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Polyphenol-rich *Ocimum gratissimum* leaf extract attenuates angiotensin-converting enzyme activity and impaired endothelial vascular function in diabetic rats

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Abstract

Endothelial vascular dysfunctions are prevalent among diabetic patients, significantly impacting their quality of life and prognosis. This study examined the effects of polyphenol-rich extracts from *Ocimum gratissimum* leaves on oxidative stress markers, serum lipid profiles, and angiotensin-converting enzyme activity in diabetic cardiac tissue. Forty *Wistar* rats (150–220 g) were divided into five groups: normal control, diabetic control, low-dose *Ocimum gratissimum* (122.46 mg/kg), medium-dose *Ocimum gratissimum* (244.98 mg/kg), and a positive control (standard diabetic drug). All rats except the normal control group were made diabetic via an intraperitoneal injection of 50 mg/kg streptozotocin, followed by 28 days of extract administration. Diabetes induction led to a significant ($p<0.05$) disruptions in lipid profiles, oxidative stress markers, and ACE activity. Treatment with *Ocimum gratissimum* extracts at both doses significantly ($p<0.05$) decreased triglyceride, low-density lipoprotein cholesterol, and very-low-density lipoprotein cholesterol levels while increasing high-density lipoprotein cholesterol. Lipid peroxidation marker, malondialdehyde, was significantly ($p<0.05$) decreased, and antioxidant enzyme activities significantly ($p<0.005$) improved. ACE activity was significantly ($p<0.05$) reduced, approaching control levels. These results suggest that the polyphenol-rich extract of *Ocimum gratissimum* exerts antioxidant and cardio-protective effects, improving vascular functions in diabetic rats by improving lipid profiles, reducing oxidative stress, and suppressing ACE activity.

Keywords: Angiotensin-converting enzyme; Diabetes; Lipid profile; *Ocimum gratissimum*; Oxidative stress

1. Introduction

Diabetes mellitus, a complex and chronic metabolic disorder marked by sustained high levels of blood sugar due to disruptions in insulin signaling pathways, is usually associated with an increased risk of various complications, including impaired vascular functions, which is among the leading causes of morbidity and mortality in diabetes. [1-3] Diabetic vascular complications are widespread and severe despite intensive glycemic control. [4,5] When exposed to risk factors such as high blood sugar, elevated blood pressure, cholesterol lipids, etc., the vascular endothelium experiences cellular damage, programmed cell death (apoptosis), and excessive deposition of extracellular matrix proteins (fibrosis) caused by the production of reactive oxygen species leading to endothelial dysfunction and impaired cardiac function. [6] Endothelial dysfunction significantly contributes to the development of diabetic vascular complications, including atherosclerosis, hypertension, microvascular damage, etc. [6,7] Therefore, targeting endothelial dysfunction is vital and crucial in preventing the development of diabetic vascular complications.

Plant-derived natural products are being extensively researched to identify potential sources of drug lead compounds [8, 9], as plant-based bioactive compounds, in contrast to synthetic alternatives, often possess significant pharmacological effects with minimal adverse side effects. [10-14] Due to its many medicinal qualities and therapeutic potential, the edible herb *Ocimum gratissimum* (OG), commonly known as African basil, which is mostly found in tropical and subtropical regions of Africa, has attracted interest in ethno-pharmacological research. Numerous studies have

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documented the pharmacological activity of *Ocimum gratissimum*, including its anti-diabetic potential. [15–22] Because of its anti-diabetic qualities, *Ocimum gratissimum* may be able to lessen the complications associated with diabetes.

This study aims to evaluate the extent to which *Ocimum gratissimum* leaf extract reduces the cardio-biochemical changes brought on by streptozotocin and a high-fat diet in a diabetic rat model. The research will specifically look into how polyphenolic compounds affect lipid profiles, endothelial function, scavenging of reactive oxygen species (ROS), and the activity of the angiotensin-converting enzyme (ACE), a key regulator of the renin-angiotensin system (RAS) that contributes to heart failure and fibrosis in diabetic patients. This will provide significant insight on *Ocimum gratissimum's* potential as a substitute for conventional treatments and could lead to the creation of more effective medications to treat the cardiovascular issues caused by diabetes mellitus.

2. Materials and Methods

2.1. Plant samples collection and preparation

Fresh leaves of *Ocimum gratissimum* were collected from the botanical garden of the faculty of pharmacy, University of Uyo town campus, and authenticated by the Department of Botany, University of Uyo. A specimen voucher number of *Ocimum gratissimum* (herbarium number 3078) was obtained from the Faculty of Pharmacy herbarium with taxonomic identification.

