RESEARCH PAPER

IN VITRO AND IN VIVO EVALUATION OF ANTITRYPANOSOMAL ACTIVITIES OF AQUEOUS ETHANOLIC EXTRACT OF LAWSONIA INERMIS

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ABSTRACT

A study was conducted to access the In vitro and In vivo antitrypanosomal activities of aqueous ethanolic extract of Lawsonia inermis on Trypanosoma brucei brucei. At the dose rate of 10mg/ml the parasites became immotile after incubating for 30 minutes while at the dose rate of 30mg/ml, the parasites became inactive immediately after incubation. Although oral administration of the extract did not clear the parasites from peripheral circulation, it reduced the level of parasitaemia and prolonged the life span of infected rats. The packed cell volume and temperature of the infected rats were not significantly (P>0.05) affected. This study demonstrates the potential of lawsonia inermis in the management of African trypanosomosis. It is recommended that each fraction of the extract be access to know the most potent component of the plant extract. The LD50 study revealed that the extract have a very wide range of safety (2000mg/kg orally).

Keywords: Extract, Infection. Lawsonia inermis, Parasitemia, Trypanocide and Trypanosoma,

INTRODUCTION

Trypanosomosis is a vector-borne devastating disease of man and animals caused by haemoparasitic protozoa, trypanosome (Hu and Akosy, 2005). The disease is one of the major constraints to livestock development in Africa as it can lead to severe production losses (FAO, 2002). The disease occurs in Sub-Saharan Africa and some of the clinical signs are fever, anaemia, weakness, weight loss, oedema, reproductive disorder and death if untreated (Ikede et al., 1988; FAO, 2002).

The control of trypanosomosis continues to rely principally on chemoprophylaxis using salts of three compounds, dianaminazine acetarate, homidium chloride and isometamidium chloride (ILRAD, 1990). However, the therapeutic and prophylactic use of trypanocide is beset by numerous limitations such as toxicity and development of resistance by the parasite (Gutteridge, 1985). Currently, there is no vaccine available for the control of the disease as a result of a phenomenon known as antigenic variation exhibited by the parasite. There is a need for more and urgent search of effective and less toxic therapeutic agents especially of plant origin for the control of the disease.

Over twenty thousand species of higher plants are used medicinally worldwide and the plants have provided the bases for traditional treatment for different types of diseases and still offer an enormous potential source of new chemotherapeutic agent (Tagboto and Townson, 2001). Lawsonia inermis is one of such plants used traditionally in the management of...
African trypanosomosis. The plant belongs to the class Magnoliophyta and Lythraceae. It is a tall shrub or small tree of about 2.6m high and is a native of tropical and Sub-tropical region of Africa, Southern Asia and Austialasia in Semi-arid Zones and produces highest dye content. It is cultivated commercially in Middle East and Northern African Countries. The extract of the plant leaves contain alkaloid such as lawsome, quinine and resin which are dyes, tannin, gallic acid, glucose, mannitol, luteolin and glucoside (Fitoterapia, 1980).

To mention but just a few of the several uses of *Lawsonia inermis*, it is used as cosmetic agent (Henna et al., 1998), as antituberculosus (Sharma, 1990), for treatment of wounds, cough, lumbago, rheumatalgia, inflammation, diarrhea, dysentery, cancer (leucoderma), scabies, boils, anemia, haemorrhages and fever (Burkhill, 1966; Vaidyara tuany, 1995). The alcoholic extract of the plant leaves also showed mild antibacterial activity against Micrococcus pyogenes and Eschericia coli (Kritikar and Basu, 1981). It is also used for the treatment of amoebiasis and headache (Leung, 1980), as antifungal agent and oral candidia albica (Prasurst et al., 2004). It is used for staining tissue in histological paraffin section of different organs (Veeresh Kumar, 2005). However, there is lack of wealth of information on the scientific validation of the potential of *Lawsonia inermis* in the treatment of trypanosomosis. This stimulated this study and it was designed to investigate the *In vitro* and *In vivo* antitrypanosomal activity of aqueous ethanolic extract of Lawsonia inermis leaf.

