



Research Paper

*Afr. J. Traditional,  
Complementary and  
Alternative Medicines*  
[www.africanethnomedicines.net](http://www.africanethnomedicines.net)

ISSN 0189-6016©2009

**ANTI-TRYPANOSOMAL EFFECTS OF AQUEOUS EXTRACT OF *OCIMUM GRATISSIMUM* (LAMIACEAE) LEAF IN RATS INFECTED WITH *TRYPANOSOMA BRUCEI BRUCEI***

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### Abstract

The anti-trypanosomal effects of aqueous extract of the leaf of *Ocimum gratissimum* were evaluated in both *in-vitro* and *in-vivo* studies. The anti-trypanosomal activity of the extract against *Trypanosoma brucei* was investigated *in-vitro*. The survival and motility of the trypanosomes were completely inhibited within two hours of incubation in various concentrations of the extract. Parasite survival time was concentration dependent being longer in lower (25 and 12.5 mg/ml) than higher (100, 75 and 50 mg/ml) concentrations of the extract. The *in-vivo* anti-trypanosomal effect of the leaf extract of the leaf extract was investigated in rats infected with *Trypanosoma brucei* and treated with the extract. The infected rats treated with the extract had less dramatic clinical manifestations and mortality, survived longer and higher PCV values than their untreated counterparts, however, parasitaemia was not significantly reduced. The results suggest that the folkloric medicinal application of the aqueous extract of *Ocimum gratissimum* has no possible pharmacological basis.

**Key words:** Anti-trypanosomal, Leaf, *Ocimum gratissimum*, Rats

### Introduction

Trypanosomoses comprises a variety of disease syndrome caused by protozoan parasites of the genus *Trypanosoma*. The disease is of immense economic importance with a recent estimate of 4.5 billion U.S. Dollars as annual losses to agricultural production in Africa alone (PAAT, 2000). Furthermore most of the drugs used for treatment are either scarce or costly when available, while the parasite is rapidly developing resistance to the available drugs (Williamson, 1976; Mansfield, 1984; Nantulya and Mooloo, 1989). In addition, no new anti-trypanosomal drug for treatment of animals have been introduced in the last fifty years (Onyeyili and Egwu, 1995), thus underlying the need for the exploration of new drugs in the treatment of this disease. Consequently, several attempts have been made to scientifically evaluate plants that are traditionally said to have anti-trypanosomal effects (Asuzu and Chineme 1990; Mbaya et al., 2007).

The plant *Ocimum gratissimum* of the family Lamiaceae has shown anti-trypanosomal activity on the trypanosomatid *Herpetomonas samuelpessoai in-vitro* (Holetz et al., 2003). Recent studies in animals showed that *O. gratissimum* has anti-ulcer (Tan, 2002) and anthelmintic (Nwosu et al., 2005) effects, while its essential oil produced anti-microbial activity (Ngossoum et al., 2003). Extract of the leaf of the plant contain alkaloids, tannins, free and combined reducing sugars, saponins, terpenes, steroids and flavonoids (Adamu et al., 2008).

In an earlier study the water extract of the leaf of this plant was observed to be potentially toxic to rats with an intra-peritoneal LD<sub>50</sub> of 120mg/kg body weight (Adamu et al., 2008). However, the plant is yet to be

scientifically evaluated for efficacy against trypanosomes. This study reports the therapeutic activity of the water extract of *Ocimum gratissimum* in experimental *Trypanosoma brucei brucei* infection.

## Materials and Methods

### Collection and preparation of plant materials

The leaves of *Ocimum gratissimum* were obtained within the University of Maiduguri campus and was identified by a plant taxonomist in the Department of Biological sciences and a voucher specimen UMB 240 deposited at the departmental herbarium. The leaves were washed with distilled water, air dried for five days and ground into fine powder using pestle and mortar. The powder was exhaustively soxhlet extracted in water using Ace Soxhlet Extractor 6730 and Condenser 6740 (Quick Fit, England ) for 10 hrs at 60°C (Mittal *et al.*, 1981; WHO, 1992). The extract was then concentrated *in vacuo* using a rotary evaporator and stored at 4°C until used.

### Source of trypanosomes

*Trypanosoma brucei brucei*, Federer strain was obtained from the Nigerian Institute for Trypanosomiasis and Onchocerciasis Research (NITOR) Vom Plateau State, Nigeria. The parasites were estimated using the method of Herbert and Lumsden (1976). The parasites were maintained by serial passage in rats.

