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Full Length Research Article

Toxicity Studies on Aqueous-Methanol Pod Extract of *Vigna unguiculata* (Cowpea) in Wistar Strain Albino Rats

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ABSTRACT
Herbal preparation of *Vigna unguiculata* (cowpea) pod has long been used by a group of Hausa people from northern Nigeria called *Yan tauri* performers and traditional healers. This study was conducted to evaluate the toxicological effect of the extract in Wistar strain albino rats using biochemical, haematological and histopathological indices of toxicity. Acute toxicity (LD_{50}) and sub-chronic toxicity studies were determined using the method developed by OECD. Twenty-five (25) rats were grouped into five(5) consisting of five(5) rats each, one of the groups served as control., Group II, III, IV and V were orally administered with the extract at a daily dose of 400 mg/kg, 800 mg/kg, 1200 mg/kg and 1600 mg/kg of the extract, respectively for 28 days. The LD_{50} of the extract was greater than 5000 mg/kg and its oral administration for 28 days did not produce significant changes (P>0.05) on biochemical and haematological indices. Histopathological evaluation revealed mild widening of Bowman's capsule of animals administered with 1200 mg/kg and 1600 mg/kg of the extract. It can thus be concluded that the pod is non-toxic.

Keywords: *Vigna unguiculata* pod, *Yan tauri* performers, Aqueous-methanol extract, Acute and sub-chronic toxicity

INTRODUCTION
Pod is the long narrow outer case holding the seeds of a legume plant such as common beans, pea or vanilla (FAOUN, 2013). Green cowpea pods are cooked as a vegetable (Chikwendu *et al*., 2014). Dried cowpea pod are rehydrated before cooking and can serve as browse for livestock (RCP, 2012). It is also taken as a diuretic to increase urine production (Weiss and Fintelmann, 2000). Cowpea pods are also believed to have antibacterial, anti-inflammatory, antioxidant (Subramanian and Leelavinothan, 2002), antiparasitic, antiviral, cleansing and detoxifying, emollient and gas-relieving properties (Rafi and Vastano, 2002). Cowpea pods are a source of dietary fibre (Romero-Arenas *et al*., 2013). It is used for the treatment of diabetes (Roman-Ramos *et al*., 1995), obesity, arthritis, gout, oedema, hypertension, constipation, kidney and bladder disorders (Verhelst, 2010). Modified and unmodified cowpea pod can be used for effluent water treatment (Adediran *et al.,* 2005; Mubo *et al.,* 2015).

Yan tauri performers (tough-skin men) found in the northern part of Nigeria are people who cannot be cut or pierced by a knife or any metal and when cut they do not bleed to death. They are known to drink concoction prepared with dry cowpea pods known as *kowa* in Hausa language. According to Gidley (1967), *Yan tauri* performers are public entertainers.

Scientific evaluation of medicinal plants is important to assess toxicity risks associated with the use of herbal preparations (Toma *et al*., 2009). Owing to the wide consumption of cowpea pod by the *Yan tauri* performers; this study was designed to examine the effect of cowpea pod on the haematological and histopathological indices of the liver and kidney of rats.

MATERIALS AND METHODS
Chemicals and Reagents
All chemicals and reagents used in this study were of analytical grade.

Sample Collection and Identification
The Cowpea pod was obtained from farmland in Gidan Tudu village near Usmanu Danfodiyo

University, Sokoto. The plant was identified by a Taxonomist in the Botany unit, Biological Science Department, Usmanu Danfodiyo University, Sokoto and a voucher specimen was deposited at the herbarium of the same department with a voucher number UDUH/ANS/0152. The dried sample was pulverized into a fine powder using a mechanical blender. It was then macerated in an aqueous-methanol solvent (70:30; methanol and water) at room temperature with shaking at regular intervals for 72 hours after which it was filtered. The filtrate was then dried using the drying cabinet.

