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Cymbopogon citratus Stapf (DC) Extract Ameliorates Atherogenic Cardiovascular Risk in Diabetes-Induced Dyslipidemia in Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Authors CEE and EEA performed the experiments and gathered the data. Authors CEE, KD and EEA analyzed the data. Author CEE wrote the first manuscript, with contributory comments from author KD. Authors CEE, KD and EEA contributed to the analyses and editing of the manuscript, and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: Diabetic dyslipidemia is a recognized risk factor for coronary heart disease (CHD). Plant medicinal agents such as *Cymbopogon citratus* (*C. citratus*) have shown potential as alternative therapies for reducing cardiovascular risk factors. The aim of this study was to investigate the effect of *C. citratus* leaf extract on the atherogenic index of plasma (AIP) in diabetic dyslipidemic rats (n=35).

Materials and Methods: A *C. citratus* extract was prepared by ethanol extraction of leaf material. Rats were divided into seven groups (n=5) as follows: (a) Normal diet control, (b) Hyperlipidemic diet (HLD) control, (c) HLD + 65mg/kg streptozotocin (STZ) control (d) HLD + STZ + 250mg/kg *C. citratus* extract (CCE), (e) HLD + STZ + 500mg/kg CCE, (f) HLD + STZ + 1000mg/kg CCE, and (g) HLD + STZ + 5mg/kg atorvastatin + 600µg/g glibenclamide. Animals were treated with HLD for 14 days and then injected intraperitoneally with 65mg/kg STZ. Confirmed diabetic dyslipidemic animals were treated intragastrically with CCE at doses of 250, 500, and 1000mg/kg, with 5mg/kg atorvastatin, and with 600µg/g glibenclamide for 30 days.

Results: The extract, which tested positive for tannins, saponins, alkaloids, flavonoids, etc. lowered fasting blood glucose and glycosylated hemoglobin levels, and dose-

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dependently decreased the serum levels of T-chol, LDL, VLDL, and β-HMG-CoA reductase, while simultaneously increasing HDL levels. The AIP was lowered in a dose-dependent manner by 33, 43.7, and 52.4% in groups treated with 250, 500, and 1000 mg/kg of CCE respectively.
Conclusion: The results indicate that the *C. citratus* extract had an ameliorative effect on hyperglycemia, hyperlipidemia, obesity, and atherogenic index of plasma.

Keywords: Cymbopogon citratus stapf; diabetes; dyslipidemia; atherogenic risk; rats.

1. INTRODUCTION

Diabetic dyslipidemia is a collection of lipid abnormalities associated with diabetes mellitus, specifically including high serum triglycerides (TG), small, dense low-density lipoprotein-cholesterol, low levels of high-density lipoprotein cholesterol (HDL-C) and normal levels of total low-density lipoprotein-cholesterol (LDL-C) [1]. Due to its association with an increased incidence of coronary heart disease, diabetic dyslipidemia has become a significant public health concern worldwide [2]. Among the abnormalities associated with diabetic dyslipidemia, changes in serum TG, LDL-C and HDL-C levels are the most commonly observed and have pronounced but antagonistic action [3]. Accordingly, the plasma levels of these lipid sub-fractions have become a common index known as the atherogenic index of plasma (AIP) that is quantitatively calculated as the logarithm of the value of plasma TG divided by HDL-C [4]. AIP has become the most useful index for predicting and quantifying coronary artery disease risk. Indeed, AIP is a single, independent predictor of morbidity and mortality in diabetic patients and is strongly associated with increased arteriosclerotic cardiovascular risk in diabetics and the general population [5]. In fact, diabetic dyslipidemia actually amplifies the effects of other common cardiovascular risk factors, including smoking, family medical history, hypertension, and obesity. In terms of other diabetes risks, dyslipidemia is associated with macrovascular disease, whereas hyperglycemia is associated with microvascular complications, with most patients dying as a result of macrovascular disease.

