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RESEARCH ARTICLE

THE NEPHROTOXIC EFFECT OF *Mucuna pruriens var utilis* SEED IN WHITE ALBINO RATS

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ABSTRACT

The toxic effects of *Mucuna pruriens var utilis* seeds on the kidney was evaluated on white albino Wistar rats in the laboratory using powdered raw and cooked *Mucuna* seeds incorporated into the feed of the rats at 10, 20 and 50% levels for four weeks, and than evaluating the serum urea and creatinine levels (kidney function tests) of the rats. The serum urea levels of the rats fed with different percentage inclusions of raw *Mucuna* seeds in the feed were significantly ($P<0.001$) increased in a dose dependent manner when compared to the negative control group. Also the serum urea and creatinine levels of the rats fed with different percentage inclusions of cooked *Mucuna pruriens* seed in the feed where significantly ($P <0.02$) increased when compared to the negative control group of the rats. These increased with the increase in the percentage inclusion in the feed. In conclusion, this study suggests that feeding *Mucuna pruriens, var utilis* seeds to animals or man may cause some levels of damage to the kidney which may be dependent on the level of inclusion in the feed and the toxic effect may also be drastically reduced by cooking the seeds before using it for feeding.

INTRODUCTION

Mucuna pruriens var utilis commonly known as velvet bean is a tropical legume of the family *Fabaceae* and genus *mucuna* with up to 100 species (Duke, 1981). It is an annual herbaceous, vigorous climbing vine that grows 3-6m in height. It is indigenous to the tropical regions especially Africa, India and West Indies (Siddhuraju *et al.,I* 2000). The leaves are mostly free and trifoliate, flowers white or dark purple and hang on long clusters, pods are sigmoid and the seeds are ovoid having 4-6 seeds per pod (Duke, 1981 and Buckles, 19995). The specie nomenclature pruriens in Latin refers to itching sensation due to the result of contact with pod hair. The itching is caused by a chemical in the hairs called "mucunain" (Buckes 1995 and Leslie, 2005).

The common names of *M. pruriens* include cow hitch plant, cow hage, velvet or devil bean and Jackbonne (German). Others includes: "Agbara" by the Igbos (South East), "Yerepe" by the Yoruba's (South West) in Nigeria. *Mucuna pruriens* have been reported to have nutritional, pharmacologic and industrial use. Mucuna seeds constitute source of food for some tribes and some ethnic groups of Asia and Africa (Iyayi and Egharevba, 1998).

Mucuna is a rich source of protein supplements of food and feed for livestock (Siddhuraju and Becker, 2001). Some ethnic groups in Nigeria use the pods and leaves as vegetables (Adebowale and Lawal, 2003) while the seeds are used as thickener of soup and as vegetable oil (Ukachukwu *et al*, 2002). Cauis (1989) reported that all parts of *Mucuna* plant are known to posses high medicinal value. *Mucuna* seeds are used in treating leucorrhea,spermatorrhoea and aphrodisiac related problems. It also possesses anabolic, analgesic, anti inflammatory hypoglyceamic, antiparasitic and diuretic properties among others (Leslie, 2005., Thomas, 2006., Sridhar and Rajeev, 2007).

Mucuna seeds also contain an anti-nutritional factor 3,4-dihydro-L-phenyl alamine (L-DOPA) which provides symptomatic relief in Parkinsons' disease treatment (Prakash and Tewari, 1999, Nagashana *et al*, 2000, Ezeagu *et al,* 2003). The above reports show that *M. Pruriens*, *var utilis* is widely utilized by humans and livestock but there is scanty information on the toxic effects of the plant hence this study was carried out to determine its effects on the kidney using white albino Wistar rats and using standard methods considering the importance of the kidney to the overall well being of both man and animals.

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MATERIALS AND METHODS

Collection and identification of material

The *Mucuna pruriens*, seeds were sourced from Apeh Ezeocha herbal garden at Awokwuru, Olido in Enugu-Ezike of Igbo-Eze North Local Government Area of Enugu State and identified as *Mucuna pruriens var utilis* by Mr. A. Ozioko of Bioresources and conservation programme, Aku road, Nsukka, Enugu State.

Preparation of the test sample

The *Mucuna* seeds were oven dried at the temperature of 40^0C for 1 hour, cooked and dehulled to separate the seed coat and the inner seed. After separation, the seeds were divided into two. One part was grinded into powdery form, the second part was boiled for 30 minutes and was also grinded with the aid of a milling machine, dried and then stored in refrigerator at the temperature of 15^0C until ready for use.

