Effect of Ethanolic Leaf Extract of *Mucunapruriens* (*fabaceae*) on Lipid Profile in Alloxan-Induced Diabetic Wistar rats

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**Abstract:** Diabetes mellitus is a global health burden leading to an increase in the search for herbal hypoglycemic agents as alternative to synthetic ones. The effect of diabetes on lipid metabolism is well established. This study was aimed at evaluating the effects of ethanolic leaf extract of *M. pruriens* on lipid profile levels in alloxan-induced diabetic Wistar rats. Diabetes was induced by injection of Alloxan monohydrate (150 mg/kg b w) intraperitoneally to rats. Diabetic rats were randomly divided into the following groups (n = 6). Group I (normal control) received distilled water, Group II (diabetic control) received distilled water, group III, IV and V received 100, 200 and 400 mg/kg b w of the extract, respectively, while group VI received 250 mg/kg b w Metformin orally once daily for 21 days. At the end of the treatment period, blood samples were collected from the animals and assayed spectrophotometrically for serum concentration of TC, TG, HDL-C and LDL-C. The study showed that there was a significant reduction (p<0.05) in the serum levels TC, TG, LDL-C and increased HDL-C in alloxan-induced diabetic treated groups. However, there was a marked reduction (p<0.01) of 96.86±10.6, 106.31±7.5, 48.09±11.0 observed in the groups treated with 400 mg/kg for TC, TG, LDL-C. While a maximum increase (p<0.01) of 23.01±1.8 and 28.52±0.90 recorded in the group treated with 200 and 400 mg/kg for HDL-C. The result shows that the plant may be useful in the management of secondary complications of diabetes (dyslipidemia) associated with diabetes mellitus.

**Keywords:** Alloxan, diabetes mellitus, dyslipidemia, lipid profile, metformin, *Mucunapruriens*

**INTRODUCTION**

Diabetes mellitus is a disease of worldwide significance and increasing prevalence. It is a multifactorial disease that has a significant impact on the health, quality of life and life expectancy of patients, as well as on the health care system (Subbiah et al., 2006; Amos et al., 1997). According to World Health Organization, the prevalence of diabetes is likely to increase by 35%. Currently there are over 150 million people living with diabetes mellitus and the numbers is likely to rise to 300 million or more by the year 2025 (Jadhav et al., 2009). Diabetes mellitus is a group of metabolic disease characterized by hyperglycemia resulting from defects in insulin secretion and insulin action or both (Kangralkar et al., 2010). Beside hyperglycemia, several other factors like hyperlipidemia and enhanced oxidative stress play a major role in diabetic pathogenesis (Saikat et al., 2008). Insulin affects many sites of mammalian lipid metabolism. It stimulates synthesis of fatty acid in liver adipose tissue and in the intestine (Suryawanshi et al., 2006). The elevated reactive oxygen species and the simultaneous decline in antioxidative defence mechanisms observed in diabetic condition could promote the development of late complications (Brown and Goodman, 1998). To reduce the risk of late complications and other deleterious consequences of diabetes mellitus, such as blindness, renal failure, limb amputation, the control of blood glucose and lipid profile levels is necessary (Subbiah et al., 2006). Diabetes produces disturbances in lipid profiles and especially, an increased susceptibility to lipid peroxidation (Li, 2007). In the last stages of diabetes, lipid metabolism is affected and seen as hyperlipidemia and hypercholesterolemia which are risk factors in atherosclerosis (Schwartz, 2006).

In regions of the world particularly in Africa, plant materials have played an important role in the traditional treatment of diabetes mellitus and herbal remedies continue to be more accessible and affordable than conventional drugs and represent the first line of treatment available to diabetic patient especially in the rural communities. From the beginning of the last century, evidence of the lipid lowering properties of medicinal plants has accumulated (Kritchevsky, 1995). Many researchers across the globe have demonstrated the role of medicinal plants in the control of hyperlipidemia (Subbiah et al., 2006). 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 a multitude 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 seeds are bitter, stimulants, purgative and diuretic. 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 neuroprotective (Manyham et al., 2004). Its analgesic and anti-inflammatory 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 testosterone 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 effects of Mucuna pruriens on lipid profile of Alloxan-induced diabetic Wistar rats.

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 collected and 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 paper (Whatmann size no. 1) and the filtrate was evaporated to dryness on water bath at 60°C to a brown dried residue of 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: The index of the acute toxicity is the LD₅₀. This was done using the method described by Lorke (1983). In the initial phase, rats were divided into 3 groups of 3 rats each and were treated with Mucuna pruriens leaf 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. The rats were also observed for 24 h for signs of toxicity including death.

