

IN VIVO ANTITRYPANOSOMAL EVALUATION OF SOME MEDICINAL PLANT EXTRACTS FROM OGUN STATE, NIGERIA.

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ABSTRACT

Aqueous extracts of 5 medicinal plants comprising of the root bark of *Morinda morindiodes* and leaves of *Tithonia diversifolia*, *Lippia multiflora*, *Ocimum gratissimum* and *Acalypha wilkesiana* were investigated for antitrypanosomal activities in albino rats infected with *Trypanosoma brucei brucei*. The plant extracts at 400mg/kg body weight (of rats) were administered once daily for 7 days in an established infection of 5×10^6 parasitaemia before starting treatment. There was significant reduction in parasitaemia ($P < 0.05$) on the 3rd day of treatment in rats treated with *Morinda morindiodes*, *Tithonia diversifolia* and *Acalypha wilkesiana* but parasitaemia later increased till survival time. *Morinda morindiodes*, a plant well known for its potent antimalarial effect, has its root bark extracts exhibiting the highest value of mean survival time (12.6±0.7) days this study. The result may probably suggest reduction in parasite virulence by *Morinda morindiodes* root bark extract.

Keywords: Antitrypanosomal, evaluation, medicinal plants, in vivo, Ogun State, Nigeria.

INTRODUCTION

Trypanosoma brucei brucei are unicellular parasites transmitted by the tse-tse fly. They are the causative agent of African Animal Trypanosomiasis (AAT) (Antia *et al.*, 2009). The disease results in acute, subacute, or chronic disease characterized by intermittent fever, anaemia, occasional diarrhea, rapid loss of condition and often terminates in death (Nurulaini *et al.*, 2007). Despite development of attempts at control, trypanosomiasis is still one of the limiting factors to livestock industry in sub-Saharan Africa (Kamuanga, 2003). Direct losses in meat production and milk yield, and the cost of programmes that attempt to control trypanosomiasis are estimated to cost between \$600 million and 1.2 billion each year. Poor clinical efficiency, drug resistance and toxicity are some of the limitations facing programmes targeted at controlling the disease (Onyeyili & Egwu, 1995; Geerts & Holmes, 1998; Legros *et al.*, 2000; Kamuanga, 2003). Currently, chemotherapeutic agents constitute the principal method of control, as development of vaccines against AAT is still in progress. Trypanosome infections are known to cause immunosuppression responsible for the host's inability to eliminate the trypanosomes even after administration of trypanocidal drugs (Godwin *et al.*, 1972; Osmo *et al.*, 1992). Diminazene aceturate and isometamidium chloride are the most currently used trypanocides, used both for prophylactics and curative purposes for the control of the disease in cattle (WHO, 1995). Unfortunately the parasite has developed resistance to these drugs (Schrevel *et al.*, 1996; Anene *et al.*, 2000; Geerts *et al.*, 2001) which makes the

search for efficacious chemotherapeutic agents from locally available ethnomedicinal plants for use as trypanocidal agents necessary.

Previous studies had documented trypanocidal effects in plants (Antia *et al.*, 2009; Biobaku *et al.*, 2009). The root bark extract of *Terminalia superba* totally inhibited the growth of trypanosomes in both rats and mice while the root bark extracts of *Azelia Africana* and *Khaya senegalensis* resulted in parasite clearance in rats only (Antia *et al.*, 2009). As part of our efforts to screen Nigeria medicinal plants for antiparasitic activities, we present herein the *in vivo* trypanocidal activities of five antimalaria plant extracts (Valentin *et al.*, 1995; Tona *et al.*, 2001; Goffin 2002; Zirih *et al.*, 2005; Udobang *et al.*, 2010).

MATERIALS AND METHOD

Plant collection and authentication: The leaves of *Tithonia diversifolia*, *Acalypha wilkesiana*, *Lippia multiflora* and *Ocimum gratissimum*; and root-bark of *Morinda morindiodes* were obtained in March, 2009 in Odeda Local Government Area of Ogun State and authenticated at the Botany Department of the University of Agriculture, Abeokuta and also at the Forestry Research Institute of Nigeria (FRIN), Ibadan.

