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AQUEOUS EXTRACT OF *TERMINALIAAVICENNIOIDES* (GUILLPERR) AMELIORATES SOME PATHOLOGICAL CONDITIONS IN *T. BRUCEIBRUCEI*-INFECTED RATS

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ABSTRACT
The study was designed to investigate the ability of the aqueous extract ofthe stem bark of *Terminaliaavicennioides*(ATA) to alleviate some pathological conditions in rats experimentally infected with *Trypanosomabruceibrucei.* The treatment groupsconsisted: Extract treated group [rats (n=9) were infected (10^4 parasite per rat) intraperitoneally (i.p.) with the parasite and treated with 200 mg/kg b.w. of ATAfor six consecutive days; Negative control [rats (n=9) were not infected but administered 300 µL 100mM phosphate buffer saline pH 7.2, for six consecutive days]; Positive control group [rats (n=9) were infected with about 10^4 T. *bruceibrucei* and left untreated but given 300 µL 100mM phosphate buffer saline pH 7.2, as placebo for six consecutive days]; Standard drug group [rats (n=9) were infected (10^4 parasite per rat) and given (i.p.) 3.5 mg/kg b.w. Diminazineaceturate] and the Extract control group [rats (n=9) in this group were not infected but were treated (i.m.) with 200 mg/kg b.w. of ATA for six consecutive days].Both extract and standard drug were administered intramuscularly (i.m.) and the mean values of the haematological and organ parameters in ATA/drug groups were compared with those of the control groups. Results revealed significant ($p<0.05$) suppression of parasitaemia by the extract. Comparative analysis of PCV, Red Cells and White Cells counts showed slight alleviation of anaemia and leucopenia. There was no variation seen with respect to the Mean ratios of kidneys and liver obtained from experimental rats relative to animals' body weights when compared among all experimental groups.However, splenomegaly seen to accompany the infection were alleviated in the extract-treated group as well as the group of infected rats treated with the standard trypanocide. Distortion of parasite morphology was seen in Giemsa-stained thin film smear of blood obtained from extract treated rats as seen via oil immersion microscopy. The findings suggest that interactionwith parasite membrane could be an early step in the mechanism of action of ATA when used as component of herbal concoction in the traditional management of African Trypanosomiasis.

Keywords: *Terminaliaavicennioides*, *Trypanosomabruceibrucei*, Albino Rats, Anaemia, Leucopenia, Splenomegaly, Traditional medicine.

INTRODUCTION

African trypanosomiasis, also known as sleeping sickness,has continued to prevail among human and domestic animals in African continent. The disease is caused by a single-celled protozoan parasite belonging to the genus *trypanosoma* and spreadsvia the bite of tsetse-fly vector during feeding.Anaemia, leucopenia and spleenomegalyare important pathological features of human and animal trypanosomiasis (Anosaet al., 1977;Tizard et al., 1978; Anosa, 1988;;) and the release of biochemicals such as proteases (McKerrow et al., 1993; Lonsdale-Eccles and Grab, 2002;), phospholipases (Nok et al., 1993), sialidases (Nok et al., 2003; Nok and Balogun, 2003)e.t.chave been associated with the presence of the parasite *in vivo* and have been demonstrated to play various roles in the pathogenesis of the disease making the management of the disease a herculean task. Infact, it is generally accepted that where treatment is not administered to infected host, the pathologies tend to persist, culminating in 100% fatality of the infected animals (Seed, 2000).

Compare toChagas disease, another protozoan parasitic infectionof South America which is caused by *Trypanosomacruzi*, the chemotherapy of African trypanosomiasis, which is currently the main management approach against the infection, has lagged behind and has been severely limited by several factors includinglimited repertoire of trypanocides, rising costs of orthodox medications, low therapeutic index of synthetic compounds and the growing incidence of drug resistance observed with various species of trypanosome (Onyeyilli and Egwu, 1995; Noket al., 1996; Seed, 2000). Therefore, there is a need to source for drugs that are cheaper, less toxic and are able to effectively reverse lesions accompanying the disease process.

