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Histology of Kidneys of West African Dwarf Rams Administered Aqueous Extracts of *Aspilia africana* Leaves

[1]*Etim, N. N., [2]Oguike, M. A. and [2]Herbert, U.

[1]Department of Animal Science, Akwa Ibom State University, Obio Akpa Campus, Akwa Ibom State, Nigeria.
[2]Department of Animal Breeding and Physiology, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

***Corresponding Author: e-mail:** etimbobo@yahoo.com, **Phone Number:** +2348020859015*

***Target Audience:** Sheep farmers, Extension agencies and researchers,*

Abstract

This study was conducted to evaluate the histology of kidneys of West African Dwarf rams administered Aspilia africana extracts. A Completely Randomized Design with 4 treatments (T_1, T_2, T_3, T_4) and each treatment replicated 3 times was adopted. Each treatment had 6 rams with 2 rams/replicate. The rams (n=24), aged 6 to 9 months, average weight of 4.65kg and 4.85 kg were used in phases 1 and 2, respectively. Rams in phase 1 were administered aqueous A. africana extract orally at 0, 1000, 2000 and 3000 mg/kg Body Weight (BW) in T_1, T_2, T_3, T_4, respectively, for 64 days. In phase 2, rams received same extract orally at 0, 11000, 12000 and 13000mg/kg BW in T_1, T_2, T_3, T_4, respectively, for 30 days. The animals were fed 2kg mixed forage and 500g concentrate throughout the trials. The kidneys excised following slaughtering were fixed in 10% formal saline for histological examination. Results showed that, in phase 1, rams in all groups had normal histological features in the kidneys, whereas in phase 2, rams in the treated groups showed different degrees of tubular necroses and degeneration in their kidneys. These indicate that 11000 - 13000mg/kg BW of the extract could be toxic to kidneys of West African Dwarf rams.

Keywords: *Aspilia africana, aqueous, histology, toxicity, rams.*

Description of Problem

Despite the fact that sheep production holds a great potential for meeting the animal protein needs of people in developing countries, high cost of concentrates can constitute serious challenge to its expected expanded production [1]. It therefore becomes imperative to substitute its feed with feed sources which are cheap or free of cost, readily available and nutritious. One of such feed is forages, such as *Aspilia africana.*

Aspilia africana is a common weed of field crops in West Africa. It is consumed as forage by cattle and sheep [2]. Several reports indicate that the plant is medicinal and possesses antimicrobial, haemostatic, anti-inflammatory and anti-fertility properties [3, 4, 5, 6]. Chemical analysis of the plant showed that it has high crude oil and protein content [7]. Another researcher [8] analyzed the plant and reported that it is a good source of calcium, phosphorus, potassium, magnesium, iron and zinc. It is also rich in saponin, tannins, glycoside, flavonoids, phenols and alkaloids, some of which exhibit phytoestrogenic activities [9, 10, 6, 11, 12, 13, 8, 14, 15]. Hydrodistillation of the leaves of *Aspilia africana* produced four essential oils [16]. These oil samples included sesquiterpenes, monoterpenes, germacrene d and alpha-pienene [6].

Furthermore, *Aspilia africana* has been reported to boost growth in animals [17, 18, 19, 20]. This may be due to its nutritional and medicinal properties. It has also been reported to improve haematological, serum biochemical, carcass and sensory characteristics of animals [21, 22, 17, 23, 24, 25, 26, 27, 28, 29]. Therefore, extracts from the plant could be offered as dietary supplements to rams.

Although, *Aspilia africana* possesses these potentials, there is no guarantee of its safety, with regards to the vital organs of the body, especially the kidneys. Nevertheless, it was stated by [30] that oral administration of up to 10,000mg/kg body weight of aqueous extract of *Aspilia africana* leaf is safe for human and animal consumption, but such conclusion was drawn from experiment conducted on male Swiss albino mice which only monitored behavioural changes and mortality of the mice in response to the extracts. Thus, minimal or no research has been conducted to investigate the degree of toxicity of aqueous extract of *Aspilia africana* leaves on kidneys of farm animals, especially rams. It is worthy of note that the kidneys are vital organs of the body that play important roles of removing waste products from the body, maintaining balanced electrolytes levels, and regulating blood pressure. Any factor that may lead to kidney disease can cause structural and functional disorders [31, 32]. Malfunctioning of the kidney can lead to serious illnesses and death [32, 33].

This study, was therefore, conducted to assess the effects of varying doses of aqueous *Aspilia africana* extract on the kidneys of rams in order to ascertain its safety for consumption by rams.

