Subacute Toxicity Studies of Ethanol Root Extract Of *Mucuna Pruriens* on Albino Wistar Rats

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**ABSTRACT**

**Objective:** The subacute toxicity of ethanolic root extract of *Mucuna pruriens* was evaluated in albino Wistar rats. 

**Methods:** The study was conducted by intraperitoneal administration of the extract at daily doses of 125, 250, 500 and 750 mg/kg body weight to groups of five (5) rats each, for a period of 28 days. Rats in the control group received only 0.5ml of normal saline. 

**Results:** Mortality and significant (p<0.05) decrease in percentage gain/loss in body weight were recorded for all the groups treated with 500 and 750mg/kg body weight of the root extract. There was a significant (p<0.05) increase in the activities of serum biomarker enzymes (Aspartate aminotransferase, Alanine amino transferase and Alkaline phosphatase) and increased serum concentrations of cholesterol, triglyceride and low density lipoprotein cholesterol (LDL-C) with concomitant decrease in high density lipoprotein cholesterol (HDL-C) concentration of rats treated with 500 and 750 mg/kg body weight of the extract, compared to the control group. There was significant (p<0.05) increase in the serum concentrations of urea and creatinine with decrease serum concentrations of total protein and albumin concentration of rats treated with 250, 500 and 750 mg/kg body weight. Treatment with 125 mg/kg body weight significantly (p<0.05) decreased the serum cholesterol concentration of the rats. No significant (p>0.05) changes were recorded in the serum electrolyte concentrations and hematological parameters of all the treated groups. Histological examination of the kidney, liver and heart revealed tissue damage and infiltrations on the groups treated with 500 and 750 mg/kg body weight of *Mucuna pruriens* root extract. 

**Conclusions:** It could therefore be concluded that the intake of high doses of this extract can cause organ and system damage.

**Keywords:** Subacute toxicity, *Mucuna pruriens*, Risk assessment, Drug toxicity

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**INTRODUCTION**

Plants and their derived products have been used extensively for the management of various ailments. Polasa and Nirmala (2003) reported that about 80% of the world population still rely on botanical medicine in their management of health and diseases. The major challenge in the use of plants and their derived products in the management of health and diseases is the lack of information on their safety/toxicity. Plants produce a wide array of toxic chemicals. These toxic chemicals are often held to evolve as defense mechanism against herbivorous animals, particularly insects and mammals (Ernest, 2004). Thus, sufficient knowledge about plant toxicity is empirical to their effective use for the management of health and diseases. *Mucuna pruriens* is an annual twinning legume commonly known as velvet beans. It belongs to the family of Fabaceae (Vermal et al., 2014). It is indigenous to tropical regions and is widely used as food, feed, nutraceutical as well as inremedying various ailments. According to Szabo and Tebbett (2002), the main drawback in the use of *Mucuna pruriens* as food sources and medicine for either human and/or livestock is associated with its chemical constituents. Various research has revealed the presence of novel sterols, flavonoids, alkaloids and saponins (Manyann et al., 2004), however, the whole plant including leaf, stem, root, and seed are known to contain relatively high concentration of 3,4-dihydroyx-L-

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phenylalanine (L-DOPA) (Szabo and Tebbett, 2002), a toxic non-protein amino acid that is a precursor of the neurotransmitter, dopamine.

*Mucuna pruriens* is widely known for the aggressive itching ability of the leaves and pods, especially, the young leaves and pods of the plant. The itching is caused by the high concentration of Mucunain, a proteolytic enzyme and serotonin, a neurotransmitter, in leaves and pods of the plant (Pretty *et al.*, 1998).

*Mucuna pruriens* is among the various medicinal plants that has found great use in the cure and management of various ailments in the traditional medical settings. Various researches has validated the use of various parts of the plants for the treatment of different medical conditions including inflammation, tumor, depression, diabetes mellitus and Parkinson’s disease (Deokar *et al.*, 2016). In this study, the subacute toxicity effect of ethanol root extract of *Mucuna pruriens* was evaluated in female albino wistar rats.

**MATERIALS AND METHODS**

**PLANT MATERIALS**
The *Mucuna pruriens* roots used for the study was collected from the native community, UKE, in Idemili-North L.G.A. of Anambra State, Nigeria. The plant was identified and authenticated by Dr.(Mts.) E.N. Ekpo of the Forest Conservation and Protection Department, Forestry Research Institute of Nigeria, Ibadan. The Herbarium Voucher number of the plant is FHI 107680.