The verse resource in African vegetation could provide the required raw material from where the sourcing of safer and more effective indigenous plant-derived products for the prevention and treatment of African trypanosomiasis can be done. It has been estimated that about 4000 million inhabitants of the world, that is about 80% of world's population, are thought to rely chiefly on traditional medicine, which is largely of plant origin, for their primary health care needs (Farnsworth et al.,1985)and that the medicinal values hidden in local plants in developing countries

are largely untapped (Olayiwola, 1993).

Our previous research efforts at screening *Terminaliaavicennioides* (Guill and Perr), a tropical medicinal herb common to North central vegetation of Nigeria, have revealed the ability of its stem bark aqueous extract to immobilize trypanosome *in vitro*and tosuppress parasitaemia in infected rats (Bulus et al., 2008;Atawodi et al., 2011). This study was designed to investigate the ability of aqueous extract of *Terminaliaavicennioides* to ameliorate some pathological conditions associated with trypanosome infection in experimental rats.

MATERIALS AND METHODS

Plant Collection and Identification

The plant, *Terminaliaavicennioides,* (Guill and Perr) was obtained from Tashan Fulani village in Zaria Local Government of Kaduna State. The plant's identity was confirmed at the Herbarium of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria. Its voucher number is 901452.

Preparation of Aqueous Extract of *Terminaliaavicennioides*

Exactly 200 g of the dried stem bark sample were ground and boiled in 1 litre of distilled water contained in a conical flask for one hour. The extract was thereafter filtered hot and the filtrate was concentrated in a water bath with its temperature set at 50^{o}C. The concentrated extract was finally exposed to air,dried and the extract thereafter stored at 4 ^{o}C until required.

Purchase and Maintenance of Experimental Rats

Fifty white male albino rats (Wistar stock) were purchased from the Department of Pharmacology and Clinical Pharmacy, Ahmadu Bello University, Zaria, Nigeria.The animals were fed on diet specially prepared from chick Grower's mash (Pfizer Company, Nigeria) and were given water *ad libitum* throughout the study period. Animals' weights ranged from 100 g to 200 g just before the commencement of the experiment.

Experimental design for *in vivo*antitrypanosomal Study with crude aqueous extract

Forty-five rats (mixed sexes) were randomly placed into five groups of nine animals each and were given the following treatment:

Group 1(positive control group): rats were infected with about 10^4 T. *bruceibrucei* but were left untreated.

Group 2: Animals in this group were not infected but were intramuscularly (i.m.) given 200 mg/kg b.w. of ATA for six consecutive days.

Group 3: Rats in this group were also infected with about 10^4 of *T. bruceibrucei* but were each treated (i.m.) with standard trypanocide (3.5 mg/kg b.w. single dose of DiminazineAceturate).

Group 4: Here, rats were infected with about 10^4 parasites and thereafter treated (i.m.) with 200 mg/kg b.w. of ATA for six consecutive days.

Group 5: (negative control group): rats in this group were kept uninfected and administered (i.m.) only a placebo (300 µL phosphate buffer saline, PBS) for six consecutive days.

The extract (ATA) was dissolved in 100 mMPBS pH 7.2, for administration to infected animals. Extract administration commenced four days post infection (p.i.) and lasted for six days.Animals in the standard drug group were given single dose of 3.5 mg/Kg b.w. Diminazine Aceturate four days post infecion. Theparasitaemia, Pack cell (PCV) volume, Red Blood cell count (RBC) and Total white blood cell count of all groups were taken from three representative rats per group at seven days interval post infection. Data taken were compared with base line values taken from five healthy rats from the onset of the experiment. Deaths in experimental groups were also observed and recorded.

