment and lifestyle too (9).

Apart from administration of insulin in management of DM, several other antidiabetic medications were shown to control hyperglycemia (10); even they may have drug related side effects along with their pharmacological properties (11). The metabolism of oral medications happens in organs such as liver, kidney and gastrointestinal tract (12). The non-metabolized content of hypoglycemic medications can also accumulate in the kidney, liver etc. (13). The presence of benzamide moiety in the oral hypoglycemic drugs like glibenclamide can inhibit the activity of K_{ATP} channel in cardiac, skeletal and smooth muscle tissues. As synthetic drugs are usually expensive in management of diabetes, it is necessary for urgent attention to alternative approaches in management of diabetes such as herbal medicine (14). The use of traditional/medicinal plants as a cost-effective therapy in DM has led to their global acceptance in treatment of diabetes (15).

Cymbopogon citratus (Lemongrass) extract was shown to be effective to control blood glucose level and lipid profile in DM. *C. citratus* belongs to the family *Poaceae*. It is a tropical grass that thrives in hot and humid regions including Malaysia, India, America and many African countries. Lemongrass can grow up to 6 inch high. The stem has bulb with terete and glabrous straightly vented sheathed leaves and is consisted of a narrow base and a short apex. The height of the leaves reaches 100 cm and width of 2 cm. The leaves contain indispensable aromatic oils, with yellow and/or amber color in the leaves when squeezed (16). This plant is widely accepted in traditional medicine and its aqueous extract has been introduced as an aromatic drink with a distinct flavor (17).

C. citratus leaves contain many biological active components like 6-methyl-5-hepten-2-one, limonene, myrcene, citral (β -citral (neral) and α -citral (geranial)) that are used in treatment of chronic

metabolic disorders. The indispensable oil content is one of the vital key constituents of *C. citratus* leaves that are applied in production of cosmetics and perfume products. Oral administration of lemongrass extract can lower serum glucose, bilirubin, urea, creatinine, albumin and total protein levels within normal range in alloxan-induced diabetic rats. It can decrease alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) levels in diabetic rats too. Aqueous suspension of *C. citratus* was found to have hypoglycemic effects, and subsequently it can reduce liver and kidney damages caused by alloxan in diabetes rats and restore liver enzyme activities to normal levels. The study determined the effect of aqueous extract of *C. citratus* on blood glucose level, lipid profile and histopathology (liver, kidney and pancreas) of diabetic rats.

Materials and Methods

Fresh leaves of *C. citratus* were collected from an Abuja home garden and identified at the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka, Enugu State, Nigeria. The chemical (alloxan) was used to induce diabetes in male albino Wistar rats purchased from a chemical store in Nsukka, Enugu State, Nigeria. Fifteen animals were purchased from the Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Enugu State, Nigeria. With a little modification of the leaf extraction method developed by Sukhder *et al.* (18), fresh leaves of *C. citratus* were harvested, sorted, washed, drained, and air dried for three days at room temperature and then ground into powder. A total of 150 g of the leaf powder was dissolved in 4500 milliliters (mL) of distilled water and put on a shaker for 30 minutes. The mixture was later allowed to stand for 5 hours and finally drained into a 5 L dry stainless-steel bowl covered with a muslin cloth. The prepared liquid extract was concentrated in a Gallenkamp hot air oven (Size One-Oven BS) at 60°C until a gummy extract was obtained. The gummy extract was then transferred into a sample bottle and stored in refrigerator (Figure 1) until use. A stock of 100 mg/mL of the leaf extract was provided by liquefying one gram (1 g) of the *C. citratus* extract in 10 mL of distilled water. The following formula was used to determine the amount of extract to be administered: Volume of extract solution = $\frac{\text{Dose} \times \text{bodyweight of rats (g)}}{1000 \times \text{Stock solution of extract}}$ (Dose is in mg/mL).

Analysis of the lemongrass extract was conducted to determine the amount of bioactive compounds (alkaloids, flavonoids, terpenoids) in the leaf extract.

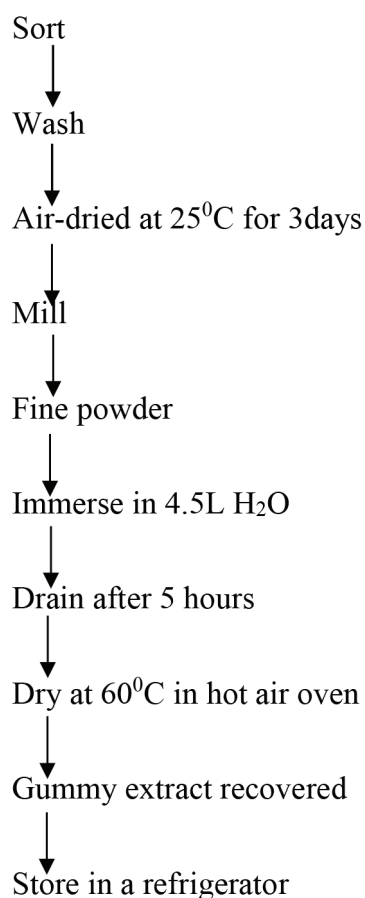
C. citratus (Lemongrass)

Figure 1: Flow chart for extraction of *C. citratus* leaves.

All analyses were carried out in triplicate, in order to validate the findings using Ngounou *et al.*'s method (19). Five gram (5 g) of the pulverized samples were macerated in 20 mL of hexane for 72 hours. The residual was filtered and air-dried, and a later treatment with 10% aqueous ammonia (NH₃) and then maceration in trichloromethane (CHCl₃) for 24 hours. The filtrate was later treated with 7.5 mL of 5% hydrochloric acid (HCl). Aqueous ammonia (NH₃) was used to change the crude extract in alkaline state, and treated with CHCl₃ three times. Distilled water was used to wash the fraction of CHCl₃ and finally the extract was put in a rotatory evaporator.

