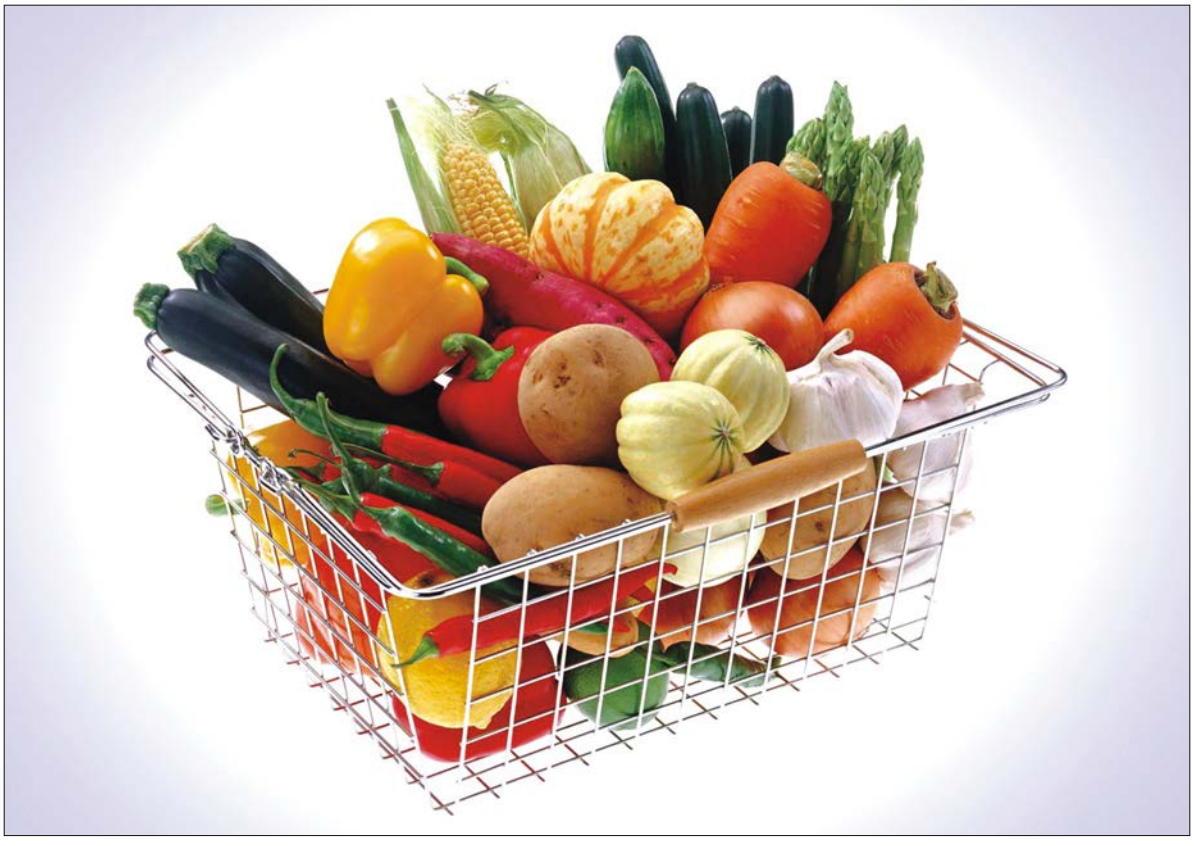

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Research Article
Effect of Mixed Aqueous Extracts of *Allium sativum*, *Annona muricata* and *Cymbopogon citratus* Leaves on the Blood Glucose and Lipid Profile of Hyperglycemic Rats

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Abstract

Background and Objective: The dilapidating action of diabetes mellitus makes it a disease of major public health importance. The study was on the effect of mixed aqueous extracts of *A. sativum*, *A. muricata* and *C. citratus*, leaves on the blood glucose and lipid profile of hyperglycemic rats. **Materials and Methods:** The study adopted an experimental study design where 15 adult male Albino rats were assigned randomly to 2 treatment groups and 1 non-treatment group, 5 rats in each group. The leaves were harvested and processed into aqueous extract using the standard method. About 1 g each of the extracts was dissolved in 10 mL of distilled water to make a stock of 100 mg mL^{-1} of extract. Alloxan was used to induce hyperglycemia in rats. Treatment commenced once diabetes was established and lasted for 21 days. Blood glucose levels and lipid profiles were evaluated using standard methods. Statistical tool (ANOVA and DMRT) was carried out, while percentage difference was used to determine study effect. **Results:** The result revealed that the initial glucose level of the rats ranged from 57.80-69.60 mg dL^{-1}, their blood glucose level after induction ranged from 253.20-293.20 mg dL^{-1}. There was 14.93, 19.19 and 25.43% fall on days 7, 14 and 21, respectively, in the blood glucose levels of the rats after treatment with mixed aqueous extracts of the leaves, while the antidiabetic drug showed a 15.14, 25.17 and 61.19% decline on same days. The LDL level showed an 80.4% fall, while the was a 28.8% increase in the HDL level of the rats treated mixed aqueous extracts of the leaves. **Conclusion:** Mixed aqueous extracts of the leaves thus, is useful in anti-hyperglycemic and anti-hyperlipidemic activity as observed in the study and then could be used in managing type 2 diabetes mellitus.

