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RESEARCH ARTICLE

TOXICOLOGICAL STUDIES OF *Mucuna pruriens* SEEDS IN ALBINO RATS USING LIVER FUNCTION TESTS

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ABSTRACT

This study evaluated the toxicological effects of raw and cooked *Mucuna pruriens* seeds using its effect on liver enzymes and serum bilirubin (total and conjugated) of white albino Wistar rats in the laboratory. Powdered raw and cooked *M. pruriens* seed meal were incorporated into the feed of rats at different percent (10,20 and 50%) inclusion levels for the test animals while normal feed was given to the negative control rats for 28 days after which blood samples were collected from each rat for serum analysis. The tests conducted were serum Aspartate Amino Transaminase (AST), Alanine Amino Transaminase (ALT), Alkaline Phosphatase (ALP), Total bilirubin (TB) and Conjugated Bilirubin (CB). The liver function tests revealed that the serum AST, ALT, ALP as well as the total and conjugated bilirubin were significantly ($P<0.03$) increased in the rats that were fed 10 and 20% raw *Mucuna* seed meal in the feed and also significantly ($P<0.0001$) increased in the rat fed with 50% raw *Mucuna* in the feed when compared to the negative control. Also the liver enzymes and serum bilirubin (total and conjugated) of the rats that received different percent (10,20 and 50%) inclusion levels of cooked *M. pruriens* seed in the feed were significantly ($P<0.05$) increased when compared to the negative control group. In all the experiments, the level of the liver enzymes and serum bilirubin increased with increase in the percent level of inclusion in the feed. In conclusion this study suggested that *M. pruriens* may have hepatotoxic potentials which may be dose dependent and may be reduced by cooking the seeds before incorporation into the feed.

INTRODUCTION

Mucuna pruriens is one of the many "under-utilized" tropical legumes that are widely used as a cover crop (Berhe, 2001). *Mucuna pruriens* belongs to the family *Fabaceae* and is indigenous to tropical areas of Asia, Caribean and Africa, and have more than 100 species (Rajeshwar *et al*, 2005). It is a vigorous, perennial, herbaceous climbing vine that has the capacity to grow up to 6m in length. The leaves are trifoliate with white or dark purple flowers that hang in long clusters. The pods are sigmoid and the seeds ovoid, having 4-6 seeds per pod. The seeds vary in colour from black, white to mottle and the pods which are thick and leathery are covered with reddish orange long stiff hairs that are readily dislodged and can cause intense irritation to the skin due to the presence of a chemical known as "*mucunain*" (Duke, 1981, Buckles, 1995 and Leslie, 2005).

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It has a variety of common names such as cowhage, velvet bean, fogarate. Locally it is known as "Agbala" in South Eastern Nigeria and 'Yerepe' by the Yorubas of South Western Nigeria.

Mucuna puriens is widely utilized by both humans and livestock for various purposes. It is a source of protein as it contains high protein level (Emenalom and Udedibie, 1998). Some ethnic groups in Nigeria use the pods and leaves as vegetables (Adebowale and Lawal, 2003). The seeds are consumed during famine or scarcity in some rural communities of Enugu state (Onweluzo and Ellita, 2003). The seeds of *M. pruriens* are also used as soup thickeners (Ukachukwu *et al*, 2002). Velvet beans are used as a source of protein in diet of fish, poultry and cattle (Vadivel and Janardhanan, 2000). Pharmacologically, *M. pruriens* seeds have been used as analgesics, anti-inflamatory, diuretics, anthelmimitics, CNS stimulant, cough suppressant, antihypertensive etc. (Leslie, 2005 and Thomas, 2006). *M. pruriens* contains an anti nutritional factor L-DOPA which is used for the treatment of Parkinson's disease (Nagashayana et al, 2000). Also within the Igbo community of South Eastern

Nigeria, *M. pruriens* seeds are used as condiments in stew, in preparation of local "moi-moi" and cooking of Bambara nut gel "Okpa". Siddhuraju and Janardhanan (1996), reported that velvet bean contains toxic substances. Also studies with poultry have shown that the raw bean is toxic to broilers (Esonu, 2001). Echo Development Notes (EDN) (1997) reported that seeds give good results in cattle and sheep but the diet are generally unsatisfactory for pigs and may even cause severe vomiting and diarrhea in this species.