It is well known that cardiovascular disease intervention protocols that focus on multiple risk factors achieve higher success than single-factor intervention strategies. To date, however, no synthetic drug monotherapies have been identified that can address multiple CVD pathologies, mandating multiple drug therapies as the only feasible option. The disadvantages of multiple drug therapies include poor compliance, drug resistance, multiple adverse effects, higher cost, and a need for regular expert consultation. Treatment can therefore become very difficult, particularly in poor communities with limited access to and usage of synthetic drugs or with a health care system already overwhelmed with managing infectious diseases.

In an attempt to address these challenges, multiple studies in developed and developing countries have focused on using herbal and natural plant products to ameliorate multiple CVD risk factors. One plant explored for this purpose is *Cymbopogon citratus* (*C. citratus*), a perennial tropical plant of the family poaceae that is widely cultivated and globally distributed. It has diverse chemical constituents that include proteins, moisture, ash, crude fiber, fat and carbohydrates [6], and it is rich in minerals, vitamins [7], phytochemicals (tannins, saponins, flavonoids, alkaloids, phenols, and anthraquinones) and anti-nutrients [8]. The leaves also contain essential oils in various concentrations (0.2–1.4% and up to 3%) [9],

of which citral is the major chemical constituent (75–80%). Other essential oil constituents include monoterpenes (citronellal, linalool, β-myrcene, limonene, geraniol, and eugenol) [6,10]. In many countries, *C. citratus* is widely consumed in aromatic drinks and used in traditional cuisine. Pharmacologically, it is used as an antimicrobial, anti-inflammatory, antioxidant, antifungal, anti-tussive, insect repellant, and anti-platelet agent [11].

Despite the well-known nutritional and therapeutic potential of this plant, there are no literature reports of its effect on AIP. We therefore sought to accurately determine the effect of *C. citratus* constituents on AIP.

2. MATERIALS AND METHODS

2.1 Plant Materials

2.1.1 Collection, identification, and preparation of *C. citratus* leaves

Fresh *C. citratus* leaves were obtained from an agricultural farm in Uyo, Akwa Ibom State, Nigeria a few days prior to utilization, and their identity was confirmed by a taxonomist (ID. No. UUH3276/UYO) in the department of botany at the university of Uyo. The leaves were rinsed, sun dried, and pulverized into powder using an electric blender to provide 400g of material. The powder was soaked in a conical flask with 2L of ethyl alcohol (98.90%). The suspension was agitated with an electric blender for 10min and stored at 4ºC for 24 h. After filtering the suspension through a Whatman No. 2 filter paper, the filtrate was evaporated to dryness by heating in a water bath at 40ºC. The final solid extract was weighed with an electric balance (ACS-ZE14, Surgifriend Medicals Ltd, England), with a total extraction yield of 35%. The prepared extract was stored in glass bottles at 4ºC and was dissolved at a concentration of 100mg/mL in physiological saline for assays.

2.1.2 Phytochemical screening of *C. citratus* leaf extract

The phytochemical analysis of extracts was carried out using standard procedures to determine the levels of saponins, phenolics, alkaloids, tannins, flavonoids, glycosides, steroids, deoxy sugars, terpenes, and anthraquinones [12]. The results are shown in (Tables 1 and 3).

Table 1. Phytochemical constituents of *C. citrates*

Phytochemical constituents	Level
Tannins	+++
Saponins	++
Flavonoids	++
Phenols	++
Anthraquinones	+
Alkaloids	+
Deoxysugars	+
Steroids	-
Cyanates	-
Phlobatannins	-

- = absent; + = low; ++ = moderate; +++ = marked

2.1.3 Determination of nutrient constituents

Ash, crude fiber, fat, and protein were determined by proximate analysis using procedures provided by the Association of Official Analytical Chemists [13]. To determine the levels of mineral constituents, extract aliquots were predigested with concentrated HNO_3, followed by digestion with a mixture (10:3) of concentrated HNO_3 and concentrated $HClO_4$. The acid samples were heated in a covered 50-mL beaker until all traces of $HClO_4$ were eliminated, as reflected by the absence of white fumes. The final liquid in the digestion beaker (about 2 mL) was brought up to a volume of 25mL with deionised water, and assayed for copper, iron, magnesium, sodium, and calcium by using an atomic absorption spectrophotometer (Jarrel-Arh model 82-362). Determination of fat and water soluble vitamins in the extract was performed according to the methods described by Ismail and Fun [14]. A reverse-phase high performance liquid chromatography (RP-HPLC) [model 582 ESA Inc. Chelmsford USA] was used to identify the water soluble vitamins using an aqueous mobile phase method [15]. The results are shown in (Table 2).