2.2. Preparation of polyphenol extract of *Ocimum gratissimum* leaves

The fresh leaves were washed and air dried, blended and macerated in 80% ethanol as the solvent, and kept for 72 hours. Thereafter it was filtered initially with cheesecloth and later under suction pressure with a Whatman's No. 4 filter paper. The polyphenol-rich extract of *Ocimum gratissimum* (PREOG) is collected, and the solvent is evaporated using a rotary evaporator under reduced pressure at a low temperature (40–50 °C) to concentrate the extract. The concentrated extract was further dried by lyophilization under reduced pressure to obtain a powdered polyphenol extract and stored in a closed glass container, and refrigerated at -20°C until use. Subsequently, the total phenolic content (TPC) of the extracts was evaluated using the Folin-Ciocâlteu technique as described by Singleton *et al.* [23] The absorbance of each mixture was evaluated with an ultraviolet-visible spectrophotometer (Beckman, DU 7400, USA) at 765 nm. The *Ocimum gratissimum* extract obtained was dissolved in 10% Tween 80 and given to rats according to the designed dosage.

2.3. Experimental animals

Forty (40) adult male *Wistar* rats obtained from the Faculty of Basic Medical Sciences animal house, University of Uyo, weighing 150 to 220 g, were used for the study. The animals were allowed to acclimatize for two weeks and given humane care in accordance with the principle of laboratory animal care and use. [24] The animals were housed in well-ventilated wooden cages and kept under controlled environmental conditions of 25 ± 5 °C and 12 hours' light/dark cycle. The rats were given standard chow, and water was provided *ad libitum* throughout the course of the study. All the studies conducted were approved by the faculty of basic medical sciences research and ethics committees.

2.4. High fat diet administration

The *Wistar* rats were commenced on a high-fat diet comprising the standard laboratory chow *ad libitum* and 5 ml/kg body weight of 100% pure olive oil administered by oral gavage once daily for 28 days, according to the method of Reed *et al*. [25] and Suman *et al*. [26] with slight modifications. The weights of the animals were recorded weekly for the 3 weeks, and the blood glucose levels were taken at the end of the 28-day period.

2.5. Diabetes Mellitus Induction

The animals treated with a high-fat diet were allowed a one-week resting period, then fasted overnight, weighed in the morning, and prepared for diabetes induction. Diabetes was induced by a single intraperitoneal injection of streptozotocin (STZ) at a dose of 50 mg/kg body weight, dissolved in freshly prepared citrate buffer (0.1 M, pH 4.5) in a sterile conical flask at a stock concentration of 50 mg/mL immediately before use. After 72 hours, fasting blood glucose levels were measured using an AccuCheck® glucometer, and rats with glucose levels above 200 mg/dL were considered diabetic as monitored in the blood from the tail vein.

2.6. Acute Toxicity Studies of *Ocimum gratissimum* Extract

Acute toxicity testing (LD_{50}) of the plant extract was carried out according to modified Lorke's method [27] with slight adjustments of increasing dosage from 10 mg/kg body weight to 2000 mg/kg body weight of extract. The animals were

observed for signs of toxicity, which include paw licking, weakness, feeling sleepy, respiratory distress, hyperactivity, coma, and death for the first 4 hours and subsequently 24 hours. The number of deaths in each group observed within 24 hours was recorded, and the final LD_{50} was calculated using the formula.

$$LD_{50} = \sqrt{DO \times Di}$$

Where

D_o = highest dose without mortality
D_i = lowest dose with mortality.

2.7. Experimental Design

Forty (40) experimental animals were weighed and then randomly divided into experimental groups of five (5) of eight (8) animals each. Administrations were by oral intubation once daily at a specific time between 7.00 and 8.00 am for 28 days.

- Group I: Rats were not induced and received 5 ml/kg 10% tween 80 (control).
- Group II: Induced diabetes with 50 mg/kg streptozotocin without treatment (diabetic control)
- Group III: Diabetic and administered a low dose (122.47 mg/kg/bw) of polyphenol-rich extract of *Ocimum gratissimum* leaves (LD-PREOG).
- Group IV: Diabetics and administered a medium dose (244.98 mg/kg/bw) of polyphenol-rich extract of *Ocimum gratissimum* leaves (MD-PREOG).
- Group V: Diabetic and administered 150 mg/kg Vildagliptin-Metformin (Vil-Met)

Fasting blood glucose levels of the animals were recorded weekly, and final fasting blood glucose was taken at the end of the treatment period, and then animals were sacrificed 24 hours after the last administration.

