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**Evaluation of Ethyl Acetate Crude Kolaviron (*Garcinia Kola* Extract) In
Trypanosoma Brucei Brucei Infected Wistar Rats**

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ABSTRACT

Trypanosoma brucei brucei are unicellular parasites transmitted by the tsetse fly. they are the causative agent of african animal trypanosomiasis (aat), which continue to pose important public health and economic problems in many parts of africa. the search for vaccination candidate against aat remains elusive and effective treatment is beset with problems of drug resistance and toxicity. this study aimed at search for effective and safe treatment of aat. after a preliminary phytochemical screening, gas chromatography mass spectrometry (gc-ms) analysis was conducted on the extract which revealed octadecenoic acid methyl ester (59%) to be the most abundant compounds. the ethyl acetate extract of *garcinia kola* (kolaviron) at dose levels of 100mg/kg and 500mg/kg body weight was assessed in *trypanosoma brucei brucei* experimentally infected wistar rats. parasitemia in all treated animals continuously increased ($p=0.0060$) till death except for the groups administered 500mg/kg and 100mg/kg per day, which maintained low parasite count ($p<0.05$) and extension of life span from 12 days in the infected untreated rats (positive control group) to 18 days in the infected but treated rats (test group). full blood count showed a significant increase in the blood parameters of test group ($p=0.0080$) when compared with positive control group ($p=0.0001$). the histopathological analysis showed no organ damage except for the degeneration of liver tissues in positive control, but regeneration in test groups. it was concluded that ethyl acetate extract of *g. kola* extract was trypanostatic. the antitrypanosomal activity exhibited by these extracts might be attributed to the chemicals detected.

Key words: ethyl acetate extract, Bitter kola., *trypanosomiasis*, gcms, histopathology

1.0 INTRODUCTION

1.1 Trypanosomiasis

Trypanosoma brucei brucei are the causative agent of African Animal Trypanosomiasis (AAT) (Antia *et al.*, 2009). The disease results in acute, subacute, or chronic disease characterized by intermittent fever, anemia, occasional diarrhea and rapid loss of condition and often terminates in death (Nurulaini *et al.*, 2007). The insect vector for *Trypanosoma brucei* is the tsetse fly (Barret, 2003). The parasite lives in the mid-gut of the fly (procyclic form), whereupon it migrates to the salivary glands for injection to the mammalian host on biting. The parasite lives within the bloodstream (bloodstream form) where it can re-infect the fly vector after biting (Roditi, 2008).

Trypanosomiasis is a debilitating disease of man, domestic and wild animals characterized by parasitaemia, anemia, loss of condition, reduced

productivity and high mortality (Coustou *et al.*, 2012). In livestock, *Trypanosoma congolense*, *T. vivax* and to a lesser extent, *T. brucei brucei* are the causal agents. An infection with one of the trypanosomes may result in a chronic, debilitating and often fatal disease (Connor *et al.*, 2004). The disease in domestic livestock has negative impact on food production and economic growth in many parts of the world, particularly in sub-Saharan Africa (Taylor, 1998). In regions where human African trypanosomiasis is endemic, several other trypanosome species, including *T. vivax*, *T. congolense* and *T. brucei brucei* are prevalent which affect animal health and production (Piccozzi *et al.*, 2002; Balogun *et al.*, 2021).

The greatest impact of the disease across the continent is on the nutrition of millions of people living in the most highly endemic areas and on the agricultural economies of their countries because it

renders vast areas of semi-arid savannah land in Africa unsuitable for raising livestock that are the source of dairy and meat products (Donelson, 2003). It is estimated that over 60 million people and 50 - 70 million animals are exposed to the infection (Geerts *et al.*, 2001).

2.0 MATERIALS AND METHOD(S)

2.1 Plant Material and Extraction

Garcinia kola was purchased from a local vendor at Mariri Market, Kano 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. 100g of the powder form was placed into a thimble and subjected to Soxhlet extraction technique using 250mL Ethyl acetate as the extraction solvent. The extracted paste was collected after evaporation process using the Rotary Evaporator.

