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Phytochemical and toxicological studies of *Mucuna pruriens* leaves

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Abstract
The study assessed the phytochemical, acute toxicity, sub-acute toxicity and blood chemistry of the aqueous leaf extract of *Mucuna pruriens*. Powdered leaf of *Mucuna pruriens* was extracted by maceration. 24 albino mice used for acute toxicity were administered between 1-8 g/kg body weight of the extract while the control group received distilled water. 12 Wistar albino rats were given oral single dose of 6 and 8g/kg of extract and water as control. For sub-acute toxicity, 20 Wistar albino rats were given 0.5, 2, 6 and 8g/kg body weight/day of the extract for 30 days. The biochemical parameters examined were Na^+, K^+, CO_3^{2-}, Cl^- , urea, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase enzymes, total and conjugated bilirubin. Phytochemical screening revealed the presence of carbohydrate, glycosides, saponins, anthraquinone, cardiac glycosides, and flavonoids in the plant. The extract at doses of 0.5, 2 and 8g/kg decreased creatinine and increased Na^+ and Ca^{++} levels. No significant differences in other parameters were found. No signs of toxicity or behavioural changes were observed at all doses. *Mucuna pruriens* leaf extract is considered safe at these doses in the short term.

Keywords: Phytochemical; Toxicity; Blood chemistry; *Mucuna pruriens*

INTRODUCTION

Various plants produce pharmacological and physiological effects and are therefore used in ethnomedicine for the treatment of various diseases. These pharmacological and physiological effects are usually attributed to the secondary metabolites present in these plants and hence a study of the phytochemical constituents of different plants is very important. These chemical constituents produced by the plant can be beneficial or harmful to man and animal. The idea that herbal medicines are safe because they are derived from nature is no longer tenable as adverse effects have been reported with the use of some herbal preparations (Basu and Anvukkarasu, 2006). It is therefore important that toxicity studies be carried out on all herbal preparations used in medicines (Akindele and Busayo, 2011).

Mucuna pruriens is a common weed of field crop found in bushes and fallow farmland. It is native to southern China and Eastern India. However, it is widely distributed in the tropics including Nigeria. It is a vigorous annual climbing legume. Young *Mucuna pruriens* are covered with hairs which disappear as the plant gets older. The leaves are trifoliate and the flowers are purple. The pods are curved and longitudinally ribbed while the seeds are black and ovoid and contact with the skin

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produces severe itching. The plant is used agriculturally to improve soil fertility (Gamaniel, 2000). Ethno medically, the plant is used for the treatment of various ailments such as, the improvement of libido and sexual performance, reverse osteoporosis, improve cholesterol profile and to strengthen the immune system (Prakkash *et al*., 2001, Malluruwar *et al*., 2006). These effects have been attributed to the presence of bioactive constituents such as mucinine, mucunadine, mucunadenine, pruriendine and other chemical substances such as lecithin, glutathione, nicotine, L-dopa and gallic acid which has been isolated from the plant (Guerranti *et al*., 2001; Raina and Khatri, 2011).

Literature survey did not reveal, to the best of our knowledge, any study on the toxicity of the plant. The present work investigates the acute and sub acute toxicity and the effect of the administration of the extract on some biochemical parameters.

EXPERIMENTAL

Collection and preparation of plant material. The leaf of *Mucuna pruriens* was collected from bushes in Eburugu, Ohaozara Local government Area, Ebonyi State, Nigeria in July 2011. The plant was identified and authenticated by the herbarium curator, Department of Pharmacognosy, Faculty of Pharmacy, University of Benin; where a voucher specimen was deposited in the herbarium. The leaves were sun-dried for four days and ground using mortar and pestle. The fine powder was preserved in airtight and moisture-free container. The powder was then used for the work.

Phytochemical studies. Standard Phytochemical screening techniques were employed to detect the presence of some secondary metabolites (Harborne, 1992, Sofowora, 1993).

Toxicological evaluation.

Extract preparation. 500g of the powdered leaf sample was macerated in 4 liters of distilled water for 2 hours. It was filtered and stripped of the solvent according to standard method. The extract weighing 107.3g (21.46% yield) was used for toxicological studies.

