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Research Article

ANTI-DIABETIC EFFECT OF CRUDE LEAF EXTRACTS OF *OCIMUM GRATISSIMUM* IN NEONATAL STREPTOZOTOCIN-INDUCED TYPE-2 MODEL DIABETIC RATS

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ABSTRACT

Ethnopharmacological relevance: Ocimum gratissimum is a herbaceous plant commonly found in the savannah, tropical rain forest and coastal areas of Nigeria and beyond where it is commonly used as a condiment in cooking. There are also reports that extracts from this plant are effective in the treatment of diabetes mellitus. However, the hypoglycaemic effects of Ocimum gratissimum have not yet been characterized in suitable animal models of type-2 diabetes mellitus. The aim of the study was to investigate the effect of crude leaf extracts of Ocimum gratissimum on the blood glucose levels in normoglycaemic rats and in neonatal streptozotocin-induced rat model of type-2 diabetes mellitus.

Materials and Methods: Dried fresh leaves of Ocimum gratissimum were ground into coarse powder and subjected to aqueous and 70% ethanol extraction, separately in a soxhlet apparatus for 10 hours. The filtrates were then slowly evaporated to dryness on an electrothermal heating mantle regulated at 60^0C. Neonatal streptozotocin-induced diabetic rat model was developed by intraperitoneal injection of streptozotocin dissolved in citrated buffer (PH 4.3) at the dose of 80mg/kg in neonatal rats on the second day after birth. After 6 weeks diabetic rats with plasma glucose level more than 180mg/dl were used for the study. A total number of 58 neonatal streptozotocin-induced diabetic rats were studied. An equal number of normal adult rats of either sex, six weeks old, weighing 120-180g with plasma glucose level 69.54-77.46 mg/dl served as controls. The aqueous and alcoholic extracts of Ocimum gratissimum leaves were tested for hypoglycaemic activity in normal and neonatal streptozotocin-induced diabetic rats over a period of three weeks. Blood samples from rats were collected from the tail vein at zero, 1, 2, 4, 8 hours after drug administration. Plasma glucose levels were determined using the glucose oxidase method.

Results: In the normoglycaemic rats, oral 100µg/g aqueous extract produced transient significant reduction in blood glucose at 2hours ($p<0.05$). Reductions in blood glucose produced by higher doses of 200µg/g and 300µg/g were more sustained compared to the controls group. Analysis of variance (ANOVA) showed that the effect produced was dose-dependent. In the hyperglycaemic rats, the aqueous extract elicited more dramatic reduction in blood glucose levels. Blood glucose was reduced significantly even at 100µg/g body weight ($P<0.01$). Administration of doses of 200µg/g and 300µg/g resulted in a highly significant reduction ($p< 0.001$). Hypoglycaemic effects were also determined to be significantly dose dependent. In both the normoglycaemic and hyperglycaemic rats, however, blood glucose reduction was significantly more in the glibenclamide treated group at all time intervals. The effects of ethanol leaf extracts in the normal and hyperglycaemic rats followed the same pattern as with the aqueous extracts except that the 100µg/g dose showed no significant reduction in blood glucose of the normoglycaemic group when compared with the untreated control group. At equivalent doses, aqueous extract of *Ocimum gratissimum* leaf produced significantly greater degree of reduction in blood glucose compared to the ethanol extract.

Conclusion: It is concluded that *Ocimum gratissimum* is a potent hypoglycaemic agent and is potentially useful in the treatment of diabetes mellitus.

Keywords: Streptozotocin, Hyperglycaemia, Type-2 diabetes, Neonatal rat, *Ocimum gratissimum*

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by hyperglycaemia and altered metabolism of lipids, carbohydrates and proteins with an increased risk of long term complications, including vascular disease[1].

The two broad categories of diabetes mellitus are designated type-1 and type-2. Type 1A diabetes mellitus result from autoimmune beta cell destruction which usually leads to insulin deficiency. Type-1B diabetes mellitus is also characterized by insulin deficiency as well as a tendency to develop ketosis. However, individuals with type-1B diabetes mellitus lack immunologic markers indicative of autoimmune destructive process of the beta cells. Type-2 diabetes mellitus is a heterogeneous group of disorders usually characterized by variable degrees of insulin resistance, impaired insulin secretion and decreased glucose utilization. Distinct genetic and metabolic defects in insulin action and/or secretion give rise to the common phenotype of hyperglycaemia in type-2 diabetes.

