Evaluation of Effect of Ethanolic Leaf Extract of *Mucuna pruriens* on Blood Glucose Levels in Alloxan-Induced Diabetic Wistar Rats

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**Abstract:** *Mucuna pruriens* is used extensively for its medicinal value for the treatment of diabetes mellitus in Nigeria. This study investigated the anti-diabetic activity of *Mucuna pruriens* on the blood glucose levels and the histopathology of the pancreas. To achieve this, acute toxicity studies of the plant extract was determined. Doses of 100, 200 and 400 mg/kg bw of the extract were administered orally daily for 21 days to alloxan-induced diabetic Wistar rats. Metformin was used as a standard anti-diabetic drug and given by gavage. The fasting blood glucose levels were determined at intervals of three days. Preminary phytochemical screening revealed the presence of flavonoids, saponin, tannins, cardiac glycosides, triterpenes and reducing sugars. The LD₅₀ of the extract of *Mucuna pruriens* was found to be 2154 mg/kg. The study showed that there was a significant reduction (p<0.05) in the fasting blood sugar levels in alloxan-induced diabetic treated groups that received 100, 200 and 400 mg/kg of the extract orally, with a maximum reduction (p<0.01) of 161.83±18.2, 153.67±13.8 and 133.83±10.4 recorded in the groups treated with 100, 200 and 400 mg/kg. The histopathological studies of the pancreas of diabetic animals revealed the degeneration of pancreatic Islet cells, but with the restoration of pancreatic Islet cells in the pancreas of diabetic group treated with various doses of the plant extract. The implications of the results obtained in the present study provide the scientific rationale for the use of *Mucuna pruriens* as an anti-diabetic agent.

**Key words:** Alloxan, blood glucose, diabetes mellitus, metformin, *Mucuna pruriens*, pancreas

**INTRODUCTION**

Diabetes mellitus (DM) currently is a major health problem of the world and is a chronic metabolic syndrome resulting from a variable interaction of hereditary and environmental factors and is characterized by abnormal insulin secretion or insulin receptor or post-receptor events, affecting metabolism involving carbohydrates, proteins and fats in addition to damaging liver, kidney and β-cell of pancreas (Baynes, 1991). The worldwide survey reported that the DM is affecting nearly 10% of the world’s population (Siddharth, 2001). The number of people suffering from the disease worldwide is increasing at an alarming rate with a projected 366 million people likely to be diabetic by the year 2030 as against 191 million estimated in 2000 (Wild et al., 2004), and has being projected as the World’s main disabler and killer in the next 25 years (Edwin et al., 2006). Diabetes mellitus is a major cause of disability and hospitalization and it results in significant financial burden (Vats et al., 2002). It is a serious illness with multiple complications and premature mortality, accounting for at least 10% of the total health care expenditure in many countries (King et al., 1998). In modern medicine, no satisfactory effective therapy is available to cure diabetes mellitus. Though insulin therapy is also used for the management of diabetes mellitus but there are several drawbacks like insulin resistance (Piedrola et al., 2001), anorexia nervosa, brain atrophy and fatty liver (Yaryura-Tobias et al., 2001) after chronic treatment. The limitations of the currently-available oral antidiabetic agents either in terms of efficacy or safety and coupled with emergence of the disease into global epidemic have encouraged a concerted effort to discover drugs that can manage diabetes more efficiently (Ranjan and Ramanujam, 2002). The use of medicinal plants in modern medicine suffers from the fact that though, hundreds of plants are used in the world to prevent or to cure diseases, scientific evidence in terms of modern medicine is lacking in most cases. However, today it is necessary to provide scientific proof as to whether it is justified to use a plant or its active principles (Singh et al., 2000). Ethnobotanical information indicates that more than 800 plants are used as traditional remedies for the treatment of diabetes (Pushparaj et al., 2000) but only a few have received scientific scrutiny. Among these plants is *Mucuna pruriens* which has been used in herbal medicine in many cultures. *Mucuna pruriens* (MP) belongs to the family **Fabaceae** and has been described as...
a multipurpose plant which is used extensively both for its nutritional and medicinal properties. All parts of *M. pruriens* possess valuable medicinal properties (Adepoju and Odubena, 2009). It is a twinning and tropical legume known as velvet bean and of common names such as: cowitch and velvet bean (English), Agbara (Igbo), Yerepe (Yoruba), Karara (Hausa), Bengal bean, Mauritius bean, itchy bean, Nescafe, and buffalo bean and many others. The roots are bitter, stimulants, purgative, and diuretic and many others. In history, *M. pruriens* has been used as an effective aphrodisiac (Amin et al., 1996). The seeds have been found to have antidepressant properties when consumed, and it has also shown to neuroprotective (Manyham et al., 2004). Its analgesic and anti-inflamatory activities have been reported (Adepoju and Odubena, 2009). And it has been studied for various activities like anti-neoplastic, anti-epileptic, anti-microbial (Sathiyanarayanan and Arulmozhi, 2007). A clinical study confirmed the efficacy of the seeds in the management of Parkinson’s disease by virtue of their L-DOPA content (Manyham et al., 2004). *M. pruriens* has been shown to increase testosteron levels (Amin et al., 1996), leading to deposition of protein in the muscles and increased muscle mass and strength (Bhasin et al., 1996). Its use as a fertility agent (in men) has been documented (Buckles, 1995). This study was aimed at evaluating the hypoglycemic effects of ethanolic leaf extract of *Mucuna pruriens* on blood glucose levels in Alloxan-induced diabetic Wistar rats and the histopathology of the pancreas.

