

Effects of ethanol extract of *Mucuna pruriens* leaves on the lipid profile and serum electrolytes of rats

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Abstract: The effects of ethanol extract of *Mucuna pruriens* leaves on the lipid profile and serum electrolytes of rats after a two-week treatment were investigated. Preliminary phytochemical analysis of the leaves of *Mucuna pruriens* revealed the presence of alkaloids, saponins, flavonoids, tannins and glycosides while cyanogenic glycosides were not detected. Oral treatment of rats with the aqueous leaf extract (400mg/kg body weight and 800mg/kg body weight) for 7 days and 14 days respectively evoked significant ($P < 0.05$) decreases in the mean body weights of rats. However, there were no significant changes in the relative liver weights of the rats compared to the control animals. There were also significant ($p < 0.05$) decreases in the levels of total serum cholesterol, low density lipoprotein (LDL) cholesterol, free fatty acids, serum phospholipids as well as serum triacylglycerol but there was a significant increase ($p < 0.05$) in the levels of high density lipoprotein (HDL) cholesterol compared to the controls. Analysis also revealed that the ethanol extract of *Mucuna pruriens* leaves brought about significant changes ($p < 0.05$) in the concentrations of the principal serum cations (Na^+ and K^+) as well as serum chloride concentrations at the same dose levels and duration. However, the serum bicarbonate ions concentration was not affected. These findings show that the ethanol extract of *Mucuna pruriens* exhibits hypolipidaemic effect on experimental animals and suggest that the extract may be beneficial and of clinical importance to individuals at risks of cardiovascular problems.

Key Words: Hypolipidaemic effect, *Mucuna pruriens*, cardiovascular disorders.

I. Introduction

Medicinal plants are now getting more attention than ever because they have potential of myriad benefits to society, especially in the line of medicine and pharmacology (Safowora, 1993). At present, the World Health Organization is taking an official interest in herbal medicines in order to facilitate its aim of making health available for all. Traditional medicine has remained popular in all regions of the world, especially for the developing countries due to accessibility, affordability and the advantage of having multiple efficacy and minimal side effects (UNESCO, 1998). There is worldwide epidemic of cardiovascular diseases, which are associated with a number of pathologies including dyslipidemia (Eckel *et al.*, 2004). Increased plasma lipids such as cholesterol and triglycerides are important risk factors in cardiovascular diseases.

Mucuna pruriens (velvet beans) is an unconventional legume commonly found in the tropical regions of Africa, India and West Indies. (Taylor, 2005). The leaves are consumed for their nutritional value and are also used in folk medicine as a therapy for various diseases such as diabetes, arthritis, dysentery, infertility, obesity and cardiovascular disorders (Nadkaru, 2001). The leaves and seeds have shown significant aphrodisiac, antispasmodic, anticataleptic, antiepileptic, anti-diabetic, antimicrobial, anti-inflammatory, pain-relieving and fever-reducing activities from various clinical researches with animals (Amin *et al.*, 1996; Hussain and Manyam, 1997; Sathiyarayanan *et al.*, 2007; Majekodunmi *et al.* 2011; Champasingh *et al.*, 2011; Lampariello *et al.*, 2012). The nutritional and toxicological potentials of the seeds have been evaluated (Enechi *et al.*, 2011). The seeds are also implicated in the treatment of Parkinson's disease (Hussain and Manyam, 1997). This study was therefore undertaken to assess the effects of *Mucuna pruriens* leaf extract on serum lipids and electrolytes with a view to giving a scientific backbone to the traditional medicinal usage of the plant in the management of obesity and its associated complications such as cardiovascular disorders.

II. Materials And Methods

Chemicals/Reagents

Total lipids, total cholesterol, and triglycerides kits are products of Randox Chemicals Hall, England. All other chemicals/reagents are of analytical grade and were obtained from BDH Limited Poole, England.

Plant material

The leaves of *Mucuna pruriens* were collected from Nsukka, Enugu State Nigeria and identified by Dr. Uche Nzekwe of the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka where a voucher specimen is also retained.

Extraction

Fresh leaves of *Mucuna pruriens* (500g) were air dried for seven days under atmospheric conditions and pulverized with the aid of an electric blender. The pulverized material was macerated in 70% ethanol to give an extract that was filtered. The filtrate was concentrated using a rotary evaporator to yield a viscous slurry with a percentage recovery of 8%.

