Mucuna urens Consumption and its Implications on the Ultrastructure of the kidney and spleen: A Laboratory Investigation.

ABSTRACT

Mucuna urens or horse-eye bean is a plant that belongs to the family fabaceae, commonly found in home gardens in the South Eastern parts of Nigeria where the seeds are used as a major soup condiment for thickening. Histomorphology, blood parameters and weight of the kidney and spleen administered with ethanolic extract of *Mucuna urens* were investigated in 24 adult male albino rats weighing between 107g-167g. The rats were divided into 4 groups of 6 rats each. Group 1 was the control, while 2, 3 and 4 were the experimental groups. The seed extract was administered orally for 28 days according to their body weight. Group 1 was administered 5 ml of distilled water, groups 2-4 were administered, 100, 200 and 300 mg/kg of extract, respectively. After sacrifice, blood sample was obtained for red blood cell count, mean corpuscular haemoglobin concentration and white blood cell count. Kidney and spleen were dissected out and fixed in 10% buffered formalin, then processed to obtain paraffin sections. The tissues were stained in H&E. Group 1 kidney showed normal cytoarchitecture. Groups 2-4 showed dose-dependent degenerated glomeruli, enlarged Bowman's space, shrunken glomerulus and eroded tubular epithelium. Group 1 of the spleen showed normal cytoarchitecture. The photomicrographs from groups 2-4 spleen also showed dose-dependent white pulp and red pulp hyperplasia, and increased fibrosis in the trabeculae. The weight of the kidney, spleen and haematological parameters were not significantly affected. Conclusively, the ethanolic extract of *Mucuna urens* led to damage of renal tubules and collapse of the glomerular capillaries and altered immune status in terms of reactive lymphocytic proliferation that was observed.

Keywords: Kidney, Spleen, *Mucuna urens*, cytoarhitecture, trabeculae

1. INTRODUCTION

Food legumes are important in many countries and serve as a useful source of needed proteins. The most domesticated legumes are valued primarily for seeds, yet the leaves of many legumes equal or exceed the protein content of the seeds on a dry weight basis [1]. The plant is a legume used also as a traditional minor food crop in many parts of the world, especially in areas around the foothills and lower hills of the Eastern Himalayas, and in Mauritius, Philippines, Java, Sri Lanka and Japan [2, 3]. Research efforts on *Mucuna urens* species as food crop are gaining momentum in Nigeria, where the leaves are used as feed for farm animals in the Northern States [4], and the beans of *Mucuna urens* are used as a soup thickener or condiment in the Eastern states [5]. In the Southern United States, the leaves of *Mucuna urens* are also used as feed for farm animals [6]. The mineral composition of *Mucuna urens* seed includes potassium, sodium, phosphorus, calcium, magnesium, iron, copper and zinc [5]. Despite the potential nutritional attributes, *Mucuna urens* species have been reported to contain some endogenous toxic factors and compounds such as phytic acid, cyanogenic glucoside, oxalate, gossypol, nicotine, physostigmine, and serotonin [7]. These toxic compounds can inhibit protein and carbohydrate digestibility; induce pathological changes in the intestine and liver tissues, thus negatively impact on the nutritive value of the horse-eye beans [8]. As the largest of the lymphatic organs, spleen participates in the body's defense system as a site of lymphocyte proliferation and of immune surveillance and response. Prenatally, the spleen is a hematopoietic

organ, but after birth it is involved primarily in identifying, removing and destruction of expended red blood cells, broken down platelets and recycling iron and globin [9].

Both acute and chronic kidney diseases are major causes of renal failure in humans and are associated with high incidence of morbidity and mortality rates linked to herbal medicinal abuse. Parts of *Mucuna urens* have been tested for several pharmacological activities, with some reports showing that its components form major constituents in extract formulations used for treating numerous ailments. The root of the plant mixed with honey is used to combat cholera [10]. The sap is rubbed on sprains, rheumatic areas, contusions, sore muscles to relief pain and used to treat fever in children [11], while the stinging hairs on the pods can be consumed to expel intestinal worms, and a tincture made from powdered beans macerated in alcohol is a soothing remedy against haemorrhoids, especially those inclined to bleed [11, 12]. Despite these merits as an ethnomedicinal plant, it has some demerits. The extract of the seeds causes degeneration of sperms and the hairs on the pod of the fruit cause itching if it comes into contact with the skin [13 11, 14]. However, other *Mucuna* species such as Mucuna pruriens improves male. Sperm count and motility were significantly recovered in infertile men [15].

2. MATERIALS AND METHOD

Collection of *Mucuna urens* Seeds: *Mucuna urens* seeds were obtained from a local market in Uyo Local Government Area, Akwa Ibom State, Nigeria. The seeds were

identified and authenticated by a taxonomist in the Department of Botany, University of Uyo, Nigeria.

Experimental Animals: Twenty-four male albino rats, weighing between 107-167 g were obtained from Animal House of Faculty of Pharmacy, University of Uyo. The rats were housed and acclimatized in the animal house for two weeks, under standard laboratory conditions. The rats were divided into four groups (Group 1, 2, 3 and 4) containing six rats each. The animals were fed daily throughout the course of acclimatization and administration with Vital Grower mash and clean water were provided *ad libitum*. All the animals were handled and cared for in accordance and in compliance with applicable guidelines and standard for the care and use of laboratory animals.