The solvent was removed from the extract and sodium sulphate was used to dry the concentrate. About 1.0 micro-liter (1 µL) of the concentrate was infused into the carrier gas stream at a flow-control-mode-pressure of 100.0 kPa, a total flow of 17.6 mL/min, a column flow of 1.33 mL/min, a linear velocity of 43.0 cm/sec, a purge flow of 3.0 mL/min, a split ratio of 10.0, an injector temperature of 220°C; and an ion-source temperature of 200°C. The temperature of the oven was set at 100°C for 2 minutes, while increased every 10°C/min, until

reaching 200°C. It was further increased to 5°C/min until reaching 220°C, and ended at 220°C at 9 min isothermal intervals. The mass-spectra were undertaken at 60-70 eV.

The analysis of flavonoids was conducted as described before (20). Briefly, 50 mL distilled water and 2 mL of HCl were added to 5 g of the sample in a conical flask. The solution was subjected to heat for 30 minutes. The heated mixture was cooled and then a Whatman filter paper (No 42) was used to filter the mixture. About 10 mL of ethyl acetate extract was then obtained. It was filtered by Whatman filter paper that was weighed initially, and the remaining part was put in the oven at 60°C to be dried. Cooling was done in a desiccator and the weight was determined. The amount of flavonoid was investigated using the formula of %flavonoid = $\frac{W_2 - W_1}{W \text{ of sample}} \times 100$ (W₁=weight of empty filter paper, W₂=weight of paper+flavonoid extract). One hundred grams (100 g) of the sample was soaked in 9 mL of ethanol for 24 hours as described before (21). The sample was filtered, and then 10 mL of petroleum ether was added in a segregating funnel. The ether was evaporated to obtain its total terpenoid content calculated as $\frac{W_i - W_f}{W_i} \times 100$.

Twenty healthy adult male albino Wistar rats weighing between 126 and 152 g were purchased from the Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria. The rats were randomly assigned to three treated groups and one untreated group, with five rats in each group. The experiment was guided by the National Institutes of Health guidelines for the care and use of laboratory animals. The experimental protocol was designed to adhere to the moral guidelines for the use and care of animals. Faculty of Veterinary Medicine Ethics Committee approved the ethical clearance of experiments. The rats were kept in a plastic cage at 25±2°C with a relative humidity (45%±5%) and 12-hours light and 12-hours dark condition in Ukwuoho, Orba road Nsukka, Nigeria. The rats were given normal pellet diet and water *ad libitum*. The animals were exposed to one-week acclimatization before experiments.

In order to induce diabetes, the rats were kept fasting for 18 hours and then alloxan (Sigma-Aldrich, St Louis, MO, USA) was injected intraperitoneally. The rats were separated into 4 groups of 5 animals each, while their weights were recorded. The dose of 100 mg/kg for alloxan was used as reported earlier (22). A total of 10 mL of distilled water was used to dissolve 1000 mg of alloxan to obtain a 100 mg/mL solution. After 2 days, blood samples were taken from each rat's tail to determine the blood glucose level and to confirm diabetes in rats. The extract was reconstituted by dissolving 1 g of the extract in 10 mL

of distilled water to reach a solution of 100 mg/mL to be used throughout the study. The treatment measure was administered orally via an intra-gastric tube (attached to a syringe) for 21 days. The group 1 was treated with 400 mg/kg of *C. citratus* extract, group 2 with 800 mg/kg of *C. citratus*, group 3 with 500 µg/kg of glibenclamide, and control group (negative control) that was left untreated.

The recto-bulbar plexus was used as the source of blood sample collection. The blood samples were centrifuged at 3000 rpm after being allowed to clot for an hour. Then, the serum was prepared and preserved for later examinations. Accu-Chek glucometer commercial kit was used to assess the fasting blood glucose level. Feeding was restricted from 7:00 pm to 6:00 am in order to determine the blood glucose level. The collected serum was also used to determine the lipid profile as described before (23). Hydrolysis and oxidative enzymatic reaction were employed for cholesterol analysis; while quinoneimine was produced in the presence of phenol peroxidase due to the action of 4-aminoantipyrine and hydrogen peroxidase. Three test tubes were labeled as sample, standard and blank and about 10 µl of serum samples was transferred into a sample test tube, 10 µl of standard specimen was put in a standard test tube, and 10 µL of distilled water was transferred into the blank test tube; while, addition of 1000 µL of cholesterol reagent was added to each of the three test tubes. Each of the samples was mixed thoroughly and incubated at ambient temperature of 20–25°C for about 10 minutes. The absorbance rate was checked for 60 minutes at 500 nm between the samples (A_{sample}) and the blank. Total cholesterol (mg/dL) = Absorbance of sample / Absorbance of standard × 202.65.

LDL-C was measured as LDL-C concentration (mg/dL) = total cholesterol (TC) × (HDL + triglycerides / 5). In the presence of divalent cations, the low and very low-density lipoproteins (LDL and VLDL) were assessed. Also, HDL in the supernatant was measured by addition of 0.1 mL of the precipitant to 0.3 mL of the serum for about 15 minutes. It was centrifuged for 15 minutes at 2000x g and the concentration of the cholesterol in the supernatant was analyzed as described previously (24). HDL-C was evaluated as HDL-C (mg/dL) = Absorbance of sample / Absorbance of standard × 202.65.

