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Effect of aqueous leaves extract of *ocimum gratissimum* (sweet basil) on alloxan induced diabetic rats.

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Abstract

Ocimum gratissiummu leaves have been claimed and used as an anti-diabetic agent by traditional healers/folk medicine practitioners. This has led to the study of anti-diabetic activity of aqueous leaves extract of this plant using a model of alloxan-induced diabetes in rats. The biochemical parameters studied are plasma glucose, serum triglyceride, cholesterol, high-density lipoprotein (HDL), and low-density lipoprotein (LDL). Extracts were orally administered daily for 7 days at doses of 50mg/kg, 100mg/kg, 200mg/kg and 400mg/kg, 48hours after alloxan injection (100mg/kg). The alloxan-diabetic rats showed significant ($p < 0.05$) reduction in plasma glucose from 10.0 ± 1.36 mmol/L in the diabetic group to 1.8 ± 0.06mmol/L in the group administered 100mg/Kg. Levels of cholesterol, triglyceride, HDL and LDL were not significantly altered ($P>0.05$). In conclusion, these results showed that *Ocimum gratissimum* leaves extract when administered orally, can reduce plasma glucose level. These results further suggest the validity of the clinical use of *Ocimum gratissimum* in the treatment of diabetes mellitus.

Keywords: *Ocimum gratissimum*, alloxan, diabetes mellitus, glucose, triglyceride.

INTRODUCTION

Diabetes mellitus is a group of syndromes characterized by hypoglycaemia, altered metabolism of lipids, carbohydrates and proteins, and an increased risk of complications from vascular disease (1). More than 400 species of plants have been reported to display hypoglycemic effects, but only a few of them have been investigated (2). *Ocimum gratissimum* is the *occimum* species most used as an anti-diabetic herbal remedy in Nigeria where it is known as "Efrinrin" (Sweet bassil) (3). This species is an erect small shrub with many branches and is usually not more than 1 meter high (4). The leaves are simple, lanceolate, usually 5-13cm long and 3-9cm wide. The herb has an aromatic smell when crushed (4)

The hypoglycaemic activity of a methanol extract of the plant was previously reported (5) but our focus is on the ant-diabetic activities of the aqueous extract and the effects of the extract on serum lipids.

MATERIALS AND METHODS

Animals

Thirty five male and female Wistar rats weighing between 178.8 ± 6 Kg were used for this study. The rats were housed in standard environmental conditions (temperature, relative humidity and light/day cycle). They were divided into seven groups comprising of 5 animals each and each of the groups were housed in standard cages. The animals were also fed with standard food and water *ad libitum*.

Plant

The leaves of *Ocimum gratisimum* were collected from an open field around the outskirts of Ilorin, Kwara State, Nigeria. Mr. T. K. Odewo carried out identification of the plant at the forestry Research institute of Nigeria (FRIN) and a voucher specimen (FHI 106934) has been deposited in the FRIN herbarium. The leaves were air dried, reduced to powder and extracted with water (100g powder/L of water) in a water bath (maintained at 90^0C) by maceration (6). A dark green solid extract was obtained with a yield of 10%. The extract was stored at a temperature of 4^o C and when needed, portions of the extract were reconstituted in saline for pharmacological studies.

Experimental design

Diabetes was induced in rats within 48 hours by the intraperitoneal administration of alloxan monohydrate dissolved in distilled water (5%) in a dose of 100mg/kg body weight (7,8). The rats were divided into 7 groups of 5 animals each. Group I: Normal control received only saline (10ml/Kg), Groups II: Diabetic control, received alloxan and saline, Groups III, IV, V, VI received alloxan and 48hours later they were treated orally with aqueous extracts of *Ocimum gratissimum* at doses of 50, 100, 200 and 400mg/kg, group vii was treated with glibenclimide (5mg/Kg) as standard. Blood samples were collected from the tail 48h after alloxan injection (to deduce the diabetic states of the animals with plasma glucose <8Mmol/L were discarded) and seven days after diabetes was confirmed.

Determination of Plasma Glucose and Lipid Concentration

Animals were anaesthetized with ether after which blood from the left ventricle of the heart was collected and the following parameters were evaluated: serum

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glucose (9), Total cholesterol (10), Low density Lipoprotein (11), High-density lipoprotein (12), Triglycerides (13).

Phytochemical Analysis
Preliminary phytochemical screening was carried out according to the methods described by Trease and Evans (14). Briefly some of the reagents and chemicals used for the study include: Dragendorffs reagents for alkaloids, $FeCl_3$ for tannins NaOH and lead acetate for flavonoids, while the frothing and benedict's tests were performed for saponins.

