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Anti-Diabetic and Lipid Profile Effect of Ethanol Extract of *Ocimum tenuiflorum* (Holy Basil) in Albino Rats

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Abstract

Background: Diabetes mellitus, a chronic metabolic disorder characterized by elevated blood glucose levels. Current diabetes management involves diverse approaches such as medications, diet, and exercise; however, these methods have limitations. *Ocimum tenuiflorum* emerges as a promising botanical in the realm of anti-diabetic therapy. This research aims at assessing the anti-diabetic and anti-hyperlipidemic potentials of *Ocimum tenuiflorum* leaf extract in rats with alloxan-induced type 2 diabetes.
Methods: Twenty-five albino rats were divided into 5 groups (n =5). Group 1 rats were not induce with diabetes and untreated, group 2 rats received mixed of alloxan monohydrate through injection in saline (120 mg/kg body weight) and water, group 3 received mixed of alloxan monohydrate through injection in saline (120 mg/kg body weight) and 200 mg/kg body weight of *O. tenuiflorum* extract once per day, group 4 received mixed of alloxan monohydrate through injection in saline (120 mg/kg body weight) and 400mg/kg body weight of *O. tenuiflorum* extract once per day, group 5 received mixed of alloxan monohydrate through injection in saline (120 mg/kg body weight) and 70 mg/kg body weight of metformin once per day, for fourteen (14) days.
Results: There were significant decreases in the concentration level of glucose (197.52±9.48, 128.78±8.08), total cholesterol (174.17±2.04 mg/dL, 161.68±1.38 mg/dL), triglyceride (73.19±2.07 mg/dL, 60.24±1.15 mg/dL), low density, lipoprotein (125.32±2.21 mg/dL, 114.86±2.14 mg/dL), and increase level of high density lipoprotein (35.91±0.46 mg/dL, 48.55±0.67 mg/dL), in a dose dependent manner in groups 3 and 4 respectively when compared with group 5.
Conclusion: The study demonstrated that ethanol extracts of *Ocimum tenuiflorum* possesses significant hypoglycemic and hypolipidemic activities in albino rats.

Keywords: *Ocimum tenuiflorum*; Anti-Diabetic; Lipid Profile; Hyperglycemia; Dyslipidemia; Metformin; Albino Rats

Abbreviations

TC: Total cholesterol; HDL: High Density Lipoprotein; LDL: Low Density Lipoprotein; NC: Normal Control, PC: Positive Control; ANOVA: One-Way Analysis of Variance.

Introduction

Diabetes mellitus is a long-term metabolic illness marked by high levels of glucose in the blood. It arises from defects in the secretion of insulin, the action of insulin, or both [1]. The consequences of unregulated diabetes encompass both microvascular and macrovascular problems, such as retinopathy, nephropathy, neuropathy, and cardiovascular illnesses [2]. Current diabetes management involves diverse approaches such as medications, diet, exercise, and insulin therapy; however, these methods have limitations. Consequently, there is growing interest in exploring

complementary and alternative therapies for diabetes, with a focus on their potential as adjunctive treatments [3]. With the global prevalence of diabetes projected to reach 700 million adults by 2045 [4], the urgency to address cardiovascular risk factors like hyperglycemia and hyperlipidemia intensifies [5].

Several studies has shown that plant-based medicinal therapies, deeply rooted in traditional practices, have gained traction for their perceived safety and efficacy [6-12]. Ocimum tenuiflorum, or holy basil, stands out with promising anti-diabetic and hypolipidemic potential in preclinical studies [13,14]. Positive outcomes could pave the way for evidence-based integrative approaches, enhancing the control of glycemia and dyslipidemia, ultimately reducing cardiovascular complications and mortality in diabetic populations. Ocimum tenuiflorum, commonly known as holy basil, emerges as a promising botanical in the realm of anti-diabetic therapy. Belonging to the Lamiaceae family, this herb has a rich history in Ayurvedic medicine, traditionally used as a general tonic and adaptogen [15]. Therefore, this study aimed to explore the anti-diabetic and anti-hyperlipidemic attributes of *Ocimum tenuiflorum* leaf extract in rats with induced with alloxan.