2.2 Test Organism and 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. The trypanosome count was determined by examination of the wet mount microscopically at x40 magnification using the "rapid matching method" of Herbert & Lumsden (1976). This method involves microscopic counting of parasites per field in pure blood or blood appropriately diluted with buffered phosphate saline (PBS, pH 7.2).

2.3 Experimental Animals and Test Groups

Twenty male and female adult Wistar Rats were purchased from the Department of Pharmacy, Ahmadu Bello University Zaria, Kaduna State. The Rats were kept in cages under recommended laboratory conditions and fed *ad libitum* until they acclimatize for seven days before the commencement of the experiment. Prior to extract administration LD₅₀ was determined using the Lorke's method (1973).

Twelve male and female Wistar rats were randomly distributed into four groups of three animals each. The first group served as the negative control (no

extract, no parasite). The second group served as the positive control (inoculated with parasite only, no extract). The third group received 100mg/kg of Ethyl Acetate extract. The fourth group received 500mg/kg of Ethyl Acetate extract. Each of these groups consists of 3 animals per group and was given $\times 10^3$ parasites intraperitoneally in 0.2 ml blood/PBS solution (with the exception of negative control group).

The parasitaemia was determined using the rapid matching method described by Herbert & Lumsden, (1976). Only animals in groups 3 and 4 were treated using 100mg/kg and 500mg/kg dose ethyl acetate extracts of *Garcinia kola*. The extracts were given per kg body weight daily via the intraperitoneal route throughout the experiment.

2.4 Laboratory Procedure

2.4.1 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.

2.4.2 Haematological Analysis

Blood was collected from the tail of each animal and was analyzed using an auto analyzer machine

2.4.3 Histopathological Analysis

The animals were sacrificed, and the heart, liver and kidney were harvested from each experimental animal. Autopsy was performed according to Avwioro, 2010.

2.5 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 Graph Pad InStat software. Values of P<0.05 were considered statistically significant.

3.0 RESULT AND DISCUSSION

3.1 Mean body weight of the experimental groups

Table 4. 1 shows the mean body weight of the experimental groups. Changes among the animals in group I to VI was observed. The group I which is the negative controls were neither infected with *T. b. brucei* nor treated with *G. kola* extract and at

the end of 21 days of exposure they indicated a weight gain from 155 ± 44.67 to 216.6 ± 32.71 ($p < 0.05$). The animals in group II which is the positive group were infected with *T. b. brucei* and treatment was not given, they indicated a drastic

weight loss from 274.3 ± 13.22 to 212.3 ± 29.03 ($p < 0.0001$). The animals in groups III and VI infected with the parasites and treated had shown weight gain that fluctuates overtime.

Table 1; Mean Body Weight of the Experimental Wistar Rats

Days	Negative control	Positive control	Ethyl acetate 100mg/kg	Ethyl acetate 500mg/kg
4	155 ± 44.67	274.3 ± 13.22	126.6 ± 29.53	148 ± 31.97
9	162 ± 32.3	212.3 ± 29.03	121 ± 27	143.3 ± 35.36
14	196 ± 31.76		129 ± 32.14	
18	216.6 ± 32.71			
P-value	$< 0.05^*$	$< 0.001^{***}$	$< 0.001^{***}$	$< 0.01^{**}$

($P < 0.05$) *significant, ***extremely significant

3.2 Mean parasitaemia of the Experimental groups

The mean parasitaemia of the experimental groups is presented in Figure 1. Parasites in the control group began to appear in day 5, at day 8 the parasitaemia level was 60×10^6 , at day 10 and 11 the level of parasitaemia rose to 250×10^6 which remained up to day 16 when all the rats died. In the group treated with 100mg/kg ethyl acetate extract (E/A1), parasitaemia began to show at day 6 and raised at day 8 to 150×10^6 , dropped to 20×10^6 at

day 12 and picked again to 50×10^6 at day 13 and the level was stable, 6×10^6 at days 14, 15 and 16. Lastly the parasitaemia rose at day 17 where the rats died. The group treated with 500mg/kg ethyl acetate extract (E/A2), started showing parasitaemia at day 7 and rose to 80×10^6 which persist at days 8, 9 and 10. At day 11 the parasitaemia level increased to 100×10^6 and remained so at 12 and 13 days before dropping to 40×10^6 at day 14 then rose again to 110×10^6 to day 15 where the rats died.

