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Acute and Sub-chronic Oral Toxicity of *Anthocleista vogelii* (Cabbage Tree) Root Hydroethanolic Extract in Albino Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author RMS performed the experiments, performed the statistical analysis and wrote the first draft of the manuscript. Author ORI supervised the work and contributed to the protocol. Author EMO provided technical assistance, supervised the work and contributed to the protocol. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The toxicity profile of *Anthocleista vogelii* ethanolic root extract was investigated in Albino rats due to its use in Nigerian ethno-medicine for the management of diabetes mellitus.
Study Design: The acute and sub-chronic toxicity of *A. vogelii* root was investigated in Albino rats by measuring daily food intake, water intake and body weight of animals. At the end of the experiment, haematological and biochemical parameters were measured.
Place and Duration of the Study: The study was carried out in the Department of Biochemistry, Faculty of Biological Sciences and in the Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria between January and May, 2014.

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Methodology: The median lethal dose (LD_{50}) was determined in acute toxicity studies and the plant extract was administered to albino rats upto 5000 mg/kg. In subchronic toxicity studies, 20 Albino rats (150-200 g) of both sexes were randomly divided into four groups of five animals each. The control group received distilled water (10 ml/kg), while the treatment groups were administered 100, 200 and 400 mg/kg extract orally (p.o.) daily for twenty-eight days. Changes in body weight, food and water intake were measured daily. At the end of the subchronic toxicity studies, biochemical parameters [cholesterol (CHOL), triglyceride (TRIG), high density lipoprotein (HDL), low density lipoprotein (LDL)], creatinine (CRT), alanine transaminase (ALT) and aspartate transaminase (AST)], haematological parameters (packed cell volume [PCV], haemoglobin concentration, red blood cell [RBC] and white blood cell [WBC]) and relative organ weight were determined while the liver and kidney tissues were examined histologically.

Results: The result showed that a single oral dose of the extract at 5000 mg/kg did not produce mortality (LD_{50} of the extract was ≥ 5000 mg/kg). However, in the sub-chronic toxicity studies, there was a significant $P < 0.05$ decrease in WBC, serum ALT, AST and CRT levels and an increase in PCV, RBC, water intake in week 3 and 4 when compared with the control. There was no pathological change in the liver and kidney tissues.

Conclusion: In conclusion from the present study, *A. vogelii* ethanolic root extract is safe when administered acutely (p.o) and it has no toxic effect when administered to Albino rats daily for twenty-eight days.

Keywords: Anthocleista vogelii; root; acute; sub-chronic; toxicity; histology.

1. INTRODUCTION

There is a recent increase in medicinal plants and herbal formulations (natural products) research in the last few decades and the demand to use herbal formulations in the treatment of a number of diseases including diabetes mellitus is also on the increase worldwide. The World Health Organization (WHO) estimated that approximately 80% of the world's population depends on herbal formulations and medicinal plants for their primary health care [1]. Despite considerable progress in the treatment of diseases with natural and synthetic products, search for plants with medicinal and safe properties is an area that draws attention of researchers. *Anthocleista vogelii* Planch. (Loganiaceae), commonly known as 'cabbage tree' in English, 'Sapo' or 'Apaoro' in Yoruba, 'Kwari' in Hausa, 'Orimi' in Benin [2] and 'Odogwu' in Igala [3] is mostly used for the treatment of diabetes, fever, pile and stomach ache in Nigeria. The leaves and stem bark are used for the treatment of leprosy, jaundice, bronchitis and venereal diseases. The stem bark and seeds are also used in Nigeria as an antipyretic, tonic and as purgative. A root decoction of this plant is also used as purgative and to induce labour. *A. vogelii is also used in* Cameroon, Sudan, Sierra Leone and Ghana for the treatment of diseases [2]. Over the years, a number of scientific studies have been undertaken to evaluate some of the biological activities of various solvent extracts of *A. vogelii*. Studies carried on the antidiabetic effect of *A. vogelii* aqueous root extract and the ethanolic extract concluded that *A. vogelli* exerted significant decrease in fasting blood glucose level (FBGL) in glucose loaded Albino rats, alloxan-induced diabetic rats and streptozotocin-induced diabetic rats [4,5]. The stem bark of *A. vogelii* possesses hypoglycemic activity in both normal and alloxan-induced diabetic animals [6]. The stem bark of *A. vogelii* possesses anti-plasmodial property [7] and the aqueous extract of *A. vogelii* stem bark was reported to have analgesic properties [8]. There is also a report that the anti-plasmodial effect of petroleum ether extract of *A. vogelii* leaves exerted a weak reduction in the parasite density when compared to chloroquine [9]. Studies carried out on the anti-bacterial and anti-fungal activities of compound isolated from the stem bark and leaves of *A. vogelii* showed that the isolates were active against *Escherichia coli* [3,10]. The results from the study on the anti-ulcer effects of the aqueous and organic extracts of the stem bark of *A. vogelii* showed that the aqueous and organic extracts of the stem bark of *A. vogelii* possess potent antiulcer properties. The findings from this study justify the ethno-medical use of the plant for the treatment of stomach ache [8].

