IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 12, Issue 5 Ver. VI (Sep. – Oct. 2017), PP 12-17
www.iosrjournals.org

In Vivo Anti-Trypanosomal Potential Of Methanol Root Extract Of *Terminalia Macroptera* (Guill. And Perr.) In *Trypanosoma Brucei Brucei* Infected Wistar Rat

Balogun, J.B.*[1]., Abubakar, Z[1]., Ibrahim,T.B.[1]., Balogun, S.U[2]., Sadiq, I.S[3]., Dogara, M. M[1]., Idoko, S.I[6]., Owolodun, H.O[4]., and Orendu, M.A[5]

[1.] *Department of Biological Sciences, Federal University Dutse, P.M.B. 7156, Jigawa State. Nigeria.*
[2]. *Department of Human Anatomy, College of Health Sciences, Faculty of Basic Medical Sciences, Kogi State University, Anyigba, Nigeria.*
[3.] *Department of Chemistry, Federal University Dutse, P.M.B. 7156, Jigawa State. Nigeria.*
[4]. *Department of Zoology, University of Ilorin, Kwara State, Nigeria..*
[5]. *Department of Human Anatomy, College of Medicine, University of Maiduguri, Borno State.*
[6]. *Department of Veterinary Pathology, University of Abuja, Abuja.*
Corresponding Author: Balogun, J.B

ABSTRACT: *Trypanosoma brucei brucei is a haemo-protozoan parasite transmitted by tsetse fly, which causes African animal trypanosomiasis (AAT), a parasitic disease that causes economic loss in cattle, sheep and goats. Existing drugs for the management of AAT have been reported to be expensive, toxic and rapidly becoming ineffective due to animals becoming resistant to the drugs. Hence the need for drugs of plant origin that are natural, easily accessible, and cheaper, with minimal side effects when compared to conventional anti-trypanocidal drugs. Terminalia macroptera (T. macroptera) is an ethnomedicinal plant used as antimicrobial and antimalarial therapies in some developing countries.The study aimed at evaluating the anti-trypanosomal potential of the methanol root extract of T. macroptera in Wistar rats. A prior Gas Chromatography Mass Spectrometry (GCMS) was carried out on the root extract. Twenty-five female Wistar rats were used for the study. These were divided into five groups (I: Therapeutically treated; II: Positive control; III: Prophylactically treated; IV: Extract control and V: Healthy control) of five animals each. Experimental groups I and III received 64 mg/kg of extract after and before a parasite dose of 10^6 respectively, via intraperitoneal route. Results obtained showed that Groups I and III had insignificant parasitaemia peaks with 40% and 20% survival respectively, at day 11 post infection (Pi), as compared to Group II which had a significant parasitaemia peak with 0% survival at day 6 Pi. The extract and healthy controls had 100% survival at day 14. Furthermore, Haematoxylin and Eosin stained spleen, liver and kidney tissues revealed distortion in the Group II as compared to Groups I and III. No significant ($P > 0.05$) change in body weight was observed. Methanol root extract of T. macroptera regulated parasite multiplication, ameliorated histopathological and hematological changes ($P < 0.05$) and extended animal survival in experimental trypanosomiasis.*

Key: *Gas Chromatography Mass Spectrometry, Terminalia macroptera, Trypanosomiasis and Trypanosoma brucei brucei*

Date of Submission: 02-10-2017 Date of acceptance: 14-10-2017

I. Introduction

Trypanosomes are blood parasites and the causative agents of parasitic disease referred to as nagana in animals, sleeping sickness and Chagas' disease in humans. In addition to *Trypanosoma brucei*, other causes of nagana include *Trypanosoma congolense* and *Trypanosoma vivax*, which are all transmitted to the mammalian host by a parasite-carrying tsetse fly during its blood meal [1,2]. Sleeping sickness and nagana are a major health problem in sub-Saharan Africa, and the economic impact of the animal disease is a severe constraint against development in Africa today [3]. A vast majority of people from less developed countries, including Nigeria depend on medicinal plants for the treatment of various diseases, due to the high cost of synthetic drugs. Most of these plants have proved to be useful sources of treatment of various diseases. Lack of cost effective drugs is a major drawback in the treatment of trypanosomiasis. Most of the anti-trypanosomal drugs currently available in market are either highly toxic to animal or the parasites rapidly becomes resistant to these drugs [4]. The search for the new chemotherapeutic agents against trypanosomiasis continues to be of great importance, a potential trypanocidal drugs without side effects is therefore urgently needed. *Terminalia macroptera* according to Guill and Perr (*Combretaceae*) is a tree which occurs widely in West Africa. In Mali *T. macroptera* is used