Materials and Methods

Location and site of the experiment

This research was conducted in the Teaching and Research Farm of the Department of Animal Science, Faculty of Agriculture, Akwa Ibom State University, Obio Akpa Campus, Akwa Ibom State Nigeria.

Collection and preparation of extracts

Fresh leaves of *A. africana* were collected from Nung Uyo Idoro village in Uyo Local Government Area of Akwa Ibom State. The leaves were sorted to remove contaminants, dead matter and sand particles. They were prepared fresh to prevent loss of bioactive ingredients which can take place during drying. The leaves were chopped into tiny pieces with chopping stick and sharp knife and ground using blender to produce *A. africana* leaf meal. 1000g of the leaf meal was measured into conical flasks and extracted with 600ml distilled water for 48 hours at room temperature. The mixture was filtered into conical flasks with Whatman paper no. 1. The solution was filtered while the filtrate was concentrated to a semi-solid form using a rotary evaporator at 40^{o}C to produce gel-like aqueous *A. africana* extracts. In phase 1, the extract was weighed and each of the solutions prepared as 1000mg, 2000mg and 3000mg for T_2, T_3 and T_4, respectively. In phase 2, the extract was weighed and each of the solutions prepared as 11,000mg, 12,000mg and 13,000mg for T_2, T_3 and T_4, respectively.

Experimental animals and management

In phases 1 and 2, twenty-four rams, aged 6-9 months with average weight of 4.65kg and 4.85kg, respectively were used for the study.

The animals were sourced from four (4) Local Government Areas (Uyo, Abak, Oruk Anam and Etim Ekpo) of Akwa Ibom State, Nigeria. The flock was managed intensively. The sheep were quarantined for two (2) weeks before the commencement of the experiment. Routine medications against endo- and ectoparasites as well as suitable vaccination, together with fumigation were performed during the pre-experimental period. The

animals were randomly assigned to 4 treatment groups, with one (1) ram per pen. The pens were constructed with concrete halved walls and iron doors in the research farm that was well ventilated. The sheep were properly identified using plastic neck-tags. The health of the animals was properly monitored and adequate treatment was given to unhealthy animals. Routine inspection and regular cleaning were carried out.

Experimental diet

In both phases, the rams were fed 2kg of forages daily. The forages included: *Panicum maximum* (guinea grass), *Pennisetum purpureum* (elephant grass) and *Cynodon nlemfuensis* (star grass). Each animal also received 500g of concentrate daily. Water was provided ad-libitum throughout the study. The quantity of forage and concentrate diet offered to the animals were weighed daily and the left-over feeds were weighed every morning using a sensitive electronic scale. Tables 1 and 2 show the composition of the concentrate diet given to the experimental animals.

Table1: Composition of concentrate diet fed the experimental animals

Ingredients	%
Maize	40.01
Soybean meal	4.31
Rice bran	41.30
Palm kernel cake	11.38
Bone meal	2.00
*Vitamin/mineral premix	0.50
Salt	0.50
Total	100

**Vitamin/mineral premixes (Growers) produced by Animal Care Product/Care Services Konsult (Nig) Ltd, Iperu Road-Ibadan Express way, Ogera Remo, Ogun State. *Vitamin Premix: Vit. A=8,000,000 I.U, Vit D_3 = 1,700,000 I.U, Vit. E = 5,000mg, Vit K_3 = 150mg, Folic acid = 200mg, niacin = 15,000mg, Vit. B_2 = 3,000mg, Vit. B_{12} = 5mg, Vit. B_1 = 1000mg, Vit. B_6 = 1000mg, biotin = 20mg. antioxidant = 125,000mg. Mineral Premix: Cobalt = 100mg, Selenium = 100mg, iodine = 100mg, Iron = 25,000mg, Manganese = 45,000mg, Copper = 3,000mg Zinc = 35, 000mg, Choline/chloride = 100,000mg.*

Table 2: Proximate composition of the concentrate diet

Parameters	Percentages
Dry matter	86.26
Crude protein	12.71
Ether Extract	7.59
Crude fibre	7.6
Ash	5.46
Nitrogen free extract	52.9
Metabolizable energy (Kcal/kg)	2529.57

Experimental Design

The experiment was in Completely Randomized Design (CRD). In phase 1, the treatment consisted of administration of aqueous *A. africana* extract at 0mg/kg body weight (control; T_1), 1000mg/kg weight (T_2), 2000mg/kg body weight (T_3), 3000mg/kg (T_4). In phase 2, the treatment consisted of administration of aqueous *A. africana* extract at 0mg/kg body weight (control), T_1), 11,000mg/kg body weight (T_2), 12,000mg/kg body weight (T_3), 13,000mg/kg body weight (T_4). In both phases, six rams were randomly assigned to each treatment and balanced for weights. Each treatment was replicated three times with two rams per replicate. The experimental model was as follows:

$Yij = \mu + T_i + eij$

Where:

Yij = Individual observation

μ = Overall mean

Ti = Treatment effect

eij = Random errors, which is assumed to be independently, identically and normally distributed with zero mean and constant variance (iind) (P=0.05).