Experimental Animals

The albino (Wistar) rats that were used in this study were purchased from the National Veterinary Research Institute (NVRI) Vom, Jos. The experimental rats were housed in cages and fed with standard pellet diet (Vital Feeds Ltd, Nigeria) and were allowed free access to feed and water (*ad libitum*) throughout the experiment. The rats were kept in the animal house of the Department of Biochemistry, Usmanu Danfodiyo University, Sokoto for two (2) weeks to acclimatize before the commencement of the experiment.

Phytochemical Screening

Phytochemical constituents of the pod were determined using the methods of Harborne (1973), Trease and Evans (1989), El-Olemyl *et al*. (1994) and Sofowora (1993).

Acute Oral Toxicity Study (Determination of LD_{50})

The acute oral toxicity study was carried out using the method developed by the Organization for Economic and Cultural Development, 2001 (OECD, 2001). Five randomly selected animals were used for the experiment. Each animal was dosed, one at a time at 72 hrs intervals. The extract was administered at 5000 mg/kg body weight in a single dose. For the first 8 hrs, 14 hrs, 24 hrs, 48 hrs and 72 hrs signs of toxicity like tremors, itching, depression, weakness, food and water refusal, salivation and death were observed. The animals were observed further for 14 days for any signs of delayed toxicity.

Sub-chronic Oral Toxicity Study

Sub-chronic oral toxicity study was carried out according to the Organization for Economic and Cultural Development (OECD, 2008) method. Twenty five albino rats were grouped into five groups containing five animals each. Group 1 served as the control while 2-5 were administered with 400 mg/kg, 800 mg/kg, 1200 mg/kg and 1600 mg/kg, respectively of the extract on a daily basis for 28 days. The extract was administered to the animals orally via cannula. At the end of the experimental period, the animals were anesthetized and blood, liver and kidney tissues were collected for biochemical, haematological and histopathological assessment, respectively. For the blood, the neck area was cleared of fur and skin to expose the jugular veins. These veins were cut sharply with a sterile scalpel blade and the rats were held head downwards and allowed to bleed into clean dry sample bottles. The serum was collected using Pasteur pipette after centrifugation at 4000 rpm for 5 minutes and kept in labelled sample bottles at 4 ^{o}C until required for analysis.

Each of the biochemical parameters was carried out using standard methods while haematological parameters were analysed using an automated haematological analyzer (Theml *et al.*, 2004).

Histopathological Study

The kidney and liver were fixed in 10% buffered formalin for 72 hours. The tissues were then dehydrated in alcohol of graded concentrations and embedded in paraffin. Embedded tissues were cut into sections of 5 µm thick and these were stained with hematoxylin and eosin for photo microscopic assessment and placed on a clean labelled microscope glass slide. The slide was mounted on an electric light microscope for examination of any possible histopathological features. Photomicrographs of the samples were then taken (Drury *et al.*, 1967).

Data Analysis

The results were expressed as mean ± standard error of the mean (SEM). Using the statistical package SPSS version 20 software, the difference between means was carried out using one-way analysis of variance (ANOVA) followed by Dunnett comparison test. Histopathological results were presented using a plate which was generated from lesion scoring: the difference between means was carried out using the Kruskal-Wallis comparison test. Values were considered statistically significant at $P<0.05$.

RESULTS AND DISCUSSION

Vigna unguiculata pod contains flavonoids, alkaloids, saponins, tannins, steroids, volatile oils, resins, glycosides, saponin glycosides, balsams and terpenes (Table 1). This agrees with the findings of Chikwendu *et al*. (2014), Tavares *et al*. (2015) and Seidu *et al*. (2014) who have reported the presence of these components and others in the members of Fabaceae family. The presence of phytochemicals in extracts of plants is responsible for pharmacological activities observed in biological systems, which may probably support claims by traditional healers. The studies of Igwo-Ezikpe *et al*. (2013) and Wonghirundecha *et al*. (2014) demonstrated the antioxidant and antimicrobial potentials of phytochemicals present in ethanol extract of *Parkia biglobosa* (African locust bean) pod and *Parkia speciosa* (stink bean) pod, respectively.