Key words: Mixed-aqueous-extract, leaves, blood glucose, lipid profile, alloxan-induced, hyperglycemic-rats

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The search for a cost-effective strategy using therapeutic and/or medicinal plants in the mitigation of hyperglycemia has continued, due to its effect and associated comorbidities in the human body. Diabetes mellitus especially type 2 diabetes has become rampart in African countries especially in Sub-Saharan Africa. The number of adults approximated to be diabetic in Sub-Saharan Africa (SSA) was 15.5 M in the year 2017, identifying a regional prevalence at 6% and the cost of healthcare rose to USD 3.3 billion. The projection showed that by the year 2045, there expected about 162.5%, totalling up to 40.7 M type 2 diabetic adults, with costs further raised to USD 6 billion[1]. The incidence of hyperglycemia and often leading to hyperlipidemia, have increased markedly in the past decades as a result of obesity, inactivity and ageing population, with a high risk of vascular comorbidities including coronary artery disease and physical inactivity and impaired cognitive function and mortality[2]. Diabetes mellitus has been reported to be a chronic disease because of inherited autoimmune and/or acquired inadequate pancreatic insulin production and insulin resistance[3]. Hyperglycemia and glucosuria were the commonest and earliest indices used to diagnose diabetes mellitus. Therefore, the abnormal metabolism of carbohydrates in diabetes and characteristic glycolytic pathway profound adjustment leads to activating alternative metabolism in polyol pathways, resulting in the accumulation of intracellular sorbitol[4]. These alterations in metabolism have been indicated in the aetiology of diabetic peripheral neuropathy, cataracts and retinopathy[5,6].

Therefore, the treatment, cure and/or management of type 2 diabetes mellitus have led to the investigation of the medicinal and therapeutic potentials of plant origin that will aid in cost-effective strategy. Several studies on individual plants have reported significant effects in lowering high blood glucose levels, total cholesterol, triglyceride and low-density lipoprotein levels in both alloxan and streptozotocin-induced diabetic rats. A study by Okorie *et al.*[2] showed a relative decline in the blood glucose levels of treated diabetic rats from 303.20 mg dL^{-1} after induction to 210.80 mg dL^{-1} after 21days treatment with aqueous extract of *Allium sativum* leaves at dose 400 mg kg^{-1} b.wt. A significant decrease was observed in the serum lipid profile in treated diabetic rats after treatment with aqueous extract of garlic leaf, except for high-density lipoprotein cholesterol which had a significant increase[7]. Sawant and Dongre[8] reported that nutrients in *A. muricata* leaves were seen to stabilize and/or normalize blood glucose levels to the normal range that is useful in managing diabetes mellitus. Adeyemi *et al.*[9] reported a significant decrease in the total cholesterol, triglyceride, low-density lipoprotein and very-low-density lipoprotein after treatment with extract of *Annona muricata* leaves at 100 mg kg^{-1}. Aqueous suspension of lemongrass was found to have an anti-hyperglycemic effect by decreasing blood glucose level, reducing the liver and renal damage due to alloxan-induced diabetes and thus, could restore activities of certain liver enzymes to normal level[10].

However, there exists little or no information on the mixed and/or combination of 2 or more individual plants extracts (both aqueous and ethanol) and the effect on hyperglycemia and hyperlipidemia. Thus, the effect of mixed *A. sativum* leaves, *A. muricata* leaves and *C. citratus* leaves on the blood glucose and lipid profile of hyperglycemic rats was evaluated.

MATERIALS AND METHODS

Study area: The study was carried out from February to March, 2021 (22/02/2021 to 21/03/2021), approximately 28 days of rat study.

Study design: Experimental study design was used to investigate the effect of aqueous extracts of *C. citratus*, *A. sativum* and *A. muricata* leave on the blood glucose and lipid profile of hyperglycemic rats. In this study design, 15 adult male Albino rats were assigned completely at random to 2 different treatment groups and 1 non-treatment group, making 5 rats in each group. However, each experimental unit had the same chance of receiving treatment as those in treatment groups. The animals (rats) were weighed individually and the ones with similar body weight were put in the same experimental unit homogenously. The 3 different groups were diabetic groups, where one group, was treated with mixed aqueous extracts of *C. citratus*, *A. sativum* and *A. muricata* leaves, another group was treated with antidiabetic drugs (Glibenclamide) and the last group received distilled water. The administration was done orally with the aid of an intra-gastric tube attached to a syringe for a period of 21 days after induction.

Experimental materials: Fresh leaves of *C. citratus*, *A. sativum* and *A. muricata* were harvested from a home garden in Abuja and identified in the Department of Plant Science and Biotechnology, University of Nigeria Nsukka, Enugu State, Nigeria. Fifteen male albino Wistar rats were purchased from the Faculty of Veterinary Medicine, University of Nigeria, Nsukka, while the chemical (alloxan) that was used to induce diabetes in the rats was purchased from a chemical shop in Nsukka, Enugu State, Nigeria.

