



Toxicological effect of *Mucuna sloanei* on biochemical indices and hematological parameters

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Abstract

Mucuna sloanei seeds, commonly known as the 'Horse-eye' or 'Hamburger' bean, which holds significance in various Nigerian ethnic groups. This research aims to assess the toxicological effects of *Mucuna sloanei* seeds on hematological and biochemical parameters in albino rats. Twenty albino rats were randomly divided into four groups (A, B, C and D) of five rats each. Group A (control) received distilled water normal rat feed only throughout the duration of the experiment while Groups B, C and D received different dosages (50 mg/kg body weight, 100 mg/kg body weight and 200 mg/kg body weight respectively) of *Mucuna sloanei* seed extract for fourteen days. Twenty four hours after the last administration, the animals were sacrificed following anesthesia. The activities of serum biomarkers aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), bilirubin (BIL), albumin (ALB), urea and uric acid (AC) were determined. Administration of all dosages of *Mucuna sloanei* seed extract (50, 100 and 200mg/kg b.w) caused significant ($P < 0.05$) decrease in the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and levels of bilirubin, urea and uric acid of the animals when compared to the control. The values obtained further showed that the extract (at all dosages) caused significant increase in albumin level when compared to the control. The results suggest the antioxidant properties of ethanolic extract of *Mucuna sloanei* seed extract, lend support to the ethnobotanical usage of the seed in the treatment of various ailments and sheds light on impact *Mucuna sloanei* seed extract on liver and renal function, health.

Key word: Hematological Parameters; Biochemical Indices; Albino Rats; *Mucuna sloanei*

1. Introduction

Plants have been used for food, medicine, building materials and fuel for many centuries until now [1]. The World Health Organization (WHO) noted that of the 119 plants-derived pharmaceutical drugs, 74 % are used in modern medicine in ways that correlated directly with their traditional uses as Herbal plant medicine by native culture. WHO estimated that about 80 % of the world population presently uses herbal medicine for some aspects of their primary health care needs while plant products also play important roles in the health care system of the remaining 20 %, who mainly reside in developed countries (Hoareau, and Da silva, 2019).

Mucuna sloanei, commonly known as the 'Horse-eye' or 'Hamburger' bean, is an annual leguminous plant extensively utilized by various ethnic groups in Nigeria [2]. Locally, it is referred to as 'ukpo' by the Ibos, 'karasuu' by the Hausas, 'Ijokun' by the Yorubas, and 'ibabat' by the Efiks. Its seeds are used as a source of vegetable oil, condiment, or soup thickener in Igbo communities across sub-Saharan Africa. Rural populations in Nigeria consume *M. sloanei* seeds during periods when other legumes are scarce [3]. These seeds are rich in protein, carbohydrates, crude fat, fiber, and amino acids, as well as bioactive substances like L-3,4-dihydroxyphenylalanine (L-DOPA), a precursor of dopamine. Additionally, they contain important phytochemicals such as alkaloids, phytic acid, tannins, flavonoids,

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haemagglutinins, and oligosaccharides. The lectin from *M. sloanei* seeds can agglutinate blood cells of various animals, acting as an effective cell receptor signal inducer [4].

A study by [5] reported that aqueous extracts of the seeds did not adversely affect the haematological parameters of normal Wistar rats. Despite its noted medicinal values, there is limited information on its effects on liver and renal function in experimental animals, and potential risks for human consumption remain unclear. Within the *Mucuna* genus (synonym: *Stizolobium*), approximately 100 varieties have been identified, including *Mucuna pruriens* and *Mucuna cochinchinensis*, which is cultivated in parts of Southern Nigeria and Senegal.

Its parts are used in traditional medicine for a wide range of ailments, including constipation, nephropathy, dysmenorrhoea, ulcers, helminthiasis, fever, and delirium. The leaves, pods, and seeds are used as aphrodisiacs, anthelmintics, and for treating inflammation, ulcers, and general debility. The seeds possess multiple medicinal properties, including analgesic, anti-inflammatory, anti-Parkinson's, antispasmodic, anti-venin, febrifuge, hormonal, hypocholesterolemic, hypoglycemic, immunomodulator, nervine, neurasthenic, antilithic, antiparasitic, and central nervous system stimulant properties [6]. The value-added phytochemicals in *Mucuna* seeds have significant medicinal importance, containing compounds such as alkaloids, arachidic acid, beta-sitosterol, dopamine, flavones, gallic acid, serotonin, and stearic acid. These compounds contribute to their antioxidant activities and potential health benefits [6]. Therefore the present study investigated toxicological effect of *Mucuna sloanei* seed in albino rats.