The use of crude extracts from traditional plants in the treatment of diabetes mellitus is widely practiced in Nigeria[2]. *Ocimum gratissimum* (Linn), family Labiaceae, is a herbaceous plant commonly found in the savannah, tropical rain forest and coastal areas of West Africa and tropical Asia. In Nigeria it is commonly used as a condiment in cooking. There are also reports that extracts from this plant are effective in the treatment of diabetes mellitus[3]. An initial study in Jos, Nigeria reported significant hypoglycaemic activity of alcoholic extract of this plant in a type-1 diabetes mellitus rat model[4]. However, the hypoglycaemic effects of *Ocimum gratissimum* have not yet been studied in suitable animal models of type-2 diabetes mellitus or in clinical trials on human subjects.

Animal model of diabetes mellitus is not identical to the human disease. However the neonatal streptozotocin-induced rat (n-STZ rat) model has several advantages over other models and is considered to be a suitable experimental model for type-2 diabetes mellitus[5]. Streptozotocin(STZ) is a deoxy-s[{methyl_nitrosamino) carbonyl}-amino}-D gluco pyranose molecule that produces a selective toxic effect on β–cells and induce diabetes mellitus in most laboratory animals[6,7]. High doses of beta cell toxins like streptozotocin and alloxan induce insulin deficiency and type 1 diabetes mellitus with ketosis. However doses calculated to cause a partial destruction of beta cells mass can be used to mimic the mild relative insulin deficiency state of type 2 diabetes mellitus without causing ketosis[8]. Streptozotocin (STZ) is preferred to alloxan and other islet cell toxins because it has more specific beta cell cytotoxicity.

The diabetic syndrome in this model is generated by injecting Wistar rats on the day of birth (n^0=birth) intravenously via saphenous vein or intraperitoneally with 100mg\kg of STZ[9]. The n-STZ rat model may be adjusted by varying the day of the STZ injection after the birth. For instance the n^2-STZ and n^5-STZ models are developed by 80mg/kg intraperitioneal STZ injection on the second day and fifth day after birth respectively[10,11]. These models have been shown to be suitable experimental models of type 2 diabetes mellitus[5,10,11].

The objective of the present study is to investigate and compare the effect of crude leaf extracts of *Ocimum gratissimum* on the blood glucose levels in normoglycaemic rats and neonatal streptozotocin-induced type-2 model diabetes rats.

MATERIALS AND METHOD

Plant material

Fresh leaves of *Ocimum gratissimum* were collected from a garden in University of Nigeria Enugu Campus and were botanically authenticated at the Department of Botany University of Nigeria Nsukka, Nigeria.

Experimental animals

Preparation of neonatal STZ-induced rat (n-STZ) model of type 2 diabetes mellitus

The neonatal rats with a body weight of 5.16 ± 0.29g were treated with streptozotocin (Sigma, Aldrich lnc. USA) dissolved in citrated buffer (PH 4.3) by intraperitoneal injection of 80µg/g on the second day after birth[10,11]. To achieve this 100mg streptozotocin was dissolved in 50ml of citrated buffer giving a concentration of 2mg/ml. Intraperitoneal administration of streptozotocin (STZ) was done using 100 I.U./ml insulin syringes and needle. The required minimum sample size was estimated using a standard formula[12]. A total of 87 neonatal rats were treated with STZ. The blood glucose was monitored weekly using a One Touch® glucometer. After 6 weeks, diabetic rats having fasting plasma glucose level above 180mg/dl were divided into 13 groups of 3 animals each. The average weight at 6 weeks was 152±3.16g. The rats were described as "fasted" after 10 – 12 hours of overnight fast.

Controls

Adult wistar rats of either sex, six weeks old, weighing 120-180g were used as controls.

Housing

The animals were housed under standard condition in the Animal Research Unit of the Department of Pharmacology and Therapeutics, College of Medicine, University of Nigeria Enugu Campus. The animals were fed ad libitum on commercially available livestock feed (Unique feeds®, Farm Associates Nigeria Ltd. Enugu, Nigeria.) and were allowed free access to clean drinking water. The animals were fed on the "starter" mesh feed within the first two weeks of life; thereafter, the "grower/finisher" mesh feed was used until the end of the study.

Preparation of extract

Fresh leaves of *Ocimum gratissimum* were kept in the oven at 80^{0}C for 10minutes to stop enzyme activity and then 60^{0}C for 30minutes to dry. They were then air dried and ground into coarse powder. The coarse powder was subjected to aqueous and 70% ethanol extraction, separately, in a soxhlet apparatus for 10hours as follows:- Fifty grams of the powdered leaves were placed in the inner thimble of the soxhlet extractor apparatus. Water was passed through the inner thimble via a condenser with reflux system, from a round bottom flask containing 450ml of distilled water and placed on a thermostatic heating mantle regulated at 100^{0}C. The alcohol extract was prepared in the same way as the aqueous extract with the exception of the use of 70% ethanol as solvent and soxhlet thermostat regulated at 60^{0}C. After filtration through Whatman filter paper No.40, the filtrate was slowly evaporated to dryness with an electrothermal heating mantle regulated at 60^{0}C. The yield of the aqueous and ethanol extracts was 13.0 and 11.0% (weight for weight in terms of dried starting material), respectively. Aliquots of the extracts were stored in screwed cap vials at 4 – 8^{0}C until further use. The extracts were re-dissolved in distilled water when used and given orally through gastric intubations.

