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Research Article
Effect of *Ocimum gratissimum* Leaf-extract on Hematological Indices and Lipid Profile of Streptozotocin-induced Diabetic Wistar Rats

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Abstract

Background and Objective: *Ocimum gratissimum* (OG) has been used in ethnopharmacology for the treatment of diabetes. The aim of the study was to evaluate the effect of *Ocimum gratissimum* leaf-extract on hematological indices and lipid profile of Streptozotocin-induced diabetic Wistar rats. **Materials and Methods:** Twenty-four rats weighing 100-160 g were randomly assigned to four treatment groups, the normal and diabetic controls, received a placebo treatment, while groups three and four were administered glibenclamide and OG leaf-extract (400 mg kg^{-1} b.wt.), respectively. The extracts were administered twice daily for 28 days. The rats were sacrificed and whole blood was collected for hematological and serum lipid profile assays. Data were analyzed using one-way ANOVA. **Results:** Diabetes induction resulted in decreases ($p \leq 0.05$) in Red Blood Cell (RBC), Hemoglobin (Hb), White Blood Cell (WBC) and increases in Mean Corpuscular Hemoglobin and Blood platelets compared to the normal control. Treatment with *O. gratissimum* extract reversed RBC (7.74 ± 0.39 µL), WBC (16.57 ± 3.02) and Platelet (804.33 ± 194.02) levels, but not Hb, towards normal levels (7.99 ± 0.04, 11.27 ± 0.69, 839.67 ± 10.17 respectively). Diabetes induction also resulted in increases ($p \leq 0.05$) in Triglyceride (TG) and Very-Low-Density Lipoprotein (VLDL), decreases ($p \leq 0.05$) in High-Density Lipoprotein (HDL) and Low-Density Lipoprotein (LDL) compared to normal control with no significant change in Total Cholesterol (TC). After administration with *Ocimum gratissimum* TC, LDL and VLDL and HDL levels were significantly ($p \leq 0.05$) reduced relative to the diabetic control. TG was however increased relative to the diabetic control. **Conclusion:** Overall, data suggests the plant holds great potential in amelioration of diabetes-induced dyslipidemia and hematological disorders.

Key words: Diabetes mellitus, *Ocimum gratissimum*, lipid profile, hematological indices. Streptozotocin-induced

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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

Diabetes mellitus remains an emerging major public health concern, especially in the developing countries[1-3]. It can be defined as a group of metabolic diseases characterized by inappropriate hyperglycemia, due either to an absolute lack of insulin secretion by the pancreas or a reduction in its biological effectiveness or both, as well as disordered metabolism of carbohydrate (glucose), lipid and protein[3-5]. If left uncontrolled, the disease can lead to increased risk of atherosclerosis, thereby leading to cardiovascular complication viz-a-vis retinopathy, nephropathy and neuropathy[2,6,7]. Its adverse effect is symptomized by polyphagia, polyurea and polydipsia[1]. There are projections that, 366 million people are likely to be diabetic by the year 2030[8]. This derives from the fact that none of the anti-diabetic drugs could give a long term glycaemic control without an attendant adverse side effect[9-11], hence the need for a continuous search for better management options.

However, leads from traditional nutritional and medicinal practices have proven that some medicinal plants and edible vegetables may be effective in controlling plasma glucose level with minimal side effects, as alternative therapy especially in developing countries[3,10,12]. Although these plants have been used for traditional management or control of plasma glucose in diabetes mellitus, not many of such medicinal plants have been scientifically validated.

One of such vegetables commonly used in traditional control of diabetes is *Ocimum gratissimum* (OG). The plant which is cultivated in many gardens around village huts in Nigeria for its medicinal and culinary uses is commonly referred to as *'Ntong'* in Efik tribe in Calabar and '*Eyazumana*' among the Bahumono tribe in Abi local government area of Cross River State and '*Nchu anwu'* in Igbo speaking tribe of Nigeria and belongs to the family Lamiaceae. Chemical evaluation of the Phyto-compounds in the plant revealed the presence of active ingredients, such as flavonoids, triterpenes, alkaloids, citral, saponins, eugenol, linalool, methyl cinnamate, camphor and thymol[13-15]. Besides the known medicinal value of these reported compounds present in the vegetable, the leaves, have found evidence-based relevance in traditional folk medicine, as a remedy against several ailments, diabetes and some infectious diseases[13,15].