2.8. Sample collection

At the end of the experiment, rats were sacrificed under chloroform anesthesia and blood collected by cardiac puncture. Blood samples were collected into ethylenediaminetetraacetic acid (EDTA) bottles and centrifuged at 2000 rpm for 10 minutes to obtain serum. The serum was immediately extracted and refrigerated until it was needed for further analysis. Each animal's heart was collected by dissecting the thoraco-abdominal walls and cleared of all adhering tissues, washed in normal saline, and blotted dry using a filter. Gross morphometric assessments were performed. Thereafter the heart was fixed in 10% buffered formalin for tissue processing and stored at 4°C until use.

2.9. Biochemical Analysis

2.9.1. Determination of malondialdehyde level

The level of malondialdehyde (MDA), a marker of lipid peroxidation, was determined using the method described by Ohkawa *et al*. [28]. This method relies on the reaction of MDA with thiobarbituric acid (TBA) to form a pink chromogen, known as the thiobarbituric acid reactive substances (TBARS). MDA standard curves were prepared using tetraethoxypropane (TEP) at five-point concentrations from 0 to 5 nmol/mL. The sample solution was put into a 1.5 mL microtube containing 400 µl of distilled water, 200 µl of 20% trichloroacetic acid (TCA), and 400 µl of 0.67% thiobarbituric acid (TBA) were added, then incubated at 96-100 °C for 10 minutes. The mixture was kept at room temperature until there were no bubbles in the tube. The absorbance of the supernatant was read at a wavelength of 540 nm against a reference blank after centrifuging for another 10 min.

2.9.2. Determination of reduced glutathione level

The level of reduced glutathione (GSH) was determined using the method outlined by Ellman (1959) [29] based on the reaction of GSH with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), also known as Ellman's reagent. In this reaction, GSH reduces DTNB to produce 2-nitro-5-thiobenzoic acid (TNB), a yellow-colored compound that can be quantified by measuring the absorbance at 412 nm. 1.0 mL of heart homogenate was added to 0.1 mL of 25% trichloroacetic acid (TCA), and the precipitate was removed by centrifugation at 5000 g for 10 min. Supernatant (0.1 mL) was added to 2 mL of 0.6 mM DTNB prepared in 0.2 M sodium phosphate buffer (pH 8.0). The reduced chromogen is directly proportional to GSH concentration, and its absorbance can be measured at the absorbance of 412 nm using a spectrophotometer.

2.9.3. Determination of glutathione peroxidase activity

The activity of glutathione peroxidase (GPx) was determined using the method described by Haque *et al.* [30] This approach measures GPx's ability to catalyze the reduction of hydrogen peroxide or organic peroxides to water or corresponding alcohols, with glutathione (GSH) serving as the electron donor. The assay mixture consists of 1.49 ml of sodium phosphate buffer (0.1 M), 0.1 ml of EDTA (1 M), 0.1 ml of sodium azide (1 M), 0.1 ml of GSH (1 M), 0.1 ml of NADPH (0.021 M), 0.01 ml of H_2O_2, and 0.1 ml of sample in a total volume of 2 ml. The oxidation of GSH to glutathione disulfide (GSSG) by GPx is coupled with its regeneration to GSH by glutathione reductase, consuming NADPH in the process. Oxidation of NADPH is recorded by a spectrophotometer at 340 nm. GPx activity is then expressed as enzyme units per milligram of protein.

2.9.4. Determination of SOD Assay

The method described by Misra and Fridovich (1972) [31] of the indirect method of inhibition auto-oxidation of epinephrine to its adrenochrome was used to assay for the activity of superoxide dismutase. Tissue homogenate of 0.5 mL was diluted in 4.5 mL of distilled water (1:10) dilution factor. An aliquot of 0.2 mL of diluted sample was added to 2.5 mL of 0.05 M carbonate buffer (pH 10.2) to equilibrate in a spectrophotometric cuvette, and the auto-oxidation of epinephrine was initiated by adding 0.3 mL of substrate (0.3 mM Epinephrine) and 0.2 mL of distilled water. The auto-oxidation was read in a spectrophotometer at 480 nm every 30 s for 5 min. One unit of SOD activity was defined as the concentration of the enzyme (mg protein/ml) in the plasma that caused a 50% reduction in the autoxidation of epinephrine. [32] activity was defined as the conc. Of the enzyme (mg protein/ml) in the plasma that caused 50% reduction in the autoxidation of epinephrine. [32]

