Journal of Agriculture and Veterinary Sciences
Volume 5, Number 2, 2013
ISSN: 2277-0062

In vivo Efficacy of Aqueous Extract of *Citrullus lanatus* Leaf on *Trypanosoma brucei* Infected Albino Rats

[1]BIU, A.A., [2]BURATAI, L.B., [2]ONWUATOGWU, J., [1]MOHAMMED, A., [2]AGADA, N.O. AND [1]KONTO, M.

[1]Department of Veterinary Microbiology and Parasitology, University of Maiduguri, Nigeria.
[2] Department of Biochemistry, University of Maiduguri, Nigeria.
E-mail: biuvet@yahoo.com

ABSTRACT

This study was conducted to determine the *In vivo* activity of *Citrullus lanatus* leaf aqueous extract on *Trypanosoma brucei* infected albino rats. Parasitaemia was evaluated using the rapid matching technique. The parasites were detected in the peripheral blood of infected albino rats 2 days post infection, with the level of parasitaemia in all graded doses of the extract decreasing significantly ($p < 0.05$) from day 4 up to the 10^{th} day post infection compared with the positive control which had a corresponding increase in parasitaemia. There was complete clearance of parasitaemia in rats administered diminazene aceturate (berenil^{R}) on day 4 post infection. In conclusion, the level of parasitaemia was positively correlated to the dose of extract.

Keywords: Efficacy, *In vivo, Citrullus lanatus*, *Trypanosoma brucei*, Albino Rats.

Introduction

Trypanosoma brucei, a haemoprotozoan parasite poses a major threat to man and his livestock and the disease it causes is characterized by anaemia, reproductive failure, endocrine, cardiac and kidney dysfunctions (Anosa, 1988). Chemotherapy and chemoprophylaxis are the most widely used methods of trypanosomosis control, with the few available trypanocides associated with problems of severe side effects, and require lengthy parenteral administration, lack efficacy, development of resistance and toxicity and high cost (Legros *et al*., 2002; Alli *et al*., 2011; Mann *et al*., 2011).

Plants and their products may provide the much needed clue for the emergence of the long awaited new generation of trypanosomal drugs, as several Nigerian medicinal plants were evaluated for their *In vivo* antitrypanosomal activity in mice (Abubakar *et al*., 2005; Ibrahim *et al*., 2008; Ogbadoyi *et al.,* 2007; Mann *et al*., 2009; 2011).

Copyright © 2013 Cenresin Publications
www.cenresinpub.com

The plant *Citrullus lanatus* from the family *Cucurbitaeceae*, with a 'least concern' conservation status (Raimondo *et al*., 2009) is restricted to Africa and parts of Asia, and widely used in traditional herbal medicine to treat bacterial and fungal diseases (Laghetti and Hammer, 2007); hence the need for this study to evaluate its antitrypanosomal potential in albino rats.

Materials and Methods

Experimental Albino Rats: The albino rats used in this study were obtained from the animal house, Federal University of Technology, Yola, Nigeria. They were allowed to acclimatize in plastic cages for 2 weeks in the Postgraduate Veterinary Parasitology Laboratory, University of Maiduguri. They were fed daily with pelletized commercial feed (Vital Feeds, Jos, Nigeria Plc.) and all had access to clean water *ad libitum* until the commencement of the experiment. All albino rats were handled in accordance to the international accepted principles for laboratory animal use and care as recommended by the declaration of Helsinki (WMA-APS, 2002).

Plant Collection and Authentication: *Citrullus lanatus* leaves were obtained from a commercial farm at Ynakari village in Borno State, and identified by a plant taxonomist of the Department of Biological Sciences, University of Maiduguri. The leaves were rinsed in clean tap water to remove dirt, and cut into small bits, and allowed to air dry under shade and ground into fine powder to obtain a 205g weight which was extracted by cold maceration for 24hrs in 1000ml of distilled water (ratio 1:1 w/v). The mixture was filtered with a muslin cloth (2mm) and later with Whatman filter paper (No. 1). The resulting solution was evaporated on a water bath at 50^0C to obtain a 76g dry powder as extract with 62.9% w/w yield. Different doses of the extracts used for this study were reconstituted using distilled water.

