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Hypoglycemic Effect of *Lippia javanica* in Alloxan Induced Diabetic Mice

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Abstract

Lippia javanica is widely distributed throughout Kenya where it is used extensively in traditional herbal preparations. An infusion of the leaves is commonly used as a decongestant for colds and coughs including diabetes, however, its efficacy profiles have not been scientifically evaluated. The aim of this study was to determine the *in vivo* antidiabetic activity of aqueous leaf extracts of this plant in white male alloxan-induced albino mice. The antidiabetic activity of the aqueous leaf extracts was orally and intraperitoneally bioscreened in alloxan induced diabetic mice at different doses of 25 mg/kgbwt, 48.4 mg/kgbwt, 93.5 mg/kgbwt, 180.9 mg/kgbwt and 350 mg/kgbwt. The treatment effects were then compared with the controls. 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 in a dose independent manner. The extracts contained tannins, flavonoids, saponins, sterols, alkaloids, and free or bound anthraquinones. The observed hypoglycemic activity could be associated with the phytochemicals present in this plant extract. In conclusion the results showed that the plant extracts were effective in reducing blood sugar levels and revealed the presence of vital phytochemicals which possess antidiabetic activities. The study therefore, confirmed the traditional use of these herbs and established their efficacy data that can guide proper use of these plants in the management of diabetes mellitus. Consideration should be made to carry out the same studies using higher animals or subject the plant to organic solvent extraction and compare activities of both aqueous and organic fractions.

Keywords: Diabetes Mellitus; *Lippia javanica;* Hypoglycemic activity; *in vivo;* Antidiabetic; Phytochemicals; Insulin Units (IU)

Introduction

Diabetes is a group of metabolic disorders characterized by a chronic hyperglycemic condition resulting from defects in insulin secretion, insulin action or both [1]. The prevalence of diabetes is increasing rapidly worldwide and the [2] has predicted that by 2030 the number of adults with diabetes would have almost doubled worldwide, from 177 million in 2000 to 370 million. Experts project that the incidence of diabetes is set to soar by 64% by 2025, meaning that a staggering 53.1 million citizens will be affected by the disease [3]. The disease has several pathogenic processes ranging from autoimmune destruction of pancreatic β-cell proteins resulting in absolute insulin deficiency (Type I) to multiple abnormalities that include a combination of genetic factors related to impaired insulin secretion, insulin resistance and environmental factors such as obesity, overeating, lack of exercise and stress, as well as aging (Type II) [4].

At the onset of overt hyperglycemia the patient manifests excessive excretion of urine (polyuria), thirst (polydipsia), constant hunger (polyphagia), weight loss, vision changes and fatigue. These symptoms are more marked in type 1 diabetics [5]. Chronic hyperglycemia causes disturbances in metabolism of carbohydrates, fats and proteins resulting in long term microvascular and macrovascular complications [6]. These complications include retinopathy with potential loss of vision; nephropathy resulting in renal failure; peripheral neuropathy associated with risk of foot ulcers, amputations, and Charcot joints; and autonomic neuropathy which is recognized by gastrointestinal, genitourinary, and cardiovascular symptoms and sexual dysfunction [6].

Normal fasting plasma glucose levels ranges between 3.5-6.7mmol/l (63-120.6 mg/dL). After a meal the blood glucose level rises to approximately 8mmoles/L and rarely exceeds this level. Repeated fasting blood glucose levels ≥ 7.0mmoles/L (126mg/dL) or 2-hour postprandial glucose values ≥ 11.1mmole/L (200mg/dL) is considered to be diagnostic criteria for diabetes and correlates with Hb A1C threshold of 6.5% [7]. .

In conventional medical practice, the current therapies of diabetes mellitus are reported to be expensive, unavailable and have side effects [8]. For example, use of insulin and oral hypoglycemic agents is associated with drawbacks such as ineffectiveness on oral administration, short shelf life, requirement of constant refrigeration and in the event of excess dosage, fatal hypoglycemia ensue. The use of oral hypoglycemic drugs like sulfonylureas and biguanides is also associated with tendency to gain weight [9]. Therefore, there is need to use effective, easily accessible and cheap means to manage diabetes mellitus.

Herbal medicines and traditional medical practitioners are receiving considerable attention from mainstream health officials, international medical research and training institutions. Traditional medicine, in the estimate of the World Health Organization is used by up to 80% of the population of most developing countries especially in Africa [10]. This is envisaged by strained economic situations of most

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African countries that drive African diabetics to seek cheaper treatment and management options [11]. Herbal medicines are thought to be effective, safe and affordable [12].

