Toxicity of *Mucuna pruriens* seed extract on the kidney of adult Sprague-Dawley rats

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**Background:** The commonly acceptable knowledge that herbal medications have little or no toxicity and are absolutely safe makes people consume them indiscriminately. All parts of *Mucuna pruriens* have been reported to possess valuable medicinal properties, but its potential toxicity on vital organs remains unexplored.

**Objective:** To determine the deleterious effect of *Mucuna pruriens* on the Kidney of Adult Sprague-Dawley Rats.

**Methodology:** Twenty Sprague-Dawley rats were used and divided into four groups of five rats per group. Group I served as control and received distilled water and groups II-IV received 50, 100 and 200 mg/kg of the extract respectively for 2 weeks. The animals were sacrificed, blood was collected for kidney function test and the kidneys were excised via ventral laparatomy. The right kidney was fixed for histological studies while the left kidney was analysed for biochemical markers of oxidative stress.

**Results:** Lipid peroxidation increased significantly while superoxide dismutase and glutathione recorded a significant decrease in activities when the treated groups were compared to control. Creatinine decreased significantly and urea increased significantly when treated groups were compared to control. Histological sections showed degenerative changes and tubular necrosis in the kidney at higher doses.

**Conclusion:** *Mucuna pruriens* causes degenerative changes in glomerular epithelia and reduced urea clearance possibly by an oxidative stress mechanism.

**Keywords:** Kidney, *Mucuna pruriens*, superoxide dismutase (SOD), creatinine, urea.

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1. Introduction

Medicinal plants are used as one of the available medicines especially in developing countries (Hashim et al, 2010). Plants used as traditional medicine contain a wide range of substances that are effective in the treatment of sicknesses and diseases. Clinical microbiologists also have great interest in screening of medicinal plants for new therapeutics (Penyasamy et al, 2010). Among the various under-utilized wild legumes, is the velvet bean called *Mucuna pruriens* that is found in tropical and sub-tropical regions of the world. It is considered a viable source of dietary proteins (Janardhanan et al, 2003; Pugalenthi et al, 2005) due to its high protein concentration of 23–35%. All parts of *Mucuna pruriens* possess valuable medicinal properties (Sathiyanarayanan and Arulmozhi 2007). *In vitro* and *in vivo* studies on *Mucuna pruriens* extracts revealed the presence of substances that exhibit a wide variety of pharmacological effects, including anti-diabetic, anti-inflammatory, neuroprotective and anti-oxidant properties, probably due to the presence of L-dopa, a precursor of the neurotransmitter dopamine (Misra and Wagner, 2007).

It is known that the main phenolic compound of *Mucuna pruriens* seeds is L-dopa (approximately 5%) (Vadivel and Pugalenthi 2008). Phytochemical screening of the plant revealed that it contains alkaloids, flavonoids, tannins, saponins, cardiac glycosides, anthraquinones and carbohydrates (Minari et al, 2016).
Available data on the toxicity of herbal drugs is limited. The commonly acceptable knowledge that herbal medications have little or no toxicity and thus are absolutely safe makes people consume herbal medicines indiscriminately. In lieu of this fact, there is a growing concern about the safety of most herbal drugs. This study was carried out to investigate the effect of *Mucuna pruriens* on the kidney in Sprague-Dawley rats.

2. Methods

2.1 Plant Materials

*Mucuna pruriens* plants with mature seeds were procured from Mushin market, a local market in Lagos State, Nigeria. They were identified and authenticated in the Department of Botany, Faculty of Science, University of Lagos, Nigeria where the voucher specimens of the fruits are kept in the herbarium with a voucher number of LUH 4922.

2.2 Preparation of Extract

The extraction was carried out in the Department of Pharmacognosy, Faculty of Pharmacy, University of Lagos. The seeds of the plants were collected in the month of August and September. Briefly, seeds were obtained from the pods, air-dried and pulverized into fine powder using a mortar and pestle. 1.5 kg of fine powder was mixed with absolute methanol and placed in the soxhlet apparatus for duration of 6 hrs at 60 °C. The solvent was removed by distillation and semisolid mass was dried by using a hot water bath at 40 °C and the % yield of the methanolic extract of *Mucuna pruriens* seed was calculated to be 147.8 g and stored at -20 °C until analysis.