Collection of blood and determination of blood glucose

Blood samples were taken via the tail vein of the rats. Initial blood glucose measurements in the neonatal rats as well as during the first six weeks of monitoring were by Trinder's glucose oxidase method using One Touch® glucometer (LifeScan Inc., Milpitas, California, USA.)[13].

After the sixth week blood samples from rats were collected via the tail vein in dried sodium fluoride and oxalate bottles. Plasma was separated within 30minutes of collection by centrifuge. Blood glucose levels were determined by the modified glucose oxidase test[14].

Acute toxicity (LD50) determination

Acute toxicity (LD50) determination was adapted from a method described by Miller and Tainter[15] as follows. Fifty adult mice of either sex weighing 15- 25g were distributed into five groups (L1, L2, L3. L4, L5), with 10 mice in each group. One group (L1) was treated with normal saline and considered as control and the other four groups were treated with the aqueous extract given orally in a dose of 1, 2, 4, and 5g/kg body weight respectively. Deaths were recorded after 72 hours and any changes in the behaviour of the animals were also noted and recorded.

Pilot study

A pilot study was done to determine the minimum dosages of extract to be administered. The hypoglycaemic activity of both extracts (aqueous and ethanol extracts) were evaluated separately. A total of 12 normal rats were divided into four equal groups. The animals of group I served as untreated control whereas the other three groups II, III, and IV, were administered with the aqueous extract at a single dose of 25, 50 and 100 mg/kg respectively. Plasma glucose was estimated at 0, 4, 8, and 12 hours calculated for each group using the following formula:

$$\% \text{ variation of glycaemic} = \frac{Gi - Gt \times 100}{Gi}$$

Where Gi and Gt were the values of initial glycaemia (0 hour) and glycaemia at 4, 8 and 12 hours respectively. The plasma glucose levels of different groups at different time intervals were also compared. The extract dose that lowered the glucose level by 25% at 4hours was considered the minimum hypoglycaemic dosage. Similar procedure was carried out for the ethanol extract.

Study of oral administration of the aqueous extract in normal and n-STZ diabetic rats

Five groups of fasted n-STZ diabetic rats were used. Three groups of three rats each (A1, A2, A3) were given orally 100, 200, 300 mg/kg body weight of the aqueous extract dissolved in 2ml of normal saline respectively, using a stomach tube. The fourth group (A4; Positive control) was given orally 5µg/g of glibenclamide in 2ml of normal saline in the same way and the fifth group (A5; negative control) was given 2ml of normal saline and was considered as a negative control group. Blood glucose levels were measured at zero, 1, 2, 4, 8, 10 hours after administration.

Another five groups of fasted normoglycaemic rats, three rats in each group (B1, B2, B3, B4, B5) were studied. Groups B1, B2, and B3 were given orally 100, 200,and 300, µg/g body weight respectively of the aqueous extract, group B4 (positive control) was given orally 5µg/g of glibenclamide while group B5 (negative control) was given orally 2mls of normal saline. Blood glucose was measured in the same way.

Study of oral administration of the alcoholic extract in normal and n-STZ diabetic rats

Similar study was carried out using ethanol extract on n-STZ rats C1, C2, C3, C4, C5, as well as on fasted normoglycaemic rats D1, D2, D3, D4, and D5.

Effect of repeated oral administration of aqueous extract

Three groups (E_1, E_2, E_3) of three fasted normoglycaemic rats in each group were used., E_1 was given orally 200µg/g aqueous extract. E_2 received 5µg/g oral glibenclamide per day (positive control group) while group E_3 was given orally 2ml of normal saline (negative control group).

Another three groups of n-STZ diabetic rats (F_1, F_2, F_3; each of three rats) were used. Group F_1 was treated orally with 200µg/g aqueous extract per day. F_2 received orally 5µg/g glibenclamide per day (positive control group) while F_3 was given orally 2ml of normal saline (negative control group). Oral administration was done using stomach tube and was continued daily for three weeks. Fasting blood glucose levels were measured daily for 7 days, weekly for 3 weeks and then 12 hourly after the last dose for 72 hours, and finally daily for 1 week.

Data analysis

Data were presented as mean ± standard error of mean (SEM). The area under the glucose tolerance curve and the mean glycaemia value during the whole glucose tolerance test were calculated using the rectangles method. Differences between groups were compared using analysis of variance and Student t-test. In order to evaluate dose dependent effect of the extract the positive and negative controls (the glibenclamide and the saline placebo groups) were excluded and data analyzed using 2- way analysis of variance. Subsequently, post hoc Bonferroni multiple comparison test was performed to identify the source of any statistically significant difference. All statistical analyses were carried out using the statistical packages for social science (SPSS Inc. Chicago Illinois) software version 11.0. Statistical test with probability values less than 0.05 were considered statistically significant.