Preparation of the plant materials: The method described by Sukhdev *et al.*[11] was adopted for the extraction of the leaves with little modification. Freshly harvested *C. citratus*, *A. sativum* and *A. muricata* leaves were sorted, washed and air-dried at ambient temperature for 3 days. The leaves were chopped into small pieces and then milled to fine powder, ready for further treatment. One hundred and fifty grams each of the powdered leaves were immersed in 4500 mL of distilled water and agitated on a mechanical shaker for 30 min. The mixtures were allowed to stand for 5 hrs and then drained using a muslin cloth into a 5 L dry stainless steel bowl. The liquid extracts were concentrated to dryness in a Gallenkamp hot air oven (Size One-Oven BS) at 60°C until a gummy extract was obtained. The gummy extracts (concentrates) were then recovered into a sample bottle and stored in the refrigerator until use for further analysis and study. One gram each of the extracts (*C. citratus*, *A. sativum* and *A. muricata*) was dissolved in 10 mL of distilled water to make a stock of 100 mg mL^{-1} of extract. The volume of extract to be administered will be determined using the formula:

$$\text{Volume of extract solution} = \frac{\text{Dose} \times \text{body weight of rats (g)}}{1000 \times \text{stock solution of extract}} \left(\text{Dose is in mg mL}^{-1}\right)$$

Acute toxicity test of aqueous extracts of *C. citratus*, *A. sativum* and *A. muricata* leaves: This was done to evaluate the toxicity of the various leaves and their lethal dose to be used for the study. The method cited by Okorie *et al.*[2] was adopted for the acute toxicity test of the aqueous extracts of *C. citratus*, *A. sativum* and *A. muricata* leave. Fifty-four Albino mice were used in the toxicity study, giving 18 mice for each sample. The test involved 2 stages. In stage one, the animals were grouped into 3 groups of three mice each and were given 10, 100 and 1000 mg kg^{-1} body weight of the extracts, respectively. In stage two, 1600, 2900 and 5000 mg kg^{-1} body of the weight of the extract was administered to the mice, respectively. The administration of the extracts was done orally. Therefore, the acute toxicity test of the aqueous extracts of the various leaves revealed no death up to a dose of 5000 mg kg^{-1} b.wt., as shown in Table 1.

Table 1: Median lethal dose of aqueous extracts the leaves

Phase I	Dosages (mg kg^{-1} b.wt.)	Mortality
C. citratus		
Group 1	10	0/3
Group 2	100	0/3
Group 3	1000	0/3
A. sativum		
Group 1	10	0/3
Group 2	100	0/3
Group 3	1000	0/3
A. muricata		
Group 1	10	0/3
Group 2	100	0/3
Group 3	1000	0/3
Phase II		
C. citratus		
Group 1	1600	0/3
Group 2	2900	0/3
Group 3	5000	0/3
A. sativum		
Group 1	1600	0/3
Group 2	2900	0/3
Group 3	5000	0/3
A. muricata		
Group 1	1600	0/3
Group 2	2900	0/3
Group 3	5000	0/3

Experimental animals: Healthy male Albino rats weighing (126-152 g) were procured from the Faculty of Veterinary Medicine University of Nigeria, Nsukka, Enugu State, Nigeria. The experiment was performed according to the principles in the guide for the care and use of laboratory animals described by the National Institute of Health. The experimental protocol was made to conform to the rules for ethical conduct within the animal's use and care. Ethical approval was sorted and obtained from the Chairman Animal Care and Use Committee of Faculty of Veterinary Medicine, University of Nigeria, Nsukka. The rats were placed and/or housed in a plastic group cage and atmospheric temperature (25±2°C) with relative humidity (45±5%) in 12 hrs light and 12 hrs dark condition in a modern private animal house located at Ukwuoho, Orba road Nsukka within the University of Nigeria, Nsukka. The rats were given a standard pellet diet and water *ad libitum*, while the rats were allowed to acclimatize to the new environmental condition.

Experimental induction of diabetes mellitus: An established quantity of alloxan (100 mg kg^{-1} body weight) by Okorie *et al.*[2] used to induce diabetes was adopted in the study. More so, the method described by Okorie *et al.*[2] was adopted to induce diabetes using alloxan. The adult rats were kept on 18 hrs fast to induce diabetes. The induction was done intraperitoneally using alloxan (Sigma-Aldrich, St Louis, MO, USA). The rats were divided and labelled appropriately into 3 groups of 5 rats each as indicated earlier. The weight of the individual animals in each of the groups was used to calculate the volume of alloxan used in the induction process. Therefore, 100 mg kg^{-1} of alloxan was used and then, 1 g of alloxan dissolved in 10 mL of distilled water to obtain 100 mg mL^{-1} was adapted from the study by Okorie *et al.*[2]. Therefore, after 2 days of induction,

blood samples of the individual animals were drawn from their tail to confirmed diabetes and thereafter, treatment commenced across the different groups with specific treatment doses as designed for each group. The different extracts were reconstituted using 1 g of each extract dissolved in 10 mL of distilled water to obtain 100 mg mL^{-1} and this (100 mg mL^{-1}) was used to calculate the actual volume given to individual rats throughout the study period. The treatments were administered orally using an intragastric tube (attached to a syringe) for 21 days after the establishment of diabetes mellitus, while the animals (rats) were fed normally and water *ad libitum*.