**MATERIALS AND METHODS**

**Plant material:** A Sample of fresh leaves of *Mucuna pruriens* were collected from the Institute for Agricultural Research Agronomy farm, ABU Samaru, Zaria in the month of August, 2010. The plant was identified and authenticated by a taxonomist, Mallam M. Musa of the herbarium unit of Biological Sciences Department A.B.U., Zaria where a voucher specimen number (0669) was deposited.

**Preparation of plant extract:** The fresh leaves were dried under shade and then ground into fine powder using laboratory mortar and pestle. The powder (460 g) was macerated in 70% of ethanol and 30% of distilled water at room temperature for 72 h. This was then filtered using a filter study (Whatmann size no. 1) and the filtrate was evaporated to dryness on water bath at 60°C to a brown dry residueof 24 g and kept in an air tight bottle until used.

**Chemical used:** All chemicals and drugs used were obtained commercially and of analytical grade. Alloxan was purchased from (Sigma chemical Company St. Louis U.S.A.).

**Phytochemical screening of the plant extract:** Preliminary phytochemical screening of the ethanolic leaf extract of *Mucuna Pruriens* was carried out by methods of analysis described by Trease and Evans (1983).

**Acute toxicity studies of the plant extract:** This was carried out by method described by Lorke (1983). In the initial phase, rats were divided into 3 groups of 3 rats each and were treated with the plant extract at doses of 10 mg, 100 mg and 1000 mg/kg body weight orally. The animals were observed for 24 h for signs of toxicity including death. Based on the results of phase one, three fresh rats were divided into 3 groups of one rat each, and were treated with 1600, 2,900 and 5,000 mg/kg body weight.

**Experimental animals:** A total of thirty healthy Wistar albino rats of both sexes between the ages of 8-10 weeks old and weighing between 150-200 g were used for the study. The animals were kept in well aerated laboratory cages in the Department of Human physiology animal house and were allowed to acclimatize to the laboratory environment for a period of 2 weeks before the commencement of the experiment. They were maintained on standard animal feeds and drinking water *ad libitum* during the stabilization period.

**Induction of diabetes mellitus:** The animals were fasted for 16-18 h with free access to water prior to the induction of diabetes. Diabetes was induced by single intraperitoneal injection of Alloxan monohydrate (Sigma St. Louis, M.S., U.S.A.) at a dose of 150 mg/kg body weight dissolved in 0.9% cold normal saline solution into 16-18 h fasted rats (Katsumata et al., 1999). Since alloxan is capable of producing fatal hypoglycemia as a result of massive pancreatic insulin release, rats were treated with 20% glucose solution orally after 6 h. The rats were then kept for the next 24 h on 5% glucose solution bottles in their cages to prevent hypoglycemic (Dhandapani et al., 2002).