Table 2. Analysis of nutrient constituents of *C. citratus* leaf (per 100g)

Nutritional constituents	Nutritional value
Constituents	
Moisture	2.36g
Total ash	4.20g
Crude protein	2.65g
Crude fibre	3.04g
Carbohydrates	36.02g
Total fat	0.86g
Cholesterol	0 mg
Electrolytes	
K^+	560mg
Na^+	16 mg
Minerals	
Ca^{2+}	56mg
Fe^{2+}	16.6mg
Mg^{2+}	40.15mg
Cu	0.486mg
Mn	8.34mg
Se	1.02mcg
Zn^{2+}	2.05mg
Vitamins	
Vitamin A	9.0g
Vitamin C	3.8mg
Folate	88mcg
Thiamine	0.84mg
Niacin	2.01mg
Pyridoxine	0.04mg
Riboflavin	0.186mg

Table 3. Chemical composition of the essential oil of *C. citratus* leaf extract

Constituents	HD%	[a]RI[b]	[b]RI[Lit]
Geranial	4	1265	1269
Neral	26.32	1234	1239
Linalool	5.29	1180	1100
Myrcene	23.05	976	988
Geraniol	10.75	1237	1369
Acetate of geraniol	10.51	1365	1379
Nerol	25.12	1225	1233
Limonene	0.51	1086	1032

Total identified: 98.5%, RI[a]: Experimental retention indices, HD: Hydrodistillation; RI[b]: literature retention indices [45]

2.1.4 Chemical composition of essential oil of *C. citratus* leaf extract

The analysis of essential oil of *C. citratus* by gas chromatography/mass spectrophotometer (GC/MS) demonstrated the presence of geranial, neral, myrcene, linalool and acetate of geraniol (Table 3).

2.2 Animals and Experimental Design

A case-control experimental study design was used to assess the effect of *C. citratus* extract on AIP in diabetic-dyslipidemic rats. A total of thirty-five 3-week-old male Wistar Albino rats weighing 160–200g, were obtained from the animal house of the faculty of Basic Medical Sciences, University of Uyo, Nigeria. Their initial weights were recorded, and they were individually housed in standard stainless steel cages, with wooden shavings as their beddings, on a 12-h light/dark cycle, at room temperature, and with a relative humidity of 55% ± 10%. All rats were fed with standard animal feed (Bendel Feed and Flour Mill Ltd, Benin) and were provided water *ad libitum*. Based on a lethality studies that determined the LD_{50} of *C. citratus* extract to be 2500.20mg/kg, non-lethal doses were chosen for further analysis.

After a 1-week adaptation period, the rats were randomly divided into seven groups of five rats each as follows:

a. Normal control (NC): received normal animal feed + citrate 0.1 M
b. Hyperlipidemic diet (HLD) control: received high HLD for 14 days + citrate 0.1 M
c. Hyperlipidemic diet + streptozotocin (HLD + STZ) control: received HLD for 14 days + 65 mg/kg STZ + citrate buffer 0.1 M
d. HLD + STZ + *C. citratus* extract (CCE): fed with HLD + 65mg/kg STZ + 250 mg/kg CCE + citrate 0.1M
e. HLD + STZ + CCE: fed with HLD + 65 mg/kg STZ + 500 mg/kg of CCE + citrate 0.1 M
f. HLD + STZ + CCE: fed HLD + 65mg/kg STZ + 1000 mg/kg CCE + citrate 0.1 M
g. HLD + STZ + atorvastatin + glibenclamide: Fed with hyperlipidemic diet + 65mg/kg STZ + citrate 0.1 M + 5mg/kg atorvastatin + 600µg/kg weight glibenclamide in aqueous solution orally for 30 days.

