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Full Length Research Paper

Effects of aqueous leaf extract of *Ocimum gratissimum* on oral glucose tolerance test in type-2 model diabetic rats

NELSON I. OGUANOBI*, CHIOLI P. CHIJIOKE and SAMUEL I. GHASI

Clinical Pharmacology Unit, Department of Pharmacology and Therapeutics, College of Medicine, University Of Nigeria, Nsukka, Enugu Campus, Nigeria.

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Preliminary studies have reported hypoglycaemic effects of leaf extracts of *Ocimum gratissimum* in type-1 diabetic animal models. However, the mechanism of its antihyperglycaemic activity remains unknown. To shed more light on this activity, we decided to investigate the efficacy of the extract in type-2 model diabetic rats, for which few reliable data are currently available. The results from this study show that aqueous extract of *O. gratissimum* leaf can significantly reduce postprandial hyperglycaemia in type-2 diabetic model rats, but without the risk of hypoglycemia. This property makes it potentially useful in type-2 diabetes human subjects with insulin resistance who are prone to high postprandial glucose surge.

Key words: Streptozotocin, hyperglycaemia, neonatal rat, type-2 diabetes, *Ocimum gratissimum.*

INTRODUCTION

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, the leaves of this plant are commonly used as a condiment in cooking.

Preliminary studies have reported hypoglycaemic effects of extracts of this plant in type-1 diabetic animal models (Igoli et al., 2005; Aguiyi et al., 2000; Egusie et al., 2006). A pilot study carried out in Jos, Nigeria, assessed the hypoglycaemic effect of methanolic extract of *O. gratissimum* leaves in normal and alloxan-induced diabetic rats. The findings of this study showed that intraperitoneal injection of the extract (400 mg/kg) significantly reduced plasma glucose levels both in normal and diabetic rat by 56 and 68%, respectively (Aguiyi et al., 2000). A similar study evaluated the hypoglycaemic property of orally administered doses of aqueous extract of *O. gratissimum* in streptozotocin (STZ) induced (type-1 model) diabetic rats.

Administration of aqueous leaf extract produced a statistically significant reduction in plasma glucose concentration in the diabetic rats. The extract had no effect on serum levels of aspartate transaminase, alanine trasaminase, alkaline phosphatase, total protein, albumin and bilirubin (Egusie et al., 2006).

However, the mechanism of its hypoglycaemic activity remains unknown. In order to elucidate the possible modes of action of the plant extract, it is necessary to investigate these findings on a suitable animal model of type-2 diabetes mellitus and also to study the effects of administration of the extract on postprandial hyperglycaemia which has been found to constitute increased risk factor for complications in diabetic patients (Cerillo, 2005).

Several animal models have been developed to investigate pharmaco-therapeutic responses in diabetes (Etuk and Muhammed, 2010; Rajalakshmi et al., 2009; Arulimozhi et al., 2004; Koffi et al., 2009). Although, none of these animal models of diabetes mellitus is exactly identical to the human disease, the neonatal STZ-induced rat (n- STZ rat) model has several advantages

*Corresponding author. E-mail: nelifyik@yahoo.com. Tel: +234803738763.

over other models and is considered to be a suitable experimental model for type- 2 diabetes mellitus (Arulimozhi et al., 2004). This study is aimed at examining the effects of aqueous leaf extract of *O. gratissimum* on oral glucose tolerance (OGT) test in type-2 model diabetic rats.

MATERIALS AND METHODS

Plant material

Fresh leaves of *O. 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 n-STZ rat model of type-2 diabetes mellitus

Neonatal rats with body weight of 5.16 ± 0.29 g were treated with STZ (Sigma, Aldrich lnc. USA) dissolved in citrated buffer (PH 4.3) by intraperitoneal injection at the dose of 80 mg/kg on the second day after birth (Junod et al., 1969; Oliver et al., 1989). To achieve this, 100 mg of STZ was dissolved in 50 ml of citrated buffer giving a concentration of 2 mg/ml. Intraperitoneal administration of STZ was done using 100 I.U./ml insulin syringes and needle. The required minimum sample size was estimated using a standard formula (Campbell and Machin, 1996). 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 180 mg/dl were divided into 13 groups of 3 animals each. The average weight at 6 weeks was 152 ± 3.16 g. The rats were described as “fasted” after 10 to 12 h of overnight fast.

Controls

Adult Wistar rats of either sex, 6 weeks old, weighing 120 to 180 g were used as controls.