Based on the research carried out on this plant and the use of different parts of this plant for the treatment of diseases, it is for this reason therefore that this study was carried out to investigate the oral toxicity profile of *Anthocleista*

vogelii hydro-ethanolic (70% ethanol) root extract in Albino rats.

2. METHODOLOGY

2.1 Plant Material

The root of *Anthocleista vogelii* was collected from the premises of National Biotechnology Development Agency, Bioresources centre, Onipanu, Ogbomosho, Oyo State and was authenticated by Mr. G. Ibhanesebhor (the Officer in charge of Ife-Herbarium) Department of Botany, Obafemi Awolowo University, Ile-Ife, Osun State. A voucher specimen (IFE 17399) was deposited at Ife-Herbarium.

2.2 Ethanolic Extraction

A. vogelii root was washed, air dried (for 7 days), pulverized and macerated in 70% ethanol for 72 hours (cold extraction). The suspension was filtered using muslin cloth and cotton wool in funnel. The filtrate was filtered again using filter paper. The filtrate was then concentrated into a solid paste *in vacuo* at 45℃ using a rotary evaporator. The solid paste was freeze dried and the percentage yield was calculated. The dry extract was stored in a refrigerator at 4℃ prior to use.

2.3 Animals

Albino rats (both sexes) weighing between 150-200 g were obtained from Animal House, Department of Pharmacology, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Osun State. They were kept in well ventilated aluminium cages and fed with Vita feed and were given water *ad libitum.* The rats were allowed to acclimatize with the environment at ambient temperature under natural day light/night conditions for two weeks before the start of the experiment.

2.4 Acute Toxicity Studies

The median lethal dose (LD_{50}) of the root extract was determined in Albino rats through oral route (p.o.) [11]. The study was carried out in two phases. In the first phase, nine Albino rats were randomly divided into three groups of three rats each. Each group (1-3) was given 10, 100 and 1000 mg extract/kg body weight (p.o.). The rats were kept under laboratory ambient conditions and observed for signs of toxicity which include but not limited to stretching, respiratory distress, change in body weight and mortality for the first critical four hours and there after daily for fourteen days. In the second phase of the study, higher doses were administered to the animals because 1000 mg/kg from Phase 1 caused no death. To another fresh set of three groups of a rat each, 1600, 2900 and 5000 mg/kg body weight (p.o.) of the extract was administered. The animals were examined at 10, 30, 60 and 120 min and at 4, 6 and 24 h for gross behavioural changes as under Phase 1 and subsequently for mortality. The animals were also sacrificed on the 15th day and the internal organs were examined macroscopically for pathological changes.

2.5 Sub-chronic Toxicity Studies in Albino Rats

Twenty Albino rats of either sex were randomly divided into four groups of five rats each. Group one, which served as the control was administered 10 mL distilled water/kg body weight (p.o.), while rats in groups two, three and four were given 100, 200 and 400 mg/kg *A. vogelii* ethanolic extract (p.o) respectively daily for 28 days [12,13]. All the rats had free access to food and water for 28 days and they were observed daily for general symptoms of toxicity. The body weight of the animals was measured daily and the dose of the drug administered was adjusted accordingly. The animals were deprived of food for 24 hours before they were sacrificed on the 29th day. On the 29th day, each rat was sacrificed under diethyl ether anaesthesia and blood collected by cardiac puncture into K^+EDTA bottles and plain bottles. Blood samples in K^+EDTA bottles were used for haematological analysis. Blood samples in plain bottles were allowed to stand for 5 minutes and then centrifuged at 2500 rpm for 25 minutes and the serum was used for biochemical analysis. The organs (liver and kidney) removed from the rats were fixed in 10% formalin (formal saline).

