

Antitrypanosomal activity of *anogeissus leiocarpus* in rats infected with *trypanosoma brucei brucei*

A.U. Wurochekke* and G.O. Anyanwu

Biochemistry Department, Federal University of Technology, Yola

Aqueous and methanol extracts of different parts of *Anogeissus leiocarpus* were analyzed for *in vitro* antitrypanosomal activity against *Trypanosoma brucei brucei*. The methanolic extract of the bark which had the highest *in vitro* activity against the parasite was selected for the *in vivo* analysis. Oral treatment with the methanolic bark extract to experimental rats at different doses (100,200mg/kg/day) for seven days did not clear the parasitemia. However, rats treated with 200 mg/kg/day survived longer than the infected control group. The results were discussed in respect to traditional treatment of trypanosomiasis.

Keywords: *Anogeissus leiocarpus*, Trypanosomiasis, *Trypanosoma brucei brucei*

INTRODUCTION

Trypanosoma brucei brucei is a haemo-protozoan parasite transmitted by tse tse fly which causes the disease called African animal trypanosomiasis (AAT). AAT can cause serious losses in cattle, sheep and goats (Moulton and Sollod, 1976), and other host animals includes pigs, horses, cats, monkeys and camels. African trypanosomiasis has serious deleterious effect on animals. The economic importance of the disease includes high morbidity rate, mortality, lower work efficiency and the high costs of treatment for animal that contract the disease (Bourn et al., 2005). It also reduces the growth rate, milk production, strength of farm animals and most of the time leads to death of the affected animal.

Some of the chemotherapeutic drugs used for the treatment of AAT are pentamidine, diminazene aceturate (Berenil), melarsoprol (Arsobal) and suramin (Fairlamb, 2003; Kuzeo, 1993). The existing drugs for AAT are unsatisfactory for various reasons, which include high level of toxicity, high cost, poor efficacy, undesirable route of administration and drug resistance (Fairlamb, 2003). Recent observation indicates that about 80% of the world's population relies solely upon medicinal plants for the treatment of diseases. This is more evident in most of the third world countries where

modern way of treatment of diseases is grossly inadequate. Most of the rural populace in north eastern part of Nigeria relies on medicinal plants for both human and animal treatment because of high level of poverty and unavailability of synthetic drugs. Although major part of chemically synthesized drugs against infectious agents is derived from natural products or from structures suggested by natural products (Kirby, 1966; Cragg et al., 1997; Soerjato, 1996), traditional healers mostly base treatments with medicinal plants on beliefs without considering the problems of over and under dosage and perhaps the toxic effects of the plants.

Anogeissus leiocarpus is a tree widely distributed in northern Nigeria. The bark and seed of the tree is used for the treatment and prevention of worm infestation in equine species (Dalziel, 1937). According to Kaboré et al. (2007), *Anogeissus leiocarpus* (DC.) Guill. and Perr. and *Daniellia oliveri* (Rolfe) Hucht. and Dalza. are commonly used to treat small ruminant gastrointestinal parasitism in the central region of Burkina Faso. Traditional healers in the north eastern part of Nigeria also believe that the bark of the plant is very effective in the treatment of African trypanosomiasis (Bizimana, 1994). This claim is not scientifically authenticated. Recent research findings have confirmed some of the claims of the traditional healers on medicinal plants while some were scientifically disapproved (Wurochekke and Nok, 2004; Wurochekke et al., 2005;

Shuaibu et al., 2008).

Already, extensive work has been done on the phytochemical components of *Anogeissus leiocarpus*. The phytochemical analysis of the extracts of *Anogeissus leiocarpus* revealed the presence of alkaloids, glycosides, phenols, steroids, tannins, anthraquinones, saponins and flavonoids (Mann et al. 2010 and Kaboré et al., 2010). Therefore, this study was aimed at determining the antitrypanosomal activity of *Anogeissus leiocarpus* against *T. brucei brucei* infection in rats.

