

Antitrypanosoma effect of methanol fruit pod extract of *Acacia nilotica* (Linn) in acute *Trypanosoma brucei* infection in wistar rats

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Abstract

Chemotherapy of trypanosomosis has been adversely affected by widespread drug resistance and toxicity. Attention has now been shifted towards the search for ethnobotanical means of controlling the disease. The aim of this study was to determine the effect of methanol extract of the fruit pod of *Acacia nilotica* on experimental *Trypanosoma brucei* infection in Wistar rats. Twenty Wistar rats were randomly allotted into four groups of 5 rats each. Rats in groups I to III were experimentally infected with an isolate of *T. brucei* while group V served as uninfected control. At day 2 post infection (PI), rats in groups I were orally treated with extract of fruit pod of *A. nilotica* at a dose of 500 mg/kg for 5 days while rats in group II were treated with diminazene aceturate at 3.5mg/kg subcutaneously once. Rats in group III served as infected untreated control. Parasitaemia, PCV and survival rates were monitored during the course of the study. Parasitaemia begins 3 days PI and progressively increased in groups I and III but was not observed in group II. There was decrease in PCV of rats in groups I and III compared to that of groups II and IV. The differences observed in parasitaemia and PCV between the groups compared were not statistically significant ($P > 0.05$). Mortality was observed in group I from day 8 PI with 2 rats (40%) surviving up to day 10 PI. All rats in group III died within 7 days post infection while mortalities were not recorded in groups II and IV. It was concluded that the methanol extract of the fruit pod of *A. nilotica* had no *In vivo* trypanocidal effect at the dose of 500mg/kg, but demonstrated some ameliorative effect on the severity of the infection in Wistar rats.

Keywords: *Acacia nilotica*, antitrypanosoma, fruit pod extract, *Trypanosoma brucei*

Introduction

African animal trypanosomosis (AAT) is a disease complex caused by tsetse-fly-transmitted haemoprotozoan parasite *Trypanosoma congolense*, *T. vivax*, or *T. brucei brucei*, or simultaneous infection with one or more of these trypanosomes (Taylor *et al.*, 2007)). The disease is most important in cattle but can cause serious losses in pigs, camels, goats and sheep. Animal trypanosomosis is a major factor retarding the growth of the livestock industry in Africa and it has constituted a major obstacle to the economic development of the rural areas affected (FAO, 2002).

The control of trypanosomosis over the years relies largely on the use of

chemotherapeutic agents and vector control (WHO, 1998). Drug resistance and toxicity are two important complicating factors in the chemotherapy of trypanosomosis and the widespread use of insecticides is not environmentally friendly. The failure of these methods has been complicated by the lack of vaccine and new drugs. Development of new antitrypanosomal drugs has been more or less static over the last three decades, due to lack of interest by the pharmaceutical industry to invest into research and development of new antitrypanosomal drugs (Welburn *et al.*, 2009). In Nigeria, several ethnobotanical studies of Nigerian medicinal plants indicated both significant *in vitro* and *in*

Antitrypanosoma effect of methanol fruit pod extract of Acacia nilotica

vivo antitrypanosomal activities (Abubakar *et al*, 2005). The present study aimed at exploring the antitrypanosomal potential of an abundant indigenous medicinal plant *Acacia nilotica* (Gum Arabic tree) with the hope of finding alternative, cheaper and safer treatment of trypanosomosis in Nigeria.

Materials and methods

Plant material

Fresh fruits of *A. nilotica* were collected from Samaru-Zaria in Kaduna State, Nigeria. The seeds of the fruits were removed and the fruit pods dried in open air in the laboratory and pounded into small particles. Fifty grams (50g) of the powder was weighed and extracted under reflux in 500 mL of 70% methanol. The extract was air dried and stored in a refrigerator at 4°C until required.

Phytochemical analysis of the extract

The methods described by Harborne (1978) were used to test for the presence of some active constituents in the extract.

Determination of the median lethal dose (LD₅₀) of the extract

The median lethal dose (LD₅₀) and hence the acute toxicity of the extract was determined using the method described by Lorke (1983).

