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Trypanocidal Properties of Aqueous Leaf Extract of *Morinda lucida*

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ABSTRACT

This study was designed to evaluate the trypanocidal properties of hot and cold aqueous leaf extracts of *Morinda lucida* in mice infected with *Trypanosoma brucei brucei*. The hot aqueous extracts of the leaf at 100, 200, 300, 400 and 500 mg kg⁻¹ body weight per day were administered to the test groups intraperitoneally 72 h post infection. The negative control was treated with normal saline. The most effective dose (400 mg kg⁻¹ b.wt.) of both hot and cold extracts were administered on another group of infected mice to re-ascertain its efficacy while 3.5 mg kg⁻¹ body weight single dose of berenil was administered on positive control group. To establish the safety of the extract at higher doses, 1000 mg kg⁻¹ body weight of both hot and cold extracts were administered on parasite-free mice, while 400 mg kg⁻¹ body weight of normal saline was given to the negative control group. The level of parasitemia, variation in weight and percentage Packed Cell Volume (%PCV) in the different groups were monitored throughout the period of study. The mice treated with 400 mg kg⁻¹ body weight of hot and cold extract lived upto 34 and 50 days post infection with minimal and zero level of parasitemia, respectively while mice in the negative control died on the 12th and 14th day post infection. There was significant (p<0.05) improvement in packed cell volume and weight of the treated mice after the period of treatment. No signs of toxicity were observed in the group administered 1000 mg kg⁻¹ body weight. The results obtained in this study show that aqueous extract of *Morinda lucida* possesses antitrypanosomal activity.

Key words: *Morinda lucida*, *Trypanosome brucei brucei*, berenil, trypanocidal, toxicity, parasitemia

INTRODUCTION

Trypanosomiasis is an important protozoan disease of domestic animals and man in most parts of Africa (Igbokwe, 1989). The malady has been categorized among the list of major diseases, facing mankind by World Health Organisation (Mhlanga, 1996). In developing countries such as Africa, sleeping sickness caused by Trypanosome is one of the major hindrances to livestock production whose abolition and control is mainly based on chemoprophylaxis and chemotherapy that is confronting with problems of drug resistance, toxicity and inadequate drugs/expensive (Gutteridge, 1985).

Decline rate with melarsoprol in southern Sudan, northern Uganda and northern Angola was about 30% (Ogbadoyi *et al.*, 2007) and most of the accessible drugs are highly toxic with about 5% of those treated with melarsoprol dying due to the high toxicity of the drug (Ogbadoyi *et al.*, 2007). In addition to these shortcomings is the problem of scarcity of the drugs in rural areas where the burden of the disease is more manifest and when the drugs are available, the cost is unaffordable.

In spite of efforts made by the World Health Organization (WHO) to assist new treatments, there have been few new drugs in recent times. Vaccination for Trypanosomiasis still remains the most excellent speculative option in the fight against a disease that is constantly hovering between its wildlife reservoir and its reservoir in man and domestic animals. While antigenic variation of the parasite surface coat has been considered the major obstacle in the development of an efficient vaccine, current research into the biology of B cells has showed that the problems might go extra than that (Magez *et al.*, 2010). As a result of these apparent impediments and the non accessibility and affordability of the few available trypanocidal drugs, majority of the world's population especially in Africa depends on traditional medical remedies for this infection and it is estimated that some 20,000 species of higher plants are used medicinally throughout the world (Phillipson and Wright, 1991).

Morinda lucida is a tropical plant of the family Rubiaceae (Asuzu and Chineme, 1990). The roots of *M. lucida* together with leaves of *Carica papaya*, *Magnifera indica* and *Cassia podocarpa* boiled together and drink twice daily are used for treatment of malaria fever in Nigeria (Gbile, 1986). Adewunmi and Adesogan (1984) have also isolated some anthraquinones from *M. lucida* such as damnacanthol, nordamnacantol, morindin and rubiacin. There are many studies that reported the antimalarial activities of *M. lucida* (Antimalarial activity of extracts of the plant parts; leaf, stem bark and root) (Asuzu and Chineme, 1990; Makinde and Obih, 1985). Antimalarial effects of the petroleum ether extract and fractions of the leaf samples against *Plasmodium falciparum* using the Rabbit *in vivo* study has been reported by Awe and Makinde (1998). However, little has been done as regard the antitrypanosomal activities of this plant as compared to its antimalarial activities despite the causative organisms are of the same class protozoa.

The objective of this study is to evaluate the trypanocidal activity of both crude hot and cold aqueous leaf extract of *M. lucida*, its periodical effect on pack cell volume and body weight in albino mice during the two weeks of treatment in three different stages of experimental set up.

