Journal of Agriculture and Veterinary Sciences
Volume 6, Number 1, 2014
ISSN: 2277-0062

Studies on the Trypanocidal and Haematological Effects of *Cassia sieberiana* DC (Caesalpinaceae) Stem Bark Aqueous Extract in *Trypanosoma brucei* Infected Albino Rats

[1]BIU, A.A., [2]BURATAI, L.B., [2]MARK, V.A., [2]SAMSON, Y., [1]MOHAMMED, A., [1]PAUL, B.T., [3]FASUYI, F.H.,[4]BADAU, J.S., [5]SAIDU, A.M., [6]COMFORT, Y. AND [7]MALANG, S.K.

[1]Department of Veterinary Microbiology and Parasitology, University of Maiduguri, Nigeria.
[2]Department of Biochemistry, University of Maiduguri, Nigeria.
[3]National Biotechnology Development Agency, Bioresources Development Centre, Ogbomosho, Oyo State, Nigeria.
[4]Department of Veterinary Pathology, University of Maiduguri, Nigeria.
[5]Department of Veterinary Surgery and Theriogenology, University of Maiduguri, Nigeria.
[6]Department of Veterinary Anatomy, University of Maiduguri, Nigeria.
[7]Department of Veterinary Parasitology & Entomology, University of Abuja, Nigeria.
E-mail: biuvet@yahoo.com

ABSTRACT

Studies on the trypanocidal efficacy and the haematological effects of *Cassia sieberiana* DC (Caesalpinaceae) stem bark aqueous extract on *Trypanosoma brucei* infected albino rats was conducted in Maiduguri, Nigeria. There was a progressive increase in parasite count *In vivo* in the infected untreated (negative control) and infected extract treated groups of rats as from the 2nd day post inoculation, but became significant ($p<0.05$) on the 8th day in these groups, while the rats infected and treated with diminazene aceturate (positive control) showed disappearance in parasites in their systemic circulation from the 12th day post treatment. Complete cessation of motility of parasites was observed *In vitro* 10 minutes post inoculation with the concentrations of 1mg/10ml and 2 mg/10ml of *Cassia sieberiana* stem bark aqueous extract respectively. At the concentration of 4 mg/10ml, the motility count was zero at 5 minutes. Motility was observed in parasites in the micro titre well used as control but ceased after 30 minutes. The haematological parameters of the rats treated with graded doses of the stem bark aqueous extract of *Cassia sieberiana* had significant ($p<0.05$) decrease in PCV, Hb, RBC and WBC, while the diminazene aceturate (Veriben®) treated albino rats had insignificant ($p>0.05$) decrease when compared to the rats in the control group. This study has indicated a decrease in mean cell haemoglobin concentration (MCHC%) with a significant ($p<0.05$) increase in mean cell volume values of extract treated albino rats compared with the normal and positive control groups.The extract had suppressive effect on monocytes, lymphocytes, eosinophils and neutrophils counts of extract treated rats when compared to the differential leucocytic counts of the control group. There was insignificant ($p>0.05$) increase in monocytes, lymphocytes and eosinophils, and a decrease in neutrophils count in albino rats treated with diminazene aceturate when compared to the differential leucocytic count of the albino rats in the control group. This study has revealed the toxic effect of aqueous extract of *Cassia sieberiana* stem bark on the haematology of albino rats and its *In vitro* trypanocidal activity on *Trypanosoma brucei*.

Keywords: **Trypanocidal Activity, Haematological Effects, Diminazene aceturate, *Cassia sieberiana,* Albino Rats.**

Introduction

Trypanosomosis, a debilitating disease condition affecting man and livestock in sub-Saharan Africa is caused by haemo - flagellates of the genus *Trypanosoma*

and is transmitted principally by tsetse fly species *Glossina*. In other regions of the world including Asia and South America where trypanosomosis also exist, the disease is maintained through mechanical transmission by other haematophagous flies such as *Tabanus, Haematopota*, *Liperosia* and *Stomoxys* (Gutteridge, 1985; Leeflang, 1995; Leeflang *et al.*, 1998). Oral transmission following ingestion of bug faeces contaminated with *T. cruzi* was also observed. The severity of trypanosomosis depends on the species of trypanosomes involved, animal infected and the geographical conditions of the area of out-break. This is of course due to the fact that certain climatic conditions may tend to enhance the multiplication, development and growth of the parasites and their vector hosts *Glossina* (Soulsby, 1982; 1994).