against a variety of ailments, and more than 30 different indications have been mentioned by the traditional healers in ethnopharmacological studies. The roots are used against hepatitis, gonorrhea and various infectious diseases, including *Helicobacter pylori* associated diseases [5]. Flavonoids [6], triterpenoids [7], ellagitannins [8] and related phenolics [7], have been identified from different parts of *T. macroptera.*

II. Materials And Method

2.1 Plant material

The root of *T.macroptera* was harvested at maturity in the month of August, 2016 at Minna (70° 26′N and 3° 54′ E), Niger State, Nigeria. It was identified and authenticated (Voucher Number: 900646) by a botanist, in the herbarium of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Kaduna State, Nigeria. The root was air dried in the laboratory and made into powder by grinding. About 65 g was extracted in 500 mL methanol using soxhlet extraction technique. The whole process, utilized 260 grams of the root powder and 1500mL. The extracts were filtered under suction pressure with a Whatman's filter paper. They were then concentrated under reduced pressure using a rotary evaporator and stored at room temperature until required.

2.2. Gas Chromatography Mass Spectrometric Analysis

The powdered form of the methanol root extract of *T. macroptera* was re-constituted in a diluent (methanol) and loaded into a GCMS machine for determination of chemical constituents.

2.3. Parasites

T. brucei brucei was obtained from stabilates maintained at the Nigerian institute for trypanosomiasis research, Kaduna State, Nigeria in the month of December, 2016; and was thereafter maintained in the Centre for Biotechnology Research and Training, Ahmadu Bello University, Zaria, by continuous passage of infected blood into the wistar rats.

2.4. Animals

Twenty five healthy female Wistar rats of approximately 7 weeks old weighing between 150-200 g were purchased from the Nigerian Institute of Trypanosomiasis Research, Kaduna State, Nigeria. They were housed under standard laboratory conditions, fed commercial feed (VITAL feeds, Zaria) and given access to clean water ad libitum. These were acclimatize for 2 weeks before the commencement of the experiment. The rats were grouped into 5 groups of 5 rats each: Group I (Therapeutic; first infected and then treated with 63.25 mg/kg), Group II (Positive control; infected but not treated), Group III (Prophylactic; first treated with 63.25 mg/kg and then infected), Group IV (Extract control; treated with 63.25 mg/kg but not infected) and Group V (Healthy control; neither infected nor treated).

2.5. Trypanosome infection and treatment

Blood from a highly parasitized donor wistar rat was obtained by tail bleeding, and collected into an EDTA-coated sample bottle, and diluted appropriately with physiological saline, served as inoculums. Rats from groups: I, II and III were infected intraperitoneally with 0.1 mL of the inoculum containing about 10^6 trypanosomes/mL. Treatment began the day parasites were first detected by microscopy in the blood stream (day 4) of the group I and lasted only for three days (i.e. 3 days of treatment), while for group III, three days of extract treatment was given to the rats followed by inoculums delivery on the fourth day (day 4). It is important to note that rats from groups: I, II and III were infected with trypanosomes on the same day (i.e. 3 days post-treatment of group III). In addition, approval was sought for all experimental protocols and guidelines of the Institutional Animal Care and Use Committee of Ahmadu Bello University, Zaria, Kaduna State, Nigeria was strictly adhered to.

2.6. Sample collection

Blood was collected from the tail of each rat to determine red blood cell (RBC) values, before the commencement of drug treatment and parasite infection, which served as base line, and at two days interval upon the first parasite detection (day 4 post infection) and the days treatments were made.

2.7. Determination of RBC values, parasitaemia, organ histopathology, body weight and percentage survival

RBC values were determined by the use of an auto analyzer and parasitaemia monitored daily by method of Herbert and Lumsden, (1976). Organ (Kidney, Liver and Spleen) were fixed in 10 % buffered formalin and subsequently embedded in paraffin, sectioned and stained with haematoxylin and eosin (H & E) stain for general tissue architecture using routine histological techniques. The sections were viewed under the light microscope and photomicrographs were obtained using a microscope eyepiece attached to a computer

monitor. Daily body weight of experimental rats were determined using a weighing balance while survival was monitored daily and expressed as number of survivor divided by total initial number in the group multiplied by one hundred (100%).

