In Vivo Antidiabetic Effect of Aqueous Leaf Extract of Azadirachta indica, A. juss in Alloxan Induced Diabetic Mice

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Abstract

There is growing interest in the potential of plant remedies to treat and manage many diseases owing to the adverse side effects, unavailability and unaffordability associated with the conventional therapy. Among the traditional plants that has been prescribed for clinical use for many ailments including diabetes mellitus is Azadirachta indica. Their continued use is largely based on their long-term therapeutic effects although this has not been authenticated scientifically. This study therefore, aims to evaluate the in vivo hypoglycemic effect of aqueous leaf extracts of A. indica in alloxan-induced white male albino mice. The blood glucose lowering effect of the extract was intraperitoneally and orally bioscreened in diabetic mice in serial dilutions of the extract at 25 mg/kgbwt, 48.4 mg/kgbwt, 93.5 mg/kgbwt, 180.9 mg/kgbwt and 350 mg/kgbwt. Qualitative analysis of phytochemicals was done using standard procedures. In both routes, the extract lowered blood glucose at all dosages in a dose independent manner. The extracts contained flavonoids, tannins, sterols, saponins, anthraquinones and alkaloids. The antidiabetic activity may be attributable to these phytochemicals present in the plant extract. The study confirms the traditional use of this plant part in the treatment of diabetes mellitus. However, organic solvent extraction of the leaves of this plant should be done to compare effects of both organic and aqueous fractions.

Keywords: Azadirachta indica; Antidiabetic; Hyperglycemia; In vivo; Phytochemical; Insulin; Glibenclamide

Introduction

Diabetes mellitus is a metabolic disorder characterized by chronic elevated blood glucose levels that could lead to mortality and morbidity [1]. The pathophysiological processes involved in etiology of this disorder may be due to abnormalities that result in insulin resistance or molecular mimicry that results in autoimmune reaction and adverse side effects, unavailability and unaffordability associated with the conventional therapy. Among the traditional plants that has been prescribed for clinical use for many ailments including diabetes mellitus is Azadirachta indica. Their continued use is largely based on their long-term therapeutic effects although this has not been authenticated scientifically. This study therefore, aims to evaluate the in vivo hypoglycemic effect of aqueous leaf extracts of A. indica in alloxan-induced white male albino mice. The blood glucose lowering effect of the extract was intraperitoneally and orally bioscreened in diabetic mice in serial dilutions of the extract at 25 mg/kgbwt, 48.4 mg/kgbwt, 93.5 mg/kgbwt, 180.9 mg/kgbwt and 350 mg/kgbwt. Qualitative analysis of phytochemicals was done using standard procedures. In both routes, the extract lowered blood glucose at all dosages in a dose independent manner. The extracts contained flavonoids, tannins, sterols, saponins, anthraquinones and alkaloids. The antidiabetic activity may be attributable to these phytochemicals present in the plant extract. The study confirms the traditional use of this plant part in the treatment of diabetes mellitus. However, organic solvent extraction of the leaves of this plant should be done to compare effects of both organic and aqueous fractions.

Materials and Methods

Collection of medicinal plant

A. indica leaves were collected from Kijauri village Nyamira County, Kenya. A traditional medical practitioner provided ethnobotanical information of the plant collected. Botanical identity of the plant was authenticated by a taxonomist and a voucher specimen deposited at the National Museums of Kenya Herbarium, Nairobi.
After collection, the leaves were dried at room temperature under a shade. The dry leaves were then ground by use of an electric mill into fine powder. The powder was kept in closed, dry plastic air tight bags at room temperature.

**Processing and extraction of the plant**

One hundred grams of the powdered plant material was weighed into a conical flask and extracted in 1 liter distilled water at 60°C for 6 hours. It was left to cool, decanted then filtered under vacuum pump. The filtrate was then lyophilized for 72 hours using a Modulyo-freeze drier (Edward England). The granules were then weighed and refrigerated at -20°C in an airtight container until used for bioassay [2,3].