The pancreas, kidney and liver tissues were manually processed for histopathological studies after fixing in 10% formalin buffer for 48 hours. The samples were dehydrated in ethanol (70%, 80%, and 90%, respectively) for 90 minutes, and then in 100% absolute ethanol for 90 minutes (two changes). They were later cleared in chloroform overnight followed

by infiltration with paraffin at 60°C for 90 minutes (two changes). Five-micrometer thick sections were prepared for each sample using a rotary microtome (Shandon, Finesse 325, ThermoFisher Scientific, Luton, England). The sections were then floated in a water bath at 40°C to spread and then the egg albumin was used to coat the samples on glass slides. Thereafter, xylene (two changes) was utilized to de-paraffinize the sections for about 15 minutes. Rehydration was done using ethanol (100%, 90%, 80% and 70%, respectively) for 10 minutes and then by using distilled water for 15 minutes. Sections were manually stained for 15 minutes in Harris hematoxylin (HHS16, Millipore Sigma), washed in tap water to 'blue' the nuclei and counterstaining in alcoholic eosin Y (515, 3,801,615; Leica Biosystems Inc., Buffalo Grove, IL, USA) for 5 minutes. Then, dehydration was carried out using ethanol, clearance in xylene, and mounting by cover slipped DPX mountant (C06522, Sigma-Aldrich St. Louis, Missouri, USA) to examine under light microscopy (Bancroft and Gamble, 2008). Photomicrographs of the sections were captured using a Motic Image plus 2.0 digital camera (Motic China Group Ltd. 1999–2004) (25).

To determine the toxicity of the lemongrass leaves and the lethal dose, toxicity (acute) test was utilized as explained before (26). Briefly, eighteen albino mice were used. The test consisted two stages. Stage one included 3 groups of 3 mice each that received 10 mg/kg, 100 mg/kg and 1000 mg/kg of the extract, respectively. Stage two covered 3 groups of 3 mice each that received 1600 mg/kg, 2900 mg/kg and 5000 mg/kg of the extract, respectively. Table 1 shows stage I and stage II of the toxicity test (LD_{50}) of the aqueous extract. Data were presented as mean and standard deviation (mean ± SD). The data on phytochemicals of the aqueous extract of the leaf was analyzed using one-way analysis of variance (ANOVA). Paired sampled t-test was applied to analyze the baseline and end-line data for blood glucose level and lipid profile. The IBM-SPSS software (Version 23, Chicago, IL, USA) was used for statistical analysis. Statistical difference and/or significance were set at $p < 0.05$, while percentage difference was also calculated.

Results

No mortality was recorded in both stages of the experiments. Figure 2 demonstrates the amount of bioactive compounds in aqueous extract of *C. citratus*. The quantity of flavonoids found in the extract was 1.37 mg/100 g, alkaloids of 0.43 mg/100 g, and terpenoids of 0.06 mg/100 g. Table 2 illustrates the mean blood glucose level of the treated diabetic rats after 21 days.

Table 1: Aqueous extract of lemongrass lethal dosage.

Stage/Group	Dose (mg/kg)	Mortality
Stage I		
Group 1	10	0.3
Group 2	100	0.3
Group 3	1000	0.3
Stage II		
Group 1	1600	0.3
Group 2	2900	0.3
Group 3	5000	0.3

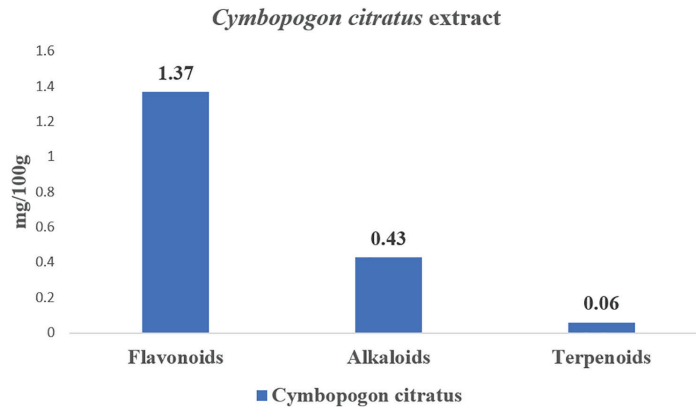


Figure 2: Phytochemical (bioactive) component of aqueous extract of *C. citratus* administered in hyperglycemic rats.

Table 2: Mean initial and final blood glucose levels of treated diabetic rats with aqueous extract of *C. citratus*.

Treatment	Initial	Final	MD	Std error	P value	%D
<i>C. citratus</i> (400 mg/kg)	278.20±25.16	114.50±18.29	163±50.67	16.24	0.002	58.8
<i>C. citratus</i> (800 mg/kg)	268.80±21.46	136.80±99.83	132.0±87.30	39.04	0.028	49.11
Glibenclamide (500 µg/kg)	293.20±24.01	113.80±9.76	179.40±29.91	13.38	0.0001	61.19
Untreated group	259.60±21.51	265.67±41.86	6.07 ±39.95	23.07	0.055	2.34

MD=mean difference; %D=percentage difference.

Table 3: Mean initial and final total cholesterol levels in diabetic rats treated with aqueous extract of *C. citratus*.

Treatment	Initial	Final	MD	Std error	P value	%D
<i>C. citratus</i> (400 mg/kg)	66.84±20.56	43.38±15.64	23.46±8.36	3.74	0.003	35.1
<i>C. citratus</i> (800 mg/kg)	54.56±4.66	28.76±6.01	25.80±5.21	2.33	0.0001	47.3
Glibenclamide (500 µg/kg)	65.00±7.72	39.40±7.52	25.60±6.11	2.73	0.001	39.4
Untreated group	48.11±0.14	54.83±0.26	6.71±0.21	0.10	0.000	13.95

MD=mean difference; %D=percentage difference.

