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

Etim, N. N.

Department of Animal Science, Akwa Ibom State University, Obio Akpa Campus, Akwa Ibom State, Nigeria.

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

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

Abstract

This 2-phase experiment was conducted to examine the degree of toxicity of Aspilia africana on the liver of West African Dwarf rams. Both phases were Completely Randomized Designed (CRD) experiments with 4 treatments (T_1, T_2, T_3, T_4) with each treatment replicated 3 times. T_1 served as control in both phases. Each treatment had 6 rams with 2 rams/ replicate. In both phases, 24 rams, aged 6 to 9 months, average weight of 4.65kg and 4.85kg for phases 1 and 2, respectively, were used. Rams in phase 1 were administered aqueous A. africana extracts 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. In both cases, control was administered 10ml of distilled water without the extract. The animals were fed 2kg mixed forages and 500g concentrate throughout the trials. At the end of each phase, 4 rams/group were randomly selected and slaughtered. In both phases, the liver obtained were fixed in 10% formal saline for histological examination. Results in phase 1 showed that liver of rams in all groups had normal histological features, while in phase 2, liver of rams in T_3 and T_4 had mild necrosis of hepatocytes and chronic liver damage, respectively. These show that 12000mg/kg BW-13000mg/kg BW of the extract could be toxic on the liver of West African Dwarf rams.

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

Description of Problem

Sheep is a major component of livestock farming. It has a relatively large flock size and higher contribution to the livelihood of livestock farmers [1]. It also holds a great potential for meeting the animal protein needs of people in developing countries. However, its production, especially on a large scale, has been constrained by high feed cost and feed scarcity [2]. It therefore becomes necessary to source for feed sources which are not only nutritious but also cheap or free of cost and readily available. Forage, such as *Aspilia africana* is one of such feed resources.

Aspilia africana, commonly referred to as African marigold plant, haemorrhage plant or wild sunflowers [3], is a forage consumed by sheep [4]. It has been reported to possess medicinal, antimicrobial, haemostatic, anti-inflammatory and anti-fertility properties [5, 6, 7, 8]. Several scientific studies have attributed the numerous medicinal properties of *A. africana* to the abundant bioactive secondary metabolites it possesses, such as alkaloids, saponins, tannins, glycosides, flavonoids, and terpenoid, some of which exhibit phytoestrogenic activities [9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19]. Chemical analysis of the plant showed that it is rich in crude oil and protein [20]. Furthermore, [17] analyzed the plant and reported that it is a good source of calcium, phosphorus, potassium, magnesium,

iron and zinc. Hydrodistillation of the leaves of *Aspilia africana* produced four essential oils [21]. These oil samples included sesquiterpenes, monoterpenes, germacrene d and alpha-pienene [8].

Empirical evidence revealed that *Aspilia africana* can enhance animals' growth 22, 23, 24, 25], probably because of its nutritional and medicinal properties. It has also been documented to improve haematological, serum biochemical, carcass and sensory characteristics of animals [26, 27, 28, 23, 29, 30, 31, 32, 33, 34]. Thus, extracts from *Aspilia africana* could be administered as dietary supplements to rams.

In spite of the fact that, *Aspilia africana* has these benefits, its safety is not guaranteed, with regards to the vital organs of the body, especially the liver. Although [35] stated 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, such conclusion was drawn from experiment carried out on male Swiss albino mice which only monitored behavioural changes and mortality of the mice in response to the extracts. Therefore, there is little or no research conducted to determine the degree of toxicity of the plant on the liver of farm animals, especially rams. The liver is considered the most vital organ for animal health, production and reproduction. The roles of the liver include detoxification, protein synthesis, and the production of chemicals that help digest food. Moreover, several metabolic activities in the body take place in the liver [36].