**Experimental animals**

Male Swiss White Albino mice weighed 21-25 g with a mean weight of 23 g were used in this study. The mice were housed in polypropylene cages, maintained under standard laboratory conditions of 12 hour light and dark sequence, at temperature of 25°C. The mice fed on rodent pellets and water ad libitum. The Principles of Laboratory Animal Care were followed.

**Induction of hyperglycemia**

Diabetes was induced experimentally by intraperitoneal administration of a freshly prepared 10% alloxan monohydrate obtained from Sigma Aldrich (Steinhein, Switzerland) [21], at 186.9 mg/kg body weight [2]. The animals were fasted for 8-12 hours, but allowed free access to water prior to use in bioassay. Blood glucose level was measured using a glucometer forty-eight hours after alloxan administration. Mice with blood glucose levels above 200 mg/dL (>11.1 mmol/L), were considered diabetic and suitable for use in the study [2].

**Experimental design**

The activity of aqueous leaf extract of *A. indica* was studied in alloxan-induced diabetic mice. The mice were divided into two portions. The first portion was used for anti-diabetic assay through intraperitoneal administration of aqueous plant extract. It consisted of the following groups of five mice each; Group I consisted of normal un-manipulated mice (the reference group of the experiment treated with the vehicle alone, 0.1 ml); Group II consisted of alloxan-diabetic negative control mice (treated with 0.1 ml vehicle alone); Group III consisted of alloxan-diabetic positive control mice treated with insulin (at 1 IU/kg body weight); Group IV through VIII consisted of alloxan-diabetic experimental mice treated with 25, 48.4, 93.5, 180.9 and 350 mg/kg body weight of aqueous plant extract. The second portion was used for antidiabetic assay through oral administration of aqueous plant extracts. The experimental design was similar to the first portion except that the reference drug used was glibenclamide (at 3 mg/kg body weight).

**Blood sampling and in vivo hypoglycemic assays**

The blood was collected by gently “milking” the tail from the body towards the tip after sterilizing the tail with 10% alcohol and then nipping to initiate bleeding. Blood sampling was repeated after 1, 2, 3, 4, 7 and 24 hours and blood glucose levels determined using a glucose analyzer model (Hypogaurd, Woodbridge, England). The tips of tail were sterilized by swabbing with 70% ethanol after the operation.

**Phytochemical screening**

Phytochemical screening of the *A. indica* was done qualitatively to determine the class of secondary metabolites present which included, saponins, tannins, anthroquinones, alkaloids, terpenoids, flavonoids and sterols using standard procedures [22,23].

**Data analysis**

The data collected was entered in the Microsoft Excel Spread Sheet where it was cleaned and then transferred to SAS statistical software version 9.1.3 for analysis. The results of statistical analysis were expressed as Mean ± Standard Deviation (SD). One-way ANOVA and post-ANOVA (Tukey's post hoc test) were used to compare the means of untreated group of normal mice with diabetic groups of mice treated with saline, conventional drug and plant extract at various dosages. Statistical significance was set at p ≤ 0.05.

**Results**

**In vivo antidiabetic effect of *Azadirachta indica* (*A. juss*) in alloxan induced diabetic mice**

*Azadirachta indica* aqueous leaf extract yielded 8% brown powder. The intraperitoneal administration of *A. indica* decreased the blood glucose levels at all the five doses of 25, 48.4, 93.5, 180.9 and 350 mg/kg body weight in a dose independent manner (Table 1). This occurred in three phases, whereby in the first hour, the extract caused a steep decline in blood glucose levels, followed by a steady decline from second to the seventh hour. A gradual increase was then observed in the twenty fourth hour. In the first hour, the extract decreased blood glucose levels to 59.1%, 70.6%, 56.8%, 61.2% and 71.4% 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 were lowered to 50.3% 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 28.2%, 34.4%, 25.6%, 24.3% and 30.4%, respectively, compared to insulin treated diabetic mice whose blood sugar levels were decreased to 35.6% within the same hour (Figure 1).

