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/kgbw, 48.4 mg/kgbw, 93.5 mg/kgbw, 180.9 mg/kgbw and 350 mg/kgbw. 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. Chronic hyperglycemia 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 3.5-6.7 mmol/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 80% of the population of most developing countries especially in Africa [10]. This is envisaged by strained economic situations of most...
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, Papea 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 accredited 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 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 0.1ml 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 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 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 25.4 mg/kg body weight in 0.1 ml physiological saline; Group VI consisted of alloxan induced diabetic mice either intraperitoneally or orally administered with 88.4 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 (Hypoguard, 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 methods [17,18] (Table 3).

**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 intraperitoneal 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

<table>
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<tr>
<th>TREATMENT</th>
<th>BLOOD GLUCOSE LEVELS AT VARYING TIMES (mmoles/L)</th>
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<tr>
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<tr>
<td>Control/Saline</td>
<td>5.26±0.05&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic/Saline</td>
<td>14.28±0.93&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic/Insulin</td>
<td>14.66±1.44&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>25(mg/kgbw)</td>
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<td>48.4(mg/kgbw)</td>
<td>14.88±2.65&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>14.74±1.73&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>180.9(mg/kgbw)</td>
<td>14.36±3.28&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>350(mg/kgbw)</td>
<td>13.36±0.80&lt;sup&gt;a&lt;/sup&gt;</td>
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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 1:** Effects of intraperitoneally administered aqueous leaf extracts of *Lippia javanica* on blood glucose levels in alloxan induced diabetic mice.
The aqueous leaf extracts of *Lippia javanica* showed insulinomimetic 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 been 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].

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- to 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 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 demonstrated insulinomimetic properties [33]. Epicatechin and its active principles have demonstrated in *vitro* and *in vivo* hypoglycemic effect of *Lippia javanica* in alloxan induced diabetic mice. The aqueous leave 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 hepatic metabolism and renal clearance. The antihyperglycemic 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].
50 mg/kg indicated that a saponin-component reduced glycemia after sugar in untreated mice. The normal glycemic rabbits at a dosage of hyperglycemia and adrenaline without affecting the levels of blood demonstrated the presence of saponins that decreased experimental Acanthopanax senticosus the leaves of glucose in alloxan-treated, genetically diabetic, and normal mice [38].

Saponins regeneration of atrophied pancreatic islets that induces the secretion hypoglycemic effect in diabetic mice due to restoration and herbs have shown the presence of terpenoids [9]. The alkaloids l-ephedrine of Ephedra distachya activities associated with this plant including antidiabetic activity. [37]. Alkaloids berberine and tetrandine have been reported to show hypoglycemic potential in diabetic mice [36]. At 50-150 mg/kg flavonoids isolated from leaf of Psidium guajava, which have been used are the effective constituents of Pterocarpus marsupium. Flavonoid glycosides such as strictinin, isostrictinin and pedunculagin could cause pancreatic beta cell regranulation.

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Lippia javanica</th>
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<tr>
<td>Alkaloids</td>
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<tr>
<td>Sterols</td>
<td>+</td>
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<tr>
<td>Terpenoids</td>
<td>+</td>
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<tr>
<td>Saponins</td>
<td>+</td>
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<td>Tannins</td>
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<td>Flavanoids</td>
<td>+</td>
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<tr>
<td>Free and Bound Anthraquinones</td>
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Key: Present phytochemicals are denoted by (+) sign, absent phytochemicals are denoted by (-) sign.

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 distachyca herbes 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 demonstrated 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**


