

# Ethanollic Extract of *Ocimum Grattissimum* Leaves (Linn.) Rapidly Lowers Blood Glucose Levels in Diabetic Wistar Rats

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## Abstract

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**Key words:** *Ocimum grattissimum*; Streptozotocin; Diabetes Mellitus.

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**Competing Interests:** The authors have declared that no competing interests exist.

**Background:** The present study was undertaken to investigate the effects of ethanollic extracts of *Ocimum grattissimum* leaves on blood glucose and weight parameters in streptozotocin induced diabetic Wistar rats.

**Aim:** The aim was to provide information on the glucose lowering potentials of ethanollic extract of *Ocimum grattissimum* and also compare this effect with that seen following administration of metformin.

**Material and Methods:** Thirty adult male rats aged between 6- 8 months weighing between 180 and 200 g were randomly divided into five groups (A, B, C, D and E) of six rats each. Group A was the control, group B received oral Metformin at 25 mg/kg, while animals in group C, D and E received *Ocimum grattissimum* at 400, 600 and 800 mg kg<sup>-1</sup> body weight orally, treatment period was for 28 days. Fasting blood glucose and weight were measured weekly throughout the treatment period. Statistical analysis was carried out using a one way ANOVA followed by the Student-Newman-Keul's test.

**Results:** Results showed that ethanollic extract of *Ocimum grattissimum* reduced blood glucose levels and body weight significantly all through the treatment period, that these effects were more rapid with increasing doses of the extract, and the glucose lowering potential of *Ocimum grattissimum* was comparable to that seen following administration of metformin.

**Conclusion:** In conclusion, this study showed that ethanollic extract of *Ocimum grattissimum* has a more potent antihyperglycaemic effect than metformin at the doses studied, however, the mechanism(s) by which it does this is not yet established, we hope to do this in our subsequent studies.

## Introduction

Diabetes mellitus (DM) is a group of metabolic disorder characterized by an underlying hyperglycemia (resulting from absolute or relative lack of insulin) with nephropathy, neuropathy, angiopathy and oculopathy as its attendant complications. Obesity, diet and sedentary life style have been named as the major causative factors for the prevalence of the disease [1]. This group of common metabolic disorders shares the phenotype of hyperglycemia [2]. Several distinct types of

DM exist and are caused by a complex interaction of genetics and environmental factors.

Worldwide, more than 140 million people suffer from diabetes, making it one of the most common non-communicable disease [3]. Based on current trends, >360 million individuals will have diabetes by the year 2030 [2]. In modern medicine, the beneficial effects of standard medications on glycemic levels are well documented; the preventive activity of medications against the progressive nature of diabetes and its

complications was modest but not always effective [4]. Insulin therapy affords glycemic control in type 1 diabetes, yet its shortcomings such as ineffectiveness on oral administration, short shelf life, the requirement of constant refrigeration, fatal hypoglycemia in event of excess dosage, reluctance of patients to take insulin injection and above all the resistance due to prolonged administration limits its usage [5]. Similarly, treatment of type 2 diabetes patients with sulfonylureas and biguanides is almost always associated with side effects [6]. Hence, search for a drug with low cost, more potential and without adverse side effects is being pursued in several laboratories around the world. Plants are one of the most important sources of medicines. Current diabetes pharmacotherapy gives fast and good control of blood glucose levels but the efficiency becomes strictly reduced after long term use due to side effects that arise not only from prolonged use but also the tendency to require increasing doses of drug to maintain blood glucose at normal levels. Herbal medicines however appear to be a potent alternative with lesser side effects from various studies reviewed. Today, a large number of drugs in use are derived from plants, like Morphine from *Papaver somniferum*, Ephedrine from *Ephedra vulgaris*, Atropine from *Atropa belladonna*, Reserpine from *Roulphia serpentine* [7].

*Ocimum gratissimum* originating in the Orient, is widespread throughout tropical countries including Brazil, where it is popularly known as "alfavacão, alfavaca and alfavaca-cravo" [8]. Extensive research has been done on the various potential scientific uses of *Ocimum gratissimum*, a plant that also has extensive therapeutic use in traditional medicines in South America and Africa, which include its use in treating bacterial infections and diarrhoea [9], respiratory-tract infections, pneumonia, fever and coughs [10], anti-diarrheal effects in experimental animals [11, 12], high antiviral indices against HIV-1 and HIV-2 [13]. The essential oil of this species also presented interesting activities such as insecticidal [14], antibacterial [15-17], antifungal [18,19], and as a relaxant on isolated ileum from guinea pig [20]. Hypoglycemic and antidiabetic activity in rats have also been studied. Egesie and his colleagues concluded that the plant was an effective antidiabetic agent [21]. Because the administration of graded doses of the aqueous leaf extract produced a statistically significant decrease ( $P < 0.05$ ) in serum glucose concentration when compared with diabetic control rats and normal control rats they also reported that twenty eight day administration of the extract to non diabetic rats did not produce any significant difference in the plasma glucose concentration of non

