JOURNAL OF VETERINARY AND APPLIED SCIENCES
2019 VOL. 9 (2): 102 - 111
Manuscript No. JVAS/2018/051; Received: 13/09/2018; Accepted: 01/11/2019
Published by: Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria

EFFICACY OF *Alstonia boonei* STEMBARK EXTRACT AND DIMINAZENE ACETURATE IN MICE EXPERIMENTALLY INFECTED WITH *Trypanosoma brucei*

Damian A. Arua[1], Nwakaego E. Nweze[1*], Aruh O. Anaga[2], Boniface M. Anene[1]

[1]Department of Veterinary Medicine, [2]Department of Veterinary Physiology and Pharmacology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Enugu State, Nigeria.

==========

ABSTRACT

The antitrypanosomal efficacy of Alstonia boonei and its combination with diminazene aceturate was assessed using mice experimentally infected with Trypanosoma brucei. An acute toxicity test to determine the lethal dose of the aqueous extract was carried out. Adult mice (126) of either sex were used. The study was conducted in three experiments each using 36 mice divided into 6 groups of 6 mice each. Group 1 was uninfected control while groups 2 to 6 were inoculated intraperitoneally with 1 x 10^6 trypanosomes. In experiment 1, mice in groups 3 to 6 were treated respectively, with 40, 80, 160 and 320 mg/kg body weight of the extract while group 2 was untreated. In experiment 2, groups 5 and 6 were treated with 3.5 and 7 mg/kg body weight of diminazene aceturate respectively. In experiment 3, combinations of diminazene aceturate and A. boonei at doses of 3.5 + 80 mg/kg and 7 + 40 mg/kg respectively, were administered. Parasitaemia, body weight and packed cell volume were monitored. Significant reductions in parasitaemia were achieved in A. boonei extract treated mice when compared with control. Diminazene aceturate (7 mg/kg) cleared blood parasites 24 to 48 h post treatment. Combination of A. boonei and diminazene aceturate cleared parasites from blood of treated mice without relapse. Combination of diminazene aceturate with A. boonei in treatment of trypanosome infection of mice appeared to improve the outcome of treatment.

Keywords: *Alstonia boonei*, Diminazene aceturate, *Trypanosoma brucei*, Mice, Combination therapy.

==========

INTRODUCTION

Trypanosomosis is a parasitic disease caused by species of flagellated protozoan parasites belonging to the genus *Trypanosoma*, which inhabit blood plasma and various body tissues and fluids of hosts [1]. The disease is transmitted mainly by tsetse flies (*Glossina spp*). Important trypanosome species that cause disease in livestock include *Trypanosoma brucei, T. vivax, T. congolense, T. simiae* and *T. evansi* .

The disease is generally an acute to chronic infection characterized by anaemia, weight loss, reduced milk yield, impairment of immune function, reproductive disorders and death if appropriate treatment is not

***Correspondence: E-mail: nwakaego.nweze@unn.edu.ng; Tel: +2348054132599**
ISSN: 2315 – 6856; e-ISSN: 2636 – 5553; *Website: www.jvasonline.com*

instituted [2]. Animal trypanosomosis is rated the most devastating and widespread disease in Africa retarding the growth of the livestock industry in a third of the continent [3].

Use of trypanocidal drugs such as diminazene aceturate, isometamidium chloride and homidium salts is the principal method of control for animal trypanosomosis in most African countries, in the absence of a vaccine against the disease [4]. These drugs have been in use for decades with no new trypanocides being introduced to replace them [5]. In the past 35 years, only cymelarsan and eflornithine are new commercially available trypanocides [6]. The former is licensed for use in camel and buffalo trypanosomosis caused by *T. evansi* while the latter is associated with toxicity and prohibitive cost. The prolonged use and misuse of the few available trypanocides have resulted in the emergence of drug-resistant parasites [5] which is a serious problem in the control of trypanosomosis.

