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**Anti-Trypanosomal Activity Of Acetone Crude Kolaviron (*Garcinia Kola*
 Fruit Extract) In *Trypanosoma Brucei Brucei* Infected Wistar Rats**

Yau, M. S¹., Balogun, J. B^{1*}., Dogara, M. M¹., Balogun, S.U² and Abubakar, Z¹.

¹Department of Biological Sciences, Federal University Dutse, P.M.B. 7156, Jigawa State-Nigeria

²Department of Human Anatomy, Faculty of Basic Medical Science, Federal University Dutse, P.M.B. 7156, Jigawa State-Nigeria

Corresponding Author's Email & Phone No.: joshufeli@gmail.com, +2348068607137

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ABSTRACT

trypanosomiasis is one of the major obstacles to livestock production in africa and chemotherapy with available drugs is less efficient. preliminary phytochemical screening was carried out, followed by gas chromatography mass spectrometry (gc-ms) analysis, which revealed 9,12-octadecanoic acid (z, z)- to be the most abundant compound. a total of twenty eight (28) rats were used for the study., ld₅₀ was determined using lorke's method. fifteen (15) rats were divided into five (5) groups of three (3) rats. groups 1-4 were inoculated with 1x10³ t. b. brucei, and group 5 was a negative control. group 1 was treated with 100mg/kg, group 2 with 500 mg/kg, group 3 with 0.2ml of acetone solvent only, group 4 was a positive control. there was a 100% mortality rate in groups 3 and 4 due to parasitemia (p<0.05). groups 1, 2 and 5 all reached the time the experiment was terminated. parasitemia in groups treated with 100mg/kg and 500mg/kg showed continuous decreased parasite level (p< 0.05) and extended the life span of wistar rats from 11 days in the positive control group to 55 days in the treated groups. blood parameters of treated groups showed a significant increase (p<0.05) compared to positive control group (p>0.05). organ histopathology showed no damages but regeneration, where damages occur in groups 1 and 2, whereas group 4 showed degeneration of organ's tissues. it was concluded that acetone extract of G. kola extract was trypanostatic. the anti-trypanosomal activity might be attributed to the chemical detected by gcms.

Key word: acetone extract, bitter kola, *trypanosomiasis*, histopathology

1.0 INTRODUCTION

1.1 Trypanosomiasis

Trypanosoma brucei brucei are extracellular haemoflagellate protozoa parasites effecting human and animals in tropical Africa (Nawayana, 2017). Tsetse fly is responsible for the transmission Of the parasite in Africa (Wamwiri and Changasi, 2016). The major veterinary species of trypanosome are *T. congolense*, *T. vivax*, *T. brucei brucei*, *T. simiae*, *T. brucei rhodesiense* and *T. brucei gambiense* are zoonotic with the people as the dominant host (Christine, 2016). The clinical signs of trypanosome *brucei* include: anaemia, weight loss, abortion, low productivity, and if not treated, possibly death (Luckin, 1988). Despite development of attempts at control, trypanosomiasis is still one of the limiting factors to livestock industry in sub-Saharan Africa (Kamuanga, 2003). Direct losses in meat

production and milk yield, and the cost of programmes that attempt to control trypanosomiasis are estimated to cost between \$600 million and 1.2 billion each year. Poor clinical efficiency, drug resistance and toxicity are some of the limitations facing programmes targeted at controlling the disease (Onyeyili & Egwu, 1995; Geerts & Holmes, 1998; Legros *et al.*, 2000; Kamuanga, 2003).

Medicinal plants have been used in different countries in the treatment of trypanosomiasis, because they contain certain component of therapeutic value and therefore medicinal plants will be the best choice to obtain variety of drugs (WHO, 2016).

Bitter Kola is a medical plant that provide raw material for innovative, useful and promising antidote to environment. It contains high content of bioflavonoid compound with a general anecdotal

effect in folk medicine in Africa. Bitter Kola constituents include flavonoid (bioflavonoid), xanthenes, benzophenones and have shown anti-inflammatory, anti-parasitic, anti-microbial and anti-viral properties (Ikpesu, 2014). According to Ekene and Erhirhie (2014), *Garcinia kola* is referred to as a wonder plant because every part of it has been found to be of medicinal importance. The seed of *Garcinia kola* is chewed as an aphrodisiac or used to cure cough, dysentery or chest colds (Yakubu and Quadri, 2012). It could serve as raw material for pharmaceutical industries. The root stem bark of *Garcinia kola* is applied to malignant tumors. The fruit prevent or relieve colic disorder or cure head or chest cold, suppressed cough and is often used in the treatment of cirrhosis, hepatitis, inflammation of the liver (Mazi, 2013).

