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Hypoglycemic Effect of *Ocimum Lamiifolium* in Alloxan Induced Diabetic Mice

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Abstract

Plant remedies are the mainstay of treatment in underdeveloped regions owing to the side effects, unavailability and unaffordability of the conventional therapy. Among the traditional plants that have been used as an alternative therapy for diabetes mellitus is *Ocimum lamiifolium*, however, it has received limited scientific and medical evaluation to assess its efficacy. In this study, the *in vivo* hypoglycemic activity of aqueous leaf extracts of this plant was determined in male swiss white albino mice. The antidiabetic activity was screened in alloxan induced diabetic mice using oral and intraperitoneal routes. The phytochemical composition was assessed using standard procedures. The extract showed hypoglycemic activity at dose levels of 25, 48.4, 93.5, 180.9, and 350 mg/kg body weight. The extracts contained tannins, sterols, flavonoids, saponins, terpenoids, and alkaloids. The observed hypoglycemic activity could be associated with the phytochemicals present in this plant extract.

Keywords: Diabetes Mellitus; *Ocimum lamiifolium*; Hypoglycemic activity; Antidiabetic; Phytochemical; Toxicity

Introduction

Diabetes mellitus (DM) is a major public health problem with an estimated global incidence of 382 million diabetics by 2014 and this number is expected to increase to over 592 million people in less than 25 years [1]. Diabetes mellitus (DM) is characterized by chronic hyperglycaemia resulting from defects in insulin metabolism and impaired function in carbohydrate, lipid and protein metabolism that leads to long-term complications [2]. DM is no longer a disease of rich developed countries [3]. Changes in dietary habits, obesity and physical inactivity are responsible for spreading this epidemic into the developing countries [3].

Diabetes mellitus may present with characteristic symptoms such as thirst, polyuria, blurring of vision, and weight loss [4]. In its most severe forms, ketoacidosis or a non-ketotic hyperosmolar state may develop and lead to stupor, coma and, in absence of effective treatment, death. Often symptoms are not severe, or may be absent, and consequently hyperglycemia of sufficient degree to cause pathological and functional changes may be present for a long time before the diagnosis is made [4]. Among the complications associated with diabetes mellitus include microvascular complications which mainly affect the retina, kidney and peripheral nervous system and may progress to more overt serious complications, and macrovascular complications, mainly atherosclerosis, that may lead to cerebrovascular ischemia and stroke [3,4].

Several pathogenic processes are involved in the development of diabetes. These include processes which destroy the beta cells of the pancreas with consequent insulin deficiency (Type I diabetes), and others that result in resistance to insulin action or both (Type II diabetes) [4]. The abnormalities of carbohydrate, fat and protein metabolism are due to deficient action of insulin on target tissues resulting from insensitivity or lack of insulin [4].

Pharmacological treatment of diabetes mellitus is based on oral hypoglycaemic agents and insulin injection which have so many side effects, coupled with its high cost which is not affordable in poor economic communities [5]. Consequently, in rural parts of worldwide societies, traditional remedies from plant sources with minimal side

effect are frequently employed to manage the disorder [5].

Ocimum lamiifolium is a perennial evergreen shrub having oblong, ovate green colored leaves (0.5-5 m), oppositely arranged having pubescent leaf surface, narrow at the base and deeply serrated (Plate 1) [6]. The genus *Ocimum* is cultivated for its extraordinary essential oil which display many therapeutic usages such as in medicinal application, herbs, culinary, perfume for herbal toiletries, aromatherapy treatment and as flavoring agent [6]. It has wide range of therapeutic effects like antimicrobial, antispasmodic, bactericide, carminative, anthelmintic, hepatoprotective, antiviral, larvicidal, remedy of coughs, colds, measles, abdominal pains, diarrhea, insect repellent, particularly



Plate 1: *Ocimum lamiifolium* (photograph taken in July 2013 at Kijauri Nyamira County)

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against mosquitoes and storage pest control. The essential oils obtained from this plant as repellent against nuisance biting insects and malaria vector [6].

The plant has also been in use successfully in the management of diabetes mellitus in some parts of Kenya. However, its increased use has not been accompanied by an increase in the quantity, quality and accessibility of clinical evidence to support traditional medicine practitioner's claims. This study therefore contributes additional knowledge to the use of *Ocimum lamiifolium* in the management of diabetes from samples collected from Nyamira County, Kenya.

