*Asian Journal of Biochemistry, Genetics and Molecular Biology*

*8(4): 25-40, 2021; Article no.AJBGMB.71631*
*ISSN: 2582-3698*

# Hypoglycemic, Hypolipidemic and Antioxidant Activities of *Ocimum gratissimum* Leaf Extract on Diabetic Rats

**Na'ima Musa Sani[1*], Abdulhamid Abubakar[1] and Nwagu Jude[1]**

*[1]Department of Biochemistry, Kebbi State University of Science and Technology, Aliero, Kebbi State, Nigeria.*

***Authors' contributions***

*This work was carried out in collaboration among all authors. Author AA designed the study, performed the statistical analysis, author NMS wrote the protocol, wrote the first draft of the manuscript and managed the analyses of the study. And author NJ managed the literature searches. All authors read and approved the final manuscript.*

***Article Information***

DOI: 10.9734/AJBGMB/2021/v8i430201
Editor(s):
(1) Dr. Arulselvan Palanisamy, Muthayammal Centre for Advanced Research (MCAR), Muthayammal College of Arts and Science, India.
(2) Dr. Theocharis Koufakis, Aristotle University, Greece.
(3) Dr. S. Prabhu, Sri Venkateswara College of Engineering, India.
(4) Dr. Ahmed Medhat Mohamed Al-Naggar, Cairo University, Egypt
Reviewers:
(1) Nahla S. Abdel-Azim, National Research Centre, Egypt.
(2) R. Kavitha, Periyar University PG Extension Centre, India.
Complete Peer review History: https://www.sdiarticle4.com/review-history/71631

***Original Research Article***

***Received 02 July 2021***
***Accepted 07 August 2021***
***Published 09 August 2021***

## ABSTRACT

**Aims:** *Ocimum gratissimum* is a medicinal plant that has been traditionally used in the management of many diseases including diabetes mellitus. The aim of this research was to evaluate hypoglycemic, hypolipidemic and antioxidant activities of *Ocimum gratissimum* leaf extracts on diabetic rats.
**Study Design:** Mention the design of the study here.
**Place and Duration of Study:** Department of Biochemistry, Faculty of Life Science, Kebbi State University of Science and Technology Aliero, Kebbi State, Nigeria. Between february 2021 and June 2021.
**Methodology:** The phytochemical screening was carried out using standard procedures. The extract was administered orally (100, 200 and 400 mg/kg body weight, for 21 days) to alloxan-

**Corresponding author: E-mail: naimamusa001@gmail.com;*

induced diabetic rats. Hypoglycemic effects, change in body weight, lipid profile and antioxidant activities of diabetic rats treated with the extract were assessed and compared with normal, diabetic control and standard drug treated rats. Histological examination of the pancreas during 21 days of treatment was also carried out.

**Results:** The extract produced a significant reduction in fasting blood glucose level in alloxan-induced diabetic rats. Significant differences were also observed in body weights, serum lipid profile and levels of antioxidant vitamins and enzymes of *Ocimum gratissimum* methanol extract treated diabetic rats, when compared with diabetic, normal and standard drug treated rats. Histopathological studies of the pancreas showed comparable regeneration of the cells by extract which were earlier necrosed by alloxan. Methanol leaf extract of *Ocimum gratissimum* exhibit significant hypoglycemic, hypolipidemic and antioxidant activities in alloxan-induced diabetes in rats. The extract could be further processed towards the management of diabetes mellitus.

**Conclusion:** *Ocimum gratissimum* extract showed a promising good hypoglycemic effect, the extracts also exhibit hypolipidemic and antioxidant activities on diabetic rats. There was regeneration of pancreatic islets of Langerhans. Therefore at acute dose the extracts can serve as an alternative in the management of diabetes mellitus.

*Keywords: Alloxan, intraperitoneal; methanol; diabetes mellitus; antioxidants; Ocimum gratissimum.*

## 1. INTRODUCTION

Diabetes mellitus is an endocrine and metabolic disease with rapid increase worldwide and being considered to be at an epidemic level by the World Health Organization [1]. Diabetes mellitus has been classified into two broad types Insulin-dependent diabetes mellitus, (IDDM) or Type 1 diabetes mellitus and non-insulin-dependent diabetes mellitus (NIDDM) or the Type 2 diabetes mellitus [2,3]. An estimated of 140 million people globally has diabetes mellitus and estimated to be 300 million by the year 2025 [4].

Recently, the use of natural products has gained more interest for remedy of diabetes and other ailments. Since ancient times mankind has used plants to cure diseases and relieve physical sufferings because of their cultural acceptability, compatibility with biological system, and lesser side effects [5]. In core northern part of Nigeria the folkloric use of scent leaf has drawn attention of many herbal practitioners. *Ocimum gratissimum* is a medicinal plant that has been traditional used in the management of many diseases including diabetes mellitus.

In northern Nigeria, especially people leaving in rural areas have limited access to conventional drugs, while those that have access to convention therapy either have financial problems or might have already developed fear for conventional drugs mainly due to their side effects.

Therefore the purpose of this dissertation was to comparatively search for more effective herbal alternative among the selected plants, which in turn would be easily accessible, generally acceptable and less expensive.

## 2. MATERIALS AND METHODS

### 2.1 Collection and Identification of Plant Samples

*Ocimum gratissimum* leaves were collected from Shuni town, Shuni Local Government Area of Sokoto State they were authenticated by a Taxonomist from Department of Plant Science and Biotechnology, Kebbi State University of Science and Technology, Aleiro. A voucher specimen (KSUSTA/PSB/H/VOUCHER NO:100) is deposited in the same herbaruim.

#### 2.1.1 Plant preparation and extraction

The leaves of *Ocimum gratissimum* were washed with clean water and allowed to dry under shade for two weeks. They were then grinded to coarse powder using mortar and pestle. Five hundred grams (500g) of the powdered sample were soaked in 2500ml of methanol for 72 hrs [6]. They were then filtered using muslin cloth and the filtrates were evaporated using an oven set at $45^0$C.The dried extract was stored separately in an air tight container and kept in refrigerator at $4^0$C. The percentage yield of the extract was calculated using the formula.

Percentage yield = (weight of extract)/(weight of ground plant material ) × 100/1

## 2.2 Phytochemical Screening of *Ocimum gratissimum*

The Phytochemical screening for the presence of saponins, tannins, alkaloids, flavonoids, tannins, steroids, saponins, glycosides, cardiac glycosides, saponin glycosides, balsams, anthraguines, and volatile oil were carried out according to the methods described by [7,8,9]

## 2.3 Acute Oral Toxicity Studies (LD50)

The acute oral toxicity studies of *Ocimum gratissimum* methanol leaf extract (OGMLE) was undertaken as per the Organization for Economic Co-operation and Development [10] guidelines for testing of chemicals by up-and-down procedure. The rats were fasted overnight and the weight of each rat used was recorded just before use. Animals were divided randomly into two treatment groups for each extract, each group consisting of three Albino rats. Each treatment group received orally the studied plant in the limit test at a rate of 3000 mg/kg body weight. Conducted and terminated after three survivals out of three animals. Again a higher dose of 5000mg/kg of all extracts were given to three groups of rats. Animals were kept under close observation for 1hr, 4hrs, 6hrs and 12hrs after administering the extracts, and then they were observed daily for 14 days for any change in general behavior and other physical activities.

## 2.4 Induction of Diabetes

Diabetes was induced in the rats by intraperitoneal injection of Alloxan in a dose of 120mg/kg body weight in Normal Saline [11]. Diabetes was confirmed in the animal after 48 hours by estimation of fasting blood glucose level and only rats with blood glucose level above 150mg/dl were used for the study.

## 2.5 Fasting Blood Glucose Monitor

Fasting blood sugar was determined using Accu-check active glucometer by Roche Diagnostic according to the method [12].

The rats were randomly divided into 6 groups (n=4) and treated as follows:

Group 1 Normal control (untreated).
Group 2 Alloxan treated (diabetic)
Group 3 Alloxan induced-diabetic rats treated with glibenclamide (0.2 mg/kg).
Group 4 Alloxan induced-diabetic rats treated with extract (100 mg/kg).
Group 5 Alloxan induced-diabetic rats treated with extract (200 mg/kg).
Group 6 Alloxan induced-diabetic rats treated with extract (400 mg/kg).