The initial blood glucose level after diabetic induction ranged from 268.80 mg/dL to 293.20 mg/dL. A significant reduction ($p<0.05$) was observed for initial blood glucose level (278.20 mg/dL) and the final blood glucose level (114.50 mg/dL) after treatment with 400 mg/kg of aqueous extract of *C. citratus* revealing 58.8% decrease in the blood glucose level in treated diabetic rats with the mean difference of 163.70 mg/dL.

There was also a significant difference ($p<0.05$) between the initial blood glucose and final blood glucose levels (268.80 mg/dL and 136.80 mg/dL, respectively) in diabetic rats after treatment with 800 mg/kg of aqueous extract of *C. citratus* revealing

49.11% reduction in blood glucose level, with a mean difference of 132.0 mg/dL. Treatment with standard antidiabetic drug of glibenclamide at a dose of 500 µ/kg showed a significant difference ($p<0.05$) between the mean initial blood glucose level (293.20 mg/dL) and the mean final blood glucose level (113.80 mg/dL) in treated diabetic rats indicating 61.19% decline in blood glucose level with the mean difference of 179.40 mg/dL. There was no significant difference ($p>0.05$) between the mean initial blood glucose level (259.60 mg/dL) and mean final blood glucose level (265.67 mg/dL) of untreated diabetic rats, with a mean difference of 6.07 mg/dL and 2.34% increase in the blood glucose level.

Table 3 displays a significant difference ($p<0.05$) between the initial mean values (66.84 mg/dL) and final mean values (43.38 mg/dL) of the total cholesterol (TC) level in diabetic rats after treatment with 400 mg/kg of aqueous extract of *C. citratus* demonstrating 35.1% decrease and a 23.46 mg/dL mean difference in the TC level. Treatment with same aqueous extract (800 mg/kg) showed a significant difference ($p<0.05$) between the initial (54.56 mg/dL) and final (28.76 mg/dL) mean levels of TC in treated rats, with mean difference of 25.80 mg/dL, and a 47.3% decrease in the TC level. Treatment with glibenclamide at a dose of 500 µg/kg showed 39.4% decrease in the TC level in treated rats. There was a significant difference ($p<0.05$) between initial (65.00 mg/dL) and final (39.40 mg/dL) mean TC levels, with the mean difference of 25.60 mg/dL. Also, a significant ($p<0.05$) 13.95% increase was observed in TC level of the untreated diabetic rats.

Table 4 shows a significant difference ($p<0.05$) between the initial and final (37.50 mg/dL and 4.90 mg/dL, respectively) levels of LDL-C with 32.60 mg/dL mean difference after treatment with 400 mg/kg of aqueous extract of *C. citratus*. There was 86.9% decrease in the LDL-C level in treated diabetic rats. Treatment with 800 mg/kg of aqueous extract of *C. citratus* resulted in a significant difference ($p<0.05$) between initial mean level (33.40 mg/dL) and final mean level (3.52 mg/dL) of LDL-C in treated rats, with a mean difference of 29.88 mg/dL. Thus, there was 89.5% decrease in the LDL-C level. Treatment with glibenclamide at a dose of 500 µg/kg caused 89.5% decrease in the LDL-C level in treated rats. There was a significant difference ($p<0.05$) between the initial mean level (34.76 mg/dL) and final mean level (3.52 mg/dL) of LDL-C in treated rats with 31.12 mg/dL mean difference. Also,

a significant ($p<0.05$) 2.92% increase in the LDL-C level was noticed in untreated diabetic rats.

Table 5 shows a significant difference ($p<0.05$) between initial HDL-C level (28.34 mg/dL) and final HDL-C level (38.48 mg/dL) with the mean difference of 10.14 mg/dL after treatment with 400 mg/kg of aqueous extract of *C. citratus* revealing 35.8% increase in the HDL-C level. Treatment with 800 mg/kg of aqueous extract of *C. citratus* showed 19.3% increase in the HDL-C level, with a significant difference ($p<0.05$) between the initial and final HDL-C levels (21.16 mg/dL and 25.24 mg/dL, respectively) in treated diabetic rats. A 4.09 mg/dL mean difference was visible. Treatment with glibenclamide at a dose of 500 µg/kg led to a significant difference ($p<0.05$) in the initial mean level (30.24 mg/dL) and final mean level (35.76 mg/dL) of the HDL-C with a mean difference of 5.52 mg/dL, and 18.3% increase in the HDL-C level. There was a significant ($p<0.05$) 32.33% decrease in HDL-C level in untreated diabetic rats.

Figure 3 shows the histopathology findings in treated diabetic rats. The microscopic image of the liver in diabetic rats treated with 400 mg/kg and 800 mg/kg of aqueous extract of *C. citratus* demonstrated a mild vacuolar degeneration in hepatocytes (black arrow), while treatment with glibenclamide at a dose of 500 µg/kg revealed a mild congestion in central vein (white arrow) in diabetic rats, and vacuolar degeneration in untreated diabetic rats. In kidneys of diabetic rats treated with 400 mg/kg and 800 mg/kg of *C. citratus* and glibenclamide at a dose of 500 µg/kg, there was evidence of mild fatty infiltration in glomerulus (white arrow), while in untreated diabetic rats, tubular degeneration was found. Regarding pancreas, lymphocytic infiltration (black arrow) was

Table 4: Mean initial and final low density lipoprotein cholesterol (LDL-C) levels in diabetic rats treated with aqueous extract of *C. citratus*.