**Experimental protocol:** Seventy two (72 h) after Alloxan treatment blood glucose was measured using glucose-oxidase principle and rats having fasting blood glucose level greater than 200 mg/dl were considered as diabetic and included in the study (Stanley and Venugopal, 2001). Thereafter, animals were randomly divided into different group as follows:

**Group 1:** Diabetic control and were administered with 1ml of distilled water orally

**Group 2:** Diabetic and received 100 mg/kg body weight of *Mucuna pruriens* orally

**Group 3:** Diabetic and received 200 mg/kg body weight of the *Mucuna pruriens* orally

**Group 4:** Diabetic and received 400 mg/kg body weight of the *Mucuna pruriens* orally
Group 5: Diabetic and received 250 mg/kg body weight of metformin orally. (Ravi et al., 2005)

All treatments were given once daily for a period of twenty one (21) days.

Histopathological studies: The pancreatic tissues were dissected out and washed on ice cold saline immediately. A portion of pancreatic tissue was fixed in 10% neutral formal-saline fixative solution for histological studies. After fixation, tissues were embedded in paraffin, solid sections were cut at 5 μm and various sections were stained with haematoxylin and eosins as described by Strate et al. (2005). The slides were viewed at magnification of X 250 and photomicrographs taken.

Statistical analysis: Data of the blood glucose were expressed as mean±SEM. The data obtained were statistically analyzed using one-way analysis of variance (ANOVA) with Tukey’s multiple comparison post hoc tests to compare the level of significance between control and experimental groups. The values of p<0.05 were considered as significant (Duncan et al., 1977)

RESULTS

Preliminary phytochemical screening of the plant extract: The results of preliminary phytochemical screening of ethanolic leaf extract Mucuna pruriens revealed the presence of flavonoids, tannins, saponins, cardiac glycosides, reducing sugars, steroids and/or triterpenoids and glycosides.

Acute toxicity studies: Signs of toxicity were first noticed after 4-5 h of the extract administration. There were decreased locomotor activity and sensitivity to touch and pain, including decreased feed intake, tachypnoea and prostration after 12 h of extract administration and subsequently deaths were recorded. The LD₅₀ was thus calculated as 2154 mg/kg.

Effect of ethanolic leaf extract of Mucuna pruriens on blood glucose levels of diabetic wistar rats: The blood glucose values on day 0 indicates the fasting blood glucose values levels in the experimental animals. The study revealed that there was no significant change (p>0.05) in the blood glucose levels in the groups administered with 100, 200 and 400 mg/kg and 250 mg/kg b w of Metformin after the 3rd day, when compared to the diabetic control group. There was also no significant difference (p>0.05) on the blood glucose

<table>
<thead>
<tr>
<th>Treatment given</th>
<th>Fasting blood glucose level (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 Day</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>406.83±52.1</td>
</tr>
<tr>
<td>Alloxan+100 mg/kg</td>
<td>403.50±33.1</td>
</tr>
<tr>
<td>Alloxan+200 mg/kg</td>
<td>407.67±40.7</td>
</tr>
<tr>
<td>Alloxan+400 mg/kg</td>
<td>414.83±55.5</td>
</tr>
<tr>
<td>Alloxan+metformin</td>
<td>412.33±40.8</td>
</tr>
</tbody>
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Values are statistically significant compared to control group at *p<0.05; **p<0.01; ns: not significant; Values are presented as mean±SEM
levels in the groups treated with 200 and 250 mg/kg of Metformin. Whereas, there was a significant reduction (p<0.05) on the blood glucose levels in the groups treated with the extract at dose extract concentrations of 100 and 400 mg/kg b w after the 6th day, when compared to the diabetic control group. The study also showed that administration of the extract at dose concentration of 100, 200 and 400 mg/kg and Metformin 250 mg/kg b w, resulted to a significant decrease (p<0.05) in the blood glucose level after 9th, 12th, 15th, 18th and 21 day, when compared to the diabetic control group, with a maximum reduction (p<0.01) in the blood glucose levels recorded with the extract 100 mg/kg b w after 15th and 18th day, 200 mg/kg b w after the 21st day and Metformin after 15th, 18th, and 21st day when compared to the diabetic control group (Table 1).