Preparation of Extract: The *Mucuna urens* seeds were crushed to peel off the epicarp and the mesocarp was removed with a knife to expose the endocarp. The seeds were grinded to obtain fine particles of powder. The particles were soaked in ethanol of 800ml for 72 h. The crude extract was separated from the suspended particles by filtration. The filtrate was concentrated in water bath at 45^{o}C for 48 h and stored in a refrigerator at 4^{o}C. The sticky concentrated ethanol free *Mucuna urens* seed extract was mixed with 20ml of water. The extract was administered by oral intubation [16].

Experimental Design: The animals were weighed before administration and distributed into 4 groups of 6 rats each, Group 1 was control. Groups (2-4) were given low, middle and high doses, respectively, of ethanolic extract of *Mucuna urens* for 28 days (Table 1).

Table 1: Schedule of administration for experimental groups

Groups	Dosage/kg body weight
Group 1	Distilled water (0.5mls)
Group 2	Low dose of *Mucuna urens*(100 mg/kg)
Group 3	Mid-dose of *Mucuna urens*(200 mg/kg)
Group 4	High dose of *Mucuna urens*(300 mg/kg)

Duration of Treatment = 28 days [16].

Body and Organ Weight: The body and organ weights of the animal were taken using an electronic weighing balance.

Haematological Parameters: The blood samples were obtained for the evaluation of some haematological parameters. The blood samples were collected in sample bottles for haematological assay. This was done using Mindray BC-5380 blood analyser to count and identify blood cells.

Animal Sacrifice: After 28 days of administration the animals were weighed again and sacrificed after inhalation of chloroform vapour. The animals were dissected. Kidney and spleen were cleaned and suspended in 10% buffered formalin for one week. The tissues were then processed for light microscopic examination.

Tissue Processing: The tissue processing was done to obtain paraffin sections and staining using haematoxylin and eosin.

3. RESULTS

Histological Analysis

Section of the kidney of the control group administered with distilled water showed normal Bowman's capsule, glomerulus and Bowman's space (Figure 1).Sections of the kidney administered with low dose (100 mg/kg b.w) of the ethanolic extract of *Mucuna urens* showed degenerated glomeruli, enlarged Bowman's space, shrunken glomerulus and eroded tubular epithelium (Figure 2). The kidney from the rats administered with mid-dose (200 mg/kg b.w.) of the ethanolic extract of *Mucuna urens* showed increase in degenerated glomeruli, enlarged Bowman's space, shrunken glomerulus and hemorrhage (Figure 3), while the high dose (300 mg/kg b.w.) of the extract caused severely enlarged Bowman's space and shrunken glomeruli (Figure 4).

The sections of the spleen of the control rat showed normal white and red pulps and lymphoid follicles with a clear peri-arteriolar lymphocytic sheath (Figure 5). The spleen administered with low dose of the ethanolic extract of *Mucuna urens* showed white pulp hyperplasia and red pulp hyperplasia (Figure 6), while the spleen from the rats given the mid-dose had white pulp hyperplasia, focal red pulp hyperplasia and increased fibrolytic activity in the trabeculae (Figure 7). The sections of the spleen of rats administered high dose of the extract of *Mucuna urens* showed extensive white

pulp hyperplasia, extensive red pulp hyperplasia and enlarged connective tissue (Figure 8).

Table 2: Effects of *Mucuna urens* on Mean Weight of Kidney and Spleen in AdultAlbino Rats

		Control Group	Group 2	Group 3	Group 4
Kidney (g)	Weight	1.10 ± 0.04	1.35 ± 0.07$^{N.S}$	1.19 ± 0.11 $^{N.S}$	1.14 ± 0.11 $^{N.S}$
Spleen (g)	Weight	0.56 ± 0.08	0.46 ± 0.03$^{N.S}$	0.67 ± 0.12 $^{N.S}$	0.68 ± 0.12 $^{N.S}$

Values are expressed as Means ± SD.
N.S. means statistically not different from the control.

Effect of *Mucuna urens* on the mean weight of Kidney and Spleen: The result of the effect of *Mucuna urens* on the weight of rat is presented in the Table 2 showing that the control group had a mean kidney weight of 1.10±0.04g; group 2 had increased kidney weight to 1.35±0.07g. The mean weight of the kidney from group 3 increased to 1.19±0.11g while group 4 was increased to 1.14±0.11g compared to the control. The mean weight of the spleen from the control group was 0.5±0.08g, group 2 administered with 100mg/kg body weight of ethanolic extract of *Mucuna urens* was 0.46±0.03g while group 3 administered with 200mg/kg body weight of ethanolic extract of *Mucuna urens* was 0.067±0.12g. Finally, group 4 administered with 300mg/kg body weight of ethanolic extract of *Mucuna urens* had a mean weight of

0.68±0.12g. The statistical analysis showed that there was no significant difference in the mean weight of the organs between the control and test groups.