Treatment	Initial	Final	MD	Std error	P value	%D
<i>C. citratus</i> (400 mg/kg)	37.50±15.55	4.90±2.53	32.60±14.61	6.53	0.008	86.9
<i>C. citratus</i> (800 mg/kg)	33.40±5.13	3.52±2.11	29.88±3.96	1.77	0.0001	89.5
Glibenclamide (500 µg/kg)	34.76±5.13	3.52±2.11	31.12±4.30	1.92	0.0001	89.5
Untreated group	29.11±0.12	29.97±0.06	0.85±0.14	0.06	0.0001	2.92

MD=mean difference; %D=percentage difference.

Table 5: Mean initial and final high density lipoprotein cholesterol (HDL-C) level in diabetic rats treated with aqueous extract of *C. citratus*.

Treatment	Initial	Final	MD	Std error	P value	%D
<i>C. citratus</i> (400 mg/kg)	28.34±13.69	38.48±15.56	10.14±5.74	2.57	0.017	35.8
<i>C. citratus</i> (800 mg/kg)	21.16±5.48	25.24±6.49	4.09±2.09	0.94	0.012	19.30
Glibenclamide (500 µg/kg)	30.24±6.09	35.76±6.60	5.52±1.92	0.86	0.003	18.30
Untreated group	22.18±0.29	15.01±0.02	7.17±0.30	0.13	0.0001	32.33

MD=mean difference; %D=percentage difference.

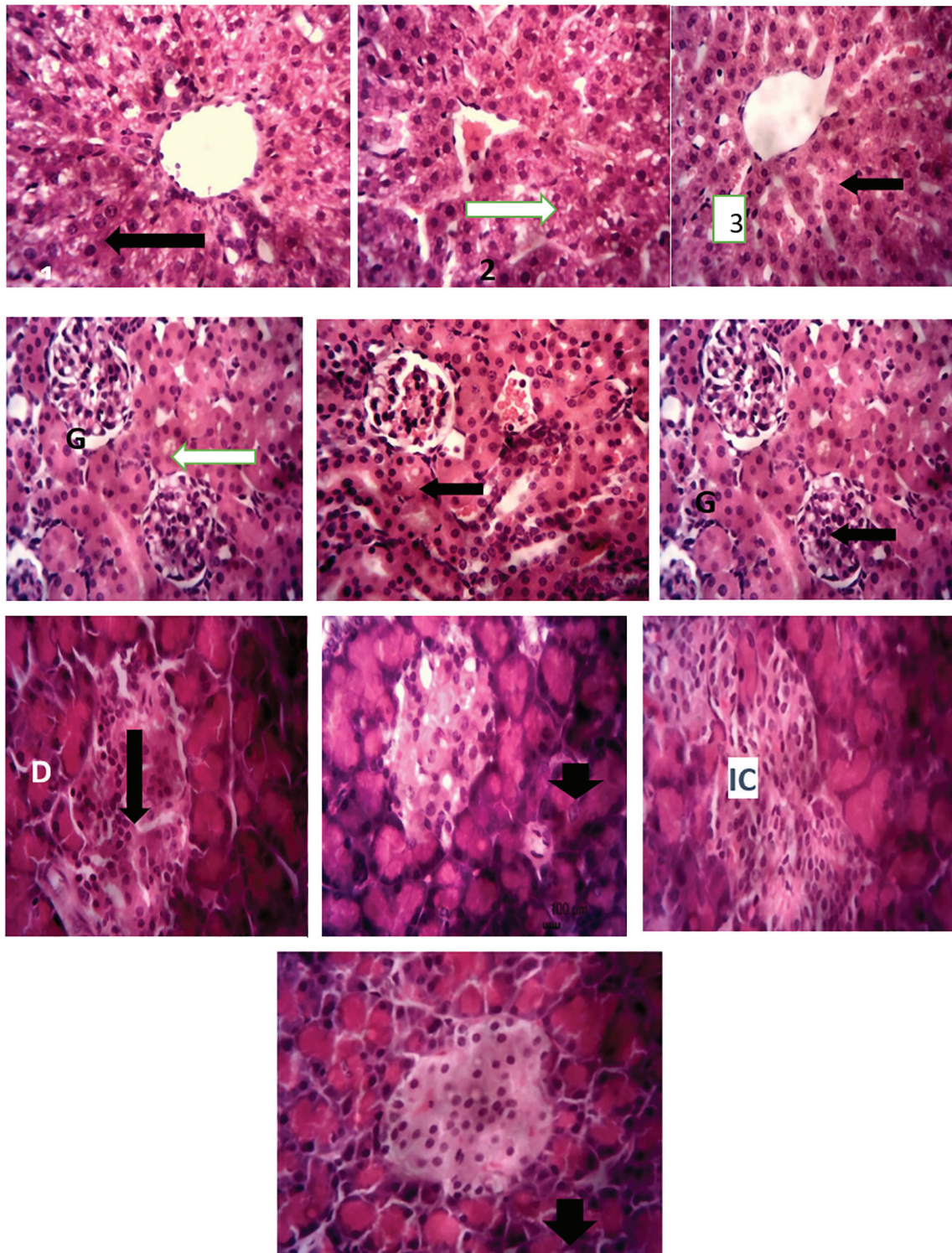


Figure 3: Liver. 1 (*C. citratus* as 400 mg/kg and 800 mg/kg); 2 (Standard drug of glibenclamide as 500 µg/kg); 3 (untreated diabetic rats); Kidney. White arrow (*C. citratus* as 400 mg/kg and 800 mg/kg, standard drug of glibenclamide as 500 µg/kg); black arrow (*C. citratus* as 400 mg/kg and 800 mg/kg, standard drug of glibenclamide as 500 µg/kg); D–black arrow (untreated diabetic rats); Pancreas: Black arrow (standard drug of glibenclamide as 500 µg/kg); arrow head (*C. citratus* as 400 mg/kg); IC (*C. citratus* as 800 mg/kg) arrow head with white rectangle (untreated diabetic rats).