Although there are preliminary reports on the glucose-lowering effect of OG[13], not much is known in terms of its contribution to the complications of diabetes, yet the health damage including cessation of life suffered by diabetics are rarely due to hyperglycemia per se but from the complications caused by chronic hyperglycaemia[1,5,6]. Moreover, given the pathophysiology and the biochemical processes of diabetes, causes of diabetes and sites of intervention are recognized to be diverse[8,16]. The dysfunctional cardiovascular process involving altered plasma lipids transport and the hematopoietic system (e.g. hemoglobin glycation and platelet aggregation) are among the key complications of diabetes[2,4,17]. This is further strengthened by the fact that, in the assessment and management of diabetes mellitus patient hematological indices are recognized as significant indicators[5,18]. Therefore, an effective diabetes intervention measure is expected to beyond blood glucose-lowering, attenuate the associated complications. It is to this end that this work was carried out to evaluate the effect of *Ocimum gratissimum* on blood and lipid parameters, indicators of diabetes-induced cardiovascular dysfunction in streptozotocin-induced diabetic Wistar rats.

MATERIALS AND METHODS

Place and duration of study: This study was conducted at the Department of Biochemistry of the University of Calabar, Nigeria, from February-April, 2014.

Reagents and chemicals: All reagents and chemicals used were of good analytical grades. The standard reagents kits for hematological indices and lipid profiles were products of Aptec Diagnostic and Randox Laboratory respectively.

Collection and identification of plant material: Fresh, mature green leaves of *Ocimum gratissimum* were purchased from Mbukpa market, Calabar South Local Government Area of Cross River State, Nigeria. The leaves were identified to be *Ocimum gratissimum* by the Botany Department, University of Calabar, Calabar, Nigeria.

Preparation of plant extracts: The leaves of the plant samples were washed, air-dried under room temperature and subsequently homogenized to powder form using a manual machine mill. The leave extract of the plant was prepared as described by Adebayo-Tayo and Odeniyi[19]. The ground pulp of the plant leaves (80 g) was soaked in 250 mL of dichloromethane and methanol respectively for 72 hrs. The extract was filtered and evaporated under a vacuum using a rotary evaporator. The residues of the extracts were stored in bottles in the refrigerator until ready for use.

Experimental animals: Twenty four albino Wistar rats of both sexes, ranging in weight between 100-160 g were obtained from the animal house, Department of Biochemistry, University of Calabar and acclimatized for one week before

commencement of the experiment. The rats were divided into four groups of six rats each. The animals were housed in wooden cages with a screen top under standard conditions of temperature (28±2°C) and relative humidity of (40±5%) with a 12 hrs light-dark cycle and proper ventilation. The animals were maintained on rats chow and provided with water *ad libitum*. Throughout the duration of experiment, hygiene was maintain by constant cleaning and removal of faeces and spilled feed from cages on a two days basis. The animal groupings and treatment of experimental animals in the respective experimental groups is shown in Table 1. The administration regiment lasted for 28 days. At the end of the administration period, the animals were sacrificed and the blood sample collected for analysis. The guideline of the National Institute of Health (NIS) publication (1985) for laboratory animal care and use (https://grants.nih.gov/grants/olaw/guide-for-the-care-and-use-of-laboratory-animals.pdf) was adopted for handling the experimental animals in the cause of the experiment and ethical approval was also obtained from the Faculty Animal Ethics Committee, Faculty of Basic Medical Science, University of Calabar, Calabar, Cross River State, Nigeria.

Induction of experimental diabetes: After one week of acclimatization, the animals were fasted for 16-18 hrs with free access to water prior to the induction of diabetes. Diabetes was induced in the albino Wistar rats by intraperitoneal injection of 150 mg kg^{-1} b.wt. of streptozotocin (STZ), using normal saline as a vehicle (Sigma St. Louis, M.S USA). Diabetes was confirmed three days later if streptozotocin-induced animals showed blood glucose (FBG) levels greater than 200 mg dL^{-1} (11.1 mmol L^{-1}) as monitored in the blood from tail vein using *Accu Check®* glucometer.