MATERIALS AND METHODS

Plant collection: Fresh leaves of *Morinda lucida* (Brimestone tree) were collected from Oba-ile, Olorunda Local Government Area of Osun State Nigeria between the months of May and June, 2008. The plant was identified by a Botanist at the National Institute for Pharmaceutical Research and Development, Abuja, Nigeria. The whole study was carried out in the Biochemistry laboratory of Federal University of Technology, Minna, Nigeria.

Experimental animals: Albino mice, used for screening, were purchased from the Biochemistry and Chemotherapy division of the National Institute for Trypanosomiasis and Onchocerciasis Research, Vom, Plateau State, Nigeria. The animals were acclimatized in the Department of Biochemistry laboratory, Federal University of Technology, Minna for minimum two weeks prior to study. All experiments involving the animals were conducted in compliance with the internationally accepted principles for laboratory animal use and care, as contained in the Canadian Council on Animal Care guidelines on animal use protocol review in 1997 and as described by Kabiru *et al.* (2010).

Parasites: *Trypanosoma brucei brucei* was obtained from the National Institute for Trypanosomiasis and Onchocerciasis Research (NITER), Kaduna, Kaduna State, Nigeria and subsequently maintained in the laboratory of the Biochemistry Department, Federal University of Technology, Minna, Niger State, Nigeria, by serial passage in mice.

Preparation of plant extracts: The hot extraction was carried out by subjecting 50 g of powdered sample to successive extraction with hexane, ethyl acetate, ethanol and water under reflux. For the cold extraction 50 g of the sample was directly soaked in 400 mL of distil water for 72 h. Both extracts were filtered using cheese cloth and solvent was removed using rotary evaporator followed by drying on steam bath. The dry extract was transferred into a sterile sample bottle and stored in a refrigerator until required for use.

Infection of animals: The animals were inoculated using the method described by Ogbadoyi *et al.* (2007). Blood from a highly infected mouse was obtained by cardiac puncture and collected with EDTA-coated syringe. The blood was appropriately diluted with physiological saline to serve as inoculums. Healthy mice of weight range 25-35 g were infected intraperitoneally with 0.1 mL of the inoculums containing about 1×10^8 trypanosomes.

Preparation of stock solution of extract: The stock solution was prepared just before use by dissolving 1 g of the aqueous extract in 10 mL physiological saline.

Administration of crude extract and monitoring the course of parasitemia: Rapid matching method of Herbert and Lumsden (1976), as described by Atawodi *et al.* (2003), Atawodi (2005), was used to estimate parasite in the blood of the infected animals. The method involves a matching technique where the microscopic fields were compared with a range of standard logarithmic values. Counting of parasites per field was done in blood approximately diluted with physiological saline. A drop of blood was obtained on a slide by pinching the tip of the pre-sterilized tail with a sterile needle, immediately covered with a cover slip and then observed under the microscope at 40X magnification. The number of trypanosomes per microscopic field was compared with the table of logarithmic values. The logarithmic values which matched the microscopic observation were then converted to antilogarithm, from where the absolute number of trypanosomes per milliliter of blood was obtained.

The first phase of the experiment was the preliminary screening for trypanocidal activity of aqueous extract of *M. lucida* (leaf) using five groups; with each consisting of three infected mice. Groups A, B, C, D and E were administered intraperitoneally 100, 200, 300, 400 and 500 mg kg⁻¹ body weight per day of the extracts, respectively for 14 consecutive days commencing 72 h post infection while group D was infected but treated with physiological saline of the highest dose equivalent (500 mg kg⁻¹ b.wt.) to serve as negative control.

The second and third phases of the experiment included the screening of the most effective dose of the extract from the first phase of the experiment. Therefore, 400 mg kg⁻¹ b.wt. of both hot and cold aqueous leaf extracts were given intraperitoneally to infected mice along with a single dose of berenil (3.5 mg kg⁻¹ b.wt., to the positive control group) and 400 mg kg⁻¹ b.wt. of physiological saline to the negative control group. Another group was left uninfected but treated with 1000 mg kg⁻¹ b.wt. of both extracts to establish the toxicity of the extract at higher dose.

Estimation of percentage packed cell volume: In the second and third phases of the experiment, the blood samples of the animals were collected into heparinized capillary tubes with one end of the tube sealed with plasticine and were spun at 2000G for 5 min in a micro-haematocrit centrifuge. The PCVs were determined in the first and second week with the aid of a micro-haematocrit reader and the values expressed as percentages.