Administration of Aqueous Extract to Experimental Animals

Phase 1: After two weeks of quarantine and acclimatization, the aqueous extract of *A. africana* was administered orally, once a day for 64 days using 10mls syringes. The control group (T_1) received 10mls of distilled water while treatments 2, 3 and 4 received 1000mg/kg, 2000mg/kg and 3000mg/kg BW of the aqueous leaf extract of *Aspilia africana*, respectively.

Phase 2: After two weeks of quarantine and acclimatization, the aqueous extract of *A. africana* was administered orally, once daily for 30 days using 10mls syringes. The control group (T_1) received 10mls of distilled water while treatments 2, 3 and 4 received 11,000mg/kg, 12,000mg/kg and 13,000mg/kg BW of aqueous extract of *Aspilia africana*, respectively.

Histological Evaluation

Phase 1: At the end of 64 days, 4 rams per treatment group were randomly selected and slaughtered. Phase 2: At the end of 30 days of administration of the extract, 4 rams per group were randomly selected and slaughtered. During both phases, the kidneys obtained were fixed in 10% formal saline. Thin cut of sections of the specimen were taken after paraffin embedding. The slides were stained with Haematoxylene and Eosin and histological features were later observed under microscope (x 400).

Results

Phase 1 of the Experiment

The results of the histological analyses of the kidney tissues of rams administered 10ml distilled water, 1000mg/kg bodyweight, 2000mg/kg BW and 3000mg/kg BW of the extract are presented in Plates 1-4 below:

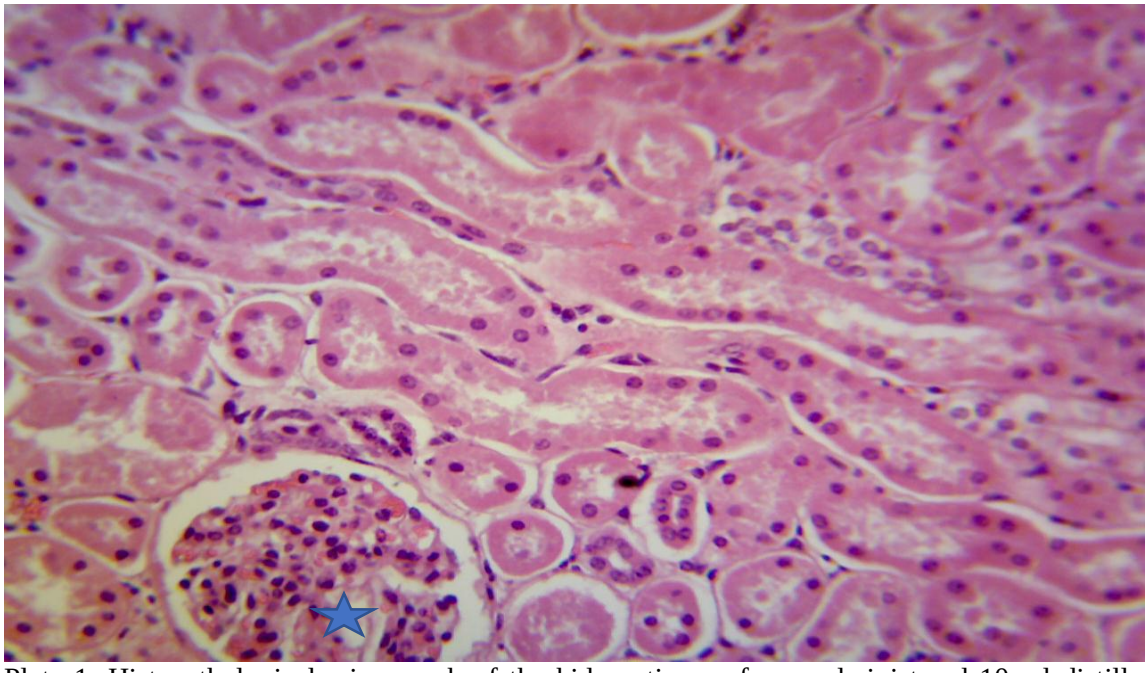

Plate 1: Histopathological micrograph of the kidney tissue of ram administered 10 ml distilled water. ★ ---**Glomerulus** (H & E, x400).

