<u>**Research Article**</u>

Cymbopogon citratus Stapf (DC) extracts alleviate gasoline vapour-induced metabolic disorders and cardiovascular disease risk in rats

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Abstract: Alternative therapies for the alleviation of chemical/xenobiotic-induced metabolic aberrations and risk factors for cardiovascular disease have been inadequately explored, despite their rich cardiovascular protective bioactive constituents. The aim of the present study was to accurately evaluate the effect of *Cymbopogon citratus* extract on gasoline-induced oxidative stress, hyperglycaemia, hyperuricemia, dyslipidaemia, and atherogenic index of plasma (AIP) in rats. Seventy-two female Wistar albino rats were segregated into six groups (n = 12 per group). The rats in group 1 served as controls, while animals in group 2 were exposed to gasoline vapour (GV) alone for 35 days. Rats in groups 3, 4, and 5 were exposed to GV and co-administered 500 mg/kg, 1000 mg/kg, and 1500 mg/kg *C. citratus* leaf extract, respectively, once daily for 14 days. Rats in group 6 were exposed to GV and co-administered 200 mg/kg vitamin C for the same time period. The results showed that group 2 had significantly higher glycaemic levels, lipid parameters (TG, T-chol, and LDL-C), uric acid (UA), MDA, and AIP and significantly lower ($p < 0.05$) HDL-C than group 1. Co-administration of *C. citratus* leaf extracts in groups 3, 4, and 5 and vitamin C to group 6 significantly lowered blood glucose levels, UA, lipid parameters MDA and AIP and simultaneously increased serum HDL-C in a dose-dependent manner. *C. citratus* leaf extract has an ameliorative effect against GV-induced oxidative stress, hyperglycaemia, hyperuricaemia, dyslipidaemia, and AIP, probably mediated through its varied bio – constituents and their activities.
Keywords: lemongrass extract, lipid peroxidation, atherosclerosis, cardiovascular risk, rat

INTRODUCTION

Gasoline is a colourless to pale brown or pink liquid. It is produced by distillation, cracking, and reforming of crude oil. Gasoline has wide domestic and industrial applications, including as a fuel in spark ignition and internal combustion engines in automobiles, trucks, and aircraft. It is a volatile liquid that evaporates easily into the atmosphere, constituting a significant environmental pollutant and hazard to the general population and in particular, to those who are occupationally exposed [1].

Acute and chronic exposure to gasoline compounds is associated with several systemic adverse health effects, including abnormalities in serum blood sugar levels, and lipid parameters (dyslipidaemia), leading to an increased risk of atherosclerotic cardiovascular diseases [2].

Available data indicate that dyslipidaemia constitutes a considerable risk factor for coronary artery disease (CAD). Dyslipidaemia is characterized by significant increases in serum levels of low-density lipoprotein cholesterol (LDL-C), triglyceride (TG), and total cholesterol (T-Chol) and low levels of high-

density cholesterol (HDL-C). Previous studies have shown that exposure to gasoline may be associated with dyslipidaemia, and thus may constitute a significant risk factor for atherosclerosis and cardiovascular diseases [2]. Several studies [3] have indicated that exposure to gasoline vapour (GV) leads to significant increases in serum levels of TG, T-Chol, LDL-C, and VLDC, and a significant decrease in serum HDL-C, as well as a corresponding increase in the TG/HDL-C ratio, otherwise known as the atherogenic index of plasma (AIP). AIP is strongly associated with increased atherosclerotic cardiovascular disease risk in the general population. In fact, it is the most useful index for predicting and quantifying coronary artery disease (CAD) risk and morbidity and mortality in those affected [4]. The pathophysiological mechanisms underlying gasoline-induced dyslipidaemia have been postulated to include induction of oxidative stress (OS) [5], and increased serum levels of C-reactive protein [6]. OS is associated with the generation of free radicals, and induction of oxidative changes in low density lipoprotein cholesterol (LDL-C) particles that initiate and promote atherosclerotic changes. Current understanding supports the use of drugs with anti-

oxidative as well as anti lipid effects to mitigate gasoline-induced dyslipidaemia and consequences.