**MATERIALS AND METHODS**

**Substance of Study:** The leaves of *Lawsonia inermis* were collected in August, within Makurdi environs, capital of Benue state, Nigeria and were identified by the chief technologist, Department of Forestry, University of Agriculture, Makurdi, Benue state. The voucher specimen was deposited at the college herbarium.

**Preparation of crude extracts:** The leaves were washed, air dried at room temperature (28°C-30°C) for two weeks, pulverized with mortar and pistol and stored in air tight container until used. The powdered material (351g) was soaked in 1,755mls of 80% ethanol and stirred intermittently for 48 hrs at room temperature (28°C-30°C). The choice of ethanol was to enhance the extraction of more constituents and the solvent is also less toxic. The material was filtered using a muslin cloth, cotton wool and Whatman filter paper No.1 respectively. The filtrate was dried under the electric fan and the extract was stored in air tight container at 4°C until used.

**Oral acute toxicity study:** The oral acute toxicity study was determined using up-and-down procedure (OECD, 2001). The rats were observed for signs of toxicity like dizziness/reflex, twitches or tremor, reaction to food or odour, reaction to noise or contact, grooming, respiratory rate, diarrhoea, recumbency/coma, lacrimation, urination and mortality within 48hrs and then 14days.

**Experimental animals:** Albino wister rats were procured from the animal house, School of Health Sciences, Benue state University, Makurdi Benue state, Nigeria. The rats were fed with pellet feed and water ad libitum. They were housed in standard rat cages and maintained under hygienic condition in well ventilated room with 12-hour and 12-hour dark photoperiod. All the rats were handled according to the international guiding principles for Bio-medical Research involving Animals (CIOMS, 1985).

**Parasites:** Stabilates of *Trypanosoma brucei brucei* were obtained from the National Institute for Typanosomosis Research, Vom, Jos, Nigeria. The parasites were maintained in the laboratory by passage in mice. Blood harvested from a donor animal at peak parasitaemia (10^7 parasites/ml of blood) was used for antitrypanosomal study.

**In vitro antitrypanosomal screening:** Different concentrations of the crude extract of *Lawsonia inermis* were prepared. Aliquots of 10ul of the extract were incubated with 10ul of infected blood in a well of micro-titer plates. For the control, the extract was replaced with phosphate buffered saline. Motility was observed under microscope (mgx400) at 10min interval for 1hr.

**In vivo antitrypanosomal study:** Ethanolic extract of *Lawsonia inermis* was administered to the rats orally using a force feeding needle for five days. A drop of tail blood from the infected rats was used to make smears on slides and the parasite were observed and counted under the microscope. The rectal temperature was determined after every other day for all the rats to determine the effect of the parasite on rectal temperature. Blood was collected to determine the packed
cell volume, differential leucocytes count and total white blood cells according to standard procedures (Schalm et al., 1975).

**Experimental Design**- Twenty rats weighing between 87 - 204g were used. They were grouped into four of five rats each. Group 1 was control (uninfected), group 2 was infected, treated with 2000mg/kg extract of *Lawsonia inermis*, group 3 was infected, treated with diminazene aceturate and group 4 was infected, untreated. 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 smear on the slides. Parasites were observed and counted under microscope as described by Herbert and Lumsden (1983).

**RESULTS**

**Toxicity study**- Oral acute toxicity study revealed that the crude extract of *Lawsonia inermis* was apparently non toxic at the dosage of 2000mg/kg body weight administered orally.

**In vitro anti-trypanosomal activity**- The result shows that in thirty minutes after incubation of the extract with the parasites, they all became inactive or immotile (Fig.1).

![Fig. I: Effect of ethanolic extract of Lawsonia inermis on the motility of Trypanosome brucei brucei.](image)

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The extract showed concentration dependent effect on parasite motility where it was observed that the parasites were completely immotile at 30% concentration (Fig.II). In vivo anti-trypanosomal activity. The results of in vivo anti-trypanosomal study are as shown in Figures III, IV, V and VI.