2.9.5. Catalase Assay Procedure

A colorimetric assay kit from Worthington Biochemical Corporation, USA, based on the principles described by Beers and Sizer (1952) [33] was used to assay for the activity of catalase. Immediately prior to use, tissue homogenate was diluted in 0.05 M phosphate buffer, pH 7.0, to obtain a rate of 0.03-0.07 absorbance/min. Spectrophotometer adjusted to 240 nm and 25 °C. Reagent-grade water (1.9 ml) and 0.059 M hydrogen peroxide (1.0 ml) were added to the cuvette and incubated in a spectrophotometer for 4-5 minutes to achieve temperature equilibration and to establish the blank rate. 0.1 ml of diluted homogenate was added, and a decrease in absorbance from 0.45 to 0.40 at 240 nm for 2-3 minutes was recorded. One unit decomposes one micromole of H_2O_2 per minute at 25 °C and pH 7.0 under the specified conditions.

2.9.6. Serum Lipid Profile Assay

Serum lipid profiles were assayed using Agappe diagnostic kits and their respective principles. High-density lipoprotein cholesterol (HDL-c) was estimated using the method described by Rifai and Warnick (1994) [34]. Low-density lipoprotein cholesterol (LDL-c) was assayed based on the principles of Crouse *et al.* [35] Very low-density lipoprotein cholesterol (VLDL-c) was estimated from the relationships established by Friedewald *et al*. [36] using triglyceride concentration. Total cholesterol (TC) was assayed based on the principle laid down by Allain *et al.* [37] Triglycerides were determined based on Bucoloy and David's (1973) [38] principle.

2.9.7. Determination of ACE Activity

The angiotensin-converting enzyme (ACE) activity was determined as described by Wanasundara *et al*. [39] based on the enzymatic hydrolysis of a synthetic substrate by ACE. Typically, ACE cleaves the substrate, hippuryl-L-histidyl-L-leucine (HHL), into hippuric acid and histidyl-leucine. The heart homogenate was centrifuged at 4°C for 15 min, and the supernatants were used for the assay. The assay was conducted in 100 mM sodium borate buffer (pH 8.3) containing 300 mM NaCl. The buffer was used to dilute samples, enzymes, and substrate. Each sample solution (50 µL) with 50 µL of 2.5 mM HHL was pre-incubated at 37 °C for 5 min, and the reaction was initiated by the addition of 50 µL of the ACE solution (50 mU/mL), and the mixture was incubated at 37 °C for 60 min. The reaction was terminated by adding 0.25 mL of 1.0 N hydrochloric acid and then 2.0 mL ethyl acetate to extract the hippuric acid formed by the action of ACE. The ethyl acetate was removed by evaporation, and the absorbance of the hippuric acid was read at 228 nm using a spectrophotometer.

2.10. Statistical Analysis

Data obtained from this study were expressed as mean ± S.D. Statistical significances were examined by one-way ANOVA followed by Tukey's multiple comparison (post-hoc test) using the software GraphPad Prism (Version 10.3). Mean values were statistically significant at $p < 0.05$.

3. Result

3.1. Medial Lethal dose of Ethanolic extract of *Ocimum gratissimum*

The *Ocimum gratissimum* extract showed no signs of toxicity at a calculated dose of 1225 mg/kg body weight. No physiological changes or mortality were observed at this dose level, indicating that it is safe and non-toxic, as indicated in Table 1.

Table 1 Medial Lethal Dose of *Ocimum gratissimum* extract

Groups (n = 3)	Dose of *Ocimum gratissimum* extract(mg/kg)	Mice Mortality
1	1000	None
2	1500	None
3	2000	Present

3.2. Effect of *Ocimum gratissimum* on fasting blood sugar

The diabetic control group had significantly higher fasting blood glucose levels ($p<0.05$) than the normal control rats, as seen in Table 2. The polyphenolic extract of *Ocimum gratissimum* significantly ($p<0.05$) lowered fasting blood glucose levels in diabetic rats, particularly in Group 4 compared to the diabetic control group (Table 3). The glucose-lowering effect of *Ocimum gratissimum* was comparable to that of vildagliptin-metformin.