![Figure 1: Mean percentage change in blood glucose levels of *Azadirachta indica* intraperitoneally administered in alloxan induced diabetic mice.](image-url)
mice but not in a dose related manner.

pancreatic beta cells through inhibition of glucokinase enzyme,

Discussion

[21,28,29].

monohydrate induces Type I diabetes in experimental animals [24,25].

therefore,

40.2%, 48.2%, 47.7%, 44.4%, and 53.1% respectively for the
glibenclamide and insulin treated groups. Glibenclamide is an orally

seventh hours at all the

blood glucose levels (5 mg/dl to 25 mg/dl) relative to the normal

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

Upon oral administration, the aqueous leaf extracts of A. indica lowered blood glucose levels appreciably from the first hour to the seventh hours at all the five doses of 25, 48.4, 93.5, 180.9 and 350 mg/kg body weight but not in a dose dependent manner (Table 2). By the second hour the extract had lowered the blood glucose levels to 40.2%, 48.2%, 47.7%, 44.4%, and 53.1% respectively for the five doses, compared to 56.6% for the conventional oral drug, glibenclamide (Figure 2).

Discussion

The alloxan-induced diabetic mice had a four to five fold elevation in blood glucose levels (5 mg/dl to 25 mg/dl) relative to the normal control mice. Studies show that chemical induction of diabetes by intraperitoneal administration of a diabetogenic agent, alloxan monohydrate induces Type I diabetes in experimental animals [24,25]. Alloxan monohydrate is derived from urea and induces diabetes by selective necrosis of pancreatic beta-cells of Langerhans [26]. This therefore, affects endogenous insulin synthesis and release making it biologically unavailable or insufficient and thus results in hyperglycemia [27]. The toxic alloxan confers its toxicological effect on pancreatic beta cells through inhibition of glucokinase enzyme, generation of free radicals, disturbances in intracellular calcium homeostasis and oxidation of essential sulphydryl (-SH groups) [21,28,29]. The underlying mechanism of action involves the selective uptake of the compound due to its structural similarity to glucose as well as highly efficient uptake mechanism of the pancreatic beta-cells [30].

Intraperitoneal administration of aqueous leaf extract of A. indica at 25, 48.4, 93.5, 180.9, and 350 mg/kg after 1, 2, 3, 4, 7 and 24 hours lowered the blood glucose levels in alloxan induced diabetic mice but not in a dose related manner. The plant extract at all doses and in both routes showed a comparable activity with the glibenclamide and insulin treated groups. Glibenclamide is an orally administered standard drug that stimulates insulin secretion from beta cells of islets of Langerhans thereby reducing the glucose concentration. It promotes insulin secretion by closure of potassium-ATP channels, membrane depolarization and stimulation of calcium ion influx, an initial key step in insulin secretion [31].

Intraperitoneal administration of insulin as a standard anti diabetic drug enhances glucose uptake across the cell membrane by ATP-dependent translocation of glucose transporter GLUT4 to the plasma membrane [32]. Insulin causes cells in the liver, skeletal muscles, and fat tissue to absorb glucose from the blood. In the liver and skeletal muscles, glucose is stored as glycogen, and in fat cells (adipocytes) it is stored as triglycerides [32].

Figure 2: Mean percentage change in blood glucose levels of Azadirachta indica orally administered in alloxan induced diabetic mice.
The presence of phytochemical constituents including, Azardirachta indica. Glucose uptake and utilization [33,34].