MATERIALS AND METHODS

Plant material

Anogeissus leiocarpus parts (root, bark and leaf) were collected from campus of the Federal University of Technology, Yola. It was identified at the Forestry Department of the same university.

Trypanosome

Trypanosoma brucei brucei (federe strain, 2001) was kindly provided by the National Institute of Trypanosomiasis Research, Vom, Jos, Nigeria. It was isolated from Cow and stored in liquid nitrogen. Each of the recipient rats were inoculated with 3.6×10^3 *T. brucei brucei* cells.

Preparation of crude extract

The leaf, bark and root of the plant collected was dried under laboratory temperature and ground into powder separately using a mortar and pestle.

Exactly 20 g of the powders of each of the plant part was macerated in 400 ml of water and 100 ml of methanol. Extracts were filtered using a Buckner funnel and Whatman No 1 filter paper. Each filtrate was concentrated to dryness under reduced pressure at 40 °C using a rotary evaporator. All extracts were then stored in the refrigerator at 4 °C until required.

Experimental animals

Adult albino male and female rats were obtained from Department of Biochemistry, National Veterinary Research Institute (NVRI), Vom, Jos, Nigeria. The rats were kept in cages in the research laboratory of the Department of Biochemistry, FUT, Yola and were allowed to acclimatize for 7 days before the study. All rats were fed with commercial pellets (Pfizer Nigeria Plc., Ikeja, Nigeria) and watered *ad libitum* throughout the duration of the study.

Trypanosome infection

Infected blood obtained from previously inoculated donor rat at peak parasitemia was serially diluted with phosphate buffered saline (pH 7.4). Experimental animals were infected intraperitoneally with approximately 10^3 parasites per ml.

In vitro screening

Infected blood obtained by cardiac puncture of a rat at peak parasitemia was put into EDTA bottle. Aqueous and methanolic extracts were dissolved separately in phosphate buffered saline (pH 7.4) in appropriate proportion. Aliquot of 10 µl of 5% crude extract preparation was then incubated with 60 µl of the infected blood in wells of microtitre plates. For control, the crude extract was replaced with phosphate PBS (pH 7.4). After 5 minutes incubation in the wells of microtitre plates, about 2 µl of test mixture was placed on microscope slides and the motility of the parasites was observed under the microscope (Mgx40) at 5 minutes interval for 1 hour. The procedure was carried out separately for the aqueous and methanolic extract. Cessation or drop in motility of the parasites in extract-treated blood compared to that of parasite-loaded control blood without extract was taken as a measure of antitrypanosomal activity. The shorter the time of cessation of motility of the parasite, the more active the extract was considered to be (Atawodi et al., 2003). Under this *in vitro* system, parasites survived for about 4 hours when no extract was present and screening was performed in triplicates in 96 wells micro titer plates (Flow laboratories Inc., McLean, Virginia 22101, USA).

In vivo analysis

Experimental design

Twenty rats weighing between 150-200g were used. They were grouped into four and each group has five rats.

Group 1: uninfected control

2: infected untreated control

3: infected treated with 100mg/kg/day.

4: infected treated with 200mg/kg/day.

Administration of extracts

The methanolic bark extract which had the highest *in vitro* activity was administered to the rats using an oral gavage tube for seven (7) days.