Plant preparation and extracts: The plants part were air-dried in the laboratory at room temperature (29°C), pulverized, finely sieved and 500gms each were separately soaked in 1 litre of distilled water per plant for 24 hours, after which they were filtered. Thereafter, the filtrates were freeze dried from which 1gm of each plant filtrate was dissolved in 20mls of distilled water to give a concentration of 50mg/ml. To ensure that the active principles are important fresh aqueous extracts make prepared every-other day.

Experimental animals: 48 adult albino rats weighing 150-220g bred within the college of Veterinary Medicine, University of Agriculture, Abeokuta were used. They were kept and acclimatized in the fly proof laboratory animal unit of the Department of Veterinary Physiology and Pharmacology, University of Agriculture, Abeokuta for 2 weeks before commencement of the experiment. They were fed with animal feed and watered *ad libitum*.

Test organism and determination of parasitaemia: *T. b. brucei* were obtained from the Department of Veterinary Pathology of the University of Ibadan, Ibadan. The parasites were maintained in the laboratory by continuous passage in rats intraperitoneally. Blood from the tail was used for the estimation of parasitaemia in wet mount. The trypanosome count was determined by examination of the wet mount microscopically at x40 magnification using the "rapid matching" method of Herbert & Lumsden (1976). This method involves microscopic counting of parasites per field in pure blood or blood appropriately diluted with buffered phosphate saline (PBS, pH 7.2).

In vivo antitrypanosomal activity of plant extracts: The 48 animals were divided into 5 experimental groups A-E, 2 positive control groups E and F and a negative control group H. Each of these groups consists of 6 animals per group and was given 1 x 10⁴ parasites intraperitoneally in 0.2 ml blood/PBS solution.

The animals were left for 5 days to develop parasitaemia and the level of parasitaemia was determined by rapid matching method (Herbert & Lumsden 1976). After 5 days post inoculation when the level of parasitaemia had reached approximately 5×10^6 per microscope field, 48 animals were grouped into 8 (A-H).

Treatments commenced on the 5th day post infection which also doubles as the day 1 of treatment. The animals in groups A-E were treated using 400mg /kg dose of the extracts from *Tithonia diversifolia*, *Acalypha wilkesiana*, *Lippia multiflora*, *Ocimum gratissimum* and *Morinda morindoides* respectively.

Animals in groups F were the control group (positive control), treated orally with Artesunate®, a commercial preparation of artesunate in 50mg tablets at dose of 100mg/kg start plus 50mg/kg for the next 5 days while group G animals (second positive control) were administered with a single dose of diminazene aceturate (Diminal®)(445mg diminazene diaceturate) at a dose of 3.2mg/kg intramuscularly. The negative control (infected) group was not treated with any known trypanocide and serve as group H; the rats in this group were administered with distilled water at 10mg/kg orally

RESULTS

Parasitaemia: The parasites were detected in the peripheral blood of infected rats 3 days post infection. The level of parasitaemia increased and reached 5×10^6 by day 5 post infection the same day when treatment commenced in the treatment groups. All the rats in the infected group showed clinical signs of pale nauseous membrane, amnesia, weakness and emaciation. Four rats in the

infected *M. morindoides* treated group (F) died due to the infection on the 12th day post infection. The remaining rats in this group died at 13th days post infection.

There was complete clearance of parasites from the blood of infected rats treated with Diminazene aceturate in group G on the 7th day post infection (i.e. 3rd day of treatment). Also, on the same 3rd day of treatment, it was observed that group A animals treated with extract from *T. diversifolia*, group B animals treated with extract from *A. wilkesiana* and group E animals treated with extract from *M. morindiodes* had their parasitaemia level significantly reduced to 2.60 ± 1.1 , 3.20 ± 1.3 and 3.80 ± 0.7 respectively compared to groups C, D treated with plant extracts (*Lippia multiflora* and *Ocimum gratissimum*) and E treated with Artesunate whose mean parasitaemia level were greater than 5.00 ± 0.0 (Table 1).

There were reductions in the activity of trypanosomes and Parasitaemia level in all the treated groups except for animals treated with *O. gratissimum* (i.e. group D) on the 3rd day of treatment compared to the infected untreated control. However after the 3rd day of treatment, parasitaemia level increased in all groups (except diminazene group) till the time of death (Table 1).

Mean survival time showed that rats treated with diminazene aceturate survived throughout the period of the study. Rats treated with *M. morindiodes* root bark extracts had the highest mean survival time of 12.60 ± 0.7 days compared to groups treated with other plant extracts (Table 1). Infected untreated control had a mean survival time of 10.00 ± 0.4 days.