2. Materials and Methods

2.1. Reagents and Chemicals

All chemicals and all other reagents used in this study were all of analytical grade.

2.2. Collection and authentication of plant samples

Mucuna sloanei seeds used for this study was obtained from a farm at Iworoko Ekiti, Ekiti State, Nigeria. A voucher sample was deposited at the herbarium section of the Department of Agricultural Technology where it was identified by a taxonomist.

2.3. Preparation of the plant material

Dried, mature nuts of *M. sloanei* were obtained from Iworoko-Ekiti, Ekiti State, Nigeria, and identified using [7] key. The seeds were de-hulled, dried to a constant weight for forty three days and pulverized with an automated laboratory blender and stored at room temperature for further use following the method of [8] method,

2.4. Preparation of the ethanol extract of *Mucuna sloanei* Seed

Mucuna sloanei seeds were air-dried at room temperature for 43 days at room temperature. The air-dried seeds were ground to fine powder using a blender. 500 g of the powdered root was soaked in 2000 ml of ethanol for 72 hours, filtered and air -dried to obtain the dried extract. The extract was kept in a closed container and kept inside the fridge at 4°C for further studies [9].

2.5. Animals' protocol

Twenty albino rats (male) weighing 120 kg – 150 g were obtained from the animal house of the Department of Biochemistry, Ladoke Akintola University Ogbomoso, Oyo State.

They were acclimatized for two weeks at the animal house of the Department of Science Technology, Federal Polytechnic, Ado Ekiti and were allowed to have free access to food (commercial pelletized diet from Vital Feed Mill) and drinking water *ad libitum* daily.

2.6. Experimental Design

Twenty male albino rats were randomly divided into five (1-4) of five animals in each group.

- Group A: Normal Control
- Group B: 50 mg/kg body weight of *Mucuna sloanei* seed extract
- Group C: 100 mg/kg body weight) of *Mucuna sloanei* seed extract
- Group D: 200 mg/kg body weight of *Mucuna sloanei* seed extract

2.7. Administration of the extracts

The ethanol and aqueous extract of *Mucuna sloanei* seed extract at the stated concentrations were administered through the oral route for fourteen days.

2.8. Dissection of the Rats

Twenty four hours after the last administration, the rats were dissected and portion of blood was collected into EDTA and plain bottles for haematology and determination of biochemical parameters.

Target organs (liver and kidney) were excised using scissors and forceps and trimmed of fatty tissues.

2.9. Preparation of Serum

Serum was prepared by centrifugation at 3000 rpm for 10 min at 25°C. The clear supernatant was collected and used for the estimation of plasma biochemical parameters.

2.10. Enzyme Biomarkers

2.10.1. Assay of Aspartate Aminotransferase (AST) Activity

AST activity was determined following the principle described by [10].

2.10.2. Procedure

Briefly, 0.1ml of diluted sample of sample was mixed with phosphate buffer (100mmol/L, pH 7.4), L-aspartate (100mmol/L), and α -oxoglutarate (2mmol/L) and the mixture incubated for exactly 30min at 37°C. 0.5ml of 2,4-dinitrophenylhydrazine (2mmol/L) was added to the reaction mixture and allowed to stand for exactly 20min at 25°C. Then 5.0ml of NaOH (0.4mol/L) was added and the absorbance read at 546 nm against the reagent blank after 5 min.

2.11. Assay of Alanine Aminotransferase (ALT) Activity

The principle described by [10].was followed in the assay of ALT using commercially available assay kit (Randox laboratories, UK) according to the instructions of the manufacturer.

2.11.1. Assay Procedure

Reagent 1 (0.5 ml) containing phosphate buffer (100mmol/l, pH 7.4), L-alanine (200mmol/l) and α -oxoglutarate (2.0mol/l) was added to 0.1ml of serum in a test tube and the mixture was incubated at 37°C for 30 minutes. Exactly 0.5ml of R2 containing 2, 4-dinitrophenylhydrazine (2.0mmol/l) was added and the solution incubated again at 20°C for 20 min. Finally, 5ml of NaOH was added and the solution was allowed to stand for 5 minutes at room temperature and the absorbance was read at 546nm.

2.11.2. Calculation

The activity of ALT in the serum was obtained from the standard curve provided in the kit.

2.12. Assay of Alkaline Phosphatase (ALP) Activity

Assay of serum ALP was based on the method [11].using commercial assay kits (Randox laboratories, UK) according to the instructions of the manufacturer.