Acute toxicity study. Twenty four healthy albino mice of either sex weighing between 19.1g to 28.4g were bought from Animal House, University of Ibadan, Oyo State, Nigeria and acclimatized in the animal house for 4 weeks. The mice were divided into 6 groups of 4 animals per group and were fed with super mix® and Top feeds grower mash® and had access to water ad libitum. *Mucuna pruriens* leaf extract was administered as a single dose orally, using orogastric tube at doses of 1000, 2000, 4000, 6000, and 8000 mg/kg body weight and the control group received 2ml each of distilled water. The animals were observed for 14 days for any signs of behavioral changes, toxicity and mortality. Wistar albino rats (12) of either sex weighing 115 to 270g were bought from the same place as above and acclimatized for 4 weeks. The rats were divided into 3 groups of 4 animals per group and were fed as above. *Mucuna pruriens* leaf extract was administered orally as a single dose using orogastric tube at doses of 6000, and 8000 mg/kg body weight and the control group received 2ml of diluent (distilled water). The animals were observed for 14 days for any signs of behavioural changes, toxicity and mortality.

Sub-acute toxicity study. Wistar albino rats of both sexes weighing between 82g to 270g from the same source were divided into 5 groups of 4 animals per group and housed under same condition as described above. *Mucuna pruriens* leaf extract were administered using orogastric tube for 30 days at doses of 500, 2000, 6000 and 8000mg/kg body weight/ day and control group received

4ml of distilled water (diluents). Toxicity signs were observed daily and body weight changes were recorded every 7 days till the end of the study.

Clinical test parameters. At the end of the 30th day, the animals were weighed and sacrificed with chloroform anesthesia and 5ml of the blood sample collected from each animal from the abdominal aorta and kept in lithium heparin bottle for blood chemistry analysis using the Systemx Kx 21 hematology analyzer at the Federal Medical centre Asaba, Delta state.

Statistical analysis. The values are expressed as mean ± standard error of mean (SEM). The result was analyzed using student t-test.

RESULTS

Phytochemical result of the phytochemical screening is presented in Table 1. It revealed the presence of carbohydrate, anthraquinones, saponins, cardiac glycosides and flavonoids.

Table 1: Phytochemical constituents of *Mucuna pruriens* leaf

Test	Class of compound	Result
Molisch	Soluble carbohydrate	+
Fehling's	Reducing sugar	+
Keller-Killani	Deoxy-sugar	-
Hager's	Alkaloid	-
Wagner's	Alkaloid	-
Mayer's	Alkaloid	-
Dragendorff's	Alkaloid	-
Bornntrager's	Anthraquinone	+
Frothing test	Saponin	+
Lieberman-Burchard's	Steroidal cardiac glycoside	+
Salkowski's	Steroidal cardiac glycoside	+
Ferric chloride	Tannins	-
Sodium hydroxide	Flavonoid	+
Lead acetate	Flavonoid	+
Ferric chloride	Flavonoid	+

Table 2: The effect of *Mucuna pruriens* leaf extract on blood chemistry of treated rats

	Group 1 Control	Group 2 500mg/kg	Group 3 2000mg /kg	Group 4 6000mg /kg	Group 5 8000mg/kg
Urea(mmol/L)	6.77±0.54	6.83±0.40	6.90±0.10	6.90±0.76	7.15±1.03
Creatinine(µmol/L)	71.33±1.76	55.25±3.07	48.50±0.50	62.00±6.03	49.00±3.24
Na^+ (mmol/L)	139.00±0.19	138±1.78	154.5±7.50	137.33±4.03	136.50±2.02
K^+(mmol/L)	5.73±0.19	6.60±0.26	7.50±0.70	7.23±0.33	7.10±0.39
Cl^-(mmol/L)	90.67±1.76	82.00±4.00	83.30±1.50	80.00±3.06	85.00±1.92
HCO_3^-(mmol/L)	23.63±1.30	26.25±0.52	24.25±2.75	24.00±0.76	25.13±0.47
Ca^+(mmol/L)	1.80±0.06	2.00±0.04	2.00±0.00	1.80±0.10	1.98±0.03
AST(µ/L)	86.67±22.26	46.25±13.09	57.00±10.00	35.00±5.03	42.50±6.20
ALT(µ /L)	41.66±18.00	17.00±2.65	28.5±7.50	16.00±3.61	17.50±1.04
Alkaline phosphatase(µ /L)	28.00±6.56	28.25±5.89	50±11.00	22.67±4.81	37.25±6.21
Total bilirubin (µ mol/L)	5.67±0.99	6.95±1.05	6.15±0.34	6.70±1.45	8.85±1.70
Conjugated bilirubin(µ mol/L)	0.93±0.18	1.40±0.30	1.55±0.32	1.77±0.34	1.70±0.44

Tests were done in triplicates and values expressed as mean ± standard error of mean.