Quality control test/Evaluation of method

The reliability and reproducibility of the plasma glucose assay were assessed in three ways:

1. Random blind repeat measurement of the plasma glucose in a reference quality control laboratory.

2. Blind random introduction of solution of known glucose concentration and comparison with the measurements obtained using the modified glucose oxidase method used in the study.

3. Comparison of the results obtained using the study One Touch® glucometer with that from a quality control laboratory.

The mean absolute differences in the plasma glucose assays are compared in table 1. The variability of measurements was analyzed by Bland-Altman plot[16]. The limit of agreement was computed as average difference in measurements ± 1.96 standard deviation of the difference. There was no statistically significant variability in measurements.

Table 1: Quality control test on methods of blood glucose measurement.

Parameter	Mean absolute difference (mg/dl)	Std error of diff. b/w means (SE- diff)	T-test	P– value
QC-lab. Versus SM-lab.	3.0866	0.8653	1.002	0.334
Known Gluc. Conc. Versus SM-lab.	4.8667	0.2446	0.507	0.620
QC-lab. versus Glucometer	2.5333	0.9139	0.831	0.420

Key:

QC-lab= Quality control laboratory, SM-lab = Study medical laboratory, Gluc. Conc. = Glucose concentration.

Degree of freedom = 14, sample size (n) = 15 in each category.

RESULTS

Baseline data

A total of 87 neonatal rats weighing 5.16 ± 0.29g were given intraperitoneal injection of streptozotocin on the second day of birth. Out of these 21(24.14%) died within the first week of follow up. Thirteen (14.94%) had mild transient hyperglycaemia, while 53 (60.92%) developed chronic diabetes mellitus. Significantly elevated fasting blood glucose was noted in the streptozotocin-treated rats by the seventh day post induction. The diabetic rats manifested increased water intake, polyuria, and polydipsia, but no significant difference in weight compared with untreated normoglycaemic rats.

Acute toxicity and LD50 determination

Figure 1 shows the mortality and lethal dose (LD 50) value following oral administration of aqueous extract of *Ocimum gratissimum* in mice (n= 10 in each group). The lethal dose value was found to be 3.90mg/g. At oral doses between 1-2 mg/g, the animals showed slowness in activity suggesting mild central nervous system depression. At doses between 2-4mg/g, rapid respiration, tremors and twitches were noticed. The plasma glucose level in the animals that died (measured just before the death) was between 25-40mg/dl.

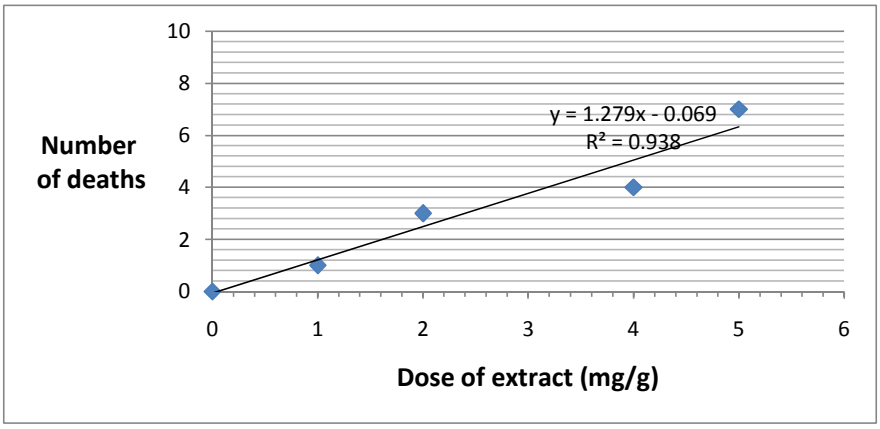

Fig. 1: Graph of mortality following oral administration of increasing doses of aqueous extract of *Ocimum gratissimum* in mice.

Pilot study/determination of minimum therapeutic dose

Significant reduction in blood glucose level by 31.43% and 26.72% at 4 hours was observed in normal rats after oral administration of water and ethanol extracts respectively at a dose of 100µg/g. Hypoglycaemic effects at doses below 100µg/g were not significant when compared with untreated control group (Table 2). The minimum hypoglycaemic (therapeutic) dose of the aqueous and ethanol extract in this study was 100µg/g.

Effect of aqueous extract in normoglycaemic and hyperglycaemic (n-STZ) rats

The mean plasma glucose of fasted animals at various time intervals after oral administration of aqueous extract of *Ocimum gratissimum* leaf in normoglycaemic and hyperglycaemic (nSTZ) rats are shown in tables 3 - 6. The glucose levels were compared to the values obtained for animals given only normal saline (negative control).

