ANALYSIS OF SOME BIOCHEMICAL AND HAEMATOLOGICAL PARAMETERS FOR *MUCUNA PRURIENS* (DC) SEED POWDER IN MALE RATS

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ABSTRACT

The biochemical and haematological effects of the seed powder of *Mucuna pruriens* in male rats were evaluated to establish some biological properties of this potential biopesticide currently undergoing investigation. The result showed that *Mucuna pruriens* seed extract produced a significant (P<0.05) increase in white blood cell (WBC) count, as well as in bilirubin concentrations, alkaline phosphatase (ALP), protein and creatinine levels measured. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were significantly reduced (P<0.05) in comparison with the experimental control. PCV, Hb, albumin level and WBC differential counts gave no significant difference between treated and control groups. The results revealed metabolic imbalance in the rats which suggests a mild cholestasis effect of the extract.

Keywords: *Mucuna pruriens*, alanine aminotransferase, aspartate aminotransferase.

INTRODUCTION

Enzymes are proteinous biomolecules that catalyze metabolic reactions (Grisham and Reginald, 1999); they are responsible for anabolic and catabolic activities in biosystems. Significantly elevated levels, for example, of Alanine aminotransferase (ALT) in the plasma signify extensive hepatic activity and possible damage (Champe, 2001). Similarly, haematological parameters are used as laboratory indices to evaluate events in biosystems; the WBC count indicates the immune status and haematologic integrity of the body defence mechanism**.** *Mucuna pruriens* seed is claimed to be used as a preservative for seed storage in some parts of Plateau and Benue states in Nigeria (Aguiyi *et al*., 1997). The plant is an annual, climbing shrub with long vines that can reach over 15 m. It bears white, lavender, or purple flowers and pods that are covered in loose orange hairs which cause a severe itch if they come in contact with the skin. They are shiny black or brown drift seeds, found in tropical Africa, India and the Caribbean (Factsheet, 2008).

The *Mucuna pruriens* seeds are contain high concentrations of levodopa, a direct precursor of the neurotransmitter dopamine (Giuliano and Allard, 2001). This explains why this plant has long been used in traditional Ayurvedic Indian medicine for diseases including Parkinson's disease (Manyam *et al.*, 2004). It has also been documented that the plant is useful in the treatment of a lot of ailments including the prevention of coagulation effects of snake bites (Aguiyi *et al*., 1996). This study is aimed at evaluating the effects of *Mucuna pruriens* seed extract for biochemical and haematological parameters using adult male rats.

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MATERIALS AND METHODS

Animals

Adult albino male rats of the Wistar strain, weighing between 130-150 g were obtained from the Animal House facility of the University of Jos, Nigeria. These animals were housed in a well ventilated and spacious room (room temperature 27±3^{o}C). All animals were fed with standard diet prepared at the facility, and were given water *ad libitum*.

Collection and identification of plant seeds

Seeds of *Mucuna pruriens* were cultivated and obtained from Benue state, Nigeria and were identified by Professor C.O. Akueshi of the Department of Botany, University of Jos, Nigeria.

Extraction procedure

The crude extract was prepared by adding 350 ml of deionized water to 35 g of *Mucuna pruriens* extract and exhaustive extraction for 72 hours at a temperature range of 65-70^{o}C, to obtain hot water extracts (HWE) of *Mucuna pruriens*. Percentage yield of the extract obtained was derived and pH of extract was measured using a pH meter.

Toxicology of plants seeds

Adult male Wistar strain rats weighing between 130-150 g were used for acute toxicity studies and the sub-chronic studies. Adopting the method developed by Lorke in 1983, acute toxicity testing was done for *Mucuna pruriens* using the male rats. The LD_{50} was later obtained for *Mucuna pruriens* in the rats used. In the case of sub-chronic studies, two dose points of 500 mg/kg and 1000 mg/kg for *Mucuna pruriens* seed powder were employed. Thirty-six (36) rats of 130-150 g weight distribution were put into labeled cages A to F and separated into six (6)

Table 1: Effect of Extract of *Mucuna pruriens* on some biochemical parameters in male rats