### Experimental animals

Adult albino rats of both sexes, weighing 100-200g, obtained from the laboratory animals unit of the Department of Biochemistry, University of Maiduguri, Nigeria were used for the study. They were housed in clean cages in a fly proof house in the Department of Veterinary Microbiology and Parasitology University of Maiduguri, Maiduguri. The animals were fed standard pelleted feed (Vital Animal Feed, Nigeria). Water was provided *ad libitum*. Before the commencement of the experiments, the rats were screened for the presence of haemoprotozoan parasites using wet mount and leishman stained blood films. The research committee of the Faculty of Veterinary Medicine University of Maiduguri, Nigeria approved this experiment and the experimental animals were handled in accordance with internationally accepted principles for laboratory animal care and use.

### *In vitro* anti-trypanosomal activity

Serial dilutions (100, 75, 50, 25 and 12.5) of the water extract of *Ocimum gratissimum* leaf were freshly prepared in normal saline (0.9% sodium chloride) and 2ml of each pipetted into different Petri dishes in duplicate. Control Petri dishes containing normal saline without the extract were included. Dilutions of fresh blood samples collected from rats infected with *T. brucei brucei* were made using phosphate buffered glucose saline solution (PH 7.4), 1ml of the diluted blood containing 300parasites/ml was added to each of the Petri dishes containing the extract dilutions (Herbert and Lumsden, 1976). The Petri dishes were thereafter incubated at 37°C (using an incubator). The presence of the parasites in the solution was assessed every 30 mins using wet mount method.

### *In vivo* anti-trypanosomal activity

Twenty albino rats were used for the study. Fifteen of the rats were inoculated intraperitoneally with 0.5ml of diluted rat blood containing 300 parasites. The number of parasites was determined using the method of Herbert and Lumsden (1976). Wet blood film and haematocrit buffy coat examination were carried out daily using blood obtained from the tail. When parasitaemia was established, the rats were separated into three groups (B, C and D). Rats in group B were infected but not treated, those in group C were treated with the water extract of the plant at the dose of 50mg/kg. Group D was treated with Berenil<sup>R</sup>. The five rats in group A were non-infected and were used to monitor the course of the disease and any other infections. All treatments were initiated 8 days post infection. The extract was giving intra peritoneally once while Berenil<sup>R</sup> (3.5mg/kg) was given once. The animals were examined daily for the presence of parasites for the first 2 days after treatment and thereafter every 2 days for 28 days.

Packed cell volume (PCV) was measured every 2 days, and was determined by a micromethod, using a Hawksley microhaematocrit centrifuge (Schalm *et al.*, 1995). All the rats that died during the study and those sacrificed at the end of the study were subjected to necropsy. The following parameters were used to assess the therapeutic activity of the water extract of the plant in rats: degree of parasitaemia and frequency of death.

## Statistical Analysis

The data obtained from the study were summarized as means  $\pm$  standard deviation and the differences between and within the means were analyzed using GraphPad Instat Version 3.05 for Windows 95 at the 5% level of significance (GraphPad, 2003).

## Results

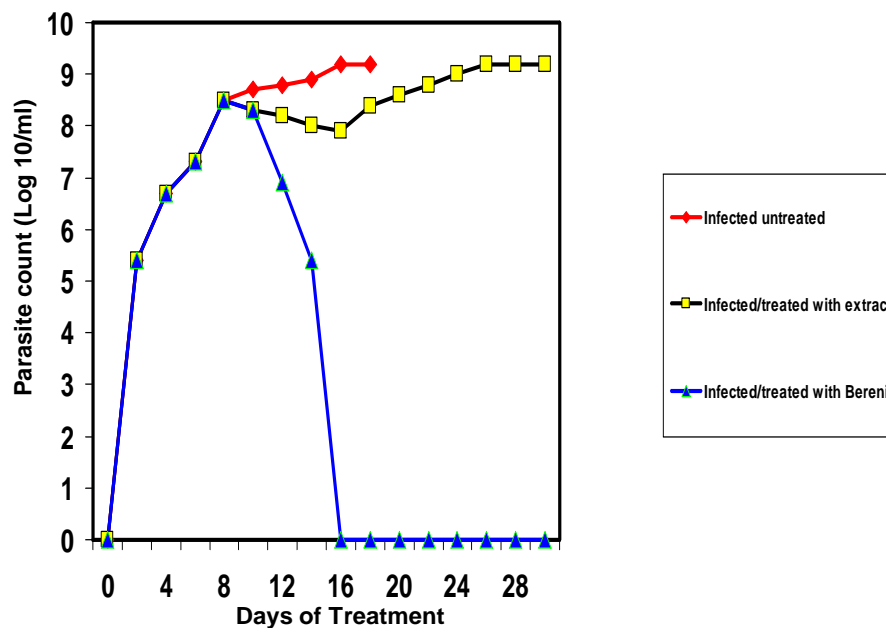
### *In vitro* anti-trypanosomal activity