diabetic extract treated rats compared with control [21]. Mohammed et al in 2007 reported that following intraperitoneal administration of *Ocimum gratissimum* there was significant reduction in blood glucose levels at the 500 mg/kg dose after 8 and 24 hours of administration and this effect was better than the effects seen with biphasic isophane insulin at this time intervals [22].

Although there have been reports on the hypoglycaemic potential, of this herb using intraperitoneal injections of methanolic extract [9], intraperitoneal injection of aqueous extract [22] or in another study oral administration of aqueous extracts [21], little has however been done to compare the hypoglycaemic effects of this herb with presently available oral hypoglycaemic agents: hence this study.

## Materials and Methods

### Animals

Thirty healthy adult male Wistar rats purchased from the Empire Animal farms in Osogbo, Osun State, Nigeria were used (age in the range of six to eight months) with weight in the range of 180 to 200 g. After being weighed on an electronic scale, the animals were randomly divided into five treatment groups. The animals were housed in plastic cages measuring 32"x18"x16" (6 rats in each cage). All animals had free access to food and water *ad libitum*. They were maintained under standard laboratory conditions i.e. a well aerated room with alternating light and dark cycles of 12 hours each and at room temperature of 25°C. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC). All rules applying to animal safety and care were observed.

### Plant material

Fresh leaves of *Ocimum gratissimum* Linn. were collected from Ogbomoso, Oyo state, Nigeria. The plant was identified by Dr Ogunkunle of the Department of Biology, Ladoke Akintola University of Technology, Ogbomoso and a voucher specimen was deposited in the herbarium of the department.

### Chemicals and drugs

Normal saline, 5% ethanol, Streptozotocin (STZ (Sigma St. Louis, USA)), 0.1M citrate buffer pH 4.5., Metformin (Bristol-Myers Squibb). All chemicals and drugs used were of analytical grade.

### Preparation of extract of *ocimum gratissimum*

*Ocimum gratissimum* leaves were first separated from the stalk, rinsed with water to remove dirt; air dried at room temperature and ground to fine powder using an electric blender (Christy and Norris - 47362, England) at the Department of Pharmacognosy of the Obafemi Awolowo University, Ile – Ife. Extraction was performed by adding 800 mg of ground powdered in 5 liters of ethanol in a sterile flask, swirled to ensure effective mixing and a stopper used to avoid loss of volatile liquid at ambient temperature ( $28 \pm 2^\circ\text{C}$ ). The mixture was extracted by agitation on a rotary shaker. After 48 hrs, the mixture was decanted. The filtrate was then poured into stainless trays and the extract was allowed to evaporate to dryness at room temperature ( $28 \pm 2^\circ\text{C}$ ) for 2-3 days by using a vacuum evaporator (RE 100B Bibby Sterilin, United Kingdom). The wet residue was freeze dried using a vacuum freeze drier (FT33–Armfield, England), and was stored until ready to use.

The percentage yield of extraction calculated as follows was 10.33%.

$$\text{Percentage yield} = \frac{\text{Weight of the dry extract}}{\text{Weight of powdered leaves}} \times 100\%$$

### Phytochemical screening of plant fraction

Preliminary screening of the extract was performed for the presence of secondary metabolites, using the following reagents and chemicals: alkaloids with Mayer's and Dragendorff's reagents [23, 24], flavonoids with the use of Mg and HCl [25, 26], tannins with 1% gelatin and 10% NaCl solutions and saponins with ability to produce suds [26].

### Acute toxicity test

The LD<sub>50</sub> determination for each of the fractions was conducted separately using modified method of Lorke (1983) [27]. For each of the fractions, the evaluation was done in two phases. In phase one, three groups of three rats each, were treated with 10, 100 and 1000 mg extract/kg body weight orally respectively. The control groups received normal saline. The rats were observed for clinical signs and symptoms of toxicity within 24 h and death within 72 h. Based on the results of phase one for the extract, fifteen fresh rats with three per group were each treated with 600, 1000, 1600 and 2900 mg extract/kg orally respectively. The control groups received normal saline. Clinical signs and symptoms of toxic

effects and mortality were then observed for seven days. The LD<sub>50</sub> were then calculated as the square root of the product of the lowest lethal dose and highest non-lethal dose, that is the geometric mean of the consecutive doses for which 0 and 100% survival rates were recorded in the second phase.