The use of synthetic lipid lowering drugs, such as fibrates and statins, has been very effective; however, they are associated with adverse health effects, such as myopathy [7], hepatotoxicity [8], and drug-drug interactions [9], which limit their use. Furthermore, long-term use of fibrates in dyslipidaemic patients is associated with increased plasma selenium [10]. High selenium levels (above the physiological limit) have been linked to hypertension [11], insulin resistance, obesity [12, 13, 14], hyperglycaemia [15], and diabetes mellitus. In addition, in some poor communities and developing countries, these drugs may not be accessible and/or affordable. Furthermore, synthetic anti-lipid and antioxidant drugs may not address the pleotropic effects of gasoline metabolite-induced dyslipidaemia, and complications such as hyperglycaemia and atherosclerotic cardiovascular disease, mandating the use of multiple drug therapies, which are associated with additional complications. Often, patients who are dissatisfied with their current treatments will seek alternative treatments [16]. Therefore, despite the large number of modern synthetic drugs, people see using complementary and alternative medicine (CAM) to prevent and treat illnesses as an attractive preposition. Thus, the search for natural products with bioactive constituents that can address health concerns is currently garnering increased attention. Many patients, healthcare providers, and environmental and biochemical toxicologists are fervently searching for CAM options to treat metabolic maladies. In one such search, Sirotkin et al. [17] found that medicinal plants can regulate ovarian cell function and alleviate the toxic effects of gasoline (benzene, toluene, ethylbenzene, and xylene [BTEX]) compounds on ovarian cells. Several reports [18, 19, 20] have demonstrated the feasibility of using plant products to treat hyperglycaemia, dyslipidaemia, and causative factors. Natural plant products are known to contain several bioactive constituents that can exert multiple anti-dyslipidaemic/anti-atherosclerotic and hypoglycaemic actions, such as antioxidative stress [21], radical scavenging [22], anti-inflammatory, and immune-modulating [23] activities, as well modify the genes of target cells and hormone secretion [24]. One such plant that has been explored for its anti-dyslipidaemic, hypoglycaemic, and antioxidant effects is *Cymbopogon citratus* (*C. citratus*) [20].

C. citratus (lemongrass) is a perennial tropical plant belonging to the Poaceae family that is known to have numerous bioactive constituents with the potential to prevent and treat various diseases through their antioxidant, anti-inflammatory, anti-protozoal, anti-fungal, anti-carcinogenic, anti-platelet aggregation, anti-bacterial, anti-tussive, anti-diabetic, and anti-malarial effects. It has also been used to treat gastrointestinal disturbances, anxiety, flu, fever, pneumonia, and hypertension. However, whether *C. citratus* extract can alleviate gasoline-induced CVD risk factors remains undocumented. In view of the National and International recommendation to increase fruit and vegetable intake, there is the need to evaluate the relation between intake of these foods and CVD protective effect [25]. Therefore, the aim of the present study was to evaluate the effect of *C. citratus* leaf extract on GV-induced hyperglycaemia, hyperuricaemia, dyslipidaemia and AIP in rats.

MATERIALS AND METHODS
Selection and Segregation of Animals

The experimental animals were 72 matured female Wister albino rats weighing 180-200g. The animals were obtained from the animal house at Faculty of Basic Medical sciences, University of Uyo, Nigeria. They were acclimatized for one week before starting the experiment. Each animal was housed in a standard wooden cage with wood shavings as bedding, which was regularly replaced. The experiment was performed under standard laboratory conditions (at room temperature [28 ± 8°C], 45% humidity, with a 12-h light/dark cycle). They were randomly divided into six groups with 12 rats per group (n=12 per group): rats in group 1 (G1) served as the control (unexposed), rats in group2 (G2) served as exposed control, while rats in groups 3,4,5 and 6 served as test groups.

All research protocols were performed at the University of Uyo according to Nigerian and international laws (Revised Helsinki Declaration, 2008) governing the acceptable use of laboratory animals.

