Research Article

In vivo antitrypanosomal activities of *Acacia nilotica* stem bark methanol extract in Wistar rats infected with *Trypanosoma brucei brucei*

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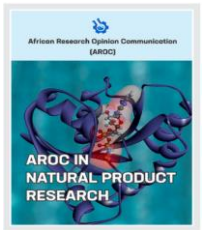

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Abstract

Background: Trypanosomiasis is a disease of vertebral animals caused by parasitic protozoa of the genus Trypanosoma. It is one of the neglected tropical diseases (NTDs) affecting about 36 countries of Sub-saharan Africa, threatening more than 60 million people and 70 million animals. Chemotherapy is the major means of controlling African trypanosomiasis is limited by rapid drug resistance, toxicity and high cost. There is an urgent need for therapeutic agents that are effective, affordable, and accessible to the rural poor people in Africa who are greatly affected by the disease. This study aimed to determine the *in vivo* effect of stem bark methanol extract of *Acacia nilotica* (*A. nilotica*) on experimental *Trypanosoma brucei brucei* infection in Wistar rats. **Methods:** Phytochemical analysis, and LD_{50} determination were carried out using standard procedures. Three (3) days pre-patent period was observed after inoculating the rats with the parasite. Parasitemia was monitored daily while the Parked Cell Volume (PCV) was determined at one-day intervals during the infection course. **Results:** The phytochemical analysis showed the presence of carbohydrates, steroid/triterpenes, saponin, alkaloid, flavonoid, tannin, glycosides, and anthraquinones. The toxicity of the stem bark methanol extract was tolerable at 1200 mg/kg body weight. Methanol extract of *A. nilotica* (stem bark) showed promising curative potential in vivo against *Trypanosoma brucei brucei* at 300, 400 and 500 mg/kg body weight. These doses completely cured the experimental T.b.b infection in Wistar rats after 3 days of treatment. Significant reduction ($p<0.05$) in the parasite burden confirmed by the absence of anaemia (PCV 48.1% ±1.5% and 46.4% ±1.3% respectively) was observed when compared with the "infected but not treated" control group (normal saline group). **Conclusion:** Based on these observations, it was therefore deduced that the methanol extract of *Acacia nilotica* stem bark extract possessed the active ingredient that cures experimental *T. brucei brucei* infections in Wistar rats.

Keyword: *Trypanosoma brucei brucei; A. nilotica;* Stem bark; anti-trypanosomal; *in vivo;* crude methanol extract

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1.0 Introduction

Trypanosoma brucei brucei is a protozoan of the genus *Trypanosoma* that causes trypanosomiasis in cattles and other domestic animals by infecting their blood plasma [1]. This parasite is transmitted by tsetse flies of the family *Glossinidae* [2]. Susceptible animals when infected become weak, emaciated, and reproductively breeding animals may abort and become infertile [1]. The *Trypanosomes*, *Trypanosoma vivax, Trypanosoma congolense* and *Trypanosoma brucei brucei* are the main species responsible for African Animal Trypanosomiasis (AAT) called Nagana in West Africa. Human African Trypanosomiasis (HAT) or sleeping sickness is a disease caused by two subspecies of *Trypanosome brucei, i.e. T. brucei gambiense and T. brucei rhodesiense.* Surra and Dourine are caused by the other *Trypanosoma species T. evansi and T. equiperdum* respectively. Sleeping sickness occurs in 36 sub-Saharan African countries where there are tsetse flies that transmit the disease [3].

In Nigeria, the disease is prevalent and the wide distribution of the disease is attributed to the abundance of its biological and mechanical transmitting vectors which are tsetse flies and biting flies, respectively [4]. It affects human and livestock production causing significant losses which range from a decrease in milk production to death [4]. All warm-blooded animals including wildlife species have been implicated in the transmission cycle of the disease. Tsetse flies cover approximately 80% of the landmass in Nigeria, hence AAT continues to thrive, and losses

incurred have not been reduced [5]. However, it seems to be re-emerging as a significant livestock disease and extending to areas that were previously designated as tsetse fly- free zones [6].