seen in rats treated with glibenclamide at a dose of 500 µg/kg; while there was a mild cellular depletion (black arrowhead) in rats treated with 400 mg/kg of *C. citratus*. However, diabetic rats treated with 800 mg/kg of aqueous extract of *C. citratus* showed no cellular depletion, while in untreated diabetic rats, cellular depletion was visible.

Discussion

It was shown that the aqueous extract of *C. citratus* contained certain quantities of flavonoids, alkaloids, and terpenoids which served as the phytochemical components and/or bioactive elements in the extract. These bioactive compounds can play an important role in addressing various clinical issues

and health concerns (27). The pathogenesis of diabetes can be due to the oxidation process (27). The alkaloid content of the extract was shown not to be in agreement with the dose of 7.20 mg/100 kg reported before (28). Alkaloids were shown to be very useful in medicine and production of several valuable drugs (29). The presence of alkaloids in the aqueous extract of *C. citratus* can control diabetes, and invariably act as an antioxidant and analgesic. Previous studies revealed that alkaloids found in various species of plant could control hyperglycemia (27, 30).

In an *in vivo* study, there was alkaloid-inhibited phosphorylase activity that could stimulate the basal glucose uptake in rats' adipocytes (31). Alkaloids can mediate the pathway of insulin signal transduction and reverse molecular defects that cause glucose intolerance and insulin resistance and reduce diabetes complications. Furthermore, consumption of the extract can impact managing of diabetes induced by oxidative stress and have hypoglycemic effects by increasing insulin secretion based on presence of flavonoids. The flavonoid content in our study is in line with a previous study (2.18 mg/100 g) (28). Therefore, oral administration of the aqueous extract of *C. citratus* had the potential to prevent many cardiovascular diseases, including hypertension and atherosclerosis. A previous study revealed the positive effect of flavonoids in increasing insulin secretion, as well as alleviating beta-cell apoptosis and modulation of its proliferation. The content of terpenoids in the aqueous extract of *C. citratus* can be helpful to control diabetes as a disorder of metabolism caused by several multifaceted factors. It was depicted by a prolonged high blood glucose level (diabetes) with upsets in the metabolism of proteins, fats and carbohydrates due to disturbances in insulin secretion.

After alloxan induction of diabetes, ablation and disruption of pancreatic beta-cells happened that resulted in an increased glucose level and/or changes in expression of genes in relation to insulin secretion. Blood glucose level is controlled by insulin secretion from pancreatic cells and apoptosis in pancreatic cells can lead to diabetes (32). Alloxan can hinder secretion of insulin, impact formation of reactive oxygen species, and lead to beta cells necrosis through inhibition of glucokinase activity and give rise to type 1 diabetes (33). In our study, alloxan was used to induce diabetes similar to Husni *et al.*'s study (32). A significant reduction in blood glucose level was observed when 400 mg/kg and 800 mg/kg of aqueous extracts of *C. citratus* were administered. This hypoglycemic effect is indicative of the presence of many bioactive components in *C. citratus* such as flavonoids, alkaloids and terpenoids

with antioxidant properties. These compounds can contribute stimulation of glucose transport and inhibit the activity of adipocyte differentiation, thereby, inducing translocation of GLUT-4. This hypoglycemic effect of aqueous extract of *C. citratus* is in agreement with other researchers too (34).

Lipid profile of our study in the treated rats with aqueous extract of *C. citratus* showed a dose-dependent decrease for TC and LDL-C levels, and a significant rise for HDL-C level that can be due to presence of many components in *C. citratus* such as flavonoids, alkaloids and terpenoids leading to a significant decline in TC and LDL-C levels and an increase in HDL-C level of alloxan-induced diabetic rats treated with *C. citratus*. Hypercholesterolemia was demonstrated to play an important role in initiation and progression of atherosclerosis that has a positive correlation with cardiovascular diseases. Our findings are in agreement with the results of others on the effect of *C. citratus* extract in treatment of diabetic rats and a decreased incidence of cardiovascular disorders (35, 36). However, our results were different from findings of other researchers whereby, the level of LDL-C was higher than HDL-C level in diabetic rats that received the extract (37).

In the present study, there was mild vacuolar degeneration of hepatocytes and congestion of the central vein, which indicate a partial damage caused by alloxan to induce diabetes as alloxan destroys the beta cells in the islets of Langerhans and reduces the amount of insulin secretion and leads to diabetes (32). Alloxan induces diabetes and causes metabolic derangement of the liver and alteration in liver enzymes (32). It was shown that *Carissa spinarum* leaf extract in alloxan-induced diabetes (38) and *Vinca rosea* extract in streptozotocin-induced diabetes results in liver and pancreatic damages. At the end of our treatment protocol, a mild fatty infiltration in the glomerulus and mild tubular degeneration in renal tubules were noticed that suggest the *C. citratus* potential as an anti-diabetic drug similar to glibenclamide at dose of 500 µg/kg. The presence of phytochemicals and/or other antioxidants in the aqueous extract of *C. citratus* can explain its healing effect. Similar outcomes were found in treatment of alloxan-induced diabetes with *Carissa spinarum* (38). Alloxan was shown to disrupt the formation of microtubules as well as destroying those already formed. The results of our pancreatic histopathology revealed lymphocytic infiltration after treatment with glibenclamide when compared to the aqueous extract of *C. citratus*. This lymphocytic infiltration can be as a result of inflammation in beta-cells of the pancreas. A mild cellular depletion was observed

in diabetic rats treated with *C. citratus* at a dose 400 mg/kg that indicates the healing effect of this aqueous extract in the pancreas. However, cellular depletion was not visible in diabetic rats treated with aqueous extract of *C. citratus* at a dose 800 mg/kg) which suggests the potential of higher doses of the extract in treatment of diabetes similar to other studies (38, 39).