Collection and Preparation of C. citratus Leaf Extracts

A day prior to utilization, fresh *C. citratus* leaves were obtained from an agricultural farm in Uyo, Akwa Ibom State Nigeria. Identification and authentication was performed by a taxonomist (ID UHH 4686/Uyo) in the Department of Botany at the University of Uyo. The leaves were rinsed, sundried, and pulverized into powder using an electric blender to provide 400 g of material. The powder was soaked with 4 L of hot water in a conical flask and allowed to stand for approximately 10 h. After filtering the solution through Whatman No. 2 filter paper, the filtrate was evaporated to dryness by heating in a water bath at 40°C. The final solid extract was weighed with an electric balance (ACS-2E14; Surgifriend Medicals, Ltd., England), with a total yield of 30%. The prepared extract was stored in glass bottles at 4°C and was dissolved in physiological saline at 100 mg/mL. The phytochemical analysis of the leaf extracts was carried out using standard procedures to determine the levels of tannins, phenolics, saponins, alkaloids, deoxysugars, and anthraquinones [26, 27].

The median lethal dose (LD_{50}) was determined using standard methods [28]. From the LD_{50} (5000 mg/kg), the low, medium, and high doses were calculated using a standard formula as 500 mg/kg, 1000 mg/kg, and 1500 mg/kg, respectively.

Exposure to GV

The animals in the test groups were exposed to unleaded gasoline purchased from a Nigerian National Petroleum Cooperation (NNPC) refuelling station on Itam-Ikot Ekpene Road in Uyo, Nigeria. The rats in the exposed groups were housed in their cages and exposed to GV in an exposure chamber ($80 \times 60 \times 100$ cm). Rats in the unexposed control group were kept in a GV-free section of the experimental house. Two calibrated 500-mL beakers each containing 100 mL of petrol were put in the modified chamber where the exposed groups were placed, and then the rats were allowed to inhale the GV in the chamber for 6 h (9 am–3 pm) daily for 35 consecutive days. At the end of the exposure period, the gasoline was removed; the initial and final volumes were recorded before and after exposure, respectively. The daily difference in volume was used to estimate the relative vapour exposure. The average exposure was approximately 80 mL/day.

Experimental treatment

Rats in groups 3,4, 5 and 6 were in addition to being exposed to GV, co-treated with 500mg/kg, 1000mg/kg and 1500mg/kg of *C. Citrates* extracts and 200mg/kg of vitamin C respectively. All animals were fed normal rat pellets and allowed free access to food and water throughout the experimental period. The *C. citratus* leaf extracts and water-solubilized vitamin C were administered by oral gavage (using an intragastric syringe) for the final 14 days of the 35-day GV exposure.

Serum bio-chemical Analysis

Serum glucose level and lipid sub-fractions including total cholesterol (T.chol), Low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C) and triglyceride (TG) were determined using a multi-channel automated system (Lipid pro TM, Model KM–001A: Info Pia Co, Ltd. South Korea). Serum uric acid (UA) levels were measured using a multi-channel automated analyser (SYNCHRON, LOS ANGELES, CA).

Statistical Analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS), version 20.0.

Data obtained were analysed using descriptive statistics and reported as the mean ± standard deviation (SD). Analysis of variance (ANOVA) was also used. Duncan's multiple range test was used to compare results between groups, determine the direction of significance, and to analyse the effect of the *C. citratus* leaf extracts on the reproductive toxicity of GV. Differences with *p* values less than 0.05 were considered statistically significant.

RESULTS

Phytochemical analysis of *C. citratus* leaf extract showed the presence of moderate concentrations of tannins, saponins, flavonoids, and phenols and low levels of anthraquinone. Table 1 shows the ameliorative effect of *C. citratus* extract on serum lipid profile markers of GV-induced dyslipidaemic models in rats.

Figures 1, 2, 3 and 4 show the ameliorative effects of C. citratus leaf extracts on serum glucose, malondialdehyde, uric acid and atherogenic index of plasma (AIP) in GV-induced hyperglycaemic, lipid peroxidation, hyperuricaemic and dyslipidaemic models in rats respectively.

Table 1: The ameliorative effect of *C. citratus* extracts on serum lipid profile markers of gasoline vapour-induced dyslipidemic models in rats.