Thus, it is important to evaluate the state of health of liver because certain medicinal herbs (parts of plants used as feed or for health benefits for animals) possess substances that can be toxic [37, 38] and can damage the liver. Any damage on this organ can disturb metabolic processes that are vital for normal health and optimum productivity [39]. This can result in malfunctioning liver and liver diseases. Liver diseases can negatively affect all kinds of meat producing animals. Therefore, constituting a major economic problem as they can lead to losses in livestock production and national income due to condemnation of great numbers of liver in the slaughter houses [36]. The consequences of liver diseases can be dangerous or even fatal. Therefore, this study, was carried out in order to examine the effects of varying doses of aqueous *Aspilia africana* extract on the liver 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, Nigeria. 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

11000mg, 12000mg and 13000mg 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 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 diets

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 body weight (T_2), 2000mg/kg body weight (T_3), 3000mg/kg body weight (T_4). In phase 2, the treatment consisted of administration of aqueous *A. africana* extract at 0mg/kg body weight (control), T_1), 11000mg/kg weight (T_2), 12000mg/kg body weight (T_3), 13000mg/kg (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 11000mg/kg, 12000mg/kg and 13000mg/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.

In both phases, the liver 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

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

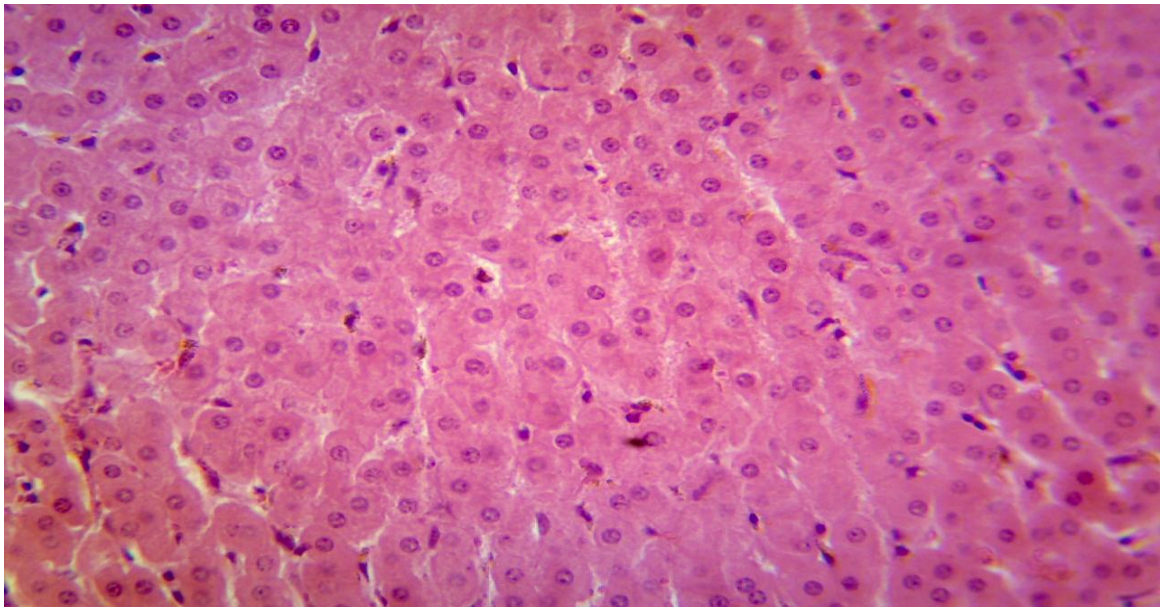

Plate 1: The liver tissue of ram given 10 ml of distilled water. (H & E, X400)