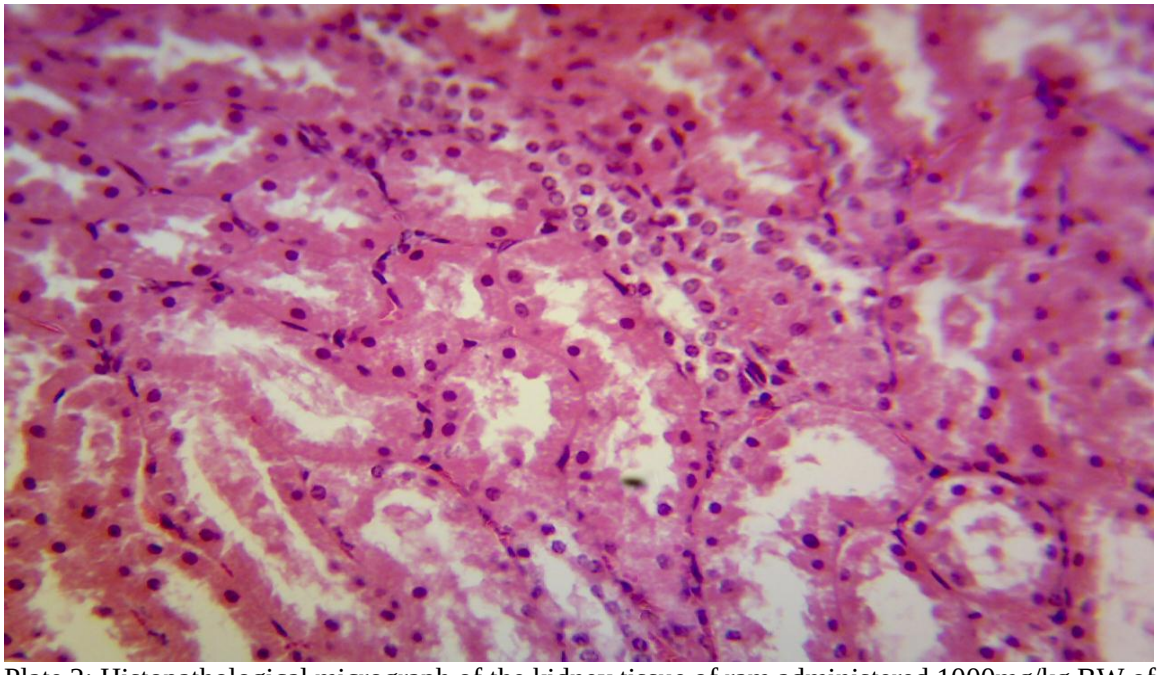

Plate 2: Histopathological micrograph of the kidney tissue of ram administered 1000mg/kg BW of aqueous *Aspilia africana* extract (H & E, x400)