Determination of Parasitaemia in Infected Rats

Parasites in blood were estimated in accordance with the rapid matching method of Herbert and Lumsden (1976). Briefly, the method employs a matching technique in which microscopic fields were compared with a range of standard logarithmic values. To count the number of parasites in blood, a drop of blood was obtained on slide from animal's tail and covered with a cover slip. The wet mount on the slide was observed under x 400 magnification. The number of trypanosomes per microscopic field was then compared with those of the standard logarithmic table provided by Herbert and Lumsden. The logarithm values which matched the microscopic observation were then converted to antilogarithm, from where the absolute number of trypanosomes per ml of blood was obtained.

Determination of Packed Cell Volume (PCV)

The blood samples from an animal's tail, after sacrifice, were collected into heparinzed capillary tubes. One end of each tube was sealed with plasticine and spun at 2000 *g* for five minutes in a microhaematocrit centrifuge. The packed cell volumes (PCVs) were determined with the aid of a micro-haematocrit reader, which gave the values as percentages (Barbara, 1980).

Red and White Blood Cell Count

The red and white blood cells were counted using standard haemocytometer method of as described by Barbara (1980). Appropriate blood dilutions (1:200 and 1:20 for RBC and WBC respectively) were prepared with respective diluting fluids and few dropsfrom the mixture were used to charged New Improved Neubauer counting chamber using Thomaor Pasteur pipette. The cells were counted under X 400 magnification and estimated through the formulae below:

Red Cells per mm^3 =Number of cells counted in five small squares X dilution factor (i.e. 200) X volume counted (i.e. 50)

White Cells per mm^3 =Number of cells counted in four large squares X correction for volume (i.e. 2.5) X correction for dilution (i.e. 20)

Preparation and Microscopic Examination of ofGiemsa-stained Thin Blood Smear

A drop of blood from rat tail (free of anticoaculant) was collected on the narrow edge of a microscopic slide. This edge is then inclined at an angle on another slide placed on a flat surface. The inclined slide is then pushed along the recumbent one so that the

blood is pulled behind it and spread in a thin layer over the stationary slide (Mulligan, 1970). The thin smear was then dried under fan and is fixed by immersion in methanol for 30 seconds. The residual methanol is thereafter shaken off the slide and the slide was placed face downward in a solution of Giemsastain (diluted, 1:11 with Phosphate buffer, pH 7.2) for 50 to 60 minutes. At the end of the staining period, the slide is removed, washed in a gentle stream of tap water and allowed to dry. The thin film prepared was examined under compound microscope with oil-immersion objective (X 100) lense.

Statistical Analysis

The statistical analyses were carried out using statistical package for social sciences (SPSS- computer package). Percentage organ-body weight ratios and rats' body weights were expressed as mean ± Standard Deviation (SD). Values in all groups were compared using the analysis of variance (ANOVA). For all analyses the level of statistical significance was fixed at $p<0.05$ (Murray, 1992).

RESULTS

The white albino rats were susceptible to experimental infection with the strain of *Trypanosomabruceibrucei* and picked up the infection as early as day 4 post infection. The infection was acute and terminated with 100 % mortality in the infected-but-untreated control groups (Table 1). ATA resulted in significant suppression of parasitaemia as observed by day 14 (p.i.) while parasite were totally eliminated from blood after treatment with diminazine-(Table 1). In addition, Giemsa-stained blood picture of animals treated with the plant extract revealed alteration of morphology (Fig. 2). Anaemia was observed to accompany infection due to trypanosma specie employed. This was revealed by drop in pack cell volume and red cell count (Table 2 and Fig. 1, respectively). Data from Tables 1 and 2, showed that the anaemia was severe at peak parasitaemia, i.e. at day 14 p.i., (Fig. 1). Leucopenia appeared in infected rats on the seventh day after inoculation and appeared to be sustained all through the study period. Figure 3 shows leucopenia during experimental infection. The percent organ-body weight ratios of Kidneys, spleen and liver (Tables 3) showed significant ($P<0.05$) enlargement of only the spleen. The study showed that both the liver and the kidneys, when compared with those of negative control rats, were not greatly affected by infection.