The survival time of *T. brucei brucei* in various concentrations of the water extract of *O. gratissimum* are presented in Table 1. The parasites in the control group survived for up to 180 mins although with diminishing activity. At the various concentrations (100, 75, 50, 25, 12.5 mg/ml) of the water extract of *O. gratissimum* the parasites survival and motility were inhibited. Parasites survival time was concentration dependent with the trypanosomes surviving for longer periods in lower concentrations (12.5 and 25 mg/ml) and for shorter periods in higher concentrations (50, 75 and 100 mg/ml) of the extract (Table 1).

### *In vivo* anti-trypanosomal activity

All the rats infected with *Trypanosoma brucei* manifested varying degree of clinical trypanosomosis that included weakness, reduced appetite, and rough hair coat. Later there was palour of the mucous membranes of the eyes and the foot pads. These signs were progressive and more severe in the infected untreated group. The survival time (Table 2) shows that the infected untreated group (Group B) had a mean survival time of  $11.6 \pm 2.4$  days as against  $22.44 \pm 3.5$  days for the group infected treated with the extract (Group C). The groups treated with Berenil<sup>R</sup> (Group D) and the uninfected untreated control (Group A) had a mean survival time of  $30 \pm 0$  days.

*Trypanosoma brucei*, parasitaemia was first detected in all the infected groups on day 2 post-infection (p.i.). Parasites counts thereafter raised sharply in a similar manner in all the infected groups by day 8 post-infection (Figure 1). Treatment with the extract resulted in a reduction in the level of parasitaemia between days 10 and 18 post-infection. The group treated with Berenil<sup>R</sup>, showed a sharp reduction in parasites count from day 10 post-infection (2 days post treatment), with the parasites completely cleared from the blood on day 16 post infection (8 days post treatment) and there was no relapse recorded in this group during the study. The uninfected control showed no signs of trypanosomosis or other infections throughout the period of experimentation.



**Figure 1:** Effects of aqueous extract of *Ocimum gratissimum*, berenil and their controls on the parasitaemia of rats infected with *Trypanosoma brucei brucei*

**Table 1:** *In vitro* anti-trypanosomal activity of *O. gratissimum* Leaf extract on *T. brucei* at 37°C

Treatment (mg/ml)	Survival time of trypanosomes in minutes.						
	0	30	60	90	120	150	180
100	+++	++	-	-	-	-	-
75	+++	++	-	-	-	-	-
50	+++	+++	-	-	-	-	-
25	+++	+++	+	-	-	-	-
12.5	+++	+++	++	-	-	-	-
Control	+++	+++	+++	++	++	++	+

No. Parasite = - ; 100 – 150 parasites = +

200 – 250 parasite ++ ; 300 parasite = + + +

**Table 2:** Survival time of rats infected with *T. brucei* and treated with either Berenil or the aqueous extract of *Ocimum gratissimum* leaf and their controls.

Group	Treatment	No in group	No(%) Dead	Range in days	Mean±SD Survival time(days)
A	Uninfected control	5	0	30	30±0 <sup>a</sup>
B	Infected/untreated	5	4(80)	8-20	11.6±2.4 <sup>c</sup>
C	Infected/treated/Extract	5	2(40)	18-30	22.44±3.5 <sup>b</sup>
D	Infected/treated/Berenil	5	0	30	30±0 <sup>a</sup>

<sup>abc</sup>Figures in same column with different superscripts are significantly different (p<0.05)