2.3 Assessment of Anti-dyslipidemia and Hypoglycemic Activity

2.3.1 Experimental induction of hyperlipidemia

Animals in groups b, c, d, e, f, and g were fed with an equal amount of high fat and high cholesterol food (g/100g of normal diet) that included sunflower oil: 20, egg yolk: 35, and

cholesterol: 0.5 [16,17] for 30 days. Animals in group a were fed standard animal feed (Bendel Feed and Flour Mill Ltd. Benin) over the same period. Blood samples were collected by retro-orbital plexus technique and assessed for hyperlipidemia using standard procedures. AIP was calculated as log (TG/HDL-C).

2.3.2 Experimental induction of diabetes

After 14 days of hyperlipidemic induction, diabetes was induced in animals in subgroups c, d, e, f, and g by a single intra-peritoneal injection of 65 mg/kg body weight of STZ (Sigma. USA) dissolved in 0.1M citrate buffer (pH 4.5). Animals in the control group received an injection of citrate buffer (0.1 M, pH 4.5) with no STZ. Seventy-two hours after STZ injection, blood samples were collected by retro-orbital plexus technique using capillary tubes [18], and the animals were considered to be diabetic for the purposes of this study if their fasting blood glucose levels were greater than 150mg/dL [19].

2.4 Administration of *C. citratus* Extract

Confirmed dyslipidemic and diabetic rats in groups d, e, and f were fed the *C. citratus* extract intragastrically at doses of 250, 500, and 1000 mg/kg daily for 30 days. Animals in group g were treated with atorvastatin 5mg/kg and glibenclamide 600µg/kg in aqueous solution orally throughout the same period. The body weights were measured daily, and the food intake was calculated weekly. After sacrifice at the end of the study, liver weights were also measured.

2.5 Biochemical Extract Analysis

After 30 days of extract administration and 8 h after the last feeding, all animals were anesthetized with a mixture of ketamine (90mg/kg) and xylazine (15 mg/kg), and whole blood was obtained by cava vein puncture and collection into specimen bottles. Serum was isolated from the blood samples by centrifugation at 3000 × *g* for 10 min at 4℃, after which analyses were completed within 2h of sample collection at the chemical pathology unit of the University of Uyo Teaching Hospital (UUTH). Parameters measured included hematological indices (PCV, RBC, and WBC), serum creatinine (SCr), urea content, liver enzymes (aspartate amonitransaminase (AST), alanine transaminase (ALT)), fasting plasma glucose, lipid profile (HDL-C, LDL-C, VLDL-C, TG, and total cholesterol), glycosylated hemoglobin (HbAic), HMG-CoA reductase activity, and electrolyte levels (Na^+, K^+ and Cl^-).

The serum creatinine levels were determined by Jaffe's method. Briefly, 1 mL of 0.75 NaOH and 1% picric acid (Sigma chemicals, India) was mixed with 1mL of each serum specimen. A standard was similarly treated. The colour change that developed within 15 min at room temperature was measured spectrophotometrically (ESA Inc., Chelmsford, USA) at 520 nm. The serum total cholesterol (T-chol), triglyceride (TG), low-density lipoprotein (LDL-C), high-density lipoprotein (HDL-C), and glucose were measured using a lipid profile/glucose automated measuring system (Lipid pro^{TM}), model ILM-0001A, Infopia Co. Ltd, South Korea. Serum urea was measured using multichannel automated analyzer (SYNCHRON, LOS Angeles, CA). Glycosylated hemoglobin (HbAic) was measured using an Automated HbAic Analyzer (CLOVER Alc^{TM} Analyzer; Infopia Co. Ltd, South Korea). Beta-hydroxyl β-methyl glutaryl-CoA (HMGCoA) reductase activity was determined. The HMG-CoA reductase to mevalonate ratio was calculated as the level of enzyme activity that catalyzes the conversion of HMG to mevalonate [17]. The hematological indices were determined using SYSMEX KX-2IN automated Hematologic Analyzer (KOBE, JAPAN).