The extract was administered to the animals orally. Body weight changes were also monitored weekly throughout the experimental period. The rats were sacrificed on the twenty-second day of the experiment. Blood samples were collected in heparinised bottles for biochemical analysis while organs (livers and pancrease) were collected for histopathology evaluation.

## 2.6 Antioxidant Assay

Lipid peroxidation was determined by measuring spectrophotometrically the level of the lipid peroxidation product, malondialdehyde (MDA) as described by Wallin et al., [13], (SOD) Activity was determined using the method of Xin et al., [14]. The activity of catalase was assayed according to the method of Aebi, [15], glutathione (GSH) was based on the method of Jollow et al., [16], Vitamin A concentration was determined using the method of Laurence and Sobel, [17] and vitamin E was determined as described by Barker and Frank, [18].

## 2.7 Serum Lipid Profile

The determinations of serum total cholesterol (TC) levels were done using kit product of Randox, UK [19]. High density lipoprotein cholesterol (HDL-C) was determined using precipitation method in the presence of phosphotungistic acid and magnesium chloride [20]. Total triglyceride was determined by the enzymatic method described by Buccolo and David, [18] using commercially available kit. Low density lipoprotein concentration was calculated using the method of Friedwald et al., [21].

## 2.8 Histopathological Examination

Histopathology was done using the method of Drury et al., [22]. Liver and pancreas of the rats were harvested and preserved in 10 % formalin. The organs were fixed in 10 % buffered formalin for 72 hours. The tissues were then dehydrated in alcohol of graded concentrations and embedded in paraffin. Embedded tissues were cut into sections of 5 µm thick and these were stained with hematoxylin and eosin for photo microscopic assessment and placed on a clean

labelled microscope glass slide. The slide was mounted on an electric light microscope for examination of any possible histopathological features. Photomicrographs of the samples were then taken.

## 2.9 Data Analysis

The data generated from the study are present as Mean ± Standard error of mean (SEM) and subjected to one-way analysis of variance (ANOVA) and statistical difference between means were separated using Duncan multiple comparison test using statistical package for social science (SPSS) version 20. Values are considered statistically significant at $P<0.05$. Graphs are plotted using Microsoft excel and Prism software, micrographs and diagrams were presented where necessary using digital camera.

## 3. RESULTS AND DISCUSSION

## 3.1 Results

### 3.1.1 Percentage Yield

The extraction of *Ocimum gratissimum* methanol leaf extract yields 13.86%. *Ocimum gratissimum* extract is dark green in colour, extremely sour in taste with a very high pleasant smell.

### 3.1.2 Results of phytochemical screening

The qualitative phytochemical screening of *Ocimum gratissimum* methanol leaf extract is presented in Table 1. The result for the phytochemical screening of *Ocimum gratissimum* revealed the presence of Alkaloids, Flavonoids, Tannins, Steroids, Saponin, Glycoside, Cardiac glycoside, Saponin glycoside, Balsams and Volatile oil While Anthraguinone is not.

### 3.1.3 Acute toxicity (LD50) profile of *Ocimum gratissimum* methanol leaf extract (OGMLE)

The results of acute oral administration of the leaf extracts in various doses indicated no mortality up to 14 days after treatment. There was also no sign of toxicity. Hence the LD50 is estimated to be greater than 3000mg/kg b.w.

### 3.1.4 Effect of *Ocimum gratissimum* leaf extract on body weight (g) of diabetic rats

The weekly effect of *Ocimum gratissimum* leaf extract on body weight of albino rats treated for 21 days was presented in (Fig. 1). Before extract administration, (week 0) there were no significant differences in the body weight of all groups ($P>0.05$). However at week 1 and 2 of the experiment there was no significant difference between standard drug, all treatment groups when compared to normal ($P>0.05$). However at week 3 of the experiment there was significant decrease in groups treated with standard control, OGMLE 100mg/kg, 200mg/kg and 400mg/kg when compared to diabetic control ($P<0.05$) but there was significant decrease of body weight in diabetic group when compared to control ($P<0.05$).

### 3.1.5 Effect of *Ocimum gratissimum* leaf extracts on fasting blood sugar

Prior to the administration of extract and standard drug (week 0), the fasting blood sugar of all the alloxan-induced groups significantly increased ($P<0.05$) compared to the normal control group (Table 2). However after week 1 of treatment there was significant difference between groups treated with OGMLE 100mg/kg and 400mg/kg when compared to diabetic control

**Table 1. Qualitative phytochemical constituents of *Ocimum gratissimum*l leaf extract**

| Phytochemicals | *Ocimum gratissimum* |
|---|---|
| Alkaloids | + |
| Flavonoids | + |
| Tannins | + |
| Steroids | + |
| Saponin | + |
| Glycoside | + |
| Cardiac glycoside | + |
| Saponin glycoside | + |
| Balsam | + |
| Anthraguinone | - |
| Volatile oil | + |

*KEY: + = Present, - = Not detected*

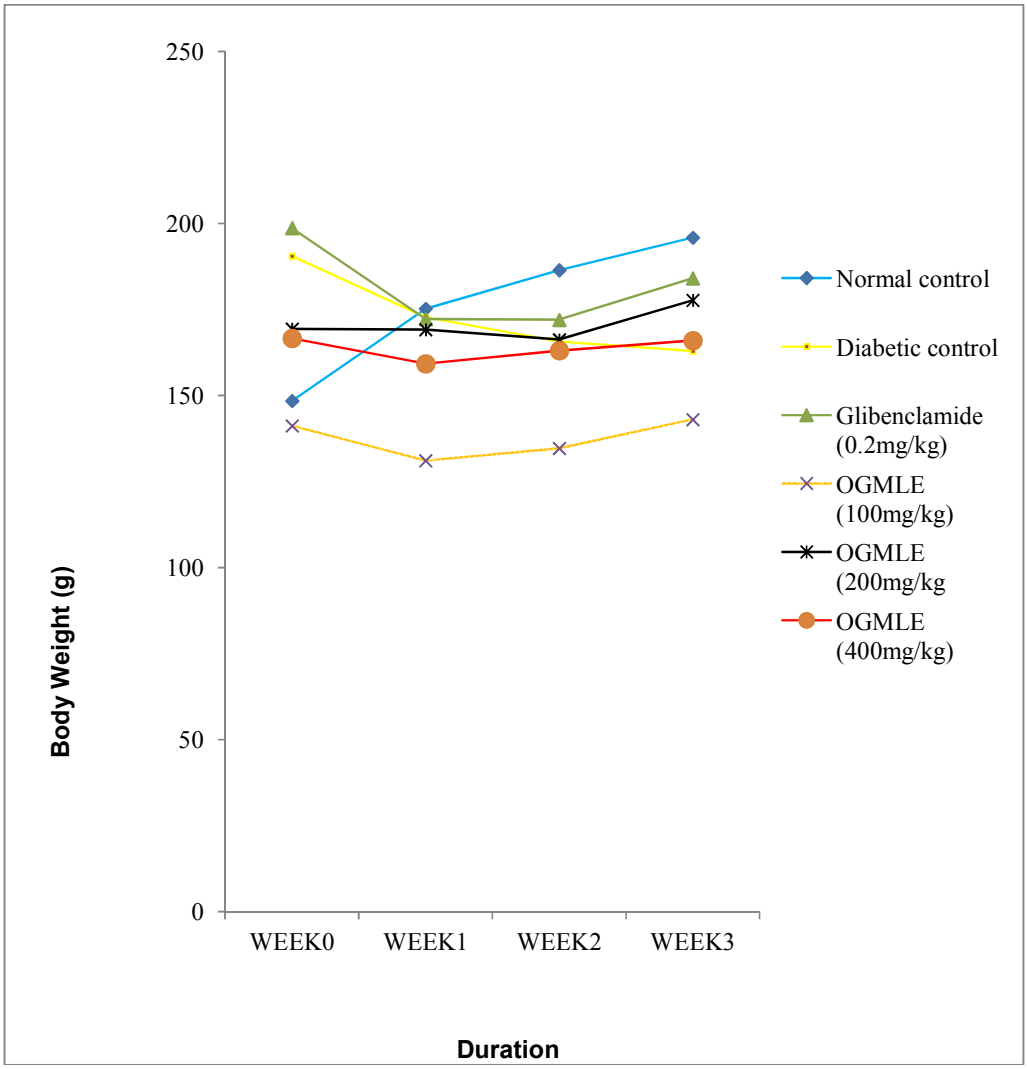


**Fig. 1. Bodyweight of animals administered with leaf extracts of *Ocimum gratissimum* for Three weeks**

group (P<0.05) but there was significant increase in diabetic control group compared to normal control (P<0.05). At week 2 and 3 of treatment the fasting blood sugar levels of groups treated with OGMLE at 100mg/kg, 200mg/kg, and 400mg/kg showed a promising non-significant decrease when compared to diabetic control group (P<0.05), But however diabetic control group significantly increase compared to normal control (P<0.05).