The reported toxic effects in livestock and poultry and because *M. pruriens* usage in food did not report any adverse effects in human health necessitated this study on the toxicological effects of *M. pruriens* in internal organs (liver) using standard liver function tests.

MATERIALS AND METHODS

Collection and Identification of Plant Materials

The white seeds of *Mucuna* were harvested from Mr Apeh Ezeocha herbal garden at Awokwuru Olido in Enugu-Ezike, Igbo-Eze North L.G.A of Enugu State and identified as *Mucuna pruriens* by Mr. A Ozioko of Bioresources Development Conservation Programme, Aku Road, Nsukka, Enugu state.

Test Sample Preparation

The seeds of *Mucuna pruriens* (velvet bean) were oven dried for 60 minutes at 40^0C. The seeds were dehulled and divided into two parts. One part was cooked for 30 minutes while the other part was left raw. Both raw and cooked seeds were pulverized separately with a mechanical grinder into powdered forms and then stored in a refrigerator at 15^0C until ready for use.

Animals

Mature Wistar albino rats of both sexes obtained from the laboratory animal facilities of Faculty of Veterinary Medicine, University of Nigeria, Nsukka were used for the experiments. The animals were kept in stainless steel cages at room temperature and relative humidity of about 45-65% and fed *ad libitum* with standard rat feed (Vital Feed®, Nigeria) until 24 hours before the experiment. Clean drinking water was provided *ad libitum*. The animals were allowed 2 weeks for acclimatization before the experiments. Ethnical rules guiding the use of laboratory animals for experiments according to Zimmerman (1983) were strictly followed.

Experiments

The experiment was done in two parts. One part was carried out using raw powdered *Mucuna* seeds while the other was with cooked powdered *Mucuna* seed meal.

Raw *Mucuna pruriens*

For the first part, 20 mature Wistar albino rats of both sexes were randomly divided into 4 groups of 5 rats per group and fed with different percent inclusion levels of raw *Mucuna* seed in the feed.

Group 1 rat were used as negative control and was fed with normal feed (vital feed® Nigeria).

Group 2 rats were fed with feed containing 10% raw *Mucuna* seed meal, groups 3 and 4 rats were fed with feed containing 20 and 50% raw *Mucuna* seed meal inclusion levels respectively.

Cooked *Mucuna pruriens*

The second part of the experiment was carried out following the above procedure but with different percent inclusion levels (10, 20 and 50%) of cooked *M. pruriens* seed meal in the feed; for groups 2, 3 and 4 respectively. All the rats in the two groups of experiments were fed for 28 days. After this period, blood was collected from each of the rats through the media cantus using capillary tubes into sterile containers. The blood samples were centrifuged at 30,000 r.p.m for 10 minutes, and later the serum were collected and used for the tests/assay.

Determination of Aspartate Amino Transferase (AST) and Alanine Amino Transferase (ALT) in serum. The method of Reitman and Frankel (1957) as described by Randox laboratories, United Kingdom using Randox kits was used for this study.

Procedure

The samples and reagents were pipetted into test tubes as follows.

	Reagent blank	Sample
Sample	-	0.1ml
Solution RI	0.5ml	0.5ml
Distilled water	0.1ml	-
Mixed and incubated for 30 minutes at 37^0C		
Solution R2	0.5ml	0.5ml
Mixed and allowed to stand for 20 minute at 25^0C		
Sodium hydroxide	0.5ml	0.5ml

Mixed thoroughly and the absorbance of samples read against the reagent blank after 5 minutes at the wavelength of 546nm.
Note: RI = Buffer (AST or ALT Buffer as the case may be)
R2 = 2, 4, Dinitrophenylhydrazine

Determination of Alkaline Phosphatase (ALP)

The method developed by kind and king (1954) and described by Randox laboratories, United Kingdom was used for the study using Randox kit.

Procedure

Reagent	Test	Test blank	Standard	Standard black
ALP Buffer	1.0ml	1.0ml	1.0ml`	1.0ml
ALP substrate	1.0ml	1.0ml	-	-
This was incubated at 37^0C for 15 minutes				
Serum	0.1ml	-	-	-
Phenol standard	-	-	0.1ml	-
Distilled water	-	-	-	1.0ml

This was then incubated at 37^{v}C for 15 minutes and read spectrophotometrically at 520nm.