Animals

Mature Wistar albino rats of both sexes sourced from the animal breeding unit of Faculty of Veterinary Medicine University of Nigeria, Nsukka. The animals were kept in a well ventilated stainless cages at room temperature with growers feed (Vital feed® Nigeria) and clean drinking water provided throughout the day. The animals were allowed 2 weeks for acclimatization before the experiments. Ethical conditions guiding the use of laboratory animals according to Zimmerman (1983) were strictly observed.

Experiments

The powdered *Mucuna pruriens* seed was added to the feed of the rats at different percentage (10, 20 and 50%) inclusion. This was done differently with both the raw and cooked *Mucuna* seeds. For the first group of experiments, 20 mature rats were divided into 4 groups (A-D) 5 rats per group. Group A rats were given 100% normal grower feed (vital feed®, Nigeria). Group B rats were given feed containing 10% raw powdered *Mucuna* seeds. Group C and group D rats were fed with feed containing 20 and 50% powdered raw *Mucuna* seeds respectively. For the second part of the experiment, the cooked and powdered *Mucuna* seeds were used. The experiment carried out above was repeated for the same group following the same procedure. All the rats in the two groups of experiment were fed for 4 weeks after which the blood was collected for serum analysis. The blood was collected from each of the rats through the media cantus using capillary tubes into sterilized containers, centrifuged at 30,000 r.p.m for 10 minute after which the serum was collected and used for analysis.

Determination of Serum Urea Level

This was done for each rat using the method of Chaney and Marbach, (1962). Test tubes for all the groups, including standard and blank were labeled. 1.0ml of urea solution was added to each of the test tubes except the blank test tube. 10 µml of samples and standard were added to appropriate tubes except the blank test tube. The tubes used were mixed thoroughly and incubated at 37^0C for 15 minutes, 5.0ml of Phenol-nitroprusside solution was rapidly added and mixed, then 5.0ml of alkaline hypochlorite solution was added and mixed. All the tubes were incubated at 37^0C for 20 minutes. The absorbance for each tube was read at 560nm against reagent blank set at zero absorbance. Concentration of urea was calculated using the formula below:

$$\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \frac{\text{concentration of standard}}{1}$$

Where concentration of standard was 50mg/dl

Determination of serum creatinine

The method of Taussky (1961) was adopted for this experiment. The serum from both the test animals and the control was deproteinized by adding 4-5ml of tumstic acid to 0.5ml of the sample, thoroughly mixed for 10 seconds and centrifuged at 1500 r.p.m for 10 minutes. Into a series of appropriately labeled tubes, 3.0ml of deporteinized serum were pipetted. Reagent blank of 3.0ml water was also set. 1.0ml of picric acid was added to each of the test tubes and mixed thoroughly. 30 seconds later, 0.5ml of NaoH, was added to each test tube and mixed thoroughly. 15 minutes after adding NaOH, absorbance of each of the test tubes were read at 500nm against the reagent blank set at zero absorbance. Concentration of creatinine was calculated using the formula below.

$$\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \frac{\text{concentration of standard}}{1}$$

Where concentration of standard was 2mg/dl

Statistical Analysis

All the results were presented as mean ±SEM and subjected to One way Analysis of Variance (ANOVA).

RESULTS

The result of different percent inclusion of raw *Mucuna pruriens* seed is shown in Table 1. From the results, it shows that there was a significant increase ($P<0.001$) in serum urea and creatinine between the control group that received the normal feed and the test groups that received different percentage inclusions of the raw *Mucuna* seed in the feed in a dose dependent manner increasing from 9.62 ±0.22 in the control group (group A) to 47.67±0.40 for serum urea in those that received 50% raw *Mucuna* seed inclusion (group D) in the feed and from 0.63±0.03 in the control group to 2.25±0.04 for serum creatinine. The result of the 10,20 and 50% cooked *M. pruriens* seed inclusion in the feed is shown in Table II. Also there was a significant increase ($P<0.02$) in serum urea and creatinnae between the control and treated groups of the rats. The serum urea increased from 9.35±0.09 in the control to 32.06± 0.07 in group D that received 50% *Mucuna* seed inclusion while serum creatinine for the negative control group was 0.62±0.13 and 0.93±0.26 1.48± 0.49 and 1.99± 0.10 for the groups that received 10,20 and 50% cooked *Mucuna* seed inclusion in the feed respectively.

DISCUSSION

The kidney (made up of functional units called nephron) which is the body's natural filtration system performs many vital role of regulating the water and electrolyte content of the body. The kidney plays this important role

by allowing the animals or persons to eat and drink according to their habits without changing the composition of the fluid compartment. The functions of

Table 1. Serum urea and creatinine levels of rats fed with different percentage inclusions of raw *M. Pruriens* seed in the feed.