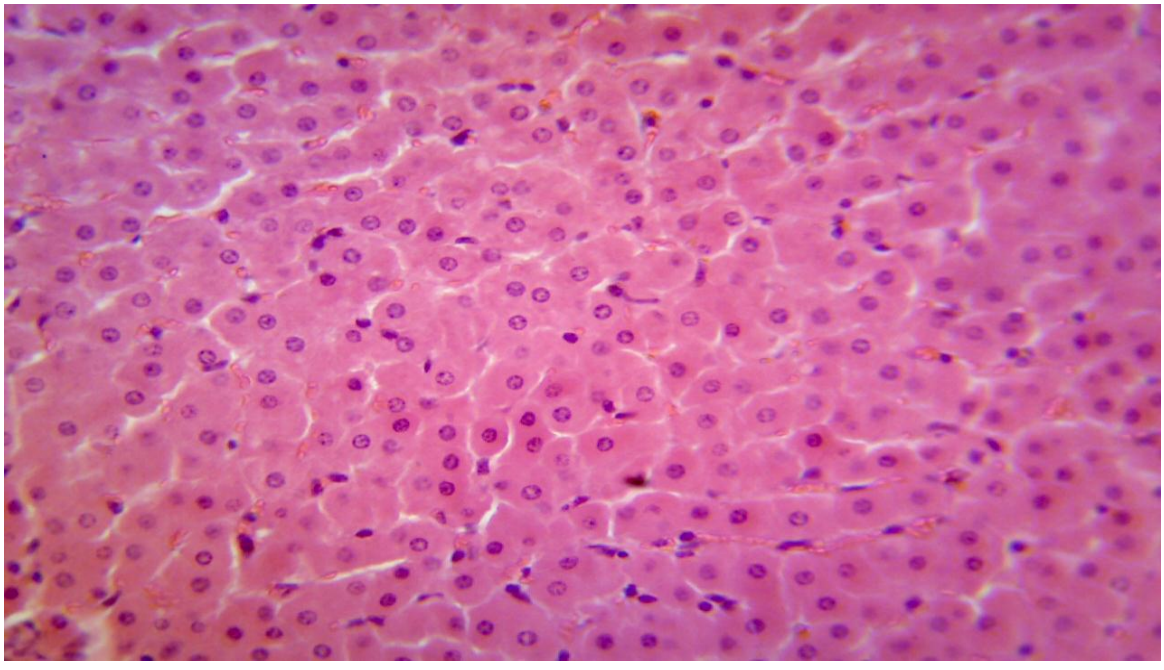

Plate 2: The liver tissue of ram administered 1000 mg/kg body weight of aqueous *Aspilia africana* extract. (H & E, X400)

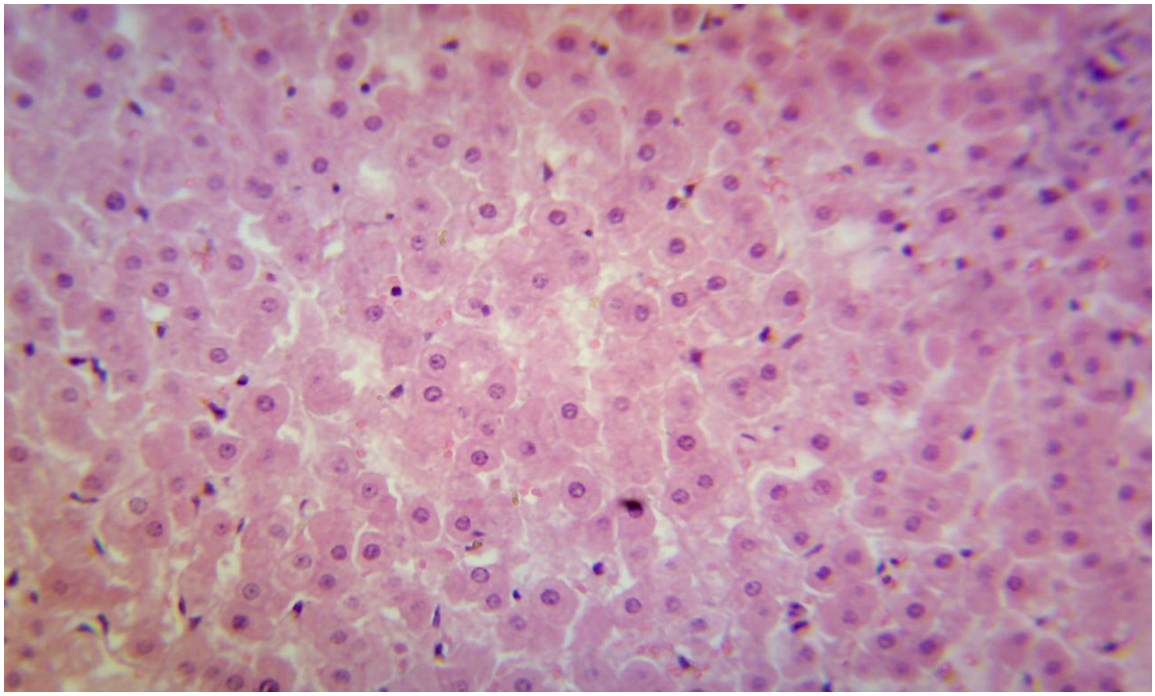

Plate 3: The liver tissue of ram administered 2000 mg/kg body weight of aqueous *Aspilia africana* extract. (H & E, X400)