**STUDY ANIMALS**
45 female albino rats weighing between 100-150 grams were obtained from the animal unit of the Faculty of Pharmaceutical Science, University of Nigeria Nsukka. The rats were fed *ad libitum* with standard commercial feed and water in a well-ventilated stainless steel cage. Prior to the study, the animals were acclimatized for a period of 2 weeks at room temperature of 25ºC and 12h light/12h dark cycle. The handling of the rats was in accordance with the standard principles of the laboratory animal care of the United States National Institute of Health (NIH, 1978).

**KITS**
Kits for the assay of AST, ALT, and ALP activities were supplied by TECO Diagnostics, UK. Kits for the determination sodium ion, potassium ion and bicarbonate ion concentrations were supplied by TECO Diagnostics, UK, while the kits for the determination of urea, creatinine, total protein, albumin triglyceride, cholesterol, HDL-cholesterol, and LDL-cholesterol concentration were supplied by Randox Laboratories, UK.

**PROCESSING AND EXTRACTION OF PLANT MATERIALS**
Fresh roots of *Mucuna pruriens* were harvested in large quantity, the roots were washed under continuous current of distilled water for 15 minutes and air-dried at room temperature undershade for 3 weeks in the laboratory. The dried samples were ground into coarse powder using an electronic grinder (Saisho 200W) and stored in an airtight container prior to extraction. 100 grams of the coarse powder was soaked in 1000ml of absolute ethanol for 48 hours. The suspension was filtered using Whatman no1 filter paper, the filtrate was concentrated in a water bath at 50ºC and dried in a vaccum dessicator.

**STUDY DESIGN AND SAMPLE COLLECTION**
20 female albino wistar rats weighing between 120-190g were randomly distributed into 5 groups (I, II, III, IV and V) each containing 5 rats. Group I was the control group and was given only the vehicle comprising 0.5 % Tween 80 in normal saline once daily for 14 days. Animals in groups II, III, IV and V were intraperitoneally administered with 125, 250, 500 and 750 mg/kg body weight of the ethanol extracts, respectively once daily for 14 days, the rats in all the groups were monitored for a further 14 days for post treatment effects.

The rats were observed daily for any signs of toxicity, and their body weights were also recorded weekly throughout the experimental period. On the 29th day of the research, the animals were fasted for about 8 hours, all animals in various groups were anesthetized under diethyl ether and blood samples were collected by cardiac puncture into EDTA and non-EDTA bottles for haematological and biochemical investigations respectively. Blood samples collected into clean non-EDTA bottles were allowed to clot and centrifuged according to groups; and serum was
separated from the clot into clean bottles for the biochemical analyses. The liver, kidneys and heart were excised from dissected rats, immediately cleaned of blood by using physiological saline and weighed. The liver and kidneys were then fixed in 10% formal saline for histopathological examination.

**WEIGHT MEASUREMENT**

The weights of the individual rats in each group were monitored on days 0, 3, 7, 10 and 14 of the study using an electronic weighing balance.

**BIOCHEMICAL PARAMETERS**

The Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) activities were determined according to the methods describe by Reitman and Frankel, (1957) using kits supplied by TECO Diagnostics. The ALP activity was determined according to the method of King (1965). The serum concentration of creatinine was determined using the method described by Bartels et al. (1972). The serum concentration of urea was determined using the method described by Fawcett and Scott (1960). The serum total protein concentration was determined using the method described by Henry et al. (1974). The serum concentration of albumin was determined using the method described by Doumas et al. (1971). Serum sodium ion concentration was determined by the colorimetric method of Maruna, (1958) Serum potassium concentration was determined by the method of Maruna, (1958). The serum concentration of bicarbonate ion was determined using the method described by Foresster, (1976). The serum concentration of cholesterol was determined using the method described by Allain et al. (1974). The serum HDL-cholesterol concentration was determined using the method described by Lopes-virella, (1977). The serum concentration of triglyceride was determined using the method described by Fossati and Prencipe, (1982).

**HEMATOLOGICAL ANALYSIS**

Hematological analysis was performed using an automatic hematological analyzer Medonic (Coulter Beckman - USA-). Parameters included: Red blood cell (RBC) count, white blood cell (WBC) count, hemoglobin (Hb), hematocrit (Hct), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV) as well as differential white blood cell counts.

**HISTOPATHOLOGY**

The histological examination was carried out according to the method described by Pieme et al. (2006). The liver, kidney and heart excised from the rats were fixed with 10% buffered formalin. The tissues were processed for histological examination. Paraffin embedded tissues were sectioned, stained with haematoxylin and eosin and mounted on glass slides. The slides were then examined under light microscope.