Table 3: Effects of *Mucuna urens* on some Haematological Parameters in Adult Albino Rats

Blood Parameters	Control Group	Group 2	Group 3	Group 4	P value
RBC (g/dl)	6.01 ± 3.67	8.08 ± 0.28	7.61 ± 0.30	8.09 ± 0.92	$0.39^{N.S}$
MCHC (%)	32.05 ± 0.69	31.38 ± 0.42	32.36 ± 1.40	31.68 ± 0.91	$0.43^{N.S}$
WBC (%)	7.91 ± 1.46	6.71 ± 1.38	8.79 ± 2.89	6.45 ± 1.43	$0.30^{N.S}$

Values are expressed as Means ± SD; Significance level is at P< 0.05; N.S means not significant

Effect of *Mucuna urens* on Haematological Parameters: The result of the effect of *Mucuna urens* on haematological parameters is presented in the Table 3. The red blood count in the control group was 6.01±3.67g/dl; from group 2 it increased to 8.08±0.28g/dl; from groups 3 and 4, the RBC count increased to 7.61±0.30g/dl and 8.09±0.92g/dl, respectively. The mean corpuscular haemoglobin concentration of the control group was 32.05±0.69%, from group 2 it decreased to 31.38±0.42%; it decreased to 32.36±1.40% from group 3, while from group 4 the MCHC decreased to 31.68±0.91g. The white blood count of the control group had 7.91±1.46%; in group 2 it increased to 8.79±2.89% while the white blood count of group 4 decreased to 6.45±1.43%. The statistical analysis showed that these changes in blood parameters brought on by the different doses of the seed extract of *Macuna urens* were not statistically significant at p<0.05.

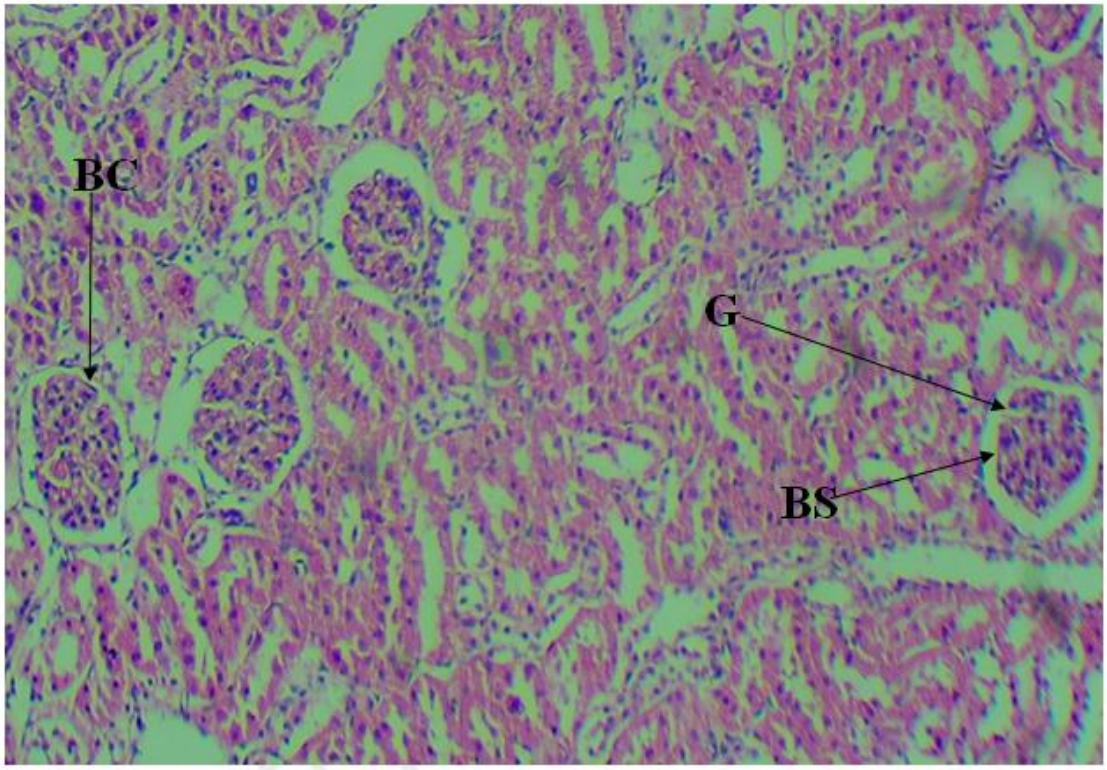

Fig. 1: Photomicrograph of kidney of control rats. Bowman's capsule (**BC**), glomerulus (**E**) and Bowman's space (**BS**) with normal cytoarchitecture. Mag X100, H&E stain.

