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Anti Trypanosomal Activity and Haematological Effects of Aqueous Extract of Leaves of *Morinda lucida* on *Trypanososma brucei brucei* Infected Rats

[1]Alli LA*, [2]Okochi VI, [3]Adesokan AA.

[1]Dept of Medical Biochemistry, Faculty of Health Sciences, University of Abuja, Nigeria.
[2]Dept of Biochemistry, College of Medicine, University of Lagos, Nigeria.
[3]Dept of Biochemistry, College of Health Sciences, University of Ilorin. Nigeria

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***Corresponding author:**

E-mail: adewale_alli@yahoo.com

ABSTRACT

Morinda lucida (Rubiaceae) is a tropical plant commonly used in western Nigeria for the treatment of malaria. Aqueous extract of leaves of *Morinda lucida* was investigated for anti trypanosomal activity, using albino rats infected with *Trypanosoma brucei brucei*. The haematological effect of the extract was also studied. Five groups comprising eight rats per group were infected with *T. brucei brucei* using 0.5ml of donor blood corresponding to 3.6 $x10^3$ parasites. Group 1 served as the control and received 10ml/kg body weight of distilled water. Group 2 received 3.2mg/kg body weight of diminazene aceturate (berenil®), while groups 3, 4 and 5 were treated with 0.2ml of extract corresponding to 100, 200 and 400mg/kg body weight respectively, starting from 24hours post infection. Hematological parameters were determined after the rats were sacrificed on 14th day post infection. There was a significant dose-dependent reduction ($p < 0.05$) in the parasite count with 5.5 observed in the dose of 400mg/kg body weight, followed by 10.5 and 22 for 200 and 100mg/kg body weight as compared with the control value of 42.8 on 14th day post infection. The red blood cell count and haemoglobin concentration were observed to be higher in treated rats than in control rats; while the white blood cell count was high in the control group. This study shows that the aqueous leaf extract of *M. lucida* possess trypanocidal properties and could be useful as a source of new trypanocidal agent from medicinal plants

INTRODUCTION

Trypanosomiasis is a protozoan disease caused by the parasite *Trypanosome* of the genus *Trypanosoma*. More than 66million people in 36 countries of sub-Saharan Africa suffer from Human African Trypanosomiasis (HAT) which is caused by either *Trypanosoma brucei rhodesiense* or *Trypanosoma brucei gambiense* [1]. *Trypanosoma brucei brucei* is a protozoan subspecie of *Trypanosoma* and it is the causative agent of African Animal Trypanosomiasis (AAT). It is an asexual digenetic protozoan flagellate with a spindle shaped body, wavelike membrane on one side, a single flagellum and a kinetoplast [2]. The infection is transmitted by the bite of infected tsetse fly of the genus" glossina".

The chemotherapy of Trypanosomiasis is associated with problems of resistance and toxicity[1, 3, 4].The search for vaccine against African Trypanosomiasis remain elusive, probably due to the variant surface glycoprotein(VSG) of the protozoan which makes it capable of evading the host immune system[5]. Four drugs (suramin, pentamidine, melarsoprol and eflornithine) are currently available for treatment of trypanosomiasis [8], with only melarsoprol and eflornithine being effective against the meningoencephalitis that develops in the late stages of the disease. In addition to problem of drug resistance, all four drugs require lengthy, parenteral administration and all except eflornithine have severe toxic side effects [6, 4] There is therefore a need for the development of new, cheap, safe, accessible and affordable drugs for the treatment of African Trypanosomiasis in both humans and animals [7]. Drugs derived from medicinal plants are natural and have been reported to be effective and safe. Moreover among the indigene of trypanosome endemic areas, there are claims of medicinal plants with therapeutic activities [4, 8]. Several plant extracts have been investigated in vivo for trypanocidal activity in mice [8-12].

Morinda lucida is a tropical plant, belonging to the family rubiaceae [9]. The roots of *M. lucida*, together with leaves of *magnifera indica, carica papaya,* and *cassia podocarba* are used for treatment of malaria fever when boiled together and drank twice daily [13]. Adewunmi and Adesogan (1983) [14] isolated some anthraquinones isolated from *M.lucida* such as damnacanthol, nordamnacantol, morindin and rubiacin.