2.0 MATERIALS AND METHOD(S)

2.1 Plant Material and Extraction

Garcinia kola was purchased from a local vendor at Lokoja Market, Kogi state. The plant was identified in the herbarium of the Department of Biological Sciences, Federal University Dutse, Jigawa State. *G. kola* was air dried and pounded using mortar and pestle into powdered form, which was used for the extraction using a Soxhlet Extractor and Rotary Evaporator machines. An initial 210g of the powder form was placed into a thimble and subjected to Soxhlet extraction technique using 420mL N-Hexane solvent as. The extract was collected after evaporation process using the rotary evaporator.

2.2 Determination of parasitaemia

Trypanosoma brucei brucei used for the entire experiment was obtained from the Protozoology laboratory, Department of Veterinary Medicine, Ahmadu Bello University, Zaria where it was maintained in a donor Wistar Rats. Blood from the tail was used for the estimation of parasitaemia in wet mount. In addition, the trypanosome count was determined by examination of the wet method microscopically at x40 magnification using the "rapid matching method" of Herbert & Lumsden (1976), which involves microscopic counting of parasites per field in pure blood or blood diluted with buffered phosphate saline (PBS, pH 7.2).

2.3 Experimental Design

Twenty eight Wistar Rats of both sexes were purchased from the Nigerian Institute for Trypanosomiasis and Onchocerciasis Research (NITOR), Kaduna State. The Rats were kept in cages under recommended laboratory conditions and fed *ad libitum* with vital feed and safe water until acclimatization period was over. Prior to extract administration LD₅₀ was determined using the Lorke's method (1973). Fifteen Wistar rats were randomly distributed into five groups of three animals each. The first and second groups were inoculated with $\times 10^3$ *T. b. brucei* per mL of blood and then treated with 100mg/kg and 500mg/kg acetone crude kolaviron respectively. The third group was inoculated and administered 0.2ml Acetone solvent. The fourth group was the positive control, inoculated with parasite but not treated with any extract, while group five served as the negative control (no extract, no parasite). The animals were left for 5 days to develop parasitaemia and the level was determined using the rapid matching method described by Herbert & Lumsden, (1976). After a 5 days post inoculation parasites had shown in all the experimental groups and treatments commenced on the same day. Only animals in groups 1 and 2 were treated using 100mg/kg and 500mg/kg dose acetone extracts of *Garcinia kola*. The extracts were given per kg body weight daily via the intraperitoneal route of drug administration throughout the experiment.

2.4 Gas Chromatography Mass Spectrophotometry analysis

The extracts collected was analyzed at the Multi-User Laboratory of the Department of Chemistry, Ahmadu University Zaria, using gas chromatogram coupled to a mass spectrometer model GCMS-Qp 2011 Plus fitted with RTX5 column. The carrier gas used was helium. The injection port and detector temperature are 25°C and 220°C respectively. The oven temperature was programmed from 60°C to 160°C at the rate of 10°C/min and ran for 3mins. The sample was injected into the spectrometer and mass spectra were acquired and processed with Gas Chromatography Mass Spectrophotometry (GC-MS) analysis.

2.5 Haematological analysis

About 20 μ L blood was collected from the tail of each animal in an Ethylene Diamine Tetra Acetate, EDTA container. The blood collected was analyzed using an auto analyzer machine to obtain some blood parameters.

2.6 Histopathological analysis

The animals were sacrificed, and the spleen, liver and kidney were harvested from each experimental animal. Autopsy was performed on the harvested organs at the Histopathology Laboratory of ABU Teaching Hospital, Shika, Kaduna State. The biopsies of liver, kidney and spleen of wistar rats were fixed with 10% formal saline, dehydrated with ascending grade of alcohol, cleared with toluene, infiltrated with molten paraffin wax. The microtome sections were stained with Haematoxylin and Eosin staining technique, and examined with Leica DM 300 microscope and

photographed with ICC 50 HD camera (Avwioro, 2010).