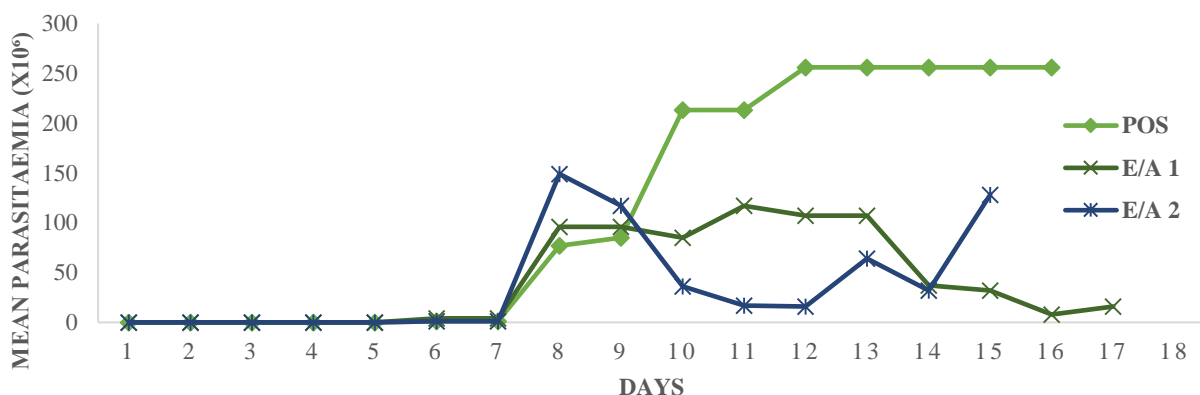


Figure 1: Mean Parasitaemia of Experimental Groups Infected with *T. b. brucei*

3.3 Percentage Survival of the Experimental Groups

Figure 2 presents the percentage survival of the experimental groups. All the animals in group I (negative control) had 100% survival rate. The rats

treated with 100 mg/kg ethyl acetate extract had 60% survival days compared with the least survival group (positive control) which had 49% survival at the end of the experiment (21 days post inoculation).

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

Table 2 revealed the effects of ethyl acetate extracts of *G. kola* on Red Blood Cell (RBC), Haemoglobin (HGB), Platelet (PLT) and Hematocrit (HCT) of infected wistar rats with *T. b. brucei*. The result

showed a 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.

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

The list of compounds presented in GC-MS of the ethyl acetate extract of *Garcinia kola* is presented in Tables 3 with Octadecenoic acid methyl ester was the most abundant (over 59%) in all the extracts of ethyl acetate.