**STATISTICAL ANALYSIS**

Data generated from the study was analyzed in a one way ANOVA using SPSS Statistical Package (SPSS version 21). Data were presented as Mean±SD. Values with p<0.05 were considered statistically significant.

**RESULTS**

Clinical signs and Mortality

All the animals in 125 mg/kg and 250 mg/kg dose categories did not exhibit any clinical signs of toxicity throughout the 28 days intraperitoneal administration of *Mucuna pruriens* root extract and no mortality was recorded from the two groups. The animals in the 500mg/kg and 750mg/kg showed clinical signs of toxicity including weakness, weight loss and restricted movement after the first week of treatment. There was significant decrease in their feed and water intake after 3 days of administration and two animals died from the 500mg/kg and 750 mg/kg dose category, each before the end of the experiment (Table 1.)

<table>
<thead>
<tr>
<th>S/N</th>
<th>DOSE</th>
<th>MORTALITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CONTROL</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>125mg/kg</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>250mg/kg</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>500mg/kg</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>750mg/kg</td>
<td>2</td>
</tr>
</tbody>
</table>
The first mortality was recorded for the 750mg/kg dose category after 7 days of intraperitoneal administration of *M. pruriens* root extract, while the second animal died after 11 days of administration. The mortalities in 500mg/kg dose category were recorded after day 10 and day 14 of administration respectively.

Figure 1 below shows the percentage gain in body weight among the various groups over the 28 days study period. There is a significant decrease (p<0.05) in percentage gain in body weight of the rats in 500mg/kg and 750mg/kg dose categories, compared to the control and other dose categories.

![Figure 1: Effect of ethanol root extract of *M. pruriens* on percentage body weight gain/loss of rats administered with different doses of *M. pruriens*](image)

Table 2 shows the effect of intraperitoneal administration of ethanol root extract of *M. pruriens* on the relative weights of some organs of albino wistar rats. The organs weighed include kidneys, liver and heart.

<table>
<thead>
<tr>
<th>ORGAN</th>
<th>CONTROL</th>
<th>125mg/kg</th>
<th>250mg/kg</th>
<th>500mg/kg</th>
<th>750mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>KIDNEY</td>
<td>0.85±0.09</td>
<td>0.87±0.11</td>
<td>0.81±0.15</td>
<td>0.78±0.06</td>
<td>0.66±0.04</td>
</tr>
<tr>
<td>LIVER</td>
<td>10.12±1.11</td>
<td>9.63±1.03</td>
<td>9.74±1.14</td>
<td>8.12±1.03</td>
<td>7.79±0.96</td>
</tr>
<tr>
<td>HEART</td>
<td>0.79±0.14</td>
<td>0.75±0.12</td>
<td>0.72±0.10</td>
<td>0.65±0.90</td>
<td>0.70±0.16</td>
</tr>
</tbody>
</table>

Each value represents mean ± SD, n = 5

Figures (3-15) show the effect of intraperitoneal administration ethanol root extract of *Mucuna pruriens* on some biochemical parameters of albino wistar rats. There were significant changes in the tested biochemical parameters (with the exception of sodium ion, potassium ion and bicarbonate ion concentrations) of rats treated with 500 and 750 mg/kg body weight when compared with the control rats.
Fig 2: Effect of ethanol root extract of *M. pruriens* on serum AST activity of albino wistar rats.

Fig 3: Effect of ethanol root extract of *M. pruriens* on serum ALT activity of albino wistar rats.
Fig 4: Effect of ethanol root extract of *M. pruriens* on serum ALP activity of albino wistar rats.

Fig 5: Effect of ethanol root extract of *M. pruriens* on serum urea concentration of albino wistar rats.
Fig 6: Effect of ethanol root extract of *M. pruriens* on serum creatinine concentration of albino wistar rats.

Fig 7: Effect of ethanol root extract of *M. pruriens* on serum total protein concentration of albino wistar rats.
Fig 8: Effect of ethanol root extract of *M. pruriens* on serum albumin concentration of albino wistar rats.

Fig 9: Effect of ethanol root extract of *M. pruriens* on serum Na⁺ concentration of albino wistar rats.
Fig 10: Effect of ethanol root extract of *M. pruriens* on serum K⁺ concentration of albino wistar rats.

Fig 11: Effect of ethanol root extract of *M. pruriens* on serum HCO₃⁻ concentration of albino wistar rats.
Fig 12: Effect of ethanol root extract of *M. pruriens* on serum total cholesterol concentration of albino wistar rats.