Materials and Methods

Study site

This study was undertaken at the Department of Biochemistry and Biotechnology, School of Pure and Applied Sciences, Kenyatta University from July 2013 to February 2015. Kenyatta University is 23 km from Nairobi off Thika Road.

Collection and preparation of the plant materials

The plant used in this study was collected from its native habitat on the basis of ethno-botanical information. It was collected with bio-conservation aspects in mind from Kijauri village Nyamira county Kenya. Information on the identity of the plant to collect, the precise locality where it grows, what part to collect, when curative potency is at maximum and the mode of preparation was provided by a traditional medical practitioner. For this study, the part of the plant collected was the leaves. Botanical identity of the plant was authenticated by an acknowledged authority in taxonomy and a voucher specimen deposited at the National Museums of Kenya Herbarium, Nairobi.

Leaves were collected while green and dried at room temperature away from direct sunlight for different periods of time depending on their succulence. The dried leaves were separately ground into fine powder by use of an electric mill. The powdered plant materials were kept at room temperature away from direct sunlight in closed, dry plastic air tight bags ready for extraction.

Preparation of the aqueous extracts

Each one hundred grams of the powdered plant material was extracted in 1 liter distilled water at 60°C for 6 hours. The mixture was left to cool at room temperature and then decanted into dry clean conical flask through folded cotton gauze stuffed into a funnel. The decanted extract was then filtered using filter papers under vacuum pump. The filtrate was then freeze-dried for 48 hours in a Modulyo freeze dryer (Edward England). The freeze-dried powder was then weighed and stored in airtight container at -20°C until used for bioassay.

Experimental animals

The study used male Swiss White Albino mice (3-4 weeks old) that weighed 21-25 g with a mean weight of 23 g. These were bred in the animal house at the Department of Biochemistry and Biotechnology of Kenyatta University. The mice were housed at a temperature of 25°C with 12 hours/12 hours darkness photoperiod and fed on rodent pellets and water *ad libitum*. The experimental protocols and procedures used in this study were approved by the Ethics Committee for the Care and Use of Laboratory Animals of Kenyatta University, Kenya.

Induction of hyperglycemia

Hyperglycemia was induced experimentally by a single

intraperitoneal administration of 186.9 mg/kg body weight of a freshly prepared 10% alloxan monohydrate (2,4,5,6 tetraoxypyrimidine; 5-6-dioxyuracil) obtained from Sigma (Steinheim, Switzerland) [7].

Forty-eight hours after alloxan administration, blood glucose level was measured using a glucose.

Analyzer model (Hypogaurd, Woodbridge, England) with glucometer strips Mice with blood glucose levels above 2000 mg/L (>11.1 mmol/L) were considered diabetic and used in this study. Prior to initiation of this experiment, the animals were fasted for 8-12 hours [8] but allowed free access to water until the end of this experiment.

Experimental design

For either intraperitoneal or oral route of drug administration, the experimental mice were randomly divided into eight groups of five animals each. Group I consisted of normal mice either intraperitoneally or orally administered with 0.1 ml physiological saline; Group II consisted of alloxan induced diabetic mice either intraperitoneally or orally administered with 0.1 ml physiological saline; Group IIIa consisted of alloxan induced diabetic mice intraperitoneally administered with 1 IU/kg body weight in 0.1 ml physiological saline; Group IIIb consisted of alloxan induced diabetic mice orally administered with (3 mg/kg body weight) in 0.1 ml physiological saline; Group IV consisted of alloxan induced diabetic mice either intraperitoneally or orally administered with 25 mg/kg body weight in 0.1 ml physiological saline; Group V consisted of alloxan induced diabetic mice either intraperitoneally or orally administered with 48.4 mg/kg body weight in 0.1 ml physiological saline; Group VI consisted of alloxan induced diabetic mice either intraperitoneally or orally administered with 93.5 mg/kg body weight in 0.1 ml physiological saline; Group VII consisted of alloxan induced diabetic mice either intraperitoneally or orally administered with 180.9 mg/kg body weight in 1 ml physiological saline. Group VIII consisted of alloxan induced diabetic mice either intraperitoneally or orally administered with 350 mg/kg body weight in 1 ml physiological saline 0.1 ml of either insulin or glibenclamide or the plant extract solution was administered either intraperitoneally or orally to each experimental mouse.