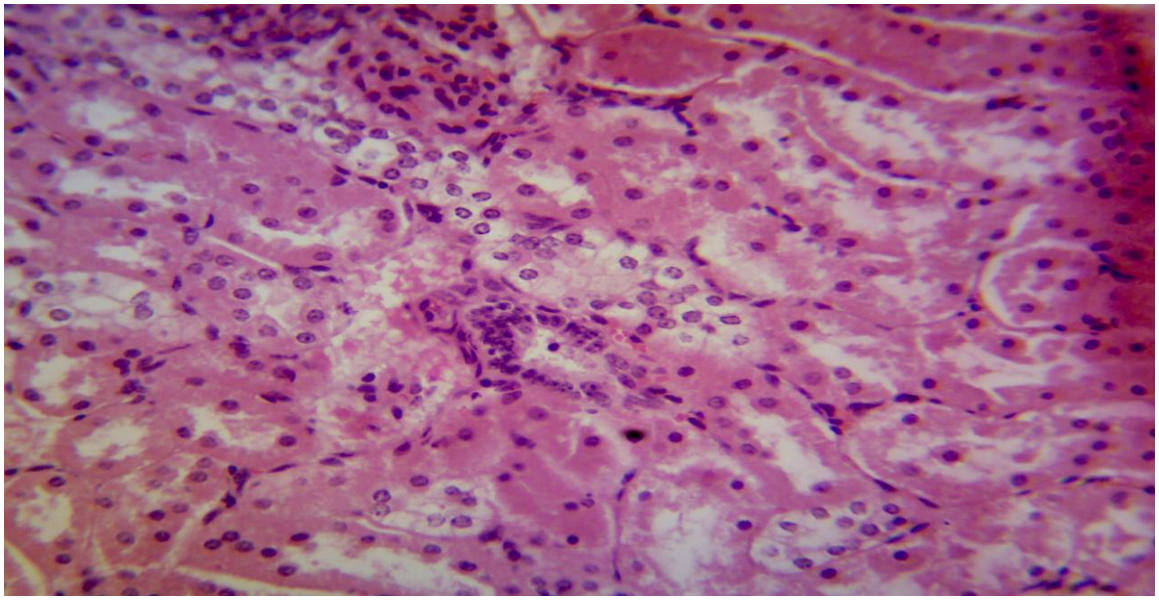

Plate 3: Histopathological micrograph of the kidney tissue of ram administered 2000mg/kg body weight of aqueous *Aspilia africana* extract (H & E, x400).

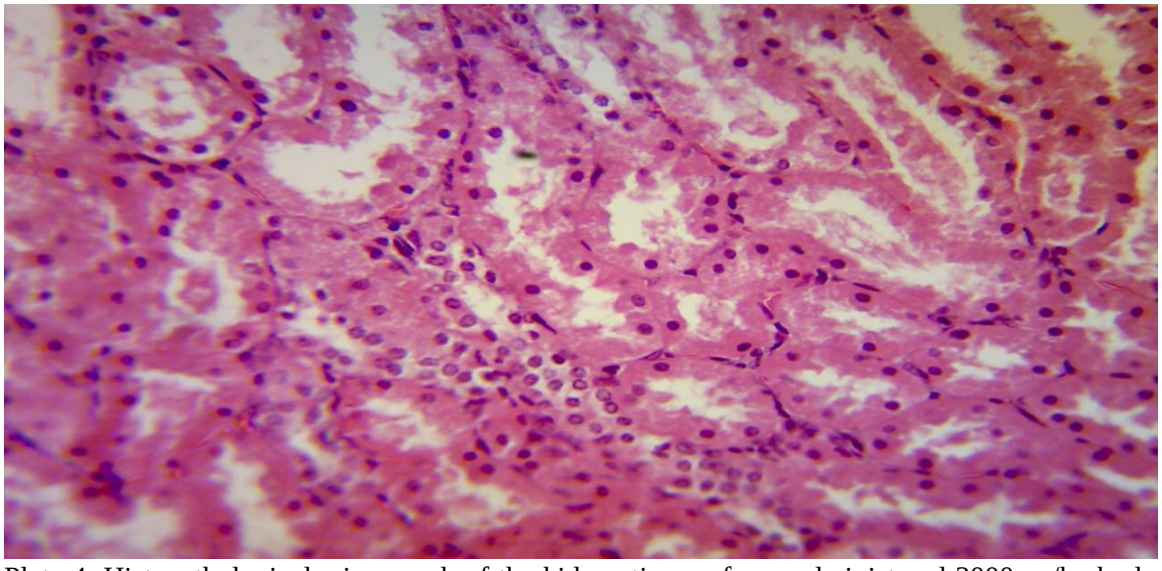

Plate 4: Histopathological micrograph of the kidney tissue of ram administered 3000mg/kg body weight of aqueous *Aspilia africana* extract (H & E, x400).

As shown in Plate. 1, the sections of the kidneys from rams in the control group revealed normal histological features. The section indicated a detailed cortical parenchyma and the renal corpuscles appeared as dense rounded structures with the glomerulus surrounded by a narrow Bowman's spaces. The section of kidneys from rams in T_2, T_3 and T_4 also showed no sign of toxicity (Plates 2-4).