Acute Toxicity Test

This was done using the Lorke's method (Lorke, 1983). A total of 25 albino rats (Wistar strain) weighing between 100–150g were fasted overnight although with access to tap water. They were divided into five groups of five rats each. Four groups were given different doses of the extract in the following order: 200, 500, 2000, 5000mg/kg body weight of the rats while the fifth group (control) received only normal saline. The rats were observed for 12 hours for any lethality or signs of overt toxicity.

Preliminary Phytochemical Analysis

The phytochemical tests on the *Mucuna pruriens* ethanol extract for the presence of alkaloids, saponins, flavonoids, tannins, glycosides and cyanogenic glycosides were carried out as described by Harboume (1973).

Animal Treatment

Twenty four albino rats (Wistar strain) of both sexes weighing averagely 150g were divided into three groups of eight rats each. They were allowed free access to water and standard diet. Group 1 served as the control and were treated with normal saline (0.4ml/100g body weight). While group 2 and group 3 were daily administered 400mg/kg body weight and 800mg/kg body weight of the plant extract respectively. After seven days, half of the animals in each group were sacrificed by chloroform anesthesia while the remaining half were sacrificed after 14 days of treatment. All administrations were orally by gastric intubation. Blood was collected by cardiac puncture and allowed to clot, centrifuged at 3000 rpm for 15mins and the serum aspirated. The livers of the animals in each case were excised, washed in normal saline, allowed to dry and then weighed.

Determination of Serum Indices of Lipid Metabolism

The following serum indices of lipid metabolism were determined spectrophotometrically using enzymatic colorimetric assay kits as follows:

Determination of Total Serum Cholesterol Concentration

Total serum cholesterol was determined according to the method of Stein (1987) using Randox kits.

Determination of low density lipoprotein (LDL) cholesterol level

Low density cholesterol level was determined according to the method of Assmann *et al.* (1976).

Determination of high density lipoprotein (HDL) cholesterol level

High density lipoprotein cholesterol level was determined according to the method of Albers *et al.* (1978) using QCA commercial kits.

Determination of free fatty acids.

Total serum free fatty acids was determined according to the method of Tietz (1990) using Randox kits.

Determination of Serum Phospholipid level

Serum phospholipid level was determined according to the method of Tietz (1990) using Randox kits.

Determination of Serum Triacylglycerol

The serum triglyceride level was determined as described by method of Tietz (1990) using Randox kits.

Determination of Serum Potassium and sodium ion Concentrations

Serum concentrations of these ions were determined using flame photometry according to AOAC (1984).

Determination of Serum Bicarbonate and Chloride ion concentrations

Serum concentrations of these ions were determined using the method described by Tietz (1990).

Statistical analysis

The data obtained in this study were evaluated using the one-way analysis of variance (ANOVA) test between two mean groups, control and test groups, followed by student's t-test. Significant levels were at $p < 0.05$. Values were expressed as means \pm standard deviation (SD).

III. Results And Discussion

Acute Toxicity Test

Oral administration of the ethanol leaf extract of *Mucuna pruriens* produced no gross behavioral and/or physical changes in lethality at doses up to 5000mg/kg body weight. The doses used in this study are therefore considered to be toxicologically safe.

Phytochemical Analysis

The results of phytochemical analysis are presented in Table 1.

Table 1: Phytochemical Screening of *Mucuna Pruriens*

Alkaloids	+
Saponins	+
Flavoniods	+
Tannins	+
Glycosides	+
Cyanogenic glycoside	ND

Key:

+ = Presence of component tested
 ND = Not detected.

Phytochemical analysis revealed the presence of alkaloids, saponins, flavoniods, tannins and glycosides as presented in Table 1 above. These bioactive compounds detected in the extract are known to have a variety of pharmacological activities (Trease and Evans, 1983).

Table 2: Effect of ethanol extract of *Mucuna pruriens* on mean change in body weights of rats.

Treatment	Mean change in body weights (g)	
	7 Days	14 Days
(a). Control (Normal saline)	82.25 \pm 4.16	90.2 \pm 11.63
(b). Group I (400mg/kg b.w)	81.23 \pm 9.19	91.6 \pm 6.00
(c). Group II (800mg/kg b.w)	83.35 \pm 6.71	*63.3 \pm 7.42

Values represent mean \pm standard deviation. *Significant at $P < 0.05$.

Table 2 shows that ethanol extract of *Mucuna pruriens* leaf elicited no significant change in the body weights of the animals when challenged with dose of 400mg/kg for the period under investigation. However, there was a significant ($P < 0.05$) change in body weights of the animals when challenged with higher doses of the extract (800mg/kg) for 14 days when compared to the control animals. This implies that the extract has effect on the body weights of the animals.