Blood sampling and blood glucose Determination

Blood sampling was done by sterilizing the tail with 10% alcohol and then nipping the tail at the start of the experiment and repeated after 1, 2, 3, 4, 6 and 24 hours. Bleeding was enhanced by gently "milking" the tail from the body towards the tip. After the operation, the tips of the tail were sterilized by swabbing with 70% ethanol. The blood glucose levels were determined with a glucose analyzer model (Hypogaurd, Woodbridge, England).

Qualitative phytochemical screening

Tannins were determined as follows; 2 ml of 5% FeCl₃ was added to 2 ml aqueous extract of each sample. Yellow brown precipitate indicated presence of tannins [9]. Alkaloids were determined as follows; 1.5 ml of 1% HCL was added to 2 ml methanolic filtrates of samples. The solution was heated and six drops of dragendroff reagent was added. Orange precipitate confirmed presence of alkaloids [9].

For saponins determination, aqueous extract of 2 g powder was made and subjected to frothing test. Frothing persistence indicated presence of saponins. Later the froth was mixed with few drops of olive oil. Formation of emulsion indicated presence of saponins [9]. For determination of flavonoids (shimodas test), 2 g material was extracted in 10 ml H₂O, few drops of HCL followed by 0.5 g of zinc turnings were

added. Tubes were boiled for a few minutes formation of pink color indicated presence of flavonoids [9].

Terpenoids and sterols were determined as follows; the *n*-hexane was stirred with 2 g of each extract to remove most coloring materials. The residue was then extracted with 2 ml dichloromethane. The dichloromethane solution was dehydrated over anhydrous sodium sulphate. Then 2 ml of the dichloromethane portion was mixed with 0.5 ml acetic anhydride followed by 2 drops of concentrated sulphuric acid. A gradual appearance of green to blue color was indicative of sterols. Color change from pink to purple indicated the presence of terpenoids [10,11].

Results

Leaf extracts yielded a 8% light brown powder. Intraperitoneally administered aqueous leaf extracts of *O. lamiifolium* decreased the blood glucose levels at all the five doses of 25, 48.4, 93.5, 180.9 and 350 mg/kg body weight (Table 1 and Figure 1). This occurred in two phases, in the first one hour the extract caused a steep decline in blood glucose levels, followed by a steady decline up to the seventh hour. After this, a gradual increase was recorded in the twenty fourth hour. However, the sugar levels were not reduced in a dose dependent manner.

In the first hour, the extracts lowered blood glucose levels to 67.8%, 59.5%, 54.4%, 65.7% and 53.9% for 25, 48.4, 93.5, 180.9 and 350 mg/kg body weight doses, respectively, compared to insulin treated diabetic mice whose blood sugar levels was lowered to 48.4% within the first hour. By the fourth hour, all the five doses (25, 48.4, 93.5, 180.9 and 350 mg/kg body weight) had lowered blood sugar levels to 37.8%, 27.0%, 27.5%, 37.2% and 26.2%, respectively, compared to insulin treated diabetic mice whose sugar levels was lowered to 36.3% within the same hour (Figure 1).

Orally administered aqueous leaf extracts of *O. lamiifolium* also lowered blood glucose levels at all the five doses of 25, 48.4, 93.5, 180.9

and 350 mg/kg body weight (Table 2 and Figure 2), from the first hour to the twenty fourth hours in a dose independent manner. By the second hour the extract had lowered the blood glucose levels to 60.0%, 34.8%,

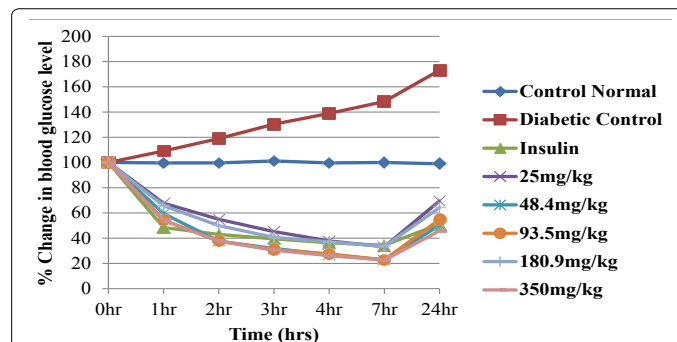


Figure 1: Mean percentage change in blood glucose levels of *Ocimum lamiifolium* intraperitoneally administered in alloxan induced diabetic mice.