***Trypanosoma brucei* Stock**: This was obtained from the Nigerian Institute for Trypanosomosis Research (NITR), Vom, Plateau State, Nigeria. The potency of *T. brucei* in albino rats was determined by wet film mount under light microscope at x40 magnification.

Experimental Infection of Albino Rats, Extracts Administration and Determination of Parasitaemia: An estimated average number of parasite/1000 RBC was obtained using the 'Rapid Matching Technique' as described by Herbert and Lumsdem, (1976). The experimental albino rats weighing between 90 and 160grams were randomly divided into 6 groups of 5 each as indicated below:

Groups A, B and C (Extract Control): Each group was inoculated intraperitoneally with 1ml of infected blood in phosphate glucose buffered saline containing 4 x 10 parasites / ml; and then were administered with graded extract doses of 200, 400 and 600mg/kg respectively.

Group D (Positive Control): This group was infected as above and treated with 3.5mg/kg of diminazene aceturate (Veriben®).

Group E (Negative Control): This group was infected as above but not treated.

Group F (Normal Control): This group was given Vital® feeds and portable water *ad libitum*.

To determine parasitaemia levels, blood films were made from the tail of each infected albino rat every 2 days post infection and trypanosome counts determined by wet mount examination microscopically at x40 magnification using the "rapid matching" technique.

Statistical Analysis: Parasitaemia counts for each experimental group was summed up as mean ± standard deviation post infection and analyzed using the one way analysis of variance (ANOVA) with values equal to or less than 0.05 regarded as significant (GraphPad, 2003).

Results

The results of this study on the *In vivo* efficacy of *C. lanatus* leaf aqueous extract in *T. brucei* infected albino rats is indicated in Table 1. Parasitaemia was first seen in all infected groups on day 2 post infection; with a significant reduction ($p < 0.05$) in parasite counts in all extract treated groups with mean ± SD values of 0.09 ± 0.03, 0.06 ± 0.08 and 0.04 ± 0.08 at 10 days post infection for 200, 400 and 600mg/kg graded doses respectively, compared with the infected untreated (E) group with 3.2 ± 0.05 ($p < 0.05$). There was a total parasite clearance on day 6 post infection in the Veriben® treated group.

Table 1: Mean ± SD (Range) of Parasitaemia Levels of Experimental Albino Rats

Groups/Graded	Days of Post Infection					
Dose ($MgKg^{-1}$)	0	2	4	6	8	10
A	0	0.98±0.29	0.80±0.48	0.17±0.03	0.11±0.01	0.09±0.03
		(0.80-1.45)	(0.55-0.63)	(0.75-1.45)	(0.10-0.12)	(0.80-0.10)
B	0	0.52±0.11	0.55±0.04	0.13±0.03	0.12±0.01	0.11±0.01
		(0.37-0.65)	(0.51-0.60)	(0.10-0.15)	(0.11-0.13)	(0.46-0.13)
C	0	0.55±0.31	0.16±0.03	0.17±0.04	0.09±0.01	0.06±0.08
		(0.15-0.85)	(0.12-0.19)	(0.15-0.21)	(0.08-0.10)	(0.08-0.10)
D	0	0	0	0	0	0
E	0	1.61±0.27	1.70±0.13	1.72±0.15	2.9±0.07	3.20±0.05
		(1.15-1.85)	(1.55-1.85)	(1.55-1.85)	(2.85-2.95)	(2.02-3.30)
F	0	0	0	0	0	0