The objective of this study is to evaluate the anti trypanosomal activity of aqueous leaf extract of *M. lucida* and also examine some haematological parameters after administration of this extract on *Trypanosoma brucei brucei* infected rats.

MATERIALS AND METHODS

Experimental animals

Swiss albino rats weighing between 90 – 120g were obtained from the animal house, college of medicine, University of Lagos. They were fed on standard mouse cubes obtained from Pfizer feeds, and water ad libitum.

The animals were observed in a well-ventilated room under 12hr light-dark cycle. All procedures involving animals in this study conform to the guiding principles for research involving animals as recommended by the declaration of Helsinki and the guiding principles in the care and use of animals (15).

Plant collection and identification

Samples of *Morinda lucida* were obtained from Idi-oro, in Mushin Local Government area of Lagos state, Nigeria. It was identified at the botany department of University of Lagos, Akoka, Lagos, Nigeria.

Plant preparation and extraction

Fresh leaves of *Morinda lucida* were collected and rinsed in water to remove dirt. The leaves were cut into small bits and allowed to dry until a constant weight is obtained. The dried leaves were ground into fine powder, 100g of the leaf powder was extracted by cold maceration for 24hrs, in 500ml of distilled water (ratio1:5 w/v). The mixture was filtered with a muslin cloth and later with Whatmann filter paper. The resulting solution was dried on a water bath to obtain a dry powder, which was kept in a clean bottle and stored in a refrigerator until required. Different doses of the extract used for this study were reconstituted from this dry sample using distilled water.

Parasites

The *T. brucei brucei* was obtained from the Nigerian Institute for Trypanosomiasis Research, Vom, Plateau state. The patency of *T. brucei brucei* in the rats was determined by wet film preparation and observed under microscope. An estimate of the average number of parasite per 1000 red blood cells was determined using the "Rapid Matching" method of Herbert & Lumsden (1976) [16]. 0.5ml of donor blood inoculated into each rat intraperitoneally contain 3.6×10^3 parasites.

Administration of extract

The rats were divided into five groups of eight rats per group. Group 1 (control) received 10ml/kg body weight of distilled water. Group 2 received 3.2mg/kg body weight of diminazene aceturate (intramuscular single dose) while groups 3, 4 and 5 were administered 0.2ml of the extract orally daily corresponding to 100, 200 and 400mg/kg body weight doses, respectively. Treatment of infected rats with the extract started 24hour post infection. Weight was monitored twice in a week and level of parasitemia was monitored daily for 14 days.

Group 1: Infected and not treated (control)

Group 2 Infected and treated with 3.2mg/kg body weight diminazene aceturate (berenil®)

Group 3 Infected and treated with 100mg/kg body weight extract

Group 4 Infected and treated with 200mg/kg body weight extract

Group 5 Infected and treated with 400mg/kg body weight of extract

Determination of parasitemia

Blood films were made from the caudal vein of each rat and trypanosome count was determined by examination of the wet mount, microscopically at × 40 magnification using the "rapid matching" method of Herbert and Lumsden (1976) [16]. Mean parasitemia and mortality was recorded on daily basis.

Analysis of haematological parameters

The white blood cell (WBC) and red blood cell (RBC) count was determined 14th day post infection, using improved Neubauer Haemocytometer counting chamber and the haemoglobin (Hb) concentration was determined by the cyanomet-haemoglobin method [16].

Statistical analysis

Data obtained were analyzed using Graph pad prism 5.0 and this were expressed as mean ± standard error of mean. The

Table No.1: Mean body weight of rats (g).

Groups	Day 1	Day 5	Day10	Day 14
Group 1	105.5 ± 2.03	95.5 ± 2.15	87.5 ± 1.61	82.5 ± 1.21
Group 2	105.0 ± 1.81	131.0 ± 1.59*	152.5 ± 1.38*	165.0 ± 1.34*
Group 3	103.8 ± 2.11	99.0 ± 1.51	97.5 ± 0.89*	90.0 ± 1.55
Group 4	105.0 ± 1.61	102.5 ± 1.21*	101.0 ± 1.75*	100.5 ± 1.65*
Group 5	105.0 ± 1.61	104.5 ± 1.35*	108.5 ± 2.01*	110.5 ± 2.01*

* = significantly different from the control at $P < 0.05$

Group 1: Infected but not treated
Group 2: Infected and treated with diminazine aceturate (3.2mg/kg).
Group 3: Infected and treated with 100mg/kg extract
Group 4: Infected and treated with 200mg/kg extract
Group 5: Infected and treated with 400mg/kg extract

differences between means were compared using One way analysis of variance (ANOVA). P< 0.05 was considered significant.