Methods

Apparatus/Equipment

The following are the apparatus and equipment use Dissecting sets, Beakers, Orogastric tube (guava), measuring cylinder, bunner, funnel, micropipette, sample bottle (fluoride bottle), conical flask, filter paper syringe and needle, test-tube and test-tube rack, curvette, centrifuge, oven, blender, spectrophotometer, hand gloves, spatula, waterbath, refrigerator, Pasteur pipette, stirring rod, weighing balance, tissue, cotton wool.

Chemicals and Reagents

Chloroform, Absolute ethanol, distilled water, methylated spirit, Total cholesterol (TC), Glucose Reagent, High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL), Triglyceride Reagent, normal saline, *Ocimum tenuiflorum* extract

Collection of Holy Basil (*Ocimum tenuiflorum*) Leaves Samples

The fresh *Ocimum tenuiflorum* leaves were obtained at a clinic garden located at Ebisame Street, Akenfa, Yenagoa, Bayelsa state, Nigeria. The leaves were identified by Prof. Inetiminebi Arrow Ogidi from the Department of Plant Science, Faculty of Agricultural Technology, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria.

Alloxan

Alloxan was gotten from pharmacological laboratory, Department of Pharmacy, Niger Delta University at Wilberforce Island Bayelsa State, Nigeria.

Experimental Animals

This experiment was carried out according to the protocol described in OECD guidelines 407 with minor modifications [16]. Twenty five (25) male albino rats weighing between 150-237 were bought from Animal house of pharmacology Department, College of Health Science, Niger Delta University and were acclimatized for two weeks in a standard condition in the animal house of the Department of Biochemistry, Niger Delta University Wilberforce Island, Bayelsa State. They were given grower mash and distilled water during the period of acclimatization. This study was approved by the Research and Ethics Committee of the Department of Biochemistry, Faculty of Basic Medical Sciences, Bayelsa Medical University, Yenagoa, Bayelsa State, Nigeria with a Reference Number FBMS/AD/BCH/REC/29/02.

Preparation of Holy Basil Ethanol Extract

The leaves were harvested, washed with clean water and was oven dried for 16 hours at 60 degree Celsius (ºC). The dried leaves were blended into a smooth powder with the use of an electric blender. Seven hundred gram (700g) of the powdered leaves were poured into a beaker containing 2000 ml of ethanol (98%) and mixed thoroughly, this mixture was left at a room temperature for three days (72 hours). Rotatory evaporator was used to separate the sample at 50ºC, the filtrate was placed in a clean beaker and taken to the water bath for further drying and preserved in an air tight container in a refrigerator for further use.

Preparation of Alloxan

The alloxan was prepared by dissolving 120 mg/kg of alloxan and 40 mg/ml normal saline together and was administered to the rats.

Experimental design

Following acclimatization, the 25 albino rats were randomly distributed into five groups in which the groups were composed of five (5) rats each, the rats were marked and their weight recorded.

Group I: Normal control: They were fed with grower mash and water for 14 days.

Group II: Positive control: induced with alloxan, fed with grower mash and received distilled water (They were induced but untreated) for 14 days.

Group III: Low dose extract: Induced with Alloxan and given 200 mg/kg of ethanol extract of *Ocimum tenuiflorum* which

was administered orally using gavage. They were also fed with grower mash and distilled water for 14 days.
Group IV: High dose extract: Induced with Alloxan and given 400 mg/kg of ethanol extract of *Ocimum tenuiflorum* which was administered orally using gavage. They were also fed with grower mash and distilled water for 14 days.
Group V: Standard control: Induced with Alloxan and received 70 mg/kg of metformin which was administered orally using gavage. They were also fed with grower mash and distilled water for 14 days.