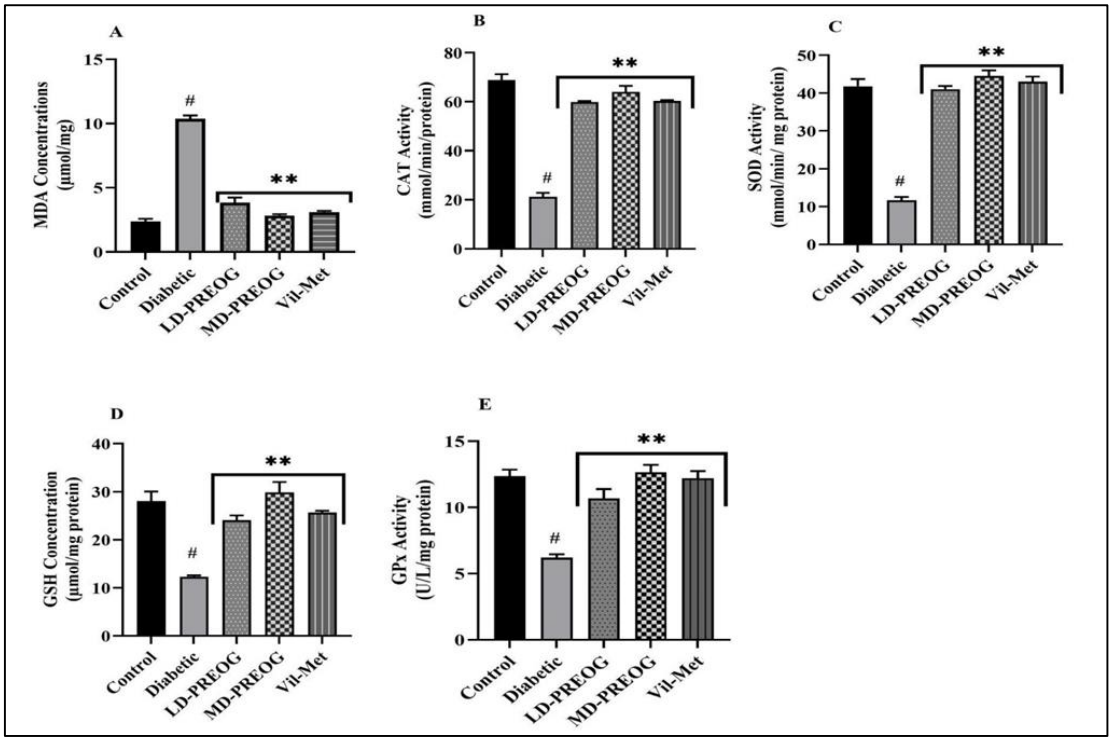
Table 2 Effect of *Ocimum gratissimum* on fasting blood sugar

FBG	Control	Diabetic	Diabetic + O.G low dose	Diabetic + O.G medium dose	Diabetic + Vildagliptin-Metformin
Initial(mg/dl)	60.00±6.59	218.6±10.91^{a}	121.00±10.75^{ab}	101 ± 10.95^{ab}	109.60± 11.86^{ab}
Final (mg/dl)	67.20±4.27	343.20±12.75^{a}	89.23± 7.51^{ab}	70.17 ± 8.45^{ab}	75.60 ± 6.95^{ab}

O.G = *Ocimum gratissimum*; FBS= Fasting blood Glucose; Values are expressed as Mean ± SEM; a= $p < 0.05$ relative to control; b = $p < 0.05$ relative to diabetic control;