Major species of trypanosomes responsible for livestock trypanosomosis are *Trypanosoma vivax*, *T. brucei*, and *T. congolense* while *T. b. gambiense* and *T. b. rhodesiense* causes human sleeping sickness in West and East-Africa, respectively (Solano *et al.*, 2003). Trypanosomosis manifests in two forms namely haemolymphatic and neurological phases with clinical signs such as anaemia, intermittent fever, progressive weight loss, generalized lymphadenitis, incoordination, high mortality, reduced productivity and economic losses due to poor hides and skin, meat and milk production in the livestock sector (Ugochukwu, 1983; Losos and Ikede, 1986; Abbott *et al.*, 2006).

Herbal preparations for the treatment of several diseases still hold a strong position in rural areas. Consequently, the toxicity associated with the use of synthetic drugs and the resistance developed against these agents by trypanosomes are constant problems while, the development of vaccine against the disease is still not tenable. These factors have increased the need to exploit the use of medicinal plants in the prophylaxis, treatment and control of trypanosomosis (ILRAD, 1990; Murray *et al.*, 1990). In northern Nigeria where trypanosomosis is prevalent, traditional healers especially Fulani people use medicinal plant decoctions from plants such as *Khaya senegalensis*, *Cassia siamaelam*, *Xeminia ameriana*, *Eucalyptus camaldulensis* (Myrtaceae) and *Cassia sieberiana* folklorically to treat trypanosomosis in livestock populations (Asuzu and Chineme, 1990; Nok *et al.*, 1993; Cragg *et al.*, 1997; Nok, 2002).

Cassia sieberiana DC (Caesalpinaceae) is a tree up to 50 ft of height found mostly in savanna regions of the world with pendulous yellow flower, black fruits flowering during the dry season and is known as African laburnum in English, Malgahi (Fulani), Gama fada (Hausa), Marga (Kanuri) and Aridan toro (Yoruba). Traditionally it has been used in the management of various ailments in human

and livestock populations in the African subcontinent especially in Nigeria. The phytochemical analysis of various parts of *Cassia sieberiana* has revealed the presence of tannins, quinines alkaloids, anthraquinones and saponins (Modusolumuo *et al.*, 1999). Decoctions from different parts of the plant has been used folklorically in the treatment of conditions such as malaria, fever, skin infections, headache, diarrhea, dysentery, vomiting, ulcer, jaundice, eczema, dysmenorrhoae, haemorrhoids, bilharziasis, leprosy, dropsy, gonorrhea, aphrodisiac, dewormer and is found to improve post parturient lactation (Madusolumuo *et al.*, 1999; Atindehou *et al.*, 2002; Wurochekke and Nok, 2004). This study was conducted to determine the trypanocidal activity of *Cassia sieberiana In vitro* and *In vivo* and its haematological effects in *Trypanosoma brucei* infected albino rats.

Materials and Methods

Plant Collection and Extract Preparation: Fresh stem bark of *Cassia sieberiana* was collected from the University of Maiduguri campus, Maiduguri, Nigeria in August, 2011. The plant was identified and authenticated as *Cassia sieberiana* DC (*Caesalpinaceae*) by a botanist in the Department of Biological Sciences, University of Maiduguri, Nigeria, and a voucher specimen with number LCMC 228 was deposited at the Department of Chemistry's herbarium. Three hundred (300) grammes of pulverised stem bark of *Cassia sieberiana* was exhaustively extracted in 1250 ml of distilled water, using Ace Soxhlet extractor 6730 and condenser 6740 (Quick Fit, England) at 60°C for 10 hours (WHO, 1992). The extract obtained was concentrated in hot air oven to remove traces of water and a product of 56.6 g was obtained and stored at 4°C for *In vitro* and *In vivo* antitrypanosomal studies.

Experimental Animals: A total of 30 adult albino rats of both sexes and weighing between 80.1g and 156.6g were used for this study. They were obtained from the Animal Breeding Centre, Department of Biochemistry, University of Maiduguri, Nigeria. They were maintained in clean plastic rat cages in the Parasitology laboratory, Faculty of Veterinary Medicine, University of Maiduguri, Nigeria. Pelleted feeds (ECWA, Nigeria Plc., Jos, Nigeria) and clean water were provided *ad libitum*. A fourteen-day acclimatization period was observed before the commencement of the experiment. Strict compliance with European Council Directive 86/609/EEC for animal experimentation was observed (Broom and Legge, 2004).