2.3 Experimental Animals

A total of twenty healthy Sprague-Dawley rats with weights ranging between 120 and 140 g were used for this study. They were housed in well standard ventilated wire mesh plastic cages in the Animal House of the Department of Anatomy, College of Medicine of the University of Lagos, Nigeria under standard room temperature (26-28 °C) and relative humidity (50-55%). The rats were exposed to twelve hours light and 10 hours dark cycle. They were allowed unrestricted access to water and commercial rat chow *ad libitum*. They were left to acclimatize for a period of two weeks before the commencement of the experiment. The animals were identified by different ear tags and the weights of the animals were taken weekly. All experimental procedures and techniques were in compliance with the guiding principles for research involving animals (Helsinki 2002).

2.4 Experimental Design

The animals were divided into four groups of five animals each. Group I received distilled water and served as control. Groups II to IV orally received varying doses of methanolic seed extract of *Mucuna pruriens* dissolved in distilled water at 50, 100 and 200 mg/kg respectively for 2 weeks. The rats were monitored for toxicity and their body weights were measured. At the end of the experiment, the rats were sacrificed using chloroform as anaesthesia, a ventral laparotomy was performed and the kidneys were dissected. The right kidney was fixed in 10% formal saline for histological studies while the left kidney was stored in -20 °C for biochemical analysis of oxidant status and blood was collected by cardiac puncture for kidney function test and analysed immediately.

2.5 Histological Procedures

Following fixation, the harvested tissues were dehydrated in graded ethanol, embedded in wax and sectioned to 5 μm thickness. The sections were stained with routine haematoxylin and eosin stains and photomicrographs were made at a magnification of 100 and 400 using Olympus and Leica microscopes (Haneia et al, 2013).

2.6 Biochemical Analysis of Oxidant status

The kidneys were washed in ice cold 1.15% KCl solution, blotted and weighed. They were then homogenized with 0.1 M phosphate buffer (pH 7.2). The tissues were introduced into mortar and laboratory sand was then added. This was crushed using a pestle. The resulting homogenate was centrifuged at 2500 rpm speed for 15 mins. Thereafter, it was removed from the centrifuge and the supernatant was decanted and stored at -20 °C until analysis.

Superoxide Dismutase (SOD) was assayed by its ability to inhibit the auto-oxidation of epinephrine, determined by the increase in absorbance at 480 nm as described by Sun and Zigma (1978). The enzyme activity was calculated by measuring the change in absorbance at 480 nm for 5 min.

Glutathione (GSH) activity was estimated according to the method described by Sedlak and Lindsay, 1968. To the tissue homogenate, 10% TCA was added, centrifuged. 1.0 ml of supernatant was treated with 0.5 ml of Elman's reagent (19.8 mg of 5, 5-dithiobisnitro benzoic acid (DTNB) in 100 ml of 0.1% sodium nitrate) and 3.0 ml of phosphate buffer (0.2 M, pH 8.0). The absorbance was read at 412 nm.

Malondialdehyde (MDA) an index of lipid peroxidation was determined using the method of Buege and Aust (1978). The supernatant was removed and the absorbance was read at 532 nm. MDA was calculated using the molar extinction coefficient for MDATBA- complex of 1.56 × 10⁵ M⁻¹ cm⁻¹.

2.7 Determination of Creatinine concentration

The creatinine concentration in serum was measured using Creatinine K commercial kits (LabtestDiagnostica SA, Lagoa Santa, Brazil), which uses a two-point optimized kinetic procedure based on the modified-Jaffe reaction. For disposing purposes, 50 µL of the serum sample was added to 50 µL of alkaline picrate, mixed and aspirated into the automatic analyser bucket set to zero at 510 nm, and then measured the absorbance at 30 seconds. The results were expressed in µmol/l.

2.8 Determination of Urea concentration

The urea concentration in serum was measured using Liquiform Urea UV test (LabtestDiagnostica SA, Lagoa Santa, Brazil) which uses an enzymatic system by UV
photometry and two-point Kinetics. For disposing purposes, 10 µL of the serum were aspirated into the photometer reservoir adjusted to 340 nm, and then measured the absorbance at 30 seconds. The results were calculated and expressed in mmol/l.

2.9 Statistical Analysis

The data obtained were analysed statistically by students’ T-test and one-way Analysis of Variance (ANOVA) test. The level of significance was at p<0.05. The data were expressed as mean ± SEM.

3. Results

No mortality was recorded during the experiment and no signs of toxicity symptoms were experienced by the animals as a result of the administered extract.

The study showed that the body weight of all the treatment groups when the initial body weights were compared to the final body weights during the course of the experiment were significantly higher. The body weights were lower in groups II and III when compared to IV in the treatment groups (Table 1).

Effect of Mucuna pruriens seed on Biochemical analysis of oxidant status

The lipid peroxidase was higher in MDA when the treatment groups were compared to the control. Furthermore, Superoxide dismutase activities and glutathione hydroxylase were significantly lower when the treatment groups were compared to control (Table 2).