### Induction of diabetes mellitus

Diabetes mellitus was experimentally induced in the animals by a single intraperitoneal injection of 80 mg/kg/body weight of streptozotocin (Sigma St. Louis, USA) dissolved in 0.1M sodium citrate buffer pH 4.5. The control (group A) animals were injected intraperitoneally with equivalent volume of the citrate buffer. The rats were fasted 16-18 before administration of STZ. 72 hours following streptozotocin injection, the rats were fasted overnight and blood taken from tail vein of the rats. Rats having hyperglycemia (that is, with blood glucose of >18mmol/L) were considered diabetic and used for the experiment.

### Experimental method

Following induction of diabetes, animals were randomly assigned into five groups A, B, C, D and E of six rats each. Group A were the control, non-diabetic group of rats, group B C, D and E had diabetes experimentally induced. Animals in group A were administered normal saline orally while animals in group B had Metformin administered orally at a dose of 25 mg/kg per day. Groups C, D and E received daily oral doses of ethanolic extracts of *Ocimum gratissimum* leaves at 400, 600 and 800 mg/kg via an oral canula. Treatment period was for 28 days. Blood glucose and body weight was measured weekly (days 1, 7, 14, 21, 28) after an overnight fast.

### Determination of blood glucose and body weight

The body weights of the animals were measured using a top loader weighing balance, the blood sugar levels for each animal was measured weekly after an overnight fast. All blood samples were collected from the tail vein of the rats. Determination of the blood glucose levels was done by the glucose-oxidase method [28], and results were initially recorded as mg/dl and then converted to mmol/l by dividing values in mg/dl by a factor of 18.

### Statistical analysis

All the data for all biochemical parameters were

analyzed by analysis of variance and post-hoc tests (Student Newman Keuls and Dunnett's) were used to determine the source of a significant effect. Results are expressed as Mean  $\pm$  S.E.M.,  $p < 0.05$  is taken as accepted level of significant difference from control.

## Results

### *Preliminary phytochemical screening*

Freshly prepared extracts were subjected to preliminary phytochemical screening test for various constituents. This revealed the presence of carbohydrates, reducing sugars, lipids, flavonoids, alkaloids, steroids, tannins, terpenes, cardiac glycosides, resin and eugenol.

### *Acute toxicity study ( $LD_{50}$ )*

The signs of toxicity were first noticed after 2 – 4 h of extract administration. There was decreased locomotor activity and decreased sensitivity to touch. Also there was decreased feed intake and prostration after 12h of extract administration. The median lethal dose ( $LD_{50}$ ) oral of ethanolic extract in rats was calculated to be 912.3 mg/kg body weight compared to 1264 (i.p) [22] for aqueous extract.

### *Physical examination of the animals*

Animals were observed for changes in their physical characteristics all through the treatment period. Animals in group A (control) showed no deterioration in their grooming, eating or drinking behaviours, they also showed no change in locomotion, sleep patterns or social behaviours whereas animals in groups B, C, D and E with experimentally induced diabetes induced showed an initial rapid reduction in grooming, eating and water drinking behaviour, this was followed by an increase in appetite, increased water consumption and an increase in urine production as evidenced by soiling of the animal coat. These changes were noticed to have abated almost immediately treatment with Metformin was commenced in the group A animals and also with commencement of *Ocimum gratissimum* extract at all doses. Animals in groups D and E were however noticed to have developed passage of bloody urine (frank haematuria) which persisted all through the experimental period.

### *Effect of *Ocimum gratissimum* on weight*

When assessing body weight variations within

the groups throughout the 28 day treatment period, there was a significant ( $F(4, 329) = 11.63, p < 0.05$ ) increase in weight seen in the animals in the control group (Group A) all through the treatment period. In groups B, C, D and E, there was however a significant ( $F(4, 29) = 63.01, p < 0.05$ ), ( $F(4, 29) = 191.96, p < 0.05$ ), ( $F(4, 29) = 211.86, p < 0.05$ ) and ( $F(4, 329) = 110.71, p < 0.05$ ) reduction in weight respectively. Compared to the animals in the metformin treatment group there was an instantaneous but significant reduction in weight in all the groups that received extract (C, D and E), best seen when comparing body weight variations on day 0 with those seen on days 7, 14, 21 and 28. The lowest body weight was seen in the animals in group E. However, animals in group B were noticed to show some increase in their weight towards the end of the treatment period compared to animals in groups C, D and E as shown in Figure 1.