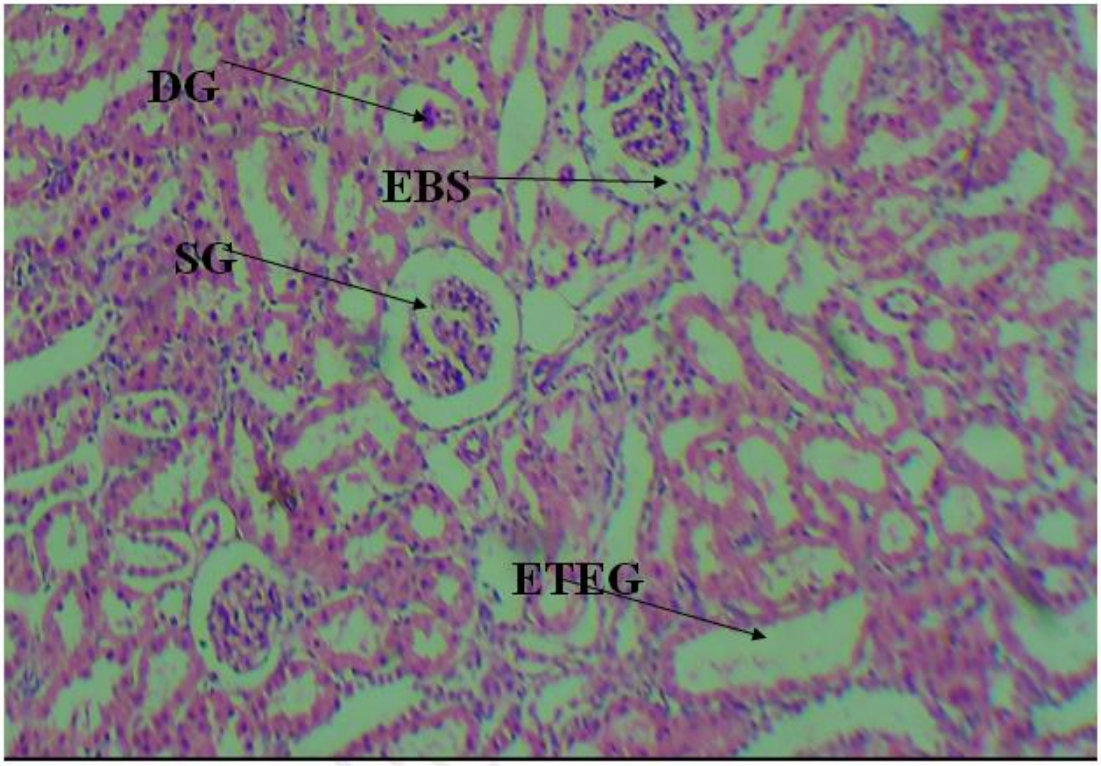

Fig. 2: Photomicrograph of kidney of group 2 rats administered with 100mg of *Mucuna urens.* Degenerated glomerulus (**DG**), enlarged Bowman's space (**EBS**), shrunken glomerulus (**SG**) and eroded tubular epithelium (**ETE**). Mag X100, H&E stain.

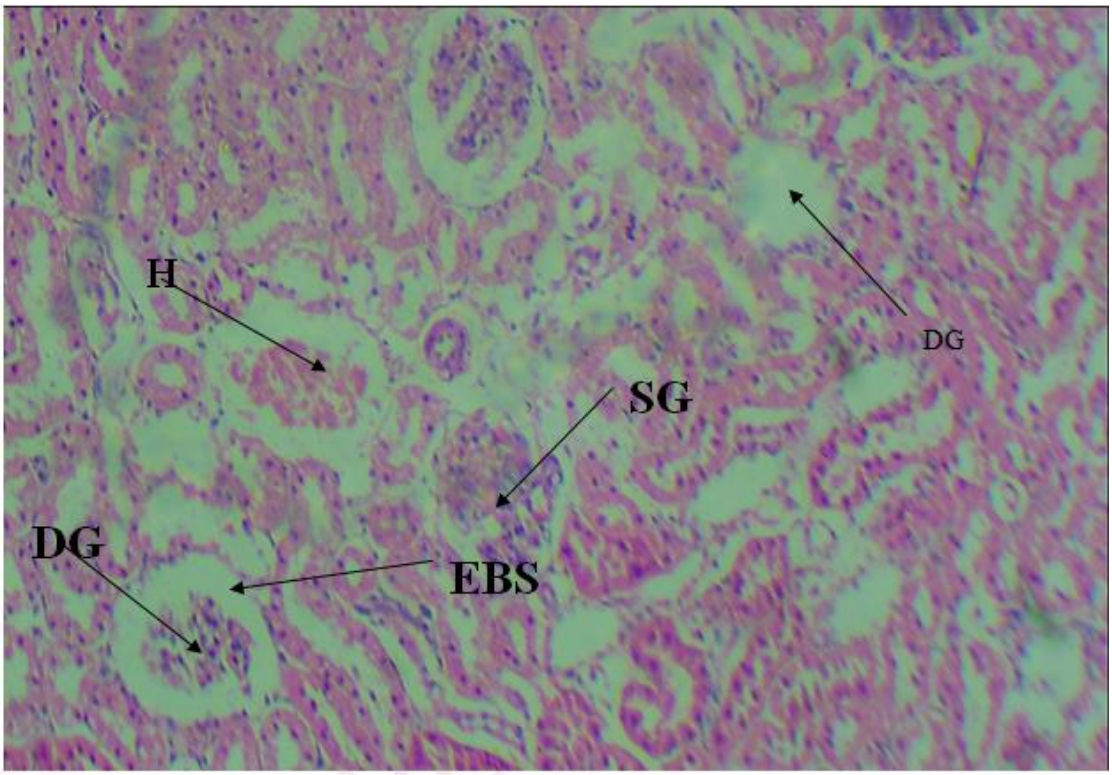

Fig. 3: Photomicrograph of kidney of group 3 rats administered with 200mg of *Mucuna urens*. Increased in degenerated glomeruli (**DG**), enlarged Bowman's space (**EBS**), shrunken glomerulus (**SG**) and hemorrhage (**H**) (**V**). Mag X100, H&E stain.

