Bioscreening for Hypoglycemic Potential of Aqueous Extract of *Tridax Procumbens* in Mice Model

Njangiru, I.K. 1, Njagi, E.M.N2, Ngeranwa, J.J.N3

1Department of Chemistry and Biochemistry, Laikipia University, P.O Box 1100, Nyahururu.
2Department of Biochemistry, Microbiology and Biotechnology, Kenyatta University, P.O Box 43844, Nyahururu
3Department of Biochemistry, Microbiology and Biotechnology, Kenyatta University, P.O Box 43844, Nyahururu

**Corresponding author:** I.K Njangiru, Department of Chemistry and Biochemistry, School of Science and Applied Technology, Laikipia University. P.O Box 1100, Nyahururu, Kenya. Tel: +254723843393, Email: iknjangiru@gmail.com.

**Abstract:** *Tridax procumbens* has been used in the treatment of several ailments in Kenya, including diabetes mellitus. However, there is limited scientific validation of its efficacy. The aim of this study was to investigate, in vivo, hypoglycemic activity of *Tridax procumbens* in alloxan induced diabetic mice. Antidiabetic activity was assessed by administering orally and intraperitoneally aqueous plants extract at doses of 50, 100, 200 and 300mg/kg body weight and determining the levels of blood glucose after 0, 1, 2, 3, 4, 6 and 24 hours. The mineral composition of the aqueous plant extract was assayed, using Total Reflection X-ray Fluorescence system (TRXF), while the phytochemicals were profiled using standard procedures. The plant extract demonstrated a dose independent antidiabetic activity in mice model. The studied plant extract contained alkaloids, flavonoids, saponins, tannins, and total phenols at varying concentration. The mineral assay confirmed the presence of detectable levels of K, Ca, Ti, V, Mn, Fe, Ni, Cu, Zn and As. The aqueous extract of the studied plant was effective in lowering blood glucose levels.

**Keywords:** Diabetes mellitus; *Tridax procumbens*; Hypoglycemic activity; Aqueous extract.

**Introduction**

Diabetes mellitus is physiological disorder of the endocrine system, associated with excessive plasma glucose levels due to hormonal imbalances or lack of cellular responses to hormones. The disease remains a fundamental concern in public health today, since it’s linked with extended term of destruction, malfunction, and impairment of various organs, moreso the kidneys, nerves, eyes, heart, and the vascular system. Studies have shown a steady increase in the prevalence of diabetes mellitus globally. The rise has been propelled by the aging population in the developed countries, increase in obesity, and stressful life style [1]. The rate of increase has been estimated to be at 6% anually. There is an increase in prevalence with age, from about 0.2% in persons less than 17 years of age, to about 10% in population aged 65 years and above [2]. Report by World Health Organization indicated that approximately 150 million persons worldwide had diabetes mellitus by1995, and the number is expected to double by 2025 [3]. Estimates by International Diabetes Federation (IDF) showed that by 2007, about 246 million people had diabetes and is projected to increase to 380 million by the year 2025 [4]. Studies by the World Health Organization (WHO) suggest that 347 million people have been diagnosed with diabetes with 80% of them living in low and middle income countries [5]. The disease manifests numerous symptoms which may occur spontaneously. These include excessive thirst (polydipsia), frequent urination (polyuria), constant hunger (polyphagia), weight loss, blurred vision and fatigue. These symptoms are more pronounced in type I diabetes than in type II diabetes. Consequently, type II DM, may be diagnosed several years after onset [6].
Excessive thirst develops due to osmotic effects; significantly elevated levels of glucose (beyond the 'renal threshold') in the blood circulation is excreted by the kidneys, which needs water to transport it and results in increased fluid loss, which must be replenished. The lost body fluid volume will be replenished with water retained inside the body cells, resulting in dehydration [7]. Uncontrolled diabetes is linked with elevated susceptibility to infections such as skin sepsis (boils) or genital candidiasis, and complains of pruritus vulvae or balanitis [8]. Diabetics can, over time, experience nerve damage, though with some, the damage may have no symptoms. Estimates by WHO indicate that diabetic retinopathy, accounts for approximately 5% of the world prevalence of blindness, with projections of 15%-17% in developed countries [9].