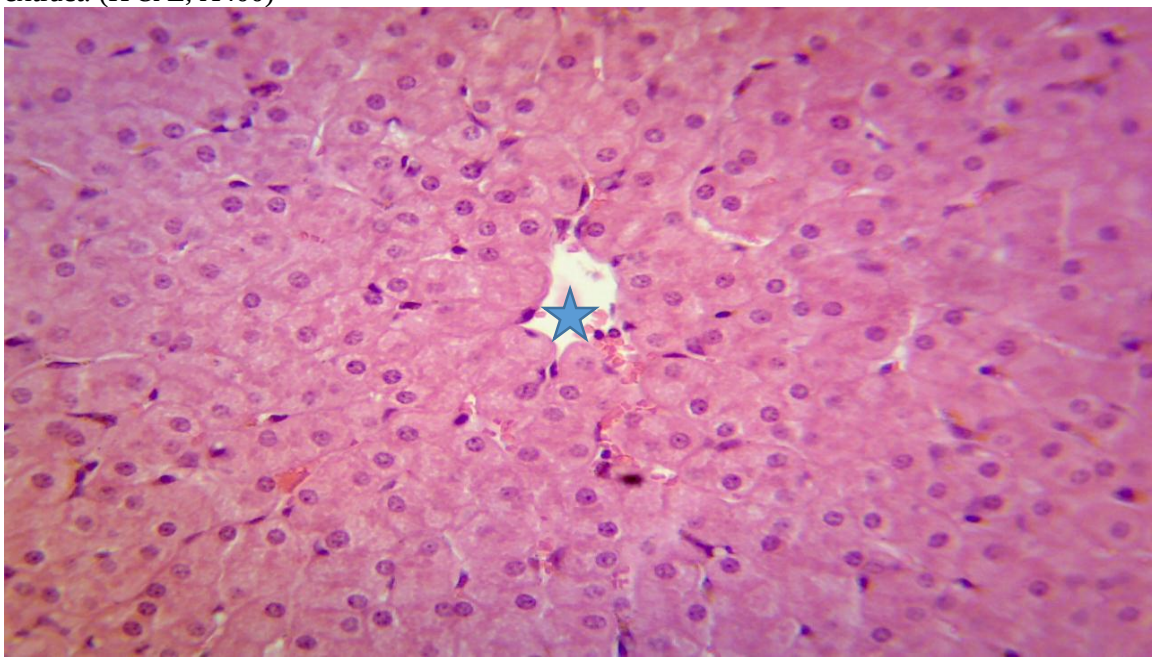

Plate 4: The liver tissue of ram administered 3000 mg/kg body weight of aqueous *Aspilia africana* extract. ★- Central vein (H & E, X400).

The section of liver from rams in T_1, T_2, T_3 and T_4 displayed normal histological features, with no sign of toxicity in phase 1 of the experiment. (Plates 1-4).

Phase 2 of the Experiment

The results of histological analyses of the liver tissues of rams administered 10ml distilled water: 11000mg/kg BW, 12000mg/kg BW and 13000mg/kg BW of the extract are presented in Plates 5-8 below:

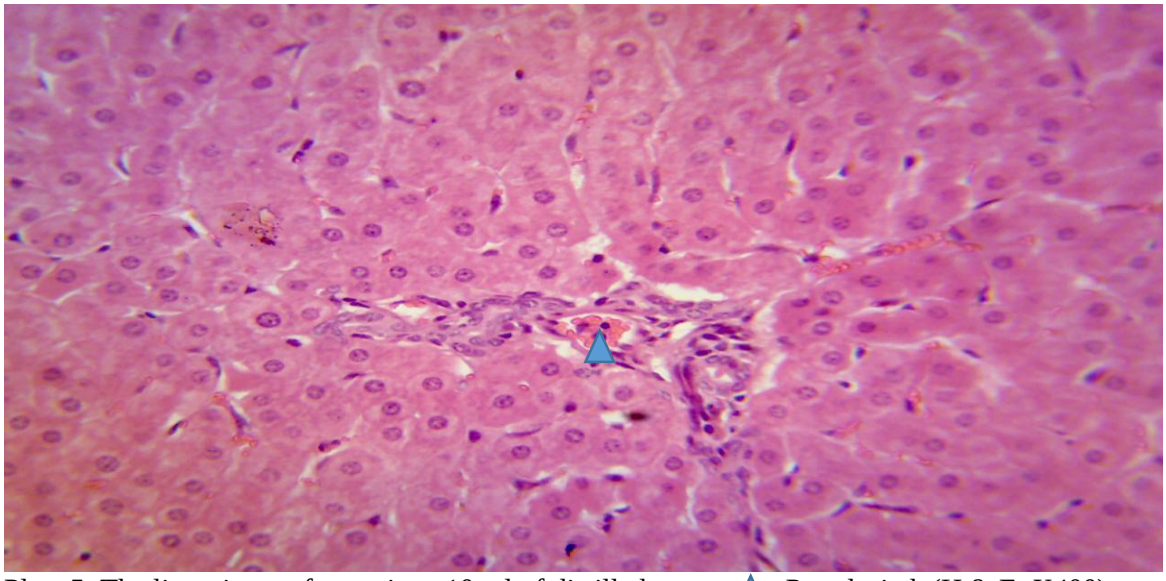

Plate 5: The liver tissue of ram given 10 ml of distilled water. ▲–Portal triad. (H & E, X400).

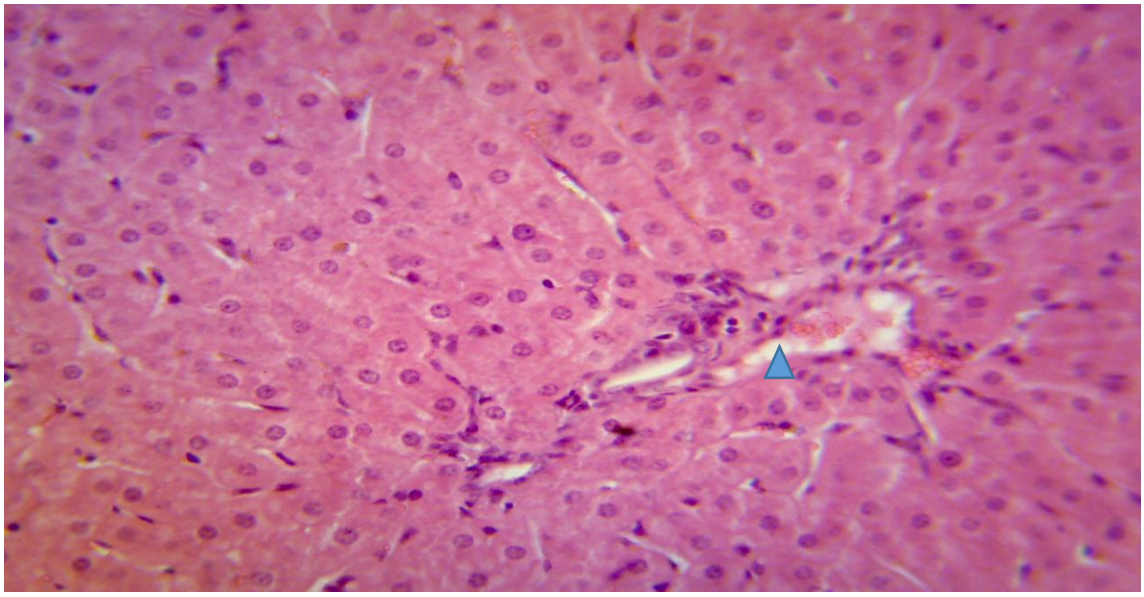

Plate 6: The liver tissue of ram administered 11000 mg/kg body weight of aqueous *Aspilia africana* extract. ▲–Portal triad. (H & E, X400)