Determination of Blood Glucose and Collection of Blood Samples

The treatments were administered daily for a period of 28 and 15 days using the ethanol extract. Daily blood glucose levels were assessed using an accucheck glucometer. Blood samples were collected by making a little incision on the lateral aspect of the tail vein using a scalpel blade. Multiple measurements were taken to confirm the glucometer readings were consistent. After the treatment was finished, the rats were terminated using a gentle anaesthetic following a 12-hour period of fasting. Subsequently, blood samples were obtained, subjected to centrifugation, and the resulting serum was stored for biochemical examination [17].

Evaluation of Hypolipidaemic Activity of the Extract

The levels of serum cholesterol, triglycerides, and High Density Lipoprotein (HDL) were assessed using enzymatic colorimetric techniques and Randox diagnostic kits. An Unicam spectrophotometer equipped with a wine light source was utilised to analyse all samples. The method established by Antia, et al. [17] was used to determine the amounts of low density lipoprotein (LDL).

Statistical analysis

Values are given as Mean ± SEM for each group where SEM is the Standard Error of Mean. Superscript 'a' 'and 'b' indicate significant difference ($p<0.05$) compared to Normal Control (NC) and Positive Control (PC) respectively. P: statistical level of significance was determined by one-way Analysis of Variance (ANOVA) followed by Tukey post-hoc test.

Results

Results of Anti-diabetic and anti-lipidemic effects of *Ocimum tenuiflorum* on albino rats

The findings of the current investigation are outlined in the Table 1 below. It shows the levels of each biochemical parameters tested for in alloxan induced-diabetic rats after *Ocimum tenuiflorum* and standard diabetic drug metformin treatment. This presents the mean serum levels of Glucose, Total cholesterol, Triglycerides, High Density Lipoprotein and Low Density Lipoprotein.

Biochemical Parameters	Groups				
	NC	PC	LD EXT	HD EXT	Metformin
Glucose	98.70±3.57^{b}	290.66±8.98^{a}	197.52±9.48$^{a\,b}$	128.78±8.08^{b}	95.31±4.62^{b}
Total cholesterol (mg/dL)	153.41±3.45b	210.62±5.54a	174.17±2.04ab	161.68±1.38b	143.42±1.14b
Triglycerides (mg/dL)	50.34±1.65^{b}	91.45±1.25^{a}	73.19±2.07$^{a\,b}$	60.24±1.15$^{a\,b}$	51.79±2.31^{b}
HDL (mg/dL)	57.25±0.76^{b}	29.79±1.12^{a}	35.91±0.46$^{a\,b}$	48.55±067$^{a\,b}$	53.46±0.70$^{a\,b}$
LDL (mg/dL)	102.03±2.99^{b}	136.38±1.45^{a}	125.32±2.21$^{a\,b}$	114.86±2.14$^{a\,b}$	117.76±1.32$^{a\,b}$

Table 1: Anti-diabetic and anti-lipidemic results of *Ocimum tenuiflorum* on albino rats.

Discussion

Diabetes mellitus is a well-known chronic condition that involves glucose metabolism. Obesity, high blood pressure, and high cholesterol are metabolic problems that can occur with type 2 and experimental diabetes; their presence indicates co-morbidities. Although diabetes mellitus is quite prevalent and has significant morbidity and death worldwide, it is considered an incurable but manageable condition. Various synthetic medications, herbal treatments, and dietary modifications effectively alleviate the associated agony. Multiple researchers have examined the potential of medicinal plants to function as hypoglycemic drugs [18-24].