Determination of acute toxicity, LD_{50} is usually the first step in the evaluation of the toxic characteristics of a substance (Ogbuehi *et al*., 2015). This study showed that the LD_{50} of the aqueous-methanol pod extract of *V. unguiculata* was greater than 5000 mg/kg as shown in Table 2. No mortality and visible signs of toxicity like tremors, itching, depression, weakness, bulging of the eye, hair loss, food and water refusal and salivation were observed over 14 days, indicating that it is non-toxic at "acute dose. In other words, acute exposure to high doses of the aqueous-methanol extract of *V. unguiculata* pod is non-toxic."

According to the toxicity scale of Hodge and Sterner, any compound with an oral LD_{50} above 5000 mg/kg should be considered practically non-toxic (Hodge and Sterner, 2005).

Table 1: Qualitative phytochemical composition of *Vigna unguiculata* pod

PHYTOCHEMICAL	STATUS
Flavonoids	+
Condensed tannins	+
Saponins	+
Alkaloids	+
Cardiac glycosides	ND
Steroids	+
Saponin glycosides	+
Balsams	+
Anthraquinones	ND
Volatile oils	+
Glycosides	+
Terpenes	+
Resins	+

Key: + = present, ND = not detected

Table 2: Acute toxicity profile of aqueous-methanol extract of *Vigna unguiculata* pod in albino rats

DOSE (mg/kg)	GROUP	NUMBER of ANIMAL	NUMBER of DEATH
5000	I	1	0
5000	II	1	0
5000	III	1	0
5000	IV	1	0
5000	V	1	0

LD_{50} >5000 mg/kg

Results presented in Tables 3, 4 and 5 indicate the non-toxicity of the pod after carrying out sub-chronic toxicity study for 28 days. Umaru *et al*. (2015) similarly reported that administration of aqueous pod extract of *Acacia nilotica* to experimental rats for 21 days did not significantly ($p > 0.05$) alter the levels of RBC, Hb and PCV. However, prolonged

administration of the extract may adversely affect the kidneys. This may explain the mild degeneration of the kidney (Table 6, Plate 1A-E) observed at high dose (1200 mg/kg and 1600 mg/kg).

Table 3: Sub-chronic Toxicity Evaluation of aqueous-methanol extract of *Vigna unguiculata* pod on Liver function indices of experimental rats

PARAMETER	CONTROL	400 mg/kg	800 mg/kg	1200 mg/kg	1600 mg/kg
ALT (U/L)	20.00±5.38	29.40±11.17	32.33±11.85	38.25±11.59	36.33±1.76
AST (U/L)	61.00±3.99	62.80±7.41	70.33±10.68	65.25±4.05	71.33±9.84
TP (g/dL)	9.58±0.41	11.65±0.91	12.09±0.76	9.51±0.87	10.32±2.17
ALB (g/dL)	5.20±0.36	5.96±0.29	5.70±0.15	5.50±0.32	4.19±0.51
G (g/dL)	4.38±0.51	5.69±1.09	6.40±0.85	4.01±1.12	6.13±2.03
A/G RATIO	1.28±0.22	1.29±0.32	0.92±0.13	1.78.±0.35	0.87±0.28
DB (mg/dL)	2.67±0.66	3.34±0.37	2.22±0.77	1.69±0.29	1.62±0.30
TB (mg/dL)	3.31±0.61	3.97±0.41	2.90±0.47	2.52±0.40	2.55±0.45

Values are mean ± standard error of the mean (n = 5). Values were not significantly different ($p > 0.05$) when compared to control group (one-way ANOVA followed by Dunnett comparison test using SPSS version 20). ALT- Alanine Amino Transferase, AST- Aspartate Amino Transferase, TP- Total Protein, ALB- Albumin, G- Globulin, A/G- albumin:globulin, DB- Direct Bilirubin, TB- Total Bilirubin.