Table 3: Effect of ethanol extract of *Mucuna pruriens* leaf on the relative liver weights of rats.

Treatment	Relative liver weight (mean \pm S.D) (g/kg)	
	7 Days	14 Days
Control (normal saline)	3.73 \pm 0.13	3.08 \pm 0.34
Group I 400mg/kg b.w.	4.00 \pm 0.73	3.00 \pm 0.22
Group II 800mg/kg b.w	3.00 \pm 0.68	3.33 \pm 0.34

Table 3 shows that there were no significant ($P < 0.05$) changes in the relative liver weights of the rats when compared to the controls. This implies that the plant extract did not exert any gross pathological effects on the livers of the animals.

Table 4: Effect of ethanol extract of *Mucuna pruriens* leaf on total serum cholesterol level in rats.

Treatment	Total serum cholesterol level (mean \pm S.D)	
	7 Days	14 Days
Control (normal saline)	431.87 \pm 25.96	369.71 \pm 37.97
Group I 400mg/kg b.w.	*307.75 \pm 43.85	*201.37 \pm 39.97
Group II 800mg/kg b.w	*272.29 \pm 28.17	*201.36 \pm 29.97

Values represent mean ± standard deviation.

*Significant at P<0.05

Table 4 above shows a significant (P<0.05) decrease in the mean total cholesterol levels in the experimental animals when compared to the control animals.

Table 5: Effect of ethanol extract of *Mucuna pruriens* leaf on serum low density lipoprotein cholesterol levels in rats.

Treatment	Serum low density lipoprotein cholesterol levels (mean ± S.D) (mg/dl)	
	7 Days	14 Days
Control (normal saline)	201.57±19.46	196.49±15.90
Group I 400mg/kg b.w.	*132.75±17.25	*125.15±20.05
Group II 800mg/kg b.w	*129.25±22.50	*115.41±20.37

Values represent mean ± standard deviation.

*Significant at P<0.05

Table 5 above shows a significant (P<0.05) decrease in the mean levels of low density lipoprotein cholesterol in the experimental animals when compared to the control animals.

Table 6: Effect of ethanol extract of *Mucuna pruriens* leaf on serum high density lipoprotein cholesterol levels in rats.

Treatment	Serum high density lipoprotein cholesterol levels (mean ± S.D) (mg/dl)	
	7 Days	14 Days
Control (normal saline)	86.22±12.11	92.34±14.40
Group I 400mg/kg b.w.	*100.25±13.06	*122.21±09.54
Group II 800mg/kg b.w	*115.27±18.32	*129.10 ±20.21

Values represent mean ± standard deviation.

*Significant at P<0.05

Table 6 above shows a significant (P<0.05) increase in the mean levels of high density lipoprotein cholesterol in the experimental animals when compared to the control animals.

Table 7: Effect of ethanol extract of *Mucuna pruriens* leaf on serum fatty acid levels in rats.

Treatment	Serum fatty acid levels (mean ±S.D) mg/dl	
	7 Days	14 Days
Control (normal saline)	56.68±5.03	46.33±8.96
Group I 400mg/kg b.w.	*26.66±7.43	*21.48±8.96
Group II 800mg/kg b.w	*24.59±14.34	*14.23±5.76

Values represent mean ± standard deviation. * Significant at P<0.05

Table 7 shows significant (P<0.05) decrease in serum fatty acid levels for the experimental animals when compared to the control animals.

Table 8: Effect of ethanol extract of *Mucuna pruriens* leaf on serum phospholipid levels.

Treatment	Serum phospholipid levels (mean ±S.D mg/dl)	
	7 Days	14 Days
Control (normal saline)	116.89±21.11	82.26±25.12
Group I 400mg/kg b.w.	*94.71±11.28	*60.58±11.28
Group II 800mg/kg b.w	*89.60±12.50	*55.47±12.50

Values represent mean ± standard deviation. *Significant at P<0.05

The ethanol extract of *Mucuna pruriens* leaf exerted a significant (P<0.05) decrease in the serum phospholipid levels for the experimental animals when compared to the controls.

Table 9: Effects of ethanol extract of *Mucuna pruriens* leaf on serum triacylglycerol levels in rat.