Conclusion

With the presence of the bioactive compounds in the aqueous extract of *C. citratus*, it can resist the formation of diabetes and hyperlipidemia. This extract was shown to play an important role in management of alloxan-induced diabetes by mitigating the elevated blood glucose, cholesterol and low-density lipoprotein levels. Furthermore, this aqueous extract had the capacity to recover and regenerate the inflamed liver, kidney and pancreas.

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Authors' Contribution

Ngozi M. Nnam: Conception/design of the work; interpretation of data for the work; and Final approval of the version to be published. Ijioma J. Okorie: Conception/design of the work; Acquisition, analysis and interpretation of data for the work; Drafting of the work; Final approval of the version to be published. Adaeze C. Okorie: Acquisition, analysis and interpretation of data for the work; Drafting of the work; Data collection; Final approval of the version to be published.

Conflict of Interest

None declared.

References

- Masoumi SJ, Nekooeian AA, Tanideh N, et al. Effect of allium porrum on streptozotocin-induced diabetes mellitus hyperglycemia and insulin resistance in male Sprague Dawley rats. *Onl J Vet Res.* 2020;24:573-7.
- World Health Organization (WHO). Non-communicable diseases Country profile. Geneva, 2014.p.1–207.
- Mamun A, Islam S, Alam AK, et al. Effects of ethanolic extract of *Hibiscus rosa-sinensis* leaves on alloxan-induced diabetes with dyslipidemia in rats. *Bangladesh Pharmaceutical J.* 2013;16:27–31.
- Tan KS, Lee KO, Low KC. Glutathione deficiency in type 2 diabetes impairs cytokine responses and control of intracellular bacteria. *J Clin Invest.* 2012;122:2289–300. DOI: 10.1172/JCI57817. PMID: 22546856.
- Asgari Q, Motazedian MH, Khazanchin A, et al. High prevalence of toxoplasma gondii infection in type I diabetic patients. *J Parasitol Res.* 2021;2021:8881908. DOI: 10.1155/2021/8881908. PMID: 33628471.
- Hosseini SE, Rezaei E, Tavakoli F, et al. Effect of pomegranate juice on lipid profile in streptozotocin-induced diabetic adult male rats. *J Exp Anim Biol.* 2013;2:13-20.
- Tan KS, Lee KO, Low KC. Glutathione deficiency in type 2 diabetes impairs cytokine responses and control of intracellular bacteria. *J Clin Invest.* 2012;122: 2289–300. DOI: 10.1172/JCI57817. PMID: 22546856.
- Hosseini SE, Mehrabani D, Rezaei E. Effects of pomegranate juice on liver enzymes (ALT, ALP, AST) in diabetic and non-diabetic rats. *J Anim Physiol Develop.* 2014;24:59-64.
- Elbandy MA, Ashoush IS. Phytochemicals in pomegranate seeds and their effect as hypolipidemic agent in hypercholesterolemic rats. *World J Dairy Food Sci.* 2012;7:85-92.
- Sher A, Fakhar-ul-Mahmood M, Shah SN, et al. Effect of garlic extract on blood glucose level and lipid profile in normal and alloxan. *Adv Clin Exp Med.* 2012;21:705-11. PMID: 23457127.
- Pandarekandy ST, Sreejesh PG, Thampi-Harikumaran BS, et al. Hypoglycaemic effect of glibenclamide: A critical study on the basis of creatinine and lipid peroxidation status of streptozotocin-induced diabetic rat. *Indian J Pharmaceutical Sci.* 2017;79:768–777.
- Abdelmoneim AS, Hasenbank SE, Seubert JM, et al. Variations in tissue selectivity amongst insulin secretagogues: a systematic review. *Diabetes Obes Metab.* 2012;14:130-8. DOI: 10.1111/j.1463-1326.2011.01496.x. PMID: 21923736.
- Arnouts P, Bolignano D, Nistor I, et al. Glucose-lowering drugs in patients with chronic kidney disease: A narrative review on pharmacokinetic properties. *Nephrol Dial Transplant.* 2014; 29:1284-300. DOI: 10.1093/ndt/gft462. PMID: 24322578.
- Saravanan R, Pari L. Antihyperlipidemic and antiperoxidative effect of Diasulin, a polyherbal formulation in alloxan induced hyperglycemic rats. *BMC Complement Altern Med.* 2005;5:14. DOI: 10.1186/1472-6882-5-14. PMID: 15969768.
- Gao D. Antioxidant Therapies for Hypolipidemia and Hyperglycemia. 2012. DOI:10.5772/47432.
- Adejuwon AA, Esther OA. Hypoglycemic and hypolipidemic effects of fresh leaf aqueous