### 3.1.6 Antioxidant activity of *Ocimum gratissimum* leaf extracts

Tabale 3 shows the antioxidant activity of *Ocimum gratissimum* leaf extract on diabetic induced albino rats. The result for superoxide dismutase (SOD) revealed increase in groups treated with OGMLE 100 mg/kg and 400mg/kg and standard control when compared to diabetic control (P<0.05) but there was significant decrease in (SOD) in diabetic control when compared normal control (P<0.05). Catalase on the other hand revealed an increase in all OGMLE treatment groups and standard control, but there was significant decrease in diabetic control compared to normal control (P<0.05). Glutathione peroxidase (GPx) showed non-significant increase between all OGMLE treatment groups (P>0.05) and standard groups when compared to diabetic control, but diabetic control group significantly decreased when

compared to normal control group (P<0.05). However Malonedialdehyde (MDA) showed a non-significant (P>0.05) decrease in groups treated with 100 mg/kg and 400mg/kg compared to standard drug control, but diabetic control showed a significant increase in (MDA) compared to normal control (P<0.05). Vitamin A showed a non-significant increase (P>0.05) in groups treated with 200mg/kg and 400 mg/kg compared to diabetic control and diabetic control group showed a significant decrease (P<0.05) in Vitamin A when compared to normal control group. There was also significant increase (P<0.05) in vitamin E between all OGMLE treatment groups and standard control when compared to diabetic control but there was significant decrease (P>0.05) in Vitamin E in diabetic control when compared to normal control group.

### 3.1.7 Effect of *Ocimum gratissimum* leaf extracts on lipid profile

The results for the effect of *Ocimum gratissimum* leaf extracts (OGMLE) on lipid profile are presented in Table 4. The serum total cholesterol showed that there was significant decrease (P<0.05) in all OGMLE treatment groups and standard control when compared to diabetic control but there was significant increase (P<0.05) in (TC) of diabetic control when compared to normal control group. Serum Total triglyceride revealed a significant decrease in all OGMLE treatment groups and standard control when compared to diabetic control (P<0.05), but diabetic control group showed a significant increase in TG when compared to normal control (P<0.05). However serum Low density lipoprotein concentration revealed a nonsignificant decrease (P>0.05) in OGMLE treatment groups at 100mg/kg and 400mg/kg when compared to diabetic control group but there was a significant increase (P<0.05) in diabetic control group when compared to normal control. Very Low density lipoprotein concentration showed a significant decrease (P<0.05) across all OGMLE treatment group and standard control compared to diabetic control, also diabetic control group significantly increased when compared to normal control. There were no significant increase (P>0.05) in serum High density lipoprotein cholesterol, (HDL-C) across all OGMLE treatment groups and standard control when compared to diabetic control and diabetic control revealed a nonsignificant reduction (P>0.05) when compared to normal control.

### 3.1.8 Histopathological examination of liver and pancreas tissues

Histopathological examination results showed that the liver of rats in normal control group, standard drug control group, alloxan control group and groups treated at 100, 200, and 400 mg/kg of *Ocimum gratissimum* methanol leaf extract exhibited normally distributed portal triad, central vein and hepatocytes (Plates 1-6) respectively. However the pancreas of rats in normal control group showed normal exocrine pancreas and islets of Langerhans (Plate 7) while alloxan control groups showed islets of Langerhans with fibrosis (Plate 8). While all groups treated with standard drug, 100, 200, and 400mg/kg of *Ocimum gratissimum* respectively showed islets of Langerhans with fibrosis and regeneration regular endometrial gland and stroma (Plates 9-12) respectively.

**Table 2. Effect of *Ocimum gratissimum* leaf extracts on fasting blood sugar**

| Treatments | Glucose Concentration (mg/dl) | | | |
|---|---|---|---|---|
| | Week0 | Week 1 | Week 2 | Week 3 |
| Normal control | 93.50±4.66$^{a}$ | 95.50±9.526$^{a}$ | 85.75±4.479$^{a}$ | 69.25±6.43$^{a}$ |
| Diabetic control | 370.00±25.93$^{b}$ | 457.50±69.03$^{d}$ | 366.25±63.72$^{b}$ | 378.25±56.23$^{b}$ |
| Standard drug (0.2mg/kg) | 407.00±83.46$^{bc}$ | 277.25±9.01$^{c}$ | 80.50±5.04$^{a}$ | 86.00±6.57$^{a}$ |
| OGMLE(100 mg/kg) | 368.75±54.62$^{b}$ | 206.25±34.92$^{abc}$ | 79.25±3.68$^{a}$ | 115.00±34.957$^{a}$ |
| OGMLE(200 mg/kg) | 528.00±8.21$^{c}$ | 146.00±33.40$^{ab}$ | 102.25±27.57$^{a}$ | 69.50±11.236$^{a}$ |
| OGMLE(400 mg/kg) | 413.50±54.24$^{bc}$ | 247.00±27.486$^{bc}$ | 78.25±9.41$^{a}$ | 91.25±14.34$^{a}$ |

*Values are presented as mean ± SEM (n = 4) value having same superscript are not significantly different at (P>0.05) analysed using One-Way ANOVA, followed by Duncan multiple comparison test with SPSS version 20.0*

*Note:OGMLE = Ocimum gratissimum methanol leaf extract*

**Table 3. Antioxidant activity of *Ocimum gratissimum* leaf extracts**

| Treatment | SOD (µmole/g) | CAT (µmole/g) | GPx (µmole/g) | MDA (µmole/g) | Vitamin A (mg/dl) | Vitamin E (mg/dl) |
|---|---|---|---|---|---|---|
| Normal control | 9.43±0.30$^{h}$ | 44.08±3.60$^{de}$ | 22.84±0.47$^{bc}$ | 1.21±0.10$^{a}$ | 6.50±0.37$^{e}$ | 1.69±0.03$^{b}$ |
| Diabetic control | 2.64±0.27$^{a}$ | 10.66±0.36$^{a}$ | 8.19±0.50$^{a}$ | 3.61±0.24$^{de}$ | 2.74±0.18$^{a}$ | 0.85±0.03$^{a}$ |
| Standard drug (0.2mg/kg) | 8.02±0.57$^{efg}$ | 38.87±4.09$^{bcd}$ | 17.19±1.54$^{b}$ | 2.30±0.10$^{abc}$ | 5.15±0.29$^{cde}$ | 1.38±0.09$^{ab}$ |
| OGMLE(100mg/kg) | 6.61±0.34$^{de}$ | 32.81±0.67$^{b}$ | 14.46±1.44$^{b}$ | 2.93±0.54$^{cd}$ | 9.50±0.35$^{f}$ | 4.48±.368$^{e}$ |
| OGMLE(200mg/kg) | 6.16±0.38$^{cd}$ | 34.87±1.86$^{bc}$ | 14.52±0.38$^{b}$ | 4.40±0.64$^{ef}$ | 5.69±0.36$^{de}$ | 2.48±.17$^{c}$ |
| OGMLE(400mg/kg) | 6.39±0.74$^{de}$ | 41.55±4.34$^{cde}$ | 17.40±2.53$^{b}$ | 2.70±0.69$^{bcd}$ | 4.21±1.45$^{abcd}$ | 3.30±367$^{d}$ |

*Values are presented as mean ± SD (n = 4) value having same superscript are not significantly different at (P>0.05) analysed using One-Way ANOVA, followed by Duncan multiple comparison test with SPSS version 20.0. SOD= superoxide dismutase, CAT= catalase, GPx= glutathione peroxidase and MDA= malondialdehide*