Treatment groups:

- **Group 1:** Diabetic rats treated with mixed/combined aqueous extracts of *C. citratus*, *A. sativum* and *A. muricata* leaves at 300 mg kg^{-1} b.wt.
- **Group 2:** Diabetic rats treated with antidiabetic drug at 500 µg body weight
- **Group 3:** Diabetic rats, administered distilled water, no treatment with extracts nor drug

Evaluation of biochemical parameters

Collection of blood: The blood sample of the rats was collected from the recto-bulbar plexus (in the eye). The blood sample was allowed to clot for about 1 hr and then centrifuge at 3000 rpm. Thereafter, the serum was collected, stored and used for further analysis of the lipid profile of the rats.

Blood glucose determination: Blood glucose estimation was done for the individual rats using Accu-Chek Glucometer commercial kit, in the following order: Initial blood glucose, blood glucose level after induction, blood glucose level on day 7, blood glucose level on day 14 and blood glucose level on day 21.

Lipid profile: The baseline and end line of the lipid profile of the rats were determined using the serum.

Determination of cholesterol: The method cited by Okorie *et al.*[2] was adopted for this study.

Principle: Cholesterol determination was done using both enzymatic hydrolysis and oxidation. The indicator quinoneimine was obtained from hydrogen peroxidase and 4-amino antipyrine in the presence of phenol and peroxidase.

Test procedure: A labelled 3 test tubes were used namely, sample blank and standard. To the blank, 10 µL of distilled water was added, 10 µL standard specimen was added to the standard test tube, while 10 µL sample (serum) was added to the sample test tube. However, 1000 µL of the cholesterol reagent was added to the 3 test tubes. Each of the samples was thoroughly mixed and incubated for 10 min at room temperature (20-25°C). The absorbance of the samples (A_{sample}) against the blank was taken within 60 min at 500 nm.

$$\text{Total cholesterol (mg dL}^{-1}) = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 202.65$$

Low-density lipoprotein (LDL): Calculation of low-density lipoprotein-cholesterol was thus:

$$\text{LDL-Cholesterol conc (mg dL}^{-1}) = \text{Total cholesterol (HDL+triglycerides/5)}$$

High density lipoprotein (HDL)

Principle: Low and very-low-density lipoprotein (LDL and VLDL) was precipitated from serum by the action of a polysaccharide in the presence of divalent cations. Then, HDL that is present in the supernatant was determined.

Procedure: The precipitant solution 0.1 mL was added to 0.3 mL of the serum and mixed thoroughly and allowed to stand for 15 min this was centrifuge at 2000×g for 15 min. The cholesterol concentration in the supernatant was determined as cited by Okorie *et al.*[2].

$$\text{HDL cholesterol (mg dL}^{-1}) = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 202.65$$

Triacylglycerol

Principle: The enzymatic reaction and/or hydrolysis of triacylglycerol was shown below:

$$\text{Triacylglycerol} + H_2O \xrightarrow{\text{lipases}} \text{Glycerol} + \text{fatty acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{GK}} \text{Glycerol} - 3 - \text{phosphate} + \text{ADP}$$

$$\text{Glycerol} - 3 - \text{phosphate} + O_2 \xrightarrow{\text{GPO}} \text{Dihydroxyacetone phosphate} + H_2O_2$$

$$2H_2O_2 + 4 - \frac{\text{aminophenazone} +}{4\ \text{chlorophenol}} \xrightarrow{\text{POD}} \text{Quinoneimine} + HCl + 4H_2O$$

Data and statistical analysis: Glucose, total cholesterol, LDL, HDL, VLDL, TAG levels were also analyzed and compared with the standard (normal range) for rats. Statistical comparisons between experimental groups were performed using one-way

analysis of variant (ANOVA) while pairwise comparisons among groups were carried out using Duncan Multiple Range Test (DMRT) and mean percentage was also calculated. Statistical analysis was performed using IBM-SPSS version 21. A level of $p<0.05$ was considered significant.

RESULTS

Table 2 showed the blood glucose of the rats across the groups. The initial blood glucose of the rats in various groups ranged from 57.80-69.60 mg dL^{-1}. The mean values of the Initial Glucose Level (IGL) of the rats in various groups were 69.60, 63.80 and 57.80 mg dL^{-1}, respectively, mixed aqueous extracts of garlic leaf+soursopleaf+lemongrass at 300 mg kg^{-1} ($G+S+L_{300\ mg/kg}$) standard drug at 500 µg ($SD_{500\ µg}$) and Diabetic No Extract (DNE). The blood glucose level after induction (GLI) ranged from 253.20-293.20 mg dL^{-1} were the mean values of the glucose level of the rats after induction included 253.20 mg dL^{-1} ($G+S+L_{300\ mg/kg}$), 293.20 mg dL^{-1} ($SD_{500\ µg}$) and 259.60 mg dL^{-1} (DNE).