DISCUSSION

The chemical constituents present in *Mucuna pruriens* leaf were carbohydrate (reducing sugar), anthraquinone, flavonoid and glycoside. These active constituents' are believed to be responsible for the observed therapeutic effect (Oduola *et al*., 2007). The result is in agreement with a previous work

which shows that there is no alkaloid present in the leaf (Udensi *et al*., 2008).

The acute toxicity study is done to give us an indication of the range of the drug that could be toxic to the animal and to establish the therapeutic index of the drug. The results show no behavioural changes, toxicity or mortality at the maximum oral dose level of 8g/kg body weight (Vadivel and Janardhanan, 2000).

Sub-acute toxicity was done to evaluate the effect of the plant on the body organs and tissues. The long term use of a drug may produce some effects which might not be detected initially and since most drugs used for various for a long period, sub-acute toxicity is more reflective of these effects. However, there were no observable changes in the general behavior of the animal and the body weight as a result of the administration of the extract.

Aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase enzymes, urea and bilirubin were assayed to determine the effect of the extract on the liver and bile. These enzymes are normally affected following liver and bile damage. Large increases of ALT and AST with small increases in alkaline phosphatase activity favour hepatocellular damage while small increase in AST and ALT with large increases of alkaline phosphatase activity favour biliary obstruction. In this study, there was a significant decrease in both AST and ALT while alkaline phosphatase was unaffected. This might infer that the liver enzymes were not induced. Hyponatreamia and reduced serum are associated with hepatic disease (Gines and Guevara, 2008). High urea with high creatinine and low urinary sodium excretion is indicative of hepatorenal failure with grave consequences (Ijeh *et al*., 2004). The study shows that these enzymes decrease in the treated animals except for alkaline phosphatase and bilirubin (total and conjugated). However this was not statistically significant at $p < 0.05$.Thus the plant extract may not have negative effects on the liver and bile since it decreased liver function tests in the treated animal compared to control.

Urea, creatinine, Na^+, K^+, Cl^- and $HC0_3^-$ were used to measure the effect of *Mucuna* leaf extract on the kidney. Decreased values of Na^+, K^+, Cl^- and HCO_3^- are indicative of renal failure, severe vomiting, diarrhea, dehydration as well as metabolic acidosis (Chukwudi *et al*., 2011). The study shows that creatinine and sodium levels were decreased by the plant. It may be safe to infer that the leaf extract does not cause renal damage. The plant increased Na^+ level in the treated group compared to control.

The toxicity of the extract on the bone was assessed using alkaline phosphatase and Ca^{2+}. Alkaline phosphatase which increases significantly in various bone disease and calcification of matrix is critically dependent on the alkaline phosphatase (WHO, 1978), which is produced by osteoblasts and degrades pyrophosphatase, a natural inhibitor of mineralization. There was no statistical difference between the plant extract and the control groups which indicates that the plant may have no effect on the bones.

Bilirubin was used to access the effect of the plant on the red blood cells and liver. Total and conjugated bilirubin increase following increase in hemolysis or liver disease or both, and can lead to jaundice on the skin or kernicterus in the brain (Majekodunmi *et al*., 2011). However, the result shows that there was no significant increase in the total and conjugated bilirubin in the treated group when compared to control. The plant can be used safely in a patient without dangers of jaundice and kernicterus as well as hemolysis and liver disease.

The plant may be considered safe at the present doses used in the study but further toxicological assessment of the plant such as

subjecting the various organs to histopathological evaluation to access the effect of the plant on various organs still need to be carried out.

Conclusion

Phytochemical test revealed that carbohydrate as reducing sugar, glycosides (saponin, anthracene derivatives, cardiac, steroidal and flavonoids) are present in the leaf of *Mucuna pruriens*. There was no evidence of acute toxicity observed at the maximum dose (8g/kg) used and is thus considered safe and free from acute toxicity. Sub-acute toxicity indicate that the *Mucuna pruriens* leaf extract do not cause toxic/ harmful effect on the kidney, liver, bile, bone and red blood cells.

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