2.12.1. Assay Procedure

Exactly 1.0ml of the reagent (1mol/l diethanolamine buffer pH 9.8, 0.5mmol/l $MgCl_2$; substrate: 10mmol/l p-nitrophenolphosphate) was added to 0.02ml of the serum sample and mixed. The absorbance was taken at 405 nm for 3 minutes at intervals of 1 minute.

2.12.2. Calculation

ALP activity was determined using the formula $U/l = 2760 \times A_{405nm}/min$

2.13. Assay of Albumin (ALB) Activity

Albumin activity was determined following the principle described by [12].

2.13.1. Procedure

Briefly, 10µl of diluted sample of sample was mixed 3000 µl of Reagent 1 (succinate buffer:75mmol/L, pH 4.2, Bromocresol green 0.15mmol/L) and the mixture incubated for exactly 20min at 20°C-25 °C. or 10 min at 37°C and the absorbance read at 578 nm within 60 min against the reagent blank.

$$\text{Albumin Concentration (g/dl)} = \frac{\text{Abs Sample}}{\text{Abs of Standard}} \times \text{Conc of sample}$$

Assay of Bilirubin (BIL) Activity

Bilirubin activity was determined following the principle described by [13].

2.13.2. Procedure

200µl of diluted sample, Reagent1(Sulphalinic acid), R2 (Nitrite) and R3 (Caffeine) were mixed and allowed to stand 10min at 20°C-25 °C. Then 200 µl Reagent R4 (tartarate) was mixed and allowed to stand for 5-30 min at 20 °C-25 °C . The absorbance of the sample against the sample blank was read at 578nm.

$$\text{Total Bilirubin (mg/dl)} = 10.8 \times A_{TB}$$

2.14. Assay of Urea

Urea concentration was determined following the principle described by [14]

2.14.1. Procedure

5µl of sample was mixed with 50 µl of Reagent 1 (EDTA 116mmol/l, Sodium nitropusside 6mmol/land Urease 1g/l) and incubated at 37°C for 10 min. 1.25ml of Reagent 2(Phenol 120mmol/l) and 1.25 ml of Reagent 3 (sodium hypochlorite 27 mmol/l and sodium hydroxide 0.14N) were mixed immediately and incubated at 37°C for 15 min. The absorbance of the sample against the blank was read at 546nm.

$$\text{Urea Concentration (mg/dl)} = \frac{\text{Abs Sample}}{\text{Abs of Standard}} \times \text{Conc of standard}$$

Assay of Uric Acid (UA)**2.14.2. Principle**

Uric acid concentration was determined following the principle described by [14]

2.14.3. Procedure

20µl of sample was mixed with 1000 µl of Reagent 1 and incubated at 20-25 °C for 15 minutes or at 37°C for 5 min. The absorbance of the sample (A, sample) and standard (A. standard) against the reagent blank was read within 30 minutes at 546nm.

$$\text{Uric acid Concentration (mg/dl)} = \frac{\text{Abs Sample}}{\text{Abs of Standard}} \times \text{Conc of standard}$$

Determination of Haematological Parameters

Haematological parameters, including total red blood cells (RBC), haemoglobin (Hb), and white blood cells (WBC) counts, were determined following [15] methods. Packed cell volume (PCV) was measured using [16] methodology. A heparinized capillary tube filled with anticoagulated blood was centrifuged at 7826g for 5 minutes, and the packed cell height was measured with a haematocrit reader. Red blood cell indices—mean cell volume (MCV), mean cell haemoglobin (MCH), and mean cell haemoglobin concentration (MCHC)—were determined according to [17]. All reagents used were of analytical grade.

3. Results

Table 1 Effect *Mucuna sloanei* Seed Extract on Serum Biochemical Parameters in Albino Rats

Parameters	A	B	C	D
ALB (g/dl)	41.53±0.23	46.14±0.25	54.19±0.15	55.21±0.10
AST (IU/l)	36.12±0.36	29.72±0.11	22.51±0.43	17.32±0.13
ALT (IU/l)	47.75±0.10	31.34±0.28	26.70±0.42	18.64±0.12
ALP (IU/l)	64.61±0.53	42.39±0.17	38.29±0.21	36.40±0.57
BIL (mg/dl)	32.54±0.24	29.63±0.40	24.31±0.51	21.87±0.20
Urea (mg/dl)	20.95±0.27	16.41±0.23	13.21±0.16	11.63±0.32
Uric Acid (mg/dl)	28.15±0.19	23.84±1.30	21.06±0.22	14.22±0.14

Key : Group A: Normal Control; Group B: 50 mg/kg body weight of *Mucuna sloanei* seed extract; Group C: 100 mg/kg body weight) of *Mucuna sloanei* seed extract; Group D: 200 mg/kg body weight of *Mucuna sloanei* seed extract

Table 1 shows the activities and levels of biomarkers enzymes and molecules: aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), bilirubin (BIL), albumin (ALB), urea and uric acid (AC).