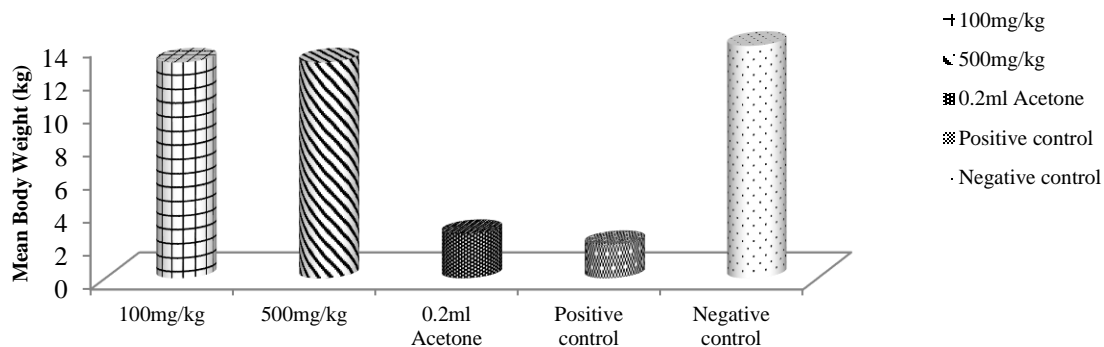
2.7 Data Analysis

Data obtained were expressed as Mean \pm SEM and were subjected to a one – way analysis of variance followed by Tukey Post hoc using SPSS version 23.0 software. Values of P<0.05 were considered statistically significant.

3.0 RESULT AND DISCUSSION

3.1 Mean body weight of the experimental groups

The mean body weight of the experimental groups showed improved mean body weight among the animals in group I and II was observed to be 13kg which is almost similar to the one in group V (14kg), compared to those in groups III and IV which 2.6kg and 2kg mean body weight because of parasite impact.



Groups

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3.2 Mean Parasitaemia ($\times 10^6$ /ml)

The parasitaemia of group I (100mg/kg acetone extract) rose to 30×10^6 /ml of blood by day 5 post infection, but pre-treatment then treatment commenced almost immediately, leading to an observed decrease in parasitaemia to 15×10^6 /ml of blood, 15×10^6 /ml of blood and 5×10^6 /ml of blood at days 6-8, 9-13 and 14-19 respectively. The parasitaemia later crashed to 0×10^6 /ml of blood by day 21 and remained so up to day 55. Similarly, the parasitaemia in group II (500mg/kg acetone extract) rose to 30×10^6 /ml of blood by day 5 post

infection, but pre-treatment then treatment commenced almost immediately, leading to an observed decrease in parasitaemia to 2×10^6 /ml of blood at day 8. The parasitaemia later crashed to 0×10^6 /ml of blood by day 10 and remained so up to day 55. Unlike groups I and II, group IV (parasite control) showed continuous increase in parasitaemia from 30×10^6 /ml of blood by day 5 post infection, to 130×10^6 /ml of blood by days 7-8 post infection and 250×10^6 /ml of blood by day 11 post infection, and have all the wistar rats killed (0% survival) by this day (Figure 2).