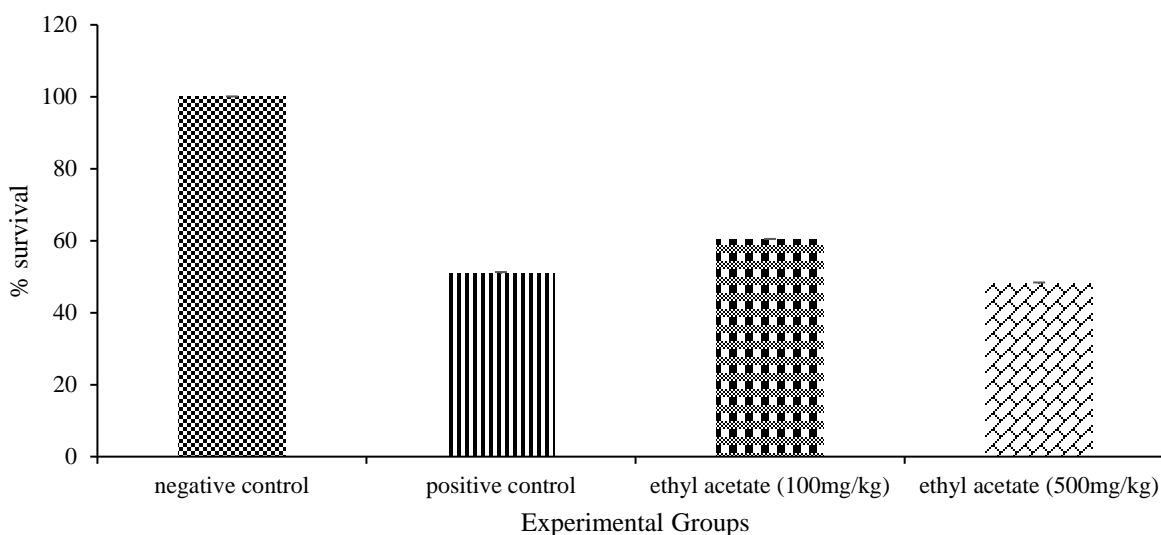


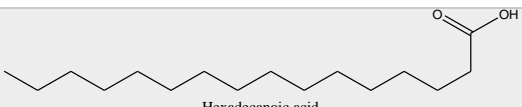
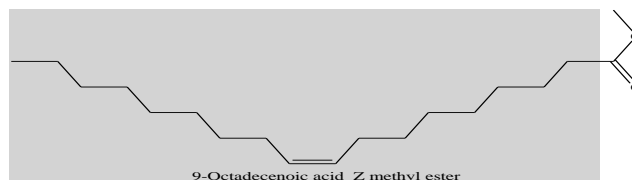
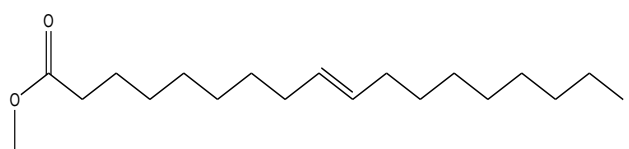
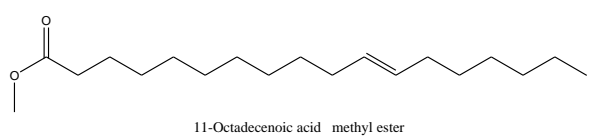
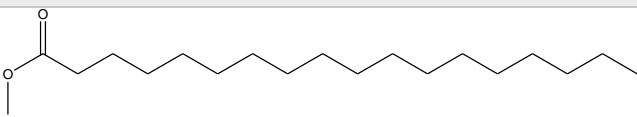
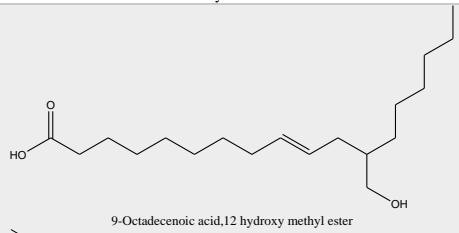
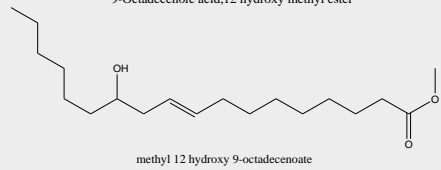
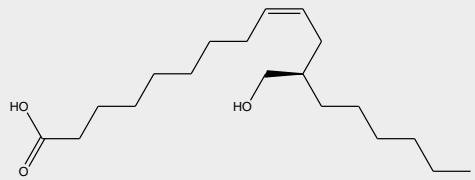
Fig. 2: Percentage Survival of the Experimental Wistar Rats infected with *T. b. brucei*

Table 2: Effect of Ethyl Acetate Extract of *G. kola* on Some Haematological Parameters of *T. b. brucei* Infected Wistar Rats.