In an attempt to discover more effective, less toxic and cheaper trypanocides, research interest in plants for their antitrypanosomal activities is currently gaining prominence [7, 8, 9]. For instance, aqueous extracts of *Acalypha hispida* leaves, *Alstonia boonei* bark, *Annona senegalensis* root, *Morinda lucida* leaves and *Picralima nitida* seeds have been tested for their *in vivo* trypanocidal activity in rodents infected with *Trypanosoma brucei* [10, 11, 12] and were found to possess some antitrypanosomal potentials. Nweze et al [13] showed that crude ethanolic extracts of *Buchholzia coriacea* exhibited antitrypanosomal activity against *T. brucei* but not *T. congolense* in experimental infection in mice.
Alstonia boonei De wild is a large deciduous evergreen tree belonging to the family Apocyanaceae and is widely distributed in the continents of Africa, Asia and America [14]. *Alstonia boonei* is known as 'Egbu-ora' in Igbo language. The stem bark has been reported to possess anti-inflammatory, analgesic and antipyretic activities [15]. The trypanocidal activity of *A. boonei* has been investigated and its stem bark decoction reduced the level of parasitaemia in mice suffering from trypanosomosis [16].

The use of increased dosages, sanative pairs and combination therapy has been proposed as a rational strategy for optimizing the usefulness of the relatively old and existing trypanocides [17]. The efficacy of combination therapy lies in the synergistic effects of the drugs. In this work, *A. boonei* extract was combined with diminazene aceturate to see if there will be improved antitrypanosomal efficacy in mice experimentally infected with *T. brucei*.

MATERIALS AND METHODS

Experimental animals

One hundred and twenty-six (126) out-bred albino mice of either sexes with an average weight of 16 g were used for the study. They were obtained from the Laboratory Animals Unit of the Department of Veterinary Medicine, University of Nigeria, Nsukka and kept in clean cages in a fly-proof animal house. They were allowed 14 days to acclimatize to their new environ before the commencement of the experiments. Feed (pelleted grower's mash) and water were provided *ad libitum*. Blood samples were collected from the median canthus of the eyes and screened for the presence of trypanosomes.

Trypanosomes

Trypanosoma brucei used was a field strain originally isolated from a pig presented for slaughter at the Nsukka Municipal Abattoir. The parasites were maintained in laboratory mice from where experimental animals were first inoculated. The parasites were identified in the Department of Veterinary Parasitology and Entomology, University of Nigeria, Nsukka using their morphological characteristics [18].

Collection of plant materials

Stem barks were cut from *Alstonia boonei* tree at Owerre-Eze Orba in Udenu Local Government Area of Enugu State, Nigeria. Samples of the stem bark and branches of the tree were taken to the International Centre for Ethnomedicine and Drug Development (Inter CEDD) Aku Road, Nsukka where they were identified and authenticated.

Extraction of stem bark
Dried stem barks of *A. boonei* were ground to a coarse powder using a milling machine. The coarse powder was sieved to obtain fine powder that was soaked in distilled water for 48 hours and filtered with Whatman filter paper to obtain an amber-coloured frothy filtrate with a characteristic pleasant odour. The concentration of the filtrate was determined by placing 1 ml on a previously weighed clean watch glass and evaporating it to a constant weight. The difference in the weight of the watch glass was taken as the concentration of the extract per mL

Acute toxicity test
Acute toxicity test was performed according to Miller and Gregory [19]. Eighteen mice were randomly allotted into 6 groups (1 – 6) of 3 mice each. Six different doses (5000, 2500, 1250, 625, 312.5 and 156.25 mg/kg) were respectively administered intraperitoneally (i.p.) to the six groups and then observed for signs of toxicity during a period of 48 hours. Percentage mortality was computed and plotted against the log dose of the extract to determine the lethal dose (LD_{50}).

Experimental design
Thirty-six mice each were used for the three experiments. Experiment 1 studied the effect of administering graded doses (40, 80, 160 and 320 mg/kg body weight) of *A. boonei* for 3 consecutive days, starting from day 6 PI, in mice experimentally infected with *T. brucei*. Experiment 2 studied the effect of single treatment with different doses of diminazene aceturate (3.5 and 7 mg/kg body weight) from day 6 PI in mice experimentally infected with *T. brucei* while experiment 3 was on the combined effects of *A. boonei* and diminazene aceturate, at the stated dose regimens above (3.5 + 160 mg/kg and 7.0 + 320 mg/kg), on *T.brucei* infection in mice. Parameters used to assess therapeutic efficacy were parasitaemia, packed cell volume (PCV), live body weight and mortality rate.

