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In vitro and *in vivo* Anti Trypanosomal Activity of the Leaf of *Lawsonia inermis* Against *Trypanosoma brucei brucei* Infection in Mice

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In vitro and *in vivo* antitrypanosomal activity of the leaf of *Lawsonia inermis* have been investigated. Crude methanolic extract had *in vitro* activity against *Trypanosoma brucei* at concentration of 8.3 mg mL^{-1} of blood. The treatment tends to ameliorate the disease condition, but did not affect the level of parasitaemia and pack cell volume. The addition of glycerol as an adjuvant did not also show any significant effect.

Key words: *Lawsonia inermis,* antitrypanosomal, *Trypanosoma brucei,* Parasitaemia

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INTRODUCTION

Trypanosomosis is one of the major obstacles to livestock production in Africa. Chemotherapy with available drugs is less efficient and tsetse fly control is faced with difficulties. There is also little or no hope for the production of anti trypanosomal vaccine in the near future due to the phenomenon of antigenic variation exhibited by the parasites[1]. This coupled with limitation of current treatment methods such as toxicity and drug resistance increase the need for urgent search of more effective and less toxic therapeutic agents against the disease.

Reports revealed several plants as potent trypanocides[2-5]. These reports suggested the need for exploring medicinal plants for efficient and cheaper trypanocides.

Lawsonia inermis is a plant commonly found in India, Egypt, Syria and Northern Nigeria. It is locally used in the treatment of jaundice, leprosy, small pox, menstrual abnormalities and nervous disorders[6].The leaves of the plant is also used traditionally in the treatment and management of trypanosomosis by herds men in Northern Nigeria[7].

This study present *in vitro* and *in vivo* activity of the leaf extract of *Lawsonia inermis* against *Trypanosoma brucei brucei* in experimental mice.

MATERIALS AND METHODS

Plant material: Leaf of *Lawsonia inermis* was collected in Zaria metropolis, identified at the herbarium unit of Biological Sciences, A.B.U., Zaria.

Extraction: Fresh leaf of the plant collected was dried under the shade. Exactly 100 g of the powdered leaf was dissolved in 400 mL of methanol or water. The filtrate obtained was concentrated on water bath at 50°C.

Trypanosome: *Typanosoma brucei brucei* (federe strain) was kindly provided by the National Institute of Trypanosomosis Research. Jos, Nigeria.

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

***In vitro* screening:** Different concentrations of the crude extract was prepared. Aliquots of 10 μL of the extract was incubated with 60 μL of infected blood in wells of microtitre plates, for control, the extract was replaced with phosphate buffered saline. Motility was observed under the microscope (Mgx 400) at 5 min intervals for 1 h.

Experimental design: 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 200 mg/kg/day
Group 4: Infected treated with 400 mg/kg/day
Group 5: Infected treated with 800 mg/kg/day
Group 6: Infected treated with 200 mg/kg/day plus 50% glycerol

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

Parasitaemia determination: A drop of tail blood from the infected animals was used to make smears on slides. Parasites were observed and counted under the microscope as described by Herbert and Lumsden[8].

Determination of pack cell volume : Pack cell volume was determined after every three days for all the animals by the microheamatocrit method.

RESULTS AND DISCUSSION

Figure 1 shows the activity of the crude extract on the motility of the parasites. Here all parasites were immotile 45 min after incubation at concentration of 8.3 mg mL^{-1} of blood. The *in vitro* activity of the extract increased with increase in concentration as shown in Fig. 2. At 16.6 mg mL^{-1} of blood and above, the parasites were immotile immediately after incubation.

There was no significant effect of the extract on the level of parasitaemia (Fig. 3). However, treated groups survived longer than the infected control group. The crude extract was also able to reduce the rapid drop in PCV of the infected treated animals although not very significant (Fig. 4).