RESULTS

There was initial reduction in body weight in the first week (day 1 and 5) in groups 4 and 5, followed by a significant increase (P < 0.05) in body weight in the second week (day 10 and 14) only in group 5. However, there was consistent reduction in body weight in group treated with 100mg/kg body weight and control group (Table No.1). All the infected rats showed progressive signs of weakness and emaciation except in the berenil® treated group.

Parasite was first detected in the blood on 3rd day post infection and continuously increased till 5th day post infection (berenil group) and 9th day post infection in groups 3, 4 and 5. This is followed by significant reduction (P < 0.05) in parasite count in all extract groups with the percentage parasitemia reduced to 3.5%, 7.5% and 15% in 400, 200 and100mg/kg body weight respectively. There was total parasite clearance on 8th day post

Table No.2: Effect of aqueous extract of *M .lucida* on *T .brucei brucei* infected rats Parasitemia

Days post infection	Group 1	Group 2	Group 3	Group 4	Group 5
3	3.0 ± 0.40	1.0 ± 0.55	2.5 ± 0.47	2.0 ± 0.58	1.5 ± 0.36
4	7.5 ± 1.52	2.5 ± 1.62	5.6 ± 0.55	4.8 ± 1.58	3.2 ± 0.45
5	13.9 ± 1.63	4.5 ± 1.83*	9.5 ± 0.51	9.0 ± 0.75	8.7 ± 0.41
6	18.7 ± 1.24	4.0 ± 1.35*	12.5 ± 0.35	5.5 ± 1.65*	4.5 ± 0.15*
7	26.4 ± 0.85	2.0 ± 1.85*	14.5 ± 0.85*	10.0 ± 1.22*	9.5 ± 0.65*
8	31.5 ± 0.52	0	11.5 ± 0.96*	12.5 ± 0.48*	6.8 ± 0.78*
9	30.6 ± 1.56	0	23.5 ± 0.75*	19.5 ± 1.36*	10.9 ± 0.55*
10	38.1 ± 2.01	0	20.5 ± 0.65*	16.0 ± 1.28*	10.6 ± 1.90*
11	33.7 ± 1.65	0	22.0 ± 1.66*	14.5 ± 0.85*	9.3 ± 0.82*
12	40.4 ± 1.50	0	16.5 ± 1.75*	12.5 ± 0.96*	7.3 ± 0.81*
13	40.8 ± 0.95	0	21.0 ± 1.85*	11.0 ± 1.36*	6.0 ± 0.56*
14	42.8 ± 1.20	0	22.0 ± 0.96*	10.5 ± 0.75*	5.5 ± 0.48*

* = significantly different from the control at P < 0.05

Table No.3: Effect of aqueous leaf extract of *M .lucida* on white blood cells (WBC) count of rats infected with *T. brucei brucei*

	WBC count (× 10^9/L)
Group 1: Infected but not treated	14400 ± 3.07
Group 2: infected and treated with diminazine aceturate drug	6500 ± 2.75*
Group 3: infected and treated with 100mg/kg extract	13500 ± 3.56
Group 4: infected and treated with 200mg/kg extract	10300 ± 2.85*
Group 5: Infected and treated with 400mg/kg extract	8400 ± 3.62*

* = significantly different from the control at P < 0.05.

Table No.4: Effect of aqueous leaf extract of *M. lucida* on red blood cell (RBC) count of rats infected with *T. brucei brucei*

	RBC count (× 10^6/L)
Group 1	3.15 ± 0.51
Group 2	6.75 ± 0.16*
Group 3	3.45 ± 0.71
Group 4	3.90 ± 0.25
Group 5	4.10 ± 0.65*

Table No.5: Effect of aqueous leaf extract of *M .lucida* on haemoglobin concentration of rats infected with *T. brucei brucei*

	Haemoglobin Concentration (g/dl)
Group 1	4.0 ± 0.85
Group 2	9.0 ± 0.42*
Group 3	4.5 ± 0.21
Group 4	5.5 ± 0.56
Group 5	7.5 ± 0.95*

infection in the berenil treated group (Table No.2).