Treatment	Protein (g/l)	Albumin (g/l)	ALT (UI)	AST (UI)	ALP (UI)
Control (untreated seeds)	70.46±0.14	44.50±0.17	40.00±0.58	131.33±0.88	445.23±0.07
Mucuna (500 mg/kg)	80.43±0.20*	40.43±0.12$^{\#}$	19.00±0.58$^{\#}$	78.66±0.88$^{\#}$	520.60±0.06*
Mucuna (1000 mg/kg)	98.66±0.33*	54.80±0.05*	18.33±0.62$^{\#}$	95.00±1.15$^{\#}$	581.46±0.18*

Significant difference, at *p<0.05 and $^{\#}p$>0.05 for treatment versus control
Control – Untreated weevil-infested *Vigna unguiculata* seeds
ALT – Alanine aminotransferase, AST – Aspartate aminotransferase, ALP – Alkaline Phosphatase

Table 2: Effect of Extract of *Mucuna pruriens* on some biochemical parameters in male rats

Treatment	T. Bilirubin (mMol/L)	D. Bilirubin (mMol/L)	Glucose (mMol/L)	Creatinine (mMol/L)
Control (untreated seeds)	1.46±0.03	0.63±0.03	7.63±0.17	145.53±0.12
Mucuna (500 mg/kg)	3.80±0.08*	2.56±0.03*	6.50±0.15$^{\#}$	144.60±0.17$^{\#}$
Mucuna (1000mg/kg)	0.83±0.06$^{\#}$	0.50±0.03$^{\#}$	4.53±0.15$^{\#}$	165.53±0.20*

Significant difference, at *p<0.05 and $^{\#}p$>0.05 for treatment versus control
T. Bilirubin – Total Bilirubin, D. Bilirubin – Direct Bilirubin

rats per group, and rats in first three cages were each administered 500 mg/kg of *Mucuna pruriens*. The rats in the remaining three cages were each administered 1000 mg/kg of *Mucuna pruriens.* Administration of the extract was daily and lasted for six weeks. Biochemical and haematological parameters were then evaluated at the end of the six weeks, using the method of Tienz (1970).

STATISTICAL ANALYSIS

All treatments were performed in triplicate using SPSS Version 16.0 2007, and each data point in the results is the mean of two or three replicate tests. All experiments were repeated at least once. The statistical significance of a treatment effect was evaluated by student's *t*-test and the values were expressed as mean ± SEM (standard error of mean).

RESULTS

Percentage yield of *Mucuna pruriens* extract was 21.57% and pH of extract measured gave 8.76. The LD_{50} was obtained for *Mucuna pruriens* in male rats as 1300 mg/kg body weight.

DISCUSSION

The measured pH of 8.76 for *Mucuna pruriens* revealed its tilt toward alkalinity which generally would not affect the action of the extract *in vivo*. There was significant difference (p<0.05) between treated and control groups, for both haematologic parameters assessed and biochemical enzymes assayed from serum samples collected from the male rats sacrificed after six weeks of daily administration of extract. However, there was a significant difference in WBC total count for rats administered with *Mucuna pruriens* as seen in table 3, compared with the control. A significantly higher average (p<0.05) WBC total count of 26,000cells/mm^2 was measured in comparison with 6233cells/mm^2 measured for the control. This increase in WBC total count was likely triggered off by the metabolic assault from alkaloidal and/or phenolic content in *Mucuna pruriens* (Rajaram and Janardhanan, 1991). L-dihydro-xyphenylacetic acid (L-dopa) is another key constituent in *Mucuna pruriens* that may likely be responsible for the observed findings in WBC total counts (Rajaram and Janardhanan, 1991). The dose-dependent and time-dependent response of *M. pruriens* on lipid peroxidation (Tripathi and Upadhyay, 2001) has found its clinical use for several free radical diseases, especially the age-related male infertility and Parkinson's disease are well documented (Vaidya *et al*., 1978). *M. pruriens* protective response on these *in vivo* models suggests two possibilities; either to be acting on the nervous system or else removes the free radicals generated due to catecholamine and iron interaction (Guliaeva *et al.*, 1988). A study carried out, however, suggested that *Mucuna pruriens* may have hepatotoxic potentials which may be dose dependent and may be reduced by cooking the seeds before incorporation into the feed. The level of liver enzymes and serum bilirubin increased with increase in the percent level of *Mucuna pruriens* inclusion in the feed prepared for albino rats (Ezeja and Omeh, 2010). These