The concentration was calculated using the formula below

concentration of test

$$\frac{\text{Test} - \text{test blank}}{\text{Standard}} \times \frac{7}{1}$$

Determination of total and conjugated bilirubin

This was done using the method described by Randox laboratories U.K using Randox kits.

Total Bilirum

Procedure

Blank and experimental tubes were set up and 200µL of reagent RI was added to both tubes. 1 drop of reagent R_2 was added into only the experimental test tube and 1µL of reagent R_3 was then added to both tubes followed by 100 µL of serum. Both test tubes were incubated in water bath for 10 minutes. The blank was used to zero the

spectrophotometer and experimental sample was read at 578nm.

Conjugated Bilirubin

Procedure

Blank and experimental test tubes were set up and 200µL of reagent R_2 was dropped into the experimental test tube only. 2ml of normal saline was pipetted into both test tubes. The blank was used to zero the spectrophotometer and experimental samples were read at 546nm.

Statistical Analysis

The results of the experiment were presented as mean ±S.E.M and were subjected to One Way Analysis of Variance (ANOVA). The difference between the means were tested using post Hoc L.SD at $P<0.05$ significance level.

RESULTS

The results of the Serum Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), Total Bilirubin (TB) and Conjugated Bilirubin (CB) of rats fed with raw *Mucuna pruriens* seed at different percent levels of inclusion in the feed is shown in Table 1. The result shows that there was a significant increase ($P<0.03$) in the liver enzymes and serum total bilirubin in groups 2 and 3 rats that were fed 10 and 20% raw. *M. pruriens* in the feed when compared to the negative control. For the conjugated bilirubin, there was no significant difference between control and group 2 rats (10% *M. pruriens* in feed), though there was a slight increase. The levels of the liver enzymes and serum bilirubin (total and conjugated) in group 4 that received 50% raw *M. pruriens* inclusion in feed was very significantly ($P<0.0001$) increased when compared to the negative control group that were given normal feed.

The result of the level of liver enzymes and serum bilirubin of rats fed with different percent inclusion levels of cooked *M. pruriens* seeds in the feed are shown in Table II. The results indicate that there was also a significant ($P<0.05$) increase in levels of liver enzymes and serum bilirubin (total and conjugated) in groups 2 and 3 rats that received 10 and 20% cooked *M. pruriens* seed in feed and greatly increased ($P<0.001$) in the rats that were given 50% cooked *Mucuna* seed meal in feed when compared to the negative control. There was no significant difference in levels of liver enzymes and serum bilirubin in group 1 rats and the negative control group.

DISCUSSION

Toxic responses of the liver to chemical agents or substances depend on both the substances involved and on the duration of the exposure of the organ to the substance. Responses to acute exposure are usually manifested by lipid accumulation in the hepatocytes, cellular necrosis or hepatobilliary dysfunction while responses to chronic exposures are usually manifested by cirrhosis or neoplasia (Braide and Anika, 2007).

The liver is an organ of many diverse metabolic activities and any assessment of its functional status is dependent upon its ability to perform specific metabolic functions. A number of tests have been devised for the detection of alterations in liver functions, among which are tests dependent upon the measurement of serum enzymes activities (non functional plasma enzyme activities) such as Aspartate. Ammotransferase (ASP), Alanine Aminotransferase (AlT), Alkaline phosphatase (ALP) and tests dependent primarily on hepatic secretion and excretion of substances like bile pigments such as serum bilirubin (Emberth, 1986), hence the employment of the above liver function tests for this study.

Generally, analysis of the activities of some basic liver function enzymes (non functional enzymes) in the plasma or serum can be used to indirectly access the integrity of tissues after being exposed to certain pharmacological agents or substance. These enzymes are usually liver markers whose plasma concentrations above the normal homeostatic limits could be associated with various forms of disorders which affect the functional integrity of the liver tissue (Friday *et al*, 2010). Also liver enzymes such as AST are commonly measured clinically as part of diagnostic liver function test to determine liver health (Gaze, 2007).

Tests for levels of serum ALT are of value in detecting liver diseases in dogs, cats and humans and serum AST levels may be increased in liver disease in all species but cannot be considered specific tests for liver damage

Table 1. Liver enzymes and serum bilirubin (Total and conjugated) levels of rats fed with different percent inclusion of raw *M. pruriens* seed in the feed.