The current chemotherapy of Trypanosomosis relies on drugs which were been used for donkey years and are expensive with toxic side effects and many studies have shown the emergence of strains that develop resistance to these drugs [7]. In view of these the development of new inexpensive, effective drugs in the treatment of Trypanosomiasis is urgently required to control the disease. However, it has been observed that natural products obtained from plants and the recent discovery of novel drugs such as artemisinin, atropine, digitoxin, digoxin, emetine, pilocarpine, quabain, quinidine, quinine, reserpine, vinblastine, vincristine, etc., from medicinal plants implies that vast potential still exists for the production of numerous more novel drugs [8]. Consequently, the area of ethnopharmacology of medicinal plants has attracted increasing attention in new drugs research and development [9]. This is the main reason why this research was designed to study the Antitrypanosomal properties of the components of Stem bark extract of *Acacia nilotica* plant.

Acacia nilotica (common name: Gum Arabic tree) is commonly found in Guinea and Sudan Savannah vegetation in Nigeria. It is locally called Bagaruwa in Hausa, Gaude in Fulfulde and Kangar in Kanuri. The plant is widely used in traditional medicine in Africa, it has been found to possess significant antimicrobial activity, antioxidant, antidiarrhoeal, anticancer, antimutagenic properties, anthelmintic activity, antiplatelet aggregatory activity and vasoconstrictor among others [10]. The aim of this study was to determine the *in vivo* anti-trypanosomal effect of *Acacia nilotica* stem bark methanol extract in Wistar rats infected with *Trypanosoma brucei brucei.*

2.0 Materials and Methods

This research is a further study on the authors' previous work carried out (on the phytochemical screening and acute toxicity studies on *Acacia nilotica* stem bark methanol extract) and re-described in sections (2.1 - 2.8, 3.1, 3.2 and 4.0) of this paper.

2.1 Animal ethical clearance

An application was submitted to the Animal Right Ethical Committee of Ahmad Bello University, Zaria and clearance/approval (Reference number: ABUCAU/2020/015) was granted upon satisfying all conditions.

2.2 Sources of plant material and identification

Fresh stem bark of *Acacia nilotica* (Linn) plant was obtained from Usmanu Danfodio University Sokoto (UDUS), Sokoto State, North-West Nigeria, between December 2019 and January 2020. The plant sample was identified at the Herbarium unit of the Department of Botany, Faculty of Life Sciences, Ahmadu Bello University Zaria, where a Voucher specimen number of ABU014634 was given and deposited.

2.3 Plant preparation

The collected stem bark was washed with tap water to remove dirt and dried under shade. The dried materials were ground using pestle and mortar. Eight hundred grams (800g) of the ground stem bark was weighed and stored in a clean tied-up polythene bag at room temperature until required.

2.4 Stem bark extraction

Standard procedures were used for the extraction of the plant materials. 500 grams of the ground stem bark was used for extraction with methanol using a Soxhlet apparatus for 24 hours. The liquid extract was concentrated to dryness under rotary evaporator to remove the solvent for 12 hours. The recovered extract was weighed and transferred to sterile universal bottle which was kept in the refrigerator until required.

2.5 Phytochemical screening

Ten (10) grams of crude stem bark methanol extract was used to evaluate the presence of some active Phyto-constituents like carbohydrates, steroids, triterpenes, cardiac glycosides, saponin, tannin, flavonoid, alkaloid, and anthracenes, using methods described by Evans [11].

2.6 Test parasite

An isolate of *Trypanosoma brucei brucei* was obtained from the Department of Veterinary Parasitology and Entomology Ahmadu Bello University Zaria and maintained in the laboratory by serial passage in rats. The culture was confirmed microscopically.

2.7 Experimental animals

A total of 100 Wistar rats of both sexes weighing 100 – 120 grams were purchased from the animal house of the Department of Pharmacology, Faculty of Pharmaceutical Science, Ahmadu Bello University, Zaria. The experiment was conducted in compliance with the internationally accepted principle for laboratory animal use and care as contained in Organisation for Economic Cooperation and Development (OECD) guidelines on animal use protocol. The animals were maintained on commercially prepared feed and housed in disinfected cages for one week to acclimatize prior to the commencement of the experiment. The experiment was conducted at the Protozoology laboratory of the Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria.