Experimental protocol: The plant extract was reconstituted in 20% dimethylsulphoxide (DMSO) before use and treatment was administered orally, via gastric intubation at a dose of 400 mg kg^{-1} b.wt. twice per day (10:00 am and 4:00 pm). The animals were maintained on commercial palletized diet purchased from Vital Feed Limited, Jos and tap water was provided ad libitum. The extract administration and animal experiment lasted for 28 days, at the end of the administration period, the animals were sacrificed after an overnight fast and blood sample collected for analyses.

Collection of blood samples for analyses: At the end of the twenty-eight days of the administration, all animals were fasted for 12 hrs but had free access to water, then anesthetized under chloroform vapor and dissected. Blood was collected by cardiac puncture, using 5 mL sterile syringe, into sample containers and centrifuged at 3,000 rpm for 10 min to remove blood cells and recover serum. The sera were pipetted using pasteurized pipette into the plain sample container, stored in a refrigerator at 4°C until when it was subjected to biochemical analysis. For the hematological analysis, a portion of the whole blood was collected into heparinized tubes and conveyed to the laboratory where evaluations were carried out within 24 hrs.

Biochemical analysis: Biochemical assays were carried out using serum and the following parameters were estimated: Total cholesterol, Triglyceride, Low-density lipoprotein, High-density lipoprotein, Very low-density lipoprotein, White blood cell, Red blood cell, Blood platelets, Hemoglobin and Mean capsular hemoglobin.

Determination of low-density lipoprotein-cholesterol (LDL-c) and very low-density lipoprotein-cholesterol (VLDL-c): Total cholesterol, Triacylglyceride and High-density lipoprotein cholesterol (HDL-c) were estimated using Randox Analytical kits, according to the manufacturer's protocol. The Low-density lipoprotein (LDL) cholesterol and very low-density lipoprotein-cholesterol (VLDL-c) were determined by calculation using the Friedewald Formula (FF) equation[20] thus:

$$\text{LDL-c (mg dL}^{-1}) = \text{TC-HDL-c-}\frac{\text{TG}}{5}$$

$$\text{VLDL-c (mg dL}^{-1}) = \frac{\text{TG}}{5}$$

Where:

LDL-c : Low-density lipoprotein cholesterol
HDL-c : High-density lipoprotein cholesterol
TC : Total cholesterol

Table 1: Animal groupings and treatment schedule (experimental design)

Groups	Number of rats	Treatments
Normal Control (NC)	6	Administered 0.2 mL 20% Dimethly-sulphoxide (DMSO) twice, daily
Diabetic Control (DC)	6	Administered 0.2 mL 20% DMSO twice, daily
Standard control (Glibenclamide (GB)	6	Administered 5 mg kg^{-1} b.wt. of Glibenclamide (GB), twice, daily
Test group (extract-treated)	6	Administered 400 mg kg^{-1} b.wt. of *Ocimum gratissimum* (OG) extract, twice, daily

TG : Triacylglyceride
VLDL-c : Very low-density lipoprotein cholesterol

Estimation of hematological indices

Hemoglobin concentration: The cyanmethemoglobin method of hemoglobin determination was used[21]. The principle of this method lies in the conversion of hemoglobin to cyanmethemoglobin by the addition of potassium cyanide and ferricyanide whose absorbance is measured at 540 nm in a photoelectric colorimeter against a standard solution. The concentration of hemoglobin in the sample was calculated by using the formula:

$$\text{Hb (g/100 mL)} = \frac{\text{Absorbance of sample} \times \text{Concentration of standard}}{\text{Absorbance of sample}}$$

Estimated of total red blood cells, platelets and white blood cells count by visual means: This involves microscopic visual identification and counting of red blood cells and white cells with appropriate diluting fluids[22]. A 1: 200 diluted blood was made in Hayem's fluid for Red Blood Cell (RBC) and Turk's fluid for White Blood Cell (WBC) in a glass (75×12 mm) tube. The tube was with tight-fitting rubber bang and titled through and angle of about 120° combined with rotation for 2 min to allow the diluted blood to mix.