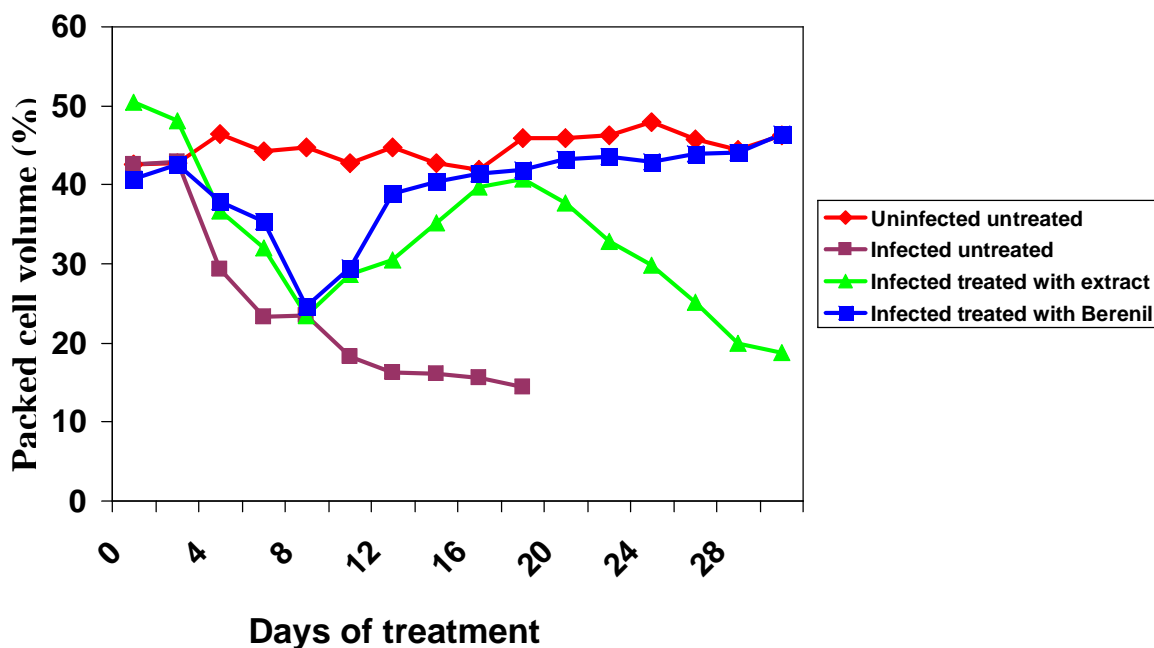
\*The study was terminated on day 30 post infection

### Packed cell volume

Figure 2 shows the mean PCV values of rats infected with *T. brucei brucei* and subsequently treated with water extract of *Ocimum gratissimum* and Berenil<sup>R</sup>. Before infection the mean PCV values for groups B, C, and D were 42.64±3.72, 50.50±6.67 and 40.70±4.78 respectively. However 8 days after inoculation the mean PCV values decreased to 23.52±1.42, 23.42±1.36 and 24.62±1.82. Following treatment the PCV levels improved and 16 days post infection (8 days post treatment), the values were 14.40±0, 40.80±2.40 and 41.98±3.02 respectively in groups B, C, and D respectively.

### Post-mortem and histopathology

The post-mortem findings in the untreated *T. brucei brucei* infected rats revealed the enlargement of the liver, kidney and especially the spleen, with the carcasses being generally pale. Histopathologically, there was marked congestion of the spleen and presence of haemosiderin pigments.



**Figure 2:** Effects of aqueous extract of *Ocimum gratissimum*, berenil and their controls on the packed cell volume of rats infected with *Trypanosoma brucei brucei*

## Discussion

The present study demonstrates that the water extract of the leaf of *Ocimum gratissimum* at the concentrations used significantly inhibited *T. brucei brucei* organisms *in vitro*. Similar results were recorded by Mikail et al., (2002) using garlic. The observed inhibition may be an indication of the possible usefulness of the leaf extract as an anti-trypanosomal agent. *Trypanosoma brucei brucei* (Federer strain) inoculated intraperitoneally to rats produces parasitaemia within 2 days associated with gradual loss of condition, facial oedema. The oedema may have resulted from release of vaso-active substances (Van den Ingh et al., 1977). The observed splenomegally resembles an earlier finding recorded in *T. brucei* in mice and rats (Anika et al., 1987).

The parasitological finding shows that when the condition was treated with the aqueous extract of *O. gratissimum*, the course of infection was modified. The extract did not significantly reduce the level of parasitaemia, however, it prolonged the survival time in the infected rats. Treatment with Berenil<sup>R</sup> resulted in the clearance of the parasites from the blood. The ability of the water extract of *O. gratissimum* to inhibit parasite activity *in vitro* and modify the course of the disease *in vivo* may be due to the presence of some active principles in the leaf extract such as alkaloids, flavanoids (Adamu et al., 2008).

The failure of the *O. gratissimum* water extract to achieve an effective therapy *in vivo* might be a reflection of no viable active components with anti-trypanosomal activity in the extract or there may be inactivation of the active components *in vivo*. In conclusion, the folkloric medicinal use of *O. gratissimum* extract has no correlation with scientific data since its intraperitoneal injection has shown no activity in rats infected with *Trypanosoma brucei brucei*.

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