Parameter	RBC ($\times 10^6/\mu\text{L}$)	HGB (g/dL)	PCV (%)	PLT ($\times 10^3/\mu\text{L}$)	P-value
Baseline	6.04 \pm 0.026	12.66 \pm 0.05	38.14 \pm 0.42	1067 \pm 5.17	P=<0.00
Pretreatment (inoculated rats)	2.88 \pm 0.34	7.46 \pm 0.08	18.36 \pm 0.06	612 \pm 30.82	P=<0.00
Post treatment Dosage: (100mg/kg)	(4.43 \pm 0.57)	(8.67 \pm 0.73)	(35.25 \pm 2.72)	(623.1 \pm 269.2)	P=0.01
(500mg/kg)	(2.66 \pm 0.10)	(7.72 \pm 0.39)	(27.86 \pm 4.89)	(638.4 \pm 233.7)	P=0.01

RBC: Red Blood Cells, HGB: Haemoglobin, PCV: Packed cell volume, PLT: Platelets (P<0.05)

Table 3: Compounds Extracted by Gas Chromatography Mass Spectrophotometry from *Garcinia kola* using Ethyl acetate as solvent

PK	RT	ARE A %	CMPD	STRUCTURE
1	13.29	14.50	Hexadecanoic acid	 Hexadecanoic acid
2	15.730	59.76	9-Octadecenoic acid (z)-methyl ester	 9-Octadecenoic acid Z methyl ester
			9-Octadecenoic acid methyl ester (E)	 9-Octadecenoic acid methyl ester E
			11-Octadecenoic acid methyl ester	 11-Octadecenoic acid methyl ester
3	16.026	12.23	Methyl stearate	 methyl stearate
4	18.552	11.30	9-Octadecenoic acid, 12-hydroxy methyl ester	 9-Octadecenoic acid, 12 hydroxy methyl ester
			Methyl 12-hydroxy-9-octadecenoate	 methyl 12 hydroxy 9-octadecenoate
			9-Octadecenoic acid, 12-hydroxy methyl ester [R-(Z)]	 9-octadecenoic acid 12-hydroxy methyl ester [R-Z]

3.6 Histopathological Organs of the Experimental Wistar Rats

Plates I, II and III displayed the livers, kidneys and hearts of the uninfected and infected wistar rats.

The histopathological analysis indicated degeneration of liver cells in the positive control group. There was disorganized liver architecture with areas of eosinophilic infiltration and dilated sinusoids. This indicated that there was liver damage in the parasite control group. The regeneration of the liver cells was observed in the

groups treated with ethyl acetate extract, where there was reduction of liver damages in the treated groups. The hearts of the experimental rats showed that the myocardium is intact, therefore there was no damage of the heart in all the untreated and treated rats. The kidneys observed also shows no damage of the renal tissues.