Parasitemia determination

A drop of blood obtained from the rats by tail

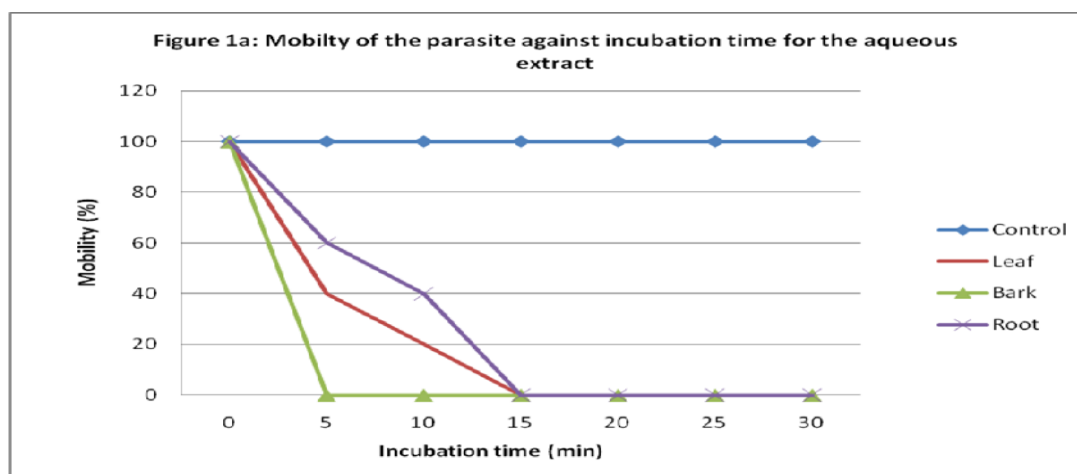


Figure 1a

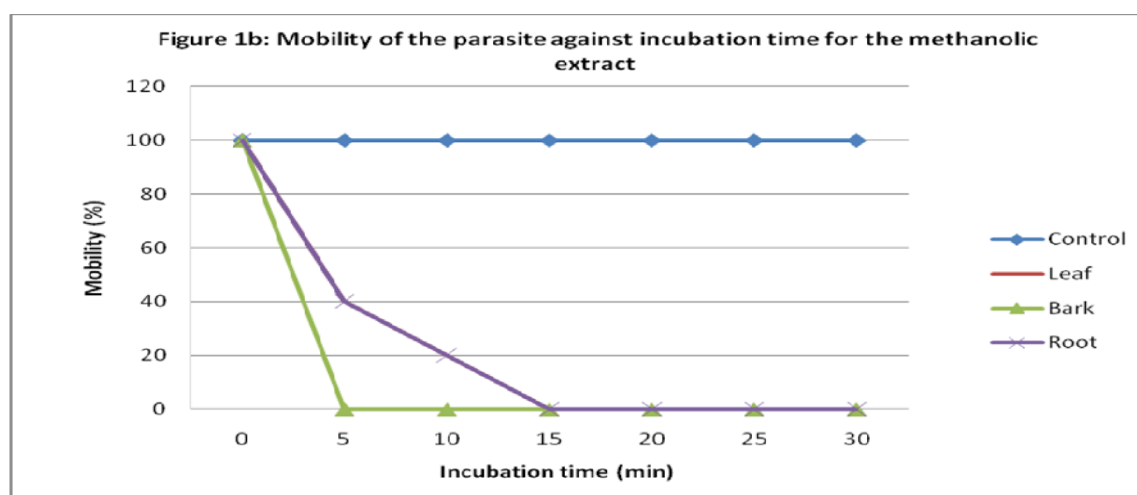


Figure 1b

snipping was used to make smears on the slides. Parasites were observed and estimated from each of the infected animals under the microscope as described by Herbert and Lumsden (1976). Parasitemia was monitored for nine (9) days.

Pack cell volume determination

Pack cell volume (PCV) was determined for five (5) alternate days using the microheamatocrit method according to Dacie and Levis (1991). All the rats died few days after the experiment with exception of the uninfected control group rats.

Statistical Analysis

The data were analyzed using analysis of variance technique (Snedecor, G.W. and W.G. Cochran (1967) and the differences in means were compared using Least Significant Differences test at 0.05 level of significance.

RESULTS

The water and methanolic extracts of the bark of the plant made parasites immotile immediately after incubation. Methanolic extracts of the leaf and root made parasites immotile 10 minutes after incubation. The aqueous extracts of the leaf and roots gradually reduced motility and 15 minutes after incubation all parasites were immotile (figure 1a and b).