In the normoglycaemic rats, the oral administration of 100µg/g aqueous extract produced transient initial significant reduction in blood glucose at 2hours ($p<0.05$). While doses of 200µg/g and 300µg/g produced more sustained blood glucose reduction at 2, 4, and 8 hours compared to the untreated negative controls (Table 3).

A combined 2-way analysis of variance (ANOVA) also showed significant difference in blood glucose concentration among the study groups at 2 hours ($F= 86.092$; $p < 0.001$), 4 hours ($F = 491.357$; $p < 0.001$), and 8 hours ($F = 209.656$; $p < 0.001$). The blood glucose concentration at the commencement of the study (time 0-hour) were similar { $F = 0.432$; $p = 0.782$ }. Significant dose dependent reduction in blood glucose level was observed, (Table 4).

In the hyperglycaemic rats, oral administration of aqueous extracts elicited more dramatic reduction in blood glucose levels. Blood glucose was reduced significantly at the dose 100µg/g at 2, 4, 8 hours ($P<0.01$). Administration of doses of 200µg/g and 300µg/g resulted in a highly significant reduction ($p< 0.001$) at 2, 4, 8 hours (Table 5). Significant dose dependent effect was also observed (Table 6). However, in both the normoglycaemic and hyperglycaemic rats blood glucose reduction was significantly more in the glibenclamide treated group at all time intervals (Tables 3 and 5).

Table 2: Pilot study: Determination of minimum therapeutic dose of aqueous and ethanol extract of *Ocimum gratissimum* in normal rats.

Treatment	Dose (µg/g)	Plasma glucose level (mg/dl) at times indicated					
		Aqueous extract			Ethanol extract		
		0-hour	2-hour	4-hour	0-hour	2-hour	4-hour
Extract	25	84.7 ±6.06	77.6 ±5.29 (8.31)	74.3 ±7.16 (12.31)	87.5 ±8.04	80.8 ±2.89 (7.71)	77.3 ±3.61 (11.71)
Extract	50	84.8 ±5.9	79.3 ±6.02 (6.49)	70.3 ±6.25 (7.11)	82.0 ±3.04	60.8 ±4.52 (5.00)	72.3 ±5.51 (15.00)
Extract	100	87.5±6.07	76.5±6.88 (12.57)	60.0±3.75 (31.43)	87.0±5.41	78.3±3.38 (10.06)	63.8±5.25 (26.72)
Untreated controls		85.5±6.00	81.5±5.68 (4.68)	72.0±8.02 (15.79)	89.5±4.58	85.5±5.41 (4.57)	79.1±3.61 (11.61)

***** Figures in bracket represent percentage reduction in blood glucose when compared with values at 0-hour.

Table 3: Effect of aqueous extract of *Ocimum gratissimum* on plasma glucose level in normal rats.

Treatment	Dose (µg/g)	Plasma glucose level (µg/g) at			
		0-hour	2-hour	4-hour	8-hour
B1 Extract	100	76.433 ±1.95	67.53**^{aaa} ±0.29	65.20^{aaa} ±0.44	61.50^{aaa} ±0.79
B2 Extract	200	77.67 ±0.88	66.70**^{aaa} ±0.38	60.03***^{aaa} ±0.20	57.40**^{aaa} ±0.70
B3 Extract	300	75.67 ±0.88	65.23***^{aaa} ±0.39	59.73***^{aaa} ±0.47	51.17***^{aaa} ±0.62
B4 Glibenclamide (Positive controls)	5	77.67 ±1.20	60.73*** ±0.38	45.63*** ±0.33	40.40*** ±0.31
B5 Controls (2ml normal saline)	-	73.33 ±1.45	69.90 ±0.37	65.77 ±0.39	62.03 ±0.58
Combined Anova (df = 4)	F-statistic	0.432	86.092	491.357	209.656
	P-value	0.783	0.000***	0.000***	0.000***

Key: * - Statistically significant compared with the control at time intervals(*< 0.05, ** <0.01, ***< 0.001).

^{a} – Statistically significant compared with Glibenclamide group at time intervals (^{a}<0.05, ^{aa}<0.01, ^{aaa} < 0.001)

n = 3 in all the treatment groups.