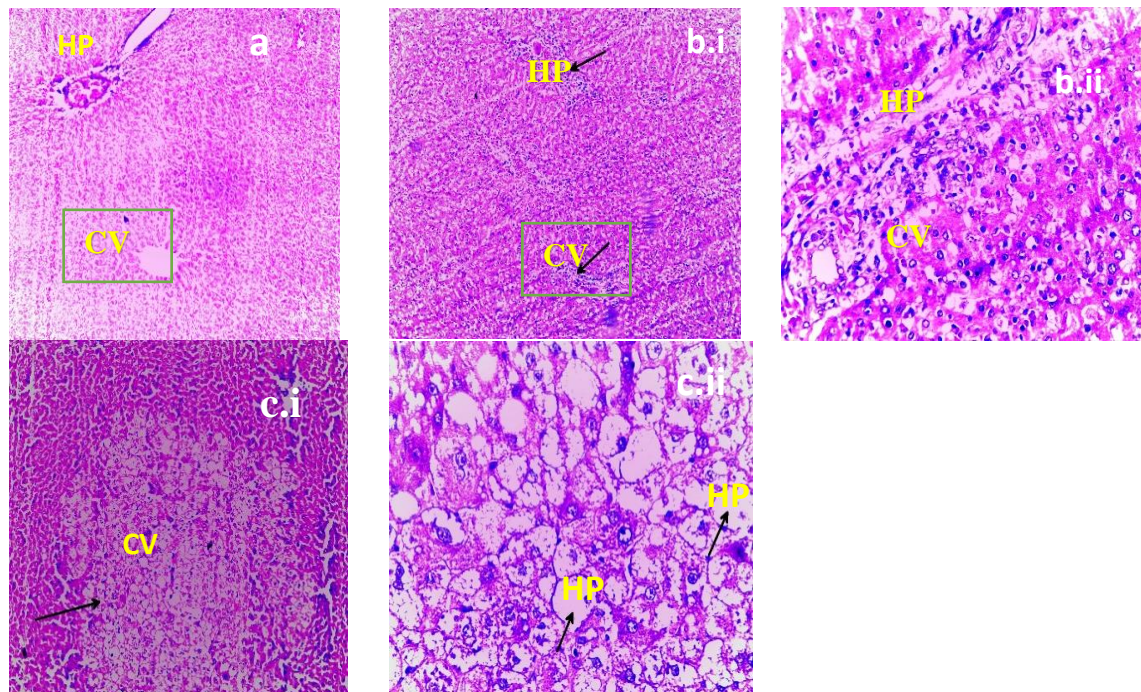
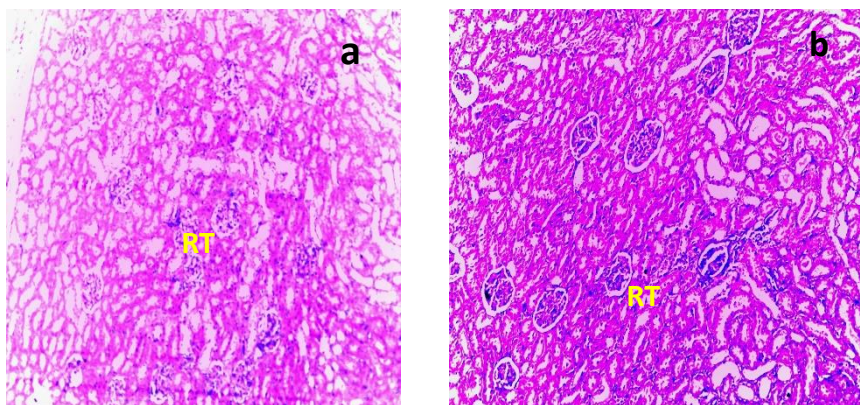


Plate I: Liver of *T. b. brucei* Untreated and Treated Wistar Rats (Magnification x100 and x400)
Key: a=negative control; b=positive control; c=ethyl acetate extract; HP=Hepatocytes; CV=Central Vein.



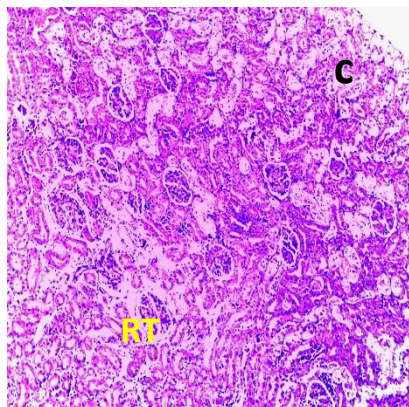


Plate II: Kidney of *T. b. brucei* Untreated and Treated Wistar Rats (Magnification x100)
 Key: a=negative control; b=positive control; c=ethyl acetate extract; RT=Renal Tissue