Care and management of experimental animals: Albino Wistar 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 two 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 experimental 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 design: In this study, thirty six (36) Wistar rats were used. The animals were fasted for 16-18 hr with free access to water prior to the induction of diabetes. 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). After induction of diabetes, the diabetic animals were randomly divided into different group as follows:

**Group 1:** Normal control rats and given 1 mL of distilled water orally

**Group 2:** Diabetic control and were administered with 1ml of distilled water orally.

**Group 3:** Diabetic and received 100 mg/kg body weight of *Mucuna pruriens* orally.

**Group 4:** Diabetic and received 200 mg/kg body weight of *Mucuna pruriens* orally.

**Group 5:** Diabetic and received 400 mg/kg body weight of *Mucuna pruriens* orally.

**Group 6:** 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 21 days.

**Collection and preparation of sera samples for lipid profile analysis:** Blood samples were collected from overnight fasted animals via cardiac puncture into plain tubes and were allowed to clot and the serum separated by centrifugation using Denley BS400 centrifuge (England) at 3000 rpm for 10 min. The serum was collected and then assayed for lipid profile.

**Lipid profile assay:** These were determined spectrophotometrically, using enzymatic colorometric assay kits (Randox, Northern Ireland) as follows:

**Assay for serum total cholesterol:** The serum level of total cholesterol was quantified after enzymatic hydrolysis and oxidation of the sample as described by method of Stein (1987). Briefly, 1000 μL of the reagent was added to each of the sample and standard. This was incubated for 10 minutes at 20-25°C after mixing and the absorbance of the sample (A_{sample}) and standard (A_{standard}) was measured against the reagent blank within 30 minutes at 546 nm. The value of TC present in serum was expressed in the unit of mg/dL.

\[
\text{TC concentration} = \frac{A_{sample}}{A_{standard}} \times 196.86 \text{mg/dL}
\]

**Assay for serum triglyceride:** The serum triglyceride level was determined after enzymatic hydrolysis of the sample with lipases as described by method of Tietz (1990). 1000 μL of the reagent was added to each of the sample and standard. This was incubated for 10 min at 20-25°C after mixing and the absorbance of the sample (A_{sample}) and standard (A_{standard}) was measured against the reagent blank within 30 min at 546 nm. The value of triglyceride present in the serum was expressed in the unit of mg/dL.

\[
\text{TGL concentration} = \frac{A_{sample}}{A_{standard}} \times 194.0 \text{mg/dL}
\]

**Assay for serum high density lipoprotein cholesterol:** The serum level of HDL-C was measured by the method of Wacnic and Alber (1978). Low-density lipoproteins (LDL and VLDL) and chylomicron fractions in the sample were precipitated quantitatively by addition of phosphotungstic acid in the presence of magnesium ions. The mixture was allowed to stand for 10 minutes at room temperature and centrifuged for 10 minutes at 4000 rpm. The supernatant represented the HDL-C fraction. The cholesterol concentration in the HDL fraction, which remained in the supernatant, was determined. The value of HDL-C was expressed in the unit of mg/dL.

**Determination of serum low-density lipoprotein cholesterol:** The serum level of (LDL-C) was measured according to protocol of Friedewald et al. (1972) using the equation below:

\[
\text{LDL-C} = \frac{TGL}{5} - \text{HDL-C}
\]

The value was expressed in the unit of mg/dL.

**Statistical analysis:** Values obtained from lipid profile assay were expressed as mean ± SEM. The data obtained were statistically analyzed using one-way analysis of variance (ANOVA) with Turkey’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., 1997).

**RESULTS AND DISCUSSION**

**Preliminary phytochemical screening of the plant extract:** The results of preliminary phytochemical
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 LD$_{50}$ was thus calculated as 2154 mg/kg.

Effect of daily oral doses of ethanolic leaf extract Mucunapruriens on serum total cholesterol level of diabetic wistar rats: The results of the study showed that the levels of total cholesterol were significantly higher (p<0.05) in the diabetic control group than in the normal control group. All doses of the extract significantly decreased (p<0.05) the serum level of total cholesterol with maximum reduction observed in the met for min-treated group when compared to diabetic control group. The extract dose of 400 mg/kg b w showed no significant difference (p>0.05), whereas 100 and 200 mg/kg b w revealed a statistically significant difference (p<0.05) on serum level of total cholesterol compared to met for min-treated group as shown in Fig. 1.

Effect of daily oral doses of ethanolic leaf extract Mucunapruriens on serum triglyceride level of diabetic wistar rats: There was a statistically significant decline (p<0.05) in the level of serum triglyceride in the diabetic groups treated with extract doses of 100, 200 and 400 and metformin 250 mg/kg b w with maximum decrease (p<0.01) recorded in the group treated with 400 mg/kg b w when compared to diabetic control group. Meanwhile 100, 200 and 400 mg/kg b w of the extract reported no statistically significant difference (p>0.05), whereas 100 and 200 mg/kg b w showed no statistically significant difference (p>0.05), whereas 100 mg/kg of the extract showed a statistically significant difference (p<0.05) compared to the metformin-treated group as shown in Fig. 2.