Determination of *In-vivo* Trypanocidal Activity: The 30 adult albino rats were randomly separated into six groups (A – D). Twenty-five of the rats in groups B,

C_1, C_2, C_3 and D received 1.0 ml of infected blood containing 4 x 10^6 trypanosomes from donor rats in phosphate buffered glucose solution (pH 7.2) intra-peritoneally,. Parasitaemia in the infected rats was checked every two days post infection by wet mount film prepared from tail blood and examined microscopically at x400. Rats in group A were used as normal control (uninfected untreated), whereas those in group B were infected and not treated (negative control). Rats in groups C_1 C_2 and C_3 were treated intraperitoneally with graded doses (100, 200 and 400 mg/kg) of the extract respectively at the onset of parasitaemia. Rats in group D were treated at the onset of parasitaemia intraperitoneally with a single standard dose (3.5mg/kg) of diminazene aceturate (Veriben®, Hoechst, Farbwerk, Germany).

Determination of *In vitro* Trypanocidal Activity: The *In vitro* trypanocidal activity was carried out in 3 replicates for each extract concentration using a microtitre plate (Flaw Laboratories, Inc., Melean, Virginia) wells. A serial dilution of the extract into graded concentrations of 1, 2 and 4 mg / 10ml in phosphate buffered saline (PBS) solution was prepared, and into each microtitre well 10µl / g of the extract concentrations were inoculated with 60 µl /g infected blood and were maintained at 37°C in a water bath for 1 hour. For a reference test 10µl extract was replaced with PBS in the microtitre wells and motility of the parasites observed at x 400 at 15 minutes intervals for a period lasting 30 minutes.

Determination of Haematological Values and Indices: Tail blood was collected every 2 days for haematology. The microhaematocrit and cyanomethaemoglobin methods (Coles, 1980) were used to determine the packed cell volume (PCV %) and haemoglobin concentration (Hb g/dl) respectively. The red blood cell (RBC x 10^6 mm ^{3}) and white blood cell (WBC x 10^3 mm^3) counts were done by haemocytometry using Dacie's fluid and 2% acetic acid as diluting fluids respectively and counted using improved Neuber's slides (Brown, 1976).

The mean cell haemoglobin concentration (MCHC) representing the percentage of haemoglobin in one decilitre of packed red blood cell expressed as %, the mean cell volume (MCV) as the average of a single cell expressed in femtolitres (fl), and the mean cell haemoglobin (MCH) expressed as the average haemoglobin concentration in picograms (pg) of a single red blood cell (Bush, 1975) were determined.

Statistical Analysis: Values obtained were expressed as mean ± standard deviation (SD) and subjected to analysis of variance (ANOVA) and p-values

equal to or less than 0.05 regarded as statistically significant (GraphPad Software, 2000).

Results

Table 1 shows *In vivo* parasite count following the intraperitoneal administration of aqueous extract of *Cassia sieberiana* stem bark in *Trypanosoma brucei* infected albino rats. There was a progressive increase in parasite counts in the infected untreated and infected treated groups (B, C_1, C_2 and C_3) which started from the 2nd day post inoculation, but became significant ($p < 0.05$) on the 8th day in these groups of rats, while the rats infected and treated with diminazene aceturate (group D) showed disappearance of *T. brucei* from rat blood on the 12th day post treatment.

Table 2 shows the In vitro effects of stem bark aqueous extract of Cassia sieberiana. Cessation of motility of parasites *In vitro* was observed 10 minutes post inoculation with the concentrations of 1mg/10ml and 2mg/10ml. At the concentration of 4mg/10ml, the motility count was zero at 5 minutes, however, motility was observed in parasites in the micro titre wells used as control but ceased after 30 minutes.