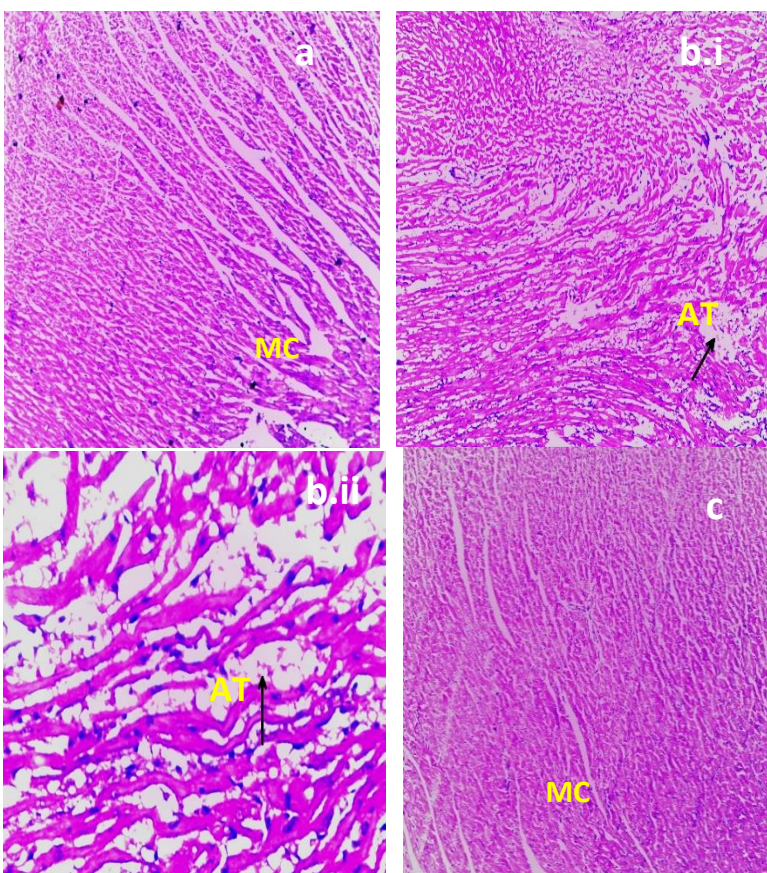


Plate III: Heart of *T. b. brucei* untreated and treated (Magnification x100 and x400)
 Key: a=negative control; b=positive control; c=ethyl acetate extract; AT= Architecture; MC=Myocardium

4.0 DISCUSSION

The prevalence of Trypanosomiasis remains alarming in sub Saharan Africa, Nigeria in particular (Balogun *et al.*, 2021), hence the need to intensify search for new but safe drugs to help eliminate this neglected tropical disease (NTD). The anti-trypanosomal activity of ethyl acetate crude kolaviron in *T. b. brucei* infected wistar rats that was assessed, showed significant ($P < 0.05$) effect. At the dose of 100mg/kg of ethyl acetate extract showed effectiveness, when compared with the 500mg/kg ethyl acetate extract which showed little effect. Though the extracts could not clear the parasite completely before the death of the rats, it however lowered parasitaemia in the treated groups. Furthermore, the extracts could not completely prevent the disease-induced anaemia thereby suggesting the involvement of other aetiological factors in the development of anaemia during experimental trypanosomiasis.

The clinical signs of pale mucous membrane, anorexia, weakness and emaciation that were observed are characteristics of trypanosomiasis in animals. These findings are in agreement with Anene *et al.* (2006). The result of these study showed that the *G. kola* extract exhibited mild to moderate anti-trypanosomal activity, but did not completely clear the parasites (Ezeokonkwo *et al.* (2007). The high level of parasitaemia attained before commencement of treatment in the infected rats could be the reason for the failure of the *G. kola* extract to completely clear the parasites in the blood (Antia *et al.*, 2009). Furthermore, the groups treated with the 500mg/kg ethyl acetate extract showed progressive parasitemia leading to the death of experimental rats. This finding is also in agreement with the finding of Atawodi (2011), which states that some extract belong to groups that act by static action, affecting growth and multiplication rather than eliminating them.