Statistical Analysis
Data were expressed as means ± S.E.M. Differences between the test and control groups of animals were evaluated using ANOVA and Duncan post-hoc test. Results were considered significant at $P < 0.05$(15).

RESULTS
Table 1. Shows the effect of oral administration of aqueous extract of *O. gratissimum* on plasma glucose and serum lipids. The treatment for 7 days with aqueous extracts of *Ocimum gratissimum* (50,100,200 and 400mg/kg) and glibenclimide produced a significant ($p<0.05$) reduction of the hyperglycemia compared to diabetic control. However, the extract did not produce any significant ($p>0.05$) differences on the serum lipids of the treated group compared to the diabetic control.

DISCUSSION
The main characteristics of diabetes mellitus are polydipsia, polyuria, and polyphagia, weight loss, muscle weakness and hyperglycemia (16). Alloxan, and streptozotocin have the ability to selectively destroy the pancreatic beta cells (17, 18). The alloxan model used for this study has been found to lead to long lasting diabetes in rats. The present work evaluated biochemical parameters such as: Serum triglyceride, cholesterol, low density lipoproteins, high-density lipoproteins and plasma glucose in experimental diabetes models caused by alloxan in rats.
The anti-diabetic properties of aqueous extract of *Ocimum gratissimum* were investigated in this study based on the claims of folk medicine practitioners that an aqueous decoction of *O gratissimum* has anti-diabetic effect.

Our result indicate that aqueous extract of *O gratissimum* significantly ($p<0.05$) reduced the plasma glucose level from the hyperglycaemic state induced by alloxan injection. This indicate that the extract exhibit ant-diabetic activity. However the optimum dose range might be between 10-100mg/kg since the 400mg/Kg dose is not as statistically significant as the 100mg/Kg dose. The result obtained from the present study further confirm those of Aguiyi *et al* (5), though these authors used a methanolic extract and only a dose of 400mg/Kg while we used an aqueous extract (the form in which the extract is used locally) and a dose range of 50-400mg/Kg

Ocimum gratissimum showed no significant alteration on plasma lipids levels, which is relevant to an experimental study on *Bauhinia forficate*, which demonstrated that there was no significant alteration in the level of serum lipids after 1month treatment with *Bauhinia forficata* decoction in streptozotocin-induced diabetic rats (18).

Phytochemical analysis of *Ocimum gratissimum* aqueous leaf extract shows the presence of alkaloids, flavonoids, saponins and cardiac glycosides. It has been reported that the effect of tertiary and quaternary alkaloids, glycoside, saponin and flavonoids components reduces the blood glucose level and also acts to reduce serum lipid levels in animal (19-20). In the present study the lipid level was not altered significantly this may be due to the short duration of the study (7 days)
In conclusion, this study has shown that, oral administration of the aqueous extract of *Ocimum gratissimum* leaves have significantly reduced blood glucose levels, but it does not significantly affect the plasma lipid imbalances associated with diabetes mellitus. These properties justify its use by traditional healers as an anti-diabetic agent.

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Table 1. *Plasma glucose and lipid profiles in Diabetic control and Alloxan-Induced Diabetic Rats after Treatment with Ocimum gratissimum.*

Variables	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg	Glibenclimide (5mg/Kg)	Diabetic Control	Normal control
Plasma glucose level	2.9 ± 0.6[a]	1.8 ± 0.1[a,C]	2.6 ± 0.1[a]	5.8 ± 0.6[b]	2.7 ± 0.1	10.0 ± 1.4	2.1 ± 0.1
Total cholesterol	96.4 ± 2.6	98.5 ± 2.9	99.3 ± 3.2	96.0 ± 2.5	96.1 ± 8.2	112.7 ± 9.8	98.7 ± 1.3
Total Triglyceride	89.6 ± 4.3	88.7 ± 5.9	86.5 ± 3.8	85.5 ± 8.5	91.0 ± 6.3	104.8 ± 5.7	77.2 ± 6.1
LDL	64.2 ± 2.8	64.1 ± 2.1	66.2 ± 3.4	61.5 ± 1.6	60.8 ± 6.0	63 ± 3.5	62.8 ± 5.3
HDL	14.4 ± 2.5	17.0 ± 1.1	16.1 ± 1.3	17.7 ±1.0	21.4 ± 5.2	29.4 ± 6.5	20.9 ± 1.0

Values of Plasma glucose are expressed as mmol/L while plasma lipids are expressed as mg/dL All values are presented as mean ± S.E.M
[b]p < 0.05, [a]p <0.01, compared to diabetic control, [C]P<0.05, compared with 400mg/Kg dose ANOVA and Duncan post hoc test.

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