Phase 2 of the Experiment

Plates 5-8 below show the results of histological analyses of the kidney tissues of rams administered 10ml distilled water: 11,000mg/kg BW, 12,000mg/kg BW and 13,000mg/kg BW of the extract

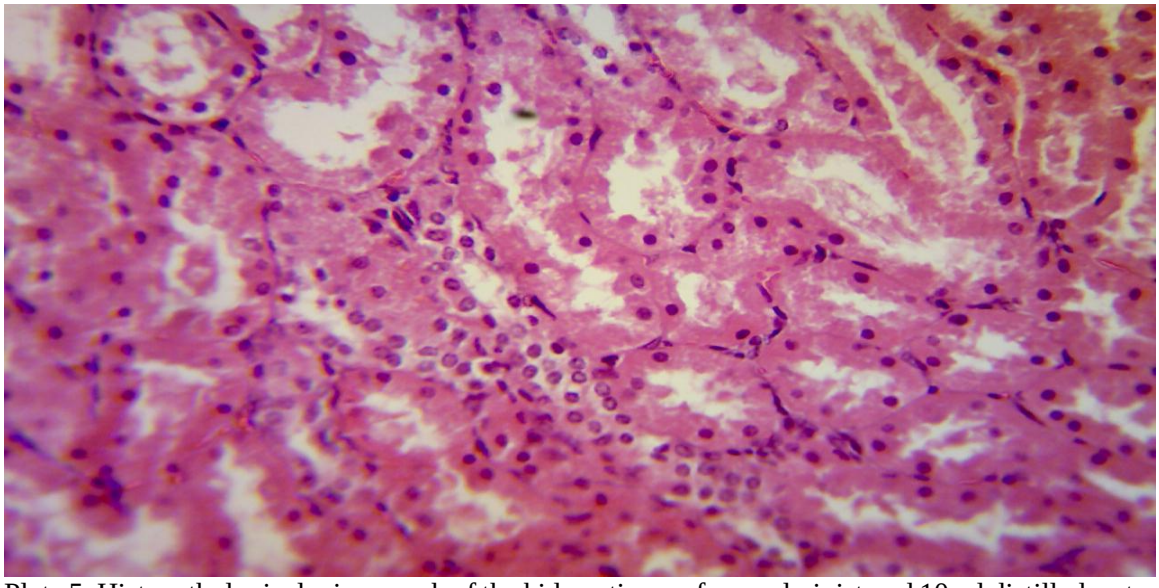

Plate 5: Histopathological micrograph of the kidney tissue of ram administered 10 ml distilled water (H & E, x400).

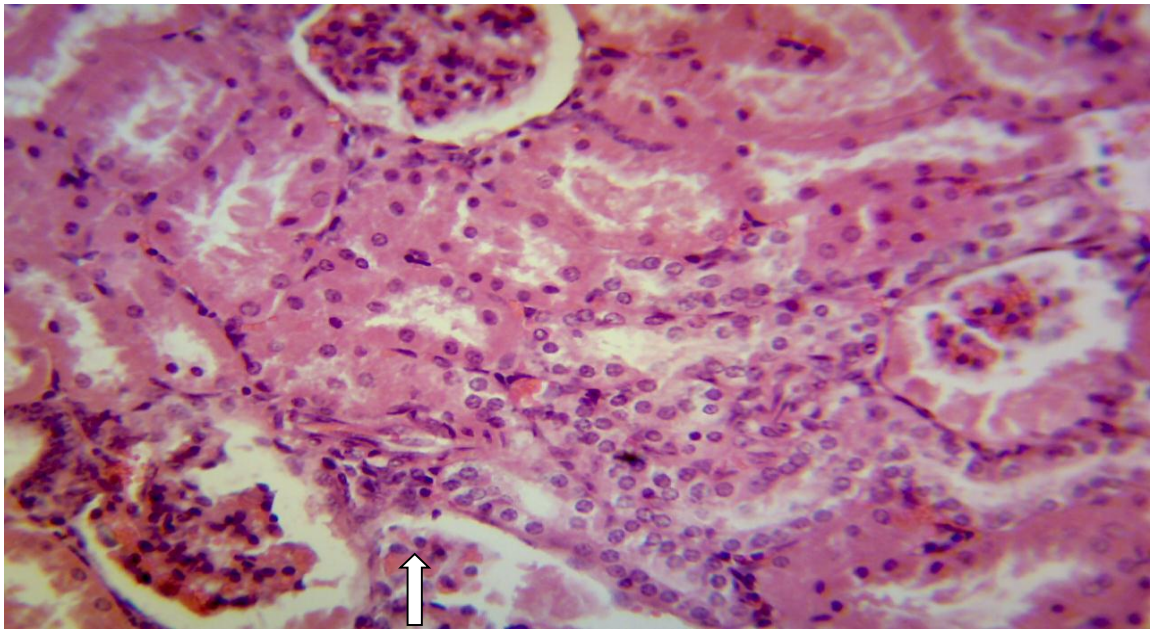

Plate 6: Histopathological micrograph of the kidney tissue of ram administered 11,000mg/kg of aqueous *Aspilia africana* extract. Mild tubular necrosis – arrow (H & E, x400).