Table 3 shows the *In vitro* effect of various concentration of *Cassia sieberiana* DC stem bark aqueous extract on the motility and survival time of *Trypanosoma brucei at* 37°C. The parasites motility was low after 5 minutes in the micro titre wells containing 1 – 2 mg / 10ml *Cassia sieberiana* stem bark aqueous extract. The parasites exposed to 4 mg/10ml showed 100% motility all through the test periods (5- 30 minutes). The parasites in the micro titre wells used as control had high, moderate and low motility from 0 - 10 minutes, 15 – 20 minutes and at 25 minutes respectively but ceased at 30 minutes.

Table 4 shows the haematological values of albino rats treated with *Cassia sieberiana.*The haematological parameters of the rats treated with graded doses (100, 200 and 400 mg / kg) of stem bark aqueous extract of *Cassia sieberiana* had a significant ($p<0.05$) decrease in their PCV, Hb, RBC and WBC mean values, while the diminazene aceturate (Veriben®) treated group had an insignificant ($p>0.05$) variation in these mean valueswhen compared to the rats in the normal control group.

Table 5 shows the leucocytic counts of *T. brucei* infected albino rats treated with *Cassia sieberiana.* The aqueous stem bark extract of *Cassia sieberiana* had suppressive effects on monocytes, lymphocytes, and neutrophils mean counts

but had a significant increase ($p<0.05$) in mean eosinophils counts when compared to the differential leucocytic counts of the rats in the control group. However, there was an insignificant ($p>0.05$) variation in differential leucocytic counts in rats treated with diminazene aceturate (veriben®) when compared to the differential leucocytic counts of rats in the control group.

Table 1: *In vivo* Parasite Count (x 10^6/µl) of *Trypanosoma brucei* Infected Albino Rats

Groups	Extract Dose (mg/kg)	Mean ± SD of Parasitaemia Counts Days Post Infection					
		2	4	6	8	10	12
A	(normal control)	0	0	0	0	0	0
B	(negative control)	3.8±0.83	20.8±7.15	146.2±3.52	304.3±5.28[a]	*	*
C_1	100	3.9±0.86	20.8±2.28	164.8±8.36	298.6±4.74[b]	*	*
C_2	200	3.7±0.80	26.3±2.20	158.6±2.60	288.6±0.50[b]	*	*
C_3	400	4.1±1.14	29.3±1.94	196.9±3.80	274.0±1.20[b]	*	*
D	(positive control)	4.4±0.50	3.4±1.16	2.9±6.80	1.9±0.60	1.84±1.67	0

Keys:

A	=	Normal control (uninfected untreated)
B	=	Negative control (infected untreated)
C_1, C_2 and C_3	=	(Infected and extract treated)
D	=	Positive control (infected and treated with diminazene aceturate)
D	=	Rats infected with *T. brucei* & treated with diminazene aceturate (Veriben®)
*	=	Indicates mortality of experimental rats

Note: Column means with different superscripts are statistically significant ($p < 0.05$)

Table 2: Shows the *In vitro* Effects of Stem Bark Aqueous Extract of *Cassia sieberiana*

Groups	Extract Concentration (mg/10ml)	Motility Count (x 106/ µl)/minute						
		0	5	10	15	20	25	30
A	1 0	26.7÷2.08	10.8±1.04	0	0	0	0	0
B	2 0	26.7÷2.08	7.3±2.08	0	0	0	0	0
C	4 0	26.7÷2.08	0 ± 00	0	0	0	0	0
D	Control	26.7÷2.08	21.5±0.50	17.5±2.50	13.5±1.50	7.0±1.00	4.7±3.05	0

Table 3: Shows the *In vitro* Effect of Various Concentration of *Cassia sieberiana* Stem bark Aqueous Extract on the Motility and Survival Time of *Trypanosoma brucei at* 37°C

Groups	Extract Concentration (mg/10ml)	Time (Minutes) Post Inoculation						
		0	5	10	15	20	25	30
A	1	+++	+	*	*	*	*	*
B	2	+++	+	*	*	*	*	*
C	4	+++	*	*	*	*	*	*
D	Control	+++	+++	+++	++	++	+	*

Key:

+++	=	High motility of parasites
++	=	Moderate motility of parasites
+	=	Low motility of parasites
*	=	No motility of parasites