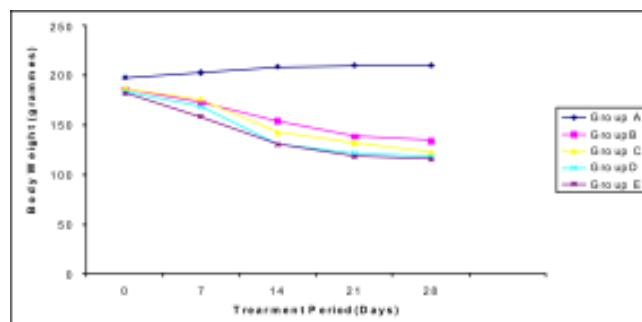


Figure 1: Effect of *Ocimum gratissimum* on Body weight. Each line represents Mean  $\pm$  S.E.M,  $p < 0.05$  group by group comparison,  $n = 6$ .

### *Effects of *Ocimum gratissimum* on blood glucose*

When assessing the blood glucose variations within and between groups throughout the 28 day treatment period, the animals in the control group were noted to have maintained a steady blood glucose level that were  $> 18$  mmol/l all through the experimental period, whereas in groups B, C, D and E there was however a significant ( $F(4, 329) = 49.08, p < 0.05$ ), ( $F(4, 29) = 230.63, p < 0.05$ ), ( $F(4, 29) = 191.75, p < 0.05$ ) and ( $F(4, 329) = 202.43, p < 0.05$ ) reduction in blood glucose levels respectively once treatment was commenced. Compared with the gradual reduction in blood glucose in the metformin treatment group, the drop in groups C, D and E were rapid and almost instantaneous this is noticeable when comparing blood glucose variations on day 0 with those seen on days 7, 14, 21 and 28. The lowest blood

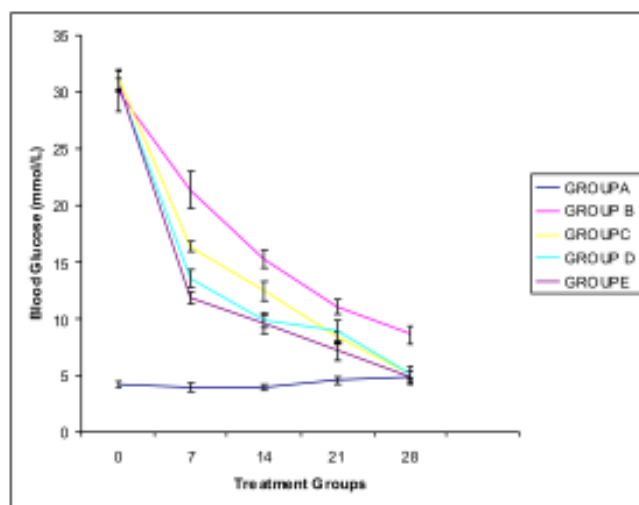


Figure 2: Effects of *O. gratissimum* on Blood Glucose. Each line represent Mean  $\pm$  S.E.M,  $p < 0.05$  group by group comparison,  $n = 6$ .

glucose levels were seen in animals in group E who received *Ocimum gratissimum* extract at 800 mg/kg as shown in Figure 2.

## Discussion

A number of studies in the past have been conducted to investigate the hypoglycaemic or possible antidiabetic effects of *Ocimum gratissimum* [9, 21, 22]. This study investigated the effects of *Ocimum gratissimum* on blood glucose levels in the diabetic state using the ethanolic extract of the leaves which contains a higher concentration of secondary constituents that are present in both extracts (ethanolic and aqueous), and has lipids and eugenol which are absent in the aqueous extract. This study also administered *Ocimum gratissimum* orally and compared its reported efficacy as a potential antidiabetic agent with a widely used oral hypoglycaemic agent (Metformin).

