Changes in Lipid Profile of Rats Administered with Ethanolic Leaf Extract of Mucuna pruriens (Fabaceae)

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Abstract: The study evaluated the effect of ethanolic leaf extract of Mucuna pruriens on some lipid profile parameters of normoglycemic Wistar rats. The acute oral toxicity studies were conducted. The animals were administered with the plant extract at graded doses of 100, 200 and 400 mg/kg bw and metformin 250 mg/kg bw orally for 21 days. Blood samples were collected from the animals at the end of the treatment period and assayed for the serum concentration of total cholesterol, triglyceride, low-density lipoprotein and high-density lipoprotein cholesterol. The results showed the extract significantly reduced (p<0.05) the levels of serum total cholesterol, triglyceride, low-density lipoprotein and elevated high-density lipoprotein in the groups treated with 100 and 200 mg/kg bw. There was no significant changes (p>0.05) in the lipid profile in the group treated with 400 and 250 mg/kg bw of metformin. In conclusion, the results of the present findings may be beneficial and of clinical importance to individuals at risks of cardiovascular problems.

Key words: Cardiovascular disease, ethanolic extract, lipid profile, metformin, Mucuna pruriens

INTRODUCTION

Cardiovascular diseases present some of the main health problems in the world today, and major ones include coronary heart diseases, stroke and hypertension (Bowman and Russell, 2001). Increased plasma lipids are risk factors in cardiovascular problems (James et al., 2010), and important lipids whose elevations are implicated in these conditions are cholesterol and triglycerides. Lipids are transported in the blood by combination of lipids and proteins complexes called lipoproteins (Nwanjo, 2004). In addition to providing a soluble means for transporting lipids through blood, lipoproteins have cell targeting signals that direct the lipids they carry to certain tissues. For this reason, there are different types of lipoproteins which are classified based on their density and charges (Gordon et al., 1989). According to the lipid hypothesis, abnormally high cholesterol levels (hypercholesterolemia), i.e higher concentration of LDL-C and lower concentration of functional HDL-C, are strongly associated with cardiovascular disease, because these promote atheroma development in arteries (atherosclerosis) and related cerebrovascular disorders. This disease lead to myocardial infarction (heart attack), Stroke and peripheral vascular disease. Because LDL-C can transport cholesterol into the artery wall, retained there by arterial proteoglycans and attract macrophages which engulf the LDL particles and start the formation of plaques. Over time vulnerable plaques rupture, activate blood clotting and produced heart attack, stroke and peripheral vascular disease symptoms and major debilitating events (Otvos, 1999). Since higher blood LDL-C, especially higher LDL particles concentrations and smaller particle size, contribute to this process more than the cholesterol content of the LDL particles (Nwanjo, 2004; Brunzell et al., 2008). LDL particles are often termed “bad cholesterol” because they have been linked to atheroma formation. On the other hand, high concentrations of functional HDL-C, which can remove cholesterol from cells and atheroma, offer protection and sometimes referred to as “good cholesterol”. HDL-C can remove atheroma within arteries and peripheral cells and transport it back to the liver for excretion or re-utilization, which is the main reason why HDL-bound cholesterol is sometimes called “good cholesterol” (National Institutes of Health Consensus Development Conference Statement, 1992; Nwanjo, 2004). A high level of HDL-C seems to protect against cardiovascular disease. These balances are mostly genetically determined, but can be changed by body build, medications, food choices and other factors (Durrington, 2003). The causal link between lipid abnormalities and cardiovascular disease (CVD) is well established. Among these abnormalities, elevated levels of low-density lipoprotein cholesterol (LDL-C) are thought to be a key determinant of cardiovascular disease risk factor (Wilson et al., 1998). LDL-C accounts for the vast majority of atherogenic particles and therefore has been
the fundamental index of atherogenic risk. Increasing evidence suggests that lipid accumulation in the liver plays an important role in the pathogenesis of cardiovascular disease (Tarantino et al., 2007). LDL particles exist in multiple subclasses, differing in size, density, and lipid content. Large and medium LDL comprise the most abundant species in plasma of the most healthy individuals, but there are two forms of small LDL, exhibiting reduced receptor binding, greater endothelial transport, greater arterial proteoglycan binding, and greater susceptibility to oxidation. Small LDL is associated with elevations in triglyceride levels. Traditional models describe a pathway from VLDL formation in the liver to formation of IDL and then of LDL particles via lipolysis. (Berneis and Krauss, 2002). There is thus, considerable interest in nutritional and pharmacological agents that are able to reduce hepatic lipid accumulation (Jeffrey et al., 2008). There is an intimate relationship between diet and dyslipidemia, and dietary manipulations may be used to understand the mechanisms of these abnormalities. 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). 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 (Adenpoju and Odubena, 2009). It is a twining and tropical legume known as velvet bean and by 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. 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 has also shown to have neuroprotective effect (Manyham et al., 2004). Its analgesic and anti-inflammatory activities have been reported (Adenpoju 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 investigating the validity of the use of the plant extract of *Mucuna pruriens* in atherosclerotic condition in folk medicine.

