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Trypanocidal Activity of the Leaf of *Guira senegalensis* Against *Trypanosoma brucei brucei* Infection in Mice

[1]A.U. Wurochekke, [2]D.B. james, [1]M.I. Bello and [2]A. Ahmodu

In vitro and *in vivo* trypanocidal activity of the leaf extract of *Guira senegalensis* against *Trypanosoma brucei brucei* have been investigated. Extract obtained from fresh leaves heated with methanol had highest *in vitro* activity against the parasite at concentration of 8.3 mg mL^{-1} of blood. Dried leaf methanolic extract also had in vitro activity at the same concentration after 30 min of incubation. Treatment with 100 mg/kg/day of the fresh leaf extract for five days tends to ameliorate the disease condition but did not clear the parasitaemia and pack cell volume values were not significantly affected. All other animals treated with the extract higher than the 100 mg/kg/day died before the infected controls. Addition of glycerol as an adjuvant did not show effect. The plant may be a promising trypanocide.

Key words: Trypanocidal, *Guira senegalensis*, *Trypanosoma brucei brucei*, parasitaemia, trypanocide

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A.U. Wurochekke
Department of Biochemistry
Federal University of Technology
Yola, Nigeria

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[1]Department of Biochemistry, Federal University of Technology, Yola
[2]Department of Biochemistry, A.B.U. Zaria, Nigeria

INTRODUCTION

Trypanosomosis is a disease syndrome that affects man and domestic animals. It is one of the major obstacles to livestock production in Africa. Control and management of the disease is principally based on vector control and chemotherapy. Chemotherapy with the available drugs is less efficient and vector control strategy is faced with difficulties. There is no hope for the production of vaccine in the near future because of the phenomenon of antigenic variation[1]. Limited and expensive drugs, toxicity and drug resistance are the major problems of chemotherapy[2]. These problems of current treatment methods increase the need for urgent search of more effective and less toxic chemotherapeutic agents from natural origin.

Recent studies revealed several plants as potent tryponocides[3-7]. These reports suggest the possibility of producing potent trypanocides from medicinal plants.

Guira senegalensis is a medicinal plant widely distributed in the West African Savannah. It grows as a shrub in dry localities where rainfall is small not well distributed over the year[8,9]. The plant is use medicinally to treat gastroenteritis, hemorrhoids, dysentery, malaria, wound and skin infections[10]. Other uses include improvement of lactation after child birth, prevention of leprosy, chest complaints and rheumatic conditions[11]. Recently Atawodi *et al.*[12] reported that herdsmen in Northern Nigeria traditionally use the leaf extract in the treatment of trypanosomiasis. Here we present *in vitro* and *in vivo* antitrypanosomal activity of the leaf extract of *Guira senegalensis* against *Trypanosoma brucei brucei*.

MATERIALS AND METHODS

Plant material: Leaf of *Guira senegalensis* was collected in Zaria metropolis and identified at the herbarium unit of Biological Sciences Department, ABU Zaria.

Extraction: Exactly 200 g of the fresh leaf collected was heated in 400 mL of methanol or water. Another 200 g of the fresh leaf were macerated in 400 mL of water overnight. 100 g of the dry powdered leaf was also heated and macerated separately in 400 mL of methanol. All filtrates were concentrated on water bath at 50°C.

Trypanosome: *Trypanosoma brucei brucei* (federe strain) was kindly provided by the National Institute of Trypanosomiasis Research, JOS Nigeria.

Trypanosome infection: Parasites were harvested from a previously inoculated donor rat at peak parasiteamia (10^7 parasites mL^{-1} of blood). The infected blood was diluted with phosphate buffered saline. Experimental mice were infected intraperitoneally with approximately 10^3 parasites mL^{-1} of blood.

***In vitro* screening:** Infected blood was harvested from the heart of a donor mouse at peak parasitaemia in to a sample bottle containing heparin as anticoagulant. Different concentrations of the crude extracts were prepared. Aliquots of 10 μL of the extract was incubated with 60 μL of the infected blood in wells of microtitre plates, for control the extract was replaced with phosphate buffered saline. Motility of the parasites was observed under the microscope (Mg x 400) at 5 min intervals for 1 h.

Animal grouping: Thirty mice weighing between 25-30 g were used. They were grouped into six of five mice each.

Group 1- Normal control
Group 2- Infected control
Group 3- Infected treated with 100 mg /kg/day.
Group 4- Infected treated with 200 mg /kg/day.
Group 5- Infected treated with 400 mg/kg/day.
Group 6- Infected with 400 mg/kg/day plus 50% glycerol.

Administration of extract: The extract was administered to the animals orally using a force-feeding needle for 5 days.

Parasitaemia determination: Blood was obtained from the tails of the infected animals and smears made on slides. Parasites were observed and counted under the microscope as described by Herbert and lumsden[13].

Determination of pack cell volume: Pack cell volume was determined after every two days for all the animals by the micro heamatocrit method.