2.5.1 Body weight, food and water intake

The percentage change in body weight in week 1, 2, 3 and 4 was calculated and used for statistical analysis. The weight of food and volume of water consumed by rats in each group were measured daily as the difference between the quantity of food and water supplied and the amount remaining after 24 hour respectively. The weekly % food intake/body weight and weekly % water intake/body weight in Week 1, 2, 3 and 4 was calculated and used for statistical analysis.

2.5.2 Haematological analysis

The packed cell volume, haemoglobin concentration, red blood cell count and white blood cell count were determined [14].

2.5.3 Biochemical analysis

Biochemical analysis include; aspartate aminotransferase (AST), alanine aminotransferase (ALT) [15], creatinine (CRT) [16], total cholesterol concentration (CHOL) [17,18,19,20], triglyceride (TG) concentration [21] and high density lipoprotein (HDL) [22].

2.5.4 Histopathology

The organs (liver and kidney) removed from the rats were fixed in 10% formalin (formal saline) for at least 48 hours. They were then processed routinely and the tissues were embedded in paraffin wax. Histological sections were cut at 5-6 µm and stained with routine Haematoxylin and Eosin. Photomicrographs of representative lesions were taken at X400 magnification.

2.6 Statistical Analysis of Data

Data for each group were collected and summarized in a tabular and graph forms for each treatment group. Data were represented as the mean ± standard error of mean (SEM) for the number (n) of animals in the group. One-way analysis of variance (ANOVA) was first used followed by Bonferroni t-test *post hoc* comparisons to determine the significant differences at 95% ($P< 0.05$) using Primer (version 3.01).

3. RESULTS AND DISCUSSION

Acute toxicity study is usually the first step in toxicological investigations and the index of acute toxicity is the LD_{50} (median lethal dose). During acute toxicity study, the animals did not show any changes in general appearance, morphological characteristics (fur, skin, eyes and nose) appeared normal and there were no tumors, convulsion, salivation and diarrhea. There was also no mortality or morbidity observed in the rats throughout the fourteen days period following single oral administration of all selected doses of *A. vogelii* ethanolic root extract. The median lethal dose (LD_{50}) of *A. vogelii* ethanolic root extract was ≥ 5000 mg/kg (p.o.). The acute toxicity result of the present study demonstrated that the LD_{50} value of the extract being greater than or equal to 5000 mg/kg body weight (b.wt) clearly suggest that the extract at the limit dose tested (5000 mg/kg) is essentially not acutely toxic when administered orally, therefore it may be considered to be substantially safe if incorporated into an oral formulation for the treatment of diseases. This observation is in line with earlier studies that showed that the aqueous stem bark of *A. vogelii* elicited an LD_{50} greater than 5000 mg/kg in an acute toxicity study [23].

Sub-chronic toxicity studies on changes in food and water intake, haematological parameters, serum liver and kidney enzymes, lipid profile, relative organ weight and histological observation of organs have long been used to investigate possible damage by medicinal agents and chemicals [12,24-26]. Acute and sub-chronic (28 day) oral toxicity of the aqueous stem bark of *Anthocleista schweinfurthii* in mice showed that there were no treatment related alterations at 250, 500 and 1000 mg/kg b.wt in the biochemical parameters investigated [27]. The acute and sub-chronic (28-day) oral toxicity studies of the ethanolic (90%) leaf extract of *Ageratum conyzoides* in rats also showed that there were no treatment related abnormalities at 500 and 1000 mg/kg b.wt in the biochemical parameters investigated [28]. Studies carried out on *A. vogelii* root bark ethanolic extract also showed no significant decrease in body weight, food intake, total fat mass and low density lipoprotein in high carbohydrate diet obese rats [29].

In sub-chronic toxicity studies, *A. vogelii* ethanolic root extract exerted no significant ($P< 0.05$) change in body weight and food intake in all the treatment groups in week 1, 2, 3 and 4 when compared with the control (Tables 1 and 2 respectively) but there was a significant ($P< 0.05$) change in water intake in week 3 and week 4 when compared with the control (Table 3). Furthermore, following 28 days of daily treatment of rats with the hydro-ethanolic extract of *A. vogelli* root at a sub-chronic concentration of 100, 200 and 400 mg/kg b.wt, there were treatment-related changes in haematological parameters. These haematological parameters tended to be dose-dependent between treated groups and control, indicating that the extract was not subchronically toxic but rather enhanced the production of circulating red blood cells and also may have interfered with the production of the white blood cells. Usually, the haematopoietic system is one of the most sensitive targets of toxic compounds and is an important index of

physiological and pathological status in man and animals [30,31]. *A. vogelii* ethanolic root extract also exerted a significant ($P< 0.05$) increase in packed cell volume at 400 mg/kg and a significant ($P< 0.05$) increase in red blood cell count at 100, 200 and 400 mg/kg when compared with the control. There was also a significant ($P< 0.05$) decrease in white blood cell count at 100, 200 and 400 mg/kg when compared with the control (Table 4). At 100, 200 and 400 mg/kg the extract elicited no significant ($P< 0.05$) effect in the relative organ weight of the kidney, liver and pancreas of rats when compared with the control (Table 5).