Determination of body weight: Body weights of all the mice in each group of the second and third phase of the experiments were taken before the commencement of the experiment, after first and second week of medications and compared with their initial weights.

Statistical analysis: Data was analyzed using analysis of variance (ANOVA). Significant differences between means were determined at 5% level using Duncan Multiple Range Test (SPSS16version).

RESULTS

Preliminary screening of trypanocidal activities of hot aqueous leaf extract of *Morinda lucida*: The trypanocidal activity of the extract at various doses used is represented in Fig. 1. It shows that the group treated with 400 mg kg⁻¹ b.wt., survived for 33 days with the least level of parasitemia (15.2×10⁶ mL⁻¹) while negative control group died five days into the study.

Trypanocidal activities of the most effective dose (400 mg kg⁻¹ b.wt.) hot aqueous leaf extract of *Morinda lucida*: The most effective dose (400 mg kg⁻¹ b.wt.) of hot aqueous leaf extract of *Morinda lucida* in Fig. 2 indicate the clearing of parasite from circulation after 2 weeks of treatment with live span of 34 days.

Trypanocidal activity of cold aqueous leaf extract of *Morinda lucida*: Trypanocidal activity of cold aqueous leaf extract of *Morinda lucida* was observed. The trypanocidal activity of 400 mg kg⁻¹ b.wt. of cold aqueous leaf extract of *Morinda lucida* is shown in Fig. 3. The parasites were cleared from circulation after 2 weeks of treatment with live span extending to 60 days post infection.

Effect of cold and hot aqueous leaf extract of *Morinda lucida* on body weight: The effect of both hot and cold aqueous leaf extracts of *Morinda lucida* on body weight of the treated animals are shown in Table 1. Significant (p<0.05) reduction in body weight was observed in the negative control group on day 7 while the treated groups experienced increase in weights after the 14th day of treatment.

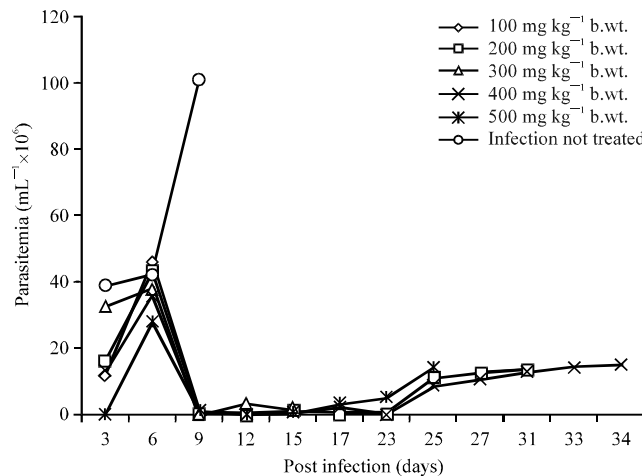


Fig. 1: Preliminary trypanocidal screening of aqueous leaf extract of *Morinda lucida*

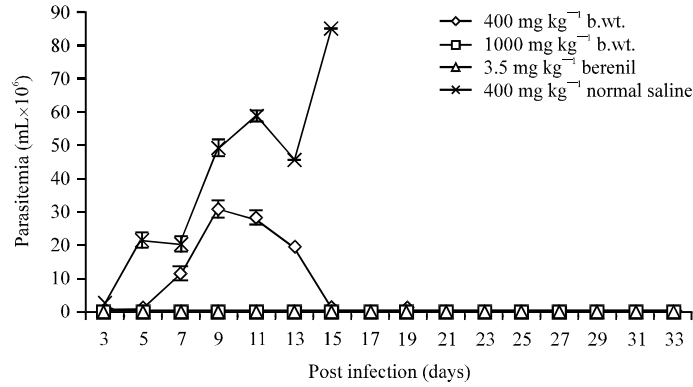


Fig. 2: Trypanocidal activity of hot aqueous leaf extracts of *Morinda lucida* in *T. brucei brucei* infected mice

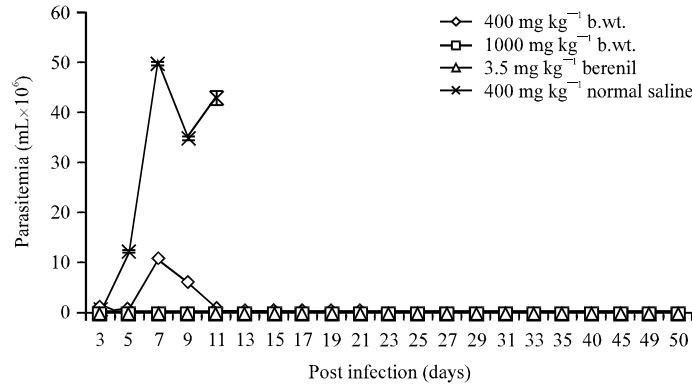


Fig. 3: Trypanocidal properties of aqueous leaf cold extract of *Morinda lucida* in *T. brucei brucei* infected mice