The pancreas is the primary organ that detects the body's food and energy status by monitoring the blood glucose levels. The pancreas releases insulin when blood glucose levels increase. Alloxan is frequently used, in conjunction with streptozotocin, to cause diabetes mellitus. Alloxan has a harmful effect on the beta cells of the pancreas. Alloxan induces hyperglycemia by triggering a substantial reduction in insulin production through the apoptosis of β-cells in the islets of Langerhans. Inadequate insulin leads to several metabolic alterations in animals, such as heightened cholesterol levels and higher levels of alkaline phosphate and transaminases [25,26].

Common symptoms observed in patients with diabetes include elevated blood glucose levels, reduced body weight, frequent urine (polyuria), excessive thirst (polydipsia), and increased appetite (polyphagia). The present investigation revealed that the induction of diabetes through the use of alloxan led to an increase in blood glucose levels. The increase in blood glucose level after administering alloxan to diabetic rats can be attributable to either a lack of insulin or a condition of insulin resistance. The ethanol extract of *Ocimum tenuiflorum* leaf shown significant efficacy in reducing blood glucose levels in diabetic rats, suggesting a potential reversal of insulin resistance or an augmentation of insulin production. The observed phenomenon can be ascribed to the restoration of damaged pancreatic β-cells in rats with diabetes caused by alloxan [27]. Previously, a multitude of plants have been analysed for their capacity to reduce blood sugar levels and induce the secretion of insulin [28-32].

The *Ocimum tenuiflorum* ethanol extract's dose-dependent glucose reduction also suggests a potential therapeutic effect on hyperglycemia. The discoveries are in concurrence with the idea that higher convergences of the concentrate might prompt more significant decreases in blood glucose levels, giving important experiences to additional investigation and possible clinical applications. Further, the reference drug, Metformin, displayed a glucose level of 95.31±4.62^{b} mg/dL as shown in Figure 1, addressing a critical decrease contrasted with both the positive control and the trial gatherings. This outcome is predictable with the deeply grounded hypoglycemic impacts of Metformin in the treatment of diabetes as referred to by Shajeela, et al. [33].

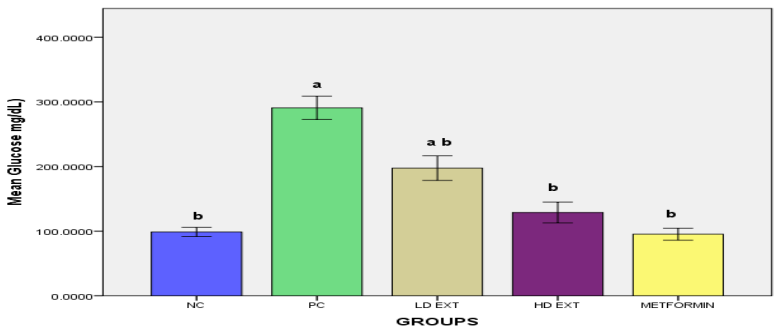

Figure 1: Glucose level of effects of ethanol extract of *Ocimum tenuiflorum* on albino rats

Researchers conducted a study to explore the potential of the plant extract in reducing high levels of lipids and glucose in the body, as there is a significant connection between hyperlipidemia and hyperglycemia [34]. The researchers selected the alloxan-induced hyperglycemia model as it is an excellent model to study the impact of the antidiabetogenic medication, as mentioned in the study by Cho, et al. [34]. Atherosclerosis, a well-documented phenomenon, occurs when elevated levels of cholesterol in the bloodstream lead to a gradual reduction in blood flow to organs, ultimately resulting in malfunction. Multiple lines of evidence indicate that lowering blood cholesterol levels is linked to an enhancement in atherosclerosis and the occurrence and seriousness of CAD. Numerous therapeutic options are available for hypercholesterolemia, although individuals are unable to adhere to them due to their contemporary lifestyle. Consequently, researchers are highly interested in natural remedies [35]. The liver is the primary organ responsible for maintaining constant cholesterol levels. Multiple sources indicate that the HC diet leads to an increase in triglyceride formation by elevating the concentration of cholesterol in the liver. In this study the typical group I, with a complete cholesterol level of 153.41±3.45 mg/dL, fills in as a gauge for the study [36-38]. This steady cholesterol level gives a reference highlight the trial gatherings. This gauge understanding is vital for contextualizing the effect of the *Ocimum tenuiflorum* concentrate on cholesterol levels in resulting gatherings [39].