Table 4: Kidney function indices of rats administered with sub-chronic doses of aqueous-methanol extract of *Vigna unguiculata* pod

Group (mg/kg)	UREA (mg/dL)	CRT (mg/dL)	UA (mg/dL)	Cl^- (mMol/L)	K^+ (mMol/L)	HCO_3^- (mMol/L)
Control	15.71±0.82	0.13±0.04	3.13±0.30	3.40±0.19	1.24±0.10	3.50±0.27
400	16.37±1.21	0.14±0.02	2.46±0.72	3.50±0.16	1.20±0.14	3.60±0.62
800	13.99±0.23	0.13±0.04	2.21±0.38	3.67±0.44	1.26±0.07	3.67±0.17
1200	12.99±0.78	0.13±0.02	2.64±0.71	3.75±0.14	1.09±0.06	3.38±0.31
1600	12.86±0.52	0.13±0.02	2.26±0.72	3.83±0.17	1.13±0.07	3.67±0.17

Values are mean ± standard error of the mean (n = 5). The values were not significantly different ($p > 0.05$) when compared with the control group (one-way ANOVA followed by Dunnett comparison test using SPSS version 20). CRT- Creatinine, UA- Uric Acid, K^+- Potassium ion, Cl^-- Chloride ion and HCO_3^-- Bicarbonate ion.

Table 5: Haematological indices of rats administered with sub-chronic doses of aqueous-methanol extract of *Vigna unguiculata* pod

PARAMETERS	CONTROL	400 mg/kg	800 mg/kg	1200 mg/kg	1600 mg/kg
WBC ($10^3/mm^3$)	12.20±1.52	8.69±1.22	8.42±1.55	8.02±1.58	10.93±1.01
RBC ($10^6/mm^3$)	5.88±0.73	7.39±0.26	7.44±0.01	7.27±0.48	6.78±0.58
HGB (g/dL)	12.18±0.84	13.96±0.47	14.13±0.19	13.38±1.07	12.73±1.27
MCHC (g/dL)	34.22±3.15	31.14±1.80	31.97±0.54	30.68±2.72	33.90±0.59
MCH (pg)	22.16±3.54	18.90±0.28	18.97±0.27	18.33±0.26	18.77±0.70
MCV (fL)	63.48±3.73	61.42±3.54	59.33±0.35	61.20±5.52	55.40±1.57
PCV (%)	41.68±1.29	42.52±1.84	44.13±0.28	40.08±3.19	37.63±3.92

PLT (10^3/mm^3)	698.60±47.14	587.60±21.22	586.33±18.68	540.75±91.69	604.67±51.56
MPV (fL)	7.34±0.47	7.04±0.16	6.83±0.09	7.05±0.20	6.80±0.23
PDW (fL)	13.14±0.57	12.86±0.47	12.47±0.52	12.95±0.16	13.00±0.42
PCT (%)	0.41±0.11	0.41±0.07	0.40±0.01	0.38±0.06	0.41±0.10
P-LCR (%)	7.72±0.49	9.84±1.41	8.10±0.69	9.08±0.67	7.80±1.84

Values are mean ± standard error of the mean (n=5). The values were not significantly ($p > 0.05$) different when compared with the control using one-way ANOVA, SPSS version 20. WBC - white blood cell, RBC - red blood cell, HGB - haemoglobin, MCHC - mean cell haemoglobin concentration, MCH - mean cell haemoglobin, MCV - mean corpuscular volume, PCV - packed cell volume, PLT - platelet, MPV - mean platelet volume, PDW - platelet distribution width, PCT- platelet haematocrit, P-LCR - platelet larger cell ratio.

Mild widening of the Bowman's capsule (Plate 1D and 1E) observed in the kidney of animals administered with 1200 and 1600 mg/kg of the extract may be that the morphological architecture of the kidney has to be extensively deranged before any significant aberration could be detected biochemically (Ghasi *et al*., 2012).

Widened Bowman's capsule may be due to tissue anoxia (Stark, 1980) or renal vasoconstriction in response to nephrotoxicant (Schellman, 1995). Akanmu *et al.* (2004) reported the histopathological examination of kidneys of rats treated with 1000 mg/kg of *Cassia fistula* pods extract showed wide Bowman's capsule and wide proximal tubules. According to Kalu *et al*. (2011), excess consumption of phytochemicals rich in saponins and alkaloids can produce undesirable side effects and could inhibit certain enzyme activities such as adenosine monophosphate.