Treatment	Serum Triacylglycerol Levels (mg/dl)	
	7 Days	14 Days
Control (normal saline)	13629±160.94	195.55±160.94
Group I 400mg/kg b.w.	*528.00±111.15	*660.74±111.15
Group II 800mg/kg b.w.	*277.67±156.67	*411.52±156.66

Values represent mean ± standard deviation. * Significant at P<0.05

Table 9 shows a significant (P<0.05) decrease in the mean serum triacylglycerol levels in the experimental groups when compared to the controls. It also shows that the marked decreases exerted by the ethanol extract on the lipid profile of the rats were concentration- dependent.

Table 10: Effect of ethanol extract of *Mucuna pruriens* leaf on serum electrolytes levels in rats after 7 days

Parameters	Treatment		
	Normal saline	Extract 400 mg/kg b.w.	Extract 800 mg/kg b.w.
Potassium (K ⁺)	0.178±0.04	0.29±0.11	0.33±0.15
Sodium (Na ⁺)	42.20±4.99	*35.87±1.94	*35.92±0.10
Bicarbonate (HCO ³⁻)	47.30±7.65	48.10±39.78	48.70±3.32
Chloride (Cl ⁻)	1.38±0.22	1.09±0.64	1.95±0.15

Table 11: Effect of ethanol extract of *Mucuna pruriens* leaf on serum electrolytes levels in rats after 14 days

Parameters	Treatment		
	Normal saline	Extract 400mg/kg	Extract 800mg/kg
Potassium (K ⁺)	0.35± 0.02	*0.80 ±0.01	*0.48±0.04
Sodium (Na ⁺)	66.30 ± 2.68	*62.35±0.16	*63.795±1.64
Bicarbonate (HCO ³⁻)	48.36 ± 2.18	44.63±3.64	49.73±3.10
Chloride (Cl ⁻)	1.65± 0.92	*3.55±1.15	*5.46±2.033

Tables 10 and 11 show no significant difference (p>0.05) in the serum concentrations of bicarbonate ions of rats after administration of 400mg/kg b.w and 800mg/kg b.w extract with respect to the controls. However, there was significant increase in serum chloride concentration (p<0.05) and decrease in sodium ions concentration after both 7 days and 14 days, when compared to the control. Also, at 400mg/kg b.w and 800mg/kg b.w respectively, the extract caused no significant difference (p>0.05) in the potassium and chloride ions concentrations after 7 days while there were significant increases (p<0.05) in the serum concentrations of potassium and chloride ions of rats with respect to the controls after 14 days of administration.

IV. Discussion

One of the efficient ways of managing the ever increasing cases of hyperlipidemia and its complications such as arteriosclerosis and hypertension is diet therapy. This is by the control of the major risk factors such as blood cholesterol and triacylglycerol that predispose to these disorders (Ghasi *et al.*, 2000) Herbal remedies are often used in folk medicine to improve the lipid profile thus preventing cardiovascular diseases (Ostlund, 2002).

The results of this study clearly indicate that the administration of extract of *Mucuna pruriens* leaves produced hypolipidemic effect in experimental animals. Phytochemical analysis revealed the presence of flavonoids alkaloid, glycosides, tannins and saponins. Previous studies showed that these phytochemicals acting wholly or partly may be responsible for the lipid-lowering action of some plant extracts (Gaamoussi *et al.*, 2010). Flavonoids prevent the oxidation of low-density lipoprotein, lower the blood levels of cholesterol and triglycerides thereby reducing the risk for the development of atherosclerosis (Subramani and Casmir, 2002). Saponins bind with bile salt and cholesterol in the intestinal tract. This binding causes a reduction of blood cholesterol by preventing its reabsorption (Oalienfill and Siddha, 1990). Cardiac glycosides have been used as diuretics and heart tonics due to their beneficial effects on the heart. They act by affecting the availability of intracellular Ca²⁺ for myocardial contraction (Walter *et al.*, 2002).The hypocholesterolemic effect of the extract may be due to a number of mechanisms including the inhibition of cholesterol biosynthesis; prevention of the oxidation of low-density lipoprotein; conversion of cholesterol into bile acids and inhibition of cholesterol absorption from the intestine due to formation of complexes with compounds such as glycosides and saponins. Furthermore, the result showed that the extract of *Mucuna pruriens* had some significant effects (P < 0.05) on

serum electrolytes (Na⁺ and K⁺) which are known to play some role in the pathogenesis of cardiovascular disorders (Ezekwesili *et al.*, 2008). The observed hypolipidaemic effect by the extract may be ascribed to the phytochemical constituents of the leaf.

These results suggest that the leaf extract of *Mucuna pruriens* might be useful in the treatment hyperlipidaemia and its associated complications such as cardiovascular diseases.

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