B = Infected treated with 400$mgkg^{-1}$ of extract

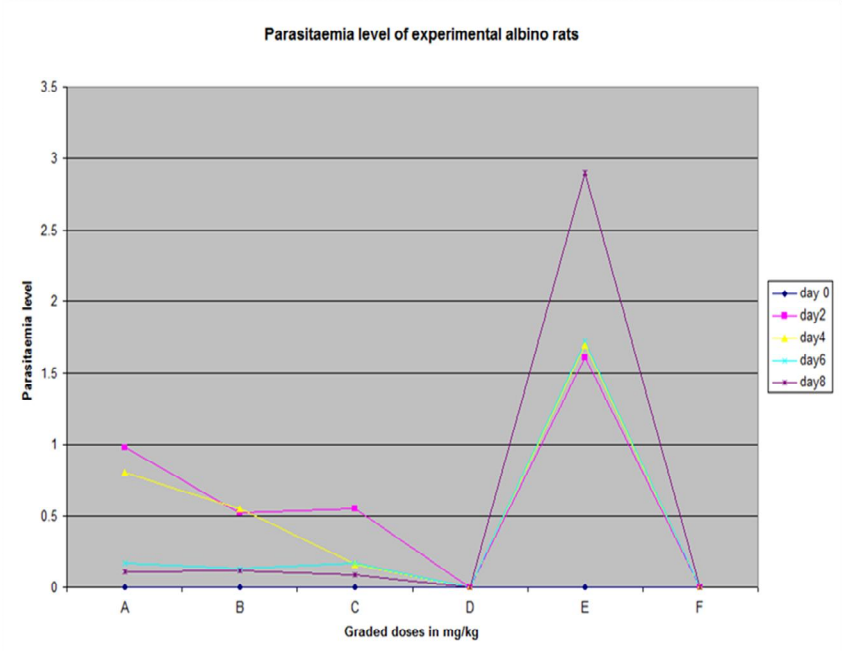
C = Infected treated with 600$mgkg^{-1}$ of extract

D = Infected treated with 1$mgkg^{-1}$ of Veriben®

E = Infected untreated

F = Uninfected untreated

N = 5



A = Infected treated with 200mgkg-1 of extract
B = infected treated with 400mgkg-1 of extract
C = infected treated with 600mgkg-1 of extract
D = infected treated with 1mgkg-1 of Veriben®
E = infected untreated
F = uninfected untreated

Figure 1:

Discussion

In this study, trypanosomes were observed in the infected albino rats from day 2 post infection. There was a significant reduction ($p < 0.05$) in parasitaemia with a mean ± SD of 0.04±0.08 observed in the extract doses of 600mg/kg, followed by 0.06±0.08 and 0.09±0.03 for 400 and 200mg/kg as compared with the negative control value of 3.2±0.05 on day 10 post infection. This has shown that *Citrullus lanatus* leaf aqueous extract contained active antitrypanosomal compounds against *Trypanosoma brucei.* Carver, (1973) reported that a 50% reduction in parasitaemia is an indication of significant activity of a trypanocide. This is also consistent with reports of Asuzu and Chineme, (1990) and Alli *et al*., (2011) who demonstrated significant reduction in parasitaemia levels following the administration of *Morinda lucida* leaf extracts. The mechanism of exhibiting antitrypanosomal activity could be attributed to interference with the redox balance of the parasites, when the extract components acts either on the respiratory chain or on their cellular defenses against oxidative stress, because these extract phytocomponents possess structures capable of generating radicals that cause peroxidase damage to trypanothione reductase which is very sensitive to disturbances in redox balance; while other phytocomponents bind to the kinetoplast DNA of the parasites (Sepulveda and Cassels, 1996; Alli *et al*., 2011).

Also the trypanocidal or trypanostatic efficacy of plant extracts have been associated with the presence of biologically active components such as alkaloids, saponins, flavonoids, anthraquinones and tannins which act individually or synergistically in trypano-suppressive activity (Atawodi and Ogunbusola, 2009; Atawodi *et al*., 2002).

References

Abubakar, A., Iliyasu, B., Yusuf, A.B., Onyekwelu, N.A., Igweh, A.C., Shamaki, B.U., Afolayan, D.O. and Ogbadoyi, E.O. (2005). Antitrypanosomal and Haematological Effects of Selected Nigerian Medicinal Plants in Wistar Rats. *Biokemistri*, 17: 95-99.

Alli, L.A., Okochi, V.I. and Adesokan, A.A. (2011). Antitrypanosomal Activity and Haematological Effects of Aqueous Extract of Leaves of *Morinda lucida* on *Trypanosoma brucei brucei* Infected Rats. *Asian J. Pharm. Hea. Sci*. 1(3): 111-115.

Anosa, V.O. (1988). Haematological and Biochemical Changes in Human and Animal trypanosomosis. Part II. *Revue Elev. Med. Vet. Pays. Trop*. 41 (2): 151-164.