Plants based herbal medicines contain a great diversity of bioactive compounds. Besides, they have been viewed by pharmaceutical industry as a source of 'qualified leads' in the production of synthetic modern drugs [13]. For example the widely used hypoglycemic drug Metformin is originally derived from the traditional medicinal plant *Galega officinalis* [11] which is a common traditional remedy for diabetes.

To date, more than 400 traditional plants have been reported to have antidiabetic effect [11]. Some of these herbs are proven to provide symptomatic relief and assist in the prevention of the secondary complication of the disease, while others were reported to help in regeneration of β-cells and in overcoming insulin resistance [14]. For instance, *Pappea capensis* [15] and *Pterocarpus marsupium* has long histories of use in treatment of diabetes mellitus. The Asian ginseng is commonly used in traditional Chinese medicine to treat diabetes. It has been shown to enhance the release of insulin from the pancreas and to increase the number of insulin receptors. It also has a direct blood sugar-lowering effect. A recent study found that 200mg of ginseng extract per day improved blood sugar control as well as energy levels in type II diabetes (NIDDM) [11]. *Ginkgo biloba* extract has proved useful for prevention and treatment of early-stage diabetic neuropathy [11]. These agents are cheap, readily available and have limited side effects. However, only a small number of these have received scientific and medical evaluation to assess their efficacy [11].

Among the traditionally used plants in the management of diabetes mellitus in Kijauri village Nyamira county Kenya is *Lippia javanica*. The plant is also used extensively in traditional medicine by both lay people and traditional healers to treat minor ailments. Many of its uses relate to microbial infections such as coughs or colds and also for skin infections or wounds. The rationale for their utilization has rested largely on long-term clinical experience. However, there is limited scientific evidence regarding the efficacy profiles to back up the continued therapeutic application of this herbal remedy. This therefore, makes it necessary to carry out a thorough scientific investigation to elucidate their hypoglycemic activities that will go a long way in validating their folkloric usage.

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 habitats on the basis of ethnobotanical information. It was collected with bioconservation 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

One hundred grams of the powdered plant material was extracted in 1 liter distilled water at 60°C for 6 hour. 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 72 hour. 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 21-25g with a mean weight of 23g. 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 (Steinhein, Switzerland) [16].

Forty-eight hours after alloxan administration, blood glucose level was measured using a glucometer. Mice with blood glucose levels above 200 mg/dL were considered diabetic and used in this study. Prior to initiation of this experiment, the animals were fasted for 8-12 hours but allowed free acess 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 0.1ml physiological saline; Group II consisted of alloxan induced diabetic mice either intraperitoneally or orally administered with 0.1ml physiological saline; Group IIIa consisted of alloxan induced diabetic mice intraperitoneally administered with 0.025 insulin units (0.25 insulin units in 1 ml) (1 IU/kg body weight) in 0.1ml physiological saline; Group IIIb consisted of alloxan induced diabetic mice orally administered with 0.075 mg glibenclamide (0.75 mg in 1 ml) (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 0.1ml 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 analyser model (Hypogaurd, Woodbridge, England).

Qualitative phytochemical screening

A phytochemical screening of alkaloids, flavonoids, saponins, tannins, terpenoids, sterols, and free and bound anthraquinones present in *Lippia javanica* extracts was performed using standard methods [17,18] (Table 3).

Data management and statistical analysis

The Data was entered in the Microsoft Excel Spread Sheet, cleaned and then exported to SAS statistical software version 9.1.3 for analysis. Results were expressed as Mean ± Standard Deviation (SD) of the number of animals used per every study point. Statistical analysis were done using ANOVA and post-ANOVA to compare the means of untreated normal control mice with diabetic mice treated with saline, diabetic mice treated with the conventional drugs, and diabetic mice treated with plant extract at doses of 25mg/kg body weight, 48.4 mg/kg body weight, 93.5 mg/kg body weight, 180.9mg/kg body weight and 350 mg/kg body weight. The values of $p \leq 0.05$ were considered to be significant.