Fig 13: Effect of ethanol root extract of *M. pruriens* on serum HDL cholesterol concentration of albino wistar rats.
Fig 14: Effect of ethanol root extract of *M. pruriens* on serum LDL cholesterol concentration of albino wistar rats.

Fig 15: Effect of ethanol root extract of *M. pruriens* on serum triglyceride concentration of albino wistar rats.
Table 3 shows the effect of 28 days intraperitoneal administration of ethanol root extract of *Mucuna pruriens* on the hematological parameters of albino rats. There were slight changes in the values of Hb, PCV, RBC, WBC, neutrophils, monocytes, lymphocytes and eosinophiles of the rats treated with 750mg/kg body of the root extract when compared with the control. There were also significant changes (p<0.05) in the WBC, neutrophil, monocyte and lymphocyte counts of the rats treated with 500mg/kg body of *M. pruriens* root extract when compared with the control. The neutrophile and lymphocyte counts of the rats treated with 500mg/kg body of *M. pruriens* root extract changed significantly (p<0.05) compared with the control.

Each value represents mean ± SD, n = 5. Values with different superscript are significantly different (P < 0.05). Values with the same superscript are not significantly different at 95% confidence level (P > 0.05)

The effect of 28 days intraperitoneal administration of ethanol root extract of *Mucuna pruriens* on the liver and kidney histology of albino wistar rats are shown in Plates (1-0). The rats administered with ethanol root extract of *M. pruriens* at 500mg/kg (Plate 4) and 750mg/kg (Plate 5) body weight showed distortion of the normal architecture of liver with constricted central vein, cellular depletion and degeneration of the hepatocytes. The kidney sections of rats treated with 500mg/kg (Plate 9) and 750mg/kg (Plate 10) of the root extract also showed distortion of the normal architecture of kidney, revealing a dilated region of glomerulus with constricted urinary space. There was also degeneration of the cells of the juxtaglomerular apparatus and renal tubules.

![Plate 1: Photomicrograph of liver section of the control rats (×400). Stain: H and E.](image)
No lesion
Plate 2: Photomicrograph of liver section of the rats administered with 125mg/kg body weight of ethanol root extract of *M. pruriens* (×400). Stain: H and E.

No lesion
Plate 3: Photomicrograph of liver section of the rats administered with 250mg/kg body weight of ethanol root extract of *M. pruriens* (×400). Stain: H and E.

Plate 4: Photomicrograph of liver section of the rats administered with 500mg/kg body weight of ethanol root extract of *M. pruriens* (×400): CV= Central vein, A= Degenerated hepatocytes, B= Infiltration of the Hepatocytes. Stain: H and E.

Plate 5: Photomicrograph of liver section of the rats administered with 750mg/kg body weight of ethanol root extract of *M. pruriens* (×400): CV= Central vein, A= Lymphocyte Infiltration, B= Degenerated hepatocytes, L= Lesion. Stain: H and E.
No lesion
Plate 6: Photomicrograph of kidney section of the control rats (×400). Stain: H and E.

No lesion
Plate 7: Photomicrograph of kidney section of the rats administered with 125mg/kg body weight of ethanol root extract of *M. pruriens* (×400). Stain: H and E.

No lesion.
Plate 8: Photomicrograph of kidney section of the rats administered with 250mg/kg body weight of ethanol root extract of *M. pruriens* (×400). Stain: H and E.

Plate 9: Photomicrograph of kidney section of the rats administered with 500mg/kg body weight of ethanol root extract of *M. pruriens* (×400): US= Urinary space, H= Hemorrhage, D= Degenerated tubules. Stain: H and E.
DISCUSSION

The current study revealed a significant (p<0.05) decrease in the percentage gain/loss in body weight of the animals treated with 500mg/kg and 750mg/kg body weight of the root extract, compared to control and other treated groups. Increment in body weight and size reflects a positive health status in young animals (Heywood, 1983). The decreased percentage gain/loss in body weight observed at higher doses of the root extract is an indication that the extract may have some harmful effect on the growth pattern of the animals at these doses. This suggestion of toxicity at high doses of the root extract was restated by the mortalities observed at these doses.