Parasitaemia was monitored daily by examination of tail blood by wet mount and buffy coat methods [20]. Levels of parasitaemia were estimated using the rapid matching method [21]. The mice were weighed using an electronic weighing balance. The microhaematocrit centrifugation method was used for the determination of the PCV [22].

Statistical analysis
Data generated were analyzed by one-way analysis of variance (ANOVA) using the SPSS software package. Variant means were separated using Duncan's multiple range tests. Significance was accepted at 5 % level of probability.

RESULTS
An LD_{50} value of 668.5 mg/kg body weight was obtained for *A. boonei* in mice.
The results of parasitaemia in mice treated with the extract alone are presented in Table 1. Parasitaemia was first detected in the infected mice between 2 and 6 days post infection (pi). Parasitaemia was sustained in infected mice with mortality of 66.7 % by day 28 pi in infected untreated control group. Following treatment with the aqueous extract of *A. boonei*, there was a relative but varied clearance of parasites (aparasitaemia) from the blood of treated mice up to 8 days pi when parasite clearance was 100, 83, 67 and 50 % in the groups treated with 0.9 mg/kg, 1.8 mg/kg of the extract or 3.5 mg/kg and 7.0 mg/kg of diminazene aceturate respectively (Table 1). Mortality was 67, 67, 50, 67 and 83 % in the infected/untreated group and those treated with 0.9 mg/kg, 1.8 mg/kg of the extract and with diminazene aceturate at 3.5 mg/kg and 7.0 mg/kg respectively, by day 28 PI.

Table 1: Parasitaemia of *T. brucei* infected mice treated with varying doses of *A. boonei* extract and diminazene aceturate

Days post infection	Experimental groups							
	Uninf/unt	**Inf/unt**	**Inf/ext40**	**Inf/ext80**	**inf/ext160**	**inf/ext320**	**inf/da3.5**	**inf/da7**
0	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
2	0/6	0/6	0/6	0/6	0/6	1/6	0/6	0/6
3	0/6	3/6	2/6	2/6	4/6	4/6	0/6	1/6
6*	0/6	6/6	6/6	6/6	6/6	6/6	0/6	2/6
7	0/6	5/5	3/6	5/6	5/6	4/6	4/6	4/6
8	0/6	5/5	0/6	1/6	2/6	3/6	4/6	4/6
9	0/6	5/5	4/6	2/6	3/5	3/6	4/6	4/6
10	0/6	5/5	5/6	5/6	3/4	5/6	0/6	0/6
11	0/6	5/5	6/6	3/6	3/4	3/6	0/6	0/6
12	0/6	5/5	5/6	3/6	3/4	6/6	0/6	0/6
15	0/6	5/5	6/6	5/6	4/4	5/5	1/6	0/6
16	0/6	5/5	6/6	5/6	4/4	5/5	0/6	0/6
17	0/6	5/5	5/6	5/5	4/4	4/4	0/6	0/6
20	0/6	4/4	4/6	4/4	2/2	4/4	1/6	0/6
23	0/6	3/3	4/6	4/4	2/2	3/3	1/6	0/6
28	0/6	2/2	2/6	3/3	2/2	1/1	1/6	1/6

* Commencement of treatment; Numerator = number of infected mice with parasitaemia; Denominator = total number of mice in a group.

On the other hand, following treatment with diminazene aceturate parasites were cleared from the blood by 10 days pi in the 3.5 and 7 mg/kg body weight groups. There was no mortality in the group treated with diaminazene aceturate at 3.5 mg/kg while a mortality of 16.7 0 % was recorded in the 7.0 mg/kg treatment group.

The results of parasitaemia in mice treated with the combination of *A. boonei* extract and diminazene aceturate are presented in Table 2. Parasites were cleared from blood of all treated mice by 9 and 8 days pi in the groups treated with 3.5 mg/kg diminazene + 80 mg/kg extract and 7.0 mg/kg diminazene + 40 mg/kg extract respectively. No relapse parasitaemia was recorded within the 28 days observation period in surviving animals for all groups but mortalities of 50 and 66.7 % occurred in groups treated with 3.5 mg/k Diminazene + 80 mg/kg extract and 7.0 mg/kg diminazene + 40 mg/kg extract respectively.