Results

Effect of oral and intraperitoneal administration of aqueous leaf extracts of *Lippia javanica* on blood glucose levels in alloxan induced diabetic mice

The aqueous leaf extracts yielded a 9.8% brown powder. Intraperitoneally administered aqueous leaf extracts of *L. javanica* 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). The pattern of decrease varied with each dose up to the seventh hour. However, the sugar levels were not reduced in a dose dependent manner. In the first hour, the extracts lowered blood glucose levels to 74.1%, 64.0%, 59.9%, 67.6% and 62.7% for 25, 48.4, 93.5, 180.9 and 350 body weight doses, respectively, compared to insulin treated diabetic mice whose blood sugar levels was lowered to 46.8% 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 42.5%, 36.0%, 36.4%, 37.1% and 37.5%, respectively, compared to insulin treated diabetic mice whose sugar levels was lowered to 37.6% within the same hour (Figure 1).

Orally administered aqueous leaf extracts of *L. javanica* decreased blood glucose levels at all the five doses of 25, 48.4, 93.5, 180.9 and 350 mg/kg body weight (Table 2), from the first hour to the twenty fourth hours in a dose-independent manner. By the second hour the extract had decreased the blood glucose levels to 66.3%, 49.3%, 48.0%, 51.8% and 50.5% respectively, for the five doses, compared to 59.2% decrease in blood sugar levels for the conventional oral drug, glibenclamide (Figure 2).

Qualitative analysis of the phytochemical composition of aqueous leaf extracts of *Lippia javanica*

The phytochemical screening of the aqueous leaf extracts of *Lippia javanica* indicated the presence of Alkaloids, Saponins, Terpenoids, Flavonoids, Tannins Free and Bound Athraquinones as shown in Table 2.

Discussion

Diabetes mellitus is a group of metabolic syndrome characterized by high blood sugar levels and altered metabolism of carbohydrates, lipids and proteins [1]. In this study, hyperglycemia was induced by administering a diabetogenic drug known as alloxan monohydrate in mice. The alloxan monohydrate destroys and reduces the pancreatic β-cells of islets of Langerhans population through the formation of reactive oxygen species such as nitric oxide [16]. The alloxan-induced diabetic mice had a three to five fold increase in blood glucose (5 mm/L to 25 mm/L) relative to the normal control mice. The intraperitonial and oral administration of the aqueous leaf extract of *Lippia javanica* demonstrated blood glucose lowering effect in mice indicating that they contained hypoglycemic constituents.

The possible mechanism of action for hypoglycemic effect of the plant extracts were either through increased utilization of glucose by peripheral tissues such as the muscle, fat and liver cells via activation of the insulin receptors [19] or direct stimulation of β-cells of islet of Langerhans to secrete insulin [20]. Increased serum insulin consequently resulted in reduced blood sugar [21] by increased facilitated uptake of glucose by peripheral tissues mediated by GLUT-4, an insulin dependent glucose transporter [22]. The plants' antihyperglycemic action might also be attributed to interference on absorption of dietary carbohydrates as well as disaccharides in small

TREATMENT	BLOOD GLUCOSE LEVELS AT VARYING TIMES (mmoles/L)						
	0hr	1hr	2hr	3hr	4hr	7hr	24hr
Control/Saline	5.26±0.05 ^{b}	5.28±0.08 ^{d}	5.30±0.07 ^{c}	5.26±0.89 ^{c}	5.30±0.07 ^{b}	5.26±0.05 ^{cb}	5.28±0.08 ^{d}
Diabetic/Saline	14.28±0.93 ^{a}	15.58±1.12 ^{a}	16.64±1.13 ^{a}	18.00±0.99 ^{a}	19.08±1.20 ^{a}	21.00±1.87 ^{a}	23.30±1.61 ^{a}
Diabetic/insulin	14.66±1.44 ^{a}	6.84±0.42 ^{cd}	6.10±0.14 ^{c}	5.78±0.15 ^{cb}	5.46±0.26 ^{b}	5.04±0.15 ^{cb}	8.12±0.54 ^{c}
25(mg/kgbw)	15.24±2.90 ^{a}	11.44±3.31 ^{b}	8.58±2.01 ^{b}	7.28±1.59 ^{b}	6.44±1.15 ^{b}	6.04±0.96 ^{b}	10.86±1.62 ^{b}
48.4(mg/kgbw)	14.88±2.65 ^{a}	9.56±2.03 ^{cb}	7.26±1.38 ^{cb}	6.00±0.90 ^{cb}	5.28±0.50 ^{b}	4.68±0.31 ^{cb}	8.88±2.28 ^{cb}
93.5(mg/kgbw)	14.74±1.73 ^{a}	8.78±0.95 ^{cbd}	7.12±0.68 ^{cb}	5.90±1.05 ^{cb}	5.34±0.82 ^{b}	4.76±0.15 ^{cb}	8.66±0.70 ^{cb}
180.9(mg/kgbw)	14.36±3.28 ^{a}	9.76±2.77 ^{cb}	7.60±1.88 ^{cb}	5.94±1.38 ^{cb}	5.24±0.72 ^{b}	4.60±0.68 ^{cb}	9.08±1.17 ^{cb}
350(mg/kgbw)	13.36±0.80 ^{a}	8.36±0.61 ^{cbd}	6.46±0.51 ^{cb}	5.50±0.44 ^{cb}	5.00±0.14 ^{b}	4.24±0.23 ^{c}	6.88±0.27 ^{cd}