In addition, the biochemical parameters investigated showed significant treatment–related alterations, when compared to control, in the levels of alanine aminotransferase, aspartate aminotransferase and creatinine which are good indicators of liver and kidney functions [32]. At 100, 200 and 400 mg/kg *A. vogelii* ethanolic root extract exerted a significant ($P< 0.05$) decrease in alanine aminotransferase, aspartate aminotransferase and creatinine levels when compared to the control (Fig. 1). Also, at 100, 200 and 400 mg/kg *A. vogelii* ethanolic root extract exerted no significant ($P< 0.05$) effect in serum cholesterol, triglyceride, high density

Table 1. Effect of *A. vogelii* ethanolic root extract on percentage change in body weight (g) of rats

Doses	Week 0	Week 1	Week 2	Week 3	Week 4
Control	100.0±7.9	101.7±8.6	105.8±9.7	107.1±6.4	109.2±6.1
100 mg/kg	100.0±4.3	101.3±4.6	103.3±4.2	106.0±4.0	107.3±3.3
200 mg/kg	100.0±7.3	101.3±7.5	103.3±7.7	105.3±6.8	108.0±6.8
400 mg/kg	100.0±8.6	103.0±4.5	105.5±5.6	107.9±4.9	109.1±6.1

Values are mean ± SEM; n = 5

Table 2. Effect of *A. vogelii* ethanolic root extract on weekly percentage food (g) intake/body weight of rats

Doses	Week 1	Week 2	Week 3	Week 4
Control	32.5±3.4	41.3±1.4	48.7±0.2	52.9±0.1
100 mg/kg	29.9±1.0	35.0±0.7	48.1±1.8	51.9±1.4
200 mg/kg	29.6±4.0	34.3±4.1	47.5±1.0	53.1±0.1
400 mg/kg	29.6±6.1	34.8±0.1	49.2±0.1	51.8±1.4

Values are mean ± SEM; n = 7

Table 3. Effect of *A. vogelii* ethanolic root extract on weekly percentage water (mls) intake/body weight of rats

Doses	Week 1	Week 2	Week 3	Week 4
Control	76.6±5.1	60.4±2.7	64.9±0.1	69.3±0.2
100 mg/kg	74.9±1.8	64.5±2.9	70.4±0.9 *	73.7±0.9 *
200 mg/kg	63.0±4.3	63.6±2.6	72.3±2.0 *	74.3±1.4 *
400 mg/kg	62.5±4.1	65.3±3.1	72.8±2.0 *	72.6±0.1 *

*Values are mean ± SEM; n = 7; *Significantly different from control at P< 0.05*

Table 4. Effect of *A. vogelii* ethanolic root extract on haematological parameters

Parameters	Control	100 mg/kg	200 mg/kg	400 mg/kg
PCV (%)	41.80±0.86	49.40±2.60	50.00±3.35	51.80±1.46 *
HAEM (g/dl)	13.90±0.38	12.80±0.29	12.80±0.37	13.30±0.51
RBCC (µl)	6.59±0.41	6.85±0.30 *	6.92±0.80 *	8.35±0.31 *
MCV (fl)	64.58±4.68	72.37±3.46	77.96±12.97	62.24±2.13
MCH (pg)	21.52±1.71	18.76±0.64	19.51±2.29	16.01±1.00
WBCC (µl)	6.98±0.30	4.10±0.69 *	4.01±0.76 *	4.12±0.69 *

*Values are mean ± SEM; n = 5; PCV: Packed cell volume; RBCC: Red blood cell count; Haem: Haemoglobin; MCV: Mean cell volume; MCH: Mean cell haemoglobin; WBCC: White blood cell count; *Significantly different from control at P< 0.05*

lipoprotein and low density lipoprotein levels when compared with the control (Fig. 2).