Groups	T-Chol (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	TL (mg/dl)
1	67.50 ± 4.84^b	43.33 ± 7.03^a	29.00 ± 3.27^b	43.17 ± 14.18^a	91.83 ± 8.89^b
2	72.50 ± 3.54^c	69.00 ± 4.24^b	18.00 ± 3.22^a	63.50 ± 10.61^a	105.50 ± 3.54^c
3	72.33 ± 3.56^c	52.33 ± 7.31^c	20.10 ± 1.27^a	48.33 ± 9.85^a	97.33 ± 2.88^b
4	65.33 ± 2.07^b	56.50 ± 3.44^c	23.25 ± 3.44^a	43.33 ± 7.94^a	85.83 ± 4.88^a
5	55.67 ± 4.32^a	56.17 ± 4.79^c	25.32 ± 1.59^b	33.33 ± 15.19^a	92.33 ± 7.53^b
6	57.50 ± 3.62^a	47.50 ± 3.02^b	27.23 ± 2.88^b	34.50 ± 5.01^a	77.33 ± 12.24^a

The different superscripts are significantly different from control at 5% (p < 0.05)
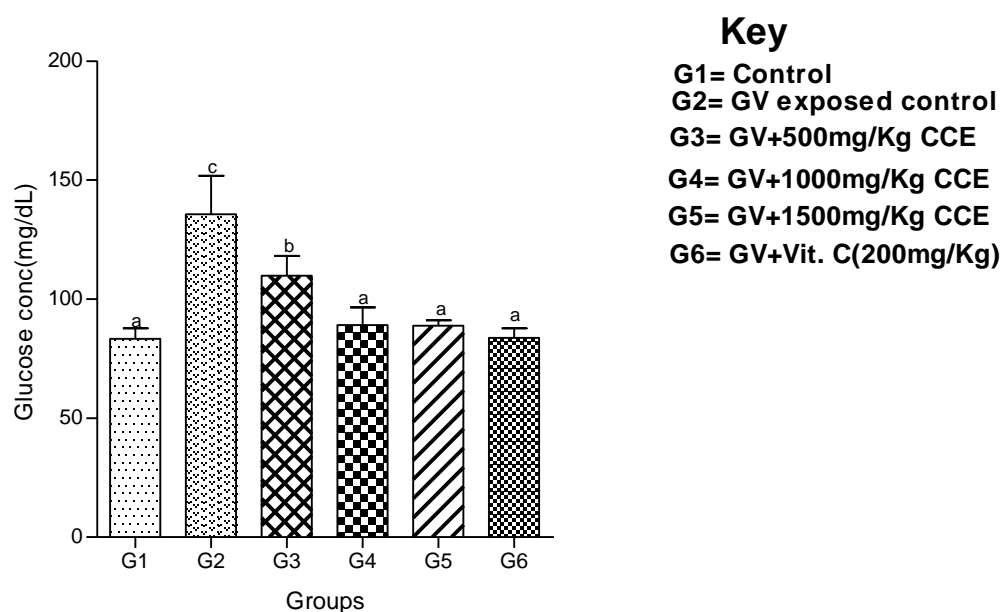
Fig-1: Effect of *C. citractus* Extract (CCE) and Vitamin C Supplementation on GV-induced Hyperglycaemia (mg/dl).
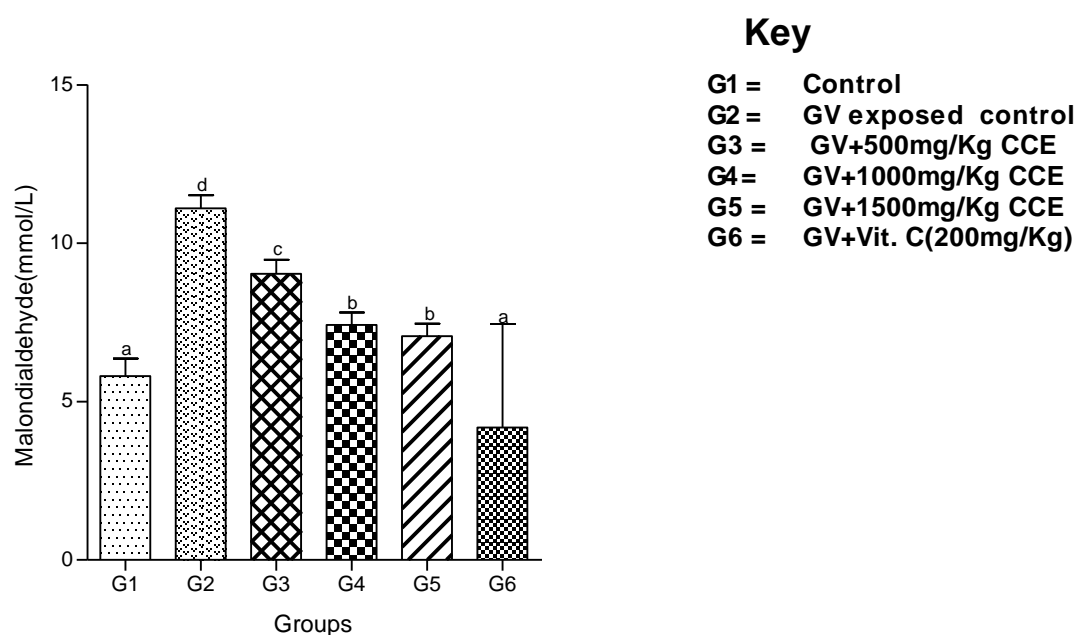