**Table 4. Effect of *Ocimum gratissimum* leaf extracts on lipid profile**

| Treatments | TC (mg/dl) | TG (mg/dl) | LDL-C (mg/dl) | VLDL (mg/dl) | HDL (mg/dl) |
|---|---|---|---|---|---|
| Normal control | 116.50±4.30$^{b}$ | 87.09±2.62$^{a}$ | 48.31±3.26$^{a}$ | 15.02±0.35$^{a}$ | 34.95±0.74$^{de}$ |
| Diebetic control | 185.61±4.75$^{d}$ | 200.31±2.86$^{e}$ | 116.70±2.22$^{d}$ | 46.14±2.21$^{g}$ | 26.06±2.057$^{abcd}$ |
| Standard drug (0.2mg/kg) | 144.71±3.94$^{c}$ | 151.23±4.55$^{c}$ | 59.64±4.29$^{bc}$ | 51.16±1.25$^{f}$ | 28.30±2.36$^{abcde}$ |
| OGMLE(100mg/kg) | 116.66±4.79$^{b}$ | 107.55±3.29$^{ab}$ | 49.90±3.97$^{ab}$ | 28.88±0.87$^{d}$ | 25.19±2.71$^{abc}$ |
| OGMLE(200mg/kg) | 89.00±2.73$^{a}$ | 99.59±2.36$^{ab}$ | 48.31±2.77$^{a}$ | 22.02±1.81$^{bc}$ | 24.95±4.13$^{abc}$ |
| OGMLE(400mg/kg) | 91.71±2.22$^{a}$ | 115.48±2.53$^{b}$ | 54.39±4.39$^{ab}$ | 21.16±0.53$^{b}$ | 24.30±1.53$^{ab}$ |

*Values are presented as mean ± SD (n = 4) value having same superscript are not significantly different at (P>0.05) analysed using One-Way ANOVA, followed by Duncan multiple comparison test with SPSS version 20.0.TC= total cholesterol, TG=triacylglycerol, LDL-C=low density lipoprotein cholesterol, VLDL= very low density lipoprotein and HDL= high density lipoprotein*

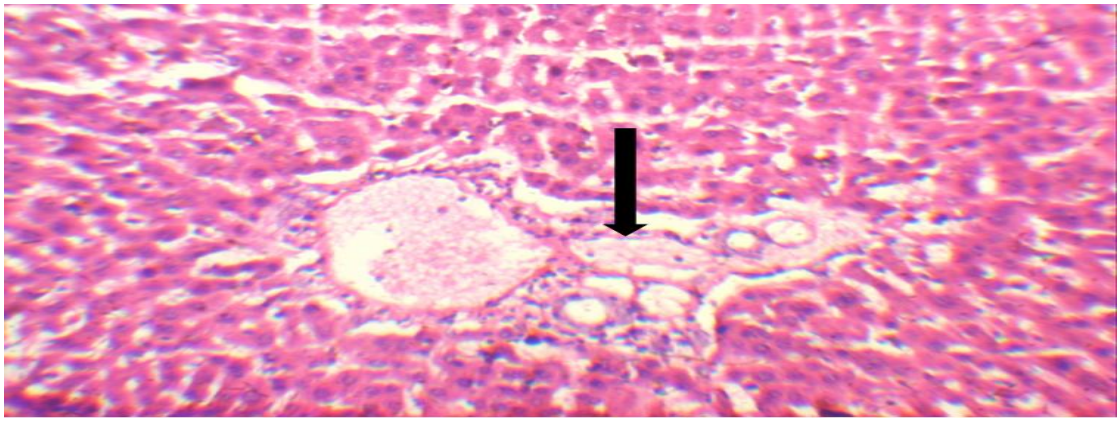

**Plate 1. Photomicrograph of rat's liver obtained from normal control**
*(H and E stain, x 200 magnification). Showing normal portal triad and hepatocyte (Black arrow)*

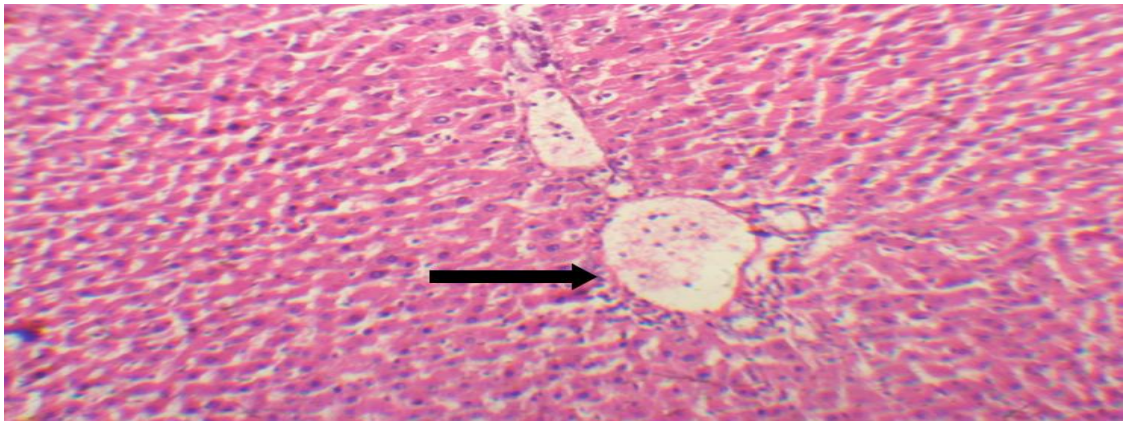

**Plate 2. Photomicrograph of rat's liver obtained from group administered with 250 µg/kg of glybenclamide**
*(H and E stain, x 200 magnification). Showing normal portal triad and hepatocyte (Black arrow)*

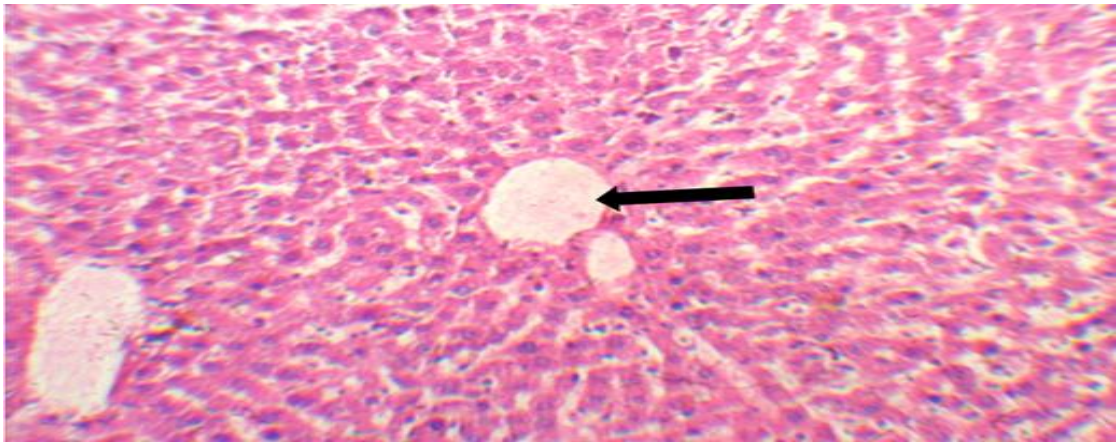

**Plate 3. Photomicrograph of rat's liver obtained from group administered with 120 mg/kg of alloxan**
*(H and E stain, x 100 magnification). Showing normal portal triad and hepatocyte (Arrow) (Arrow)*