Present results showed that methanolic extract of *Lawsonia inermis* had *in vitro* activity against *trypanosma brucei* and the activity increased with increase in concentration of the crude extract suggesting that the plant may be a potent trypanocide. However, administration of the extract at different doses for five days did not clear the parasites nor affected the onset and

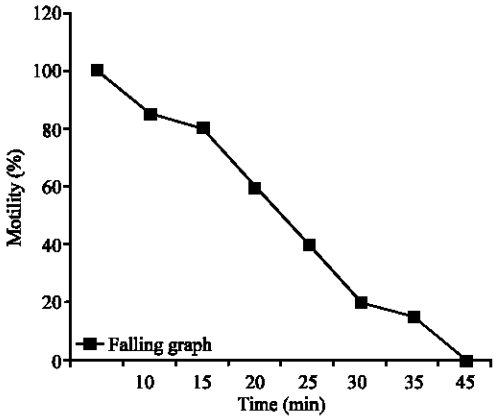

Fig. 1: Motility of the parasites against incubation time for the crude extract at concentration of 8.3 mg mL^{-1} of blood

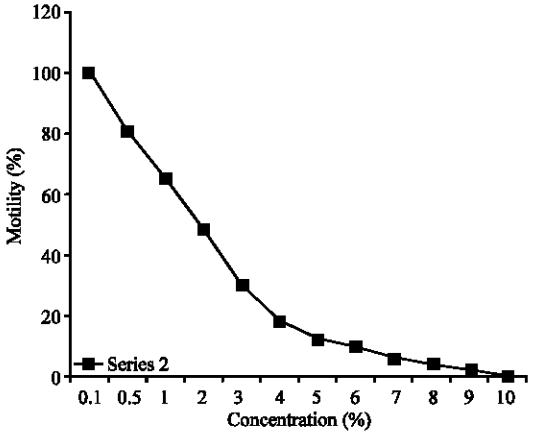

Fig. 2: Effect of concentration of the extract on the motility of the parasites

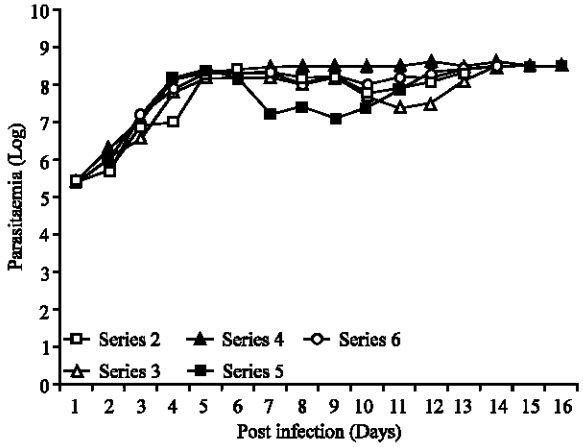

Fig. 3: Parasitaemia (Log 10 organisms mL^{-1} of blood) against days post infection
Series 2: Infected control, Series 3: 200 mg
Series 4: 400 mg, Series 5: 800 mg
Series 6: 200 mg+50% glycerol

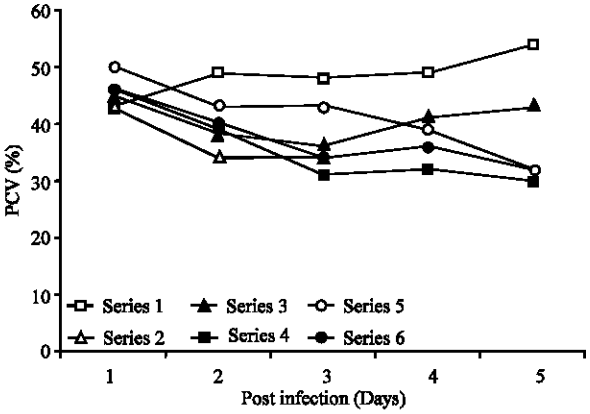

Fig. 4: Pack cell volume against date post infection for normal and infected groups
Series 1: Normal control Series 2: Infected control
Series 3: Infected+200 mg Series 4: Infected+400 mg
Series 5: Infected+800 mg
Series 6: Infected+200 mg+glycerol

level of parasitaemia. Pack cell volume values were also not affected.

The inactivity of the extract *in vivo* suggests a transformation in multicellular organisms which may convert inactive molecules to active ones, or vice versa. There is also dearth on information concerning the chemical effect of crude extract which may act by more than one mechanism and in which different compounds may potentiate or antagonize the anti trypanosomal activity. It may also be possible that albumin binds to the extract and consequently, reduce its concentration at the target site. Reduced concentration of extract at target site can contribute to emergence of drug resistance[9,10].

Although the extract tends to ameliorate the disease condition by prolonging the life span of the infected animals, the over all results suggested that the methanolic extract of the leaf of *Lawsonia sinermis* has activity against *Trypanosoma brucei in vitro* but not *in vivo*. Determination of the components of the crude extract, by fractionation and testing the different components on different species of trypanosomes using other animal models will certainly provide conclusive information on the trypanocidal activity of the plant.

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