Effect of daily oral doses of ethanolic leaf extract Mucunapruriens on serum HDL-C level of diabetic wistar rats: On the other hand, serum HDL-C level was significantly elevated (p<0.05) in the groups administered with the extract doses of 100, 200, 400 and met for min 250 mg/kg b w, with a marked increase (p<0.01) obtained in the group the received extract dose of 200 and 400 mg/kg b w when compared to the diabetic control group. However, 200 and 400 mg/kg dose of the extract showed no statistically significant difference (p>0.05), whereas 100 mg/kg of the extract showed a statistically significant difference (p<0.05) compared to the metformin-treated group as shown in Fig. 3.

Screening of ethanolic leaf extract Mucunapruriens revealed the presence of flavonoids, tannins, saponins, cardiac glycosides, reducing sugars, steroids and/or triterpenoids and glycosides.
Effect of daily oral doses of ethanolic leaf extract *Mucunapruriens* on serum LDL-C level of diabetic wistar rats: In addition, there was a significant reduction (p<0.05) in the serum level of LDL-C in the group administered with doses of 100, 200 and 400 and metformin 250 mg/kg b w, respectively, with marked decrease (p<0.01) recorded in the group treated with 400 mg/kg and metformin 250 mg/kg b w, respectively compared to the diabetic control group as shown in Fig. 4.