Effect of Mucuna pruriens seed extract on Kidney function test

The value of creatinine was lower when treatment groups were compared to control and the decrease was in a dose dependent manner. Urea was significantly higher when groups II and III were compared to control and was lower when group IV was compared to control (Table 3).

Table 1: Effect of Mucuna pruriens seed extract on body weight in S-D rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Initial Weight (g)</th>
<th>Final Weight (g)</th>
<th>Weight Difference (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>122.7 ± 1.1</td>
<td>146.3 ± 1.3</td>
<td>16.13</td>
</tr>
<tr>
<td>II</td>
<td>126.2 ± 1.8</td>
<td>148.0 ± 1.6</td>
<td>14.73</td>
</tr>
<tr>
<td>III</td>
<td>127.9 ± 1.9</td>
<td>150.0 ± 1.7</td>
<td>14.73</td>
</tr>
<tr>
<td>IV</td>
<td>130.1 ± 1.7</td>
<td>151.8 ± 1.4</td>
<td>14.30</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± Standard Error of Mean (SEM), N = 5. *p<0.05 significant when initial weight is compared to final weight

Table 2: Effect of Mucuna pruriens seed extract on oxidative stress markers in S-D rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>SOD (µ/mg protein)</th>
<th>GSH(µ/mg protein)</th>
<th>MDA (µ/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>101.75 ± 2.80</td>
<td>22.36 ± 1.60</td>
<td>4.46 ± 0.37</td>
</tr>
<tr>
<td>II</td>
<td>90.75 ± 0.57*</td>
<td>9.04 ± 0.96*</td>
<td>5.81 ± 0.41*</td>
</tr>
<tr>
<td>III</td>
<td>67.05 ± 1.55ab</td>
<td>14.10 ± 1.69ab</td>
<td>4.58 ± 0.40b</td>
</tr>
<tr>
<td>IV</td>
<td>93.08 ± 0.74ac</td>
<td>13.39 ± 0.60ab</td>
<td>5.19 ± 0.22ac</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± Standard Error of Mean (SEM), N = 5. a,b,p<0.05 significant compared to group I; a,b,p<0.05 significant compared with group II; a,b,p<0.05 significant compared with group III; a,b,p<0.05 significant compared with group IV.

Table 3: Effect of Mucuna pruriens seed extract on Kidney function test in S-D rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Urea (mmol/l)</th>
<th>Creatinine (µmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>4.48 ± 0.24</td>
<td>62.00 ± 4.39</td>
</tr>
<tr>
<td>II</td>
<td>6.60 ± 0.76a</td>
<td>58.60 ± 1.50</td>
</tr>
<tr>
<td>III</td>
<td>6.88 ± 0.16a</td>
<td>51.58 ± 0.70a</td>
</tr>
<tr>
<td>IV</td>
<td>3.62 ± 0.51bc</td>
<td>37.60 ± 0.40abc</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± Standard Error of Mean (SEM), N = 5. a,b,p<0.05 significant compared to group I; a,b,p<0.05 significant compared with group II; a,b,p<0.05 significant compared with group III; a,b,p<0.05 significant compared with group IV.
Plate 1: Photomicrograph of kidney section of control showing the renal cortex with glomeruli (white arrow). The renal tubules (blue arrow) and interstitial spaces (slender arrow) appear unaffected. Medullary ray are seen with the tubules.

Plate 2: Photomicrograph of kidney section of low dose showing the renal cortex (black arrow). The glomerulus (white arrow) appeared unaffected. Also, the renal tubules (blue arrow) and interstitial spaces (slender arrow) appeared normal.

Plate 3: Photomicrograph of kidney section of medium dose showing mesangial expansion and hypercellularity of the glomeruli (white arrow). Renal tubules (blue arrow) and the interstitial spaces contain mild haemorrhage (red arrow).

Plate 4: Photomicrograph of kidney section of high dose showing tubular necrosis (green arrow). The interstitial spaces contain severe haemorrhage (red arrow). There is swelling of the renal corpuscles (white arrow) and the interstitial spaces appears abnormal (slender arrow).
Effect of *Mucuna pruriens* seed extract on Histology

The photomicrograph of kidney sections of control group showed normal histology. The cortex with glomeruli and mesangial cells were seen. Capsular spaces, renal tubules, interstitial spaces and medullary ray were also seen (Plate 1). Histological sections from group II also appeared unaffected when compared to the control group (Plate 2). However, groups III and IV showed epithelial degeneration with mild to severe haemorrhage in the interstitial spaces when compared to the control (Plate 3 and 4).