Table 6: Histopathological results of the kidney of rats administered with sub-chronic doses of aqueous methanol extract of *Vigna unguiculata* pod

DOSES (mg/kg)	LESION SCORE (Mean ranks)	
	DEGENERATION	HAEMORRHAGE
Control	5.50^a	9.50^a
400	5.50^a	9.50^a
800	15.00^b	12.83^a
1200	15.00^b	12.00^a
1600	16.67^c	9.50^a

Mean ranks having different superscript in the same column are significantly different ($p < 0.05$) (Kruskal-Wallis comparison test using SPSS version 20).

CONCLUSION

The aqueous-methanol pod extract of *Vigna unguiculata* was studied for its acute and sub-chronic toxicity in Wistar strain albino rats. The results showed that the extract was non-toxic and it has no effect on biochemical and haematological parameters but the mild widening of the Bowman's capsule was observed in the kidney of animals administered with 1200 and 1600 mg/kg of the extract. Further studies on the chronic toxicity of aqueous-methanol pod extract of *V. unguiculata* should be carried out.

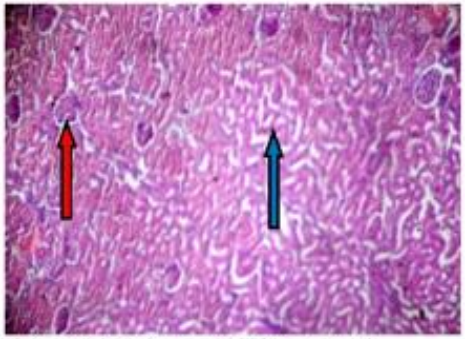

Plate 1A (control): normal kidney structure

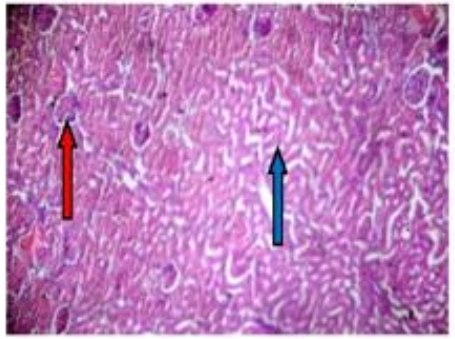

Plate 1B (400 mg/kg): normal kidney structure

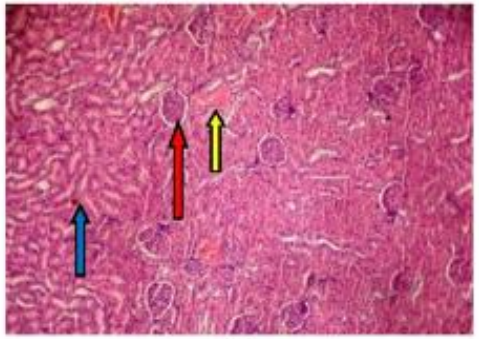