Table 1: Effect of hot and cold aqueous leaf extract of *Morinda lucida* on body weight

Group	Weight (g)		
	Day 0	Day 7	Day 14
Hot aqueous leaf extract			
A	25.00±0.60	23.18±0.92 ^a	25.23±0.97 ^a
B	25.75±0.24	27.02±1.00 ^b	29.80±0.95 ^b
C	25.20±0.92	26.34±0.98 ^c	27.20±0.95 ^c
D	25.15±0.97	21.15±0.98 ^d	Dead
Cold aqueous leaf extract			
A	25.05±0.61	23.18±0.53 ^c	25.20±0.56 ^c
B	26.00±0.92	27.02±0.57 ^a	29.80±0.56 ^a
C	25.30±0.75	26.34±0.57 ^b	27.20±0.55 ^b
D	25.20±0.69	23.15±0.56 ^d	Dead

Values are Mean±SD, n = 3. Values with different alphabetical superscripts along the same column are significantly different at p<0.05. A: 400 mg kg⁻¹ b.wt., B: 1000 mg kg⁻¹ b.wt. (not infected), C: 3.5 mg kg⁻¹ b.wt. berenil, D: 400 mg kg⁻¹ b.wt. Normal saline (Negative control)

Table 2: Effect of hot and cold aqueous leaf extract of *Morinda lucida* treatment on percentage packed cell volume (%PCV)

Group	PCV (%)		
	Day 0	Day 7	Day 14
Hot aqueous leaf extract			
A	43.50±1.00	31.00±1.00 ^b	40.00±1.50 ^b
B	45.00±0.50	43.00±2.00 ^{ac}	46.00±3.00 ^a
C	45.00±0.50	38.00±2.00 ^{c,d}	39.00±1.50 ^c
D	43.00±1.00	30.00±1.50 ^d	Dead
Cold aqueous leaf extract			
A	44.00±0.57	42.83±0.16 ^b	43.50±0.50 ^b
B	45.50±0.28	47.33±0.44 ^a	46.50±1.00 ^a
C	40.00±0.58	41.00±0.57 ^c	40.50±0.50 ^c
D	48.67±0.92	24.67±0.44 ^d	Dead

Values are Mean±SD, n = 3. Values with different alphabetical superscripts along the same column are significantly different at p<0.05.

A: 400 mg kg⁻¹ b.wt., B: 1000 mg kg⁻¹ b.wt. (not infected), C: 3.5 mg kg⁻¹ b.wt. berenil, D: 400 mg kg⁻¹ b.wt. Normal saline (Negative control)

Effect of hot and cold aqueous leaf extract of *Morinda lucida* treatment on percentage Packed Cell Volume (%PCV): The percentage packed cell volume of the animals treated with hot and cold aqueous leaf extracts of *Morinda lucida* are shown in Table 2. The %PCV was drastically reduced on day 7 compared with the initial level but there was significant improvement on the 14th day of the treatment. The %PCV stabilized afterwards.

DISCUSSION

The results obtained in this study, for both the first and second stages, demonstrated the efficacy of the hot and cold aqueous extracts of *Morinda lucida* in clearing the parasites from the blood of *Trypanosoma brucei brucei*-infected mice, especially when administered at a dose of 400 mg kg⁻¹ body weight/day. It also demonstrated the fact that the treatment period must be extended to two weeks to accomplish complete clearance of parasites and elongation of life span (Fig. 1-3). We could infer from these observations that the efficacy of the extracts is dose and time dependent. Antitrypanosomal activities of plants of different families have been reported by Freiburghaus *et al.* (1996).

The extracts were also found to reverse two principal symptoms of trypanosomiasis, viz., weight loss and anemia (characterized by reduction in %PCV). Mice treated with 400 mg kg⁻¹ body weight per day had their body weights and %PCV stabilized after treating them for two weeks. Although, the extracts did not perform to the same level with the standard drug, their activity against trypanosomes in circulation is appreciable. It provides a lead to further investigation of the plant for drug development.