Table 1. Effect of 6 days treatment (i.m.) with 200 mg/kg b.w. of aqueous extract of *Terminaliaavicennioides*(stem bark) on the Parasitaemia of *T. bruceibrucei*-infected rats

Days post infection	Number of trypanosomes per ml of blood (X 10^6)				
	1*	2	3	4	5
0	0.00±0.00	0.00	0.00	0.00±0.00	0.00
7	(25.2±1.20)^a	0.00	(30.70±18.50)^a	(63.2±13.2)^a	0.00
14	(474.00±183.00)^a	0.00	(2.65±4.58)^b	0.00	0.00
28	Dead	Dead	Dead	(7.92±11.20)^c	0.00
Treatment outcome	0/3	0/0	2/3	3/3	0/0

*Treatment groups: Group 1 (infected but not treatment); 2 (not infected but treated with 200 mg/kg b.w. of Terminaliaavicennioides aqueous extract for 6 days); 3 (infected and treated with 200 mg/kg b.w. of aqueous extract of Terminaliaavicennioides for 6 days); 4(infected and treated with 3.5 mg/kg b.w. of Diminazine; 5(not infected and untreated).Values (expressed as mean ± SD) were compared btween groups; values with different superscript indicate significant difference ($p<0.5$).

Table 2. Effect of 6 days treatment (i.m.) with 200 mg/kg b.w. of crude aqueous extract of *Terminaliaavicennioides*(stem bark) on Packed cell Volume (PCV) in rats infected with *T. bruceibrucei*.

Days post infection	PCV (%)				
	1*	2	3	4	5
0	43.30 ±4.64$^{a\,\Psi}$	43.40 ±4.64^b	43.33 ±4.64^c	43.30 ±4.64^d	43.30 ±4.64^e
7	39.00 ±5.57^a	39.33 ±9.45^b	40.67 ±2.31^c	32.67 ±7.51^d	47.67 ±4.04^e
14	36.00 ±2.00^a	45.00 ±1.40^b	36.50 ±1.63^c	40.50 ±4.95^d	38.00 ±2.65^e
28				40.00 ±11.27^d	42.67 ±2.52^e

* Treatment groups: Group 1 (infected but not treated); 2(not infected but treated with 200 mg/kg b.w. of ATA for 6 days); 3 (infected and treated with 200 mg/kg b.w. of ATA for 6 days); 4 (infected and treated with 3.5 mg/kg b.w. of Diminazineaceturate); 5 (not infected and not treated).

Ψ Values (Mean±SD) were compared between days. Same superscript means no significant difference ($p>0.05$)