Numerous blood test techniques are routinely used in diagnosis of diabetes mellitus. Repeated fasting plasma glucose greater than 126 mg/dL, is strongly suggestive of diabetes, with values from 100 to 126 mg/dL, been suggestive of impaired fasting glucose ([10]. Two postprandial tests with glucose levels of 200 mg/dL or higher at two hours are suggestive of diabetes. Glycosylated hemoglobin between 5.7% and 6.4% is indicative of elevated chances of diabetes. Levels ranging from 6.5% and above denote diabetic state. The conventional antidiabetic agents are either expensive or unavailable and may still exhibit adverse side effects which are linked with diabetes complications. Large population in developing countries has therefore embraced traditional and herbal medicines since they are locally available, cheap, and purported to be safe. Statistics by the World Health Organization (WHO) indicate that about 80% of the world populations rely on traditional medicine for their primary health care. [11]. Aqueous extract of *Tridax procumbens* has been reported to manage diabetes mellitus even though the claim has not been scientifically proven. Moreover, there is considerable evidence indicating that many of the plant products are toxic to humans, [12] hence every herbal product requires thorough testing. As many conventional drugs trace their origin from medicinal herbs, research on these plants may aid in discovery of new drugs with better efficacy and improved safety. Moreover, human activities are rendering many plants extinct, which make it crucial to study them, and advocate for their conservation. Therefore, this study was executed to determine in vivo hypoglycemic activity of aqueous plant extract of *Tridax procumbens* in alloxanized diabetic male Swiss albino mice.

### Materials and methods

#### Study site

This study was performed at the Department of Biochemistry, Microbiology and Biotechnology, School of Pure and Applied Sciences, Kenyatta University, situated about 23 km from Nairobi off Thika Road.

#### Collection and processing of plants materials

Whole plants of *T. procumbens* were collected from their natural habitat from Teret location, Njoro Division in Nakuru County, Kenya. The local identification of the plant, the exact locality where it grows, the part to be collected, time when activity is at peak and the method of preparation was provided by a Traditional Health Practitioner. The plant was validated by a taxonomist at the East African Herbarium, National Museum of Kenya.

#### Preparation of the plant sample

The plants samples were sliced into small pieces, dried at room temperature under a shade till completely dry. The dried plants materials were ground by a mechanical grinder into fine powder and sieved through a 40mm mesh sieve. The obtained powders were kept in air tight plastic bags. One hundred grams of each powdered plant sample was extracted in 1000ml of distilled water at 60°C for six hours. The extracts were decanted into a clean conical flask and filtered through a Whatman filter paper into another conical flask. The filtrates were then freeze dried and stored in a freezer at -20°C till time for bioassay.

#### Experimental animals

The study employed male Swiss albino mice (6-7 weeks old) weighing 25-29g. The animals were bred at the Department of Biochemistry, Microbiology and Biotechnology of Kenyatta University. The experimental mice were housed at room temperature with 12hours/12hours darkness photoperiod, and provided with rodent standard pellets and water *ad libitum*. The study was certified by the ethics committee for the care and use of Laboratory Animals of Kenyatta University, Kenya.

#### Experimental design

Hypoglycemic activity of the aqueous plant extracts was assayed in alloxan-induced diabetic mice. The mice were randomly divided into two categories. The first category was employed for hypoglycemic study through oral administration of the plant extract. It composed of the following groups of five mice each; Group I composed of normal mice...
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Data analysis

The data obtained was transferred to the Microsoft Excel Spread Sheet where it was cleaned and then transferred to Statistical Package for Social Sciences Software (SPSS) for statistical 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 normal saline, conventional drug and plant extract at various dosages. Statistical significance was set at p ≤ 0.05.

Results

Effects of orally and intraperitoneally administered whole aqueous plant extract of T. procumbens at four therapeutic doses in alloxan induced diabetic mice on blood glucose levels

Whole plant aqueous extract of T. procumbens yielded 5.89% (w/w) brown powder. Intraperitoneal administration of aqueous extracts of T. procumbens at four therapeutic doses in alloxan induced diabetic mice lowered blood glucose levels from the 1st hour to the 4th hour in a dose independent manner (Table 1; Figure 1).