The actual mechanism by which the extract exhibited their trypanocidal activity is not known but Sepulveda-Boza and Cassels (1996) suggested that many natural products exhibit their trypanocidal activity through interference of redox balance of the parasite acting on their respiratory chain or their cellular defense against oxidative stress. This may be so because some natural products possess constituents, capable of generating radicals that may cause peroxidative damage to trypanothione reductase that is very sensitive to alteration in the redox balance. It is also known that some agent act by binding with the kinetoplast DNA of the parasite.

The extract was found to be safe and free of toxicity at a dose level of 1000 mg kg⁻¹ body weight, thus justifying its local use by traditional herbalists at doses that are unquantified.

CONCLUSION

The results of this study demonstrated that *Morinda lucida* possesses considerable trypanocidal activity with no observable toxicity. The plant can be further exploited for use in the treatment of trypanosomiasis.

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REFERENCES

- Adewunmi, C.O. and E.K. Adesogan, 1984. Anthraquinones and oruwacin from *Morinda lucida* as possible agents in fascioliasis and schistosomiasis control. *Fitoterapia*, 55: 259-263.
- Asuzu, I.U. and C.N. Chineme, 1990. Effects of *Morinda lucida* leaf extract on *Trypanosoma brucei brucei* infection in mice. *J. Ethnopharmacol.*, 30: 307-313.
- Atawodi, S.E., 2005. Comparative *in vitro* trypanocidal activities of petroleum ether, chloroform, methanol and aqueous extracts of some Nigerian savannah plants. *Afr. J. Biotechnol.*, 4: 177-182.
- Atawodi, S.E., T. Bulus, S. Ibrahim, D.A. Ameh, A.J. Nok, M. Mamman and M. Galadima, 2003. *In vitro* trypanocidal effect of methanolic extract of some Nigerian savannah plants. *Afr. J. Biotechnol.*, 2: 317-321.
- Awe, S.O. and J.M. Makinde, 1998. Evaluation of sensitivity of *Plasmodium falciparum* to *Morinda lucida* leaf extract sample using rabbit *in vitro* microtest techniques. *Indian J. Pharmacol.*, 30: 51-53.
- Freiburghaus, F., R. Kaminsky, M.H.N. Nkunya and R. Brun, 1996. Evaluation of African medicinal plants for their *in vitro* trypanocidal activity. *J. Ethnopharmacol.*, 55: 1-11.
- Gbile, Z.O., 1986. Ethnobotany, Taxonomy and Conservation of Medicinal Plants. In: *The State of Medicinal Plants Research in Nigeria*, Sofowora, A. (Ed.). University of Ibadan Press, Nigeria, pp: 13-29.
- Gutteridge, W.E., 1985. Existing chemotherapy and its limitations. *Br. Med. Bull.*, 41: 162-168.
- Herbert, W.J. and W.H.R. Lumsden, 1976. *Trypanosoma brucei*: A rapid matching method for estimating the host's parasitemia. *Exp. Parasitol.*, 40: 427-431.
- Igbokwe, I.O., 1989. Dyserythropoiesis in animal trypanosomiasis. *Rev. Elev. Med. Vet. Pays Trop.*, 42: 423-429.
- Kabiru, A.Y., A.A. Salako and E.O. Ogbadoyi, 2010. Therapeutic effects of annona senegalensis pers stem bark extracts in experimental African trypanosomiasis. *Int. J. Health Res.*, 3: 45-49.
- Magez, S., G. Caljon, T. Tran, B. Stijlemans and M. Radwanska, 2010. Current status of vaccination against African trypanosomiasis. *Parasitology*, 137: 2017-2027.
- Makinde, J.M. and P.O. Obih, 1985. Screening of *Morinda lucida* leaf extract for malarial action on *Plasmodium berghei berghei* in mice. *Afr. J. Med. Med. Sci.*, 14: 59-63.
- Mhlanga, J.D., 1996. Sleeping sickness: Perspectives in African trypanosomiasis. *Sci. Progr.*, 79: 183-214.

- Ogbadoyi, E.O., A.O. Abdulganiy, T.Z. Adama and J.I. Okogun, 2007. *In vivo* trypanocidal activity of *Annona senegalensis* Pers. Leaf extract against *Trypanosoma brucei brucei*. *J. Ethnopharmacol.*, 112: 85-89.
- Phillipson, J.D. and C.W. Wright, 1991. 1. Medicinal plants against protozoal diseases. *Trans. R. Soc. Trop. Med. Hygiene*, 85: 18-21.
- Sepulveda-Boza, S. and B.K. Cassels, 1996. Plant metabolites active against *Trypanosoma cruzi*. *Planta Medica*, 62: 98-105.