Plate 1b, 1c and 1d (100, 200 and 400 mg/kg group liver respectively) showed normal hepatic architecture around the central vein when compared with the control (Plate 1a). Plate 2b, 2c and 2d (100, 200 and 400 mg/kg group kidney respectively) showed normal cortical architecture with normal glomerulus when compared with the control (Plate 2a). The overall trend, however, suggests that sub-chronic administrations of *A. vogelli* extract adversely did not alter the hepatocytes and the nephron of rats as observed in the histological analysis of the liver and kidney respectively.

Table 5. Effect of *A. vogelii* ethanolic root extract on relative organ weight of rats

Groups	Liver	Kidney	Pancreas
Control	4.41±0.20	0.95±0.02	0.30±0.04
100 mg/kg	4.32±0.21	1.01±0.01	0.31±0.03
200 mg/kg	4.10±0.32	0.99±0.04	0.30±0.02
400 mg/kg	4.20±0.41	0.98±0.05	0.28±0.03

Values are mean ± SEM; n = 5

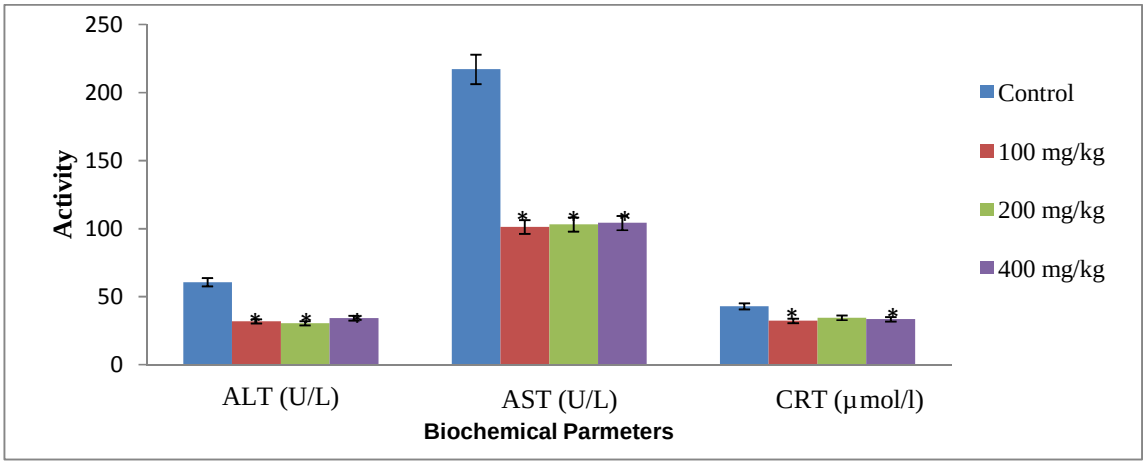

Fig. 1. Effect of *A. vogelii* ethanolic root extract on liver and kidney enzymes (serum ALT, AST and CRT) of rats

*Values are mean ± SEM; n = 5; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; CRT: Creatinine; *Significantly different from control at P< 0.05*

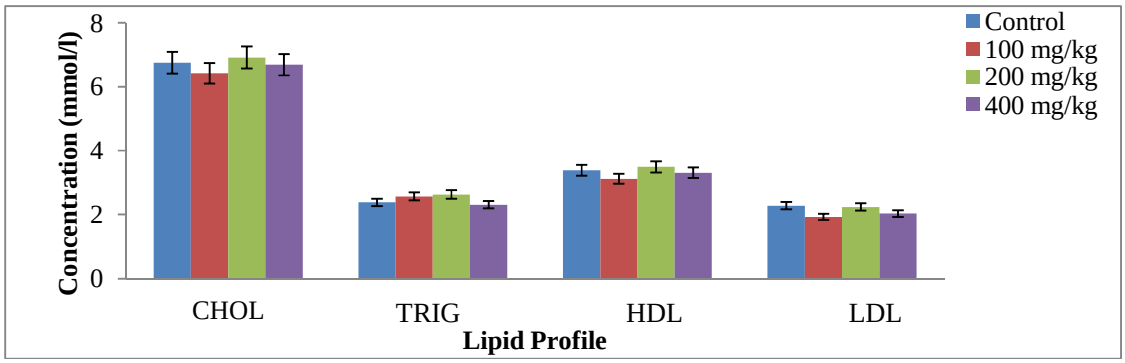

Fig. 2. Effect of *A. vogelii* ethanolic root extract on lipid profile (serum CHOL, TRIG, HDL and LDL) of rats