Table 4: Haematological Values of Albino Rats Treated with *Cassia sieberiana*

Groups	Dose of Extract (mg/kg)	Mean ± Standard Deviation						
		PCV (%)	Hb (g/dl)	RBC ($x10^6 mm^3$)	WBC ($x10^3 mm^3$)	MCV (fl)	MCH (pg)	MCHC (%)
A	Normal Control	43.0±1.00[a]	14.0±0.74 [a]	8.0±0.37[a]	11.8±0.34[a]	53.8	17.5	32.6
B	Positive Control	42.7±2.07[a]	13.8±1.29 [a]	8.0±1.00[a]	10.6±1.24[a]	53.4	17.3	32.3
C_1	100	30.0±6.55 [b]	9.4±1.91 [b]	6.9±2.2 [b]	8.3±1.71[b]	43.5	13.6	31.3
C_2	200	29.7±2.51 [b]	8.9±1.20[b]	5.0±1.07[b]	7.2±1.15 [b]	59.4	17.8	29.9
C_3	400	27.7±2.30[b]	7.0±0.75[b]	4.1±0.96[b]	6.3±10.3[b]	67.6	17.1	25.3

Note: Column means with different superscripts are statistically significant (p < 0.05)

Table 5: Leucocytic Counts of *Trypanosoma brucei* Infected Albino Rats Treated with *Cassia sieberiana*

Groups	Dose of Extract (mg/kg)	Mean ± Standard Deviation			
		Monocytes	Lymphocytes	Eosinophils	Neutrophils
A	Normal control	2.6±1.34[a]	70.2±6.87[a]	3.6±1.94[a]	23.6±7.76[a]
B	Veriben treated	2.7±0.57 [a]	71.6±7.43[a]	3.8±2.16 [a]	22.8±4.76[a]
C_1	100	1.3±0.57[b]	52.0±11.26[b]	7.0±1.73[b]	14.3±8.14[b]
C_2	200	1.1±1.00 [b]	50.0±9.53[b]	6.0±4.00 [b]	13.0±10.0[b]
C_3	400	0.7±0.57 [b]	42.0±9.94[b]	5.0±2.00 [b]	11.3±11.8[b]

Note: Column means with different superscripts are statistically significant (p < 0.05)

Discussion

This study has observed mortality in *T. brucei* infected albino rats treated with graded doses of *C. sieberiana* stem bark aqueous extract, which may be due to its inability to inhibit the virulence of *T. brucei In vivo*, as indicated by the acute parasitaemia observed 8 days post infection in groups C_1, C_2 and C_3 with the corresponding drop in PCV, Hb, RBC and WBC mean counts. These finding agrees with Tamboura *et al* (2005) and Obidah *et al* (2009) that *C. sieberiana* is very toxic with the hematopoietic system as one of its most sensitive targets (Diallo *et al.*, 2010).

This study has indicated a decrease in mean cell haemoglobin concentration (MCHC %) with a significant increase in mean cell volume values of extract treated rats compared with the normal and positive control groups. This is indicative of macrocytic red blood cells and agrees with Anosa (1983) that characteristics of anaemia in *T. brucei* infections are macrocytosis.

Despite the toxicity of *C. sieberiana* in this study, it has been reported to be effective in the treatment of diseases such as malaria, fever, skin infections, headache, diarrhea, dysentery, vomiting, ulcer, jaundice, eczema, dysmenorrhoae, haemorrhoids, bilharzias, leprosy, dropsy, gonorrhea, aphrodisiac, dewormer and is found to improve post parturient lactation (Burkill, 1995; Madusolumuo *et al.*, 1999; Atindehou *et al.*, 2002; Wurochekke and Nok, 2004).

This study has also indicated a significant decrease in mean counts of monocytes, lymphocytes and neutrophils but an increase in eosinophils counts in the extract treated groups. It has been reported that the monopenia, neutropenia and lymphopenia are usually associated with acute inflammatory conditions, indicative of bone marrow damage by toxins and eosinophilia in allergic reactions due to proteins or drugs (Jain, 1993).

It has generally been accepted too that a major disease promoting factor in trypanosomosis in hemolytic anaemia, with a complex and multifactorial aetiology which may include destruction of mature erythrocytes, leucocytes and thrombocytes by macrophages in the bone marrow, spleen, liver and haemolymph nodes (Anika *et al.*, 1995; Mbaya *et al.*, 2008; Anil, 2012).