The result showed that serum AST, ALT and ALP activities and , BIL, ALB, urea and uric acid levels were found to be decreased by treatment with *Mucuna sloanei* seed extract (50 mg/kg, 100 mg/kg and 200 mg/kg) when compared with the control. The decreases observed in 50 mg/kg, 100 mg/kg and 200 mg/kg-treated rats were dose-dependent.

In the same vein, treatment of experimental animals with *Mucuna sloanei* seed extract had pronounced effect on the albumin level of the serum. Significant decrease in the level of albumin was recorded when compared with the control.

Table 2 Effect *Mucuna sloanei* Seed Extract on Haematological indices in Albino Rats

Parameters	A	B	C	D
PCV(%)	31.38±0.4	35.40±0.5	37.22±0.1	38.58±0.2
Hgb(g/dl)	11.13±0.1	12.45±0.1	13.06±0.2	13.94±0.1
RBC(pg)	6.62±0.2	7.17±0.3	7.37±0.1	7.52±0.3
platelet/Mm3	162.67±0.3	148.3±0.2	133.05±0.3	117.01±0.2
WBC/Mm3	79.3±0.2	82.3±0.2	89.00±0.2	91.67±0.3
MCV(ml)	40.66±0.4	43.83±0.4	47.44±0.1	49.06±0.4
MCH(Pg)	14.62±0.1	15.87±0.3	15.88±0.3	16.87±0.2
MCHC(g/dl)	28.08±0.4	30.53±0.3	29.32±0.2	31.45±0.2
% Neutrophils	24.00±0.2	33.00±0.2	38.00±0.3	39.00±0.4
% Lymphocytes	34.33±0.3	40.33±0.1	40.57±0.2	41.67±0.1
Monocytes%	7.67±0.2	8.67±0.4	9.33±0.1	11.30±0.3
Eosinophils %	1.00±0.3	2.00±0.2	2.67±0.1	2.89±0.2
Basophils %	2.00±0.3	2.00±0.3	2.00±0.3	2.00±0.1
RDWC %	14.13±0.4	14.18±0.1	15.10±0.1	15.31±0.1

Key: Group A: Normal Control; Group B: 50 mg/kg body weight of *Mucuna sloanei* seed extract; Group C: 100 mg/kg body weight) of *Mucuna sloanei* seed extract; Group D: 200 mg/kg body weight of *Mucuna sloanei* seed extract

The table presents the mean and standard deviation of various haematological indices, such as packed cell volume (PCV), haemoglobin (Hgb), red blood cell (RBC) count, platelet count, white blood cell (WBC) count, mean corpuscular

volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and differential leukocyte count. The results indicate that *Mucuna sloanei* seed extract had a positive effect on most of the blood parameters, when compared with the control group.

4. Discussion

Biomarkers (enzyme) are located in the different cells, tissues and organs in the body system and leak out and make their way into the general circulation when these cells, tissues and organs are damaged [18]. Detection of injury in a cell, tissue or an organ could be afforded by measuring serum levels of known marker enzymes such as AST, ALT, ALP activities. Several studies have reported elevation in the activities of serum concentration on the normal rats AST, ALP and ALT in disease states [18]. This might possibly be due to the release of these enzymes from the cytoplasm into the blood circulation rapidly after rupture of the plasma membrane and cellular damage.

Aspartate aminotransferase (AST) is present in large amount in the heart muscle tissues but is normally found in liver, red blood cells, pancreas and kidney and is among the most sensitive markers employed in the diagnosis of organ damage [19]. The serum activity of alkaline phosphatase (ALP) is elevated in a large number of disorder that affects the damage of bile, such as gall stone or tumor blocking the flow of bile in smaller bile channels within the liver [20]. ALT is one of the most commonly used markers of hepatocytes injury, and elevation in serum level is usually observed in cases of hepatotoxicity [21]. Serum urea and uric acids levels are important biomarkers as they play a pivotal role in diagnosis and follow up of kidney failure. Urea, produced by the liver in the urea cycle as a waste product of metabolism of protein (either from the oxidation of amino acids or from ammonia), is dissolved into the blood and transported and excreted by the kidney as a component of urine. It is a sensitive biomarker used in the assessment of renal tissue damage. Therefore, in renal tissue injury, there is retention of urea. Increase urea level is associated with nephritis, renal ischemia, urinary tract obstruction, and extrarenal diseases ([22]; [23]).