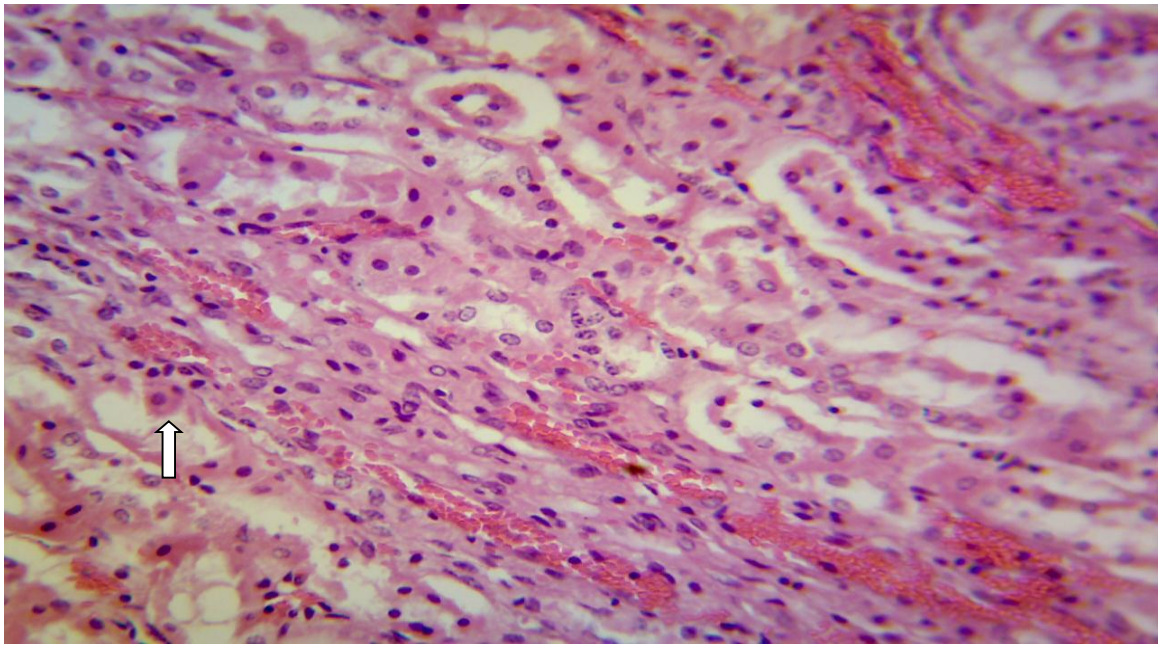

Plate 7. The kidney tissue of ram given 12,000 mg/kg body weight of aqueous *Aspilia africana* extract. Tubular necrosis – arrow. (H & E, x400)