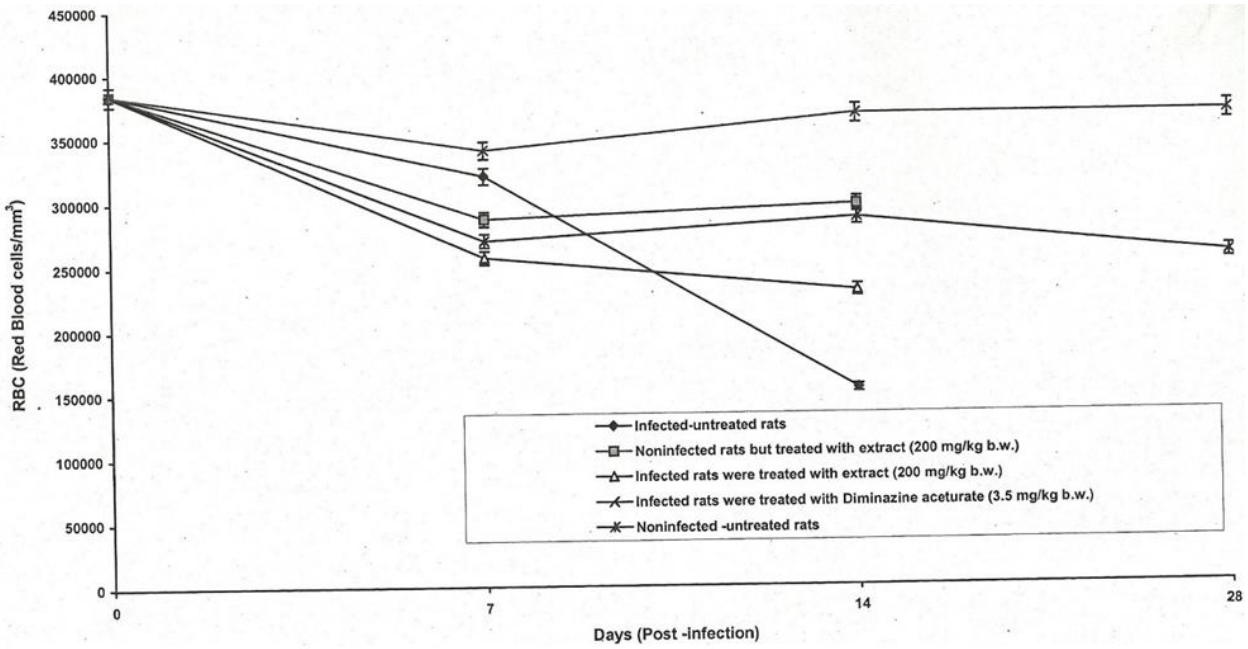

Figure 1. Efffect of i.m. treatment with aqueous extract of *Terminaliaavicennioides* on Red blood cell count of rats experimentally infected with *T. bruceibrucei*

(a) **(b)** **(c)**

Fig. 2 Photographs depicting Giemsa-stained blood film of rats infected with *T. brucei brucei* (arrowed): (a) before treatment (b) after six days treatment with 200 mg/kg b.w. of aqueous extract of *Terminalia avicennioides*. Note also the distortion of parasite morphology. (c) after treatment with 3.5 mg/kg b.w. of Diminazine aceturate.