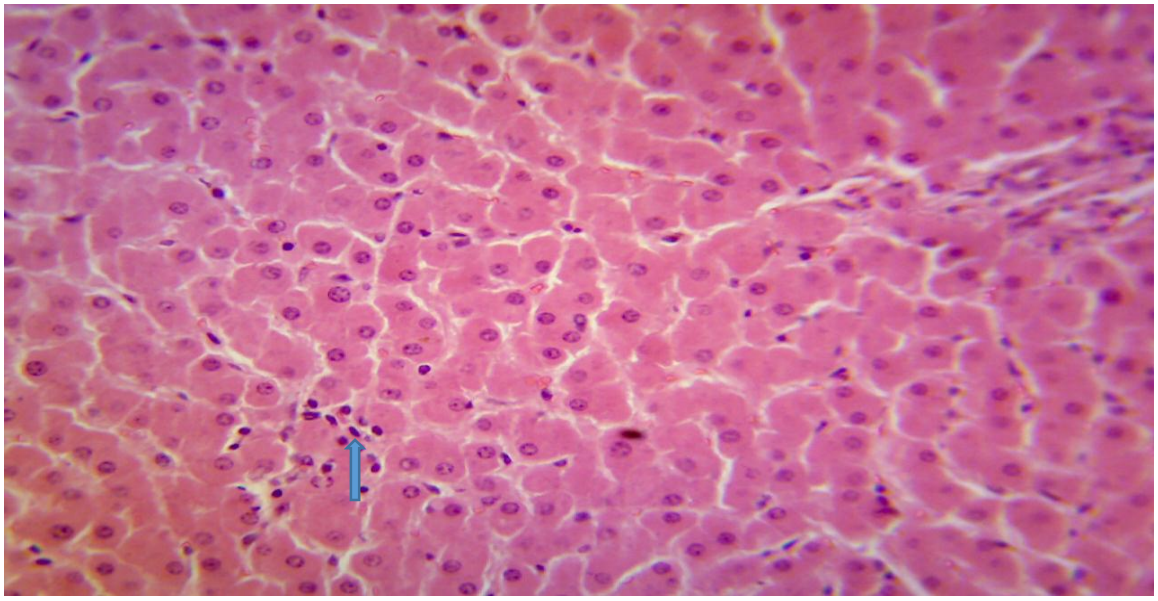

Plate 7: The liver tissue of ram administered 12000 mg/kg body weight of aqueous *Aspilia africana* extract. Mild necrosis of hepatocytes – arrow (H & E, X400)