2.8. Statistical analysis

Data obtained were expressed as percentage, mean and mean ± standard error of the mean (mean±SEM) and analysed using EZAnalyze version 3.0. Data were subjected to one way analysis of variance. Values of $P < 0.05$ were regarded as statistically significant.

III. Results

Table 1 shows the chemical compounds present in the *T. macroptera* root and their respective elution order and retention time on the chromatogram. Propanol was the first to elute with retention time of 1.8972 followed by Cyclohexane; Toluene; P-Xylene; Benzene; Carene; D-Limonene; Phenole; 2-Methoxy-3,2-propenyl acetate; Caryophyllene and Humulene with 2.3952; 3.1777; 4.0313; 4.8133; 5.2051; 5.3474; 7.0546; 8.2995 and 8.5485 retention time respectively.

Table 1: Compounds revealed by Gas Chromatography Mass Spectrophotometry in *T. macroptera* Methanol Root Extract

Chemical Compounds	Retention Time (min)
Propanol	1.8972
Cyclohexane	2.3952
Toluene	3.1777
P-Xylene	4.0313
Benzene	4.8133
Carene*	5.2051
D-Limonene*	5.3474
Phenole, 2-Methoxy-3,2-propenyl acetate	7.0546
Caryophyllene*	8.2995
Humulene*	8.5485

Keys: *Terpenoids.

The body weight pattern revealed in Figure 2 generally showed an initial body weight gain, between day 1 and 4 after which a decline was noticed in groups II and III, while groups I, IV and V maintained a slightly constant body weight gain after the first four days.