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.

Atawodi, S.E. and Ogunbusola, F. (2009). Evaluation of Antitrypanosomal Properties of Four Extracts of Leaves, Roots and Stem Barks of *Prosopis africana* in Laboratory Animals. *Biokemistri*, 21 (2): 101-108.

Atawodi, S.E., Ameh, D.A., Ibrahim, S., Andrew, J.N., Nzelibe, H.C., Onyike, E., Anigo, K.M., Abu, E.A., James, D.B., Njoku, G.C. and Sallau, A. (2002). Indigenous Knowledge System for Treatment of Trypanosomiasis in Kaduna State of Nigeria*. J. Ethnopharmacol*. 79: 279-282.

Carver, R. (1973). *Chemotherapy of helminthiasis*. Pergamon Press, 1: 101-115.

GraphPad Instat (2003). *Guide to Choosing and Interpreting Statistical Tests*. GraphPad Software Inc. San Diego, California, USA. 154pp.

Herbert, W.J. and Lumsden, W.H.R. (1976). *Trypanosoma brucei*: A "Rapid Matching" Method for Estimating the Host's Parasitaemia. *Experimental Parasitology*, 40:427-431.

Ibrahim, M.A. Njoku, G.C. and Sallau, A.B. (2008). *In vivo* Activity of Stem Bark Aqueous Extract of *Khaya senegalensis* Against *Trypanosoma brucei*. *Afr. J. Biotech.* 7 (5): 661-663.

Laghetti, G. and Hammer, K. (2007). The Corsican citron melon [*Citrullus lanatus* (Thunb.) Matsumura and Nakai *subsp. lanatus var. citroides* (Bailey) Mansf.ex.Greb.] A Traditional and Neglected Crop. *Genetic Resources and Crop Evolution.* 54: 913-916.

Legros, D., Ollivier, G., Gastellu- Etchegorry, M., Paquet, C., Burri, C., Jannin, J. and Buscher, P. (2002). Treatment of Human African Trypanosomiasis-Present Situation and Needs for Research and Development. *The Lancet Infection Diseases*. 2: 437-440.

Mann, A., Egwim, E.C., Banji, B., Umar, A.N., Gbate, M. and Ekanem, J.T. (2009). Efficacy of *Dissotis rotundefolia* on *Trypanosoma brucei brucei* Infection in Rats. *Afri. J. Biochem. Res*. 3 (1): 005-008.

Mann, A., Forajimi, O.R., Adewoye, A.T., Ukam, C., Udeme, E.E., Okorie, I.I., Sakpe, M.S., Ibrahim, D.R., Yahaya, Y.A. and Ogbadoyi, E.O. (2011). *In vivo* antitrypanosomal Effects of Some Ethnomedicinal Plants from Nupeland of North Central Nigeria. *Afr. J. Tradit. Complement. Altern. Med*. 8(1): 15-21.

Ogbadoyi, E.O., Akinsunbo, A.O., Adama, T.Z. and Okogun, J.L. (2007). *In vivo* Trypanocidal Activity of *Annona senegalensis* leaf extract against *Trypanosoma brucei brucei. J. Ethnopharmacol*. 112: 85-89.

Raimondo, D., Von Staden, L., Fodem, W., Victor, J.E., Helme, N.A., Turner, R.C., Kamundi, D.A. and Manyama, P.A. (eds) (2009). Red List of South African Plants. *Strelitzia 25.* South African Natural Biodiversity Institute, Pretoria.

Sepulveda-Boza, S. and Cassels, B.K. (1996). Plant Metabolites Active Against *Trypanosoma cruzi. Planta med*. 62: 98-105.

World Medical Association- American Physiological Society WMA (2002). Guiding Principles for Research Involving Animals and Human Beings. *Am. J. Physiol. Regul. Intergr. Comp. Physiol*. 283 (2): R281-R283.

References to this paper should be made as follows: Biu, A.A. *et al.,* (2013), *In vivo* Efficacy of Aqueous Extract of *Citrullus lanatus* Leaf on *Trypanosoma brucei* Infected Albino Rats. *J. of Agriculture and Veterinary Sciences,* Vol. 5, No. 2, Pp. 57 – 63.