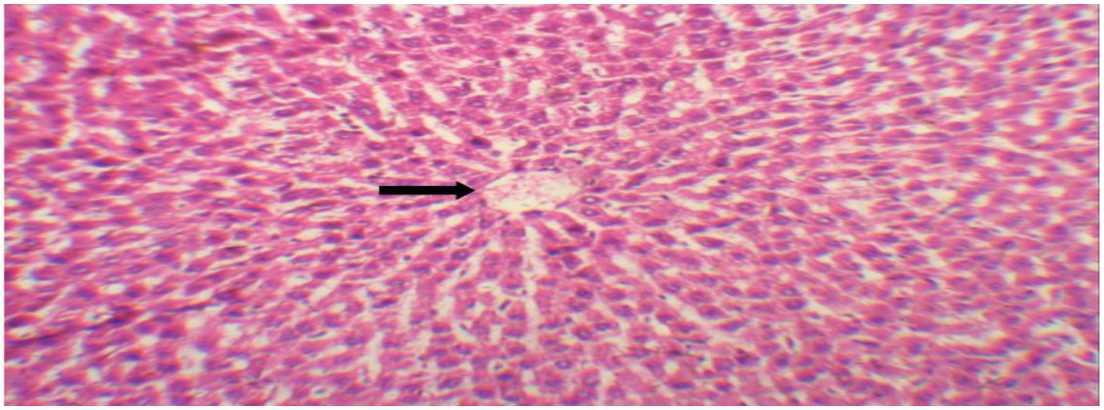

**Plate 4. Photomicrograph of rat's liver obtained from group administered with 100 mg/kg of methanol leaf extract of *Ocimum gratissimum***
*(H and E stain, x 200 magnification). Showing normal portal triad and hepatocyte (Arrow)*

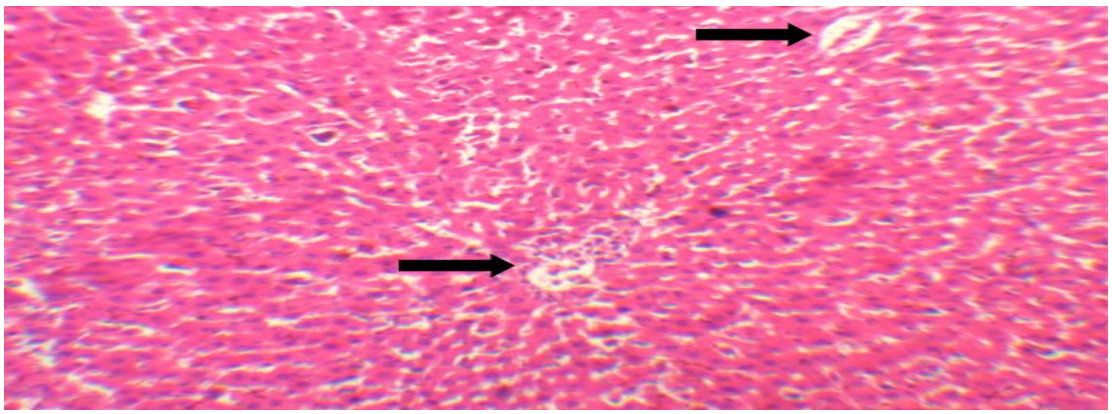

**Plate 5. Photomicrograph of rat's liver obtained from group administered with 200 mg/kg of methanol leaf extract of *Ocimum gratissimum***
*(H and E stain, x 200 magnification). Showing normal portal triad and hepatocyte (Arrow)*

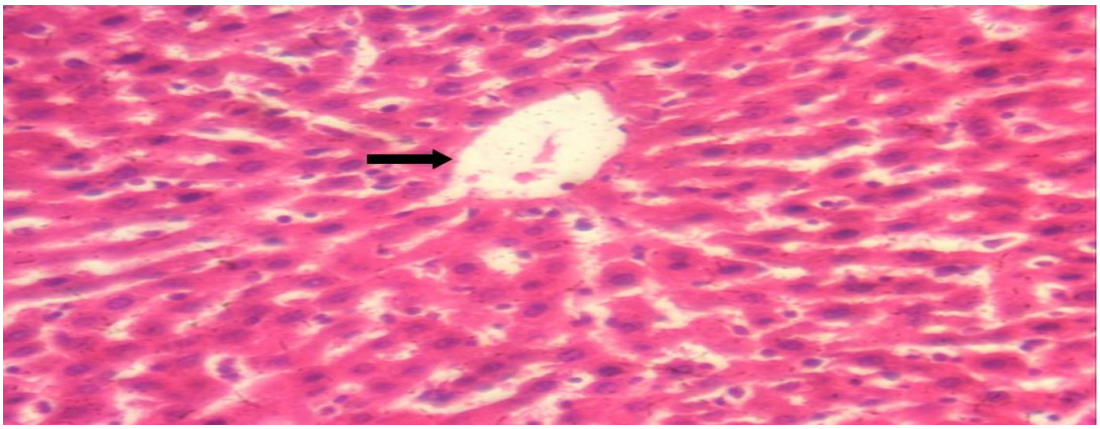

**Plate 6. Photomicrograph of rat's liver obtained from group administered with 400 mg/kg of methanol leaf extract of *Ocimum gratissimum***
*(H and E stain, x 200 magnification). Showing normal portal triad and hepatocyte (Arrow)*

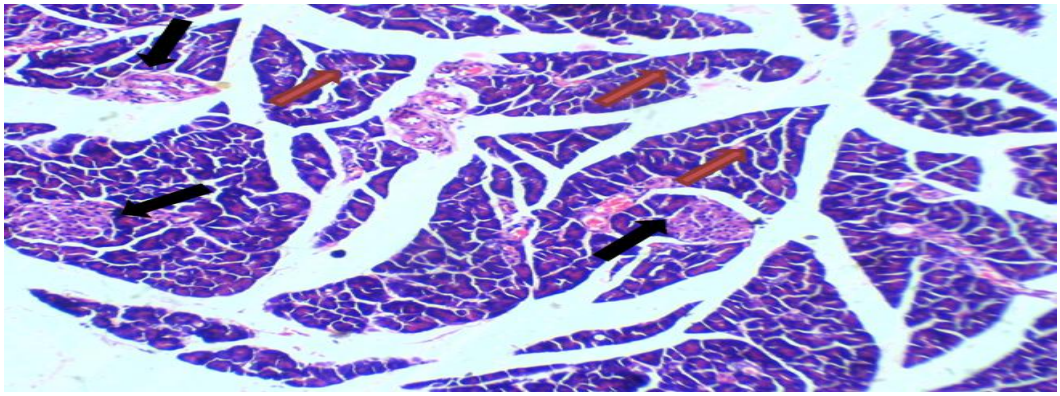

**Plate 7. Photomicrograph of rat's pancreas obtained from normal control**
*(H and E X 200 magnification) showing the endocrine cells (Islets of Langerhans black arrow) and the dark stains cells of the surrounding as the exocrine pancreas (Red arrow)*

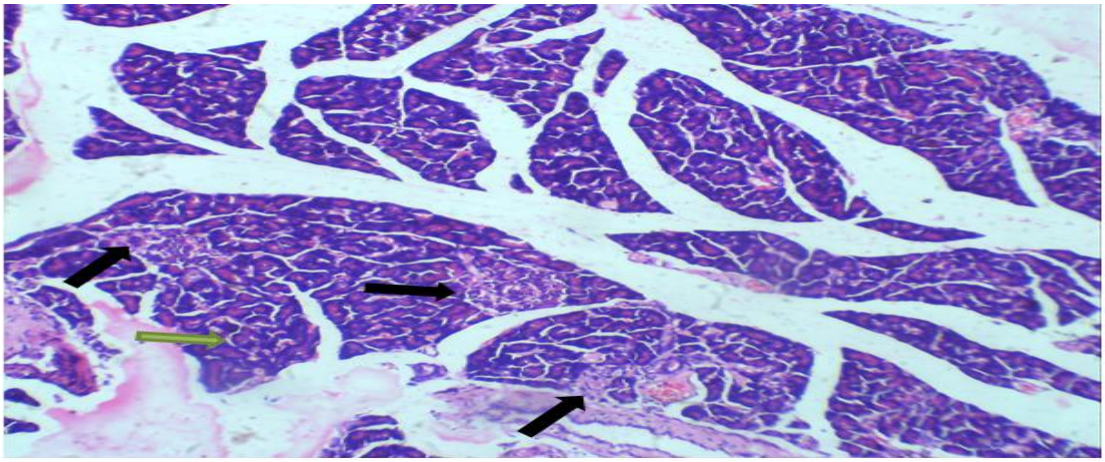

**Plate 8. Photomicrograph of rat's pancreas obtained from group administered with 120mg/kg of alloxan**
*(H and E stain, x 200 magnification). Showing the endocrine cells as pale stained (Islets of Langerhans black arrow with fibrosis) and the dark stains cells of the surrounding as the exocrine pancreas (green arrow)*