Fig-2: Effect of *C. citractus* Extract (CCE) and Vitamin C Supplementation on GV-induced increase Serum Malondialdehyde (mmol/L).
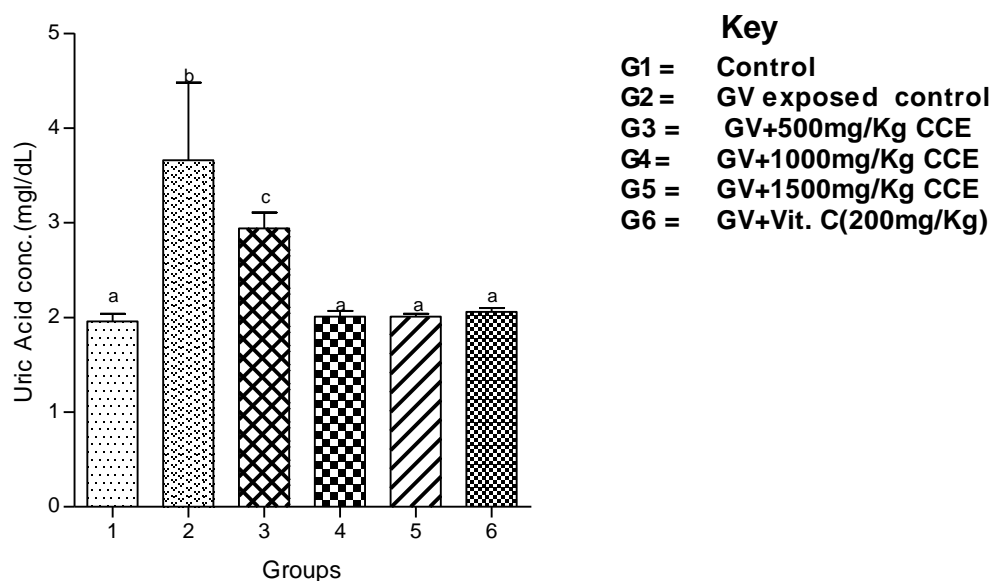
Fig-3: Effect of *C. citractus* Extract (CCE) and Vitamin C Supplementation on GV-induced increase Serum Uric Acid Concentration (mg/dl).
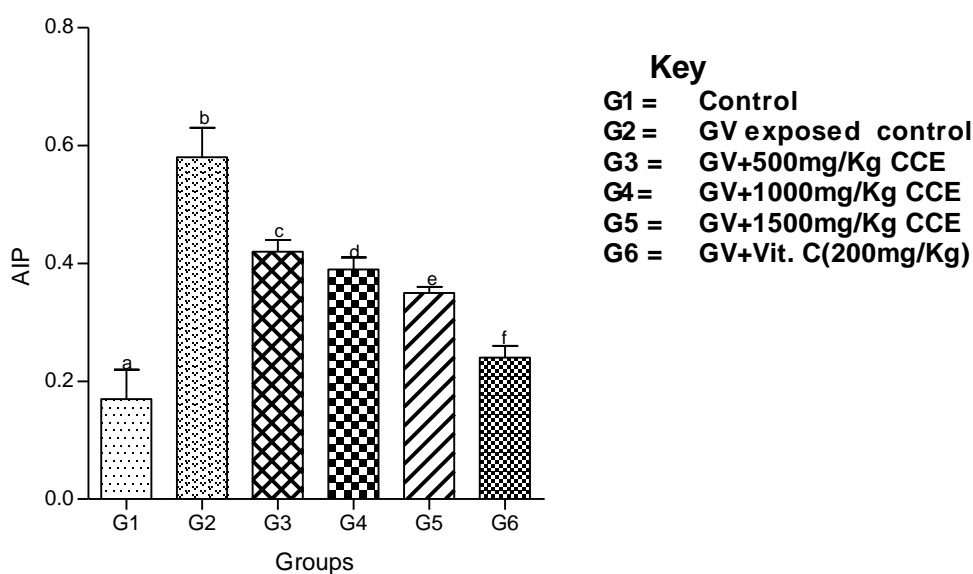