Fig. II: Effect of concentration of ethanolic extract of *Lawsonia inermis* on *Trypanosoma brucei brucei*
Fig. III: Effect of ethanolic extract of *Lawsonia inermis* on the parasite count.

Keys: A = Normal control; B = Infected treated with extract; C = Infected treated with diaminazine aceturate; D = Infected control.
Fig. IV: Packed Cell Volume (PCV) of the Wister albino rats

Keys: A= Normal control; B= Infected treated with extract; C= Infected treated with diaminazine aceturate
D= Infected control
Fig V: Total WBC count of the Wister Albino rats

Keys: A= Normal control; B= Infected treated with extract; C= Infected treated with diaminazine aceturate; D= Infected control
Fig. VI: Daily differential leucocytes count of Wister albino rats infected with *Trypanosoma brucei brucei* and treated with ethanolic extract of *Lawsonia inermis*.

Keys: A= Neutrophils; B= Lymphocyte; C= Eosinophils; D= Basophils; E= Monocytes
From the result in fig. III, the extract only reduced the level of parasitaemia but did not clear the parasites from the peripheral circulation. The extract slightly improved the PCV of the rats when compared with the infected-untreated group (Fig. IV).

**DISCUSSION**

This study demonstrates the potential of *Lawsonia inermis* as a natural agent in the management of African trypanosomosis. The oral acute toxicity study of the aqueous ethanolic extract of *Lawsonia inermis* indicates its wider safety of margin. Clarke and Clarke, (1977) suggested that, any substance whose LD50 in rats falls between 50-500mg/kg body weight should be regarded as very toxic, above 500mg/kg and below 1000mg/kg as moderately toxic and above 1000mg/kg as non toxic. This extract could be administered orally with wide margin of safety where absorption might not be complete due to factor limiting absorption in the gastrointestinal tract (Denis, 1984). The study showed concentration-dependent increase in its effect on the trypanosomes such that the parasites were completely immotile immediately after incubation at 30mg/ml of blood.

In a study by Wurochekke *et al.* (2004), showed that all parasites were immotile 45min after incubation at concentration of 8.3mg/ml and at 16.6mg/ml, the parasites were immotile immediately after incubation. There was significant effect of the extract on parasitaemia (Fig. III). However, the group treated with aqueous ethanolic extract of *Lawsonia inermis* survived longer than the infected-untreated control group. The slight difference observed in the present study was due to the reagent (ethanol) used compared to methanol used by Wurochekke and co-investigators.

The extract ameliorated, though very significant, the rapid drop in packed cell volume of the infected and extract-treated group which is often a feature of trypanosome infection. This finding is consistent with the reports of Mpiana *et al.* (2007).

There was considerable increase in the total white blood cells from day 1-4 post infections but later dropped from day 5 post-infection of the crude extract treated rats.

Leucocytosis and lymphopenia as observed in the present study is in agreement with waves of parasitaemia (Losos and Ikede, 1972) and the wax and wear syndrome on the host's immune system (Anosa, 1988).

Although oral administration of aqueous ethanolic extract of *Lawsonia inermis* for six days post-infection did not cleared the parasite completely, the life span of the rats were extended. This observation agrees with the findings of Abubakar *et al.* (2005).

The slight inactivity of the crude extract might be due to conversion of inactive molecules to active one by multicellular organism. One or more mechanism may act to enhance the chemical effect of the extract of *Lawsonia inermis* in which different compounds may potentiate or antagonize the antitrypanosomal activity.

In conclusion, this study revealed that aqueous ethanolic extract of *Lawsonia inermis* leaf has antitrypanosomal activity. However, the crude extract should be fractionized and further tested on different trypanosome species using rats and other animal models; this will provide conclusive information about the trypanocidal activity of *Lawsonia inermis* leaf.

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AUTHORS CONTRIBUTIONS

All the authors involved in this study played significant synergistic roles in the processes leading to the successful completion of the study, and subsequently, its publication.