There was a decrease in the blood glucose levels in diabetic treated rats (253.2±31.43-188.8±188.66 mg dL^{-1}) after treatment with mixed aqueous extracts of G+S+L at 300 mg kg^{-1}. There were significant percentage fall (14.93, 19.19 and 25.43%) on day 7, day 14 and 21 in the blood glucose levels of the treated diabetic rats and thus, bringing blood glucose level from 253.2-188.8 mg dL^{-1}, although a little above the normal range for the rat. However, Table 2 also showed the result of treatment on diabetic rats with standard diabetic drug (Glibenclamide) at 500 µg. A significant fall in blood glucose levels of diabetic treated rats was observed (293.2±24.01-113.8±9.76 mg dL^{-1}) after treatment with Glibenclamide at 500 µg. There was a significant percentage decrease (15.14, 25.17 and 61.19%, respectively) in the blood glucose level of treated diabetic rats after treatment on 7, 14 and 21 days. However, on day 21 the blood glucose level came down to normal (113.80 mg dL^{-1}) from 293.20 mg dL^{-1} after induction with alloxan. More so, in diabetic rats with no treatment given, there was an 18.8% increase in the blood glucose level from 259.60-308.40 mg dL^{-1} on day 7 after induction with alloxan. However, there was a decrease in the blood glucose level to 237.4 and 159.4 mg dL^{-1} on day 14 and 21, respectively. This showed an 8.55 and 38.60% fall in the blood glucose levels both on day 14 and 21, respectively, in the non-treated diabetic rats.

Table 3 reveals the baseline (after inducing diabetes) and end-line levels of Total Cholesterol (TC) and triglyceride (TAG) of treated and non-treated diabetic rats. There were significant decreases in TC and TAG levels were seen in treated diabetic rats (67.56±19.23-47.52±22.88 mg dL^{-1} and 84.16±41.75-73.78±46.20 mg dL^{-1}, respectively) after treatment with mixed aqueous extracts of *A. sativum*, *A. muricata* and *C. citratus* at dose 300 mg kg^{-1} b.wt. This further revealed about 29.7 and 12.3% fall, respectively, of the TC and TAG levels of the treated diabetic rats on day 21. Furthermore, treatment with a standard diabetic drug (Glibenclamide) at dose 500 µg revealed 39.4 and 21.6% decrease in the TC and TAG levels, respectively, of the treated diabetic rats on day 21, thereby, bringing down their TC level (65.00±7.72-39.40±7.52 mg dL^{-1}) and TAG level (71.40±41.07-56.00±44.04 mg dL^{-1}). There was also a decrease in the TC and TAG levels of the non-treated diabetic rats (48.26±3.02-16.26±15.06 mg dL^{-1} and 69.68±29.86-35.00±36.87 mg dL^{-1}, respectively), which showed about 66.3 and 49.8% fall, respectively in their TC and TAG levels on 21 days.

In Table 4, there was also a significant decrease in the LDL-C level (36.16±9.11-7.08±4.16 mg dL^{-1}) of the treated diabetic rats after treatment with mixed aqueous extract of *A. sativum*, *A. muricata* and *C. citratus* at dose

Table 2: Glucose level of the animals across the groups over the treatment periods

Groups	IGL (mg dL^{-1})	GLI (mg dL^{-1})	GL7D (mg dL^{-1})	D (%)	GL14D (mg dL^{-1})	D (%)	GL21D (mg dL^{-1})	D (%)
$G+S+L_{300\ mg/kg}$	57.80±10.78	253.20±31.43	215.40±40.97	-14.93↓	204.60±41.76	-19.19↓	188.80±188.66	-25.43↓
$SD_{500\ µg}$	69.60±14.54	293.20±24.01	248.80±16.39	-15.14↓	219.40±8.68	-25.17↓	113.80±9.76	-61.19↓
DNE	66.80±7.92	259.60±21.51	308.40±33.36	18.80↑	237.40±134.34	-8.55↓	159.40±148.49	-38.60↓

IGL: Initial glucose level, GLI: Glucose level after induction, GL7D: Glucose level after 7 days, GL14D: Glucose level after 14 days, GL21D: Glucose level after 21 days, G+S+L: Garlic+soursop+lemongrass, SD: Standard drug, DNE: Diabetic no extract, D (%): Percentage difference, ↑: Increase and ↓: Decrease

Table 3: Lipid profile of treated alloxan-induced hyperglycemic rats

	Total cholesterol (mg dL^{-1})			Triglyceride (mg dL^{-1})		
Groups	Baseline	End-line	D (%)	Baseline	End-line	D (%)
$G+S+L_{300\ mg/kg}$	67.56±19.23	47.52±22.88	-29.7↓	84.16±41.75	73.78±46.20	-12.3↓
$SD_{500\ µg}$	65.00±7.72	39.40±7.52	-39.4↓	71.40±41.07	56.00±44.04	-21.6↓
DNE	48.26±3.02	16.26±15.06	-66.3↓	69.68±29.86	35.00±36.87	-49.8↓