Housing

They were housed under standard condition in the Animal Research Unit of the Department of Pharmacology and Therapeutics, University of Nigeria Teaching Hospital Enugu. The animals were fed *ad labium* on tap water and commercially available livestock feed (Unique feeds®, Farm Associates Nigeria Ltd. Enugu, Nigeria). The animals were fed on the “starter” mesh feed within the first 2 weeks of life; thereafter, the “grower/finisher” mesh feed was used until the end of the study. The research was conducted in accordance with the internationally accepted principles for laboratory animal use and care as found in the European Community guidelines {EEC Directive of 1986; 86/609/EEC} (EEC, 1986).

Preparation of extract

Fresh leaves of *O. gratissimum* were kept in the oven at 80°C for 10 min to stop enzyme activity and then 60°C for 30 min 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 10 h as follows: Fifty grams (50 g) 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 450 ml of distilled water and placed on a thermostatic heating mantle regulated at 100°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°C.

After filtration through Whatman filter paper No.40, the filtrate was slowly evaporated to dryness an electrothermal heating mantle regulated at 60°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. The extracts were stored in screwed cap vials at 4 to 8°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 from rats were collected from the tail vein. Initial blood glucose measurements in the neonatal rats as well as during the first 6 weeks of monitoring were by Trinder’s glucose oxidase method using One Touch® glucometer {LifeScan lnc., Milpitas, California, USA), (Trinder, 1969).

After the 6th week, blood samples from rats were collected from the tail vein in dried sodium fluoride and oxalate bottles. Plasma was separated within 30 min of collection by centrifuge. Blood glucose levels were determined by the modified glucose oxidase test (Dubois et al., 1956).

OGT test in diabetic rats

Prior to an OGT test, rats were fasted for 15 h. Distilled water (control), a reference drug, glibenclamide or the water extract of *O. gratissimum* was orally administered to groups of three rats each. 30 min later, glucose (1.25 g/kg) was orally administrated to each rat. Blood samples were taken from tail veins at 30 (just before the water extract administration), 0 (just before glucose the administration), 30, 60, 90, 120, 150 and 180 min for the assay of glucose.

OGT test in normal rats

OGT tests were performed on the normal rat in the same way as described for the diabetic rats.

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 and post hoc multiple comparison (Bonferroni test). 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.

Table 1. Effect of aqueous extract of *O. gratissimum* on OGT in normal rats.

Treatment (Dose)		Plasma glucose levels (mg/dl) at						AUC (mg)
		-30 min	0	+30 min	+60 min	+120 min	+180 min	
F1 extract (100 mg/kg)		72.40 ± 1.83	73.61 ± 0.88	154.00^{d} ± 3.46	131.33^{f} ± 2.40	117.33^{f} ± 1.45	112.00^{f} ± 2.31	23616 ± 28.51
F2 extract (200 mg/kg)		71.93 ± 1.31	70.33^{d} ± 0.33	145.00^{a} ± 2.65	112.00^{e} ± 1.53	101.67^{af} ± 0.88	96.00^{d} ± 3.06	20840^{a} ± 28.53
F3 glibenclamide (5 mg/kg)		74.67 ± 1.45	72.77 ± 0.79	140.33^{b} ± 2.19	102.33^{b} ± 1.45	88.00^{b} ± 1.20	84.00^{b} ± 0.58	19000^{a} ± 17.71
F4 control (2 ml normal saline)		73.00 ± 1.73	71.70 ± 1.18	160.67 ± 3.53	129.00 ± 3.79	114.33 ± 3.84	104.67 ± 2.91	23680 ± 38.6
ANOVA	F=	0.559	2.841	9.137	49.237	37.357	24.739	8.373
	P=	0.657	0.106	0.006*	0.000*	0.000*	0.000*	0.001*

a, b and c, Statistically significant compared with the control at time intervals (P< 0.05, 0.01 and 0.001, respectively); d, e and f, statistically significant compared with glibenclamide group at time intervals (P< 0.05, 0.01 and 0.001, respectively); *, statistically significant; AUC, area under the curve of glucose tolerance test.

RESULTS

OGT test in normal and diabetic rats

The plasma glucose levels of the normal rats reached a peak at 30 minutes after the oral administration of glucose and gradually decreased to the pre-prandial level, (Table 1 and Figure 1). The water extract of *O. gratissimum* leaves at dose of 200 mg/kg produced plasma glucose levels significantly lower than those of the control group at 30, 60 and 120 min after the glucose administration. The area under the curve (AUC) during the OGT test was significantly decreased. Glibenclamide (5 mg/kg) produced a significant reduction in the plasma glucose level at 30, 60, 120 and 180 min after oral glucose administration (Table 1).

In the diabetic rats glibenclamide (5 mg/kg) and water extract of *O. gratissimum* at dose of 100 and 200 mg/kg produced significant reduction in plasma glucose levels compared with those of the controls at 30, 60, 120 and 180 min after oral administration as well as a decrease in the area under the OGT curve, (Table 2 and Figure 2).