Statistical analysis: Data was presented as Mean±standard error of the mean. Data were computed and analyzed using.

One-way ANOVA and unpaired Student's t-test with the help of a statistical package, SPSS version 18.0 for Windows. Values were considered significant at $p<0.05$.

RESULTS

Description of the Hematological indices of the experimental groups: Results of the hematological indices of the various experimental groups are shown in Table 2. Induction of diabetes led to a decrease in the WBC level in the diabetic control as compared to the normal control, however, following a 28th day OG leaf-extract administration, a significant ($p<0.05$) increase was observed in the treatment groups. The altered levels of RBC, HB and MCH were not statistically significant across the study groups. However, a significant reduction in Platelets (PLT) was observed in the OG treated group compared to the diabetic control and comparing favorably the value obtained in the Glibenclamide-treated group.

Lipid profile of the experimental groups following treatments: Table 3 shows the lipid profile of diabetic rats treated with OG leaf-extracts compared to the controls. From the data, induction of experimental diabetes was found to cause a significant decrease in total cholesterol (123.8±4.04 mg dL^{-1}) and HDL-c (27.25±2.05) levels compared to the normal control (124.21±4.38 and 31.06±3.15 mg dL^{-1}, respectively). Contrariwise, TG (197.85±7.86 mg dL^{-1}) and VLDL-c (39.57±1.57 mg dL^{-1}) increased in the untreated diabetic animals compared to the normal control (100.81±5.56 and 20.16±1.11 mg dL^{-1} respectively). However, following 28-day administration of OG there was a significant decrease in TC (98.53±20.17 mg dL^{-1}), LDL-c (46.19±17.50) and VLDL-c (35.83±5.35 mg dL^{-1}) in the extract-treated rats compared to the diabetic control ($p<0.05$);

Table 2: Treatment effects on hematological parameters of experimental groups

Parameters	Normal control	Diabetic control	Glibenclamide	*O. gratissimum* crude extract
RBC (10^6/µL)	7.99±0.04	7.37±0.18	7.59±0.22	7.74±0.39
Hb (g dL^{-1})	15.20±0.47	14.90±0.06	14.30±0,70	14.46±0.58
MCH (pg/cell)	19.17±0.38	19.30±0.25	19.37±0.82	18.83±0.54
PLT (10^3/µL)	839.67±10.17	970.67±3.28	683.00±283.36	804.33±194.02
WBC (10^6/µL)	11.27±0.69	9.10±0.06	15.23±0.52	16.57±3.02*

RBC: Red blood cell, HB: Hemoglobin, MCH: Mean capsular hemoglobin, PLT: Platelets, WBC: White blood cells, Values are presented as Mean±SEM, n = 5, $p<0.05$ is considered significant, *Mean difference is statistically significant from control

Table 3: Effect of Treatments on Lipid profile of the various experimental groups

Parameters (mg dL^{-1})	Normal control	Diabetic control	Glibenclamide	*O. gratissimum* extract
TG	100.81±5.56	197.85±7.86	190.61±10.71	217.16±41.07*
TC	124.21±4.38	123.8±4.04	84.15±9.13	98.53±20.17*
HDL	31.06±3.15	27.25±2.05	22.89±3.89	16.51±2.39*
VLDL	20.16±1.11	39.57±1.57	38.12±2.14	35.83±5.35*
LDL	72.98±3.69	56.87±5.74	23.13±11.57	46.19±17.50*

TG: Triglyceride; TC: Total cholesterol, HDL: High-density lipoproteins, VLDL: Very low-density lipoproteins, LDL: Low-density lipoproteins, Values are expressed as Mean±SEM, n = 5, $p<0.05$ is considered significant, *Mean difference is statistically significant from diabetic control

this suggested a positive attenuation of diabetes-induced dyslipidemia. The HDL-c was not impacted by the extract-treatment as much as other lipid parameters.