Test organisms

An isolate of *Trypanosoma brucei* obtained from the parasites bank of the Department of Vet Parasitology and Entomology, Ahmadu Bello University, Zaria, Nigeria.

Experimental animals

Twenty (20) albino rats of both sexes were randomly allocated into four groups (I-IV) of five rats each in separate cages in the animal house of the Department of Vet Parasitology and Entomology, ABU, Zaria. The rats were fed *ad libitum* with rat chow

and water. They were allowed to acclimatize for one week before the commencement of the experiment.

Inoculation of experimental rats with the T. brucei isolate

About 3 mL of blood was obtained from a donor rat at massive parasitaemia by sacrificing the rat via jugular venesection into a vial. The blood was diluted with PBS until 2×10^6 trypanosomes/ml of blood was obtained (Herbert and Lumsden, 1976). Rats in groups I, II and III were each inoculated intraperitoneally with 0.5 mL of the diluted blood. Rats in group IV served as uninfected control.

Treatment of the infected rats

At day 2 post infection, rats in group I were orally treated with the extract at a dose of 500mg/kg body weight for 5 days. Rats in group II were treated with diminazine aceturate (standard drug) at a single dose of 3.5 mg/kg intraperitoneally, while rats in group III and IV served as positive and negative controls respectively.

Blood sample collection and analysis

Blood was aseptically collected from each rat by vein puncture of the tail vein into heparinized capillary tubes daily for parasitaemia estimation and every other day for packed cell volume (PCV) determination.

Determination of survival rate of the experimental animals

The number of animals that survive the infection were monitored and recorded daily during the course of the experiment.

Statistical analysis

The data obtained were summarized as means \pm SE and subjected to repeated measure ANOVA followed by Tukey's post-hoc test using GraphPad Prism Software version 5.01 for windows. Values of $P < 0.05$ were considered significant.

Results

The phytochemical analysis showed the presence of carbohydrates, anthracene derivatives, steroids, triterpenes, cardiac glycoside, saponic glycosides, flavonoids, tannin, and alkaloid (Table 1). While the LD₅₀ study revealed that methanol extract of the fruit pod was relatively safe as the animals given the highest dose of 5000 mg/kg survived the acute toxicity test. Parasitaemia monitoring indicated a pre-patent period of 3-5 days. As also observed in Group III (untreated control), parasitaemia was not cleared in group I (extract treated group) throughout the course of the study (day 9 post-infection). Group II (diminazene teated) showed no parasitemia throughout the study. There

was no statistical difference (p>0.05) in the level of parasitaemia between group I and group III (figure 1). The mean PCV values of groups I and III rats showed progressive drop from day 2 PI to day 6 PI. The mean PCV values in II and IV (uninfected control) remained relatively within same range throughout the period of the study. However, there was no significant statistical difference (p>0.05) between the PCV values of the infected and uninfected groups. (Figure 2). Mortality started in group I rats from day 8 post infection with 2 rats (40%) surviving up to day 10 post infection. All rats in group III (untreated control) died within 7 days PI while mortalities were not recorded in groups II and IV throughout the study (Figure 3).

Table 1: Qualitative phytochemical analysis of methanol fruit pod extract of *Acacia nilotica*

Active principle	Leave extract
Carbohydrate	+
Anthracene derivatives	+
Steroids and Triterpenes	+
Cardiac glycosides	+
Saponic glycosides	+
Flavonoids	+
Tannin	+
Alkaloid	+