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

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

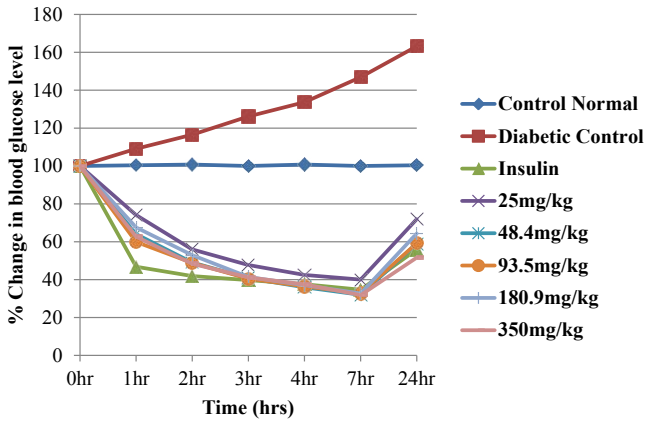

Figure 1: Mean percentage change in blood glucose levels of *Lippia javanica* intraperitoneally administered in alloxan induced diabetic mice.

intestines of mice by slowing gastric motility and emptying [23]. It can also be as a result of regeneration of β-cells [24,25], and/or restored insulin sensitivity [26]. The plant extracts might also have improved liver functions such as uptake of glucose, facilitated transport of blood glucose to peripheral tissue and utilization [27].

The blood glucose lowering effect of these plants was similar to that reported of other plants investigated in early researches. The aqueous leaf extracts of *Helichrysum odoratissimum* demonstrated to possess antidiabetic activity by increasing either the pancreatic secretion of insulin from the β cells or the release of bound insulin [28]. Phytoconstituents present in garlic also showed antioxidative property evidenced by scavenging of reactive oxygen species and increasing cellular antioxidant enzymes: superoxide dismutase, catalase, and glutathione peroxidase [28]. Murugi et al. [8] Observed that aqueous leaf extracts of *Caesalpinia volkensii* showed hypoglycemic activity in alloxan-induced diabetic mice through administration of the dosage at 50,100 and 150mg/kg body weight**.** The oral administration of *Momordica charantia* at a dose level of 300 mg/kg body weight significantly decreased the fasting blood glucose levels in mice [29].

The higher antidiabetic effect demonstrated by antihyperglycemic plant extracts administered via intraperitoneal route relative to the oral route could be due to the fact that in the oral route the constituents may have been transported more slowly across the intestinal wall while the intraperitoneal route immediately provided their constituents into the systemic circulation [30]. The route of administration therefore brings the difference in activity attributable to the much higher bioavailability of the extract in blood when administered intraperitoneally (three-four more times bioavailable than oral route). However, there exists a possibility that when the extracts are intraperitoneally administered a direct inhibitory effect on glucose transporters in peritoneal mesothelial cells might be experienced forming a barrier to glucose absorption across the peritoneum [31].

The observed dose independent hypoglycemic action of the plant extracts in this study suggests that the extracts 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 [32]. The observed low levels of blood glucose in mice orally and intraperitoneally administered with plant extracts can be as a result of increased glycolysis [30].

The aqueous leave extracts of *Lippia javanica* showed insulinemimetic activity and at times worked better than conventional drugs in both oral and intraperitoneal administration, these may have been due to the fact that it increased uptake of glucose by peripheral tissues mediated by GLUT-4 or the extracts might have being easily absorbed in the intraperitoneal cavity and gastro intestinal mucosa. For the intraperitoneally administered extracts, the active principles could have been more active because they were immediately bioavailable into the systemic circulation as opposed to orally administered extracts whose active principles may have been slowly transported across the intact intestinal wall [9].