Figure 1: Mean percentage change in blood glucose levels by aqueous extract of T. procumbens at four therapeutic test doses intraperitoneally administered in alloxan induced diabetic mice

During the 1st hour the four therapeutic doses of the aqueous extract had lowered blood glucose levels by 36%, 43%, 48%, and 51%, respectively, compared to insulin which had lowered blood sugar levels by 38% within the same hour. By the 4th hour the percent blood glucose reductions by the four therapeutic doses of the extract were 69%, 69%, 72% and 77%, respectively, compared to insulin.

Blood glucose assay

Blood used for glucose assay was obtained by sterilizing the tail (with 10% alcohol) prior to nipping the tail at the onset of the experiment and repeating the same after 1, 2, 3, 4, 6 and 24hrs. The glucometer model (HYPOGAURD, ENGLAND), was used to determine blood glucose levels.

Phytochemical screening

Phytochemical assay of T. procumbens was performed using standard procedures to quantitatively determine the levels of saponins, tannins, flavonoids, alkaloids and total phenols.

Induction of hyperglycemia

Diabetes was induced through intraperitoneal administration of freshly prepared 10% alloxan monohydrate at 186.9mg/kg body weight. Forty eight hours after administration of alloxan monohydrate, sugar levels were determined using a glucometer. Mice with sugar levels above 11.1mmol/litre were considered diabetic and appropriate for use in the bioassay. The animals were fasted for 8-12 hours prior to initiation of the experiment [13], though were allowed free access to water.

Blood glucose assay

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Phytochemical screening

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Group I comprised of normal mice (reference) administered with 0.1ml of normal saline; Group II comprised of diabetic mice dosed with 0.1ml of normal saline; Group III composed of diabetic mice (positive control) administered with Glibenclamide (reference drug at 3mg/kg body weight) in 0.1 ml normal saline; Group IV composed of diabetic experimental mice dosed with plant extract at 50mg/kg body weight in 0.1 ml normal saline; Group V composed of diabetic experimental mice administered with plant extract at 100mg/kg body weight in 0.1 ml normal saline; Group VI composed of diabetic experimental mice administered with plant extract at 200mg/kg body weight in 0.1 ml normal saline and Group VII composed of diabetic experimental mice, administered with plant extract at 300mg/kg body weight in 0.1 ml normal saline.

The second category was employed for hypoglycemic assay through intraperitoneal administration of the plant aqueous extract. A similar experimental design as that employed with the first group was used except that Insulin was used as a reference intraperitoneal drug (at 1 IU/kg body weight).

Phytochemical screening

Phytochemical assay of T. procumbens was performed using standard procedures to quantitatively determine the levels of saponins, tannins, flavonoids, alkaloids and total phenols.
which had lowered blood sugar levels by 72% by the same hour. By this hour, the four tested dose levels lowered blood glucose levels to normal and were as effective as insulin. By the 6th hour the four therapeutic doses of the extract had lowered blood glucose levels by 59%, 63%, 59% and 77%, respectively.

Oral administration of aqueous extracts of *T. procumbens* at four therapeutic doses in alloxan induced diabetic mice decreased the blood glucose levels in dose independent manner. During the 1st hour the percent reduction in the blood glucose levels by the four therapeutic doses were 29%, 35%, 38%, and 43%, respectively, compared to the Glibenclamide (reference drug) which lowered blood sugar levels by 31% within the same hour (Table 2; Figure 2).

By this hour however, the four tested doses did not lower blood glucose levels to normal. By the 4th hour, the percent blood glucose had reduced by 71%, 62%, 73% and 73% in the four tested doses, respectively, compared to Glibenclamide which lowered blood sugar levels by 71% by the same hour. By this hour, the four tested doses had lowered blood glucose levels to normal and were as effective as Glibenclamide. After the 6th hour, a steady increase in blood glucose level across all the test doses was recorded, that persisted through to the twenty-fourth hour.