Table 3: Effect of Extract of *Mucuna pruriens* on some haematological parameters in male rats

Treatment	PCV (%)	HB (d/l)	WBC (cell/mm^2)	LYMPH (%)
Control (untreated seeds)	36.67±0.8	13.68±0.9	6233.33±16.6	83.33±0.8
Mucuna (500 mg/kg)	40.33±0.3$^{\#}$	15.46±0.2$^{\#}$	26250.00±28.8*	83.00±0.6$^{\#}$
Mucuna (1000mg/kg)	46.00±0.5$^{\#}$	16.92±0.3$^{\#}$	6516.67±16.1*	86.00±0.6$^{\#}$

Significant difference, at *p<0.05 and $^{\#}p$>0.05 for treatment versus control, using Student's t-test, (n=6 per group).
Values are expressed as mean ± S.E.M
PCV - Packed Cell Volume, HB – Haemoglobin concentration, WBC – White Blood Cells, LYMPH – Lymphocytes

Table 4: Effect of Extract of *Mucuna pruriens* on some haematological parameters in male rats

Treatment	NEUTR (%)	EOSINO (%)	MON (%)	BFP
Control (untreated seeds)	16.00±0.58	0	0.33±0.3	Normal
Mucuna (500 mg/kg)	16.00±0.58$^{\#}$	0.33±0.3$^{\#}$	0	Normal
Mucuna (1000mg/kg)	13.00±0.58$^{\#}$	0.33±0.3$^{\#}$	0	Normal

Significant difference, at *p<0.05 and $^{\#}p$>0.05 for treatment versus control
NEUTR – Neutrophils, EOSINO – Eosinophils, MON – Monophils, BFP – Blood Film Picture

results were inconsistent with the findings from this work and could not be corroborated with other studies done. These findings of Ezeja and Omeh could have been linked to the method of extract preparation or methodology adopted by the scientific team. The results of this present research show that the biomarker enzymes were decreased and were detected rapidly, hence, can be used for the prediction and diagnosis of metabolic insults. The membrane bound target enzymes, Aspartate Amino transaminase (AST) and Alanine Amino transaminase (ALT) significantly decreased in liver tissues when measured after six weeks of treatment in relation to the control. The reason for this decreased observation in enzyme level has been attributed to the fact that *M. pruriens* is a known antioxidant (Aguiyi *et al*., 1996; Tripathi and Upadhyay, 2001). The results from this current study were consistent with a study that was obtained from treatment with methanolic extract of *Mucuna pruriens* that showed a decrease in the levels of lipid peroxidation and an increase the levels of glutathione, superoxide dismutase and catalase. These results suggest that the extract of *Mucuna pruriens* seeds exhibits significant antitumor and antioxidant effects in mice (Rajeshwar *et al.*, 2005). Therefore, the suppression of liver enzymes to significant amounts could be explained by the enhanced suppressive effect displayed by some components in *Mucuna pruriens* extract, thereby, preventing over-sensitization of enzymes to the metabolism of various substances foreign to the normal system in the rats used for the study.

CONCLUSION

Haematological and biochemical investigations carried out showed no significant difference (p>0.05) in the parameters measured for packed cell volume (PCV), haemoglobin (Hb), Albumin level and WBC differential counts. However, *Mucuna pruriens* caused an increase in total WBC count that was significant (p<0.05), as well as in ALP, protein, creatinine levels and bilirubin concentrations measured. Alanine Amino transaminase and Aspartate Amino transaminase levels were reduced in comparison with their controls respectively. The results revealed metabolic imbalance in the rats, suggesting a mild cholestasis effect from the extract.

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