The changes observed in the level of parasitemia of the infected animals are shown in Table 1. The level of parasitemia increased progressively in all the infected groups to reach a peak 7 days post infection. Treatment did not directly affect the course of parasitemia. However, the rats treated with 200 mg/kg body weight/day lived beyond Day 9 while that of the

Table 1: Parasitemia profile

| Days | Infected control | Infected treated 100mg/kg/day | Infected treated 200mg/kg/day |
|------|------------------|----------------------------------|----------------------------------|
| 3 | 8.0513±0.07902 | 7.9048±0.21188 | 7.77±0.23693 |
| 4 | 8.2275±0.08267 | 8.1619±0.18639 | 8.1056±0.17029 |
| 5 | 0.3505±0.06464 | 8.5225±0.02147 | 8.3981±0.08335 |
| 6 | 8.5964±0.06287 | 8.5556±0.04345 | 8.4891±0.07760 |
| 7 | 8.7250±0.03747 | 8.7267±0.05593 | 8.6840±0.06249 |
| 8 | 7.0538±1.76348 | 8.7975±0.06482 | 8.7891±0.03087 |
| 9 | 0 | 0 | 8.8491±0.01646 |

Table 2: Pack cell volume (PCV) against days post infection

| Days | Control | Infected control | Infected treated 100mg/kg/day | Infected treated 200mg/kg/day |
|------|------------|------------------|----------------------------------|----------------------------------|
| 3 | 59.60±1.70 | 58.60±2.11 | 58.60±1.89 | 58.80±1.07 |
| 5 | 58.80±1.59 | 39.80±1.77 | 41.60±2.16 | 41.60±1.63 |
| 7 | 53.80±1.69 | 37.80±2.20 | 39.00±2.05 | 39.20±0.80 |
| 9 | 52.60±1.63 | 0 | 0 | 36.00±1.64 |

infected untreated control died on Day 8.

Table 2 show the changes observed in the level of the pack cell volume (PCV). The PCV of the infected control group dropped faster compared to the gradual decrease observed in the treated groups

DISCUSSION

Extracts of the different parts of the plant were found to possess *in vitro* antitrypanosomal activity against *Trypanosoma brucei brucei*. The activity varied among the different parts of the plant. Methanolic and aqueous extracts of the plant bark had the highest *in vitro* activity against the parasite. This suggests that the bark of the plant may have higher concentration of the active component responsible for the activity observed. The active components in the stem bark of *Anogeissus leiocarpus* and *Terminalia avicennoides* were hydrolysable tannins (Shuaibu et al., 2008). It also implies that the active ingredient responsible for the activity observed in this work can easily dissolve in the solvents (methanol and water) used for the extraction. *In vitro* activity shown by the various parts of the plant produce evidence to support the local use of the plant, since bioactive screening is a useful method for pre-selection of plants for bioassay

guided isolation and identification of active principles.

In vivo activity was carried out with the methanolic extract of the plant bark which had the highest *in vitro* activity to support the antitrypanosomal activity of the plant. Treatment by the extract did not show any significant difference compared to the infected untreated control ($p < 0.05$). However, animals treated with 100 mg/kg/day died earlier than those treated with 200mg/kg/day. This suggests that the antitrypanosomal effect of the extract might be dose dependent, as higher dosage may mean higher concentration of the active phytochemical components, which could be responsible for the survival of the rats treated with 200mg/kg/day for four (4) days after the experiment was concluded.

The inability of the extract to clear the parasite from the blood could be because of their failure to reach the site of action or rapid metabolism (Dwivedi, 1997; Wurochekke et al., 2005). The extract was orally administered and lack of activity could be due to biotransformation within the GIT and liver. Notwithstanding, the extract contains active phytochemicals which might be acting individually or synergistically to inhibit the antitrypanosomal activity observed in the *in vitro* test.

In conclusion, the plant has shown *in vitro* antitrypanosomal activity against *T.brucei brucei*,

and *in vivo* higher doses of the extracts ameliorated the disease condition. Further work has to be done with doses higher than those in this study to sufficiently support the work reported by Shuaibu et al. (2008), that the extracts from the stem bark of *Anogeissus leiocarpus* possessed remarkable trypanocidal activity. Nonetheless, this work could be evidenced for the local use of the plant by the herdsmen for the treatment of trypanosomiasis.

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