The results of PCV are presented in Tables 3 and 4. The PCV of the infected untreated mice was significantly ($p<0.05$) lower than that of the control by day 14 pi. Following treatment with *A. boonei*, no significant ($p>0.05$) difference occurred between PCV of uninfected and treated groups by day 14 pi except in group treated with 160 mg/kg of the extract that had a significantly ($p<0.05$) lower PCV. From 21 to 28 days pi, PCV was significantly ($p<0.05$) lower in extract treated groups compared to the uninfected/untreated control. Treatment with diminazene aceturate alone resulted in significant ($p<0.05$) improvement in PCV of mice on day 14 pi which was comparable to that of control by day 21 pi (Table 3). After treatment with combination of extract and diminazene aceturate no significant ($p>0.05$) difference in PCV was detected on day 14 pi between the groups. On day 28 pi, PCV was significantly ($p<0.05$) lower in the infected/untreated groups and that treated with diminazene aceturate at 3.5 mg/kg but was not significantly ($p>0.05$) different in the other treatment groups when compared with the uninfected/untreated control.

Tables 5 and 6 show the mean body weight, respectively, of infected mice treated with *A. boonei* alone and combinations of diminazene aceturate plus *A. boonei*. Following treatment with *A. boonei* extract, there was no significant ($p>0.05$) difference in mean body weight of all the groups. Also, after treatment with combinations of *A. boonei* and DA, body weights were similar in all groups except that it was significantly ($p<0.05$) lower in group 2 when compared to group 6 on day 21 pi (Table 6).

DISCUSSION

Parasitaemia with *T. brucei* infection was established 2 to 6 days post inoculation. The infections had relatively short prepatent periods and ran acute courses with parasitaemia terminating in death of infected mice. These findings agree with the report of Kubata et al. [23] who reported a similar prepatent period of 2 days and others [5, 24, 25] who reported a prepatent period of 3 to 8 days. Immune status of animals, infective dose and parasite strains [23] may be responsible for length of prepatent period.

Trypanocidal effects were observed as significant reductions in parasitaemia on administration of aqueous extract of *A. boonei* at the tested doses. For unknown reasons, perhaps toxicity, duration of parasite clearance was inversely proportional to the dose. At 40 mg/kg body weight, time of parasite clearance was shortest while at 320 mg/kg body weight, it was longest. Mortality rate of 50 – 83 % in treated mice was comparable to that of the infected untreated control (67 %).

Treatment with diminazene aceturate alone eliminated the parasites in a dose-dependent manner. The combinations of extract and diminazene aceturate appeared to produce some trypanocidal action as there were no post treatment relapses within the 28 day experimental period. This could be due to the combined trypanocidal activities of diminazene aceturate and *A. boonei*. However, higher mortalities (33.3 – 66.7 %) occurred in treated groups when compared with the infected untreated control.

Table 3: Mean packed cell volumes (%) ± SE of *T. brucei* infected mice treated with *A. boonei* extract and diminazene aceturate

Days	Experimental groups							
	Control Uninf/unt	**Control Inf/unt**	**Extract Inf/40 mg/kg**	**Extract Inf/80 mg/kg**	**Extract inf/160 mg/kg**	**Extract inf/320 mg/kg**	**Da inf/3.5 mg/kg**	**Da inf/7.0 mg/kg**
0	$49.3^{a} \pm 1.58$	$50.3^{a} \pm 1.38$	$51.5^{a} \pm 0.89$	$50.8^{a} \pm 1.25$	$50^{a} \pm 1.37$	$51.67^{a} \pm 2.03$	$45^{a} \pm 2.35$	$46.67^{a} \pm 0.88$
7	$36.3^{a} \pm 2.59$	$36.6^{a} \pm 2.06$	$34.2^{a} \pm 2.34$	$33.2^{a} \pm 1.14$	$33.3^{a} \pm 3.69$	$39.5^{a} \pm 1.52$	$42.5^{a} \pm 1.03$	$42.5^{a} \pm 1.57$
14	$35.2^{a} \pm 2.30$	$26^{b} \pm 1.48$	$32.7^{ab} \pm 2.60$	$32.8^{ab} \pm 1.22$	$29.0^{bc} \pm 1.58$	$32.3^{ab} \pm 1.28$	$46.75^{b} \pm 1.8$	$49.4^{bc} \pm 2.11$
21	$40.2^{a} \pm 2.04$	$29.6^{bc} \pm 1.33$	$31.7^{bc} \pm 1.89$	$33.8^{b} \pm 1.30$	$29.3^{bc} \pm 1.60$	$28.2^{c} \pm 1.77$	$49.8^{c} \pm 2.04$	$44.8^{bc} \pm 1.39$
28	$41.7^{a} \pm 0.84$	$32.5^{b} \pm 0.50$	$29.0^{b} \pm 2.17$	$31.5^{b} \pm 2.02$	$35.5^{ab} \pm 2.50$	$28.7^{b} \pm 2.96$		