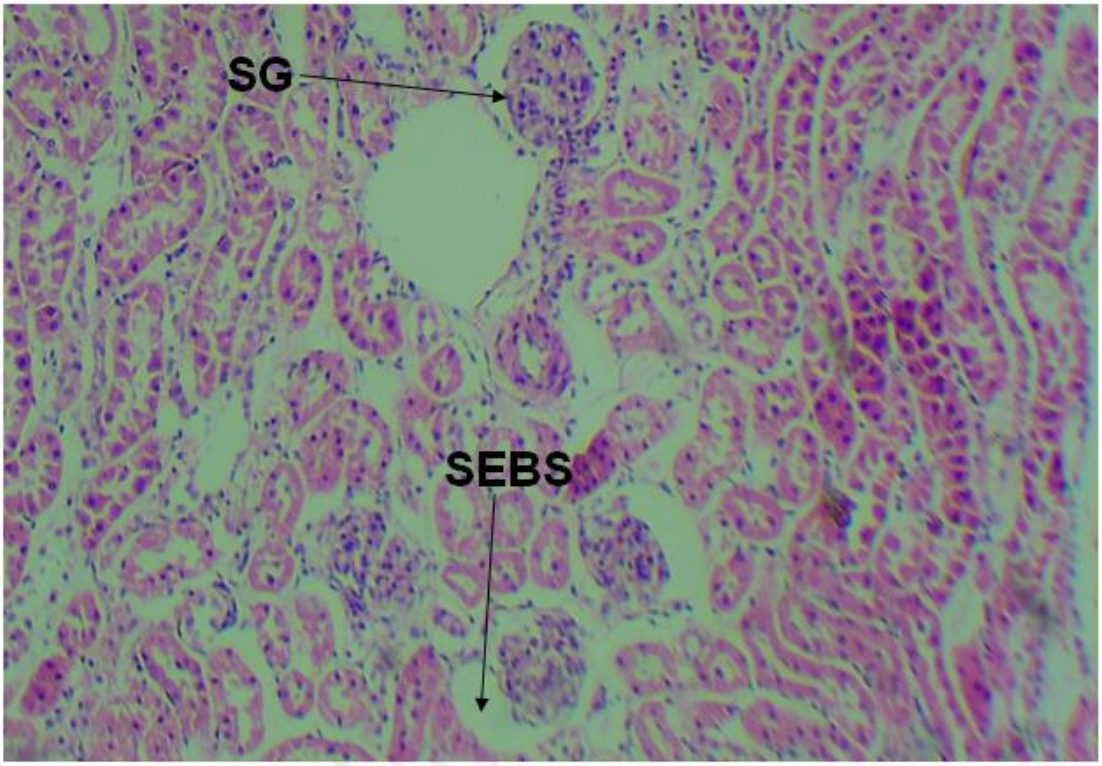

Fig. 4: Photomicrograph of kidney of group 4 rats administered with 300mg of *Mucunaurens. S*everely enlarged Bowman's space (**SEBS**), shrunken glomerulus (**SG**)Mag X100, H&E stain.

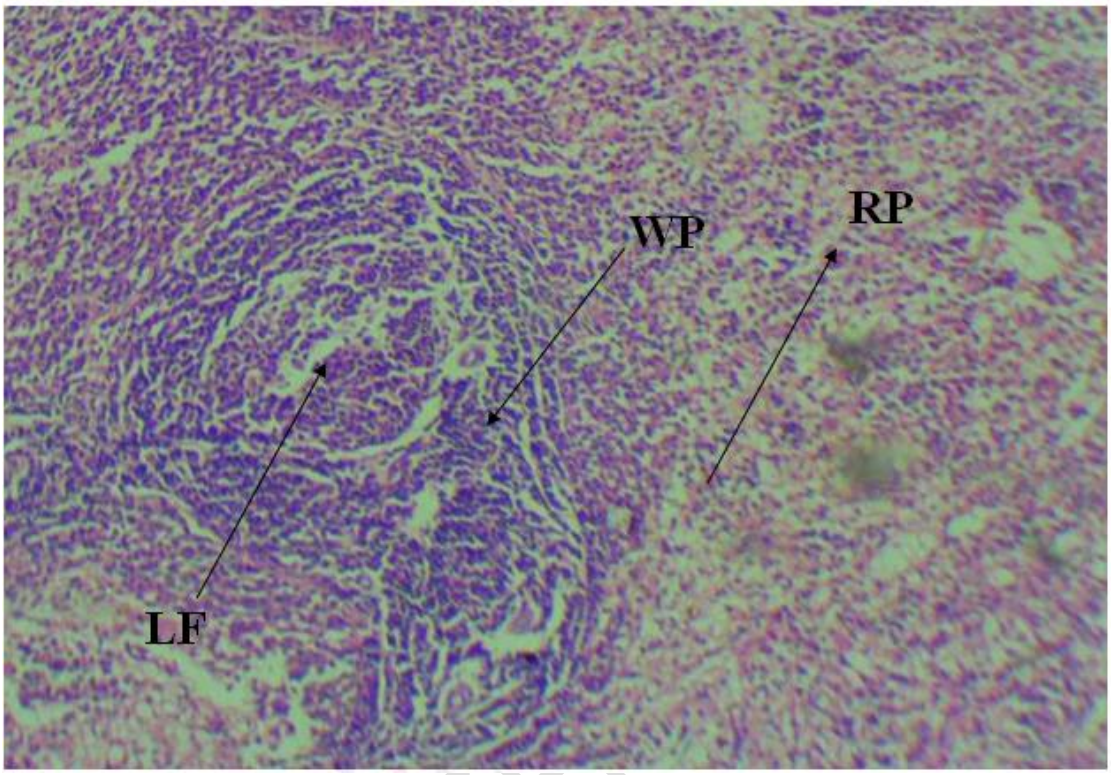

Fig. 5: Photomicrograph of spleen of control rats showing white pulp (**WP**), Red Pulp (**RP**) and lymphoid follicle (**LF**) with a discernible peri- arteriolar lymphocyte sheath of normal cytoarchitecture . Mag X100, H&E stain.