Uric acid is the major product of the catabolism of purine nucleotides, however, the bulk of purine are ultimately excreted as uric acid from degradation of endogenous nucleic acids. Elevation of serum uric acid levels have been implicated in various disorders including gout, increased nuclear breakdown and renal diseases possibly by inhibiting pyrimidine nucleotide formation and cellular growth [23]. Proteins circulate throughout the blood to help the body maintain fluid balance. Albumin is a type of protein the liver makes. It's one of the most abundant proteins in the blood. Proper balance of albumin is needed to keep fluid from leaking out of blood vessels. Albumin gives the body the proteins it needs to keep growing and repairing tissue. It also carries vital nutrients and hormones. A serum albumin test is a simple blood test that measures the amount of albumin in the blood.

Oral administration of the ethanolic extracts of *Mucuna sloanei* seed extract significantly reduced the activities of AST, ALT, ALP and levels of BIL, urea and uric acid while the level of albumin was increased when compared with the control (Table 1). This is an indication that the extracts might prevent kidney and liver damage by maintaining the integrity of the plasma membrane and suppressing the leakage of the enzymes through the membrane.

The examination of hematological parameters including the red cells (erythrocytes), white cells (leucocytes) and the platelets (thrombocytes) and factors relating to them, provide information on inflammation, necrosis, various infections of visceral organs and the presence of stress factors ([25]; [26]). It also plays a vital role in the physiological, nutrition and pathological status of an organism [27]. The observed significant increase in PCV, RBC counts, and MCHC of the test rats at dose of 50mg/kg, 100 mg/kg and 200 mg/kg may suggest that the *Mucuna sloanei* seed extract has haematopoietic property and might enhance erythropoiesis of animals and may increase resistance to oxidative damage to red blood cells membranes. This is in agreement with previous studies [28] which reported that *M. indica* stem bark extract has potential for boosting the haematopoietic system of rats, and it was also earlier reported that *M. indica* stem bark extract has antianaemic properties, so might improve erythropoiesis and probably reduce oxidative damage to RBC membranes. The major functions of the white blood cell and its differentials are to fight infections, defend the body by phagocytosis against invasion by foreign organisms and to produce or at least transport and distribute antibodies in immune response [29]. The significant increase in WBC counts in the test animals suggests that the extract may have immunological properties, by stimulating increased production of white blood cells, thereby boosting the defense system of the animals. This is in agreement with earlier findings [30] that *M. indica* extract might have immune stimulating properties, which may account for the use of the plant for medicinal purposes by traditional medical practitioners. Lymphocytes use the blood to travel round the body but can wander freely in other types of tissues using the lymphatic channels. The lymphocytes are also known to play key roles in the immune defense system as a function of the immune status. High lymphocytes in animal blood shows ability to resist viral, parasitic and bacterial infection. The concentrations of lymphocytes was significantly ($p < 0.05$) increased in the *Mucuna sloanei* seed extract treated groups compared to the control. Herbal extracts have potential application as immune stimulants and could act against

a broad spectrum of pathogenic microorganisms. The effect is dose dependent and there is the tendency for overdose to occur, therefore dosage optimization is strongly recommended

5. Conclusion and Recommendation

In conclusion, the administration of *Mucuna sloanei* seed extract to albino rats resulted in decreased activities of AST, ALT, and ALP, as well as levels of bilirubin, urea and uric acid while the level of albumin was increased. The treatment with the ethanolic extract of *Sida acuta* showed promising renoprotective effects in these rats.

The study also showed that *Mucuna sloanei* seed extract may increase the rate of erythropoiesis, slow down the natural process of oxidative breakdown of RBCs and may also promote the immune stimulatory activities of WBC.

It is therefore recommended that further studies should be carried out to ascertain the effect of prolonged treatment with the plant extract in order to ascertain its chronic effect on the vital organs of rats. Also, characterization of the active phytochemicals responsible for the observed effect of the seed extract, and their individual contributions, might be necessary.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

Ethical approval was obtained from the Federal Polytechnic Research Ethics Committee.

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