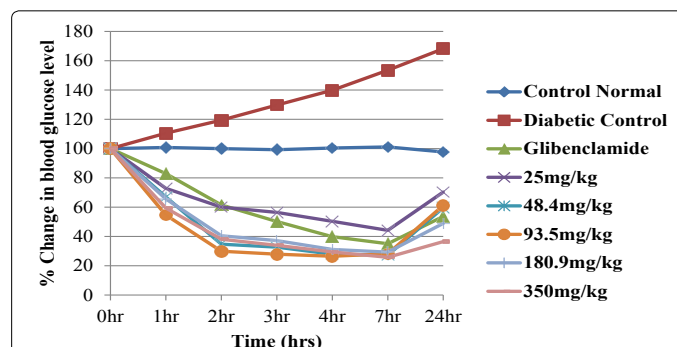


Figure 2: Mean percentage change in blood glucose levels of *Ocimum lamiifolium* administered orally in alloxan induced diabetic mice.

Treatment	Blood Glucose Levels at Varying Times (Mmoles/L)						
	0 hr	1 hr	2 hr	3 hr	4 hr	7 hr	24 hr
Control/Saline	5.28 ± 0.19 ^b	5.26 ± 0.15 ^b	5.26 ± 0.09 ^b	5.34 ± 0.09 ^b	5.26 ± 0.09 ^b	5.28 ± 0.13 ^b	5.22 ± 0.08 ^c
Diabetic/Saline	15.18 ± 4.65 ^a	16.46 ± 4.63 ^a	17.74 ± 4.27 ^a	19.40 ± 4.42 ^a	20.58 ± 4.46 ^a	21.92 ± 4.41 ^a	25.26 ± 3.67 ^a
Diabetic/insulin	15.60 ± 4.95 ^a	7.06 ± 0.21 ^b	6.20 ± 0.25 ^b	5.64 ± 0.47 ^b	5.20 ± 0.41 ^b	4.94 ± 0.28 ^b	7.46 ± 0.65 ^{cd}
25(mg/kgbw)	16.82 ± 4.53 ^a	11.78 ± 5.66 ^{ba}	9.28 ± 3.10 ^b	7.58 ± 2.11 ^b	6.26 ± 1.24 ^b	5.46 ± 1.01 ^b	11.64 ± 2.90 ^b
48.4(mg/kgbw)	15.18 ± 2.81 ^a	9.16 ± 3.27 ^b	5.60 ± 0.74 ^b	4.72 ± 0.49 ^b	4.00 ± 0.58 ^b	3.40 ± 0.49 ^b	7.56 ± 1.39 ^{cd}
93.5(mg/kgbw)	15.32 ± 3.06 ^a	8.30 ± 1.59 ^b	5.72 ± 0.80 ^b	4.68 ± 0.54 ^b	4.16 ± 0.65 ^b	3.46 ± 0.69 ^b	8.48 ± 2.59 ^{cd}
180.9(mg/kgbw)	15.90 ± 5.49 ^a	10.60 ± 4.66 ^{ba}	7.92 ± 2.63 ^b	6.38 ± 1.89 ^b	5.70 ± 1.13 ^b	5.12 ± 0.93 ^b	9.88 ± 2.33 ^b
350(mg/kgbw)	17.94 ± 3.42 ^a	9.58 ± 1.30 ^{ba}	6.62 ± 0.61 ^b	5.38 ± 0.70 ^b	4.62 ± 0.71 ^b	4.04 ± 0.84 ^b	8.04 ± 1.04 ^{cd}

Results are expressed as Means ± SD for five mice per group. Values followed by the same superscript are not statistically different ($P \leq 0.05$; analysed by ANOVA followed by Tukey's post hoc test).