That the aqueous leaf extracts of *Lippia javanica* at all dose levels significantly lowered the blood glucose levels in both oral and intraperitoneal routes from the, first to seventh hour may be due to the fact that the extracts could have been absorbed quickly through the intraperitoneal cavity and gastro-intestinal mucosa or there could be no compound in the extract that can act as a pro-drug thus the active principles in the extracts did not require biotransformation so as to become antihyperglycemic. That in the intraperitoneal route 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, the extracts may have been prone to fast hepartic metabolism and renal clearance.

The antihyperglcemic effect of the aqueous plant extracts might also be attributed to the presence of various phytoconstituents including: tannins, flavanoids, saponins, alkaloids, terpenoids, sterols, free and bound anthraquinones that have been associated with antidiabetic activity [33].

The antidiabetic effect of *Lippia javanica* could have been due to the observed presence of flavonoids. The polyhydroxylated flavonol enhances lipogenesis and glucose uptake in the adipocytes and flavanoid, myricetin has demostrated insulinomimetic properties [33]. Epicatechin and its active principles have demonstrated *in vitro*

TREATMENT	BLOOD GLUCOSE LEVELS AT VARYING TIMES (mmoles/L)						
	0hr	1hr	2hr	3hr	4hr	7hr	24hr
Control/Saline	5.34±0.11 ^{b}	5.34±0.13 ^{c}	5.36±0.05^{c}	5.34±0.11^{c}	5.34±0.15^{b}	5.32±0.08^{cb}	5.36±0.09 ^{e}
Diabetic/Saline	14.26±1.43^{a}	15.50±1.49 ^{a}	16.36±1.38^{a}	17.48±1.50^{a}	18.80±1.47^{a}	20.26±1.07^{a}	23.56±0.87 ^{a}
Diabetic/ Glen	14.14±1.42 ^{a}	10.78±1.17^{b}	8.38±0.90^{cb}	6.82±0.52^{cb}	5.60±0.46 ^{b}	5.14±0.17^{cb}	9.32±0.71 ^{cbd}
25(mg/kgbw)	15.36±2.82 ^{a}	12.68±3.27^{ba}	10.38±3.32^{b}	7.50±1.91^{b}	6.06±0.89 ^{b}	5.52±0.54^{b}	12.28±2.46 ^{b}
48.4(mg/kgbw)	14.92±4.69 ^{a}	10.38±2.81^{b}	7.12±1.52^{cb}	5.36±0.61^{cb}	4.84±0.49 ^{b}	4.60±0.41^{cb}	10.12±2.71^{cb}
93.5(mg/kgbw)	14.16±1.98 ^{a}	8.90±2.15^{bc}	6.88±2.23^{cb}	5.34±0.83^{c}	5.02±0.82 ^{b}	4.38±0.65^{c}	9.32±0.61 ^{cbd}
180.9(mg/kgbw)	14.00±2.50 ^{a}	9.88±2.41^{b}	7.26±1.45^{cb}	5.78±1.00^{cb}	5.22±0.92 ^{b}	4.54±0.60^{cb}	8.90±1.53 ^{cd}
350(mg/kgbw)	14.18±1.02 ^{a}	9.08±1.04^{bc}	7.16±0.65^{cb}	5.72±0.79cb	5.02±0.30 ^{b}	4.20±0.23^{c}	6.74±0.43^{ed}

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

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

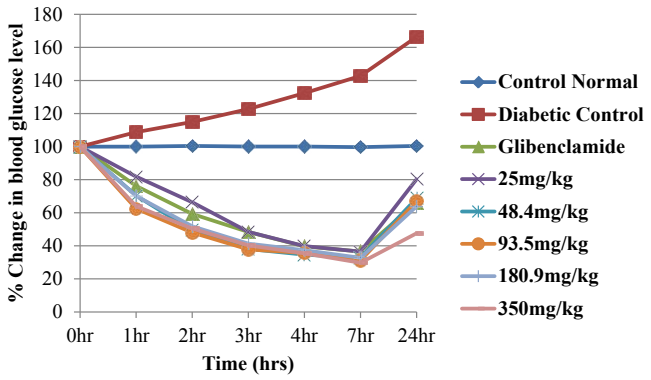

Figure 2: Mean percentage change in blood glucose levels of *Lippia javanica* orally administered in alloxan induced diabetic mice.