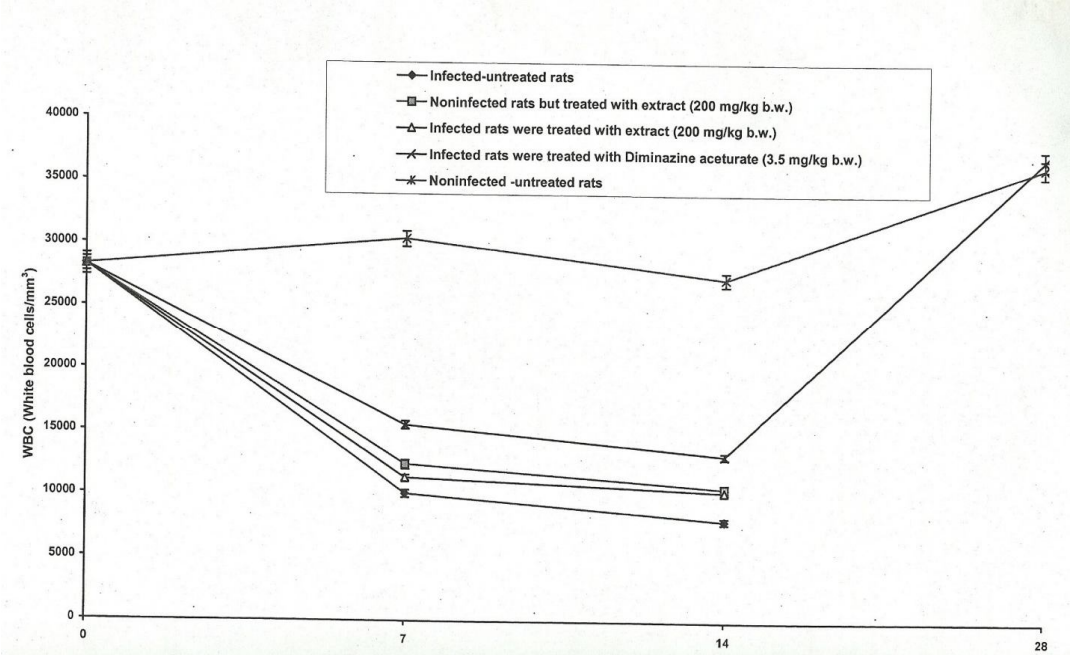

Fig. 3. Efffect of i.m. treatment with aqueous extract of *Terminaliaavicennioides* on white blood cell count of rats experimentally infected with *T. bruceibrucei*

Table 3. Effect of six days treatment (i.m.) with 200 mg/kg b.w. of aqueous extract of Terminaliaavicennioides (stem bark) on percent organ-body weight ratios in rats infected with T. bruceibrucei

Organs	Days (post Infection)	Organ/body weight (%)				
		1*	2	3	4	5
Liver	0			3.184 ±0.333[f]		
	7	3.206[c] ±0.964	2.347[c] ±0.295	3.634[b] ±0.435	2.679[a] ±0.962	2.777[a] ±0.442
	14	4.193[b] ±0.242	2.871[b] ±0.943	4.188[b] ±0.420	3.436[b] ±0.722	3.051[b] ±0.754
	28				3.729[c] ±0.671	3.679[c] ±0.124
Kidneys	0			0.313 ±0.040		
	7	0.320[a] ±0.840	0.308[a] ±0.064	0.340[a] ±0.540	0.291[a] ±0.510	0.313[a] ±0.029
	14	0.301[a] ±0.021	0.426[c] ±0.037	0.428[c] ±0.037	0.352[b] ±0.048	0.293[a] ±0.015
	28				0.317[a] ±0.010	0.309[a] ±0.024
Spleen	0			0.459 ±0.081		
	7	1.230[b] ±0.376	0.262[a] ±0.033	1.221[b] ±0.314	0.976[b] ±0.490	0.421[a] ±0.078
	14	1.659[b] ±0.532	0.318[a] ±0.081	0.805[a] ±0.188	0.530[a] ±0.229	0.354[a] ±0.105
	28	Dead	Dead	Dead	1.382[a] ±1.018	0.356[a] ±0.013

* Treatment grroups: Group1 (infected but not treated); Group2 (not infected but treated with 200 mg/kg b.w. of *Terminaliaavicennioides* extract for six days); Group 3 (infected and treated with 200 mg/kg b.w. of *Terminaliaavicennioides*extract for six days); Group 4 (infected and treated with 3.5 mg/kg b.w. of Diminazineaceturate); Group 5 (not infected and not treated).

t Values (mean±SD) were compared between groups (within a row). Different superscripts in the same row differed significantly (P<0.05).

DISCUSSION

Previous *in vitro* and *in vivo* studies with *Terminaliaavicennioides* (Guill and Perr), a tropical medicinal herb common to North central vegetation of Nigeria, revealed that its extracts are able to immobilize trypanosome and to suppress parasitaemia in infected rats (Bulus et al., 2008; Atawodi et al., 2011). The study is part of the in vivo research and it aimed at investigating the ability of the aqueous extract of the plant's stem bark (ATA) to alleviate some pathological conditions in rats experimentally infected with *Trypanosomabruceibrucei.*

Treatment of infected rats with the ATA extract resulted in significant suppression of parasitaemia as observed by day 14 (p.i.) even though this is not as good as the complete elimination of parasite from animal blood seen with theDiminazineAceturate-treated group (Table 1). In addition, Giemsa-stained blood picture revealed alteration of morphology (Fig. 2) of residual parasite found after extract treatment. Aqueous extract of *Terminaliaavicennioides* has been previously shown to be rich in saponin (Bulus, et al., 2008), a phytochemical known to have a strong surface action on erythrocyte membrane (Godwin and Theodore, 2001). It is possible that ATA exerts its inhibitory effect via interaction with parasite membrane,as supported by changes inparasite external structure (Fig. 2).