3.3. Effect of *Ocimum gratissimum* on cardiac oxidative stress biomarkers

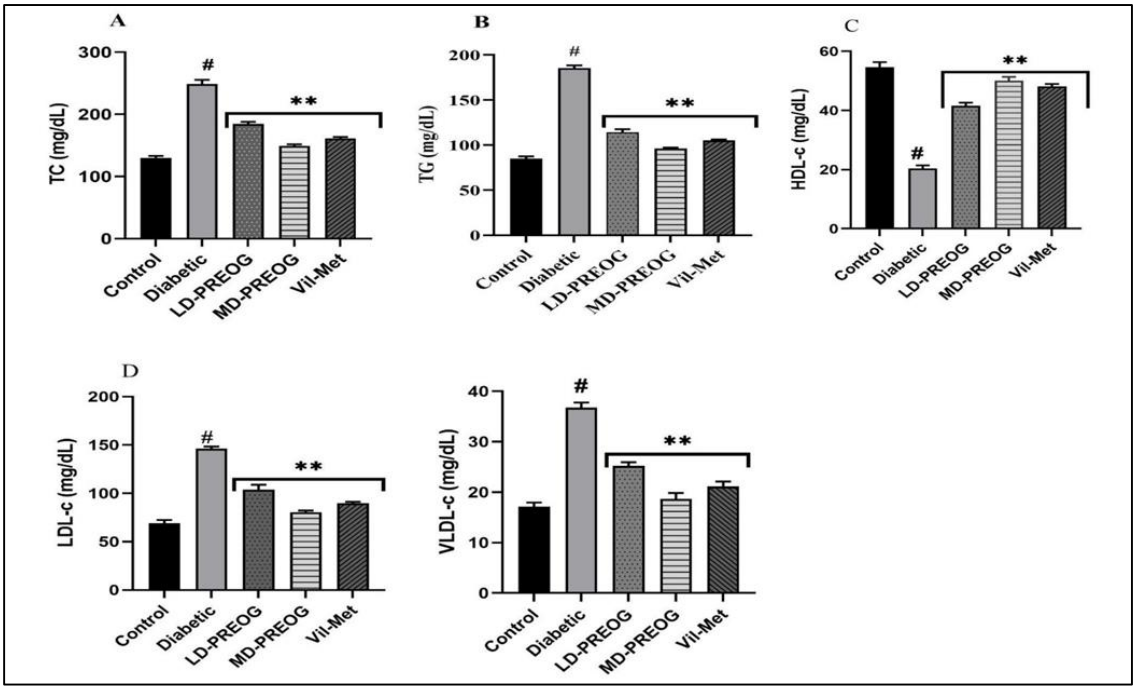
The results shown in Fig. 1. demonstrate that diabetic rats had significant ($p<0.05$) elevated levels of malondialdehyde (MDA), a marker of lipid peroxidation, indicating a significant ($p<0.05$) increase in oxidative stress. Additionally, reduced levels of important antioxidant enzyme activity like glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) in diabetic rats indicate a compromised antioxidant defense system. Treatment with *O. gratissimum* at both low and medium doses significantly ($p<0.05$) reduced MDA levels and considerably restored the activities of GSH, SOD, CAT, and GPx significantly ($p<0.05$), almost completely normalizing them to control levels.



Values are expressed as mean ± S.D; # = Relative to normal control, ** = Relative to diabetic control. **Legend:** Control: Normal Control, Diabetic: Diabetic Control, LD-PREOG: Diabetic rats administered a low dose (122.47 mg/kg bodyweight) polyphenol rich extract of *Ocimum gratissimum* leaves, MD-PREOG: Diabetic rats administered a medium dose (244.98 mg/kg bodyweight) polyphenol rich extract of *Ocimum gratissimum* leaves, Vil-Met: Vildagliptin-Metformin, MDA: malondialdehyde, CAT: Catalase, SOD: Superoxide dismutase, GSH: Glutathione, GPx: Glutathione peroxidase.

Figure 1 Effect of *Ocimum gratissimum* on cardiac oxidative stress biomarkers activities and levels in diabetic rats

3.4. Effects of *Ocimum gratissimum* on Lipid profile



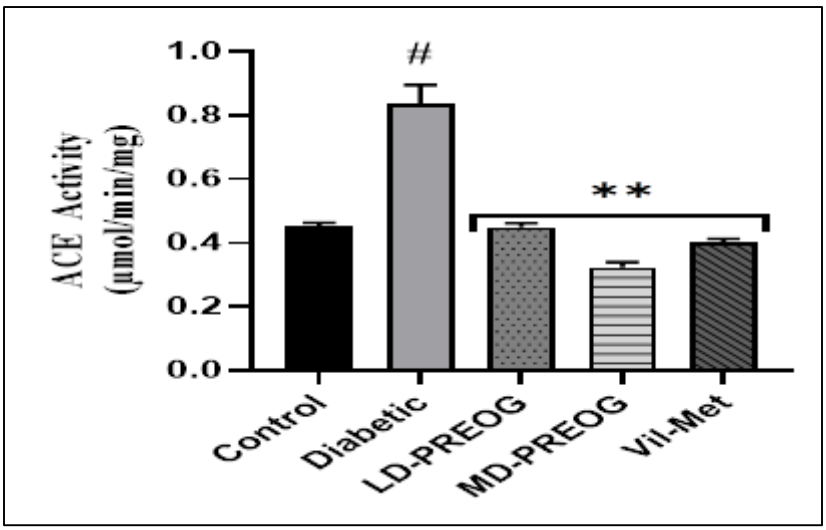
Values are expressed as mean ± S.D; # = Relative to normal control, ** = Relative to diabetic control. **Legend:** Control: Normal Control, Diabetic: Diabetic Control, LD-PREOG: Diabetic rats administered a low dose (122.47 mg/kg bodyweight) polyphenol rich extract of *Ocimum gratissimum* leaves, MD-PREOG: Diabetic rats administered a medium dose (244.98 mg/kg bodyweight) polyphenol rich extract of *Ocimum gratissimum* leaves, Vil-Met: Vildagliptin-Metformin, ,TC = Total cholesterol; TG = Triacylglycerol; LDL-c = Low density lipoprotein cholesterol; HDL-c = High density lipoprotein cholesterol; VLDL-c = Very low-density lipoprotein cholesterol.