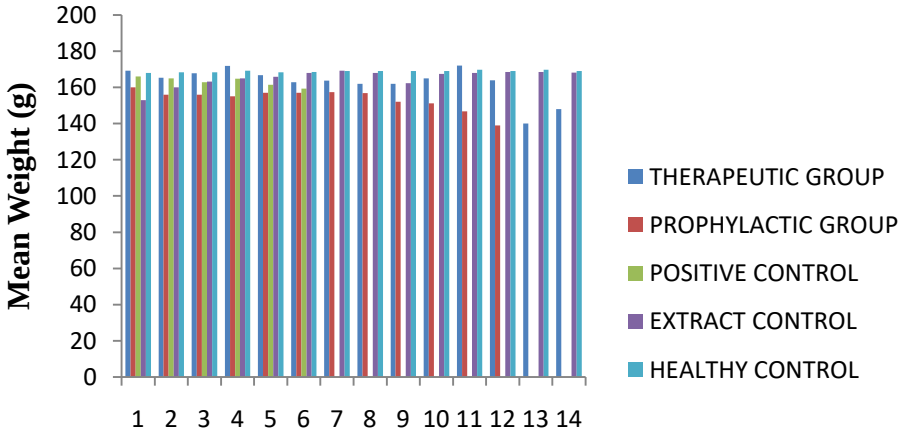

Fig. 2: Mean Body Weight of Experimental Rats

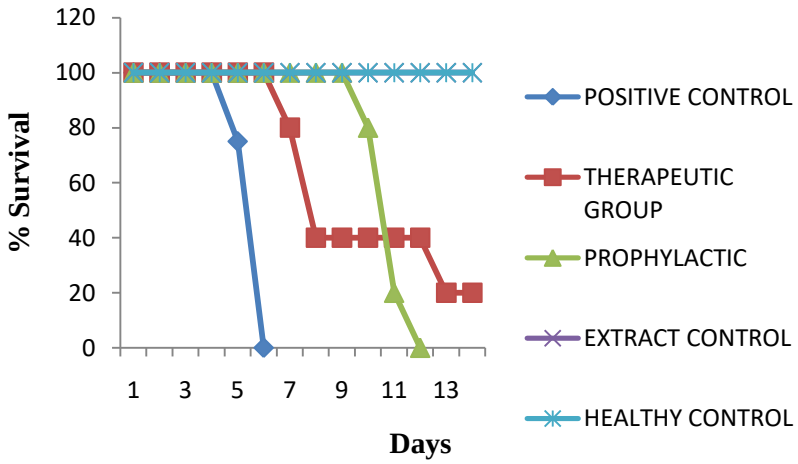

Figure 3: Percentage Survival of Experimental Wistar Rats

Table 2 shows the mean red blood cell volume in which the healthy control showed increased RBC, with a baseline, post infection (refers to days after infection of groups I, II and III) and post treatment (groups I, III and IV) values of 5.39±0.08, 6.12±0.04 and 6.32±0.02 respectively. Similarly, the extract control showed increased RBC, with a baseline, post infection (refers to days after infection of groups I, II and III) and post treatment (groups I, III and IV) values of 5.84±0.01, 6.04±0.02 and 5.95±0.01 respectively. The RBC values for the positive control showed significant decrease from a baseline value of 5.20±0.03 to 4.35±0.03 during parasitaemia progression and finally 3.38±0.03 at peak of parasitaemia. Whereas, the therapeutic group showed decrease in the RBC values from a baseline value of 4.45±0.02 to 4.04±0.01 during parasitaemia progression and finally 4.07±0.07 at peak of parasitaemia, the prophylactic group showed increased RBC value from a baseline value of 4.40±0.12 to post treatment value of 4.99±0.07 and penultimate post infection value of 5.96±0.01.

The positive control group showed a peak with mean parasitaemia of 1000 x 10^6 trypanosomes/mL at day 6 where 100% (Fig. 3) of the rats were killed by the parasite without a single parasitaemia wave. The therapeutic group experienced two parasitaemia waves, the first major peak with mean parasitaemia of 1000 x 10^6 trypanosomes/mL at day 8 where 40% of the rats were still surviving and a second minor peak with mean parasitaemia of 50 x 10^6 trypanosomes/mL at day 13 where 40% of the rats survived (Fig. 3). The prophylactic group had two minor peaks, the first with mean parasitaemia of 450 x 10^6 trypanosomes/mL at day 10 where 80% of the rats were still surviving (Fig. 3) and the second peak with mean parasitaemia of 50 x 10^6 trypanosomes/mL at day 12.

Table 2: Mean ±SE of red blood cell volume $10^{12/}$L of experimental groups

Description	Healthy Control	Therapeutic Group	Prophylactic Group	Parasite Control	Extract Group
Base Line	5.39±0.08	4.45±0.02	4.40±0.12	5.20±0.03	5.84±0.01
Post Infection	6.12±0.04	4.04±0.01	5.96±0.01	4.35±0.03	6.04±0.02
Post Treatment	6.32±0.02	4.07±0.07	4.99±0.07	3.38±0.03	5.95±0.01

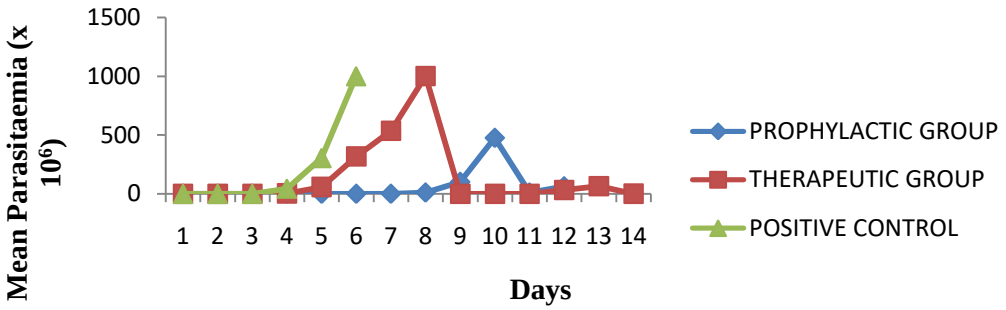

Figure 1: Mean Parasitaemia of Experimental Wistar Rats

Plate 1 (A-E), shows the kidney histology of groups I-V. Groups I and II showed some level of tissue degeneration, whereas the group III only exhibited a mild tissue degeneration, no degeneration in IV and V.

Similarly, some form of degeneration in spleen tissue (Plate 2, A-E), was also observed in Groups I and II, whereas whereas the group III only exhibited a mild tissue degeneration, no degeneration in IV and V.
Plate 3 shows liver histology of all the groups, where major tissue degeneration was observed in group II with only minor degeneration in groups I and III. While the groups IV and V showed no tissue damage.