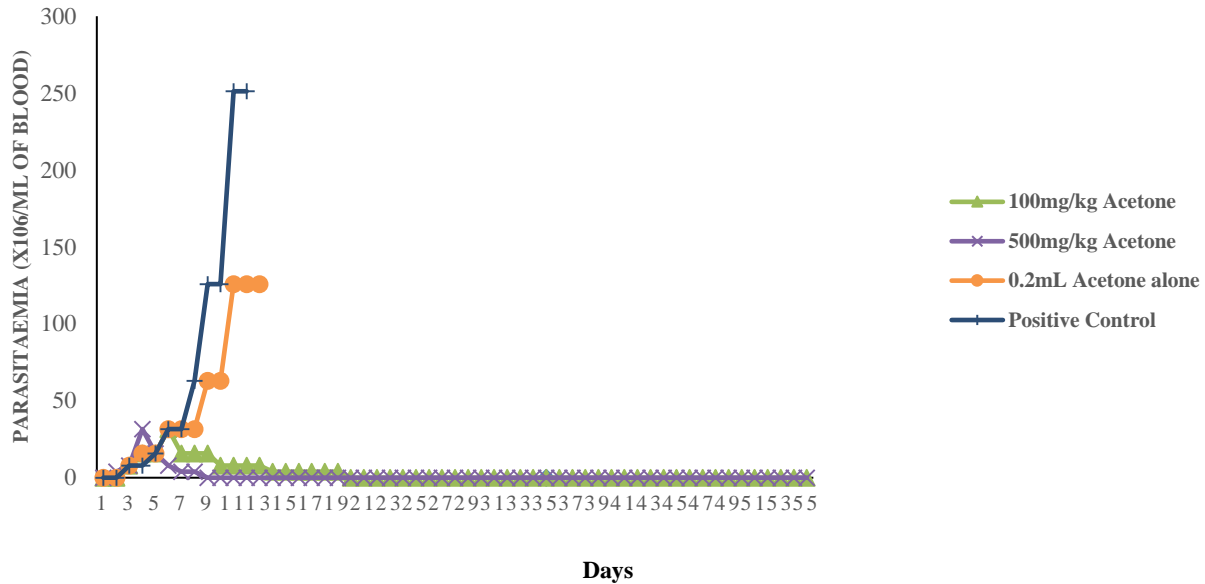


Figure 2: Mean Parasitaemia of Experimental Animals

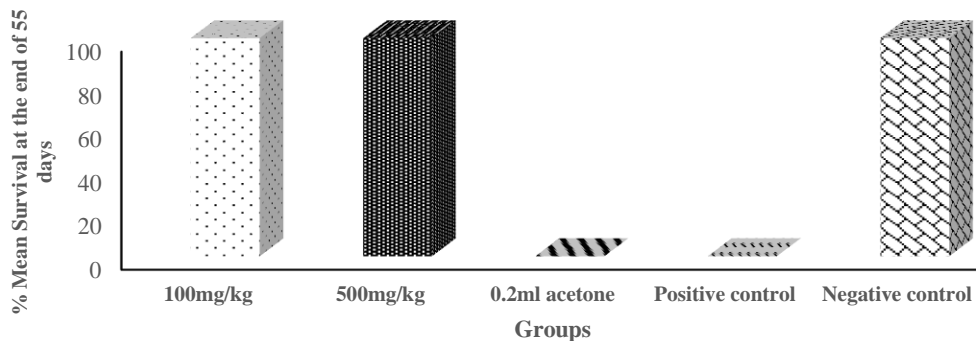


Figure 3: Mean Percentage Survival at the End of 55 Days Post Inoculation

3.3 Percentage Survival of the Experimental Groups at the End of 55 Days

The mean survival time in days and % survival was 55 and 100% respectively, for groups I (100mg/kg kolaviron), II (500mg/kg kolaviron) and V (Negative control). The mean survival time in days and % survival was 13 and 100%; 55 and 0% respectively for group III (0.2ml acetone solvent). Also the mean survival time in days and % survival

was 11 and 100%; 55 and 0% respectively for group IV (positive control).

3.4 Effects of *G. Kola* extract on Some Haematological Parameters

Table 1 revealed the effects of Acetone extracts of *G. kola* on RBC and PLT of infected wistar rats with *T. b. brucei*. The result showed that there was no significant difference ($p > 0.05$) between the

baseline, the pretreatment and the post treatment rats infected with *T. b. brucei* in both the extracts used for treatment. However, the result showed that there was significant difference ($p>0.05$) in RBC between the pretreatment and the post treatment

rats infected with *T. b. brucei*. Similarly, the platelets (PLT) of groups I, II and III showed significant difference ($p>0.05$), which is indicative of tissue injury and inflammation due to parasitaemia.

Table 1: Effect of Acetone Extract of *G. kola* on RBC and Platelets (PLT) of *T. b. brucei* Infected Wistar Rats.