^{abc}Means with different superscripts in the same row are significantly difference (p<0.05); Da = Diminazene aceturate.

Table 4: Mean packed cell volumes (%) ± SE of *T. brucei* infected mice treated with combinations of diminazene aceturate and *A. boonei* extract

Days	Experimental groups			
	Uninfected/untreated	**Infected/untreated**	**80 mgAb+3.5 Da mg/kg**	**40 extract+7.0 Da mg/kg**
0	$49.3^{a} \pm 1.58$	$53.2^{a} \pm 1.66$	$54.7^{a} \pm 2.48$	$52.0^{a} \pm 0.93$
7	$36.3^{a} \pm 2.59$	$41.0^{b} \pm 1.38$	$39.8^{ab} \pm 1.99$	$42.3^{b} \pm 2.06$
14	$35.2^{a} \pm 2.30$	$32.6^{a} \pm 1.08$	$33.0^{a} \pm 1.79$	$33.8^{a} \pm 1.72$
21	$40.2^{a} \pm 2.04$	$32.2^{b} \pm 1.20$	$33.8^{ab} \pm 1.62$	$37.3^{ab} \pm 2.75$
28	$41.7^{a} \pm 0.84$	$33.0^{bc} \pm 1.68$	$29.3^{c} \pm 1.86$	$36.0^{ab} \pm 0.00$

Ab = *A. boonei*; Da = Diminazene aceturate; ^{abc}Means with different superscripts in the same row are significantly difference (p<0.05)

Table 5. Mean body weight (g) ± SE of *T. brucei* infected mice treated with various doses of *A. boonei* extract or diaminazene aceturate.

Days	Experimental groups							
	Control Uninf/unt	Control Inf/unt	Extract Inf/40 mg/kg	Extract Inf/80 mg/kg	Extract inf/160 mg/kg	Extract inf/320 mg/kg	Da inf/3.5 mg/kg	Da inf/7.0 mg/kg
0	$24.8^{a} \pm 1.99$	$28.8^{a} \pm 1.87$	$25.2^{a} \pm 2.06$	$27.0^{a} \pm 1.66$	$25.6^{a} \pm 2.24$	$27.0^{a} \pm 1.66$	22.8 ± 1.05	22.0 ± 1.28
7	$25.7^{ab} \pm 2.40$	$31.6^{c} \pm 1.96$	$26.7^{abc} \pm 0.47$	$31.2^{bc} \pm 1.31$	$24.9^{a} \pm 1.98$	$29.2^{abc} \pm 1.84$	23.6 ± 1.50	22.4 ± 1.25
14	$25.9^{a} \pm 2.47$	$31.3^{a} \pm 2.13$	$26.8^{a} \pm 1.91$	$30.1^{a} \pm 1.49$	$25.8^{a} \pm 2.78$	$27.1^{a} \pm 1.65$	23.7 ± 1.52	23.0 ± 1.45
21	$25.2^{a} \pm 2.35$	$30.9^{a} \pm 2.16$	$23.4^{a} \pm 2.33$	$27.9^{a} \pm 1.48$	$26.0^{a} \pm 3.26$	$28.0^{a} \pm 1.41$	$23.1^{ab} \pm 1.21$	$20.9^{a} \pm 1.70$
28	$24.0^{a} \pm 2.10$	$32.1^{a} \pm 2.10$	$24.6^{a} \pm 2.06$	$24.1^{a} \pm 2.03$	$30.8^{a} \pm 5.30$	$24.3^{a} \pm 2.8$	21.2 ± 1.67	23.0 ± 1.55

^{abc}Means with different superscripts in the same row are significantly difference ($p<0.05$); Da = Diminazene aceturate.