- extract of *Cymbopogon citratus* Stapf in rats. *J Ethnopharmacol.* 2007;112:440-4. DOI: 10.1016/j.jep.2007.03.034. PMID: 17513076.
- 17 Figueirinha A, Paranhos A, Perez-Alonso JJ, et al. *Cymbopogon citratus* leaves. Characterisation of flavonoids by HPLC-PDA-ESI/MS and an approach to their potential as a source of bioactive polyphenols. *Food Chem.* 2008;110: 718-728.
 - 18 Sukhdev SH, Suman PSK, Gennaro L, et al. Extraction technologies for medicinal and aromatic plants. 2008.p.22.
 - 19 Ngounou FN, Manfouo RN, Tapondjou LA, et al. Antimicrobial Diterpenoid Alkaloids from *Erythrophleum Suaveolens* (Guill and Perr.) Brenan. *Chem Society Ethiopia.* 2005;221-226.
 - 20 Onwuka GI. *Food Analysis and Instrumentation, Theory and Practice.* 2005;5:1333-1334.
 - 21 Indumathi C, Durgadevi G, Nithyavani S, et al. Estimation of terpenoid content and its antimicrobial property in *Enicostemma litorale*. *Int J Chem Tech Res.* 2014;6:4264-4267.
 - 22 Okorie I, Adesanmi RA, Nnam N. Blood glucose and lipid profile of Alloxin- induced hyperglycemic Albino Rats treated with aqueous extract of *Allium Sativum* leaf. *World J Pharmaceutic Res.* 2021;10:25-34.
 - 23 Abell LL, Levy BB, Brodie BB, et al. A semi-automated procedure for the determination of serum cholesterol. *J Biological Chem.* 1952;195:357-366.
 - 24 Kameswara RB, Kesavulu MM, Giri R, et al. Antidiabetic and hpyolipidemic effects of *Momordica cymbalaria* Hook. Fruit powder in alloxandiabetic rats. *J Ethanopharmacol.* 1999;67:103-109.
 - 25 Strate T. Microcirculatory function and tissue damage is improved after the repeated injection of bovine haemoglobin in severe acute rodent pancreatitis. *Pancreas.* 2005;30:254-259. DOI: 10.1097/01.mpa.0000157481.22155.2d. PMID: 15782104.
 - 26 Lorke D. A new approach to practical acute toxicity testing. *Arch Toxicol.* 1983;54:275-287.
 - 27 Ojiako OA, Chikezie PC, Ogbuji AC. Blood glucose level and lipid profile of alloxan-induced hyperglycemic rats treated with single and combinatorial herbal formulations. *J Tradit Complement Med.* 2016;6:184-192. DOI: 10.1016/j.jtcme.2014.12.005. PMID: 27114943.
 - 28 Muhammad A, Ibrahim IS. Phytochemical screening and proximate analysis of garlic (*Allium sativum*). *Archive Organic Inorganic Chem Sci.* 2019. Lupinepublishers.com/chemistry-journal/pdf/AOICS.MS.ID.000180.pdf
 - 29 Dalhat MH, Adefolake FA, Musa M. Nutritional composition and phytochemical analysis of aqueous extract of *Allium cepa* (onion) and *Allium sativum* (garlic). *Asian Food Sci Jo.* 2018;3:1-9.
 - 30 Prabhakar PK, Doble M. A target based therapeutic approach towards diabetes mellitus using medicinal plants. *Curr Diabetes Rev.* 2008;4:291-308. DOI: 10.2174/157339908786241124. PMID: 18991598.
 - 31 Christodoulou MI, Tchoumtchoua J, Skaltsounis AL, et al. Natural alkaloids intervening the insulin pathway: New hopes for anti-diabetic agents. *Curr Med Chem.* 2019;26:5982-6015. DOI: 10.2174/0929867325666180430152618. PMID: 29714135.
 - 32 Husni A, Purwanti D, Ustadi. Blood glucose level and lipid profile of alinate from *Sargassum crassifilium*. *J Biologic Sci.* 2016;16:58-64.
 - 33 Lenzen S. The mechanisms of alloxan and streptozotocin-induced diabetes. *Diabetologia.* 2008;51:216-26. DOI: 10.1007/s00125-007-0886-7. PMID: 18087688.
 - 34 Ademuyiwa AJ, Elliot SO, Olamide OY, et al. The Effects of *Cymbopogon citratus* (Lemon grass) on the Blood Sugar Level, Lipid Profiles and Hormonal Profiles of Wistar Albino Rats. *Merit Res J Med Med Sci.* 2015;3:210-216.
 - 35 Adeyemi OS, Akanji MA. Iron and nitric oxide play key role in the development of cardiovascular disorder. *J Toxicol Environ Health Sci.* 2011;3:249-253.
 - 36 Sekh AN, Bhupinder D, Rashmi K, et al. Alliin obtained from leaf extract of garlic grown in situ conditions possess higher therapeutic potential analyzed in alloxan-induced diabetic rats. *Pharm Biol.* 2011;49:416-421. DOI: 10.3109/13880209.2010.521163 PMID: 21391887.
 - 37 Adeyemi OS, Orekoya BT. Lipid profile and oxidative stress makers in Wistar rats following oral and repeated exposure to Fijk herbal mixture. *J Toxicol.* 2014;2014:876035. DOI: 10.1155/2014/876035. PMID: 25386188.
 - 38 Sharma S, Rana A. Histopathological alterations in alloxan induced diabetic mice liver and kidney after *Carissa spinarum* methanolic leaf extract treatment. *Int J Pharm Sciences Res.* 2020;11:1777-1783.
 - 39 Jannu V, Vishal DS, Babu VR, et al. Antidiabetic activity of hydro-alcoholic extract of *Cissampelos pareira* leaves in streptozocin induced diabetic rats. *Int J Pharm Tech.* 2011;3:3601-11