Table No.3 shows significant reduction ($P < 0.05$) in WBC count at doses of 200 and 400mg/kg body weight. However, the dose of 100mg/kg body weight did not alter the WBC count when compared to the control.

The red cell count (Table No.4) and Haemoglobin concentration (Table No.5) was significantly increased ($P < 0.05$), only at the dose of 400mg/kg. The dose of 100 and 200mg/kg body weight did not produce any significant change in red cell count and haemoglobin concentration when compared to control.

DISCUSSION

Table 1 shows the weight of the rats from day 1 to day 14 post infection. Rats in group 1 and group 3 showed consistent weight loss. However, treated rats in groups 2 and 5 showed consistent increase in weight which is most pronounced in berenil treated (group 2). This is in agreement with the report of mann et al (2008) [18] in which infection with *T. brucei brucei* is associated with weight loss in rats. Nwaorgu (1981) [19] reported histopathologic changes after *T. brucei brucei* infection manifesting as lesions in various tissues and organs, anemia, emaciation and eventually death.

Trypanosomes were observed in the infected rats from the 3rd day post infection. There was a significant dose-dependent reduction ($P < 0.05$) in the parasite count with 5.5 observed in the dose of 400mg/kg body weight, followed by 10.5 and 22 for 200 and 100mg/kg body weight as compared with the control value of 42.8 on 14th day post infection. Also the level of parasitemia in the control group increased progressively up to day 14 post infection. This result showed that aqueous leaf extract of *M. lucida* contain active antitrypanosomal agent against *T. brucei brucei*. Carver (1973) [20] reported that a 50% reduction in parasitemia is an indication of significant activity of a trypanocide. This is also consistent with the result of Asuzu and Chineme (1990) [9], that demonstrated significant reduction in level of parasitemia with intraperitoneal and oral administration of *M. lucida* leaf extract. Also the best trypanocidal activity was obtained when treatment with extract commenced simultaneously with trypanosome inoculation. The initial increase in the level of parasitemia in group 3 and 4 could be attributed to inadequate dose of the extract administered orally or enzymatic inactivation of active compounds in the gut or reduced absorption from the gut or a combination of all the above factors [18]. The initial increase and fluctuation in the level of parasitemia could also be attributed to the variant surface glycoprotein (VCG) coat of the parasite which makes them elusive to host immune system and chemotherapy [5]. The mechanism by which these extract exhibit trypanocidal action was not determined. However, Sepulveda and Cassels (1996) [21] suggested that many natural products exhibit their trypanocidal activity through interference with the redox balance of the parasites, by acting either on the respiratory chain or on the cellular defenses against oxidative stress. This is because natural products possess structures capable of generating radicals that may cause peroxidative damage to trypanothione reductase that is very sensitive to alterations in redox balance. It is also known that some agents act by binding with the kinetoplast DNA of the parasite.

The effect of aqueous leaf extract of *M. lucida* on haematological parameters such as white blood cell (WBC), red blood cell (RBC) and haemoglobin concentration is shown in tables 3, 4 and 5. WBC count was found to be highest in group 1, followed by group 3. This is consistent with normal body response to infection as WBC is the primary defense against infection [5]. RBC count was found to be lowest in group 1 followed by group 3, however microscopic examination of blood film revealed marked red cell changes with hypochromia, microcytosis, and fragmented cells. These features are in keeping with trypanosome infection.

High haemoglobin concentration in group 2 and 4; with 7.5 g/dl and 9.0g/dl respectively; when compared with 6.0g/dl for control showed that the extract possess haematopoetic property. This beneficial effect on haemoglobin concentration can be used to correct the anaemia induced by trypanosome infection. This in agreement with the report of Asuzu and Chineme (1990) [9] that *M. lucida* leaf extract increases packed cell volume and haemoglobin concentration.

CONCLUSION

The results obtained from this study provides evidence that aqueous extract of *M. lucida* posses anti trypanosomal activity and can also correct anaemia induced by Trypanosomiasis. It also provides a scientific basis for its continuous use in traditional medicine for the management of African trypanosomiasis. Further investigations are needed to identify and isolate the active agent(s) in *M. lucida* determine the mechanism of action.

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