Table 6. Mean body weights (g) ± SE of *T. brucei* infected mice treated with combinations of diminazene aceturate and *A. boonei* extract

Days	Experimental groups			
	Uninfected/untreated	Infected/untreated	80 mgAb+3.5 Da mg/kg	40 extract+7.0 Da mg/kg
0	24.8 ± 2.00	25.3 ± 1.74	22.8 ± 1.05	22.0 ± 1.28
7	25.7 ± 2.40	27.4 ± 2.00	23.6 ± 1.50	22.4 ± 1.25
14	25.9 ± 2.47	27.4 ± 2.03	23.7 ± 1.52	23.0 ± 1.45
21	$25.2^{ab} \pm 2.35$	$27.1^{b} \pm 2.53$	$23.1^{ab} \pm 1.21$	$20.9^{a} \pm 1.70$
28	24.0 ± 2.01	24.4 ± 3.14	21.2 ± 1.67	23.0 ± 1.55

Ab = *A. boonei*; Da = Diminazene aceturate; ^{abc}Means with different superscripts in the same row are significantly difference ($p<0.05$).

Treatment with diminazene aceturate alone eliminated the parasites in a dose-dependent manner. The combinations of extract and diminazene aceturate appeared to produce some trypanocidal action as there were no post treatment relapses within the 28 day experimental period. This could be due to the combined trypanocidal activities of diminazene aceturate and *A. boonei*. However, higher mortalities (33.3 – 66.7 %) occurred in treated groups when compared with the infected untreated control.

Trypanosoma brucei infection resulted in significant ($p<0.05$) progressive reduction in PCV of infected mice from day 14 PI. This steady decline in PCV is an indication of anaemia. This finding is similar to those of other workers [26, 27, 28, 29, 30]. Anaemia is a consistent finding in trypanosomosis and has been attributed to accelerated destruction of red blood cells through phagocytosis by activated macrophages [31, 32].

Administration of *A. boonei* significantly ($p<0.05$) improved PCV to the level of the uninfected control by day 8 post treatment but was not sustained probably due to recrudescence of parasitaemia after initial reduction. Treatment with diminazene aceturate alone, improved PCV significantly ($p<0.05$) up to the level of the uninfected control group. This was due to the trypanolytic effect of diminazene aceturate. Combinations of diminazene aceturate and *A. boonei* extract at levels of 7 mg/kg DA + 40 mg/kg extract and 3.5 mg/kg DA + 80 mg/kg extract improved PCV to the level of the control. The observed red blood cell improvement was clearly superior to that of extract treatment alone but was similar to that of diminazene treatment. This improvement in PCV recorded was due to clearance of parasites in blood by these combinations.

Administration of aqueous extract of *A. boonei* did not lead to any significant variations in body weights of mice perhaps because the infection ran an acute course. In conclusion, combination of aqueous stem bark extract of *A. boonei* with diminazene aceturate cleared parasitaemia within the 28 day experimental period without relapse in *T. brucei* infected mice.