Table 1: Effects of intraperitoneally administered aqueous leaf extracts of *Ocimum lamiifolium* on blood glucose levels in alloxan induced diabetic mice.

Treatment	Blood Glucose Levels at Varying Times (mmoles/L)						
	0hr	1hr	2hr	3hr	4hr	7hr	24hr
Control/Saline	5.22 ± 0.08 ^b	5.26 ± 0.05 ^a	5.22 ± 0.08 ^{cd}	5.18 ± 0.08 ^{cd}	5.24 ± 0.05 ^{cbd}	5.28 ± 0.08 ^{cb}	5.10 ± 0.10 ^c
Diabetic/Saline	13.52 ± 2.22 ^a	14.86 ± 1.80 ^a	16.04 ± 1.89 ^a	17.36 ± 1.61 ^a	18.74 ± 1.84 ^a	20.56 ± 2.10 ^a	22.56 ± 2.72 ^a
Diabetic/ Glen	15.56 ± 2.78 ^a	12.90 ± 2.47 ^{ba}	9.62 ± 2.55 ^b	7.80 ± 1.57 ^b	6.18 ± 1.25 ^{cb}	5.38 ± 0.60 ^{cb}	8.22 ± 0.86 ^b
25(mg/kgbw)	13.66 ± 2.46 ^a	9.86 ± 1.62 ^{bcd}	9.86 ± 1.62 ^b	7.56 ± 0.50 ^b	7.56 ± 0.50 ^b	6.72 ± 0.30 ^b	5.92 ± 0.68 ^c
48.4(mg/kgbw)	13.52 ± 2.36 ^a	9.06 ± 1.95 ^{cd}	4.70 ± 1.58 ^{ed}	4.42 ± 1.11 ^{cd}	3.78 ± 0.81 ^d	3.74 ± 1.01 ^c	8.00 ± 1.47 ^{cb}
93.5(mg/kgbw)	12.60 ± 1.04 ^a	6.84 ± 0.94 ^{ed}	3.68 ± 1.21 ^e	3.44 ± 1.08 ^d	3.28 ± 1.16 ^d	3.48 ± 1.08 ^c	7.66 ± 2.01 ^{cb}
180.9(mg/kgbw)	17.38 ± 3.52 ^a	11.32 ± 1.88 ^{bc}	6.94 ± 0.96 ^{cbd}	6.36 ± 0.86 ^{cb}	5.28 ± 0.54 ^{cbd}	4.92 ± 0.22 ^{cb}	8.26 ± 1.08 ^b
350(mg/kgbw)	14.32 ± 2.47 ^a	8.52 ± 1.70 ^{ecd}	5.50 ± 1.97 ^{cd}	4.88 ± 1.32 ^{cd}	4.20 ± 0.93 ^{cd}	3.70 ± 0.96 ^c	5.12 ± 0.66 ^c

Results are expressed as Means ± SD for five mice per group. Values followed by the same superscript are not statistically different ($P \leq 0.05$; analysed by ANOVA followed by Tukey's post hoc test).

Table 2: Effects of orally administered aqueous leaf extracts of *Ocimum lamiifolium* on blood glucose levels in alloxan induced diabetic mice.

Phytochemicals	<i>Ocimum lamiifolium</i>
Alkaloids	+
Sterols	+
Terpenoids	+
Saponins	+
Tannins	+
Flavonoids	+

Table 3: Qualitative phytochemical screening of aqueous leaf extract of *Ocimum lamiifolium*

30.0%, 40.4% and 38.1%, respectively for the five doses, compared to 61.5% for the conventional oral drug, glibenclamide.

Table 3 shows qualitative phytochemical composition of aqueous extracts of *Ocimum lamiifolium*. Results show that the plant extracts contained alkaloids, sterols, terpenoids, flavonoids, tannins and saponins.

Discussion

Diabetes mellitus has a significant impact on the health, quality of life and life expectancy of patients as well as healthcare expenditure [12]. With increasing incidence and mortality from its complications, prompt and adequate glycemic control in diabetes is paramount if management can meaningfully improve the quality of life and increase life expectancy. Diet and physical activity remains to be the mainstay in the management of diabetes mellitus [5]. In conventional medical practice, prescription of oral hypoglycemic agents and injectable drugs such as insulin has been used in the management of diabetes. However, due to the unaffordability, unwanted side effects and unavailability associated with the conventional therapy, underdeveloped and developing countries have given traditional medicine considerable attention towards managing of this disorder [13].