G+S+L: Garlic+soursop+ lemongrass, SD: Standard drug, DNE: Diabetic no extract, D (%): Percentage difference, ↑: Increase and ↓: Decrease

Table 4: LDL and HDL profile of treated alloxan-induced hyperglycemic rats

	Low density lipoprotein (mg dL^{-1})			High density lipoprotein (mg dL^{-1})		
Groups	Baseline	End-line	D (%)	Baseline	End-line	D (%)
G+S+$L_{300\ mg/kg}$	36.16±9.11	7.08±4.16	-80.4↓	31.40±12.09	40.44±18.97	28.8↑
$SD_{500\ \mu g}$	34.76±3.72	3.64±2.04	-89.5↓	30.24±6.09	35.76±6.60	18.3↑
DNE	31.30±29.86	6.62±7.40	-78.8↓	19.96±9.61	9.64±9.68	-51.7↓

G+S+L: Garlic+soursop+lemongrass, SD: Standard drug, DNE: Diabetic no extract, D (%): Percentage difference, ↑: Increase and ↓: Decrease

300 mg kg^{-1} b.wt., which indicated 80.4% fall on day 21. The diabetic rats were also treated with standard diabetic drug at dose 500 µg and this showed about 89.5% fall in the LDL-C level (34.76±3.72-3.64±2.04 mg dL^{-1}) of the treated diabetic rats at the end of the study, while the LDL-C level (31.30±29.86-6.62±7.40 mg dL^{-1}) of non-treated diabetic rats showed also about 78.8% fall at the end of the study. There was a 28.8% increase in the HDL-C level (31.40±12.09-40.44±18.97 mg dL^{-1}) of the treated diabetic rats after treatment with mixed aqueous extracts of *A. sativum*, *A. muricata* and *C. citratus* at dose 300 mg kg^{-1} b.wt., at the end of the study, while treatment with a standard diabetic drug at 500 µg revealed an 18.3% increase in the HDL-C level (30.24±6.09-35.76±6.60 mg dL^{-1}) of the treated diabetic rats at the end of the study. However, a significant percentage decrease (51.7%) was observed in the HDL-C level (19.96±9.61-9.64±9.68) of the non-treated diabetic rats at the end of the study mentioned in Table 4.

DISCUSSION

The term diabetes mellitus describes a metabolic disorder of multiple aetiology characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from a defect in insulin secretion, insulin action or both[2]. The initial blood glucose level of the animals (rats) were within normal range before diabetes was induced. The chemical (alloxan) was used to induce diabetes mellitus in the rats.

In this study, the rats were diabetic induced using alloxan. Diabetes mellitus was established amongst the rats after 48hours of alloxan induction, where the blood glucose level varied above 200 mg dL^{-1}. These variations in blood glucose level may have resulted due to the effect of the chemical (alloxan-induced diabetes) on the individual animal response to partly the rate of apoptosis of cells of the pancreas or their gene expression of insulin resulting in reduced insulin synthesis. Cells of the pancreas normally maintain blood glucose concentration within a narrow range by modulating their insulin secretion rate in response to the blood glucose concentration[12]. However, apoptosis of pancreatic cells could be the primary factor that ultimately results in hyperglycaemia[13,12].

The rats in group 1, that were treated with mixed aqueous extract of *A. sativum*, *A. muricata* and *C. citratus* at a dose of 300 mg kg^{-1} b.wt., (G+S+$L_{300\ mg/kg}$) revealed that a combination of aqueous extracts of *A. sativum*, *A. muricata* and *C. citratus* leaves indicated lowering potentials by recording a reduction in high blood glucose of the alloxan-induced hyperglycemic rats. This lowering effect of the mixed aqueous extracts of the leaves may be attributed to the presence of phytochemicals such as flavonoids and alkaloids, tannins, which may have contributed to the anti-hyperglycemic activity of the leaves. One would have thought that the mixed aqueous extracts of the leaves could have been the best in the hypoglycemic effect than the individual aqueous leave extracts in ameliorating high blood glucose levels in diabetic rats. However, the unstable activities of the mixed aqueous extracts to lower alloxan-induced hyperglycemia in rats may be attributed to nutrient and/or chemical interactions amongst the constituent phytonutrient and/or phytochemical of the various individual extract dosage, which could either be synergistic or antagonistic in exhibiting their various potentials and capacity to decrease elevated blood glucose level of diabetic rats and thus, may have contributed to the rate of decline in the blood glucose levels of the treated hyperglycemic rats after treatment with mixed aqueous extracts of *A. sativum*, *A. muricata* and *C. citratus* leaves at dose 300 mg kg^{-1} b.wt., as observed in the study. The result, therefore, corroborated with previous reports by other authors[4,14-16] in their various studies on the effect of combination and/or mixed extracts on the blood glucose level of alloxan-induced diabetic rats. However, when compared to treatment with a standard antidiabetic drug (Glibenclamide) at 500 µg kg^{-1} b.wt., the elevated blood glucose levels of the rats had a better outcome by mitigating high blood glucose levels to normal range, than the mixed aqueous extracts of the leaves, the indication of its blood glucose-lowering effects. Although there was a gradual decline in the elevated blood glucose in treated alloxan-induced hyperglycemic rats, the aim of the study on the effect of the mixed aqueous extracts of the leaves on the blood glucose of hyperglycemic rats was