Table 4: Post Hoc multiple comparison of blood glucose levels at varying doses of aqueous extract in normoglycaemic rats using Bonferroni test

Dosage groups	Mean diff. in blood glucose	Std. error	P-value
B1 versus B2 at 2-hours	0.8333	0.5048	0.450
B1 versus B3 at 2-hours	2.3000	0.5048	0.012*
B2 versus B3 at 2-hours	1.4667	0.5048	0.081
B1 versus B2 at 4-hours	5.1667	0.5471	0.0001*
B1 versus B3 at 4-hours	10.4667	0.5471	0.0001*
B2 versus B3 at 4-hours	5.300	0.5470	0.0001*
B1 versus B2 at 8-hours	4.100	1.0015	0.0001*
B1 versus B3 at 8-hours	10.333	1.0015	0.0001*
B2 versus B3 at 8-hours	6.2333	1.0015	0.002*

Key: B1 = 100µg/g, B2 = 200µg/g, B3 = 300µg/g.

*= Statistically significant.

Table 5: Effect of aqueous extract of *Ocimum gratissimum* on plasma glucose level on n-STZ rats.

Treatment	Dose (µg/g)	Plasma glucose level (µg/g) at			
		0-hour	2-hour	4-hour	8-hour
A1 Extract	100	196.67 ±1.76	130.93*** ±2.13	114.8***aaa ±1.60	111.00***aaa ±1.53
A2 Extract	200	195.87 ±1.99	139.00*** ±3.25	111.00***aa ±3.06	108.67***aaa ±1.20
A3 Extract	300	198.00 ±1.15	138.00*** ±2.04	107.35***a ±3.43	101.67***aaa ±1.20
A4 Glibenclamide (Positive controls)	5	198.7 ±1.73	125.00*** ±4.04	91.83*** ±1.36	88.00*** ±1.15
A5 Controls (2ml normal saline)	-	198.73 ±2.11	181.67 ±3.48	153.17 ±1.59	140.33 ±1.45
COMBINED ANOVA (df = 4)	F-statistic	0.424	42.031	83.12	212.929
	P-value	0.788	0.000***	0.000***	0.000***

Key: * - Statistically significant compared with the control at time intervals(*< 0.05, ** <0.01, ***< 0.001).

[a] – Statistically significant compared with Glibenclamide group at time intervals ([a]<0.05, [aa]<0.01, [aaa] < 0.001)

n = 3 in all the treatment groups.

Table 6: Post Hoc multiple comparison of blood glucose levels at varying doses of aqueous extract in nSTZ rats using Bonferroni test.

Dosage groups	Mean diff. in blood glucose	Std. error	P-value
A1 versus A2 at 8-hours	2.333	1.8659	0.773
A1 versus A3 at 8-hours	9.333	1.8659	0.006*
A2 versus A3 at 8-hours	7.000	1.8659	0.022*

Key: A1 = 100µg/g, A2 = 200µg/g, A3 = 300µg/g.

*= Statistically significant.

Effect of ethanol extract in normoglycaemic and hyperglycaemic (n-STZ) rats.

The effect of oral administration of ethanol extract of *Ocimum gratissimum* leaf on blood glucose level of normoglycaemic and neonatal streptozotocin induced hyperglycaemic rats are shown in tables 7 and 8.

In the normal and n-SZT rats, oral administration of ethanol at 100µg/g showed no significant reduction in blood glucose at 2, 4, and 8 hours when compared with the untreated control group. At a dose of 200µg/g, significant reduction was found in the normal rats at 4 and 8 hours (p< 0.05) while dose of 300µg/g produced significant reduction at 2, 4, and 8 hours (p< 0.01; Table 7).

In the hyperglycaemic rats, significant reduction in blood glucose was observed at 4 and 8 hours (p< 0.05) at oral dose of 100µg/g. Oral administration of 200µg/g and 300µg/g produced significant lowering of blood glucose at 2, 4 and 8 hours (Table 8).

Greater reduction in blood glucose was noted in the glibenclamide treated groups (Tables 7 and 8). Significant dose dependent effects were noted in both the normoglycaemic and hyperglycaemic rats using ANOVA.

At equivalent doses, aqueous extract of *Ocimum gratissimum* leaf produced significantly greater degree of reduction in blood glucose when compared with the ethanol extract.

Table 7: Effect of ethanol extract of *Ocimum gratissimum* on plasma glucose in normal rats.

Treatment	Dose (µg/g)	Plasma glucose level (mg/kg) at			
		0-hour	2-hours	4-hours	8-hours
Extract (D1)	100	83.00 ±2.08	78.27aaa ±0.69	75.43aaa ±0.87	70.00aaa ±0.58
Extract (D2)	200	80.33 ±0.88	76.03aa ±0.58	70.63**aaa ±0.58	66.37**aaa ±0.54
Extract (D3)	300	180.00 ±1.15	78.70* ±0.85	70.20**aaa ±0.46	62.83**aaa ±0.80
Glibenclamide (D4)	5.0	81.67 ±1.64	71.03** ±0.61	63.20*** ±0.59	50.10*** ±0.89
Controls (D5) {2ml normal saline}	-	79.33 ±2.40	75.50 ±1.32	75.13 ±0.59	71.70 ±0.89
COMBINED ANOVA (df = 4)	**F-statistic**	0.695	13.706	60.443	156.684
	P-value	0.612	0.000*	0.000*	0.000*

Key: * - Statistically significant compared with the control at time intervals(*< 0.05, ** <0.01, ***< 0.001).