The occurrence of anaemia during the infection as revealed by drop in pack cell volume and red cell count (Table 2 and Fig. 1, respectively) is a common feature of both human and animal trypanosomiasis (Tizard, 1978; Esievo, 1983; Nok et al., 1993). Data from Tables 1 and 2, showed that anaemia(as shown by PCV values, Fig. 1) were severe at peak parasitaemia (day 14 p.i.). The inverse relationbetween PCV and paraistaemia agrees with previous studies (Fiennes, 1954; Jenkins et al., 1980) and several theories have already been put forward to explain the occurrence of anaemia in trypanosomiasis, some of which include the enzymatic rolesof phospholipases and sialidases (Mellors and Samad, 1989; Esievo, 1983; Nok et al., 1993). Infected rats that were treated with ATA had significantly (P<0.05) higher PCV values than those in the positive control (infected-untreated) group indicating improvement of PCV with ATA-treatment. Again, the possibility of haemolytic action by the ATA extract was tested by administering the extract to healthy rats for the same period of treatment. The mean PCV of healthy ratstreated strictly with extract (Table 2) were found to be slightly lower than that of uninfected group which have not received the extract nor the standard drug, thus supporting the presence of haemolytic substance in the extract.

Leucopenia appeared in infected rats on the seventh day after inoculation and appeared to be sustained all through the study period. Figure 3 shows leucopenia during experimental infection. Leucopenia has been reported in rabbits infection with *T. brucei* and *T. congolense* (Iliyasu, 2000), *T. brucei* infection in mice (Anosa, 1980) and Deer mice infected with *T. brucei* (Anosa and Kaneko, 1983). The sustained leucopenic state associated with period of high parasitaemia (day 7-14 p.i.) is in agreement with the findings of Wellde and his colleagues (1974) and has been attributed to lymphocytosis, neutrophilia, eosinopenia, basophilia and monocytosis (Anosa, 1988). Extract administration to infected rats seems to have initiated the process of alleviating the observed leucopenia even though the animals did not stay alive long enough for white cells to be counted on the 28th day after infection. This alleviation can be attributed, in part,to the observed lowering of systemic blood parasites.

The percent organ-body weight ratios of Kidneys, spleen and liver (Tables 3) showed significant (P<0.05) enlargement of only the spleen. Though hepatomegaly has been reported in deer mice infection of *T.brucei* (Anosa and Kaneko,1983), the studyshowed that both the liver and the kidneys, when compared with those of negative control rats, were not greatly affected by infection. Spleen enlargement, which is the main observed organ pathology, and which has been associated with sequestration of erythrocytes by spleen during infection (Anosa et al ., 1977), was found to improve after ATA treatment (Table 3).

Single dose administration of the extract even beyond5000 mg/kg b.w. was previously found to be safe to experimental animals (Bulus et al., 2011). However, continuous administration ofhigher doses to rats could present signs of toxicity (Bulus et al., 2011). The death of rats in the ATA-treated group may partly be accounted for by possible toxicity posed by consecutive administration of extract. Of particular importance is the route of administration of the extract; Extract administered orally may be exposed to the action of gastrointestinal enzymes which could reduce their toxic potential. The present study, however, employed intramuscular route since it allows for direct and faster extract delivery.

In conclusion, considering the phytochemical nature of ATA extract as previously observed, and the death seen in ATA-treated rats, it is easier to think that effective usage of *Terminaliaavicennioides* extract in traditional treatment of African trypanosomiasiscould be as part of component of combine herbal preparation. This is actually the common traditional practice in Northern Nigeria (Atawodi et al 2002). Currently,we are focusing on the possible isolation of saponinsfron*Terminaliaavicennioides* and other related plants of the *Terminalia* family, with the hope that their antitrypanosomal efficacy would be subsequently ascertained.

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