**Effect of ethanolic leaf extract of *Mucuna pruriens* on histopathological studies of diabetic rats:** The histopathological studies of the endocrine region of pancreas of the diabetic revealed degenerated pancreatic islet cells in the diabetic rats as shown in Plate 2, when compared to normal control (Plate 1), showing intact architecture of pancreatic Islet cells with no observable microscopic lesions. But the administration of various doses of plant extract showed restoration of pancreatic islet cells in all diabetic extract treated groups as shown in Plate 3, 4 and 5, suggesting the antidiabetic potential of the plant extract.

**DISCUSSION**

Medicinal plants are widely used in the management of diseases all over the world (Adewunmi and Ojewole, 2004). In Nigeria, several thousands of plant species have been claimed to possess medicinal properties and employed in the treatment of many ailments (Iweala and Oludare, 2011). Ethnopharmacological surveys indicate that more than 1200 plants are used in traditional medicine for their alleged hypoglycemic activity (Kesari et al., 2007) of which *Mucuna pruriens* is one of them. Management of diabetes without side effects is still a challenge to the medical system and has led to search for plants with hypoglycemic properties and their employment in the management of diabetes (Iweala and Oludare, 2011). However, several species of medicinal plants used in traditional treatment and management of diabetes have been evaluated, but only a few of such plants have received scientific scrutinizing (Godwe et al., 2008). The World Health Organization has recommended and encouraged the use of alternative therapy especially in developing countries where access to the conventional treatment of diabetes is not adequate (Claudia et al., 2006). Alloxan induces diabetes in experimental animals.
by destroying the beta cells of the Islet of Langerhans in the pancreas leading to reduction in the synthesis and release of insulin thereby inducing hyperglycemia (Szkudelski, 2001). Alloxan has been shown to induce free radical generation and cause tissue injury. The pancreas is especially susceptible to action of alloxan-induced free radical damage. Therefore, alloxan-induced diabetes is one of the frequently used models for the study of IDDM in experimental animals (Babu et al., 2002). The present study evaluated the effects of ethanolic leaf extract of *Mucuna pruriens* on blood glucose levels in Alloxan-induced diabetic Wistar rats. Preliminary phytochemical screening of the extract revealed the presence of flavonoids, tannins, saponins, cardiac glycosides, reducing sugars, glycosides, steroids and triterpenes. The results of this study showed that the extract at all doses caused a significant decrease on the blood glucose levels in Alloxan-induced diabetic Wistar rats. The mechanism by which the extract exert the hypoglycemic effect appear to be related to the presence of flavonoids among other secondary metabolites or bioactive chemical constituents found in the plant extract which may be an active constituents in a group or as an individual responsible for the hypoglycemic activity of the plant extract (Marles and Farnsworth, 1995). Flavonoids have been shown to exert their antioxidant activity by scavenging or quenching free radicals or by inhibiting enzymatic systems responsible for free radical generation (Lukacinova et al., 2008). Apart from being antioxidants, flavonoids have been reported to inhibit sodium-dependent vitamin C transporter 1 (SVCT 1) and glucose transporter Isoform 2 (Glut 2), the intestinal transporters for vitamin C and glucose, leading to a decrease in the intestinal absorption of glucose, hence decrease in the blood glucose concentration (Song et al., 2004). Several researchers have also demonstrated that flavonoids act as reducer of hyperglycemia by causing inhibition of renal glucose reabsorption through inhibition of the sodium-glucose symporters located in the proximal renal convulated tubule (Lukacinova et al., 2008). This may probably be possible mechanisms by which the plant extract exert its hypoglycemic effects in the diabetic animals and lend credence to the use of this plant in the management of diabetes mellitus in this part of the country.

In conclusion, the results of this study clearly demonstrated that the LD$_{50}$ of *Mucuna pruriens* as determined by acute toxicity study by oral route was found to be 2154 mg/kg body weight. The research work also showed that oral administration of the plant extract at all doses resulted to a significant decrease on the levels of blood glucose in alloxan-induced diabetic. The histopathological studies carried out indicated that the ethanolic leaf extract of *Mucuna pruriens* caused a restoration of pancreatic islet cells in alloxan-induced diabetic Wistar rats.

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