[a] – Statistically significant compared with Glibenclamide group at time intervals ([a]<0.05, [aa]<0.01, [aaa] < 0.001)

n = 3 in all the treatment groups.

Extended oral administration of aqueous extract of *ocimum gratissimum* in normal and diabetic rats

Extended oral administration of 200µg/g of aqueous extract in normal rats produced no significant change in the fasting plasma glucose level from day 1 to the 3rd week of treatment when compared with both the pre-treatment value as well as with untreated group at same time intervals.(Table 9)

In the diabetic rats, daily administration of glibenclamide (5µg/g) and aqueous extract of *Ocimum gratissimum* (200µg/g) produced significant reduction in fasting plasma glucose level from day-one (after commencement) on ward, when compared with pre-treatment Day-0-level, as well with that of untreated control group. Greater blood glucose reduction was observed in the glibenclamide treated group, (Table 10).

Table 8: Effects of ethanol extract of *Ocimum gratissimum* on plasma glucose in n-STZ rats.

Treatment	Dose µg/g	Plasma glucose level (mg/dl) at			
		0-hour	2-hours	4-hours	8-hours
C1 Extract	100	210.07 ±1.22	198.00^{aaa} ±2.52	153.17**^{aaa} ±3.04	149.67**^{aaa} ±2.89
C2 Extract	200	204.67 ±1.76	183.33*^{aa} ±2.03	146.97*^{aa} ±3.66	124.67*^{aaa} ±4.37
C3 Extract	300	206.67 ±2.03	166.00**^{aa} ±4.16	136.35** ±2.30	95.67***^{aa} ±2.60
C4 Glibenclamide	5.0	208.00 ±1.53	139.67*** ±3.28	123.00** ±5.13	79.50*** ±1.89
C5 Controls (2ml normal saline)	-	208.00 ±1.15	193.33 ±2.40	178.00 ±2.74	165.27 ±3.76
COMBINED ANOVA (df = 4)	**F-statistic**	0.783	63.674	32.442	123.580
	P-value	0.562	0.000***	0.000***	0.000***

Key: * - Statistically significant compared with the control at time intervals(*< 0.05, ** <0.01, ***< 0.001).

^{a} – Statistically significant compared with Glibenclamide group at time intervals (^{a}<0.05, ^{aa}<0.01, ^{aaa} < 0.001)

n = 3 in all the treatment groups.

Table 9: Effect of prolonged administration of aqueous extract on the plasma of blood glucose in normal rats

Treatment (Dose)	Plasma glucose levels (mg/dl) at								
	Days				Weeks		Hours after last dose		
	0	1	2	5	2ndwk	3rd wk	12hrs	24hrs	72hrs
Extract (200µg/g)	81.33 ±1.53	80.33^{a} ±1.52	80.00^{a} ±1.00	79.00^{a} ±1.58	81.00^{a} ±1.00	80.67^{a} ±1.52	79.67^{a} ±0.58	82.00^{a} ±2.00	81.33^{a} ±1.33
Glibenclamide (5µg/g)	75.67 ±2.08	59.60*$^{+}$ ±2.65	57.33*$^{+}$ ±2.52	51.00*$^{+}$ ±1.00	51.67*$^{+}$ ±1.53	54.33*$^{+}$ ±2.08	62.67*$^{+}$ ±3.06	54.00*$^{+}$ 2.00	60.67*$^{+}$ 1.53
Controls (normal saline)	76.00 ±2.00	79.00 ±1.00	79.00 ±1.00	80.00 ±0.58	77.00 ±1.00	77.67 ±1.00	77.67 ±0.88	76.67 ±1.15	77.33 ±1.55

Key: * - Statistically significant compared with the control at time intervals

^{a} – Statistically significant compared with Glibenclamide group.

+ Statistically significant compared with values at day 0.

Table 10: Effect of prolonged administration of aqueous extract of ocimum gratissimum on the plasma blood glucose level in n-STZ rats.