TABLE 1. EFFECT OF AQUEOUS PLANT EXTRACTS ON RATS TREATED DAILY AT 400Mg/Kg ON PARASITAEMIA AND MEAN SURVIVAL PERIOD

	Parasitaemia level Day 5 P.I. /Day 1 of treatment	Parasitaemia level Day 7 P.I. /Day 3 of treatment	Parasitaemia level Day 9 P.I. /Day 5 of treatment	Parasitaemia level Day 11 P.I. /Day 7 of treatment	Parasitaemia level Day 13 P.I. /Day 9 of treatment	Mean survival Time (Days)
A- <i>Tithonia diversifolia</i>	5.40±0.3	2.60±1.1	5.80±0.2	8.20±0.8	9.00±0.0	11.6±0.40
B- <i>Acalypha wilkesiana</i>	6.00±0.0	3.20±1.3	5.60±0.3	9.00±0.0	9.00±0.0	11.00±0.0
C- <i>Lippia multiflora</i>	5.60±0.3	5.80±0.8	6.20±0.7	8.20±0.8	9.00±0.0	11.00±1.0
D- <i>Ocimum gratissimum</i>	6.00±0.0	6.60±0.6	6.60±0.6	9.00±0.0	9.00±0.0	10.40±0.8
E- <i>Morinda morindiodes</i>	6.00±0.0	3.80±0.7	6.00±0.0	7.80±0.7	7.80±0.7	12.6±0.7
F-Artesunate	5.60±0.3	5.40±0.3	5.20±0.2	8.40±0.0	8.40±0.0	11.6± 0.4
G-Diminazene Aceturate	6.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0	15.00±0.0
H-Untreated control	5.20±0.2	6.00±0.0	6.00 0.0	9.00±0.0	9.00±0.0	10.00±0.4

Mean parasitaemia value 9.00= Death
 Mean survival Period of 15.00= Lived
 P. I. = Post Infection

Discussion

The clinical signs of pale mucous membrane, anorexia, weakness and emaciation in this study are characteristic and typical of trypanosomosis in animals. These findings are in agreement with Ezeokonkwo & Agu (2003, 2004) and Anene *et al.*, (2006).

The result of this study shows that all the plant extracts except *Ocimum gratissimum* and *Lippia multiflora* exhibited mild to moderate antitrypanosomal activity *in vivo* but did not completely clear the parasite. On the other hand, diminazene aceturate had total clearance of parasitaemia from the 2nd day post commencement of treatment without relapse of infection throughout the study. Complete clearance of parasitaemia in rats treated with the control drug diminazene aceturate 7 days post infection supports the findings of Ezeokonkwo *et al.*, (2007).

The high level of parasitaemia attained before commencement of treatment in the infected rats could be the reason for the failure of the plants extract to completely clear the parasites in the blood

(Antia *et al.*, 2009). Previous studies had shown that the medicinal plants used in this study are known to possess other medicinal properties; *Tithonia diversifolia* possess antimalarial and mosquito repelling properties in experimental animals and humans (Oyewole *et al.*, 2008). Antimalarial properties of *Acalypha wilkesiana* had also been reported by Udobang *et al.*, (2010), and *Morinda morindiodes* enjoys considerable reputation in traditional medicine in some African countries, frequently used against malaria and fever, diarrhoea, amoebiasis, haemorrhoids, gonorrhoea and rheumatic pains (Kambu, 1990). *Ocimum gratissimum* and *Lippia multiflora* showed no antitrypanosomal properties. *Ocimum gratissimum* had the least value of mean survival time. Similar weak trypanocidal activity of *Ocimum gratissimum* was reported by Adamu *et al.*, (2009) where no significant reduction in parasitaemia was observed. For the plant *Lippia multiflora*, it's antitrypanosomal activity could not be ascertain in this study even though Abena *et al.*, (2003) observed it to have antimalarial, analgesic, antipyretic and anti-inflammatory properties.

The antitrypanosomal properties of the medicinal plants presented in this study was observed under only one concentration of extracts. Further study at varying concentrations and doses may likely show if the antitrypanosomal properties is dose dependent and also starting treatment at a much lower parasitaemia level may lead to clearance of the parasite in the blood before the infection get fully established.

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