The results of this study indicated that an ethanolic extract of *Ocimum gratissimum* like the aqueous and methanolic extracts that have been previously reported [9, 21, 22] has antidiabetic potentials. This is evident in the rapid reduction in blood glucose levels that were noticed once administration of the extract commenced as well as the alleviation of all symptoms of diabetes that were initially noticed before commencement of treatment. Mohammed and his colleagues [22] reported that at a dose of 500 mg/kg, *Ocimum gratissimum* significantly ( $P < 0.05$ ) lowered the blood glucose level

when compared to control after 8 and 24 h of extract administration. The dose of 500 mg/kg was found to be more effective with percentage glycaemic change of 80.6 and 81.3% after 8 and 24 h, respectively, this compares with the effect we saw at 400, 600 and 800 mg/kg, although the effects seen at the 600 and 800 mg/kg doses was more marked and more rapid than that seen at 400 mg/kg dose very unlike the response they noticed at 1000 mg/kg [22].

Egesie *et al* in 2007 [21] reported that in streptozotocin induced diabetic rats, administration of graded doses of the aqueous extract produced a gradual decrease in plasma glucose levels from 1 to 14 and 28 days. This is also comparable to the effects seen from this study although the effects rather than been gradual was rapid, this could be attributed to the higher concentrations of secondary metabolites in the ethanolic extract compared to the aqueous extract since both extracts were administered via the same route (oral). The decrease in plasma glucose levels may be due to the fact that *Ocimum gratissimum* could be facilitating utilization of glucose by peripheral tissue. It is important to note that the plant extract contains flavonoids and other phytochemical constituents believed to be responsible for its hypoglycaemic property. The extract also contains major mineral elements e.g. Calcium, chloride, manganese, magnesium, zinc and potassium which might also play a contributory role in enhancing medicinal properties such as the hypoglycaemic property [29].

Diabetes is a disorder of carbohydrate, fat and protein metabolism attributed to the diminished production of insulin or mounting resistance to its action. Besides the use of insulin, oral hypoglycaemic drugs are widely used for controlling hyperglycemia. Among these, Sulphonylureas and Biguanides are popular [30]. Metformin improves hyperglycemia primarily through its suppression of hepatic glucose production (hepatic gluconeogenesis) [31]. The "average" person with type 2 diabetes has three times the normal rate of gluconeogenesis; metformin treatment reduces this by over one third [32]. Metformin activates AMP-activated protein kinase (AMPK), a liver enzyme that plays an important role in insulin signaling, whole body energy balance, and the metabolism of glucose and fats [33]. Activation of AMPK is required for metformin's inhibitory effect on the production of glucose by liver cell. Metformin also increases low-affinity and high-affinity receptors of insulin, and improves insulin resistance [34]. Results of fasting blood glucose done weekly showed a significant

reduction in blood glucose which was almost instantaneous at 600 and 800 mg/kg doses. The anti hyperglycemic effect of the extract was noticed to be higher than what was seen with metformin at all doses tested. Since the ethanolic extract of *Ocimum gratissimum* lowered hyperglycemia in streptozotocin induced diabetic rats better than metformin, it is possible that its mechanism are similar to that seen with metformin; the more dramatic effects however could be due to other mechanisms as reported in the case of some other herbs with similar secondary constituents. Kobayashi *et al.* [35] reported that an aqueous extract from the flowers of *I. britannica* subsp. *I. japonica* (IB) reduced the degree of insulinitis and destruction of beta-cells induced by streptozotocin, inhibited IFN-g production, and had a preventive effect on autoimmune diabetes by regulating cytokine production. Mechanisms by which *Ocimum gratissimum* lower blood glucose levels in diabetic rats may have been by increasing glycogenesis, inhibiting gluconeogenesis in the liver, or inhibiting the absorption glucose from the intestine. There are studies underway for further elucidation.

The results of this study also showed that *Ocimum gratissimum* leave extract at the doses tested compared with vehicle caused a continued reduction in weight of the animals regardless of its ameliorative effect on the symptoms of the diabetic state, this reduction in weight is comparable to that seen with metformin. Metformin is used to treat diabetes, but several studies show that it also helps non-diabetics to lose weight by reducing hunger [36]. The administration and the withdrawal of metformin produced changes in body weight similar to those provoked by restriction of calories, or selective carbohydrate restriction and re-alimentation, respectively. Since such rapid shifts in weight are largely due to changes in water-balance, it is highly probable that metformin reduces the amount of body water. The mechanism involved may concern a decrease in carbohydrate metabolism as well as an increase in that of fat [37]. It stands to reason that considering *Ocimum gratissimum* also caused a similar reduction in weight the likelihood of similar mechanism of action is strong and worth studying.

In conclusion, this study showed that ethanolic extract of *Ocimum gratissimum* has a more potent antihyperglycaemic effect than metformin at the doses studied, however, the mechanism(s) by which it does this is not yet established, we hope to do this in our subsequent studies.

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