Fig- 4: Effect of *C. citractus* Extract (CCE) and Vitamin C Supplementation on GV-induced increase Atherogenic Index of Plasma (AIP).

DISCUSSION

The results from the present study showed that exposure to GV alone significantly increased serum glucose, UA, TG, LDL-C, T-chol, MDA, and AIP, and significantly decreased serum HDL-C.

When rats in group 3, group 4, and group 5 were co-administered 500 mg/kg, 1000 mg/kg, and 1500 mg/kg of *C. citratus* leaf extract, respectively, we observed significant dose-dependent decreases in the aforementioned lipid parameters, blood glucose, UA, MDA, and AIP, and a significant increase in HDL-C. These changes were similar to those observed in rats

(group 6) co-administered vitamin C (an antioxidant reference). The present results highlight the etiopathogenic role of oxidative stress in GV-induced dyslipidaemia, hyperglycaemia, hyperuricaemia and atherosclerosis, as was previously documented [29, 3, 2].

Numerous established studies have confirmed that GV is readily absorbed when inhaled, and after a series of biotransformative and toxicokinetic processes, the GV is transformed into reactive metabolites, leading to the generation of toxic reactive oxygen species (ROS), such as superoxide (O^-), hydroxyl radical (OH^-),

hydrogen peroxide (H$_2$O$_2$), peroxyl hydroxyl, and organic hydrogen radical [30, 2]. These reactive oxygen intermediates are known to induce oxidative stress that is a critical pathophysiological step in GV-induced abnormalities in serum lipid parameters (dyslipidaemia) [31], such as significantly increased levels of serum glucose, LDL-C, VLDL-C, TG, and T-chol and a simultaneous decrease in HDL-C, as was observed in the present study. The mechanisms underlying GV-induced dyslipidaemia and subsequent atherosclerosis may include, in part, the inactivation of metabolic enzymes and damage to important cellular components and oxidation of nucleic acids [30]. These are associated with disordered protein, nucleic acid, carbohydrate, and lipid metabolism, leading to alterations in intracellular and extracellular homeostasis and subsequent loss of cell integrity, enzyme function, and genomic stability [32]. For example, enhanced lipid peroxidation [31], including the oxidation of LDL-C, is a necessary step in the development of atherogenesis [33]. GV-induced dyslipidaemia has also been thought to be due to GV-induced liver toxicity, since the liver functions in lipid metabolism and homeostasis [2]. Interestingly, these processes can be mitigated by the use of nutritional antioxidants [30], particularly green leafy vegetables and vitamin-rich fruits [34, 35] such as *Cymbopogon citratus* [36, 37, 38, 20], which is known to decrease the toxic events of lipid peroxidation in both serum and heart tissue [39]. The significant increase in serum levels of MDA in rats exposed to GV alone (G2) indicates increased lipid peroxidation, a reaction between free radicals and polyunsaturated fatty acids in the cell membrane [31] following oxidative stress.