DISCUSSION

Extracts and reference hypoglycaemic drug (glibenclamide) showed hypoglycaemic effect in the OGT test in normal and n-STZ-diabetic rats. This is evident in the significant decrease in the hyperglycaemic peak as well as the decrease in the AUC during the glucose tolerance test.

Previous studies have documented a lack of activity of glibenclamide in the OGT test in STZ-induced diabetes model in adult animals due to the absolute insulin deficiency that is characteristic of this model (Peungvicha et al., 1998; Chattopadhyay, 1993).

In the n-STZ induced diabetes model of the present study, there is partial destruction of the pancreatic islet cells with consequent relative insulin deficiency. The findings of this study suggest that extracts of *O. gratissimum* leaves exhibited action similar to glibenclamide, that is, stimulation of the surviving beta- cells to release more insulin. Similar observation has been made in a study with *Ocimum sanctum,* a species of herbal plant closely related to *O. gratissimum* (Chattopadhyay, 1993). However, it is possible that increased peripheral utilization of glucose may play some role in the hypoglycaemic action of *O. gratissimum*. This hypothesis is supported by findings from two independent studies in Nigeria of significant hypoglycaemic action of leave extracts of this plant in type-1 model diabetic rats (Aguiyi et al., 2000; Egusie et al., 2006). However, further investigation is necessary to evaluate this.

The findings from this study shows that extracts of *O. gratissimum* leaves can significantly reduce postprandial hyperglycaemia in type-2 diabetic rat models. This property makes it potentially useful in human type-2 diabetes subjects with insulin resistance prone to high postprandial glucose surge. Postprandial hyperglycaemia has been demonstrated to be associated with increased risk of microvascular and macrovascular complications in diabetes mellitus (Cerillo, 2005). The mechanisms through which acute hyperglycemia exerts its multi-organ toxic effects may be due to the production of free radicals (Ceriello et al., 1999; Shige et al., 1999). This alarmingly suggestive body of evidence for a harmful effect of postprandial hyperglycemia on diabetes

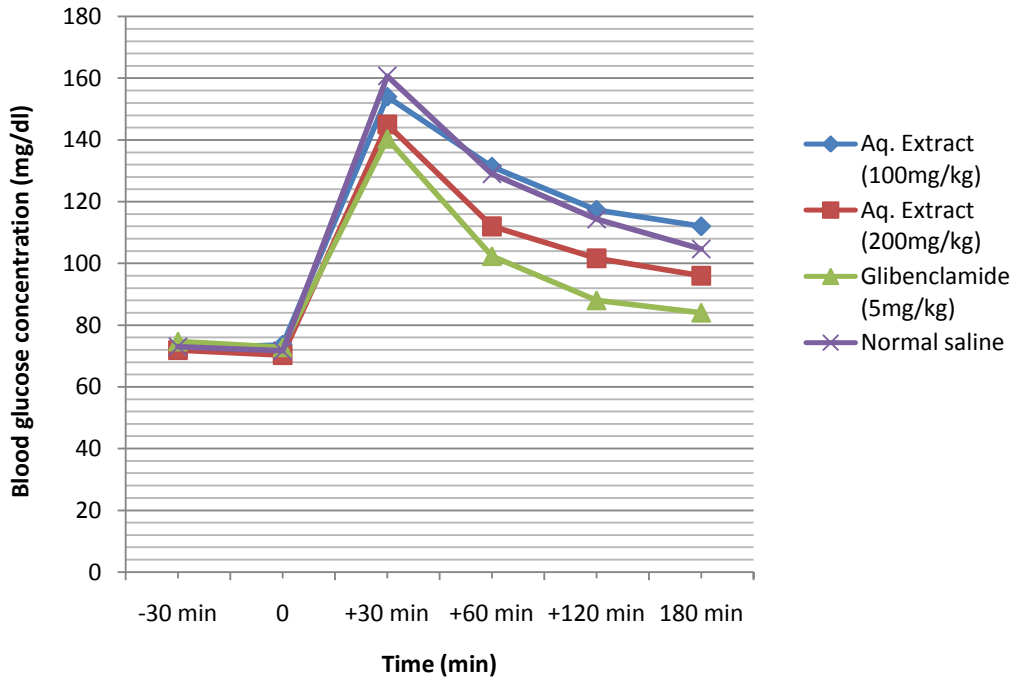

Figure 1. Graph showing the effect of aqueous extract of *O. gratissimum* on OGTT in normal rats.

Table 2. Effect of aqueous extract of *O. gratissimum* on OGT test in n-STZ rats.