2.6 Statistical Analysis

Values are reported herein as the mean ± SEM (standard error of mean). Statistical comparisons between experimental groups was performed using one-way analysis of variance (ANOVA) while pair-wise comparisons among groups (post-hoc test) were carried out using the least significance difference test (LSD). Statistical computations were conducted with the use of Statistical Package for Social Sciences (SPSS version 20.0), and statistical significance was established at 5%.

3. RESULTS AND DISCUSSION

In the present study, we found that the analysis of the bioactive natural constituents from *C. citratus* leaf extracts revealed the presence of a wide range of phytochemical, nutritional, and essential oil constituents (Tables 1, 2 and 3 respectively). We also observed that the extract lowered the fasting plasma glucose concentrations and glycosylated hemoglobin (HbA_{ic}) levels in diabetic dyslipidemic rats (Table 4) and also dose-dependently decreased their plasma levels of T-chol, TG, LDL-C, VLDL-C, and HMG-CoA reductase, while increasing HDL-C levels. Additionally, the AIP of the animals treated with *C. citratus* leaf extract decreased in a dose-dependent manner by 33, 43.7, and 52.4 % for low, medium, and high extract doses, respectively (Table 5). The effects observed with *C. citratus* treatment were comparable to the effects observed with a conventional hypoglycemic and hypolipidemic chemotherapy regimen of atorvastatin and glibenclamide.

These findings support the anti-atherogenic effects of *C. citratus* leaf extract as previously documented [20], and are attributed in part to the synergistic actions of its component phytochemicals, nutritionals, and essential oils on lipid biosynthesis [21]. The isoprenoid derivatives from *C. citratus* essential oils (geranol, linalool, limonene, β-ionone, and citral) have been shown to lower serum cholesterol and inhibit lipogenesis by post-transcriptional repression of HMG-CoA reductase, with no effect on the mRNA levels of the gene [22]. This enzyme is a polytopic transmembrane microsomal enzyme that catalyzes the conversion of HMG-CoA to mevalonate, which is necessary for cell growth and is involved in the synthesis of sterols, isoprenoids, and lipids. Since HMG-CoA reductase catalyzes the rate-limiting step of cholesterol biosynthesis, it is the major cellular target of many lipid-lowering drugs [23].

In the present study, *C. citratus* extract lowered the plasma levels of HMG-CoA reductase in a dose-dependent manner in diabetic dyslipidemic rats, a finding that is consistent with previous reports of similar activity [21,22,24]. Ewenighi et al. [24] showed that treatment with *C. citratus* extract over the course of several weeks significantly reduced body weight, blood glucose, TG, T-chol, and LDL levels in diabetic rats. The treated animals also exhibited significantly higher HDL levels than untreated control animals. In a similar study, Elson et al. [25] reported the hypocholesterolemic effect of *C. citratus* essential oil in human subjects. In their study, Middleton and Hui [22] concluded that the action of *C. citratus* on hepatic HMG-CoA reductase is independent of the diurnal cycle of the enzyme and is independent of hormones likes insulin, glucagon, glucocorticoids, and triiodothyronine. It is also thought that the cholesterol-lowering potential of *C. citratus* extract may be ascribed to the modification of intestinal cholesterol uptake, increased conversion of cholesterol to bile acids, and increased biliary excretion [20].

Table 4. Effects of low, medium, and high doses of *C. citratus* extract on plasma glucose, glycosylated hemoglobin and HMG-CoA reductase

Measured parameters	Control	HDL	HDL + STZ	HLD + STZ + CCE (250mg/kg)	HLD + STZ + CCE(500mg/kg)	HLD + STZ + CCE(1000mg/kg)	HLD + STZ + Atorvastatin(5 mg/kg) +Glibenclamide (600 µg/kg)
Plasma glucose (mmol/L)	6.45±1.01	9.32±1.02	13.06±1.52[a]	10.36±105[a]	9.82±1.01[a]	7.16±2.08	7.04±1.12
Bood $HbA1_c$ (%)	5.65±1.22	6.21±1.52	8.64±02.51[ab]	7.02±2.14	7.67±2.02	6.84±1.52	6.02±1.46
HMG-CoA reductase (ratio of HMG-CoA/mevalonate)	4.24±1.02	6.8±1.01	8.02±2.06	6.04±1.04	4.02±1.03	3.96±1.32	4.01±1.24

a=significantly different from group a (normal control); b=significantly different from group b (HLD control); c=significantly different from group c (HLD + STZ control). Values are reported as mean ± standard deviation