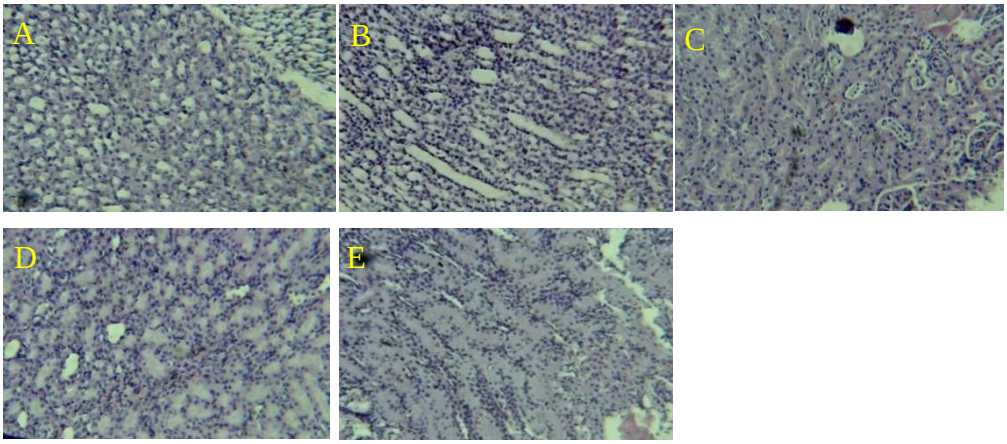

Plate 1: Kidney of *T. b. brucei* infected and non-infected rats (Haematoxylin and Eosin stain), (Magnification ×40). Key: A= Therapeutic; B= Parasite control; C= Prophylactic D=Healthy control ;E= Extract control; TBG=TubularDegeneration.

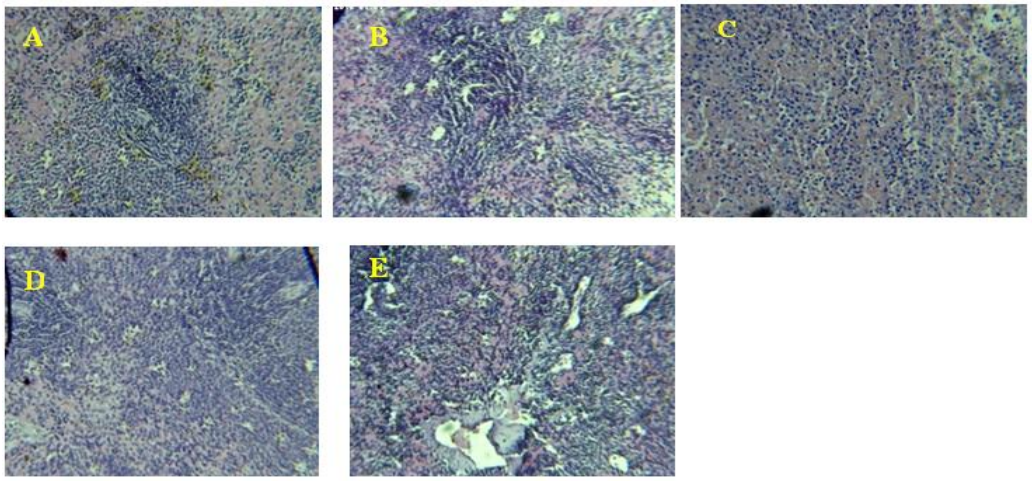

Plate 2: Spleen of *T. b. brucei* infected and non-infected rats (Haematoxylin and Eosin stain), (Magnification ×40). Key: A= Therapeutic; B= Parasite control; C= Prophylactic D=Healthy control ;E= Extract control.