The *In vitro* assay has indicated that the *C. sieberiana* extract in this study has trypanocidal activity and agrees with the findings of Wurochekke and Nok (2004), who also reported that plant bioactive products are responsible for

most physiological and chemotherapeutic effects *In vitro* and that bioactive screening *In vitro* remains a useful tool for pre – selection of plants and bioassay - guided fractionation for the isolation and identification of active principles.

Conclusively, the aqueous extract of *Cassia sieberiana* stem bark was found to exhibit side effects on the haematology of albino rats. Therefore, application of this plant in the treatment of human and animal trypanosomosis in Nigeria should be done with extreme caution. However, its remarkable *In vitro* trypanostatic and trypanocidal activity approves that its use in the folkloric treatment of trypanosomosis has a pharmacological basis.

Reference

Abbott, C.R. Small, C.J., Sajedi, A., Smith K.L., Parkinson, J.R., Broadhead, L.L., Ghatei, M.A. and Bloom, S.R. (2006). The Importance of Acclimatization and Habituation to Experimental Conditions When Investigating the Anorectic Effects of Gastrointestinal Hormones in the Rat. *International J. Obesity*. 30:288–292.

Anika R.E., Ohore B.O., Ogunsanmi A.O., Michael J.I., Awolaje O.A., Ajuwape A.T.P., Adeneye J.O. and Oyejide A. (1995). Clinical Pathological Changes Associated with Trypanosoma Antigen-anaemia in Nigeria Cattle. In O.A.U/S.T.R.C. **18(**9123**)**:136.

Anil, J.P. (2012). A Candle of Medicinal Herb's Identification and Usage Medicinal Herbals Team http://www.indianmedicinalplants.info.

Anosa, V.O. (1983). Mammalian Blood Cells in Health and in Trypanosomiasis. *Trop. Vet*. **1**: 177-199.

Asuzu, I.U. and Chineme, C.N. (1990). Effects of *Morinda lucida* Leaf Extracts on *Trypanosoma brucei brucei* Infection in Mice. *J. Ethnopharmacol.* **30**: 307-313.

Atindehou, K.K., Kone, M., Terreaux, C., Troare, D., Hostettmann, K. and Dosso, M. (2002). Evaluation of the Antimicrobial Potential of Medicinal Plants from Ivory Coast. *Phytotherapy Res*. 16(5): 497 – 502.

Broom, S and Legge, D. (2004). Animal Welfare U.S.A Free Trade Wins: An Examination of the Animal Welfare Implications. Ministry of Agriculture, Fisheries and Food Compassion Farming. *Animal Welfare.* 9: 81 – 85.

Brown, B.A. (1976). *Haematology, Principles and Procedures*, 2nd ed., Lea and Febiger, Philadelphia. Pp. 56-81

Bush, B.M. (1975). *Veterinary Laboratory Manual*. William Heinemann Medical Books Ltd, London Pp. 130-131.

Coles E.H. (1986). Veterinary Clinical Pathology, 14th Edn. W. B. Sanders Company Philadelphia. Pp.475-477.

Cragg G.M, Newman D.J. and Snader K.M, (1997). Natural Products in Drug Discovery and Development. *J. Nat. Prod.* **60**: 52 – 60.

Diallo, A., Eklu-Gadegkeku, K., Agbonon, A., Aklikokou, K., Creppy, E.E. and Gbeassor, M. (2010). Acute and Sub Chronic (28 Days) Oral Toxicity Studies of Hydroalcoholic Extract of *Lannea kerstingii Engl.* and *K. kraus* (Anacardiaceae) stem bark. *J. Pharmacol Toxicol*. **5**:343-349

GraphPad Software Instat Version 3.05 (2000). Guide to Choosing and Interpreting Statistical Tests. GraphPad Software Inc. 5757 Oberlin Drive, San Diego, California 92121, USA Pp. 153.

Gutteridge, W.E. (1985). Existing Chemotherapy and Its Limitations. *Br. Med. Bull.* **41**: 162-168.

ILRAD, (1990). Chemotherapy for Trypanosomosis. Intern. Lab. Res. Anim. Dis. 8 (3): 1 – 6.

Jain, N.C. (1993). Essentials of Veterinary Haematology, 1st ed. Lea and Febiger, Philadelphia, 417pp.

Leeflang, G.P. (1995). Bovine Trypanosomosis in Northern Nigeria. A Contribution to the Epidemiology, Host-specific and Drug Sensitivity of *Trypanosoma vivax*. *Protechrift Rukuniever siteitle Utrct*. 2: 1–39.