REFERENCES

1. Hutchinson, O. C., Picozzi, K., Jones, N. G., Mott, H., Sharma, R., Welburn, S. C., Corrington, M. (1996). Variant surface glycoprotein gene repertoires in *Trypanosoma brucei* have diverged to become strain specific. *BMC Gemenomics*, 8: 234.
2. Blom-Potar, M. C., Chamond, N., Cosson, A., Jouvion, G., Bergere, S. D., Huerre, M., Minoprio, p. (2010) *Trypanosoma vivax* infections: Pushing ahead with mouse models for the study of *nagana* immunobiological dysfunctions. *PLOS Neglected Tropical Diseases,* 4: (8) e793 DOI 10.1371.
3. Omotainse, S. O., Kalejaiye, J. O., Dede, P. M., Dadah, A. J. (2004). The current status of tsetse and animal trypanosomiasis in Nigeria.. *Vom Journal of Veterinary Science*. 1 (1): 1 – 7.
4. Barrett, M. P., Boykin, D. W., Tidwell, R. R. (2007) Human African trypanosomiasis: Pharmacological re-engagement with a neglected disease. *British Journal of Pharmacology,* 152 (8): 1155 – 1171.
5. Anene, B. M., Ifebigh, A. O., Igwilo, I. A., Umeakuana, P. U. (2011) Prevalence and haematobiochemical parameters of trypanosome infected pigs at Nsukka, Nigeria. *Comparative Clinical Pathology*, 20: 15 – 18.
6. Bacchi, C. J., Nathan, H. C., Livingston, T., Valladiminazeneres, G., Saric, M., Sayer, P. D., Njogu, A. R., Clarkson, A. B. (1990). Differential sensitivity of D-alpha-difluoromethylornithine in clinical isolates of Trypanosoma brucei rhodesiense *Antimicrobial Agents Chemotherapy* 34: 1183 – 1188.

7. Atawodi, S. E., Bulus, T., Ibrahim, S., Ameh, D. A., Nok, A. J., Mamman, M, Galadima, M. (2003). In vitro trypanocidal effect of methanolic extract of some Nigerian savannah plants. *Afrcan Journal of Biotechnology*, 2 (9): 317 – 321.
8. Mann, A., Ifarajimi, O. R,. Adewoye, A. T., Ukam, C., Udeme, E. E., Okorie, I. I., Sakpe, M. S., Ibrahim, D. R., Yahaya, Y. A., Kabir, A. Y., Ogbadoyi, E. O. (2011). In vivo antitrypanosomal effects of some ethnomedicinal plants from Nupeland of North Central Nigeria *African Journal of Traditional Complementary and Alternative Medicines*, 8 (1): 15 – 21.
9. Mbaya, A. W., Ibrahim, U. I. (2011). In vivo and in vitro activities of medicinal plants on haemic and humoral trypanosomes. *International Journal of Pharmacology*, 7 (1): 1 – 11.
10. Igwe, A. C., Onabanjo, A. O. (1989). Chemotherapeutic effects of Annona senegalensis in *T. b. brucei* infection in mice. *Journal of Ethnopharmacology*, 30: 307 – 313.
11. Asuzu, I. U., Chineme, C. N. (1990). Effect of Morinda lucida leaf on Trypanosoma brucei brucei infection in mice. *Journal of Ethnopharmacology*, 30: 307 – 313.
12. Ibrahim, H. M., Ogbadoyi, E. O., Adamu, K. Y., Bello, M. U., Yemisi, I.J. (2012). Evaluation of antitrypanosomal activity of ethyl acetate extract of Adansonia digitata seed extract in T. brucei brucei infected albino mice. *International Journal of Drug Research Technology*, 2 (7): 454 – 460.
13. Nweze, N. E., Anene, B. M., Asuzu, I. U. (2011). Investigation of the antitrypanosomal activity of *Buchholzia coriacea* seed extract against a field strain of *Trypanosoma congolense*. *African Journal of Traditional Complementary and Alternative Medicine*, 8 (55): 175 – 180.
14. Akinloye, O. A., Oshilaja, R. T., Okelanfa, O. A., Akinloye, D. I., Idowu, O. M. O. (2013). Hypoglycaemic activity of Alstonia boonei stem bark extract in mice. *Agriculture and Biology Journal of North America*, 4 (1): 1-5..
15. Osadebe, P. O. (2002). Anti-inflammatory properties of the root bark of Alstonia boonei. *Nigerian Journal of Natural Product and Medicine*, 6: 39-41.
16. Asuzu, I. U., Anaga, A. O. (1991). Pharmacological screening of the aqueous rxtract of Alstonia boonei stem bark. *Fitoterapia*, 63: 411-417.
17. Anene, B. M., Onah, D. N., Nawa, Y. (2001). Drug resistance in pathogenic African trypanosomes: what hopes for the future? *Veterinary Parasitology*, 96: 83-100.
18. Soulsby, E. J. L. (1982). *Helminth, Arthropods and Protozoa of Domesticated Animals*. 7th Edn., Balliere Tindall, London.
19. Miller, R., Gregory, W. (1996). Technology and industrial performance. *OECD observer* Retrieved June 4, 2016 from https://www.oecd.observer.org/news/getfile.
20. Murray, M. (1988). Trypanotolerance: its criteria, genetic and environmental influences. In: *Livestock Production in Tsetse Affected Areas of Africa*: Proceedings of a meeting held in Nairobi, Kenya from the 23rd to 27th November 1987, ILCA/ILRAD. ILCA/ILRAD, Nairobi, Kenya.
21. Herbert, W. J., Lumsden, W. H. R. (1986). *Trypanosoma brucei*: A rapid matching method for estimating the host's parasitaemia. *Experimental Parasitology*, 40: 427 – 432.
22. Alan, H. F. (2002). Metabolic pathway analysis in trypanosomes and malaria parasites. *Transactions of the Royal Society for Tropical Medicine and Hygiene*, 85: 107.
23. Kubata, B. K., Nagamune, K,, Murakami, N., Merkel, P., Kabututu, Z., Martin, S. K., Kalulu, T. M., Mustakuk, H., Yoshida, M. (2005). *Kola acuminata* proanthocyanidins: a class of antitrypanosomal compounds effective against *Trypanosoma brucei*. *International Journal for Parasitology*, 35: 91 – 103.
24. Umar, I. A,, Toma, I., Akombum, C. A., Nnadi, C. J., Mahdi, M. A., Gidado, A., Igbokwe, I. O., Buratai, L. B. The role of intraperitoneally administered vitamin C during *T. congolense* infection of rabbits. *African Journal of Biochemical Research*, 2 (3): 88 – 91.
25. Eze, J. I., Agwubilo, C. I., Anene, B. M. (2012). Efficacy of increasing doses of diminazene diaceturate or isometamidium chloride in treatment of mice infected with isometamidium chloride resistant *Trypanosoma congolense*. *Solia Veterinaria*, 56 (1): 5 – 11.