Plate 1C (800 mg/kg): mild haemorrhage

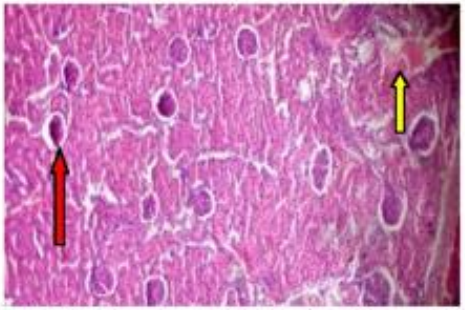

Plate 1D (1200 mg/kg): mild widening of Bowman's capsule

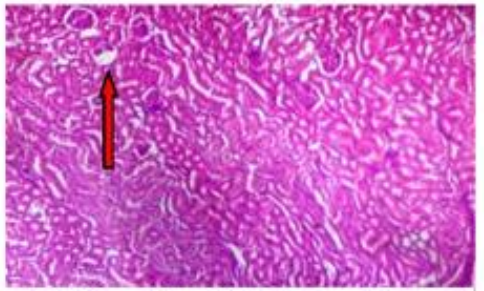

Plate 1E (1600 mg/kg): mild widening of Bowman's capsule

Plate 1A-E: photomicrograph of rat kidney administered with sub-chronic doses of aqueous-methanol extract of *Vigna unguiculata* pod (H&E stain, x 100 magnification). Red arrow – glomerulus, blue arrow – collecting duct, yellow arrow – haemorrhage

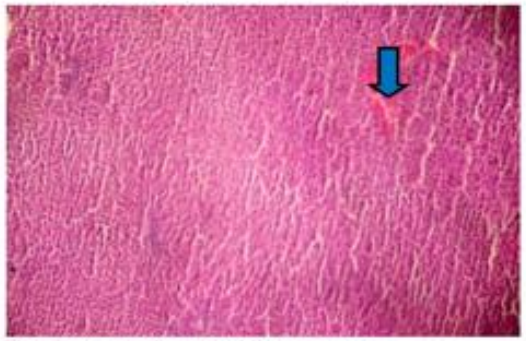

Plate 2A (control): normal liver structure

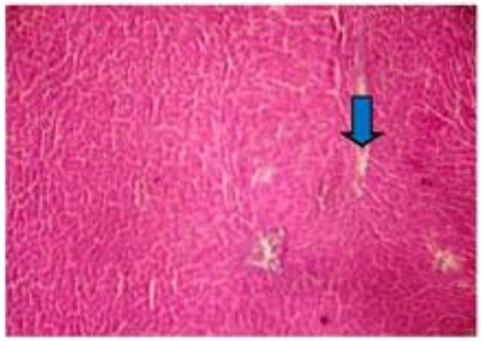

Plate 2B (400 mg/kg): normal liver structure

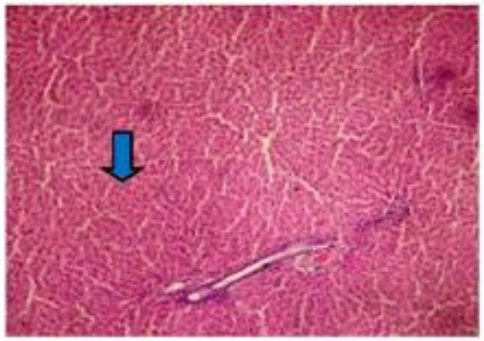

Plate 2C (800 mg/kg): normal liver structure

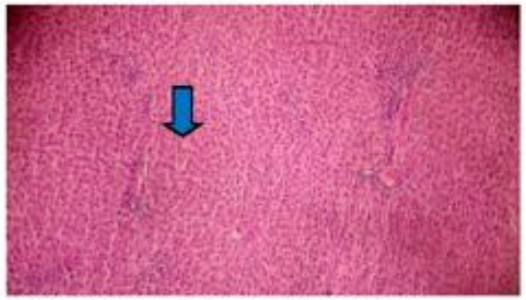

Plate 2D (1200 mg/kg): normal liver structure

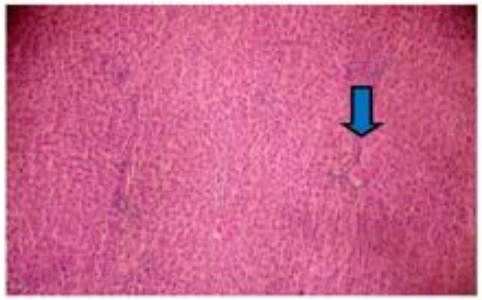

Plate 2E (1600 mg/kg): normal liver structure

Plate 2A-E: photomicrograph of rat liver administered with sub-chronic doses of aqueous-methanol extract of *Vigna unguiculata* pod (H&E stain, x 100 magnification). Blue arrow – hepatocyte

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