thus achieved. More so, search for a cost-effective strategy in ameliorating diabetes was also achieved as the leaves are very much available for use at a minimal and/or insignificant cost compared to the cost of buying antidiabetic drugs (Glibenclamide). Therefore, regular consumption of the leaves in a meal plan would play an important role in lowering blood glucose levels. The decrease in the blood glucose of the non-treated diabetic rats suggested the effect of the diet fed to the rats, although their blood glucose levels were not within the normal range, it does suggest that an appropriate diet both in quality and quantity could improve blood glucose level and thereby, would play an important role in the management of type 2 diabetes mellitus.

The significant decrease in levels of TC, TAG and LDL-C obtained in the study were indicative of the potentials of mixed aqueous extracts of *A. sativum* leaves, *A. muricata* leaves and *C. citratus* to mitigate serum levels of these parameters and invariably increase serum levels of HDL-C compared to treatment with standard antidiabetic drugs. This, therefore, suggests an enhanced cardiovascular system of the treated alloxan-induced diabetic rats devoid of related disorders. This is in line with previous studies[17-19], earlier reported that elevated level of HDL-C is linked to reduced occurrences of cardiovascular disorder while the increased level of LDL-C has been associated with a higher risk of atherosclerosis. The mixed aqueous extracts of *A. sativum*, *A. muricata*, *C. citratus* at a dose of 300 mg kg^{-1} b.wt., had also a positive effect on the HDL-C level of the rats, which indicated anti-hyperlipidaemia activity of the mixed aqueous extracts.

Therefore, the normal and/or high ratio of HDL-C to LDL-C observed in the present study due to various treatments administered, could implicate reduced occurrences of cardiovascular disorder (atherosclerosis). However, the result of this study is in contrast with the study by Adeyemi and Orekoya[20] where the level of LDL-C was higher than HDL-C level of alloxan-induced diabetic rats after oral and repeated administration of their herbal mixture. This synthetic pathway plays a vital role in mitigating cholesterol levels in the blood and peripheral tissues as well inhibit the formation of atherosclerotic plaque in the aorta[21]. Also, the present study conforms with the study by Ademuyiwa *et al.*[22] whereby the serum cholesterol levels were found to be down-regulated after treatment. The display of synergistic effect by the mixed aqueous extracts in lowering hyperglycemia could be depended upon the type and number of individual extracts involved in constituting the experimental doses. Thus, the mixture of the aqueous extracts may have contributed to the readjustment in the complete concentrations of the bioactive components and by extension may influence the nature and outcome of their interactions as observed in the study, in dictating dose-dependent therapeutic potentials of the various aqueous leaves extracts.

CONCLUSION

Therefore, it can be inferred that the regular intake of the mixed aqueous extracts of *C. citratus*, *A. muricata* and *A. sativum* leaves have the capacity and potentials to lower high blood glucose (hyperglycemia), hyperlipidaemia and/or hypercholesterolemia caused by alloxan-induced diabetes mellitus and more so, could have the ability to mitigate the complications of disorders of carbohydrate, protein and lipid metabolism, thereby enhancing insulin secretion.

SIGNIFICANCE STATEMENT

This study discovered the potentials of mixed aqueous extracts of *A. sativum*, *A. muricata* and *C. citratus* leaves that can be beneficial for the management of type 2 diabetes, lowering of blood glucose level and blood lipids as was observed *in vivo* (alloxan-induced hyperglycemic rats). This study helped the researchers to further uncover the critical areas in disease management especially non-communicable diseases (such as diabetes mellitus), that many researchers were not able to explore or had little or no input is done. Thus, a new theory on the biological action of extracts and/or mechanism of actions of these aqueous extracts in both *in vivo* and *in vitro* may be arrived at.

REFERENCES

1. Zimmermann, M., C. Bunn, H. Namadingo, C.M. Gray and J. Lwanda, 2018. Experiences of type 2 diabetes in Sub-Saharan Africa: A scoping review. Global Health Res. Policy, Vol. 3. 10.1186/s41256-018-0082-y.
2. Okorie, I., R.A. Adesanmi and N. Nnam, 2021. Blood glucose and lipid profile of alloxan-induced hyperglycemic Albino rats treated with aqueous extract of *Allium sativum* leaf. World J. Pharm. Res., 10: 25-34.
3. Yazdanparast, R., M.A. Esmaeili and J.A. Helan, 2005. *Teucrium polium* extract effects pancreatic function of streptozotocin diabetic rats: A histopathological examination. Iran. Biomed. J., 9: 81-85.
4. Ojiako, O.A., P.C. Chikezie and A.C. Ogbuji, 2015. Blood glucose level and lipid profile of alloxan-induced hyperglycemic rats treated with single and combinatorial herbal formulations. J. Traditional Complementary Med., 6: 184-192.