Phytochemicals	*Lippia javanica*
Alkaloids	+
Sterols	+
Terpenoids	+
Saponins	+
Tannins	+
Flavanoids	+
Free and Bound Anthraquinones	-

Key: Present phytochemicals are denoted by (+) sign, absent phytochemicals are denoted by (-) sign.

Table 3: Qualitative phytochemical screening of aqueous leaf extract of Croton macrostachyus.

that they facilitate insulin release through conversion of pro-insulin to insulin [34]. It has been shown that the flavonoid fraction from *Pterocarpus marsupiu* could cause pancreatic beta cell regranulation. Flavonoid glycosides such as strictinin, isostrictinin and pedunculagin are the effective constituents of *Psidium guajava*, which have been used in clinical treatment of diabetes due to improved sensitivity of insulin [35]. At 50-150 mg/kg flavonoids isolated from leaf of *Ipomoea batatas*, reduced blood glucose level and lipid parameters in alloxan induced diabetic mice [36].

The aqueous leaf plant extracts of *Lippia javanica* contained alkaloids, which are known to have blood glucose lowering activity. Alkaloid fraction from *C. decidua* showed hypoglycemic potential in mice [37]. Alkaloids berberine and tetrandine have been reported to demonstrate antioxidant activity responsible for various biological activities associated with this plant including antidiabetic activity. The alkaloids l-ephedrine of *Ephedra distachya* herbs have shown hypoglycemic effect in diabetic mice due to restoration and regeneration of atrophied pancreatic islets that induces the secretion of insulin [1,32].

The aqueous leaf extract of *Lippia javanica* contained saponins. Saponins have been shown to have antihyperglycemic activity. For instance, ginseng and its saponins have been shown to lower blood glucose in alloxan-treated, genetically diabetic, and normal mice [38]. The intraperitoneal administration of 100, 200 mg/kg body weight of the leaves of *Acanthopanax senticosus* in alloxan induced diabetic mice demostrated the presence of saponins that decreased experimental hyperglycemia and adrenaline without affecting the levels of blood sugar in untreated mice. The normal glycemic rabbits at a dosage of 50 mg/kg indicated that a saponin-component reduced glycemia after 1, 2, 3 and 6 hour after an oral administration of *Citrullus colocynthis*. Saponin extracts graded at 10, 15 and 20 mg/kg caused a marked hypoglycemic effect in alloxan-induced diabetic rabbits [39]. In elderly patients with hyperglycemia, saponins were shown to have reduced serum glucose [40].

The aqueous leaf extract of *Lippia javanica* contained tannins that are known to contain antidiabetic activity. Kumari et al. [41] demonstrated that *Acacia nilotica* contained 50% tannins that that exhibited hypoglycemic activity in diabetic subjects. Tannins are polyphenols from multiple species classified into two broad groups; hydrolysable tannins and condensed tannins. In clinical terms, all forms of tannins may participate in the management of glucose level in blood. Tannin has been shown to stimulate the receptor cells to utilize carbohydrate [41].

The aqueous leaf extracts of *Lippia javanica* contains terpenoids which are heart-friendly antidiabetic phytoconstituents [1]. Terpenoids have shown to reduce diastolic blood pressure and lower the sugar level in blood in hypertensive and diabetic patients respectively [1]. Terpenoids also improves the skin tone, increases the concentration of antioxidants in wounds, and restore inflamed tissues by increasing blood supply [1]. Terpenoids also improve lung function. The leaves and seeds of *S. spectabilis* are used in the treatment of diabetes due to the presence of terpenoids [9].

The aqueous leaf extracts of *Lippia javanica,* contained steroids that make them a good source of steroidal compounds which are potent precursors for the synthesis of sex hormones [42].

Conclusion

The aqueous leaf extracts of *Lippia javanica* had antidiabetic activity and revealed the presence of vital phytochemicals which possess antidiabetic activities. The intraperitoneal route was more effective in reducing the blood glucose levels than the oral route. The antidiabetic activity of the studied plant may have resulted from its phytochemical constituents. The study therefore, confirmed the traditional use of these herbs and established their efficacy data that can guide proper use of these plants in the management of diabetes mellitus. Consideration should be made to carry out the same studies using higher animals or subject the plant to organic solvent extraction and compare activities of both aqueous and organic fractions.