Key: + = Present

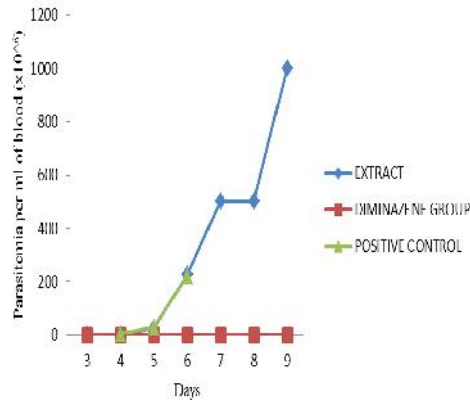


Figure 1

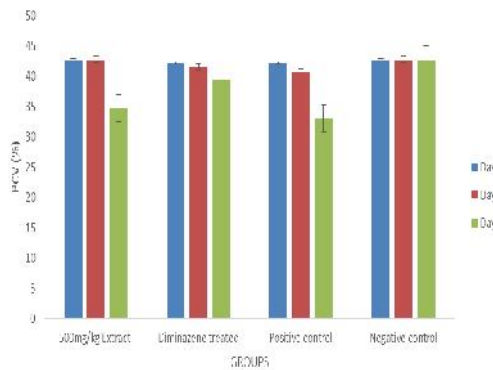


Figure 2

Figure 1: Parasitemia values of *T. brucei* infected rats treated with methanol fruit pod extract of *Acacia nilotica*

Figure 2: Changes in packed cell volume values of rats infected with isolates of *Trypanosoma brucei brucei* and treated with fruit pod extract of *Acacia nilotica*.

Antitrypanosoma effect of methanol fruit pod extract of Acacia nilotica

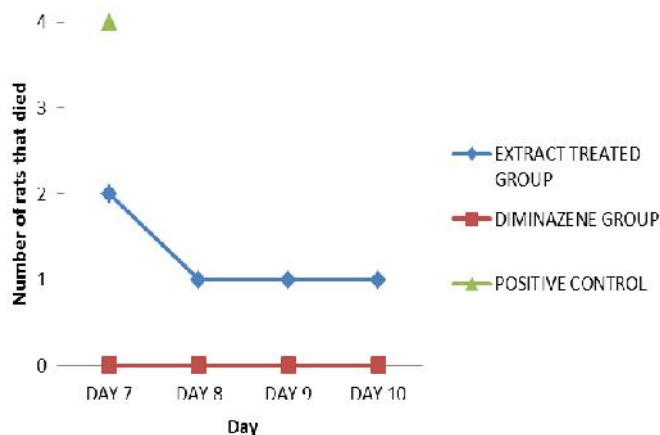


Figure 3: Effect of methanol fruit pod extract of *Acacia nilotica* on survival period in the extract treated group.

Discussion

The phytoconstituents observed in the methanol extract of the fruit pod of *Acacia nilotica* in the present study agrees with the report of Ogbadoyi *et al.* (2011) who also reported similar phytoconstituents in the stem bark extract of the plant in Niger state, Nigeria. Of the phytoconstituents observed in the present study, alkaloids, terpenes, quinones and polyphenols have been shown to be potent growth inhibition of *T. cruzi* (Wright and Phillipson, 1990). Triterpenoids and sterols from the plants are reported to possess antitrypanosomal activity (Hoet *et al.*, 2007). However, despite observing these active ingredients in this study, the extract could not clear or inhibit the progression of parasitemia in the infected rats. This could be attributed to the fact that different parts of the plant might have different concentrations of the active ingredients (Ogbadoyi *et al.*, 2011). It could also be due to the strain or species differences on the part of the parasite as different strains or species of Trypanosome might respond differently to the effect of the phytoconstituents. The observed progressive drop in PCV of the extract

treated rats further question the antitrypanosoma potential of the extract as anemia is a major clinical feature of trypanosomosis. However, the present study had shown that the fruit pod extract of the plant prolonged the survival period of the treated group compared to the infected untreated group. This observation conforms to the previous report by Ogbadoyi *et al.* (2011) and could be attributed to either the extracts ability to reduce the parasite load or its ability to neutralize the toxic metabolites produced by trypanosomes (Abubakar *et al.*, 2005).

We recommend that further studies be carried out using the extract at higher doses to ascertain the effective dose that may elicit antitrypanosomal effect on experimental *T. brucei brucei* infection in rats since some of the phytoconstituents tested in the extract have been previously reported to have antitrypanosomal activities.

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