Table 5. Effects of low, medium, and high dose of *C. citratus* extract on plasma lipid profile and atherogenic index in plasma (AIP)

Measured parameters	Control	HLD	HLD + STZ	HLD + STZ + CCE (250 mg/kg)	HLD + STZ + CCE (500mg/kg)	HLD + STZ + CCE (1000 mg/kg)	HLD + STZ + Atorvastatin (5 mg/kg) + Glibenclamide (600 µg/kg)
Triglyceride (TG) (mmol/L)	3.02±0.02	10.64±1.02[a]	10.96±1.06[a]	8.24±0.98[abc]	5.40±0.72[abc]	6.02±0.83[abc]	5.00±0.62[abc]
HDL (mmol/L)	1.53±0.05	1.18±0.02[a]	1.02±0.08[ab]	1.68±0.03[abc]	1.86±0.12[abc]	1.95±0.15[abc]	1.98±0.12[abc]
LDL (mmol/L)	0.86±0.03	3.64±0.14[a]	2.02±0.01[ab]	1.08±0.06[abc]	1.06±0.07[abc]	1.04±0.05[abc]	0.98±0.004[abc]
VLDL (mmol/L)	0.69±0.01	1.98±0.06[a]	1.64±0.09[ab]	1.02±0.12[abc]	1.04±0.14[abc]	0.98±0.06[abc]	0.86±0.04[abc]
Cholesterol (mmol/L)	2.8±0.12	6.04±1.05[a]	5.06±1.02[a]	4.64±0.92[a]	4.12±0.96[a]	3.08±0.14[b]	3.02±0.11[b]
Atherogenic index (logTG/HDL-C)	0.30±0.01	0.96±0.02[a]	1.03±0.01[ab]	0.69±0.04[abc]	0.58±0.01[ab]	0.49±0.01[abc]	0.40±0.02[bc]

a=significantly different from group a (normal control); b=significantly different from group b (HLD control); c=significantly different from group c (HLD + STZ control). Values are reported as mean ± standard deviation

Table 6. Effects of low, medium, and high doses of *C. citratus* extract on hematological indices, serum electrolytes, food intake, and body and liver weight

Measured parameters	Control	HLD	HLD + STZ	HLD + STZ + CCE (250 mg/kg)	HLD + STZ + CCE (500 mg/kg)	HLD + STZ + CCE(1000mg/kg)	HLD + STZ + Atorvastatin (5 mg/kg) + Glibenclamide (600µg/kg)
Body weight (g)	160±4.21	180±6.17[a]	172 ± 5.22	176±4.72[a]	155±2.85[abc-]	153±5.96[abc]	158±2.47
Food intake/rat/day (g)	16.32±1.22	18.02±2.51	19.06 ±3.63	18.2±2.88	16.02±3.02	17.05±3.44	18.06±2022
Liver weight (g)	3.6±0.22	3.8±0.34	3.61 ± 0.82	3.58±0.63	3.54±0.74	3.45±0.85	3.62±0.92
Hematological indices							
PCV (%)							
RBC (mm^3)	38.34±3.14	38.87±3.05	37.02 ±2.71	37.08±2.82	38.42 ± 2.88	39.02 ± 3.02	38.01±3.11
WBC (mm^3)	4.38±0.42	4.20±0.53	4.42 ± 0.61	4.61±0.38	4.7 ± 0.12	4.30 ± 0.22	4.4±0.28
	2.64±0.06	2.46±0.01[a]	2.48 ± 0.02[a]	2.67±0.01[bc]	2.86 ± 0.05[abc]	2.02 ± 0.01[abc]	2.34±0.03[abc]
Serum electrolyte							
Na^+ (mmol/L)	140.0±2.85	142±2.77	140.00±3.11	138.00±2.57	144.00 ± 2.18	142.00 ± 2.95	142.00±2.87
K^+ (mmol/L)	4.02±0.81	4.28±0.98	3.88 ±0.72	3.86±0.82	4.40 ± 0.79	4.00 ± 0.83	4.3±0.97
Cl^- (mmol/L)	98.06±5.22	99.42±4.31	97.00±4.22	98.00±3.22	97.00 ± 3.14	99.04 ± 3.58	97.00±3.82