References

1. Piero NM, Kimuni NS, Ngeranwa NJ, Orinda OG. Njagi MJ, et al. (2015) Antidiabetic and Safety of Lantana rhodesiensis in Alloxan Induced Diabetic Rats. J Develop Drugs 4: 129.
2. World Health Organization & International Diabetes Federation (2004) Diabetes action now.
3. Rowley WR, Bezold C (2012) Creating public awareness: state 2025 diabetes forecasts. Popul Health Manag 15: 194-200.
4. American Diabetes Association (2012) Diagnosis and Classification of Diabetes Mellitus. Diabetes care 35: S64- S71.
5. World Health Organization (2015) Diabetes Fact Sheet NO.312.
6. Piero MN, Njagi JM, Kibiti CM, Ngeranwa JJN, Njagi ENM (2012) Metabolic Complications of Diabetes Mellitus: A Review. South Asian Journal of Biological Science 2: 37- 49.
7. Kerner W1, Brückel J2; German Diabetes Association (2014) Definition, classification and diagnosis of diabetes mellitus. Exp Clin Endocrinol Diabetes 122: 384-386.
8. Murugi NJ, Piero NM, Mwiti KC, Ngeranwa JJN, Njagi ENM, et al. (2012) Hypoglycemic effects of Caesalpinia volkensii on alloxan-induced diabetic mice.