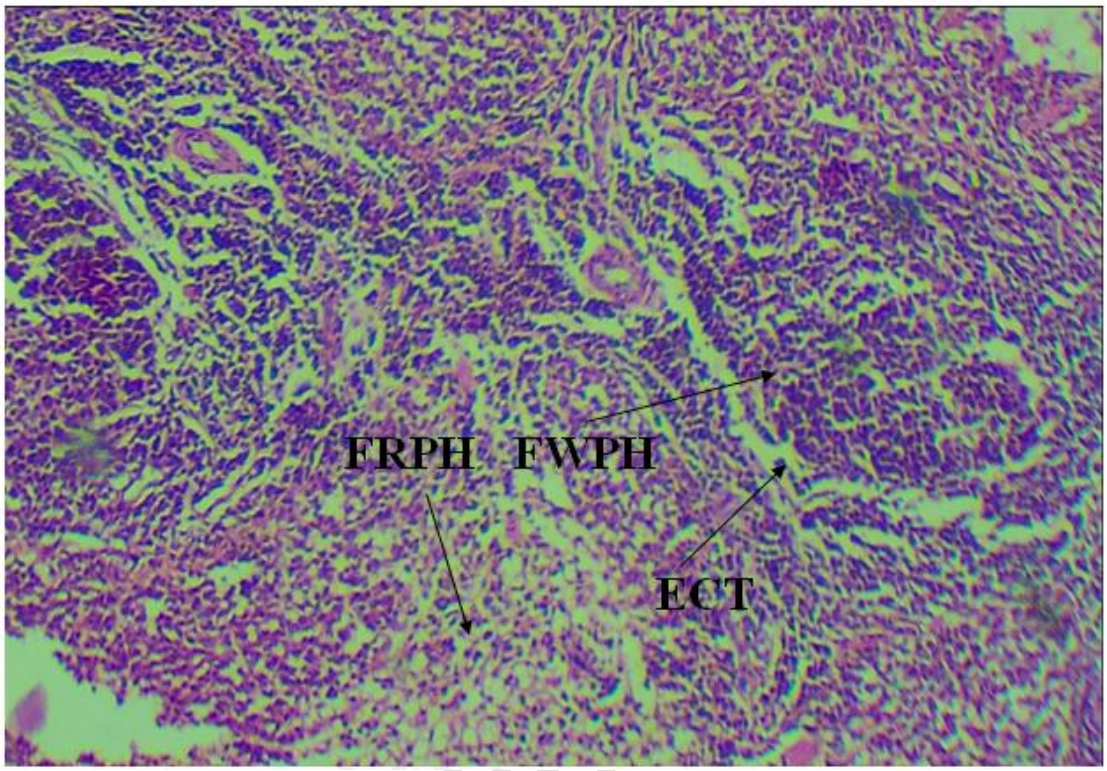

Fig. 6: Photomicrograph of spleen of group 2 rats administered 100mg body weight of *Mucuna urens* showing white pulp hyperplasia (**HWP**), red pulp hyperplasia (**RPH**) and enlarged connective tissue (**ECT**) trabeculae. Mag X100, H&E stain.