The significant dose-dependent decreases in some lipid sub-fractions (LDL-C, TG, and T-chol), the lipid peroxidation marker MDA, and CAD-risk indicator AIP, and the simultaneous increase in the good lipid sub-fraction (HDL-C) in groups co-administered *C. citratus* leaf extracts or vitamin C compared with the corresponding levels in the control group confirmed the antioxidant effect of *C. citratus* leaf extract, which is likely due to its rich and varied antioxidant constituents and their activities, as was reported in previous studies [40]. Several of the *C. citratus* constituents, including some phytochemicals (flavonoids, tannins, polyphenols, alkaloid, and saponins), vitamins (A, C, and E), and minerals and trace elements (zinc, selenium, magnesium, and copper) [20], have documented antioxidant activities that likely contribute to their anti-dyslipidaemic, hypouricaemic, and hypoglycaemic effects [41,42,43]. In our earlier study [20] on the effect of *C. citratus* leaf extract on AIP in diabetic dyslipidaemic animals, administration of *C. citratus* extract dose-dependently abated the rise in serum levels of T-chol, LDL-C, VLDL-C, β-hydroxyl β-methyl glutaryl-CoA (HMGCoA) reductase, and AIP and simultaneously increased HDL-C. These results support those of a previous study by Adeneye

and Agbaje [44], which was designed to investigate the hypoglycaemic and hypolipidaemic effects of *C. citratus*; the authors found that *C. citratus* leaf extract lowered fasting plasma glucose and lipid parameters dose-dependently, while raising plasma HDL-C. Accordingly, Ewenighi et al. [45] found that a four-week treatment with *C. citratus* leaf extracts mitigated the increases levels in serum TG, T-chol, and LDL-C in diabetic animals compared with the levels in the untreated group. In a parallel study, Agbafor et al. [46] confirmed the anti-dyslipidaemic effect of *C. citratus* leaf extract, which was traceable to its phytochemical constituents. In addition, the presence/effect of these bioactive antioxidants in *C. citratus* leaf have been used to abate drug/xenobiotic-induced oxidative stress and adverse health endpoints, leading to increased levels of the key antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) and simultaneously decreasing the level of the lipid peroxidation marker MDA [40]. Koh et al. [47] found that prophylactic treatment with *C. citratus* dose-dependently ameliorated carbon tetrachloride-mediated hepatic oxidative damage in animals. This effect was attributable to the antioxidant and free radical-scavenging properties of the extract. In a parallel study, Ojo et al. [36] reported the antioxidative properties of *C. citratus* in paracetamol-induced oxidative stress in animals. Likewise, a study by Olorunsanya et al. [37] reported that *C. citratus* inhibited lipid oxidation in raw pork patties under refrigeration. Besides the antioxidant effect of some *C. citratus* constituents, the essential oil (EO) constituents myrcene, geraniol, linalool limonene, β-ionone, and citral (α and β) have been shown to possess antioxidant activities in human cells [48] and cholesterol-reducing activities in mice [49,50]. Soares et al. [51] compared the antioxidant capacity of *C. citratus* EO with that of a synthetic antioxidant butylated hydroxytoluene (BHT), and found that *C. citratus* EO has greater antioxidant activity than the methanolic extracts and similar antioxidant activity to that of BHT. Several other authors have reported the antioxidant activity of *C. citratus* EO [52,53,54,55] and evaluated the application of *C. citratus* EO as a replacement for the commercial antioxidant BHT in food preservation. In addition, citral, an EO constituent, has been shown to lower serum cholesterol and inhibit lipogenesis by post-transcriptionally inhibiting HMG-CoA reductase, an enzyme that catalyzes the rate-limiting step of cholesterol biosynthesis and a major cellular target of many anti-dyslipidaemic drugs [56]. These rich antioxidant constituents of *C. citratus* leaf extract not only prevent direct damage due to gasoline compounds but also may inhibit the signalling pathways leading to inflammation as a result of its anti-inflammatory actions [57,58], thereby inhibiting the inflammatory component of gasoline-induced atherosclerosis. Support for this view comes from the observation by Gbenou et al [58], that *C. citratus* EO displayed significant dose dependent anti-inflammatory,

analgesic and antipyretic effects when orally administered to rats. Furthermore, numerous established studies have confirmed the hypoglycaemic effect of *C. citratus* phytoactive constituents [59,20].