9. Mukundi MJ, Piero NM, Mwaniki NE, Murugi NJ, Daniel AS, et al. (2015) Antidiabetic Effects of Aqueous Leaf Extracts of Acacia nilotica in Alloxan Induced Diabetic Mice. J Diabetes Metab 6: 568.
10. Musila W, Kisangau D, Muema J (2002) Conservation Status and Use of Medicinal Plants by Traditional Medical Practitioners in Machakos District, Kenya. National Museums of Kenya.
11. Piero NM, Njagi MJ, Kibiti MC, Ngeranwa JJN, Njagi NM, et al. (2012) Herbal management of diabetes mellitus: A rapidly expanding research avenue.
12. Surendran S, Eswaran MB, Vijayakumar M, Rao CV (2011) In vitro and in vivo hepatoprotective activity of Cissampelos pareira against carbon-tetrachloride induced hepatic damage. Indian J Exp Biol 49: 939-945.
13. Mahmood A, Mahmood A, Qureshi RA (2012) Antimicrobial activities of three species of family mimosaceae. Pak J Pharm Sci 25: 203-206.
14. Pandey A, Tripathi P, Pandey R, Srivatava R, Goswami S (2011) Alternative therapies useful in the management of diabetes: A systematic review. J Pharm Bioallied Sci 3: 504-512.
15. Karau GM, Njagi ENM, Machocho AK, Wangai LN, Kamau PN (2012) Hypoglycemic Activity of Aqueous and Ethylacetate Leaf And Stem Bark Extracts of Pappea capensis in Alloxan-Induced Diabetic BALB/C Mice. British Journal of Pharmacology and Toxicology 3: 251-258.
16. Szkudelski T (2001) The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiol Res 50: 537-546.
17. Hosseinzadeh H, Younesi HM (2002) Antinociceptive and anti-inflammatory effects of Crocus sativus L. stigma and petal extracts in mice. BMC Pharmacol 2: 7.
18. Houghton PJ, Raman A (1998) Laboratory Handbook for the Fractionation of Natural extracts. Chapman and Hall 154-187.
19. Ayodhya S, Kusum S, Anjali S (2010) Hypoglycaemic activity of different extracts of various herbal plants Singh. International Journal Ayurveda Resources Pharmaceutical 1: 212-224.
20. Shafighi M, Amjad L (2013) Evaluation of Antioxidant Activity, Phenolic and Flavonoid Content in Punica granatum var. Isfahan Malas Flowers. International Journal of Agriculture and Crop Sciences 5-1133.
21. Obatomi DK, Bikomo EO, Temple VJ (1994) Anti-diabetic properties of the African mistletoe in streptozotocin-induced diabetic rats. J Ethnopharmacol 43: 13-17.
22. Ngugi MP, Njagi JM, Kibiti CM, Miriti PM (2012) Pharmacological Management of Diabetes Mellitus.
23. Esmaeili MA, Yazdanparast R (2004) Hypoglycaemic effect of Teucrium polium: studies with rat pancreatic islets. J Ethnopharmacol 95: 27-30.
24. Sharma SB, Nasir A, Prabhu KM, Murthy PS (2006) Antihyperglycemic effect of the fruit-pulp of Eugenia jambolana in experimental diabetes mellitus. J Ethnopharmacol 104: 367-373.
25. Lombardo YB, Chicco AG (2006) Effects of dietary polyunsaturated n-3 fatty acids on dyslipidemia and insulin resistance in rodents and humans. A review. J Nutr Biochem 17: 1-13.
26. Kaliwal BB, Sanakal RD, Shetti AA (2012) Antidiabetic effect of ethanolic leaf extract of Phyllanthus amarus in alloxan induced diabetic mice. Asian journal of plant science and research 2: 11-15
27. Syed-Ikmal SIQ, Zaman-Huri H, Vethakkan SR, Wan Ahmad WA (2013) Potential biomarkers of insulin resistance and atherosclerosis in type 2 diabetes mellitus patients with coronary artery disease. International journal of endocrinology.
28. Njagi JM, Ngugi MP, Kibiti CM, Ngeranwa J, Njue W, et al. (2015) Hypoglycemic effect of Helichrysum odoratissimum in alloxan induced diabetic mice.
29. Xu X, Shan B, Liao CH, Xie JH, Wen PW, et al. (2015) Anti-diabetic properties of Momordica charantia L. polysaccharide in alloxan-induced diabetic mice. Int J Biol Macromol 81: 538-543.
30. Meezan E, Meezan EM, Jones K, Moore R, Barnes S, et al. (2005) Contrasting effects of puerarin and daidzin on glucose homeostasis in mice. J Agric Food Chem 53: 8760-8767.
31. Shafighi M, Amjad L (2013) Evaluation of Antioxidant Activity, Phenolic and Flavonoid Content in Punica granatum var. Isfahan Malas Flowers. International Journal of Agriculture and Crop Sciences 5: 1133.
32. Elliot M, Chithan K, Theoharis CT (2000) The effects of plant flavanoids on mammalian cells: Implications for inflammation, heart disease and cancer. Pharmacol Rev 52: 673-751.
33. Modak M, Dixit P, Londhe J, Ghaskadbi S, Devasagayam TP (2007) Indian herbs and herbal drugs used for the treatment of diabetes. J Clin Biochem Nutr 40: 163-173.
34. Chauhan A, Sharma PK, Srivastava P, Kumar N, Duehe R (2010) Plants having potential antidiabetic activity: a review. Der Pharm Lett 3: 369-387.
35. Li F, Li Q, GAO D, Peng Y (2009) The optimal extraction parameters and anti-diabetic activity of flavonoids from Ipomoea batatas leaf. Afr J Tradit Complement Altern Med 2: 195-202.
36. Shukla S, Mehta A, Mehta P, Bajpai VK (2012) Antioxidant ability and total phenolic content of aqueous leaf extract of Stevia rebaudiana Bert. Exp Toxicol Pathol 64: 807-811.
37. Sharma B, Salunke R, Balomajumder C, Daniel S, Roy P (2010) Anti-diabetic potential of alkaloid rich fraction from Capparis decidua on diabetic mice. J Ethnopharmacol 127: 457-462.
38. Kimura M, Suzuki J (1985) The pharmacological role of ginseng in the blend effect of traditional Chinese medicines in hyperglycemia. Advances of Chinese Medicinal Materials Research, World Scientific, Singapore.
39. Abdel-Hassan IA, Abdel-Bary JA, Tariq MS (2000) The hypoglycemic and anti-hyperglycemic effect of Citrullus colocynthis fruit aqueous extract in normal and alloxan diabetic rabbits. J Ethnopharmacol 71: 325-330.
40. Chen KJ, Zhang WP (1987) Advances on antiageing herbal medicines in China. Ab Chin Med 1: 309-330.
41. Kumari M, Jain S, Dave R (2014) Babul (Acacia nilotica), A. potential source of tannin and its suitability in management of type II diabetes. Nutrition & Food Science 2: 122-124.
42. Piero NM, Njagi NME, Kimuni NS, Orinda OG, Njagi JM, et al. (2015) In Vivo Antidiabetic Activity and Safety In Rats of Cissampelos pareira Traditionally Used In The Management of Diabetes Mellitus In Embu County, Kenya. J Drug Metab Toxicol 6: 184.