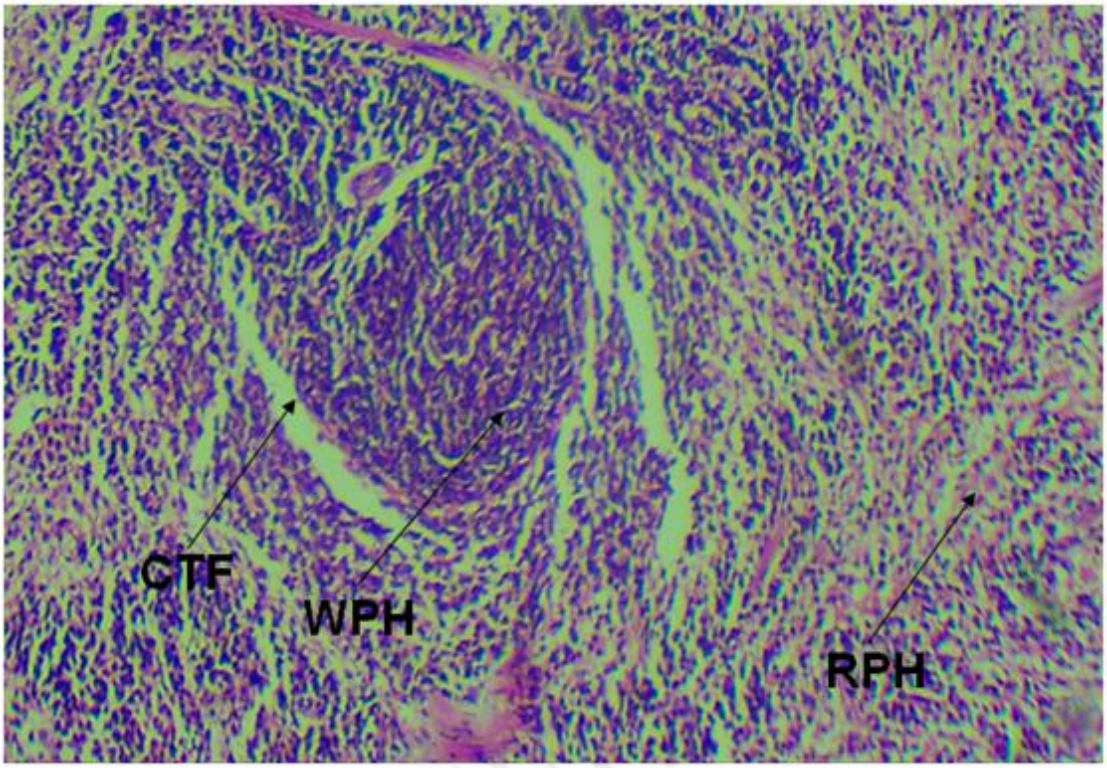

Fig. 7: Photomicrograph of spleen of group 3 rats administered 200 mg of *Mucuna urens* showing white pulp hyperplasia (**HWP**), red pulp hyperplasia (**RPH**)) and connective tissue fibrosis (**CTF**). mag X100, H&E stain.

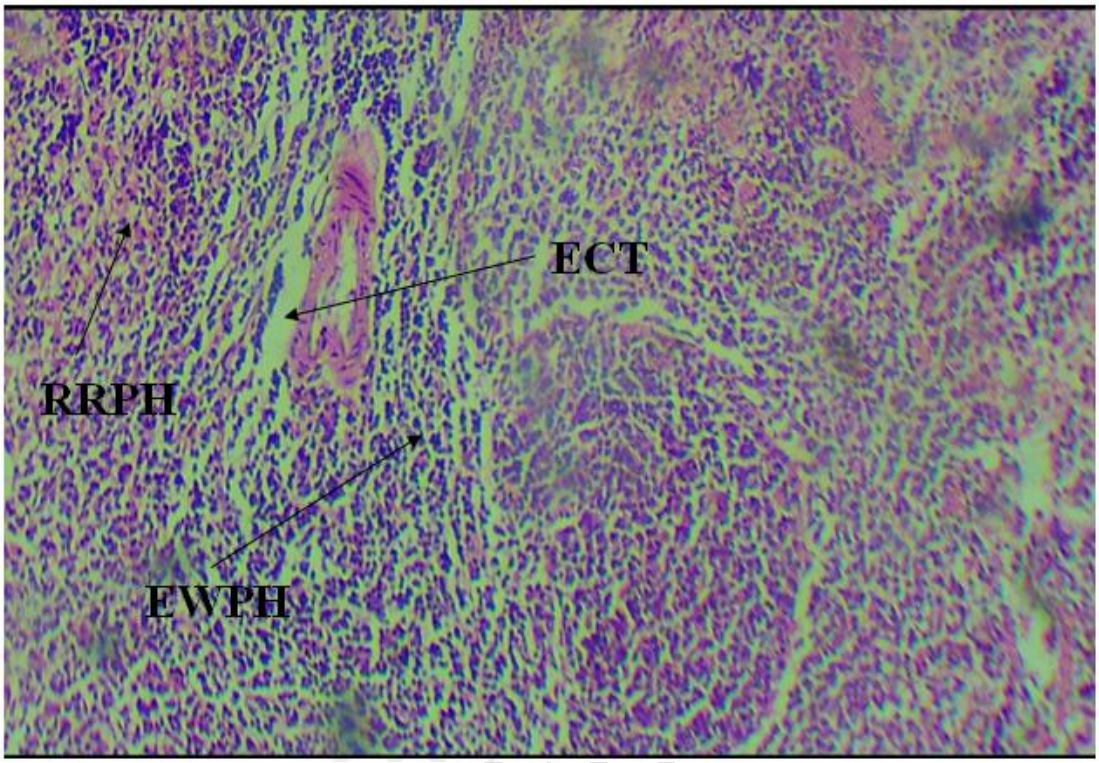

Fig. 8: Photomicrograph of spleen of group 4 rats administered with 300mg of *Mucunaurens*showing extensive white pulp hyperplasia (**EHWP**), extensive red pulp hyperplasia (**ERPH**) and enlarged connective tissue (**ECT**). Mag X100, H&E stain.