Figure 2 Effect of *Ocimum gratissimum* on Lipid profile in diabetic rats

Figure 2. revealed that diabetic rats had a significant ($p<0.05$) higher total cholesterol, triglyceride, low density lipoprotein cholesterol, and very low density cholesterol levels, as well as lower high density lipoprotein cholesterol levels, compared to the control group. The polyphenolic extract of *Ocimum gratissimum*, especially at higher doses, significantly ($p<0.05$) improved lipid profiles in diabetic rats. In comparison to the standard drug Vildagliptin-metformin,the polyphenol extract of *Ocimum gratissimum* demonstrated competitive efficacy in improving lipid profile.

3.5. Effect of *Ocimum gratissimum* leaf extract on Angiotensin-Converting Enzyme Activity

Figure 3. shows that the activity of angiotensin-converting enzyme (ACE) was significantly ($p<0.05$) higher in diabetic control rats compared to normal control rats, indicating increased activity in diabetic conditions. The administration of *Ocimum gratissimum* extract to diabetic rats resulted in a significant decrease in ACE activity comparable to the standard antidiabetic drug. Notably, the group receiving the medium dose exhibited a more significant ($p<0.05$) reduction in ACE activity, indicating a dose-dependent effect.



Values are expressed as mean ± S.D; # = Relative to normal control, ** = Relative to diabetic control. Legend: Control: Normal Control, Diabetic: Diabetic Control, LD-PREOG: Diabetic rats administered a low dose (122.47 mg/kg bodyweight) polyphenol rich extract of *Ocimum gratissimum* leaves, MD-PREOG: Diabetic rats administered a medium dose (244.98 mg/kg bodyweight) polyphenol rich extract of *Ocimum gratissimum* leaves, Vil-Met: Vildagliptin-Metformin, ACE: Angiotensin-Converting Enzyme

Figure 3 Effect of *Ocimum gratissimum* leaf extract on Angiotensin-Converting Enzyme Activity

4. Discussion

Medicinal plants have a long history of use in diverse cultures to treat ailments, and their effectiveness has been widely investigated via both experimental and clinical studies. [40,41]. Therefore, this study evaluated the ability of the polyphenol-rich extract of *Ocimum gratissimum* (PREOG) leaves to improve endothelial function and provide cardiac protection in people with diabetes, using streptozotocin-induced diabetic rats as an experimental model. Endothelial dysfunction is greatly influenced by chronic hyperglycemia, and confirmatory assessment in this study confirmed that both low and medium doses of PREOG resulted in a significant ($p<0.05$) decrease in blood glucose levels in diabetic rats. The medium dose brought the glucose levels down to almost normal levels, similar to the standard diabetic drug vildagliptin-metformin, in agreement with other studies [42, 43] that also found that *Ocimum gratissimum* leaf extract effectively reduced glucose levels in diabetes.

Findings from this study revealed that in diabetic conditions, oxidative stress is heightened, in agreement with prior studies [44-46] demonstrating the association between diabetes and elevated oxidative stress, a key contributor to endothelial dysfunction caused by the excessive production of reactive oxygen species (ROS). The untreated diabetic group exhibited significantly higher levels of malondialdehyde (MDA), a marker of lipid peroxidation, as well as a significant lower activities of key antioxidant enzymes, catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), and reduced glutathione (GSH), indicating heightened oxidative stress. MDA is known to have cytotoxic effects, particularly when oxidative stress is present. Furthermore, reactive oxygen species (ROS) will accumulate as a result of decreased SOD, CAT, and GPx enzyme activity, as well as decreased GSH levels. This buildup can lead to cellular damage, which accelerates the deterioration of many tissues, particularly vascular tissues. [47,48] The administration of phenolic extracts from *Ocimum gratissimum* significantly improved oxidative stress

biomarkers in diabetic rats, with the medium dose showing superior efficacy in reducing oxidative damage comparable to the antidiabetic drug vildagliptin-metformin. The extracts' antioxidant properties most likely stem from their ability to chelate metal ions, donate electrons, and scavenge free radicals, all of which protect against oxidative damage. This is in line with research showing that, in situations of increased oxidative stress, plant phenolic extracts offer substantial protection and cardioprotective advantages. [49–56] Antioxidants are reducing agents and can neutralize reactive oxygen species (ROS) and free radicals. By neutralizing ROS and free radical intermediates, they effectively stop a variety of harmful biochemical reactions, including oxidative chain reactions, which helps to prevent ROS-induced cellular damage. [57] This neutralizing effect suggests that *Ocimum gratissimum* leaf phenolic compounds possess potent antioxidant qualities that lower lipid peroxidation and provide protection against oxidative stress in diabetes patients.