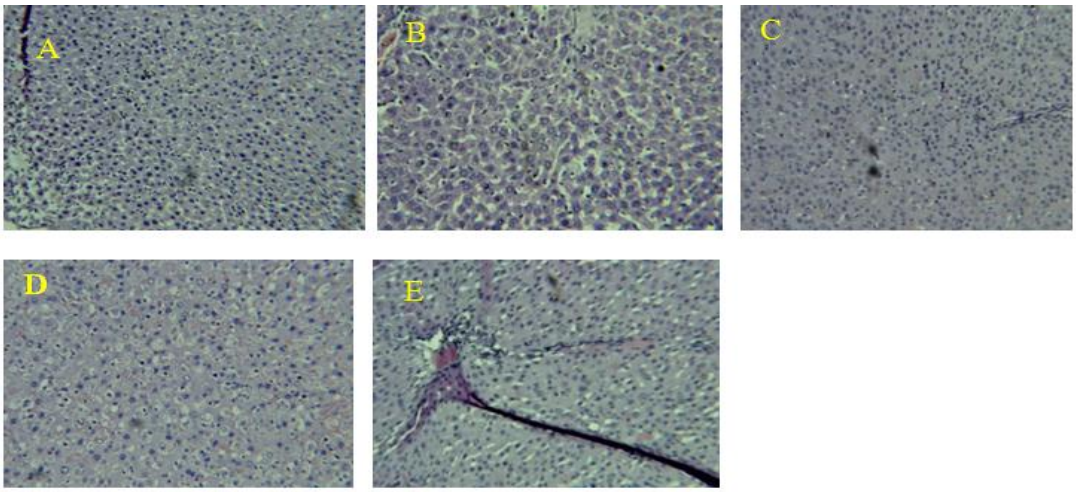

Plate 3: Liver of *T. b. brucei* infected and non-infected rats (Haematoxylin and Eosin stain), (Mag ×40). Key: i = Therapeutic; ii = Parasite control; iii = Prophylactic iv =Healthy control ;
v = Extract control. Hepatocellular degeneration. VCU= Vaculation.

IV. Discussion

Anaemia is a key feature of trypanosome infections and the severity had been linked to the level of parasitaemia [9], A measurement of anaemia often provides useful information on the severity of disease like trypanosomiasis. The significant decrease in RBC of the *T. brucei brucei* infected rats is in agreement with earlier reports [10, 11] in trypanosome-infected animals. This had been attributed to the release of hemolytic factors by dead trypanosomes into the animals blood thereby causing destruction of erythrocytes and hence, reduction in RBC [12, 13]. It has also been reported that anaemia may be caused by erythrocyte injury caused by lashing action of trypanosome flagella, undulating pyrexia, platelet aggregation, toxins and metabolites from trypanosomes, lipid peroxidation and malnutrition [14]. The increase in RBC after treatment with 64 mg/kg in the therapeutic group may be due to its ability to eliminate parasites from the blood, probably by reaching the site of action or rapid metabolization [10, 15]. Also, the antioxidant activity of the plant [16] may have contributed to the increase, as studies have demonstrated the ability of vitamins to ameliorate anaemia in trypanosome-infected rat.

Oxidative stress is one of the earliest events in the pathogenesis of trypanosomiasis, however, triterpenoids have been found to facilitate antioxidant response and reduce inflammation in several models [17], hence the triterpenoid contained in the plant used might have been responsible reduced inflammation in the organs examined. The regulation of parasitaemia in both the therapeutic and prophylactic groups could be as a result of the chemical components of plant been able to suppress parasite multiplication as opposed to what was seen in the positive control group, which had high parasitaemia and early mortality of rats.

V. Conclusion

In conclusion, the plant had shown *in vivo* antitrypanosomal activity against *T.brucei brucei* as evidences from the results revealed it`s efficacy on the parasitaemia level, increased life span of the treated rats beyond that of the untreated rats. Thus, the anti-trypanosomal activity of *T. macroptera* may be attributed to its high antioxidant activity and its terpenoids, phenol, flavonoids and alkaloid contents as revealed by gas chromatography mass spectrometer,

Reference

[1] Jamonneau, V., Ravel, S., Koffi, M., Kaba, D., Zeze, D.G., Ndri, L., Sane, B., Coulibaly, B., Cuny, G. and Solano, P. (2004). Mixed infections of trypanosomes in tsetse and pigs and their epidemiological significance in a sleeping sickness focus of Cote d'Ivoire. Parasitology, 129, 693–702.

[2] Krafsur, E.S. (2009). Tsetse flies: genetics, evolution, and role as vectors. Infect. Genet. Evol. 9, 124–141.