Parameter	RBC ($\times 10^{12}/L$)				
	100mg/kg	500mg/kg	0.2ml Ac	Positive	Negative
Baseline	9.64 \pm 2.71	11.08 \pm 2.51	10.45 \pm 1.21	11.09 \pm 2.52	10.92 \pm 2.11
P-value	0.753	0.136	0.136	0.225	0.425
Pretreatment (inoculated rats)	7.18 \pm 4.50 0.009	7.10 \pm 8.80 0.000	7.46 \pm 2.49 0.000	5.68 \pm 1.99 0.032	11.26 \pm 1.79 0.042
Post treatment	(4.43 \pm 0.57) 0.831	(8.67 \pm 0.73) 0.273	(5.45 \pm 0.39) 0.375	(3.62 \pm 0.29) 0.862	(11.45 \pm 1.55) 0.035
Pretreatment (inoculated rats)	PLT($10^3/mm^3$)				
P-value	(276.87 \pm 3.37) 0.026	(229.33 \pm 17.0) 0.024	(323.67 \pm 7.9) 0.009	(424.67 \pm 2.91) (0.018)	(266.87 \pm 2.4) 0.150
Post treatment	(27.5 \pm 1.48) 0.009	(45.2 \pm 2.18) 0.000	(3.9 \pm 1.55) 0.000	(89.1 \pm 1.21) 0.032	(266.87 \pm 2.4) 0.047

3.5 Gas Chromatography Mass Spectrometric analysis of *Garcinia kola* extract

The list of compounds presented in GC-MS of the Acetone extract of *Garcinia kola* is presented in

Tables 2, with 9,12 –octadecadienoic acid (Z,Z) , being the most abundant compound with area of 26.43, retention time is 38.659 and peak is 4.all the extracts of acetone (Table 2).

Table 2: Compounds Extracted by Gas Chromatography Mass Spectrophotometry from *Garcinia kola* Using Acetone as solvent

PK	RT	AREA	COMPOUND
1	31.991	1.32	1,3,5-Benzenetriol
2	37.314	1.98	1,2-Benzenedicarboxylic acid, buty 1,8- Methylnoyl ester 1,2-Ethylhexyl ester
3	37.477	4.78	Phthic acid, buytl 2-pentyl ester n-Hexadecanoic acid Tetradecanoic acid
4	38.659	26.43	9,12-Octadecadienoic acid (Z,Z)
5	38.909	25.14	9,12-Octadecadienoic acid (Z,Z)- 3,4-Octadiene, 7- methyl-
6	39.028	6.21	9,12-octadecadienoic acid (Z,Z)- 2-Chloroethyl Linoleate
7	39.122	20.44	9,12-Octadecadienoic acid(Z,Z) 7- Hexadecyne 3,4-Octadiene, 7-methyl-

8	40.755	13.70	Phthalicacid,di(hept-3-yl) ester Phthalic acid, hept-3-yl Isohexyl ester Phthalicacid,octyl 2,4,4-trimethylpenty ester.
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3.6 Histopathological Analysis of the Experimental Wistar Rats Organs

3.6.1 Liver Histology

Animals in group III infected with *T. brucei* parasite and treated with 0.2ml of Acetone solvent and group 4, (positive control) showed visible degenerated central vein (CV),with have highly inflamed glandular apparatus (G) with no capsular space and tubular space (T). But, the kidney

histopathology of Animals group I infected with *T. brucei* parasite and treated with Acetone 100mg of kolaviron extract, animals in group II infected with *T. brucei* parasite and treated with Acetone 500mg of kolaviron extract, animals in group V (negative control) showed well out line tubular (T), and well defined capsular space (C) the cuboidal epithelium of the tubules is well demonstrated (Plate I).