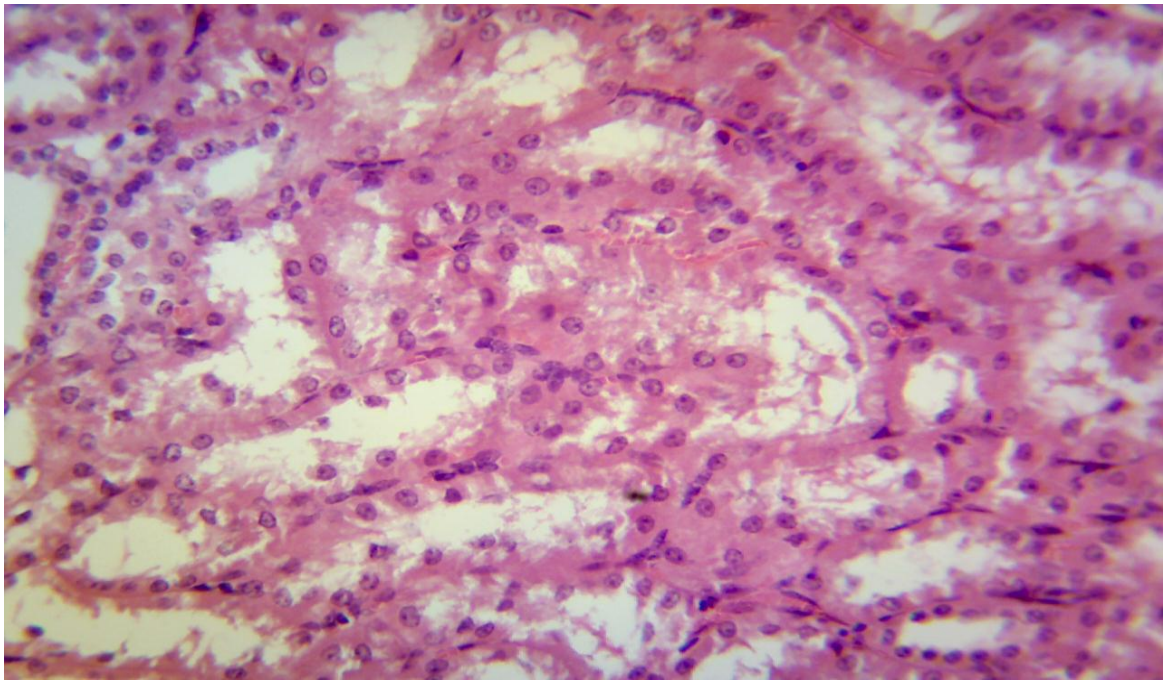

Plate 8. The kidney tissue of ram given 13,000 mg/kg body weight of aqueous *Aspilia africana* extract. Tubular degeneration and necrosis (H & E, x400)

Histopathological evaluation of kidney tissues of rams administered 10 ml distilled water showed no pathological changes. Normal histological features were observed, with no trace of damage to the kidney tissues (Plate 5). However, tubular necrosis and tubular degeneration (as shown by the arrows in Plates. 6-8) were observed in the kidney tissues of rams administered 11,000mg/kg -13,000mg/kg BW of the aqueous *Aspilia africana* extract. The pathological changes were observed to be more severe with increase in the dosage of the extract.

Discussion

Normal features observed in the kidneys of rams in all the treatment groups during the first phase of the experiment (Plates 1-4) indicated that there was no damage done to the kidneys of the rams. These showed that the extract posed no risk to the organ at 1000-3000mg/kg body weight. This finding is consistent with the report by [34] that *Aspilia africana* leaves can be classified among substances with low toxicity. The observation corroborates earlier report by [30] that oral administration of up to 10,000mg/kg BW of aqueous *Aspilia africana* extract is safe for animal consumption.

However, the distortion of cyto-architecture of the renal cortical structures, as well as the varying degree of tubular necroses and degenerative changes observed in the kidney tissues of the treated rams (T_2, T_3 and T_4) in the second phase of the experiment (Plates 5-8), might be attributed to the test extract administered at different doses to the experimental animals. These could be indications that administration of the extract at 11,000mg/kg – 13,000mg/kg BW could be toxic to rams. This is apparent when sections of the kidneys of rams in the control groups in both phases of the experiment and the treated groups in phase 1 of the experiment (Plates 1-5) are compared with those of the treated rams in phase 2 of the experiment which received higher doses of the extract (Plates 6-8).

The observed changes in the kidney of the treated rams in phase 2 may imply that at higher concentrations, the secondary metabolites, which are largely responsible for therapeutic or pharmacological activities of medicinal plants [35], may also cause such plants or their extracts to become toxic when administered at excessive dose(s). This is consistent with the report by [36]. These findings suggest that at higher concentrations of up to 11,000mg/kg BW and above, some secondary metabolites in the aqueous extract of *Aspilia africana* such as alkaloids, flavanoids, saponins, glycosides, tannins, and terpenoids [3, 9, 10] might have caused low blood flow to the kidneys and might be nephrotoxic [36, 37]. This is evident in tubular necroses observed in the kidney of rams in T_2, T_3 and T_4. The observed deleterious effects suggest that 11,000-13,000mg/kg BW aqueous *Aspilia africana* extract may lead to kidney disease, which can cause structural and functional problems in the kidney [5]. Such malfunctions may lead to severe illnesses or even death as was reported by [31], [32] and [33].

Conclusion and Application

1. Normal histological features observed in the kidney tissues of rams administered varying doses of aqueous *Aspilia africana* extract in phase 1 of the experiment is an indication that up to 3000mg/kg body weight of the extract is not toxic to the kidneys of West African Dwarf rams.
2. Tubular degeneration and necroses observed in the treated rams during phase 2 of the experiment may imply that high doses of the extract (11,000mg/kg-13,000mg/kg BW) could be toxic and impair the

functionality of kidney tissues of West African Dwarf rams.

3. Therefore, up to 3000mg/kg body weight of the extract is safe and is recommended for administration to rams, whereas doses as high as 11,000mg/kg BW of the extract is discouraged from being administered to West African Dwarf rams.

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