In all likelihood, the hypoglycaemic effects of these constituents are due, at least in part, to their antioxidative activities. For instance, tannic acid stimulates glucose transport and induces GLUT translocation [60]. Polyphenols stimulate insulin release from the beta cells of the pancreas and inhibit α-glucosidase [61]. In related study, Bharti et al [62] showed that *C. citratus* EO significantly decreased glycosylated haemoglobin (HbAIc) and insulin levels as well as insulin resistance and oxidative stress markers in diabetic rats, while simultaneously increasing β-cell mass and islet number.

Furthermore, exposure of the experimental animals to GV alone caused significant increase in serum level of uric acid (UA), suggesting the hyper-uricaemic effect of GV. Hyper-uricemia is an independent risk factor for essential hypertension, metabolic syndrome and cardiovascular diseases [63,64]. Other diseases associated with hyper-uricemia which could have directly or indirectly impacted cardiovascular endpoints include autoimmune, inflammatory, endocrine and matastatic disorders [4]. Interestingly, when the GV exposed animals were co-treated with C. citratus extracts, a dose-dependent significant (p<0.05) decreases in serum levels of UA were observed, and were similar to the effect of vitamin C, a reference antioxidant and hypouricaemic agent [4].

These findings are similar to those previously observed by Mirghani et al [65] and confirm the hypouricaemic effect of C. citratus leaf extract. In their overall assessment of the xanthine oxidase (XO) inhibitory potential of C. citratus extract, Mirghani et al [65] concluded that C. citratus has a high potentiality to be an alternative source of antigout drug, as it has a potential compound containing XO inhibitor. This idea is supported by the detection of high levels of polyphenol-compounds (tannins, flavonoids and phenols), electrolytes (potassium (K^+), Magnesium (Mg^{2+})), vitamin C in C. citratus leaf extracts in the present and previous studies [4]. The hypouricaemic effects of these compounds have been extensively documented [4]. For instance, polyphenol compounds have been shown to possess high XO inhibitory activity, and their ability to decrease serum UA levels has also been reported [66]. XO is an enzyme that catalyzes the hydroxylation of hypoxanthine to xanthine and UA in purine metabolic pathway [67] according to the following reaction:

$$xanthine + O_2 + H_2O - UA + 2O_z + H^+ ... \ (i)$$
$$xanthine + O_2 + H_2O - UA + H_2O \ ... (ii) [4].$$
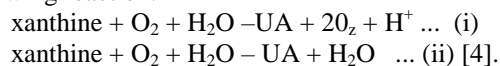
Polyphenol compounds such as flavonoids block these reactions by competitively binding to the specific active site of XO altering its stereochemistry and hence biochemical effects [4]. This inhibitory action is enhanced by the presence of Olefins at carbon 2 (C2) and Carbon 3 (C3) that maintains the planar structure of flavonoids, the hydroxyl moiety at C7 and C5, and the carbonyl group at C4 in the flavonoid structure.

The presence of these functional groups has been shown to enhance favourable hydrogen bonds and electrostatic interactions between flavonoids and the XO active site, which are the prerequisites for the potent inhibitory action of flavonoids on XO [68]. Additionally, the increase in serum UA levels in the GV alone group could , in part, be attributed to the effect of GV on renal endpoints, impairing excretion as documented previously [69,4]. The kidneys excrete about 70% of UA generated daily to maintain homeostasis [70], therefore impaired renal function can lead to increase serum UA level as observed previously following exposure to GV [69,71]. The observed improvement in the groups (G3, G4, G5) and G6 co-treated with various doses of C. Citrates extracts and vitamin C respectively, could partly be due to the ameliorative effect of C. citratus leaf extracts and vitamin C on GV induced renal function impairment as reported in previous studies [72,73].

CONCLUSION

In conclusion, *C. citratus* leaf extract has the potential to abate GV-induced hyperglycaemia, hyperuricaemia, dyslipidaemia, and atherosclerotic cardiovascular disease risk. Its action is comparable to known synthetic antioxidants, and it may possess a wider spectrum of actions due to its numerous components with various activities that can address the pleotropic effects of GV-induced detrimental health effects.

Conflicts of Interests

The authors declare that they have no competing interest.

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