There was appreciable decline in parasitemia especially in animals treated intraperitoneally with 100 mg/kg ethyl acetate extract compared with those treated with 500 mg/kg ethyl acetate extract. Also the culmination of death of animals even at low levels of parasitemia, may be connected to the findings of (Boitigon *et al.*, 1990) which showed that extracellular factors release by the trypanosomes have pathological effect on the host animal. Furthermore, the toxins released into the mammalian system by the parasite makes the host

to generate antibodies against the parasite, which in turn undergo antigenic variations, by changing the surface glycoprotein (Sternberg, 2004) to bypass host defences.

It has been shown in previous studies that different parts of *Garcinia kola* could show varying levels of anti-trypanosomal activity (Atawodi *et al.*, 2011). In the present study the extracts had varying degrees of anti-trypanosomal activity. The positive effect of *G. kola* extract can further be deduced from the weight status of the experimental rats. Even though the treated and untreated rats lost weight as a result of parasitic infection, the weight of the treated rats remained consistently higher than those of the untreated rats indicating that the groups treated was in better physical state to eat more than the untreated ones. They were, therefore, more able to contain certain weight loss due to trypanosomiasis (Urbina, 1993).

Mean survival time showed that rats treated with 500mg/kg ethyl acetate crude Kolaviron had least survival at higher doses of the extract compared to 100mg/kg.

Haematological parameters such as RBC, PCV, HGB and PLT, have been shown to be important indices of livestock health and production status. This is supported by the report of Ekanem *et al.* (2006) which documented that the measurement of anaemia gives a reliable indication of the disease status and productive performance of trypanosome infected animals. PCV is a measure of anaemia, and the reduction in PCV observed in this study particularly in the positive control group suggests that the anaemia in this infection is haemolytic and that it involves a significant increase in the rate of erythrocyte destruction. The findings in this study is in agreement with the reports Igbokwe and Nwosu (1997) who concluded in their studies that trypanosomiasis may cause anaemia as a result of massive erythrophagocytosis by an expanded and active mononuclear phagocytic system (MPS) of the host. In addition, several authors have reported that severity of anaemia usually reflects the intensity and duration of parasitemia in trypanosomal infection (Ekanem *et al.*, 2008; Saleh *et al.*, 2009). The reduced haematological values of RBC, HGB and PLT obtained from this study agree with previous studies reported by Sulaiman and

Adeyemi (2010), which reported such trend to be indicative of acute phase infection, parasitic infection and immunological reaction due to persistent intensity of parasitaemia, respectively. 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 ethyl acetate extracts of *G. kola* exhibited antitrypanosomal activity which could be linked to the presence of bioactive compounds, found in *G. kola* especially Octadecenoic acid methyl ester. Damage of the liver cells were seen in the parasite control group with evident degeneration of the liver tissues. The negative control shows no damage, this is in line with findings of (Charity *et al.*, 2012) while the liver in ethyl acetate extracts showed regeneration of liver tissues. This also revealed that ethyl acetate 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

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The deduction from this study was that the chemical constituents found in kolaviron as revealed by Gas Chromatography Mass Spectrometry being octadecenoic acid methyl ester, which was in abundance, could be responsible for the positive effect observed in the treatment of *T. b. brucei* conducted.

The crude ethyl acetate *G. kola* extracts had revealed efficacy on parasitaemia level, weight and survival of the infected treated rats compared to infected untreated rats. This is crucial in saving lives of animals suffering from trypanosomiasis. The haematological analysis showed that lower dose, 100mg/kg ethyl acetate worked best for the treatment of *T. b. brucei* infection. The histopathological analysis revealed liver of the infected untreated rats were damaged by the parasite, while there was visible regeneration of the liver cells in the infected treated rats, while the hearts and kidneys of the experimental rats showed no damages.

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