Leeflang, G.P., Buys J. and Blotkamp, P.C. (1998). Studies of *Trypanosoma vivax* Infectivity and Serial Maintenance of Natural Bovine Isolates in Mice. *International J. Parasitol*. 65: 413–417.

Losos, G. J. and Ikede, B. O. (1986). Review of Pathology of Domestic and Laboratory Animals Caused by *Trypanosoma congolense, T. vivax, T. brucei, T. rhodiense* and *T. gambiense. J. Vet. Pathol.*, 9: 1–71.

Madusolomuo, M.A., Nadro, M.S. and Wurochekke, A.A. (1999). Antihepatotoxic Properties of *Cassia sieberiana* in Acetaminophen Treated Rats. *Nig. J. Biochem. Mol. Biol.,* 14:21 – 25.

Mbaya, A.W., Aliyu, M. M., Nwosu, C. O. and Ibrahim, U.I. (2008). Captive Wild Animals as Potential Reservoirs of Haemo-ectoparasitic Infection of Man and Domestic Animals in the Arid Regions of Northern Nigeria. *Vet. Arhiv* **78**: 431-442.

Murray, M., Trail, J.C.M., D'iteren, G.D.M., (1990). Trypanotolerance in Cattle and Prospects for the Control of Trypanosomosis by Selective Breeding. Revue Scientifique et Technique Del' Office International Des Epizooties. 9(2): 369 – 386.

Nok A.J., (2002). Azaanthraqinone Inhibits Respiration and *In vitro* Growth of Long Slender Blood Stream Forms of *T. congolense. Cell Bio Chem. Funct*. **20**: 205 – 212.

Nok, A.J., Esievo, K.A.N., Lingdet, I., Arowosafe, S., Onyenekwe, P.C., Gimba, C.E. and Kagbu, J.A. (1993). *In vitro* Activity of Leaf Extracts Against *Trypanosoma brucei brucei. J. Clin. Biochem. Nutr.* **15**: 113-118.

Obidah, W., Sa'ad, U.A. and Wurochekke, A.U. (2009). Toxic Effects of Aqueous Stem Bark Extract of *Cassia sieberiana* on Some Biochemical Parameters in Rats. *African J. Biochem. Res*. 3(5): 229-231

Solano, P., Dela-Roques, S., Duvalet, G. (2003). Biodiversity of Trypanosomes Pathogenic for Cattle and Their Epidemiological Importance. *Ann. Soc. Pathol.* 68(1), 169 – 171.

Soulsby, E.J.L. (1982). Helminths, Arthropods and Protozoa of Domesticated Animals, 7th Edition Bailliere Tindal, London. 46–49.

Soulsby, E.J.L. (1994). How Parasites Tolerate Their Hosts. *British Vet. J.* 19: 150–312.

Tamboura, H.H., Bayala, B., Lompo, M., Guissou, I.P. and Sawadogo, L. (2005). Ecological Distribution, Morphological Characteristics and Acute Toxicity of Aqueous Extract of *Holarrhena floribunda* (G. Don) Durand and Schinz, *Leptadenia hastata* (PERS.) DECNE and *Cassia sieberiana* (D.C.) Used by Veterinary Healers in Burkina Faso. *African J. Trad. Compl. Altern. Med.* **2(1)**:13-24.

Ugochukwu, E. (1983). Caprine Trypanosomiasis Caused by T. Congolense. A Case Report. *Nig. Vet. J.* 12: 24–27.

WHO (1992). Quality Control Methods for Medicinal Plant Materials Geneva. Pp.26.

Wurochekke, A.U. and Nok, A.J. (2004). The *In vitro* Trypanocidal Activity of Some Medicinal Plants Used by Local Herdsmen in Northern Nigeria. *Afri. J. Biotechnology*, 3(9): 481 – 483.

References to this paper should be made as follows: Biu, A.A. *et al.* (2014), Studies on the Trypanocidal and Haematological Effects of *Cassia sieberiana* DC (Caesalpinaceae) Stem Bark Aqueous Extract in *Trypanosoma brucei* Infected Albino Rats. *J. of Agriculture and Veterinary Sciences,* Vol. 6, No. 1, Pp. 112 – 123.