4.0 Discussion

Body weights increased significantly in all the treatment groups when initial body weights were compared to final body weights. This suggests that the extract does not interfere with appetite, digestion and/or nutrient uptake. The positive correlation with body weight may be attributed to its micronutrient and phytochemical composition (Minari et al, 2016).

Sections of groups I and II showed unaffected histology with renal cortex having glomeruli and tubules within the medullary rays. Groups III and IV showed interstitial spaces containing haemorrhage. Furthermore, hypercellularity of the glomerulus was observed in group III and tubular necrosis in group IV. The presence of haemorrhage can be as a result of rupture in the capsule, damage to the glomerulus and epithelial degeneration (Suchismita et al, 2015). The epithelium plays essential role in the mechanisms involved in blood clearance and recovery of essential metabolites from the glomerulus by active transport. Degeneration of epithelium will result in severe reductions in glomerular filtration leading to renal dysfunction (Smith et al, 2006) and ultimately to renal failure.

The role of free radicals in normal cellular functions and different pathological conditions has been a focus of pharmacological studies in the recent times. When there is an imbalance between activities of ROS and antioxidant/scavenging defence systems, oxidative stress (OS) occurs (Aprioku, 2013). A good number of studies have shown OS is involved in the development of several disease conditions. MDA results from lipid peroxidation of polyunsaturated fatty acids. Lipid peroxidation is a physiological event that occurs in normal cells to some extent (Olayinka et al, 2015). It is a well-established mechanism of cellular injury in animals, and commonly used as marker for the induction of oxidative stress in cells (Qasim and Mohmood, 2015). This present work showed an increase in MDA activity in the kidneys of treated rats. An increase in the level of MDA will increase the generation of free radicals and increase the cells susceptibility to oxidative stress which will invariably alter cell membrane integrity, permeability and function of the kidneys (Donaldson and Knowles, 1993; Yin and Lin, 1995; Little and Gladen, 1999; Saber and Wael, 2012; Laamech et al, 2016). Superoxide dismutase (SOD) constitutes an important link in the biological defence mechanism through dismutation of endogenous cytotoxic superoxide radicals to H₂O₂ and O₂ which are deleterious to polyunsaturated fatty acids and proteins (Fridovich, 1975). SOD catalyses the dismutation of superoxide anions and prevents the subsequent formation of hydroxyl radicals. (Halliwell and Gutteridge, 1984; Imlay and Linn1988). From this study, SOD was observed to be significantly reduced in the renal tissues. It can be inferred therefore that overproduction of ROS overwhelmed the antioxidant defence system provided by SOD thereby resulting in oxidative stress.

One of the most important antioxidant systems is the glutathione redox cycle (Sharma and Paliwal, 2013). Glutathione is highly abundant in cytosol, nuclei and mitochondria, and is the major soluble antioxidant in these cell compartments (Sharma et al, 2010). Glutathione (GSH), the main component of the endogenous non protein sulphhydryl pool is known to be a major low molecular weight scavenger of free radicals in the cytoplasm (Toklu et al, 2013). This study reported a significant decrease in GSH activities. It is a general knowledge that decreased levels of GSH promotes generation of ROS and OS with a cascade of effects, thereby affecting functional as well as the structural integrity of cell and organelle membranes (Singh et al, 2000). From our study, it can be deduced therefore that the damage on the kidney resulted in reduction in the antioxidant defence system.

Creatinine is synthezised in the liver and it passes into circulation where it is taken up almost entirely by the skeletal muscles. It is commonly measured as an index of glomerular function (Treasure, 2003). It is excreted exclusively through the kidney. Our study showed reduction in creatinine levels. Although low levels of serum creatinine indicate normal kidney function however, it cannot be completely ruled out from this study that the kidneys are under some stress.

Urea is a nitrogenous compound and the main end product from protein breakdown. Urea is made predominantly from ammonia and bicarbonate and about 90% of urea produced is excreted through the kidney (Ranjna, 1999; Walmsley et al, 2010). Increase in serum urea level indicates diminished ability of the kidneys to filter waste products from the blood and can be used as indicators of nephrotoxicity (Pagana et al, 1998, Bremer and Floyd, 1999, Burtis and Edward 1999, Wallach, 2000, Henry, 2001). Our result showed an increase in the level of urea which may suggest impairment in the normal kidney function.

5.0 Conclusion

This study showed that *Mucuna pruriens* seed extract at high doses causes epithelial degeneration and reduces renal urea clearance probably by an oxidative stress mechanism.

Conflict of Interest declaration

The authors declare no conflict of interest.
References