a=significantly different from group a (normal control); b=significantly different from group b (HLD control); c=significantly different from group c (HLD + STZ control), bc= significantly different for HDL and HLD +STZ. Values are reported as mean ± standard deviation

Table 7. Effects of low, medium and high doses of *C. citratus* extract on serum urea, creatinine, liver enzymes, and proteins

Measured parameters	Control	HLD	HLD + STZ	HLD + STZ + CCE (250 mg/kg)	HLD + STZ + CCE (500 mg/kg)	HLD + STZ + CCE (1000 mg/kg)	HLD + STZ +atorvastatin (5 mg/kg)+ glibenclamide (600 µg/kg)
Liver enzymes							
AST (µ/L)	14.00±1.20	13.8±1.41	16.00±1.22	15.06±1.01	17.00±1.05	16.00±1.07	18.00±1.02
ALT (µ/L)	17.00±2.15	18.00±2.27	17.00±2.31	18.00±2.45	18.00±2.52	19.00±2.28	17.00±2.89
ALP (µ/L)	45.00±3.55	47.00±4.22	48.00±4.01	47.00±3.47	46.00±3.22	48.00±3.82	47.00±2.93
Organic substances							
Total protein (g/L)	38.20±4.31	50.08±5.22	43.06±4.02	57.00±3.56[a]	56.00±4.22[a]	57.00±5.01[a]	56.00±3.88[a]
Albumin (g/L)	27.18±2.02	28.00±1.93	27.00±1.54	29.00±1.92	26.00±1.6	28.00±1.78	29.00±1.63
Globulin (g/L)	25.02±1.47	26±1.25	25.00±1.01	26.00±1.52	28.00±1.08	27.00±1.92	30.00±1.45[ac]
Urea (mmol/L)	4.40±0.52	5.8±0.18[a]	5.7±0.22[a]	5.40±0.31	5.60±0.27	5.8±0.22[a]	4.8±0.21[b]
Creatinine (mmol/L)	62.52±3.88	68.0±4.22	78.02±2.15[a]	74.00±3.08[a]	70.00±2.22[c]	73.00±2.51[a]	72.00±1.01[ac]

[a]=significantly different from group a (normal control); [b]=significantly different from group b (HLD control); [c]=significantly different from group c (HLD + STZ control). Values are reported as mean ± standard deviation

Several of the *C. citratus* bioactive constituents like flavonoids, tannins, saponins, polyphenols, alkaloids, and vitamins A, C, and E, have recognized antioxidative activities that are likely to contribute to their anti-diabetic and lipid-lowering effects. It has been demonstrated that flavonoids stimulate the restoration of damaged beta cells in diabetic rats and enhance insulin secretion [26]. Flavonoids and other *C. citratus* bioactives were also shown to exert a cardioprotective effect by inhibiting the oxidation of LDL-C, which is a critical step in atherogenesis [27,28]. Similarly, saponin reduces gastrointestinal uptake of glucose and cholesterol via intraluminal physiochemical reaction, leading to hypoglycemia and hypocholesterolemia [29,30]. Likewise, tannic acid stimulates glucose transport by activating the insulin-mediated signaling pathway [31] and can also prevent adipocyte differentiation by inhibiting the expression of key adipogenesis genes [32]. Tannins have also been shown to inhibit the expression of the peroxisome proliferator-activated receptor (PPAR)-y gene, which is an essential gene for adipogenesis [32,33]. Other diabetes mellitus-associated proteins that have documented interactions with *C. citratus* phytoconstituents include: protein tyrosine phosphate 1β (PTP 1β) [34] and retinaldehyde hydrogenase [35]. Modulation of these proteins is associated with alleviation of hyperglycemia in diabetic patients [33,36].