In the present study, biochemical examination of the serum was carried out following 28 days intraperitoneal treatment with *Mucuna pruriens* root extract. Serum biomarkers such as AST, ALT, ALP, cholesterol, LDL-C, HDL-C, triglyceride, total protein, albumin, creatinine, urea, sodium ion, potassium ion and bicarbonate ion were analysed to evaluate any toxic effect on the liver and/or kidney as well as on the lipid metabolism. The present study revealed a significant (p <0.05) increase in the activities of the liver marker enzymes, AST, ALT and ALP. Liver plays an important role in the metabolism and excretion of xenobotics, thus, it is highly exposed to the toxic effect of these compounds (Ghosh, 2004). The liver enzymes AST, ALT and ALP leak into the blood circulation following damage to the hepatocytes, thus, they are good indicators of liver integrity and functionality (Rahman et al., 2001). ALT is a better indicator of liver damage than AST, as the latter is also found to some extent in heart, skeletal muscles, kidney, brain, pancreas and blood tissues (Akhtar et al., 2012). These observed elevation in activities of these biomarkers suggest a hepatoxic effect of this extract at high doses.

The liver is an important organ involved in the metabolism of various lipids including cholesterol, cholesterol lipoproteins (HDL-C and LDL-C) and triglycerides. In the present study, treatment with 125mg/kg body weight of the root extract significant (p<0.05) decreased the serum concentrations of cholesterol and LDL-C with concomitant increase in the serum concentration of HDL-C. This suggests that *Mucuna pruriens* root extract at its low non-toxic doses could affect the lipid metabolism in a positive manner. Treatment with 500mg/kg and 750mg/kg body weight of the root extract however, significantly (p<0.05) increased the serum cholesterol, LDL-C and triglyceride concentrations with concomitant decrease in the serum HDL-C concentration. This observation could be due to the inability of the liver to metabolise and excrete cholesterol following treatment with high doses of *Mucuna pruriens* root extract. These significant changes in the activities of the liver biomarker enzymes and the concentrations of some lipid metabolic products of the liver observed at 500mg/kg and 750mg/kg body weight of *Mucuna pruriens* suggests that the subacute treatment with high doses of the root extract altered the integrity and functionality of the liver. This suggestion was confirmed by the the histological examination of the liver tissues from these groups, showing distorted and degenerated liver architecture.

In the current study, treatments with 500mg/kg and 750mg/kg body weight of *Mucuna pruriens* root extract significantly (p<0.05) increased the serum concentrations of urea and creatinine when compared with the control and other treated groups (125mg/kg and 250mg/kg body weight). Urea and creatinine are elevated in cases of kidney dysfunction, thus are important biomarkers of renal damage and dysfunction (Mukinda and Eagles, 2010). Creatinine is a nitrogenous product from kidney and its the most common clinically used biomarker for kidney damage (Hayes, 2008). The kidney is the major organ involved in the excretion of xenobotics, thus it is heavily
affected by toxic exogenous substances. The observed increase (p<0.05) in the serum concentrations of urea and creatinine at 500mg/kg and 750mg/kg body weight of *Mucuna pruriens* ethanol root extract suggests that the plant extract may have caused damages to the kidney at these doses. This suggestion was made stronger by the histological examination of the kidney tissues from these groups, showing degenerated cells. The kidney is also vital in ensuring an effective electrolyte homestasis. Serum electrolyte concentrations could therefore be informative in evaluating the state of the kidney. In the current study, however, there is no significant difference (p>0.05) in the serum concentrations of sodium ion, potassium ion and bicarbonate ion, an indication that if any injury was caused to the kidney due to the treatment with the root extract, it did not affect the integrity of the organ for an effective electrolyte metabolism.

In the current study, the histopathological examination of the liver, rats treated with doses of 125mg/kg and 250mg/kg of the ethanol root extract of *M. pruriens* showed no changes in the microscopic structure of the liver. The general architecture of the liver, appearance of the hepatocytes, the hepatic sinusoids, portal triads and central veins are normal as compared with controls. But there were distortion in the liver cells architecture and cell degeneration in the liver sections of animals treated with 500mg/kg and 750mg/kg body weight dose categories. The result were also accompanied by the adverse effects of the extract in the biochemical markers (such as ALT, and AST), which showed statistically significant changes compared with the control group. In the histopathological study of the kidney, rats treated at both doses (500 and 750mg/kg) of the extract showed degeneration of the cells and urinary system as compared to controls, the normal appearance of glomeruli, tubules and macula densa were distorted compared to normal control and other dose categories (125 and 250mg/kg). The result can be further supported by the values of biochemical parameters of the blood (such as urea, creatinine, and total protein), which are main indicator of kidney damage.

**CONCLUSION**

The data from the study indicated that though the ethanol root extract of *Mucuna pruriens* is relatively safe at low doses, high doses of the root extract is potentially very toxic to liver and kidney, this is evident by the marked changes in the liver and kidney function parameters at high doses of the extract. These results therefore suggest that caution should be applied in the use of the plant products, especially at high doses.

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