**DISCUSSION**

Medicinal plants are widely used in the management of diseases all over the world (Adewunmi and Ojewole, 2004; Aliyu et al., 2007). 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). 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 leaving residual or less active β-cells (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. The resultant effect which is insulin deficiency, leads to various metabolic alterations in the animals such as, increase in blood glucose, total cholesterol and triglyceride levels (Vivek et al., 2010). Preliminary phytochemical screening of the ethanolic leaf extract of *Mucuna Pruriens* revealed the presence of flavonoids, tannins, saponins, cardiac glycosides, reducing sugars, glycosides, steroids and triterpenes. Hyperglycemia and dyslipidemia as well as oxidative stress generally coexist in diabetes subjects. Dyslipidemia which includes not only quantitative but also qualitative abnormalities of lipoprotein plays a significant role in the pathogenesis of vascular complications in diabetes mellitus (Sobngwi et al., 2001; Beckman et al., 2002; Rotimi et al., 2011). High cholesterol levels and hyperlipidemia are associated consequences of diabetes mellitus (Mironova et al., 2000; Odetala et al., 2006; Iweala and Oludare, 2011). Abnormalities of lipid profile are one of the most common metabolic complications of diabetes mellitus which is found in about 40% of diabetics (Ganhi, 2001; Ravi et al., 2005) The present study showed an increase in the concentration of total cholesterol, triglyceride, low-density lipoprotein cholesterol (LDL-C) and a decrease in HDL-C in diabetic rats. This results support the findings of Mendez and Balderas (2001) and Mitra et al. (1995) that who have reported increased plasma cholesterol, triglycerides, LDL-C and decreased HDL-C in streptozocin-induced hyperglycemia in rats. Daisy et al. (2009) had reported insulin deficient associated hypercholesterolemia and hypertriglyceridemia in streptozocin induced diabetes in rats. Mathe (1995) reported that hypercholesterolemia in streptozocin results from increased intestinal absorption and synthesis of cholesterol. Diabetic-induced hyperlipidemia is attributable to excess mobilization of fat from the adipose due to under utilization of glucose (Krishnakumar et al., 2000; Nimenibo-uadia, 2003). The lack of insulin and elevations of the counter-regulatory hormones lead to activation of enzymes (hormone-sensitive lipase) that stimulate lipolysis and enhanced release of free fatty acids from adipose tissue (Subbiah et al., 2006; Rotimi et al., 2011). The fatty acids from adipose tissues are mobilized for energy purpose and excess fatty acids are accumulated in the liver, which are converted to triglyceride (Suryawanshi et al., 2006). The marked hyperlipidemia that characterizes the diabetic state may therefore be regarded as a consequence of unlimited actions of lipolytic hormones on the fat depots (Claudia et al., 2006). Lowering of serum lipid levels through dietary or drugs therapy seems to be associated with a decrease in the risk of vascular disease in diabetes (Claudia et al., 2006). In this study, administration of all doses of the ethanolic leaf extract of *Mucunapruriens* significantly reduced serum levels of total cholesterol, triglyceride, low-density lipoprotein and increased serum levels of high-density lipoprotein in Alloxan-induced diabetic Wistar rats. However, significantly decrease on serum lipid profile levels observed on treatment with the ethanololic leaf extract of *Mucuna pruriens* may presumably be mediated by a control of lipid metabolism by some of the phytochemicals present in the plant. Many nutritional factors such as saponins and tannins have been reported to contribute to the ability of herbs to improve dyslipidemia (Nimenibo-uadia, 2003; Rotimi et al., 2011). Preliminary phytochemical screening of the extract revealed the presence of saponin among other polyphenolic compounds. This may be responsible for the lipid-lowering effect of *Mucunapruriens* on plasma lipid. Saponins are known antinutritional factors, which lower cholesterol by binding with cholesterol in the intestinal lumen, preventing its absorption and/or by binding with bile acids, causing a reduction in the enterohepatic circulation of bile acids and increase its fecal excretion (Nimenibo-uadia, 2003; James et al., 2010; Rotimi et al., 2011). Increased bile acid excretion is offset by enhanced bile acid synthesis from cholesterol in the liver and consequently lowering of the plasma cholesterol (Rotimi et al., 2011). Hence, saponins have been reported to have hypocholesterolemic effect (James et al., 2010). Kumarappen et al. (2007) reported that administration of polyphenolic compounds to alloxan-induced diabetic rats
reduced hyperlipidemia and attributed this to a reduction in the activity of hepatic HMG-CoA reductase, which is the first committed enzymatic step of cholesterol synthesis. This lowers elevated LDL cholesterol levels, resulting in a substantial reduction in coronary events and deaths from CHD that occurs in diabetics (Richard and Pamela, 2009). Thus, the observed hypolipidemic effect of *Mucunapruriens* can be therefore, linked to the synergistic actions of phytochemicals like saponins and polyphenolic compounds contained in the plant extract. It is reported that the derangement of glucose, fat and protein metabolism during diabetes, results into the development of hyperlipidemia (Brown and Goldstein, 1983; Austin and Hokanson, 1994). In this study, all doses of the plant extract used produced a significant beneficial effect on serum lipid profile in alloxan-induced diabetic rats. This beneficial effect on the lipid profile may be secondary to glycemic control. The significantly lowered cholesterol level may have contributed to the observed significant high serum high-density lipoprotein cholesterol in the animals. About 30% of blood cholesterol is carried in the form of HDL-C. Significant lowering of total cholesterol and rise in HDL-C is a very desirable biochemical state for prevention of atherosclerosis and ischemic conditions (Schwenke and Carew, 1989; Luc and Fruchart, 1991; Mitra et al., 1995). HDL-C function to remove cholesterol antheroma within arteries and transport it back to the liver for its excretion or reutilization, thus high level of HDL-C protect against cardiovascular disease (Kwiterovich, 2000; James et al., 2010). Therefore, the observed increase in the serum HDL-C level on administration of various doses of the extract in alloxan-induced diabetic rats, indicates that the extract have HDL-C boosting effect. Moreover, the stabilization of serum triglyceride and cholesterol levels in rats by the plant extract may be attributed to glucose utilization and hence depressed mobilization of fat (Momo et al., 2006; Iweala and Oludare, 2011). This implies that the plant extract may be useful in reducing the complications of hyperlipidemia and hypercholesterolemia which often coexist in diabetics (Sharma, 2003). The study also revealed that administration of the extract at various doses significantly lowered the serum LDL-C in alloxan-induced diabetic Wistar rats. Studies have shown that chronic insulin deficiency as observed in alloxan-induced diabetes in experimental animals is associated with diminished levels of LDL-C receptors. This results to an increase in LDL particles and consequently increases serum level of LDL-C (Suryawanshi et al., 2006). Metformin produces a beneficial effect on lipid profile mainly by correcting abnormal glucose metabolism (Defronzo and Goodman, 1995). In addition to its role as an antihyperglycemic agent, metformin has an important property of its ability to modestly reduce hyperlipidemia (Richard and Pamela, 2009). It also produces a moderate reduction in serum triglyceride levels as a result of decreased hepatic synthesis of very low-density lipoprotein (Chehade, 2000). A similar effect was observed for metformin in this present study.

In conclusion, the results of this study clearly demonstrated that the LD₅₀ of *Mucunapruriens* determined by acute toxicity study by oral route was found to be 2154 mg/kg body weight. The study also showed that oral administration of all doses of the extract resulted to a significant decrease on the levels of lipid profile in alloxan-induced diabetic mellitus as well as improved hyperlipidemia associated with diabetes. In present study, all doses of *Mucunapruriens* leaf extract appeared to be effective and were comparable to the standard drug (met for min). The result obtained from this work showed that the plant may be useful in the management of secondary complications of diabetes (dyslipidemia) associated with diabetes mellitus.

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Cuminumcyminum L.