[3] Fenwick, A., Molyneux, D. and, Nantulya, V., (2005). Achieving the millennium development goals. Lancet 365, 1029–1030

[4] Atawodi. S.E, Ameh. D.A. Ibrahim. S, Andrew. J.N, Nzeilibe. H.C, Oniyike. E., (2002). Indigenous knowledge system for treatment of trypanosomiasis in Kaduna state of Nigeria. Journal of Ethnparmarmacol.,**79**:279-282.

[5] Pham, A. T., Malterud, K. E., Paulsen, B. S., Diallo, D.and Wangensteen, H. (2011). DPPH radical scavenging and xanthine oxidase inhibitory activity of Terminalia macroptera leaves. Journal of Natural Product Communication,**6**:1125–1128.

[6] Nongonierma, R., Proliac, A., Raynaud, J., (1990). O-glycosyl flavonoids from the flowers of Terminalia macroptera Guill and Perr (Combretaceae). Pharmaceutica Acta Helvetica 65, 233–235.

[7] Conrad, J., Vogler, B., Klaiber, I., Reeb, S., Guse, J. H., Roos, G.and Kraus, W.(2001). Vanillic acid 4-O-β-D-(6'-O-galloyl) glucopyranoside and other constituents from the bark of Terminalia macroptera Guill. et Perr. National Product Letters journall1**5**:35–42

[8] Fenwick, A., Molyneux, D. and Nantulya, V.(2005). Achieving the millennium development goals. Lancet, 365, 1029–1030.

[9] Pham, J. L., Glaszmann, J. C., Sano, R., Barbier, P., Ghesquiqre, A., and Second, G. 1990. Isozyme markers in rice: genetic analysis and linkage relationships. Genome, 33,348-359.

[10] Coustou, V., Plazolles, N., Guegan, F. and Baltz, T. (2012). Sialidases play a key role in infection and anaemia in Trypanosoma congolense animal trypanosomiasis. Cell Microbiol; 14(3): 431-445.

[11] Oduola, T., Bello, I., Adeosun, G., Ademosun, A., Raheem, G. and Avwioro, G. (2010). Hepatotoxicity and nephrotoxicity evaluation in Wistar albino rats exposed to Morinda lucida leaf extract. N Am J Med Sci; 2(5): 230-233.

[12] Faremi, A.Y, Ekanem J.T. (2011). Haematological parameters and enzyme studies in Trypanosoma brucei-infected rats reared on Nigella sativa oil-based diet. Asian J Biochem; 6: 90-97.

[13] Atawodi., S.E, Bulus, T. and Mamman, M. (2011). Bioassay guided fractionation and anti-trypanosomal effect of fractions and crude aqueous and methanolic extracts of Terminalia avicennioides (Guill. & Perr.) parts. Int J Biol; 3(3): 19-30.

[14] Olukunle JO, Abatan MO, Soniran OT, Takeet MI, Idowu OA, Akande FA, et al. (2010) In vivo antitrypanosomal evaluation of some medicinal plant extracts from Ogun State, Nigeria. Sci World J; 5(10): 17-19.

[15] Mbaya, A., Kumshe, H. and Nwosu, C.O. (2012). The mechanisms of anaemia in trypanosomosis: A review. In: Silverberg S, editor. Anemia. InTech: Croatia; p. 269-282.

[16] Wurochekke AU, James DB, Bello MI, Ahmodu A. Trypanocidal activity of the leaf of Guira senegalensis against Trypanosoma brucei brucei infection in rats. J Med Sci 2005; 5: 1-4.

[17] Janakiraman, N., Sahaya, S.S. and Johnson, M. (2012). Anti-bacterial studies on Peristrophe bicalyculata (Retz.) Nees. Asian Pac J Trop Biomed 1: 2.

[18] Antia, R.E., Olayemi, J.O., Aina, O.O. and Ajaiyeoba E.O. (2009). In vitro and in vivo animal model antitrypanosomal evaluation of ten medicinal plant extracts from southwest Nigeria. Afr J Biotechnol, 8(7): 1437-1440

Balogun, J.B. "In Vivo Anti-Trypanosomal Potential Of Methanol Root Extract Of Terminalia Macroptera (Guill. And Perr.) In Trypanosoma Brucei Brucei Infected Wistar Rat." IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) , vol. 12, no. 5, 2017, pp. 12–17.