DISCUSSION

This study investigated the effect of the crude extract of *O. gratissimum* on hematological parameters and lipid profile of diabetes-induced Wistar rats. Diabetic induction was found to cause a significant reduction in Red Blood Cell (RBC) and hemoglobin levels in the diabetic rats. This is in consonance with reports that anemia and increased erythrocyte fragility are some of the conditions that have been associated with diabetes[12,18,23-25]. This reduction could be as a result of Reactive Oxygen Species (ROS) generated in the diabetic condition, damaging the RBC with a concomitant reduction in the hemoglobin level. Similar observations have been made by other studies[12,26,27]. Moreover, anemia is known to occur in uncontrolled diabetes mellitus due to the increased non-enzymatic glycosylation of RBC membrane proteins[17,18,28]. The decrease in RBC in this study across groups was also observed in an earlier study Colak *et al.*[29]. In this study, the authors reported that diabetes mellitus causes the development of hypochromic anemia due to a fall in the iron content of the body, a consequence of diabetes-associated oxidative stress[2,5,6]. However, treatment with *O. gratissimum* extract for 28 days ameliorated the decrease by causing an increase in these parameters. This observed amelioration effect could be as a result of the antioxidant in the plant scavenging the free radicals, thus protecting the red blood cells. This result was similar to that reported by Mohammed *et al.*[26] and Edet *et al.*[27]. Also, the hitherto decreased WBC upon diabetes induction, indicating compromised immunity in diabetes, was attenuated after the 28-day administration of the OG leaf-extract. It is clear from this observation that the extract could prevent the various complications of diabetes, such as that of compromised immune function[3,5]. The increased WBC counts following the administration of *Ocimum gratissimum* suggested that the plant might have the potential to boost the animals' immune system. For the increased platelet count occasioning diabetes, it is known that insulin is a natural antagonist of platelet hyperactivity which suggests that reduced insulin sensitivity may account for platelet hyperactivity in diabetes[30,31].

Dyslipidemia in diabetes is characterized by hypertriglyceridemia, increased plasma total (TC) cholesterol, very low-density lipoprotein cholesterol (VLDL-c) and low-density lipoproteins cholesterol (LDL-c) and decrease in high-density lipoproteins cholesterol (HDL-c)[32-35]. This usually is a consequence of increase in fat synthesis and mobilization orchestrated by decreased glucose utilization by insulin-dependent tissues[36,37]. In the present study, there was observed dyslipidemia following diabetes induction indicated in VLDL-c and HDL-c. This is similar to earlier work of Mgbeje *et al.*[35] on other plants. There was, however, no change in TC and rather a decrease in LDL-c in this study. This finding is in consonance with the findings of Ephraim, *et al.*[38] and Obianime *et al.*[14]. The LDL-c is not always increased in diabetes and in part, this may represent a balance of factors that affect LDL-c production and catabolism[16]. A necessary step in LDL-c production is the hydrolysis of its precursors VLDL-c by lipoproteins lipase deficiency[34,36]. A reduction in this step due to lipoprotein lipase deficiency or excess surface apo-proteins, under the influence of the extract, may cause decrease LDL-c synthesis, hence the observed decreased plasma concentration. Considering that *O. gratissimum* extract reversed the elevated TC, LDL-c and VLDL-c levels, the extract might be appropriate for modulation of the diabetes-induced hyperlipidemia (lipid metabolizing disorders) and thus, prevent progression of cardiovascular disease. The observed null effect of the extract that depressed HDL-c warrants further study.

CONCLUSION

The results of this study revealed that *Ocimum gratissimum* leaf-extract was capable of reversing some of the effect of diabetes on hematological indices and lipid profile in the diabetic rats suggesting that the plant can be used to improve diabetes management outcomes. This thus implies that OG can provide amongst other things, a natural, safer and cost-effective alternative, to the conventional diabetic drugs in the developing countries including Nigeria.

SIGNIFICANCE STATEMENT

This study validated the ethnopharmacological use of *Ocimum gratissimum* leaf extract in the treatment of diabetes. It provides a basis for elucidating the active principles responsible for the antidiabetic activities of the plant.

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