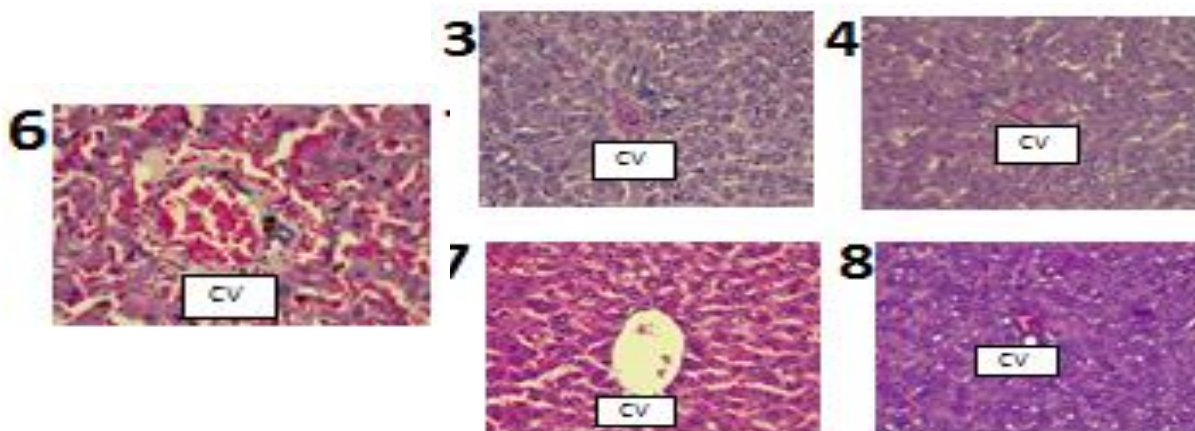


Plate I: Liver photomicrograph of groups: I (3), II (4), III (6), IV (7) and V (8). Pathological Conditions with Visible Degenerated Central Vein (CV) are seen in 6 and 7.

3.6.2 Kidney Histology

Group III infected with *T. brucei* parasite and treated with 0.2ml of Acetone solvent Group IV (positive control), showed apparently unhealthy highly inflamed glandular apparatus (G) with no capsular space and tubular space (T). But, the kidney histopathology of Animals Group I infected with *T. brucei* parasite and treated with Acetone

100mg/kg of kolaviron extract, animals in Group II infected with *T. brucei* parasite and treated with Acetone 500mg of kolaviron extract, animal in Group V (negative control) showed healthy and well out line tubular (T), and well defined capsular space (C) the cuboidal epithelium of the tubules is well demonstrated (Plate II).

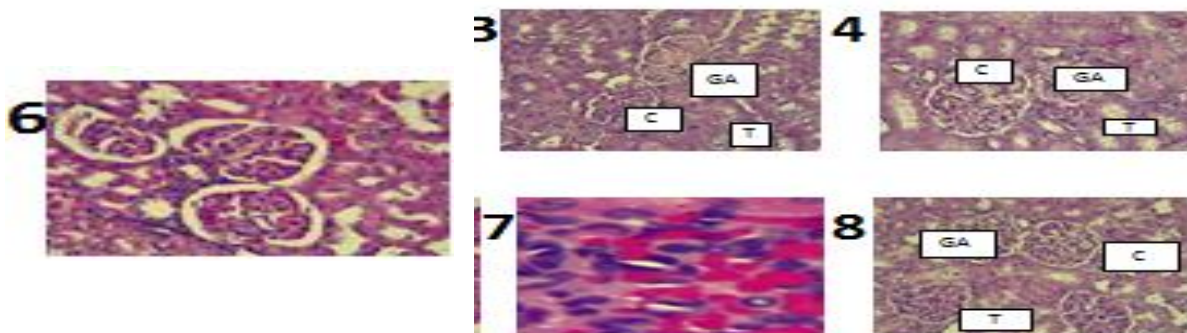


Plate II: Kidney Photomicrograph of Groups: I (3), II (4), III (6), IV (7) and V (8). Pathological Conditions with Visible Degenerated Capsular Space (C) are Seen in 6 and 7.

3.6.3 Spleen Histology

Animals in groups; I infected with *T. brucei* parasite and treated with 100mg/kg of acetone kolaviron extract, IV infected with *T. brucei* parasite and treated with 500mg/kg of Acetone extract, V (negative control), showed health granular layer (GL) with normal nucleus, red pulp

layer (RP) for blood flow characteristics and a large blood vessels (BV) was visible. Whereas, animals in groups; III infected with *T. brucei* parasite and treated with 0.2ml of Acetone solvent, and IV (positive control), showed unhealthy hemorrhage (H), dark staining pyknotic nuclei (p) and necrosis (N) in their spleen (Plate III).