Values are mean ± SEM; n = 5; CHOL: Cholesterol; TRIG: Triglyceride; HDL: High density lipoprotein; LDL: Low density lipoprotein

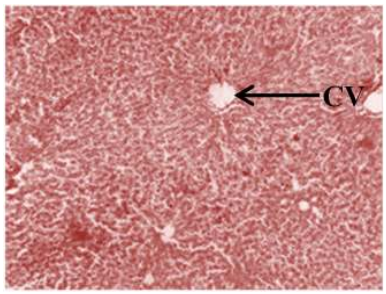

Plate 1a. Liver (control)

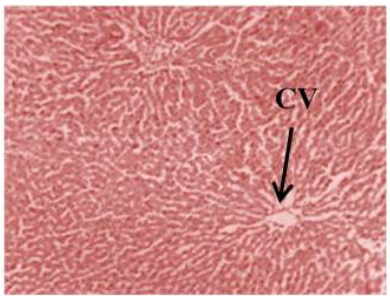

Plate 1b. Liver (100 mg/kg)

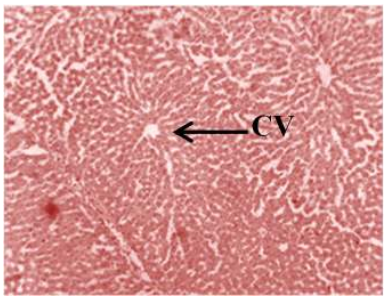

Plate 1c. Liver (200 mg/kg)

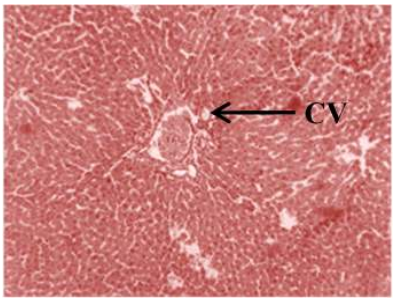

Plate 1d. Liver (400 mg/kg)

Plates 1a-1d. The histopathological effect of *A. vogelii* ethanolic root extract on the liver of rats
Magnification x 400; CV: central vein

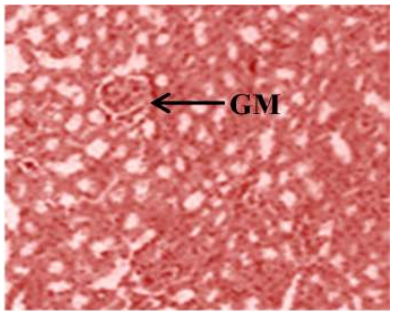

Plate 2a. Kidney (control)

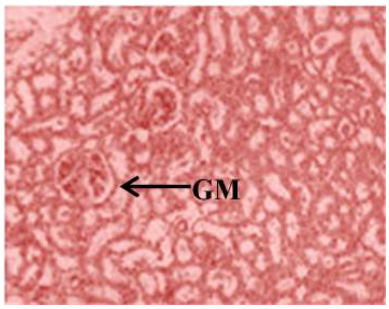

Plate 2b. Kidney (100 mg/kg)

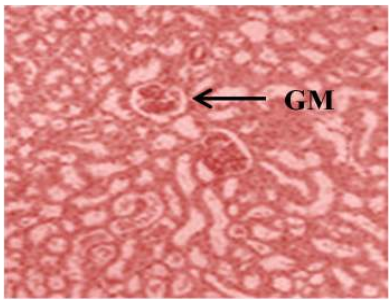

Plate 2c. Kidney (200 mg/kg)

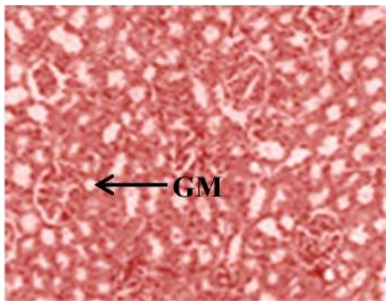

Plate 2d. Kidney (400 mg/kg)

Plates 2a-2d. The histopathological effect of *A. vogelii* ethanolic root extract on the kidney of rats
Magnification x 400; GM: glomerulus

4. CONCLUSION

In conclusion from the present study, *A. vogelii* ethanolic root extract is safe when administered acutely (p.o) and it has no toxic effect when administered to Albino rats (p.o.) daily for twenty-eight days.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The animal experiments were performed according to the approved guidelines of Obafemi Awolowo University Research ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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