Qualitative phytochemical composition of Azardirachta indica A. Juss

As depicted in Table 3, A. indica contained free and bound anthraquinones, saponins alkaloids, tannins, terpenoids, sterols and flavonoids.

| Phytochemicals       | Azardirachta indica |
|----------------------|---------------------|
| Alkaloids            | *                   |
| Sterols              | *                   |
| Terpenoids           | *                   |
| Saponins             | *                   |
| Tannins              | *                   |
| Flavonoids           | *                   |
| Free and Bound Anthraquinones | *     |

Key: Present phytochemicals are denoted by (+) sign

Alkaloids that have been associated with antidiabetic activity [40]. As reported by [41], flavonoids enhances lipogenesis and glucose transport in the adipocytes hence lowering blood sugar [40]. The alkaloids promotes the regeneration of pancreas islets thereby restoring insulin secretion [40]. Tannins and saponins have also been shown to have hypoglycemic activity [42] and [43]. The plants contained terpenoids which are heart-friendly because they help to reduce diastolic blood pressure and lower the sugar level in blood [44]. Anthraquinones which have earlier been reported to lower blood glucose are used also in the treatment of peripheral neuropathy [43].

The observed dose independent hypoglycemic action of the plant extract in this study suggests 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 [34]. A gradual increase of blood sugar levels observed from 7th, 24th hrs following the oral and intraperitoneal administration of A. indica may have been due to the extract having a short half-life or it might have been prone to fast hepatic metabolism and renal clearance [45].

Conclusion

The results from this study indicated that Azadirachta indica had hypoglycemic effects in alloxan induced diabetic mice, thus scientifically verifying its folkloric use in the management of diabetes mellitus. These actions were exhibited due to cumulative effect of phytocomponents present in the extract including free and bound anthraquinones, alkaloids, tannins, terpenoids, flavonoids, saponins and sterols. However, further investigation should be done in order to isolate the constituents responsible for the antidiabetic effect of this plant through bioassay guided fractionation. Moreover, the organic solvent extraction for this plant should also be done to compare the antidiabetic activities of both aqueous and organic fractions.

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Blood Glucose Levels at Varying Times (mmoles/L)

| Treatment | 0 hr       | 1 hr       | 2 hr       | 3 hr       | 4 hr       | 7 hr       | 24 hr      |
|-----------|------------|------------|------------|------------|------------|------------|------------|
| Control/Saline | 4.94 ± 0.18b | 5.04 ± 0.21c | 5.06 ± 0.11c | 5.14 ± 0.09b | 5.12 ± 0.08bd | 5.12 ± 0.08bd | 5.10 ± 0.10d  |
| Diabetic/Saline | 14.92 ± 2.16a | 17.54 ± 2.39a | 20.02 ± 2.59a | 22.48 ± 2.61a | 24.42 ± 2.15a | 25.76 ± 2.15a | 27.30 ± 1.63a  |
| Diabetic/ Glen | 14.44 ± 3.08b | 10.88 ± 2.94b | 8.12 ± 1.58b | 6.70 ± 1.18b | 5.14 ± 0.55b | 4.98 ± 0.34b | 7.14 ± 1.11b   |
| 25 (mg/kgbw)  | 14.56 ± 0.57a | 9.68 ± 1.19b | 5.86 ± 0.79ib | 3.88 ± 0.80c | 3.70 ± 0.46ib | 3.36 ± 0.40ibd | 6.44 ± 0.95ib   |
| 48.4 (mg/kgbw) | 14.18 ± 2.33a | 9.86 ± 1.11ib | 6.76 ± 0.73ib | 4.28 ± 0.43ib | 4.30 ± 0.45ib | 4.22 ± 0.79ib | 7.96 ± 1.98ib   |
| 93.5 (mg/kgbw) | 13.94 ± 0.74a | 9.78 ± 1.03ib | 6.68 ± 1.54ib | 3.40 ± 0.79c | 3.06 ± 0.86ib | 2.80 ± 0.75ib | 6.44 ± 1.61ib   |
| 180.9 (mg/kgbw) | 15.20 ± 1.61a | 10.16 ± 1.36b | 6.74 ± 1.24b | 4.24 ± 0.64b | 3.84 ± 0.74b | 3.18 ± 0.80b | 7.38 ± 1.60b   |
| 350 (mg/kgbw)  | 14.88 ± 2.84a | 10.12 ± 0.97b | 7.48 ± 1.44b | 5.44 ± 2.20b | 4.32 ± 1.25b | 3.12 ± 0.70b | 7.40 ± 1.73b   |

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