Groups	Serum Urea	Serum Creatinine
Group A Negative control	9.62±0.22	0.63±0.03
Group B 10% *M.p* inclusion	25.65±0.28*	1.19±0.10*
Group C 20% *M.p* inclusion	38.17±0.09*	1.87±0.07*
Group D 50% *M.p* inclusion	47.07±0.40*	2.25±0.04*

*P<0.001 When compare to negative control

Table 2. Serum urea and creatinine levels of rats fed with cooked *M. Pruriens* seed at different levels of inclusion.

Groups	Serum Urea	Serum Creatinine
Group A Normal feed (negative control)	9.35±0.09	0.62±0.13
Group B 10% *M.p* inclusion	16.12±0.41*	0.93±0.25*
Group C 20% *M.p* inclusion	26.73±1.0*	1.48±0.49*
Group D 50% *M.p* inclusion	32.86±0.07*	1.99±0.10*

*P<0.02; When compared to negative control

the kidney can be classified as excretory, regulatory and endocrine functions (Klahr, 1985) and therefore, any substance that is toxic to the kidney affects the overall well being of the person or animal. Nephrotoxicity after therapeutic application of drugs or feeding with some substances has been well documented. The cellular response of kidney to toxic injury varies from minor biochemical abnormalities to cell death and the effects usually reported following toxic injury to the kidney reflect decreased elimination of wastes such as an increase in blood urea nitrogen (BUN) or an increase in plasma creatinine (Braide and Anika, 2007), hence the employment of serum urea and creatinine levels (standard kidney function tests, Henry, 2001) for this study. Serum urea level measures the amount of urea nitrogen in the blood (BUN). Urea is formed in the liver and represents the principal end product of protein catabolism excreted in the urine by the kidneys (Emberth, 1986). High BUN levels can indicate kidney dysfunction (Wallach, 2000). From the experiment, there was a significant increase ($P < 0.001$) in the serum urea levels in all the groups given feed containing different percentage of *Mucuna pruriens* seed when compared to the negative group (those given normal feed). The serum urea increased with increase in the percentage inclusion in both the raw and cooked seed containing feed, although the serum urea levels were lower in the group of rats that were given feed containing cooked *Mucuna* seeds. Increased levels of urea is seen in kidney diseases such as early nephritis, polycystic kidney, damaged renal function such as acute neptrotoxicity caused by ingestion of certain toxic substances and in certain infections causing renal insufficiency and nephrosis (Bernard, 1965).

Since there was a marked increase in the serum urea levels after feeding the rats with raw and cooked *Mucuna* seeds for 4 weeks and this increased with increase in the percentage inclusion of the seeds in feed but lower in cooked seed when compared to the control, it is therefore possible that the *Mucuna* seed may have nephrotoxicity effect which may be dependent on the level of inclusion and the state it is consumed (raw or cooked). Creatinine is a non-protein nitrogenous substance formed during muscle metabolism of creatine and phosphocreatine (Emberth, 1986). It is synthesized in the kidney, liver and pancreas by two enzymatically mediated reactions; firstly by tansamination of arginine and glycine to form guanidinoacetic acid and the methylation of guanidinoacetic acid which occurs with 5-adenosyl methionine as a methyl donor (Narayanan and Appleton, 1980). It is then transported in the blood to other organs such as muscle, brain and then excreted in urine through the kidney. Creatinine is not influenced by diet, its daily production from muscle is relatively constant and its production is not easily influenced by catabolic factors, therefore conditions such as fever, toxaemia, infection and drug administration do not readily influence creatinine levels and as such it has had the reputation of being a more specific test for diagnosis of progressive renal disease than serum urea (Emberth, 1986).

From the result there was a significant increase ($P < 0.02$) in serum creatinine in the groups of rat that received different levels of *M. pruriens* seeds (raw and cooked) when compared to the control group. The level of increase was lower in the lower percentage inclusion and also lower in the cooked *M. pruriens* seeds than in the raw seeds. This suggests that the source of kidney damage as indicated by increase in serum creatinine, and was proportional to the percentage incorporation feed, in this experiment may be inclusion of *M. pruriens* seed in the diet. Also Bernard (1985) reported that few pathological conditions that have been associated with elevation of serum creatinine include renal injury subsequent to trauma, anuria or toxins. In conclusion, *Mucuna pruriens var utilis* seeds which are fast gaining recognition and acceptance as a source of feed for livestock and food for humans may have some level of nephrotoxic effects, though more work should be carried out to determine its effects in other organs such as liver and heart. Care should also be taken in using *M. pruriens* as part of diet and when the usage is unavoidable, it should be included in a small percentage and should be well cooked.

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