Treatment (Dose)	Plasma glucose levels (mg/dl) at								
	Days				Weeks		Hours after last dose		
	0	1	2	5	2ndwk	3rd wk	12hrs	24hrs	72hrs
Extract (200µg/g)	211.00*^{a} ±3.61	183.33*$^{a+}$ ±4.93	132.17*$^{a+}$ ±2.57	123.17*$^{a+}$ ±1.76	128.00*$^{a+}$ ±2.00	131.67*$^{a+}$ ±2.08	148.00*$^{a+}$ ±2.00	135.33*$^{a+}$ ±1.15	160.67*$^{a+}$ ±3.06
Glibenclamide (5µg/g)	205.33 ±5.03	172.60*$^{+}$ ±1.51	155.33*$^{+}$ ±3.06	138.00*$^{+}$ ±2.00	130.07*$^{+}$ ±3.06	128.00*$^{+}$ ±2.00	138.00*$^{+}$ ±2.00	132.67*$^{+}$ 2.30	163.33*$^{+}$ 3.06
Controls (normal saline)	206.00 ±2.00	208.33 ±1.53	207.83 ±1.61	198.67 ±3.21	200.33 ±1.53	208.67 ±1.15	211.67 ±2.89	200.00 ±2.89	202.67 ±3.06

Key:* - Statistically significant compared with the control at time intervals

^{a} – Statistically significant compared with Glibenclamide group.

+ Statistically significant compared with values at day 0.

DISCUSSION

Traditionally, various in-vivo models (e.g. diazoxide, alloxan or streptozotocin-induced diabetic rats) are used in evaluating medicinal plants with suspected hypoglycaemic potentials[17,18]. In this study, diabetes mellitus was induced using intraperitoneal injection of streptozotocin at a single dose of 80mg/kg of body weight in two-old neonatal rats. This dose reliably established diabetes mellitus in the treated rats seven days post induction. Previous studies have reported that neonatal rats treated with streptozotocin exhibited hyperglycaemia 5-7 days after administration[10,11,19]. The absence of any significant difference in body weight between the streptozotocin-treated rats and untreated rats is explained by the non-obese characteristic of this experimental type-2 diabetic model[5].

The present study has demonstrated significant hypoglycaemic action of aqueous and ethanol extracts of *Ocimum gratissimum* leaf in normal and neonatal streptozotocin-induced diabetic rats. The minimum hypoglycaemic dose from this study was 100µg/gbody weight of the rat and a dose dependent increase in activity was observed in both extracts. These findings are consistent with the report from an earlier study by Aguiyi *et al* in Jos, Nigeria which showed significant reduction in plasma glucose concentrations following intraperitoneal injection of methanol extract of *Ocimum gratissimum* (400mg/kg) in normal and alloxan-induced diabetic rats[4]. However, the study did not evaluate the effect of the aqueous extract on the experimental animals. Two other separate studies by Egesie et al[20] and Mohammed et al[21] have reported significant blood glucose reduction following oral administration of aqueous extract of *Ocimum gratissimum* (500mg/kg) in streptozotocin-induced diabetic rats. All these studies were conducted on type-1 diabetic rat models. The relatively lower hypoglycaemic doses recorded in our study could at least in part be explained by the following factors: Firstly, the more refined soxhlet extraction technique employed in this study ensured a greater yield of active ingredients in extracts obtained. Secondly, the relative insulin deficiency present in the

type-2 diabetic rats used in this study would require lower doses of active extract to produce significant hypoglycaemic effect.

The aqueous extract of *Ocimum gratissmum* showed significantly greater reduction in blood glucose level than the ethanol extract in both normoglycaemic and diabetic rats indicating that the active substance is more soluble in water than in alcohol. The repeated daily administration of the aqueous extract and glibenclamide for three weeks decreased the plasma glucose level in the diabetic rats, and the percentage reduction in plasma glucose was less in the extract treated group.

Repeated administration in the normoglycaemic rats produced significant reduction in morning non fasted plasma glucose level in the glibenclamide group but no significant reduction in the extract treated group. At least two mechanisms could explain this observation. Firstly, the half life of the active substance in the extract might be shorter than that of glibenclamide, and as such would require multiple daily dosing in order to achieve sustained glycaemic control. Secondly, it is possible that the extract has an intrinsic less propensity of provoking hypoglycaemia compared with glibenclamide. This property may be inherent on its possible synergistic action on peripheral glucose utilization making it potentially useful in diabetics with insulin resistance. However, the effect of the extract of *Ocimum gratissimum* on insulin resistance has not been investigated. Inhibition of cortisone activity has been postulated as possible mechanism of its anti-diabetic action[22]. Further studies are suggested with insulin resistant type-2 model obese diabetic rats.

CONCLUSION

Aqueous and ethanol extracts of *Ocimum gratissimum* leaf possess hypoglycaemic effects on normoglycaemic and neonatal streptozocin-induced diabetes model. The minimum therapeutic (hypoglycaemic) dose is 100µg/g for both the aqueous and ethanol extracts while the lethal dose 50 (LD_{50}) for the aqueous extract is 3.9mg/g. It is recommended that further studies to identify and characterize the active components of this part of the plant be undertaken with a view to determining the molecular bases for the hypoglycaemic action. Furthermore, controlled clinical trials are required to confirm its hypoglycaemic actions in human subjects.

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