In diabetes, triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-c), and very low-density lipoprotein cholesterol (VLDL-c) are elevated, while high-density lipoprotein cholesterol (HDL-c) levels are reduced, as observed in diabetic models in agreement with other research confirming the link between diabetes and disturbances in lipid profile. [58-62] The disruptions in lipid levels seen in diabetes are mainly due to metabolic imbalances, which are characterized by insulin resistance, elevated fatty acid flux, and impaired lipid metabolism, all of which are pivotal in the development of diabetes and contribute to endothelial dysfunction and atherosclerosis. [58,60,62] Haffner *et al*. [59] discovered that diabetics have elevated levels of atherogenic lipoproteins even in the presence of controlled blood glucose. This underscores the significance of addressing metabolic dysregulation beyond hyperglycemia, making lipid management a critical component of diabetes care. Based on the findings of this study, phenolic extracts from *Ocimum gratissimum* significantly improved lipid parameters in diabetic rats at low and medium doses, demonstrating results that are comparable to vildagliptin-metformin. The extract demonstrated a significant ability to raise levels of HDL-c, also known as "good cholesterol" because of its role in reverse cholesterol transport, while also lowering serum levels of TG, TC, LDL-c and VLDL-c. This lipid-modifying effect of the phenolic extract of *Ocimum gratissimum* is consistent with previous reports indicating that plant-derived phenolic compounds have significant potential in improving lipid profiles. [63-65].

In cardiac tissues, STZ-induced diabetes triggered an elevated angiotensin-converting enzyme (ACE) activity; this finding is consistent with previous reports. [66-70]. Elevated ACE levels are common in diabetes and contribute to vascular complications by converting angiotensin I to angiotensin II, a potent vasoconstrictor. The development of hypertension and other vascular problems in persons with diabetes is significantly influenced by this conversion. [66-70] Furthermore, angiotensin II stimulates fibroblasts and increases extracellular matrix deposition, which leads to cardiac fibrosis and worsened cardiac function and heart failure. [66-70] Angiotensin II enhances the formation of reactive oxygen species (ROS) in diabetes by elevating NADPH oxidase subunit activity, which in turn depletes nitric oxide (NO), which is essential for vasodilation and endothelial function. [71,72] Atherosclerosis and other vascular problems are encouraged by reduced NO bioavailability, which also raises vascular tone, aggravates endothelial dysfunction, and hinders vasodilation. Additionally, oxidative stress caused by NADPH oxidase triggers inflammatory pathways, promoting leukocyte migration and adherence into the endothelium and fostering an environment that is pro-atherogenic. [73,74] Administration of *Ocimum gratissimum* phenolic extracts significantly suppresses ACE activity in cardiac tissue and plasma, in agreement with other studies that demonstrate the ability of plant-derived phenolics to act as natural ACE inhibitors. [75-78] ACE inhibition is vital for regulating the renin-angiotensin system (RAS), which plays a central role in blood pressure regulation and cardiovascular health. Findings from this study suggest that the polyphenolic extract of *Ocimum gratissimum* may have an impact on ACE inhibitory activity, reduce oxidative stress, and improve lipid profiles in diabetic conditions, which may lead to an improvement in cardiovascular health. However, although phenolic extracts from plants exhibit strong ACE and oxidative stress inhibitory effects *in vitro*, a study by Kwon et al. [79] reveals that the extract's translation in *in vivo* conditions in actual human biological systems may be constrained by variables including concentration, bioavailability, and extract processing. Therefore, further research is encouraged to confirm these results in larger and clinically relevant studies, concentrating on the human bioavailability and pharmacokinetics of these compounds, as well as figuring out the optimal dosages for therapeutic efficacy in the management and prevention of cardiovascular disease.

5. Conclusion

The findings suggest that the phenolic compounds in *Ocimum gratissimum* may be effective in promoting endothelial repair and reducing vascular complications, supporting the development of these extracts as adjunctive therapies for diabetic cardiovascular complications.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that there is no conflict of interest.

Statement of ethical approval

The experimental design was approved by the Faculty of Basic Medical Science, University of Uyo research and ethics committee in accordance with the principle of laboratory animal care and use outlined in the 8th edition of the guide for the care and use of laboratory animals by the institute for laboratory animal research, national research council of the National academies press, Washington D.C.

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