The positive control group, treated with a standard cholesterol-changing specialist, exhibited a critical expansion in all our cholesterol levels (210.62±5.54 mg/dL), approving its viability in prompting hypercholesterolemia. This compares to the study by Robert, et al. [40]. The huge increment affirms the unwavering quality of the exploratory model and the picked positive control. Albino rats treated with a low portion of the *Ocimum tenuiflorum* ethanol extract showed a complete cholesterol level of 174.17±2.04 mg/dL, lining up with studies proposing a likely effect of the plant remove on cholesterol guideline [41].

Suanawnsawat, et al. [39] hypothesis that higher concentrations of the extract may have a more pronounced effect on lowering cholesterol is supported by the fact that the high dose extract group, on the other hand, had a lower total cholesterol level of 161.68±1.38b mg/dL as shown in

Figure 2. The group treated with metformin, a known anti-diabetic prescription, showed lower cholesterol level of 143.42±1.14 mg/dL, which is in agreement with the studies by Robert, et al. [40].

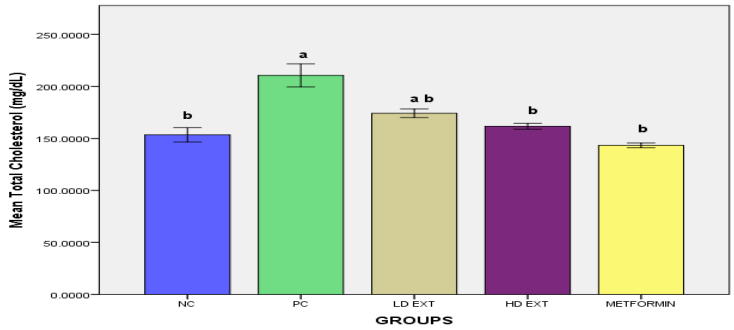

Figure 2: Total Cholesterol level of effects of ethanol extract of Ocimum tenuiflorum on albino rats.

The normal control group had Triglyceride levels of 50.34±1.65 mg/dL, while the positive control group had 91.45±1.25 mg/dL, a significant increase that is consistent with previous studies that have shown a rise in Triglyceride levels in either untreated or treated hyperlipidemia [42]. The more significant levels in the positive benchmark group approve the model's acceptance of hyperlipidemia. The critical raised Triglyceride levels in the positive group propose effective enlistment of hyperlipidemia, giving a standard to assessing the effect of the *Ocimum tenuiflorum* concentrate on lipid guideline.

The low dose extract group showed Triglyceride levels of 73.19±2.07 mg/dL, while the high dose extract group displayed lower levels at 60.24±1.15 mg/dL. These findings are in accord with the studies of Suanawnsawat, et al. [39], which detailed the lipid-bringing down impacts of Ocimum tenuiflorum. The Triglyceride levels in the metformin-treated group were 51.79±2.31 mg/dL as shown in Figure 3, tantamount to the normal group. This finding is in agreement with Albert's, et al. [43] research, which features the lipid-managing impacts of metformin.

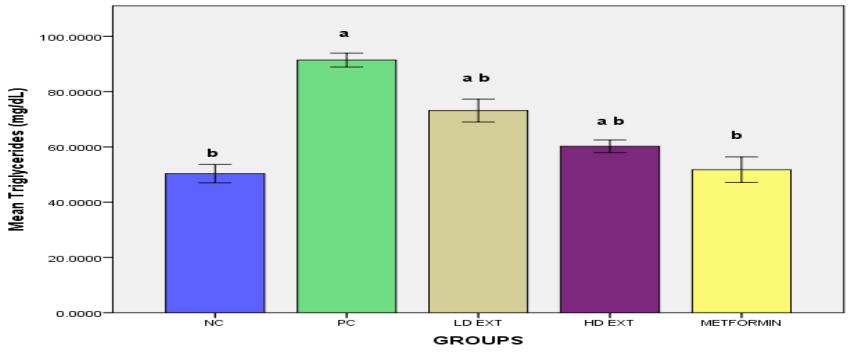

Figure 3: Triglycerides level of effects of ethanol extract of *Ocimum tenuiflorum* on albino rats.