4. DISCUSSION

Histopathologically, in the control group showed normal cytoarchitecture of the kidney with well-defined Bowman's capsule, glomerulus and Bowman's space. The renal corpuscle consists of glomerulus, the glomerular capsule with a parietal and a visceral layer, between this layer is the capsular space with podocytes located in the surface of the visceral layer. At the capsular pole of the renal corpuscle, blood vessels enter and leave the renal corpuscle [17]. This research showed that the administration of ethanolic seed extract of *Mucuna urens* produced some pathological changes in the cytoarchitecture of the sections of the kidney compared to the control group. The photomicrograph of the kidney of an adult albino rats administered with 100 mg/kg body weight of ethanolic extract of *Mucuna urens*, showed degenerated glomeruli, enlarged Bowman's space, shrunken glomerulus and eroded tubular epithelium which may be due to weakening of the tubules caused by alkaloids present in *Mucuna urens* which according to Manfred (2002)[18] are vasolidators. These conditions are associated with prolonged reduction in the percentage of cardiac output to the kidney which causes reduced glomerular pressure and collapse of the glomerular capillaries [19]. Dilation of vessels all over the body for a prolonged time can cause cardiac depression which can eventually lead to reduced glomerular capillary pressure and increase urinary space [20]. The messengial cells of the kidneys are contractile and modify the diameter of the glomerular capillaries in response to vasoactive substance. This is in agreement with Ojewole and Obebiyi (1980) [21] who reported that

tetramethy/pyrazine (TMPZ) isolated from stems of Jatropha species possesses specific spasmolytic and vasodilator activity. The photomicrograph of kidney administered with 200 mg/kg body weight of ethanolic extract of *Mucuna urens* showed increased degenerated glomeruli, enlarged Bowman's space, shrunken glomerulus and hemorrhage. The hemorrhage may be due to the presence of alkaloids which is vasodilator [18]. These conditions, are associated with decreased renal blood flow due to bleeding and when glomerular filtration rate is decreased. General reduction in body fluid and extracellular fluid caused by hemorrhage, vomiting and diarrhea can cause glomerular hypotension, which can also result in glomerular shrinkage, distortion and increased peri-glomerular spaces [20]. The photomicrograph of kidney administered with 300 mg/kg of ethanolic extract of *Mucuna urens*, showed severely enlarged Bowman's space and shrunken glomerulus which may be due to spasmolytic and vasodilatory effects of *Mucuna urens* which are associated with capillary pressure, which increases tensile stress to the capillary wall and collapse of the glomerular capillaries [22]. Spleen is an ovoid, usually pulplish, pulpy mass about the size and shape of one's fist. The spleen is located in the superolateral part of the left upper quadrant or hypochondrium of the abdomen, as the largest of lymphatic organ, It participates in body's defense system as a site of lymphocyte proliferation and immune surveillance and response. The control group showed normal cytoarchitecture of the white pulp, red pulp and lymphoid follicles with a peri-ateriolar lymphocyte sheath. The photomicrograph of spleen of group 2 administered

with 100 mg/kg body weight of ethanolic extract of *Mucuna urens* showed white pulp hyperplasia, red pulp hyperplasia and enlarged connective tissue trabeculae. These may be due to the weakening of tubules caused by alkaloids present in *Mucuna urens* resulting in the compression of adjacent splenic tissue and distortion of the capsule and thereby causing abnormal bleeding, this is in agreement with the work of Stefanski *et al.* (1990) [23] who found out the effect of hyperplasia of the red pulp in aging fischer rats. The photomicrograph of spleen of group 3 administered with 200 mg/kg of ethanolic extract of *Mucuna urens,* showed extensive white pulp hyperplasia, enlarged connective tissue fibrosis and reduced red pulp hyperplasia, which may be due to the high toxicity of the *Mucuna urens* and these might alter immune status and thereby causing abnormal bleeding and chronic lymphocyte leukemia [24]. The photomicrograph of the spleen of group 4 administered with 300 mg/kg of ethanolic extract of *Mucuna urens* showed extensive white pulp hyperplasia, increased red pulp hyperplasia and enlarged connective tissue resulting in altering immune status. However, chronic *Mucuna urens* treatment could produce cytological shifts in the lymphocyte population which might be due to selective stimulation of specific lymphocyte subpopulation. The shifts in the lymphocyte subpopulation subsequent to *Mucuna urens* administration might reflect and altered immune status. Yamano (2009) [25] studied the effect of cadmium chloride on the spleen. In this study, white blood cell, (WBC), Mean corpuscular hemoglobin concentration (MCHC) red blood count (RBC) proved no Significant. That is, WBC, MCHC and

RBC were significantly not affected compared to the control group. In the MCHC, animals administered with ethanolic extract of *mucuna urens e*xerted the highest value (32.36±1.40g) compared to the control group (32.05±0.69g) although not significant. This may be due to haematopoietic activity caused by some minerals in *Mucuna urens* which enhanced haemoglobin formation such minerals include copper and iron [5]. It also contains ascorbic acid an erytropoietic factor [26]. These together enhances increase percentage of MCHC. In the RBC, animals administered with ethanolic extract of *Mucuna urens e*xerted the highest value (8.09±0.92g) compared to the control group (6.0±3.67g). The increase in RBC above the control may be due to anti-anemic effect of *Mucuna urens* leading to polycythemia [27]. In the WBC, animals administered with ethanolic extract of *Mucuna urens e*xerted the highest value (8.79±2.89g) compared to the control group (7.91±1.46g). The weight of the kidney, spleen and hematological parameters were significantly not affected compared to the control group.

5. CONCLUSION

The results obtained from this research showed that *Mucuna urens* have degenerative changes on the kidney and spleen thereby producing pathological changes such as shrunken glomeruli, degenerated glomerulus, enlarged Bowman's space, and hemorrhage in the kidney, white and red pulp hyperplasias, and increase fibrosis in the spleen respectively. Therefore, the seeds of *Macuna urens* should be consumed with caution.

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