Group	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	TB (µmol/L)	CB (µmol/L)
Negative control	12.27±0.38	9.49±0.34	36.35±0.33	11.65±0.34	2.35±0.37
2 10% *M.p*	18.62±0.49*	11.71±0.68*	39.44±0.45*	13.98±0.62*	2.39±0.14
3 20% *M.p*	27.37±0.91**	20.57±0.57**	47.99±0.22*	16.68±0.38*	4.90±0.20*
4 50% *M.p*	42.74±0.32**	29.53±1.12**	52.98±0.84**	30.70±1.85**	6.69±0.42**

* $P<0.03$ when compared to negative control

** $P<0.0001$ when compared to negative control

Table 2. Liver function parameter and serum bilirubin levels of rats fed with different percent inclusions levels of cooked *M. Pruriens* seed meal in the feed.

Group	ALT	AST	ALP	TB	CB
1(Negative control)	12.27±0.38	9.48±0.34	36.35±0.33	11.65±0.34	2.35±0.37
2 (10% *M.p*)	15.752±0.43	10.151±0.14	37.68±0.43	11.91±0.14	2.14±0.05
3 (20% *M.p*)	21.04±0.41*	16.00±0.43*	41.49±0.27*	14.64±0.47*	3.03±0.15*
4 (50% *M.p*)	29.55±0.42**	18.70±0.44**	44.67±0.12**	16.69±0.35*	4.04±0.14*

* $P< 0.05$ when compared to negative control

** $P< 0.001$ when compared to negative control

(Emberth, 1986) but liver cell damage can be distinguished from other billiary problems by measuring Alkaline phosphatase (Giboney, 2000).

In the present study, the level of all liver enzymes (AST, ALT and ALP) were significantly ($P < 0.03$) increased in groups 2 and 3 that were fed with 10 and 20% raw *M. pruriens* seed inclusion in the feed. The increase was even more in the rats that received 50% raw *M. pruriens* seed in the feed when compared to the negative control group. Also there was a significant ($P<0.05$) increase the liver enzymes levels of rats fed with different percent inclusion rates of cooked *M. pruriens* seed in the feed. The liver enzyme levels increased with increase in the percent inclusion in the feed of *M. pruriens*, though the levels were lower in the cooked *Mucuna* seed fed rats.

Non functional plasma enzymes perform no known physiologic function in the blood. Their presence in plasma at levels elevated above normal values suggests an increased rate of tissue destruction (Harper *et al,* 1997). Tissue (liver) damage or necrosis resulting from injury or disease is generally accompanied by increases in the levels of several non-functional plasma enzymes (Robert *et al,* 2006). Also AST and ALP are two of the most reliable markers of hepatocellular injury or necrosis. Their levels can be elevated in a variety of hepatic disorders. (Giboney, 2005).

The above assertion and the elevation in the rats liver enzymes levels that was dose dependent in rats that were fed different percent inclusions of *M. pruriens* seed in the feed (raw and cooked) in this experiment suggests that *M. pruriens* seeds may have a hepatotoxic potential, the severity of which may be dependent on the level of inclusion in the feed. Also the reduction in the liver enzyme levels in cooked *M. pruriens* seed fed rats also indicates that cooking may have effect in reducing the toxicity.

Bilirubin is the yellow breakdown product of normal heme catabolism. It is the chief bile pigment found in the serum. The renal threshold of both total and conjugated bilirubin is low and elevation of serum bilirubin (total and conjugated) is indicative of hepatocellular damage (Cronwall *et al,* 1980). From the result of this experiment, there was a significant increase ($P<0.05$) in the serum bilirubin (total and conjugated) in the rats that were fed with different percent inclusions in feed of both raw and cooked *M. pruriens* seed which was also dose dependent but was lower in cooked *Mucuna* seed fed rats. This also suggests that *M. pruriens* seeds may caused hepatocellular destruction as suggested by Cronwall *et al* (1980) above.

In conclusion, *Mucuna pruriens* seed fed to rats at different percent inclusion levels in feed in this experiment caused significant elevation of liver enzymes and serum bilirubin (total and conjugated) which increased with increase in the percent level of inclusion and also reduced with cooking and this suggests that *M. pruriens* seed have some levels of hepatotoxic potential which is lower when the seeds are cooked. More work is however needed to be done to determine its toxic effect on other internal organs such as the heart and also to determine its exact mechanism of action. Also care should be taken in the use of *M. pruriens* as food and feed for livestock and when it is inevitable should be cooked and included in small percentages.

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