In this study, experimental evaluation of the antidiabetic potentials of *Ocimum lamiifolium* showed that the oral and intraperitoneal administration of the aqueous leaf extract to alloxan induced diabetic mice reduced fasting blood glucose in dose independent manner which suggests their inherent hypoglycemic effect. The alloxan-induced diabetic mice had a three to four fold increase in blood relative to the normal control mice. Administration of diabetogenic drug, alloxan monohydrate, caused a selective massive destruction of the β cells of islets of Langerhans resulting in hyperglycemia [14,15]. The lowering effect of blood sugar levels by *Ocimum lamiifolium* in the same manner regardless of the dosage might suggest that the extract may have been absorbed in the cell system through active transport where a particular concentration saturation of the extract occurred resulting to the rest of extract being excreted [16]. Similar results have been reported on the hypoglycemic activity of plant extracts of *L. wightiana* and *L. camara* Linn [17,18], *Artemisia herba* [19] *Colocynthis citrullus* [20] and *Ocimum gratissimum* [21,22].

The antidiabetic effect of *Ocimum lamiifolium* extract could be linked to more than one mechanism. The possible mechanism includes the stimulation of β cells and subsequent release of insulin and activation of the insulin receptors [23]. The plants antihyperglycemic action may be by stimulation of pancreatic secretion of insulin. In this context a number of other plants have also been reported to have antihyperglycemic and insulin release stimulatory effect [24-26]. Hence, in the present study the *Ocimum lamiifolium* extract also may act as a hepatoprotective agent so this evidently, improves the function of liver and maintains glucose uptake, enhanced transport of blood glucose to peripheral tissue and utilization, which may be another mechanism of action [5].

The blood glucose lowering effect of this plant extracts may be attributed to the presence of saponins, flavonoids, tannins, alkaloids, terpenoids and sterols that have been associated with hypoglycemic activity [27]. The presence of flavonoids, sterols and saponins has previously been reported in ethanolic fruit extracts of *L. camara* Linn which demonstrated hypoglycemic activity in streptozotocin induced diabetic male wistar rats [18]. Saponin fraction isolated from *Momordica charantia* reduced blood glucose levels and increased insulin secretion and glycogen synthesis in alloxan induced diabetic mice [28]. The alkaloid 1-ephedrine promotes the regeneration of pancreas islets following destruction of the beta cells, hence restores the secretion of insulin, and thus corrects hyperglycemia [27]. Intraperitoneally administered alkaloids isolated from leaves of *Acanthus montanus* at doses of 100, 200 and 400 mg/kg body weight showed hypoglycemic action in alloxan-induced diabetic rats [29]. The aqueous leaf extracts of the same plant contained tannins that are known to have hypoglycemic activity [30]. Condensed tannins extracted from some Kenyan foods showed antihyperglycemic action due to inhibition of α -amylase and α -glucosidase enzymes [31]. Terpenoids are very popular among patients with high blood pressure and diabetes because they help to reduce diastolic blood pressure and lower the sugar level in blood [2]. Due to the presence of terpenoids, the leaves and seeds of *E. officinalis* are used in the treatment of diabetes [32].

That the aqueous leave extracts of *Ocimum lamiifolium* in both oral and intraperitoneal routes the sugar levels started rising from the seventh hour in all dosage levels may have been due to the extracts having a short half-life or the extracts may have been prone to fast hepatic metabolism and renal clearance [5].

Conclusion

The present observation provide evidence that aqueous leaf extract of *Ocimum lamiifolium* exhibited antidiabetic activity on experimental alloxan induced diabetic mice when therapeutic doses were administered through intraperitoneal and oral routes. This effect might be due to the presence of phytochemicals such as saponins, flavonoids, tannins, alkaloids, terpenoids and sterols which could act synergically or independently in enhancing the hypoglycemic activity of the aqueous leaf extracts of *Ocimum lamiifolium*. However, further, comprehensive chemical and pharmacological investigation should be carried out to isolate the active compound and appropriate elucidation of its mechanism.

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