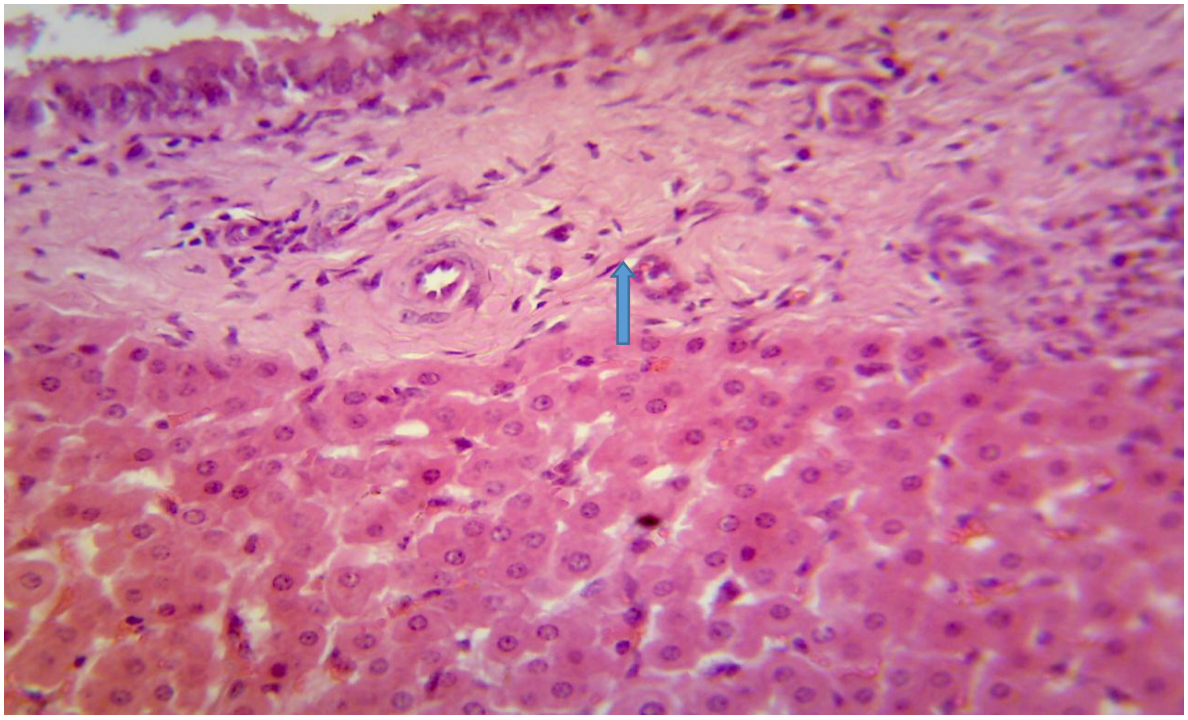

Plate 8: The liver tissue of ram administered 13000 mg/kg body weight of aqueous *Aspilia africana* extract. Chronic liver damage – arrow. (H & E, X400).

In the second phase of the experiment, although normal histological features were also observed in liver tissues of rams in the control group (T_1) and T_2 (Plates 5 and 6), histopathological evaluation of the liver tissues of rams administered higher doses of the extract (12000 mg/kg BW-13000mg/kg BW) revealed signs of toxicity in the form of mild necrosis of hepatocytes (Plate 7) and Chronic liver damage (Plate 8). The pathological changes were observed to be more severe with increase in the dosage of the extract [37].

Discussion

In the first phase of the experiment, the normal features observed in the liver tissues of rams in the control (T_1) and treated groups (T_2, T_3, and T_4) (Plates 1-4) suggested that the experimental extract at the dose of 1000mg/kg BW-3000mg/kg BW had no deleterious effects on the organ. This agrees with earlier report by [40] that *Aspilia africana* is a plant with low toxicity. This finding is also in conformity with the position that animals can safely consumed up to 10,000mg/kg BW of *Aspilia africana* [35].

On the other hand, the pathological changes observed in the liver tissues of rams in (T_3, and T_4) (Plates 7-8) might be associated with the aqueous *Aspilia africana* extract administered to the rams. These showed that although the *Aspilia africana* leaf has been categorized as substances with low toxicity, higher doses of 12000mg/kg BW-13000mg/kg BW of its aqueous extract could be toxic to the liver of WAD rams. These may also imply that the secondary metabolites, which are largely responsible for therapeutic or pharmacological activities of medicinal plants [41], may also make such plants or their extracts to become toxic when administered at higher concentrations as was posited by [42]. This might have activated the necrosis of hepatocytes and chronic damage to the liver [43]. The findings of this study supported earlier reports by [37] and [38] that certain medicinal herbs (parts of plants used as feed or for health benefits of animals) possess substances that can be toxic to the liver [36, 43, 44]. This indicates that at higher concentrations of up to 12000mg/kg BW, some secondary metabolites in the aqueous extract of *Aspilia africana* such as alkaloids, flavanoids, saponins, glycosides, tannins, and terpenoids (3, 9, 10) could induce hepatoxicity in dose-dependent manner [43, 44]. This is evident in the mild necrosis of hepatocytes observed in rams T_3 which received 12000mg/kg BW of the extract compared to the chronic liver damage observed in liver tissues of rams in T_4 that received the highest dose of 13000mg/kg BW of same extract.

Conclusion and Applications

1. From the findings of this study, as much as 3,000mg/kg body weight of aqueous extract of *Aspilia africana* leaves is non-toxic to the liver of WAD rams since sections of the livers in rams of all treatment groups showed normal histological features in the first phase of the experiment.
2. The mild hepatic necrosis and chronic liver damage observed in rams in T_3 and T_4 in the second phase of the experiment indicated that higher doses of the extract (12,000mg/kg-13,000mg/kg BW) could induce hepatotoxicity in the liver of WAD rams.
3. Therefore, up to 3,000mg/kg body weight of the aqueous *Aspilia africana* extract is safe and recommended for administration to rams, whereas dose as high as 12,000mg/kg BW may be deleterious to the liver of WAD rams.

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