A comparison of the effectiveness of oral and intraperitoneal routes of drug administration at the four test doses (50, 100, 200 and 300mg/kg body weight), showed that the two routes were generally, equally effective in lowering blood glucose level throughout the experimental period. However, significantly higher blood glucose lowering effect was observed, in the first hour, following intraperitoneal administration of the plant extract at 200mg/kg body weight when compared to oral route. By the 6th hour, intraperitoneal route was still more effective in lowering blood glucose levels following administration of the plant extract at 50 mg/kg body weight and 100mg/kg bodyweight (Table 3).

**Table 1: Effects of intraperitoneal administration of aqueous plant extract of *T. procumbens* at four therapeutic doses in alloxan induced diabetic mice on blood glucose levels**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Glucose Levels at Varying Times (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0hr</td>
</tr>
<tr>
<td>Normal/Saline</td>
<td>5.50±0.37 IA</td>
</tr>
<tr>
<td>Diabetic/Saline</td>
<td>20.14±2.20 ME</td>
</tr>
<tr>
<td>Insulin</td>
<td>18.48±1.43 HA</td>
</tr>
<tr>
<td>Extract dose (mg/kg body weight)</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>200</td>
</tr>
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<td></td>
<td>300</td>
</tr>
</tbody>
</table>

Values are expressed as Means ± SD for five animals per group. Means within respective columns followed by similar lower case letters are not significantly different at ρ≤0.05 by ANOVA and post ANOVA. Means along each row followed by similar upper case letters are not significantly different at ρ≤0.05 by ANOVA and post ANOVA.
Table 2: Effects of oral administration of aqueous plant extract of *T. procumbens* at four therapeutic doses in alloxan induced diabetic mice on blood glucose levels

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Glucose Levels at Varying Times (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0hr</td>
</tr>
<tr>
<td>Normal/Saline</td>
<td>5.00±0.57</td>
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<tr>
<td>Diabetic/Saline</td>
<td>19.52±2.64</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>22.22±1.13</td>
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<tr>
<td>Extract dose (mg/kg body weight)</td>
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<tr>
<td>50</td>
<td>17.18±0.58</td>
</tr>
<tr>
<td>100</td>
<td>16.98±2.18</td>
</tr>
<tr>
<td>200</td>
<td>21.98±2.18</td>
</tr>
<tr>
<td>300</td>
<td>16.10±1.26</td>
</tr>
</tbody>
</table>

Values are expressed as Means ± SD for five animals per group. Means within respective columns followed by similar lower case letters are not significantly different at p≤0.05 by ANOVA and post ANOVA. Means along each row followed by similar upper case letters are not significantly different at p≤0.05 by ANOVA and post ANOVA.

Table 4.6: Comparison of the activity of *T. procumbens* extract administered orally and intraperitoneally in alloxan induced diabetic mice

<table>
<thead>
<tr>
<th>Dose (mg/kg bw)</th>
<th>Route</th>
<th>Glucose Levels at Varying Times (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0hr</td>
</tr>
<tr>
<td>50</td>
<td>Oral</td>
<td>17.18±0.58</td>
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<tr>
<td></td>
<td>ip</td>
<td>17.48±4.72</td>
</tr>
<tr>
<td>100</td>
<td>Oral</td>
<td>16.98±2.18</td>
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<tr>
<td></td>
<td>ip</td>
<td>17.70±1.68</td>
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<tr>
<td>200</td>
<td>Oral</td>
<td>21.98±2.18</td>
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<td></td>
<td>ip</td>
<td>17.52±3.77</td>
</tr>
<tr>
<td>300</td>
<td>Oral</td>
<td>16.10±1.26</td>
</tr>
<tr>
<td></td>
<td>ip</td>
<td>18.18±1.37</td>
</tr>
</tbody>
</table>

Results are expressed as Mean± Standard Deviation (SD) for five animals per group; *p ≤0.05 is considered statistically significant when the means of oral and intraperitoneal groups are compared by T-test.

Quantitative estimation of phytochemicals present in *T. procumbens* aqueous plants extracts

The quantitative estimation of phytochemicals in the plants extract under study is shown in Table 3. Results indicate that the plant extract of *T. procumbens* contained alkaloids, total phenols, flavonoids, tannins and saponins at varying concentrations.