Vitamins A and C have demonstrable antioxidant activity that may contribute to the anti-dyslipidemic activity of *C. citratus*. Vitamin C supplementation reduces fasting plasma glucose levels and glycosylated hemoglobin in diabetic patients. It is also posited that vitamin C preserves and improves β-cell mass and insulin content [37]. Finally, supplementation with vitamins A and C decreases lipid peroxidation and increases the activity of anti-oxidant enzymes [38,39]. The presence of these bioactive substances in *C. citratus* may explain why its treatment can increase the levels of key antioxidant enzymes (superoxide dismutase [SOD], catalase [CAT], glutathione peroxidase [GPx]) and can decrease the level of lipid peroxidase in animals with induced oxidative stress [40].

Oxidative stress plays an important role in the pathologies leading to diabetes mellitus, including enhanced lipid peroxidation, insulin resistance, lipolysis, beta-oxidation of fatty acids, and dyslipidemia. Each of these metabolic perturbations can be reduced by preventing the formation of free radicals in tissues [41]. Mechanistically, production of free radicals decreases the activities of the enzymes associated with these perturbations and vice versa [42]. *C. citratus* should therefore dampen these perturbations through its antioxidant activities. Furthermore, the activity of *C. citratus* to scavenge reactive oxygen species (ROS) should contribute to this activity. Indeed, plant-derived antioxidants with ROS scavenging activities may have broad therapeutic applicability for oxidative stress-related diseases, particularly diabetes mellitus [42].

In the current study, we observed an inverse relation between the dose of the extract administered and the body weight of the experimental animals (Table 6). Importantly, we did not observe differences in food intake among any of the groups, underscoring that the weight reducing effect was attributable to the *C. citratus* active components. Several other studies have also reported that *C. citratus* can affect weight gain. A study by Thayalini et al [43] showed that broilers fed *C. citratus* had decreased body weight, and that the weight decrease correlated with the amount of *C. citratus* ingested. Similarly, Ewenighi et al. [24] observed a similar effect in diabetic rats treated with *C. citratus* extract. The consistent findings from multiple studies provide significant evidence for the anti-obesogenic effects of *C. citratus* extract [43-45]. An anti-obesogenic effect would be beneficial for diabetes management because obesity and dyslipidemia are central to the problem of diabetes in

both men and women. Evidently, weight loss can reduce hyperglycemia, dyslipidemia, and hypertension, and vice versa.

We also observed that the liver weights were decreased by *C. citratus* extract treatment in diabetic dyslipidemic rats, perhaps because the extract inhibited either cholesterol deposition in the liver tissues or hepatic HMG CoA reductase activity [23]. There is evidence that *C. citratus* extract can lower blood cholesterol level by acting as a bile acid sequestrant. This would cause the liver to synthesize and excrete more bile acid, utilizing more blood and liver cholesterol in the process [20]. The lack of a significant change in the plasma levels of liver enzymes associated with *C. citratus* treatment (Table 7) is consistent with findings by previous investigators [40] and was attributed in part to the hepato-protective antioxidative effect of the extract. Oxidative stress has been shown to be associated with destruction of hepatic cells leading to a significant increase in levels of these enzymes and vice versa [40].

A significant increase in total serum protein levels were also observed in the treated groups compared to the control groups, although the albumin and globulin sub-fractions were not significantly different between the two groups (Table 7). This finding is consistent with a report by Arhogro et al. [40] in which rats treated with *C. citratus* leaf extract had a dose- and time-dependent increase in plasma protein compared to untreated groups. This effect can be attributed in part to the rich protein content of the leaf extract, as demonstrated in the present study. By extension, the increased plasma protein could have increased ureagenesis in the liver [46], explaining the increase in plasma urea that we observed.

4. CONCLUSION

In conclusion, the results of our study indicate that *C. citratus* extract had an ameliorative effect on hyperlipidemia, obesity, diabetes mellitus, and atherogenic index of plasma and may be potential therapeutic agent for atherogenic cardiovascular diseases.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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