Treatment (Dose)	Plasma glucose levels (mg/dl)						AUC (mg)
	-30 min	0	+30 min	+60 min	+120 min	+180 min	
F1 extract (100 mg/kg)	182.67 ± 2.03	178.67 ± 1.01	352.67 ± 1.76^{a,e}	326.33 ± 2.73^{a,e}	255.67 ± 3.38^{c,f}	205.33 ± 6.72^{b,f}	52320^{a} ± 81.71
F2 extract (200 mg/kg)	188.67 ± 2.03	180.00 ± 2.31	342.67 ± 1.76^{b,d}	316.17 ± 3.61^{b,e}	246.00 ± 3.64^{c,f}	184.33 ± 2.60^{b,f}	50672^{a} ± 72.88
F3 glibenclamide (5 mg/kg)	186.67 ± 1.76	178.17 ± 3.31	338.67 ± 2.40^{b}	296.67 ± 1.76^{c}	171.67 ± 3.38^{c}	168.67 ± 4.06^{c}	45472^{a} ± 71.01
F4 control (2 ml normal saline)	187.67 ± 1.76	185.33 ± 2.91	360.00 ± 1.15	341.67 ± 3.28	305.67 ± 2.03	269.00 ± 0.58	58912 ± 54.10
ANOVA F=	1.915	2.357	28.033	41.460	313.598	112.113	6.029
ANOVA P=	0.206	0.148	0.000*	0.000*	0.000*	0.000*	0.004*

a, b and c, Statistically significant compared with the control at time intervals (P< 0.05, 0.01 and 0.001, respectively); d, e and f, statistically significant compared with Glibenclamide group at time intervals (P< 0.05, 0.01, 0.001 respectively); *, statistically significant; AUC, area under the curve of glucose tolerance test.

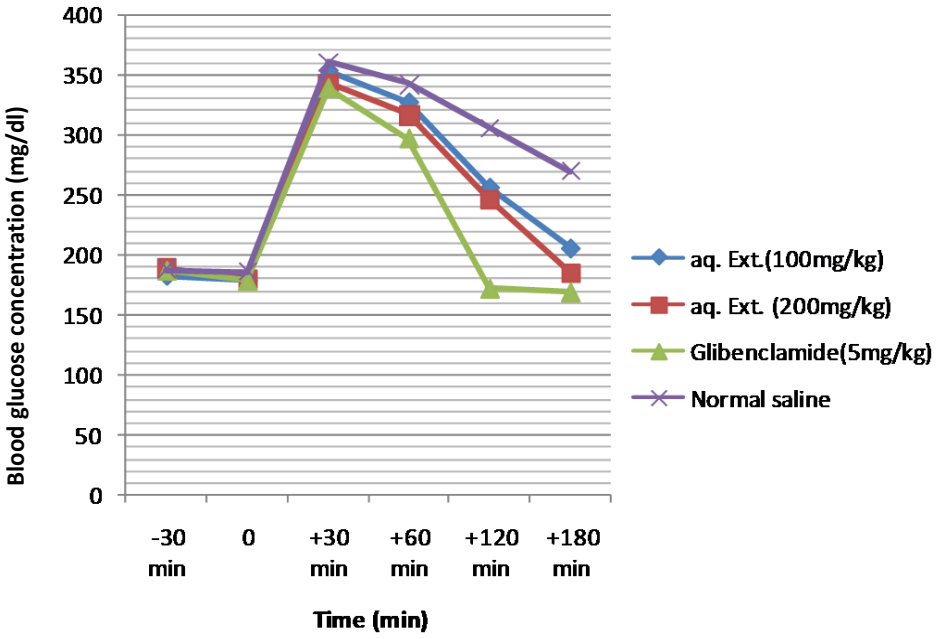

Figure 2. Graph showing the effect of aqueous extract of *O. gratissimum* on OGTT in n-STZ rats.

complications has been sufficient to influence guidelines from key professional scientific societies (American Diabetes Association, 2004; American College of Endocrinology, 2002; Alberti and Gries, 1988; Canadian Diabetes Association Clinical Practice Guidelines Expert Committee, 2003; De Backer et al., 2003). Correcting postprandial hyperglycemia forms part of the strategy for the prevention and management of complications in diabetes (American Diabetes Association, 2001).

Conclusion

These findings show that aqueous extract of *O. gratissimum* leaf can significantly reduce postprandial hyperglycaemia in type-2 diabetic model rats, but without the risk of hypoglycemia, which may occur after glibenclamide. These studies need to be followed up with structure – activity relationship analyses and clinical trials to further establish the value of *O. gratissimum* in human type-2 diabetes mellitus. In particular, one should also ascertain whether the risk of frank hypoglycemia is also lacking in humans.

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