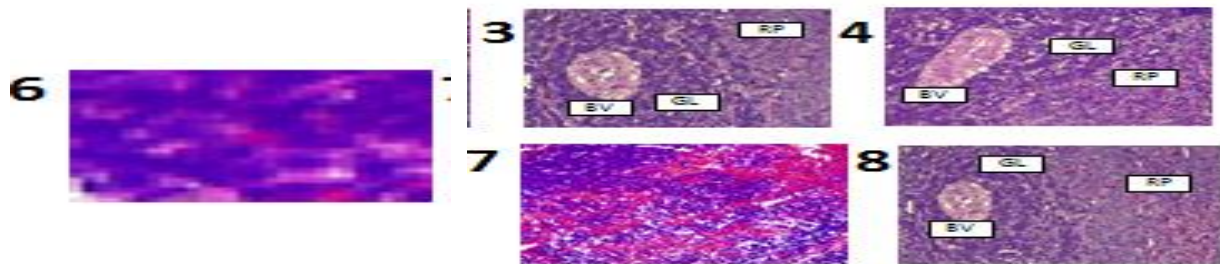


Plate III: Spleen photomicrograph of groups: I (3), II (4), III (6), IV (7) and V (8). Pathological conditions with visible degenerated capsular space (C) and blood vessels (BV) are seen in 6 and 7

4.0 DISCUSSION

Trypanosomiasis remained one of the neglected tropical diseases with high prevalence in Nigeria and parts of sub Saharan Africa (Balogun *et al.*, 2021). The anti-trypanosomal activity of acetone crude kolaviron in *T. b. brucei* infected wistar rats that was assessed, showed significant ($P < 0.05$) effect. The doses of 100mg/kg and 500mg/kg of acetone extract showed significant difference in the parasitaemia of the infected treated groups ($P < 0.05$) as it caused continuous parasite decrease. Although the acetone extracts could not clear the parasite completely in the rats, it however lowered parasitaemia in the treated groups to microscopically observed $0 \times 10^6/\text{ml}$ of blood. In addition, the extracts could not completely prevent anaemia during experimental trypanosomiasis, it however, reduce the magnitude in the treated groups as compared to the untreated group.

Signs such as pale mucous membrane, weakness, anorexia and emaciation that were observed are characteristics of trypanosomiasis in animals which is in agreement with Anene *et al.* (2006).

In terms of weight gain and loss, the treated group exhibited constant mean weight gain ($P < 0.05$) as compared to the groups that were administered 0.2ml acetone and the positive control groups, this is in agreement with Awotunde *et al.* (2002).

Mean survival time showed that rats treated with both 100mg/kg and 500mg/kg acetone crude Kolaviron had improved mean survival time compared to positive control groups.

Haematological parameters such as RBC and PLT, have been shown to be important indices of livestock health and production status. The significant decrease ($P < 0.05$) in the blood parameters of the *T. b. brucei* infected rats is in agreement with earlier reports of Balogun *et al.* (2017).

The acetone extracts of *G. kola* exhibited antitrypanosomal activity which could be linked to the presence of bioactive compounds, found in *G. kola* especially 9,12 –octadecadienoic acid (Z,Z). Hepato-Splenomegaly was a prominent feature of infection with *T. b. brucei* (Aremu *et al.*, 2017). This could be due to acute or parasitemia phase of trypanosome infection and attributed its cause to red blood cell sequestration (Hunter *et al.*, 1991; Aremu *et al.*, 2017). The negative control showed no damage, this is in line with findings of (Charity *et al.*, 2012). This also revealed that acetone extracts are effective on the parasite by regulating the parasitaemia level, though it did not clear the parasites completely. The Kidney of all experimental rats showed no damage, thus showing that ethyl acetate extracts of *G. kola* prevented the parasite from causing lesions. The heart of all experimental rats also showed no damages.

5.0 CONCLUSION

The study revealed that 9, 12 –octadecadienoic acid (Z, Z) found in kolaviron, which was in abundance,

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could be responsible for the protective effects observed in the treatment of *T. b. brucei* infected wistar rats. The crude acetone *G. kola* extracts had revealed efficacy on parasitaemia level, weight and survival of the infected treated rats compared to infected untreated rats, which is crucial in livestock agriculture. The histopathological analysis revealed liver, spleen and kidney of the infected untreated rats were damaged by the parasite, while there was visible protection of hepatic, splenic and kidney tissues from pronounced damages in the infected treated rats. It is crucial that further studies be carried out to isolate 9, 12 –octadecadienoic acid (Z, Z), do molecular docking first and then administer it alone to *T. b. brucei* infected wistar rats.

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