26. Onyeyili, P. A., Anika, S. M. (1989). Effects of trypanosomal infection on the pharmacokinetics of diminazene aceturate in dogs. *Tropical Medicine and Parasitology*, 40 (4): 419 – 421.
27. Anene, B. M., Chukwu, C. C., Chime, A. B., Anika, S. M. (1989). Comparative study of chemotherapy, haematology and clinical manifestations of *Trypanosoma brucei brucei* and *Trypanosoma congolense* infections in dogs. *Zariya Veterinarian*, 4: 11 – 18.
28. Abenga, J. N., Ezebuiro, C. O., David, K., Akinyemi, O. F., Samdi, S. (2005). Studies on anaemia in Nigeria local puppies infected with *Trypanosoma congolense*. *Veterinarski Arhiv*, 75 (2): 165 – 174.
29. Akpa, P. O., Ezeokonkwo, R. C., Eze, C. A., Anene, B. M. (2008). Comparative efficacy assessment of pentamidine isethionate and diminazene aceturate in the chemotherapy of *Trypanosoma brucei brucei* infection in dogs. *Veterinary Parasitol*ogy, 151 (2 – 4): 139 – 149.
30. Obi, C. F., Obidike, R. I., Ezeh, I. O., Omoja, V. U., Iheagwam, C. N., Idika, I. K., Ezeokonkwo, R. C. (2013). Effects of *Trypanosoma brucei* infection and diminazene aceturate therapy on testicular morphology and function of Nigerian local dogs. *Veterinary Parasitology*, 196 (3 – 4): 283 – 288.
31. Holmes, P. H., Eisler, M. C., Geert, S. (2004). Current chemotherapy of animal trypanosomiasis. In: *The trypanosomiases.* Maudlin, I., Holmes, P. H., Miles, M. A. (Eds.,). CAB, London, UK.
32. Noyes, H. A., Alimohammadian, M. H., Agaba, M., Brass, A., Fuchs, H. (2009). Mechanisms controlling anaemia in *Trypanosoma congolense* infected mice. *Plos One*, 4 (4): e5170.doi: 10.1371.