RESULTS AND DISCUSSION

Extract of fresh leaf heated with methanol has the highest *in vitro* activity against *Trypanosoma brucei* (Fig. 1). Parasites incubated with 8.3 mg of the extract were immotile 20 min after incubation. The extract obtained from dry leaf macerated with methanol was also able to prevent motility 30 min after incubation at the same concentration. The three other extracts were not very

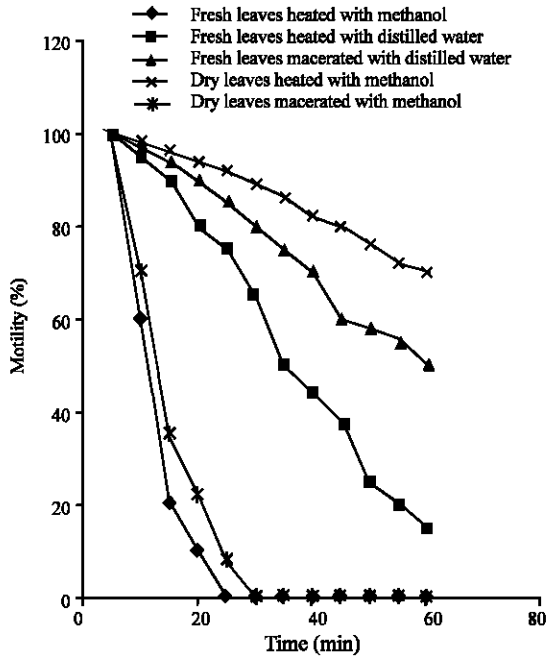

Fig. 1: Motility of the parasite against incubation time for the different extracts

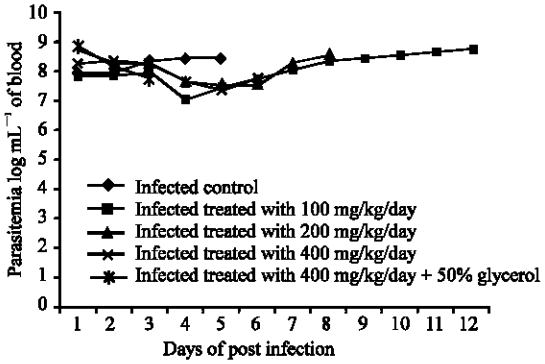

Fig. 2: Parasitemia profile

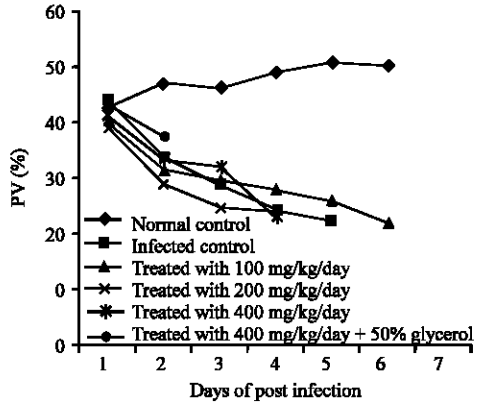

Fig. 3: Pack Cell Volume (PCV) against days of post infection

active (Fig. 1). This finding confirms the efficacy of the plant since *in vitro* activity of crude plant extracts produces evidence to support the use of such plants. However, administration of the most active extract *in vitro* to experimental mice at different doses for five days did not clear the parasites. The level of parasiteamia and pack cell volume values were not significantly affected (Fig. 2 and 3). This suggests that the crude extract is not very active *in vivo*. Although animals treated with 100 mg/kg/day survived longer than the infected control suggesting disease amelioration effect.

The inactivity of the extract *in vivo* could be as a result of biotransformation in multicellular organisms where active molecules may be converted to inactive once or vice-versa. Different compounds in the crude may potentiate or antagonise anti trypanosomal activity. Binding to albumin might have possibly reduced the concentration of the extract at the target site. Reduced concentration of drug at the target site could contribute to emergence of drug resistance[14,15].

All animals treated with more than 100 mg/kg/day of the extract died before the infected control (Fig. 3). The PcV values of these animals did not reduced significantly (Fig. 3) suggesting that higher concentrations of the extract might have contributed to the early death of the animals. Studies on the phytochemical constituents of the plant revealed the presence of tannins, alkaloids, saponins and flavonoids[16,17]. High concentration of these constituents could be toxic and may be responsible for the death of the animals. However, therapeutic benefits of traditional remedies might depend upon combination of these constituents. Amos *et al.*[18] reported that aqueous extract of guira senegalersis have sedative effect and attributed the effect in part to the presence of alkaloids and saponins.

The over all results revealed that leaf extract of *Guira senegalensis* have *in vitro* activity against *Trypanosoma brucei* and treatment with low concentration ameliorated the disease condition in experimental mice. Further studies are in progress to fractionate and identify the possible active component.

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