**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 paper (Whatmann size no. 1) and the filtrate was evaporated to dryness on water bath at 60ºC to a brown dry residue of 24 g and kept in an air tight bottle until used.

**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 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.
Experimental design: In this study, thirty six wistar rats were used. The animals were randomly divided into different group as follows:

**Group 1:** Normal control rats and given 1ml of distilled water orally and served as the negative control.

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

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

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

**Group 6:** Normal and received 250 mg/kg body weight of metformin orally

All animals were subjected to a daily oral doses of the plant extract for a period of twenty one (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 ten minutes and the supernatant (serum) collected were then subjected to lipid profile analysis.

Lipid profile assay: These were determined spectrophotometrically, using enzymatic colometric 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 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 total cholesterol present in the serum was expressed in the unit of mg/dL.

TC concentration = A\_sample/A\_standard × 196.86 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.

TGL concentration = A\_sample/A\_standard × 194.0 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 min at room temperature and centrifuged for 10 min 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:

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LDL-C = TGL/5 - HDL-C
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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.*, 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. The LD₅₀ was thus as the square root of the product of the
Effects of ethanolic leaf extract of mucuna pruriens on serum total cholesterol: There was no significant change (p>0.05) in the serum total cholesterol levels in the group treated with 400 mg/kg and 250 mg/kg b w of metformin when compared to the control group. But there was a significant reduction (p<0.05) in the serum total cholesterol level in the groups administered with 100 and 200 mg/kg b w, with a maximum reduction (p<0.01) recorded in the group that received 200 mg/kg when compared to the control group as shown in Fig. 1.

Effects of ethanolic leaf extract of mucuna pruriens on serum triglyceride: There was a significant decrease...
Effects of ethanolic leaf extract of *Mucuna pruriens* on serum high-density lipoprotein cholesterol: However, serum HDL-C level was significantly increased (p<0.05) in a dose dependent fashion in all groups that received 100, 200 and 400 mg/kg b w and metformin 250 mg/kg when compared to the control group (Fig. 3). In addition, there was no significant change (p>0.05) in the serum LDL-C level in the groups that received 400 mg/kg b w of the extract and 250 mg/kg b w of metformin when compared to the control group.

Effects of ethanolic leaf extract of *Mucuna pruriens* on serum low-density lipoprotein cholesterol: In addition, the serum LDL-C level was significantly (p<0.05) depleted in the groups that received 100 and 200 mg/kg b w with a marked reduction (p<0.01) recorded in the group treated with 100 mg/kg b w when compared to the control group as shown in Fig. 4.

**DISCUSSION**

Cardiovascular diseases present some of the main health problems in the world today, and major ones include coronary heart diseases, stroke and hypertension (Bowman and Russell, 2001). Increased plasma lipids are risk factors in cardiovascular problems (James et al., 2010), and important lipids whose elevations are implicated in these conditions are cholesterol and triglycerides. In the present study, effect of daily oral administration of ethanolic leaf extract of *Mucuna pruriens* on the levels of some serum lipid profile was assessed in albino Wistar rats. The results showed that all doses of the plant extract significantly reduced the serum lipids and elevated the serum concentration of HDL-C in the animals. 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. Many nutritional factors such as saponins and tannins have been reported to contribute to the ability of herbs to improve hyperlipidemia (Nimenibo-uaadia, 2003; Rotimi et al., 2011). The presence of these phytochemicals especially saponins among polyphenolic compounds, may be responsible for the lipid-lowering effect of the plant extract that was observed in this present study. 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-uaadia, 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 consequent 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 in 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 coronary heart disease (Richard and Pamela, 2009). Thus, the observed hypolipidemic effect of *Mucuna pruriens* can be therefore, linked to the synergistic actions of phytochemicals like saponins and polyphenolic compounds contained in the plant extract. However, 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 high-density lipoprotein cholesterol. 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 the animals indication that the extract have HDL-C boosting effect. The study also revealed that administration of the extract at various doses significantly lowered the serum LDL-C in the animals. 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).

In conclusion, the results obtained from this study showed that oral administration of all doses of the plant extract resulted to a significant decrease on the levels of serum lipid profile. This therefore, may suggest that the plant extract possess hypolipidemic activity, and may be useful in the management of dyslipidemia which is one of the risk factors in patients with cardiovascular diseases.

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