2.8 Acute Toxicity Studies/Determination of LD_{50}

Before experimenting with the animals, normal females, nulliparous and non-pregnant were randomly selected and grouped into five groups (n=4) and then kept in their cages for 7 days prior to dosing to allow acclimatization to the laboratory conditions. All groups of the rats fasted overnight prior to administration. Following the fasting period, all animals were weighed, and the doses were calculated based on their body weight. The animals were given 80% methanol stem bark crude extracts (prepared in distilled water) orally at different doses of 600, 800, 1000 and 1200 mg per kilogram body weight (kg^{-1}bw) respectively while distilled water was given to fifth group (control group). After the administration, the animals were kept under close observation continuously for 1 hour and intermittently for 4 hours and thereafter once every 24 hours for the next 2 days. During this study period, clinical observations were made for mortality, behavioral changes, unnecessary noises and any other abnormalities and their weight were measured.

The LD_{50} was calculated using the formula:

$LD50 = \sqrt{(Do \times D100)}$ Where:

D_0 = Highest dose that gave no mortality and D_{100} = Lowest dose that produced mortality [12].

2.9 Inoculation of experimental rats with test organism isolate

A 3 ml of blood was collected by cardiac puncture with EDTA coated syringe from heavily infected rats followed by dilution with normal saline to serve as inoculum. Healthy rats were infected intraperitoneally with 0.2 ml of the inoculum containing about 10^6 trypanosome cells. Infection was monitored daily by microscopic examination of blood samples taken from tails of the infected animals. The degree of Parasitaemia was determined using the rapid matching method [13].

2.10 Treatment of the infected rats with crude stem bark methanol extract

To determine the bioactivity of the crude methanol extract, initial experiments were carried out. On day 3 post-infection (PI), three groups of rats (1 – 3) were orally administered with the extract at doses of 300, 400, and 500 mg per kilogram body weight (kg^{-1}bw) respectively for 7 days. The fourth group formed the negative control (infected but not treated), while the infected and treated with 3.5 mgkg-1bw of the standard drug diminazene (fifth group) served as the positive control [14].

2.11 Determination of parasitaemia

Parasite count was monitored daily in the infected groups and determined microscopically at 400 magnification using the "Rapid Matching" method of Herbert and Lumsden, [15]. Briefly, this involved microscopic counting of parasites per field in pure blood or blood appropriately diluted with phosphate-buffered saline (PBS, pH 7.4). Logarithmic values of these counts obtained by matching with the table of Herbert and Lumsden is converted to antilog to provide an absolute number of trypanosomes per ml of blood [16]. The pre-patent period was determined when the first *T. brucei brucei* parasite was seen in the blood of the infected animals.

2.12 Packed Cell Volume (PCV)

The PCV of the animals from each group were determined on 0, 2, 4, 6, 8, 10, 12 and 14 days' post-infection by the Microhaematocrit method described by Coles [17]. The blood was directly taken from the ocular vein into heparinized capillary tubes by capillary action to about 75% of its length. The ends of the tubes were sealed with a flame using a Bunsen burner. The sealed tubes were placed in a microhaematocrit centrifuge, with the sealed ends near the outside rim of the centrifuge (touching the rim). The cover of the centrifuge was tightened to prevent blood spillage and centrifuged for 5 minutes. Thereafter, the capillary tubes were placed in a graphic reader and the packed cell volume (PCV) were directly read in percentage.

2.13 Statistical Analysis.

All values were shown as mean values along with their standard deviations (±SD). SPSS software was used to analyze data. Statistical comparisons were carried out by Analysis of Variance (ANOVA), Tukey's multiple comparison test, and p values <0.05 were considered significant.

3.0 Results

3.1 Phytochemical compositions

Table 1 shows the results of *Acacia nilotica* stem bark preliminary phytochemical analysis. The compounds present include carbohydrate, flavonoid, saponin, alkaloid, cardiac glycosides, triterpenes, unsaturated steroids, Anthraquinones. These compounds were physiologically active phytoconstituents of most medicinal plants possessing great potential for therapeutic and prophylactic uses.

3.2 Acute toxicity studies

The Acute Toxicity studies of the showed no evidence of toxicity in Group 1 (600mgkg^{-1}) and Control group (distilled water). Conversely, there was slight increase in observable toxicity effect in the groups (animals) with increase in the administered dose however, animals fully recovered and regained their activeness after few hours and no single death (Table 2).