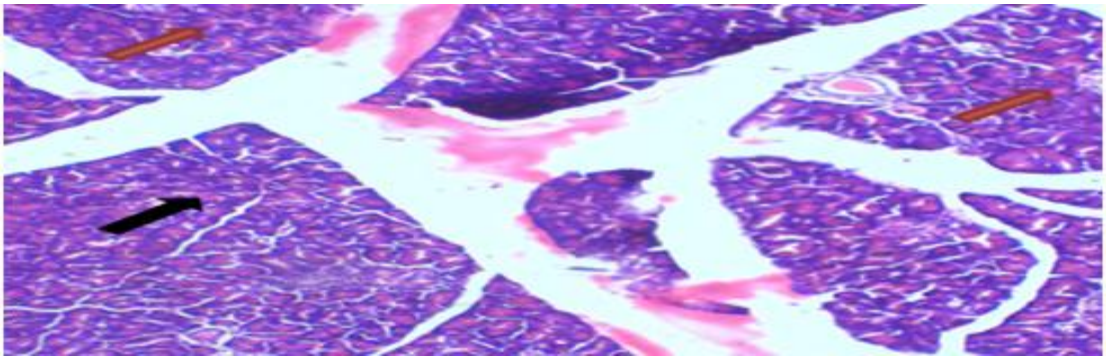

**Plate 9. Photomicrograph of rat's pancreas obtained from group administered with glybenclamide 250 µg/kg**
*(H and E stain, x 200 magnification). Showing (Islets of Langerhans black arrow with regeneration) and the dark stains cells of the surrounding as the exocrine pancreas (green arrow)*

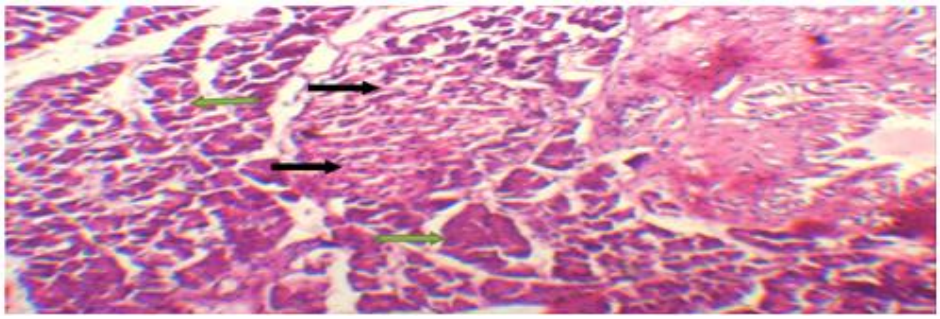

**Plate 10. Photomicrograph of rat's pancreas obtained from group administered with 100mg/kg of methanol leaf extract of *Ocimum gratissimum***

*(H and E stain x 200 magnification). Showing islets of Langerhans (black arrow) with fibrosis and regeneration and the dark stains cells of the surrounding as the exocrine pancreas (green arrow)*

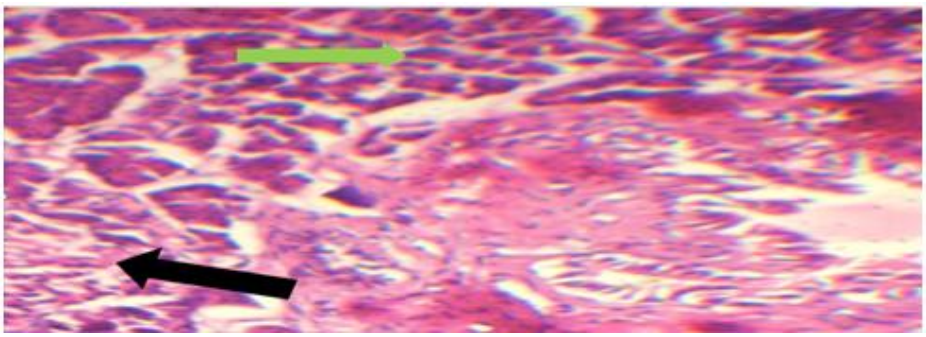

**Plate 11. Photomicrograph of rat's pancreas obtained from group administered with 200mg/kg of methanol leaf extract of *Ocimum gratissimum***

*(H and E stain x 200 magnification). Showing few endocrine cells as pale stained ( islets of Langerhans black arrow with fibrosis and regeneration) and the dark stains cells of the surrounding as the exocrine pancreas (green arrow)*

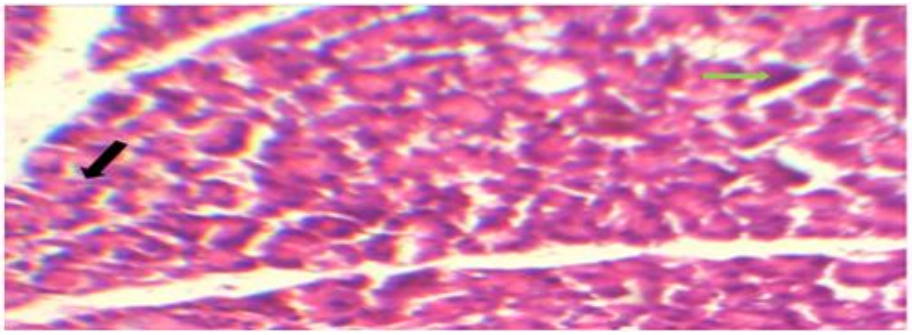

**Plate 12. Photomicrograph of rat's pancreas obtained from group administered with 250 mg/kg of methanol leaf extract of *Ocimum gratissimum***

*(H and E stain x 200 magnification). Showing the endocrine cells as pale stained ( islets of Langerhans black arrow with fibrosis) and the dark stains cells of the surrounding as the exocrine pancreas (green arrow)*

## 3.2 Discussion

Phytochemicals are relevant in medicine, food and the dye industry. Some of them have biological activities [23]. Others have pharmacological effects for example, flavones and tannin form important ingredients of several laxative medicines [24]. Mukheji et al. [25] reported that these secondary metabolites are known to be bioactive anti-diabetic agents.

Flavonoids have been reported to exert a wide range of biological effects such as hypolipidaemic, antibacterial, antioxidant, anti-inflammatory, antibacterial, hepatoprotective, antiviral, antiallergic, anticancer and cytoprotective activities [26]. Saponins are also known to exhibit antimicrobial, immunostimulant, hypocholesterolaemic, anticarcinogenic activities and also protect plants from microbial pathogens [27,28]. Alkaloids have several pharmacological activities including; antihypertensive, antiarrhythmic, antimalaria, anticancer and antiseptic effects [29]. Tannins act as a defense mechanism in plants against pathogens, herbivores and hostile environmental conditions [30]. In other plants used in folk medicine for diabetes mellitus management, phytochemical constituents like alkaloids, glycosides, flavonoids, and tannins have variously been reported to be contributory [31,32,33].

Toxicological evaluation is carried out to determine the safety of drugs and plant products for human use [34]. Determination of LD50 (lethal dose that would kill 50% of the tested population) is usually the first step in the evaluation of the toxic characteristics of a substance [35]. This study showed that the LD50 of the leaf extract of *Ocimum gratissimum* is greater than 5000 mg/kg indicating that they are nontoxic at “acute dose”.

Alloxan is a known diabetogenic agent that selectively and permanently destroys the pancreatic β-cells through production of free radicals and excessive calcium concentrations in cell cytoplasm. [36,2].This pancreatic β-cells destruction invariably leads to hyperglycemia through absolute insulin deficiency, hepatic glucose over production and reduced muscles uptake of glucose [37].

Weight loss or gain in animals has major financial and medical implications, as witnessed by the plethora of popular diets and the relationship of weight change to health and disease [38]. Body weight loss is one of the most dramatic and consistent changes resulting from chronic social stress and considerably more indicative for underlying toxicity than is weight gain [38]. Weight loss is commonly observed during diabetic condition. This is likely due to the breakdown of adipocytes and muscle tissues to replace energy lost from the body due to frequent urination and increased glycogenolysis (breakdown of glycogen to glucose) [39]. In this study, the diabetic rats treated with *Ocimum gratissimum* leaf extract showed bodyweight lost during the first week of the experiment however a relatively steady body weights was observed subsequently. These steady body weights observed with rats agrees with the work of Davis and Humphrey, [40], where n-hexane extract of Annona squamosa had similar effects on diabetic animal models. This also agrees with the reports of Viswanathaswamy et al., [41] and Gutierrez et al., [42], whom noticed weight loss in diabetic rats during the first and second week of administration.