The normal control group had a mean HDL level of 57.25±0.76 mg/dL, which is the typical HDL concentration in the absence of treatment and serves as the study's baseline. The expected range for HDL in healthy subjects is consistent with the elevated HDL level in the normal control [44]. The positive control group, regulated a standard treatment, exhibited an essentially diminished HDL level of 29.79±1.12 mg/dL. This huge reduction is reliable with the expected impact of the positive control, conceivably instigating an unfavorable effect on HDL levels as shown by Michael, et al. [45].

The noticed decrease in HDL in the positive group upholds the legitimacy of the plant extract. Compared to the normal and positive control groups, the low dose extract group had an HDL level of 35.91±0.46 mg/dL, indicating a moderate effect on HDL. The high group exhibited a higher HDL level of 48.55±0.67 mg/dL contrasted with the low group, showing a portion subordinate impact on HDL levels. This corresponds to the study by Albert, et al. [43], indicating that herbal extracts may cause a more pronounced physiological response at higher concentrations. This finding compares

to the study by Adli, et al. [46], who recommended a group subordinate reaction of plant extracts on lipid profiles. The moderate diminishing in HDL at the low group proposes a nuanced connection between *Ocimum tenuiflorum* concentrate and lipid digestion. The metformin group, a standard anti-diabetic treatment, displayed a HDL level of 53.46±0.70 mg/dL as shown in Figure 4, exhibiting a huge increment contrasted with other study groups. According to Bright, et al. [47], the existing body of research on metformin's beneficial effect on lipid profiles is in line with this finding.

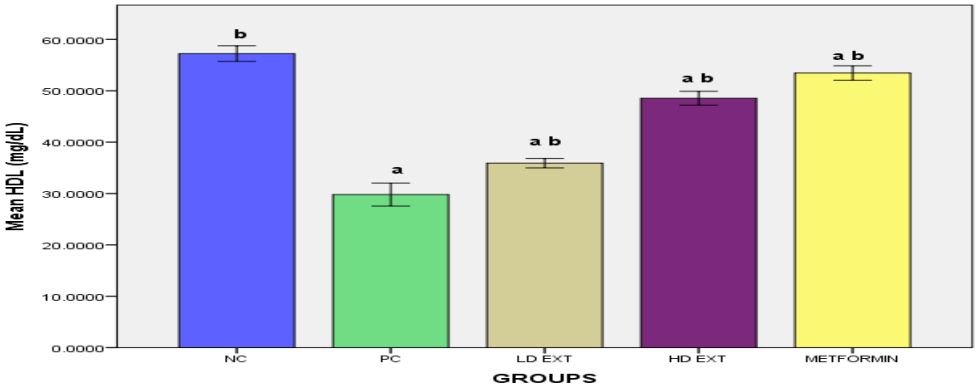

Figure 4: HDL level of effects of ethanol extract of *Ocimum tenuiflorum* on albino rats.

The normal control group showed a LDL level of 102.03±0.76 mg/dL. The LDL level in this group serves as a benchmark for assessing the effects of Metformin and *Ocimum tenuiflorum* extract. The steady LDL level in the control group proposes that the exploratory circumstances prompted no unfavorable impacts on lipid digestion. The positive control group, treated with a standard intercession, showed a LDL level of 136.38±1.45 mg/dL. This critical height lines up with discoveries from previous studies, demonstrating that the positive control prompts an expansion in LDL [48]. The higher LDL level in the positive group affirms the unwavering quality of the trial arrangement in actuating the normal lipid profile changes.