Table 3: Phytochemical composition of *Tridax procumbens* plants extract

<table>
<thead>
<tr>
<th>Phytochemical Content (mg/g)</th>
<th>Tannins</th>
<th>Total Phenols</th>
<th>Flavonoids</th>
<th>Saponins</th>
<th>Alkaloids</th>
</tr>
</thead>
<tbody>
<tr>
<td>32.16±0.03</td>
<td>45.15±0.70</td>
<td>319.91±0.40</td>
<td>71.96±3.39</td>
<td>97.00±3.11</td>
<td></td>
</tr>
</tbody>
</table>

Results are expressed as Mean± standard deviation (SD).

**Discussion**

The alloxan-induced diabetic mice showed a two- to five-fold rise in blood glucose (191.88mg/dL to 399.6mg/dL) compared to the normal control mice. Alloxan monohydrate destroys and diminishes the number of β-cells of the pancreas through the formation of free radicals such as like nitric oxide [14]. The aqueous plant extract of *T. procumbens*...
exhibited blood glucose lowering potential when administered orally and intraperitonially. The blood glucose lowering potential of these plants was in tandem with reported literature of other plants already researched. Ethanolic and water extracts of *Caesalpinia bonduc* exhibited hypoglycemic activity through enhancement of insulin secretion in isolated inslets, when administered in chronic type II diabetics [15]. The aqueous extract of *Tribulaks terrestris* significantly lowered the blood glucose levels in both normal and alloxan-induced diabetic mice, through enhanced secretion of serum insulin [16]. Several mechanisms of action have been suggested for these plant extracts. Some hypotheses link their effects on increase in insulin sensitivity, increased protective /inhibitory effect against insulinate, while other suggestions link their activity on enhancement of the synthesis, release, regeneration or revitalization of the pancreatic β-cells. Improved glucose homeostasis has also been proposed as a possible mechanism of action.

The blood glucose lowering effect of the plant extracts could be due the presence of the saponins, polyphenols, alkaloids, tannins, flavonoids and steroids [17]. The phytochemical profile of *T. procumbens* confirmed the presence of flavonoids, alkaloids, saponins, total phenols and tannins. Saponins-glycosides of triterpenes, steroids and alkaloids, have been shown to be antidiabetic. Triterpenoid glycosides have been reported from some studies to exhibit antidiabetic activity [18]-[19]. In other studies, saponins have been shown to lower blood glucose levels in elderly diabetic patients [20]. Ginseng containing saponins has been reported to exhibit blood glucose lowering effects in normal mice, genetically diabetic and alloxan-treated mice [21]. Tannins whether hydrolysable or condensed, may take part in management of serum glucose levels. Tannins aid in utilization of carbohydrate through stimulation of glucose receptor cells [22]. Flavanoids have been extensively employed in clinical management of diabetes. Myricetin a polyhydroxylated flavanol has portrayed insulinomimetic properties through stimulating the synthesis of fatty acids and triglycerides as well enhancing glucose transport in the adipocytes. The blood sugar lowering effect of the aqueous extracts of *T. procumbens* in a non-dose dependent manner may imply uptake of the active ingredients through saturable active transport, where a specific concentration saturation of the extract occurred resulting to the rest of extract being excreted [23], or it may also reflect maximum hypoglycemic activity at the lowest dose used (50mg/kg body weight).

**Conclusion**

The outcome of this study demonstrated that *Tridax procumbens* exhibited hypoglycemic effects in alloxanized diabetic mice, thus scientifically validating its folkloric use in the management of diabetes mellitus. The hypoglycemic activity was due to cumulative effect of secondary metabolites present in the plant extract including alkaloids, tannins, flavonoids, saponins and total phenols. However, further research should be executed aimed at isolating the bio-constituents responsible for the hypoglycemic effect of this plant through bioassay guided fractionation. Moreover, the antidiabetic activity of the organic solvent extraction of this plant should also be profiled in order to compare the activities of both aqueous and organic fractions.

**Acknowledgements**

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**References**