In this study, diabetic rats were diagnosed with extremely high fasting blood glucose levels above 150mg/dl. However, treatment with *Ocimum gratissimum* leaf extracts successfully reduced the FBGLs, just as was observed with the standard drug. This agrees with the works of Viswanathaswamy et al., [41], where Plectranthus lowered BGLs in diabetic rats. This reduction in FBGLs is also in agreement with the works of Nirmala et al., [43], where n-hexane extract of Cassia fistula barks reduced blood glucose levels and Gutierrez et al., [42] where n-hexane extract of Phalaris canariensis reduced the blood glucose levels of streptozotocin-induced diabetic mice.

Superoxide dismutase (SOD) protects tissues against oxygen free radicals by catalyzing the removal of superoxide radical (O2-), which damages the membrane and biological structures [44]. Catalase (CAT) has been shown to be responsible for the detoxification of significant amount of hydrogen peroxides (H2O2) [45]. CAT and SOD are the two major scavenging enzymatic antioxidant that removes the toxic free radical in vivo. In the liver and kidney, it has been reported that there is reduction in the activation of CAT and SOD in diabetes mellitus. This causes some of the deleterious effect due to the elevation of superoxide radicals and hydrogen peroxide [46]. GSH is another enzymatic antioxidant that support and defend against ROS. They form the first line of antioxidant defense mechanism to protect the organism from ROS-mediated oxidative damage. MDA is a major product of lipid peroxidation and thus an index for measuring the degree of lipid peroxidation and alteration in the cellular redox status. In agreement with the previous studies of Kakkar et al., [47], the induction of diabetes in rats with alloxan results to an increase in lipid peroxidation, an indirect evidence of intensified free radical production. In the present study there

was increased activity in SOD, GSH and CAT in all treatment groups with of *Ocimum gratissimum* leaf extracts as well as standard drug group.

The non-enzymatic antioxidant molecules play excellent roles in preserving the cells from oxidative damage, thus maintaining membrane integrity [48]. Benammar et al., [49] reported that the concentration of different vitamins (A, and E) in diabetic rats were found to have an increase in there dose dependent manner by leaf and root of Zizphus lotus aqueous extract in diabetic rats. Vitamin E is the most lipholic antioxidant and resides mainly in the cell membrane. In the present study *Ocimum gratissimum* leaf extracts revealed a promising result in vitamin A and E .

Increased LDL-C level leads to accumulation of fat deposit in the arteries, thus, obstructing the free flow of blood and this could be fatal. This is the major risk factor for the development of atherosclerosis and several lipid-associated ailments like obesity, coronary heart disease (CHD), stroke and kidney failure [50]. Cholesterol is a lipid that combines with protein to form lipoprotein for adequate circulation in the blood. When the lipoprotein has more protein than cholesterol, it is called high density lipoprotein cholesterol (HDL-C) and its circulation in the body till it gets to the liver is faster. However, when the lipoprotein has more cholesterol than protein, it is called low density lipoprotein cholesterol (LDL-C) and its circulation in the body is slower leading to the obstruction of blood vessels. HDL-C is the good cholesterol and LDL-C is the bad cholesterol [51].

The LDL-C is the major carrier of cholesterol in the blood. The role of LDL-C is to transport cholesterol to peripheral tissues and regulate cholesterol synthesis at the sites [52]. When there is high cholesterol, the HDL-C and LDL-C levels are reversed making LDL-C level higher than HDL-C. High concentration of serum cholesterol and total triglyceride (TG) in diabetes may be attributed to inhibition of cholesterol catabolism [53]. This may be also result from mobilization of fatty acids from adipose tissue by lipolysis (lipid breakdown) due to insulin deficiency. In the present studies *Ocimum gratissimum* leaf extracts showed reducing potential on LDLC, TC, TG, VLDL, suggesting the extracts hypolipidaemic properties and agreed with the works of Gutierrez et al., [43].

The photomicrographs in the post sub-chronic study in groups treated with 200 and 400mg/kg of *Ocimum gratissimum* leaf extracts showed pancreatic cells Islets of Langerhans with fibrosis and regeneration this suggesting that the extracts have mild generational pancreatic effects. Histopathological studies of diabetic control group, standard control group showed multiple vacuolation, reduction in size and number of islet cells, hypertrophy of pancreatic lobules, oedematoid islet cells (resulting from lesion). This is because alloxan caused β-cell necrosis (permanent damage) and since β-cells make up about 80% of the islet cells, its alloxan-induced necrosis and obliteration resulted in reduction in islet size and number [54].

## 4. CONCLUSION

*Ocimum gratissimum* extract showed a promising good hypoglycemic effect, the extracts also exhibit hypolipidemic and antioxidant activities on diabetic rats. There was regeneration of pancreatic islets of Langerhans. Therefore at acute dose the extracts can serve as an alternative in the management of diabetes mellitus.

## ETHICAL APPROVAL

Animal ethics committee approved the use of experimental animals for this study.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES

1. Petal M, Rybczynski P. Treatment of non-insulin dependent diabetes mellitus. Expert Opin.Inveslig. Drugs.2003;12(4):623-633.
2. Ozturk Y, Aydin S, Beis R, Başer KHC, Berberoglu H. Effects of *Hypericum perforatum* L. and *Hypericum calycinum* L. extracts on the central nervous system in mice. Phytomedicine. 1996;3(2):139-146.
3. Judd RL, Raman P. Comparative Effects of troglitazone and insulin on glucagon-stimulated glucose production in isolated rat hepatocytes. Diabetes. 1999;48(5):SA263-SA263.
4. Smith SA, Tadayyon M. Insulin sensitisation in the treatment of Type 2 diabetes. Expert opinion on investigational drugs. 2003;12(3):307-324.
5. Giwa A. Contraceptive decision-making in north-central Nigeria (Doctoral dissertation, University of Sheffield); 2015.