5. Jung, M., M. Park, H.C. Lee, Y.H. Kang, E.S. Kang and S.K. Kim, 2006. Antidiabetic agents from medicinal plants. Curr. Med. Chem., 13: 1203-1218.
6. Dewanjee, S., A.K. Das, R. Sahu and M. Gangopadhyay, 2009. Antidiabetic activity of *Diospyros peregrina* fruit: Effect on hyperglycemia, hyperlipidemia and augmented oxidative stress in experimental type 2 diabetes. Food Chem. Toxicol., 47: 2679-2685.
7. Nasim, S.A., B. Dhir, R. Kapoor, S. Fatima and A. Mujib, 2011. Alliin obtained from leaf extract of garlic grown under *in situ* conditions possess higher therapeutic potency as analyzed in alloxan-induced diabetic rats. Pharmaceut. Biol., 49: 416-421.
8. Sawant, T.P. and R.S. Dongre, 2014. Biochemical compositional analysis of *Annona muricata*: A miracle fruit's review. Int. J. Pharma Biosci., 3: 82-104.
9. Adeyemi, D.O., O.A. Komolafe, S.O. Adewole and E.M. Obuotor, 2009. Anti hyperlipidemic activities of *Annona Muricata* (Linn). Int. J. Altern. Med., Vol. 7. 10.5580/293b.
10. Atiku, M.K., D. Adamu, A. Gadanya and M. Shehu, 2009. The effect of "gadagi" tea on liver function and serum glucose concentration in Albino rats. Bayero J. Pure Appl. Sci., 2: 125-128.
11. Sukhdev, S.H., P.S.K. Suman, L. Gennaro and D.R. Dev, 2008. Extraction Technologies for Medicinal and Aromatic Plants. International Centre for Science and High Technology, Italy, Pages: 260.
12. Husni, A., R. Wijayanti and Ustadi, 2014. Inhibitory activity of α-Amylase and α-Glucosidase by *Padina pavonica* extracts. J. Biol. Sci., 14: 515-520.
13. Patel, R., A. Shervington, J.A. Pariente, M.A. Martinez-Burgos, G.M. Salido, E. Adeghate and J. Singh, 2006. Mechanism of exocrine pancreatic insufficiency in streptozotocin-induced type 1 diabetes mellitus. Ann. N. Y. Acad. Sci., 1084: 71-88.
14. Liu, R.H., 2003. Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. Am. J. Clin. Nutr., 78: 517S-520S.
15. Niwano, Y., K. Saito, F. Yoshizaki, M. Kohno and T. Ozawa, 2010. Extensive screening for herbal extracts with potent antioxidant properties. J. Clin. Biochem. Nutr., 48: 78-84.
16. Milugo, T.K., L.K. Omosa, J.O. Ochanda, B.O. Owuor, F.A. Wamunyokoli, J.O. Oyugi and J.W. Ochieng, 2013. Antagonistic effect of alkaloids and saponins on bioactivity in the quinine tree (*Rauvolfia caffra* sond.): Further evidence to support biotechnology in traditional medicinal plants. BMC Complement. Altern. Med., Vol. 13. 10.1186/1472-6882-13-285.
17. Adeyemi, O.S. and M.A. Akanji, 2011. Iron and nitric oxide play key role in the development of cardiovascular disorder. J. Toxicol. Environ. Health Sci., 3: 249-253.
18. Palacios, M.C., M. Haros, C.M. Rosell and Y. Sanz, 2005. Characterization of an acid phosphatase from *Lactobacillus pentosus*: Regulation and biochemical properties. J. Applied Microbiol., 98: 229-237.
19. Olukanni, O.D., O.T. Akande, Y.O. Alagbe, O.S. Adeyemi, A.T. Olukanni and G.G. Daramola, 2013. Lemon juice elevated level of reduced glutathione and improved lipid profile in Wistar rats. Am.-Eurasian J. Agric. Environ. Sci., 13: 1246-1251.
20. Adeyemi, O.S. and B. Orekoya, 2014. Lipid profile and oxidative stress markers in Wistar rats following oral and repeated exposure to Fijk herbal mixture. J. Toxicol., Vol. 2014. 10.1155/2014/876035.
21. Kim, H.Y., D.M. Jeong, H.J. Jung, Y.J. Jung and T. Yokozawa *et al*., 2008. Hypolipidemic effects of *Sophora flavescens* and its constituents in poloxamer 407-induced hyperlipidemic and cholesterol-fed rats. Biol. Pharm. Bull., 31: 73-78.
22. Ademuyiwa, A.J., U. Usunomena, G. Olubunmi and O.O. Iyere, 2016. Comparative studies on the effect of extracts of *Cymbopogon citratus* (lemongrass) on the lipid profile of Wistar Albino rats. World J. Pharm. Res., 5: 141-150.