The group treated with a low group of *Ocimum tenuiflorum* concentrate showed a LDL level of 125.32±2.21 mg/dL. This outcome compares to studies by Kaliaperumal, et al. [49] recommending that lower portions of *Ocimum tenuiflorum* may impact lipid levels decidedly. The high group showed a LDL level of 114.86±2.14 mg/dL. This finding is in concurrence with Suanawnsawat, et al. [39] work, proposing that higher centralizations of *Ocimum tenuiflorum* extract might prompt a more articulated decrease in LDL levels. The huge decrease in LDL at the high portion suggests an expected helpful impact in overseeing lipid problems. The Metformin-treated group displayed a LDL level of 117.76±1.32 mg/dL as shown in Figure 5. This outcome compares to the studies of Alfred, et al. [48], supporting Metformin's realized impact in regulating lipid profiles.

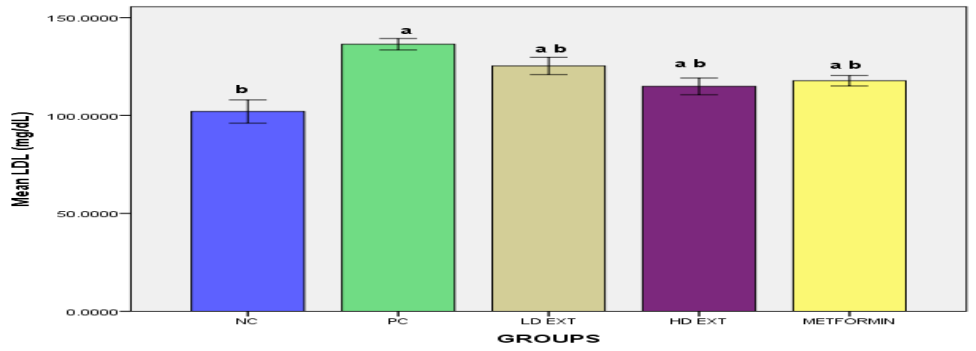

Figure 5: LDL level of effects of ethanol extract of *Ocimum tenuiflorum* on albino rats.

Conclusion

This study demonstrated that ethanol extracts of *Ocimum tenuiflorum* possess significant hypoglycemic and hypolipidemic activities in albino rats based on lowered levels of blood glucose, total cholesterol, LDL and other measured biomarkers. The effects approached or surpassed that of the clinically-used Metformin for some biochemical parameters. Based on the experimental findings, we recommend further large-scale studies to elucidate the active hypoglycemic and hypolipidemic compounds in *O. tenuiflorum* and standardize extract doses. Continued in-vivo experimentation is also recommended to reproduce effects in additional animal models of type II diabetes and metabolic syndrome to establish mechanisms of action, bioavailability, toxicity, and therapeutic ranges of *O. tenuiflorum* preparations.

Ethics Approval and Consent to Participate

This study was approved by the Research and Ethics Committee of the Department of Biochemistry, Faculty of Basic Medical Sciences, Bayelsa Medical University, Yenagoa, Bayelsa State, Nigeria with a Reference Number FBMS/AD/BCH/REC/29/02.

Consent for Publication

Not applicable

Availability of Data and Materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing Interest

The authors declare to have no financial and non-financial competing interests.

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Authors' Contribution

Concept – O.I.O., O.S.O; Design – O.I.O, D.A; Supervision – O.I.O., D.A; Resources – O.S.O., O.I.O., D.A; Materials – O.S.O; Data Collection and/or Processing – O.I.O.; Analysis and/or Interpretation – D.A.., O.I.O.; Literature Search – O.S.O; Writing – O.I.O., D.A., O.S.O; Critical Reviews – O.I.O, D.A.

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