6. Dupont É, Falardeau P, Mousa SA, Dimitriadou V, Pepin MC, Wang T, Alaoui-Jamali MA. Antiangiogenic and antimetastatic properties of Neovastat (AE-941), an orally active extract derived from cartilage tissue. Clinical & experimental metastasis. 2002;19(2):145-153.
7. Harborne JB. Phenolic compounds. In Phytochemical methods. Springer, Dordrecht. 1973;33-88)
8. Trease GE, Evans WC. Pharmacognsy. 11th edn. Brailliar Tiridel Can; 1989.
9. Sofowora A. African medicinal plants. University of Ife Press (Nig). 3rd Edition. 1993;21-30.
10. Organisation for Economic Co-operation and Development. Test No. 407: Repeated Dose 28-day Oral Toxicity Study in Rodents. OECD Publishing; 2008.
11. Chougale PA, Boylan JM, Posner BI, Faure R, Brautigan DL. Hepatic protein phosphotyrosine optimization of alloxan dose is essential to induce stable diabetes for prolonged period. Asian Journal of Biochemistry. 2007;2:402–408.
12. Marks V, Dawson A. Rapid stick method for determining blood glucose concentration. British Medical Journal. 1965;1:25-29.
13. Wallin B, kosengreen B, Shertzer HG, Camejo G. Lipoprotein oxidation and measurement of transformation in single micro liter plate its use for evolution of antioxidants. Journal of Analytical Biochemistry. 1993;208:10-15.
14. Xin Z, Waterman DF, Henken RM. Effect of copper status on neutrophil function,superoxide dismutase and copper distribution In steers. Journal of Dairy Science. 1991;74:28-31.
15. Aebi HE. Catalase: In methods of enzymatic analysis. 3rd. Ed. Academic Press, New York. 1983;673-684.
16. Jollow D, Michel JR, Zampagtionic N, Gillete JR. Bromobenzene included liver necrosis: Protective role of glutathione and evidence for 3,4-Bromobenzene oxide as the hepatotoxic Metabolite.Pharmacology. 1974;11:151-169.
17. Laurence PA, Sobel AE. Changes in serum vitamin A level during the human menstrual cycle. The Journal of Clinical Endocrinology and Metabolism. 1953;13(10):1192-1200.
18. Barker H, Frank O. Clinical vitaminology, methods and interpretation. New York7 Interscience Publishers John Wiley and Sons. 1968;172-4.
19. Trinder P. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Annals of clinical Biochemistry. 1969;6(1):24-27.
20. Buccolo G, David M. Estimation of lipids by enzymatic method. Clin Chim Acta. 1973;19:476.
21. Friedwald WT. Estimation of the concentration of low density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. Clin. Chem. 1972;18:499-502.
22. Drury RA, Wallington EA, Cameron R. Carletons histological technique. 4th Edition. Oxford University press.New York. 1967;48-66.
23. Sparg SG, Light ME, Staden VJ. Biological activities and distribution of plant saponins. Journal of Ethnopharmacology. 2004;94:219-243.
24. Ansel HC, Popovich NG, Allen LV. Pharmaceutical dosage forms and drug delivery systems. 1995;120-125.
25. Mukherji PK, Maiti K, Mukherjee K, Houghton PJ. Leads from Indian medicinal plants with hypoglycemic potentials. Journal of ethnopharmacology. 2006;106(1):1-28.
26. Kumar S, Pandey AK. Chemistry and biological activities of flavonoids: An overview. The scientific world journal; 2013.
27. Desai SD, Desai DG, Kaur H. Saponins and their biological activities. Pharma Times. 2009;41(3):13-16.
28. Francis G, Kerem Z, Makkar HP, Becker K. The biological action of saponins in animal systems: a review. British journal of Nutrition. 2002;88(6):587-605.
29. Roberts MF, Wink M. Biochemistry, ecology, and medicinal applications. Alkaloids. Plenum Press, New York, London. 1998;1-7.
30. Levi CA. Anti-diabetic properties of n-hexane fruit extract of dacryodes edulis on alloxan-induced diabetic rats (*Rattus novergicus*) (Doctoral dissertation); 2017.
31. Akah PA, Uzodinma SU, Okolo CE. Antidiabetic activity of aqueous and methanol extract and fractions of Gongronema latifolium (Asclepidaceae) leaves in alloxan diabetic rats. Journal of Applied Pharmaceutical Science. 2011;1(9):99.

32. Akubue PI, Bagchi D, Ihm WJ, Stohs SJ. Excretion of malondialdehyde, formaldehyde, acetaldehyde, acetone and methyl ethyl ketone in the urine of rats given an acute dose of malondialdehyde. Archives of toxicology. 1994;68(5):338-341.
33. Gyang SS, Nyam DD, Sokomba EN. Hypoglycaemic activity of *Vernonia amygdalina* (chloroform extract) in normoglycaemic and alloxan-induced hyperglycaemic rats. Journal of Pharmacy and Bioresources. 2004;1(1):61-66.
34. Gautam AK, Avasthi S, Bhadauria R. Colletotrichum sansevieriae on Sansevieria trifasciata–a report from Madhya Pradesh, India. Plant Pathol. Quar. 2012;2:190-192.
35. Ogbuehi IH, Ebong OO, Obianime AW. Oral acute toxicity (LD50) study of different solvent extracts of Abrus precatorius Linn leaves in wistar rats. European Journal of Experimental Biology. 2015;5(1):18-25.
36. Aguwa CN, Ndu OO, Nwanma CC, Udeogaranya PO, Akwara NO. Verification of the folkloric diuretic claim of *Hibiscus sabdariffa* L. petal extract. Nigerian Journal of Pharmaceutical Research. 2004;3(1):1-8.
37. Okonta J. Evaluation of the antidiabetic activity of the seed extract of picralima nitida stapf (Apocyanaceae) (Doctoral dissertation); 2015.
38. Wing RR, Phelan S. Long-term weight loss maintenance–. The American journal of clinical nutrition. 2005;82(1):222S-225S.
39. Ene AC, Nwankwo EA, Samdi LM. Alloxan-induced diabetes in rats and the effects of black caraway (*Carum carvi* L.) oil on their body weight. Res J Med Med Sci. 2007;2(2):48-52.
40. Davis SK, Humphrey N. The influence of emotional intelligence (EI) on coping and mental health in adolescence: Divergent roles for trait and ability EI. Journal of adolescence. 2012;35(5):1369-1379.
41. Viswanathaswamy AHM, Koti BC, Gore A, Thippeswamy AHM, Kulkarni RV. Antihyperglycemic and antihyperlipidemic activity of Plectranthus amboinicus on normal and alloxan-induced diabetic rats. Indian journal of pharmaceutical sciences. 2011;73(2):139.
42. Gutierrez-Valdez AL, Anaya-Martínez V, Ordoñez-Librado JL, García-Ruiz R, Torres-Esquivel C, Moreno-Rivera M, Avila-Costa MR. Effect of chronic L-dopa or melatonin treatments after dopamine deafferentation in rats: Dyskinesia, motor performance, and cytological analysis. International Scholarly Research Notices; 2012.
43. Nirmala PG, Gowda BT, Foro S, Sowmya BP, Fuess H. 2, 2-Dichloro-N-(phenylsulfonyl) acetamide. Acta Crystallographica Section E: Structure Reports Online. 2008;64(8):o1522-o1522.
44. Arivazhagan P, Thilakavathy T, Panneerselvam C. Antioxidant lipoate and tissue antioxidants in aged rats. The Journal of nutritional biochemistry. 2000;11(3):122-127.
45. Cheng F, Zhao Q, Zhou L, Liu J, Cao Z, Du X, Huang F. Involvement of CAT in the detoxification of HT-induced ROS burst in rice anther and its relation to pollen fertility. Plant Cell Reports. 2018;37(5):741-757.
46. Saravanan G, Ponmurugan P. Ameliorative potential of S-allyl cysteine on oxidative stress in STZ induced diabetic rats. Chemico-biological interactions. 2011;189(1-2):100-106.
47. Kakkar R, Mantha SV, Radhi J, Prasad K, Kalra J. Increased oxidative stress in rat liver and pancreas during progression of streptozotocin-induced diabetes. Clinical science. 1998;94(6):623-632.
48. Gastaldelli A, Baldi S, Pettiti M, Toschi E, Camastra S, Natali A, Ferrannini E. Influence of obesity and type 2 diabetes on gluconeogenesis and glucose output in humans: A quantitative study. Diabetes. 2000;49(8):1367-1373.
49. Benammar C, Baghdad C, Belarbi M, Subramaniam S, Hichami A, Khan A. Antidiabetic and antioxidant activities of Zizyphus lotus L aqueous extracts in Wistar rats. Nutrition and Food Sciences; 2014.
50. Ahmadi SS, Svensson AM, Pivodic A, Rosengren A, Lind M. Risk of atrial fibrillation in persons with type 2 diabetes and the excess risk in relation to glycaemic control and renal function: A Swedish cohort study. Cardiovascular diabetology. 2020;19(1):1-12.
51. Grundy SM, Cleeman JI, Bairey Merz CN, Brewer HB, Clark LT, Hunninghake DB, Coordinating Committee of the National Cholesterol Education Program. Implications of recent clinical trials for the national cholesterol education program adult treatment panel III guidelines. Journal

of the American College of Cardiology. 2004;44(3):720-732.

52. Okolo CA, Ejere VC, Chukwuka CO, Ezeigbo II, Nwibo DD, Okorie AN. Hexane extract of Dacryodes edulis fruits possesses anti-diabetic and hypolipidaemic potentials in alloxan diabetes of rats. African Journal of Traditional, Complementary and Alternative Medicines. 2016;13(4):132-144.

53. Gotto Jr, AM. High-density lipoprotein cholesterol and triglycerides as therapeutic targets for preventing and treating coronary artery disease. American heart journal. 2002;144(6):S33-S42.

54. Esimone CO, Okonta JM, Ezugwu CO. Blood sugar lowering effect of Anacardium occidentale leaf extract in experimental rabbit model; 2001.



*Peer-review history:*
*The peer review history for this paper can be accessed here:*
*https://www.sdiarticle4.com/review-history/71631*