Table 1: Phytochemical constituents present in the methanol crude stem bark extract of *A. nilotica*.

Phytochemicals	Test	Observation	Result
Carbohydrate	Molisch	Reddish colour	+
Flavonoid	Sodium hydroxide	Yellow colouration	+
Saponin	Frothing test	Foam formation	+
Alkaloid	Wagner's test	White precipitate	+
Cardiac Glycosides	Keller-killani test	Pale green	+
Triterpenes	Lieberman bucchard	Pink colour	+
Unsaturated steroid	Lieberman bucchard	Blue-green colour	+
Anthraquinones	Bontrager's test	Bright pink colour	+

KEYS: + = present

Table 2: Determination of median lethal dose (LD_{50}) from crude methanolic stem bark extract of *Acacia nilotica*.

Group (n=4)	Treated Dose	Number of Dead Animal	Observation
Group 1	600mgkg^{-1}	0	No sign of toxicity; animals remain active.
Group 2	800mgkg^{-1}	0	Animals remain sluggish for a while and later become normal again.
Group 3	1000mgkg^{-1}	0	Breathing was slow, there was the closing of eyes and erection of fur but animals later recovered fully.
Group 4	1200mgkg^{-1}	0	Sluggishness, closing of eyes, depressed breathing but later becomes active after some hours.
Group 5	Distilled water	0	No signs of toxicity observed

KEY: n= Number of Animal per group

3.3 Test for Antitrypanosomal activities of stem bark crude methanol extracts

The crude methanol extract of *Acacia nilotica* plant was subjected for bioassay to determine its curative effects in rats infected with *T. brucei brucei*. The crude methanol extract of *Acacia nilotica* at the doses of 300, 400, and 500 mg/kg bw inhibited the *T. brucei brucei* parasites proliferation (Figure 1), and improve the body packed cell volume of the infected rats (Figure 2) when compared with the group administered with normal saline. In the presence of the antitrypanosomal agents (CME and DMZ), the load (Estimated Marginal Means) of the *T. brucei brucei* parasites (Figure 1) was indirectly proportional to the Average PCV of the animals observed (Figure 2). There was no positive therapeutic effect observed in groups administered with normal saline.

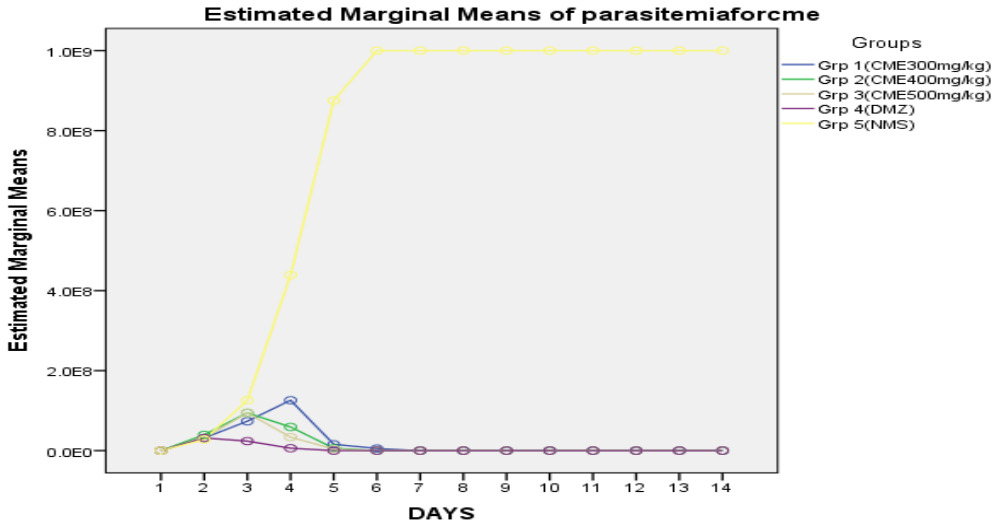

Figure 1: Screening for the antitrypanosomal effects of stem bark crude methanol extract (CME) of *A. nilotica.* Key: Grp= group, CME= crude methanol extract, DMZ= diminazene, NMS= normal saline

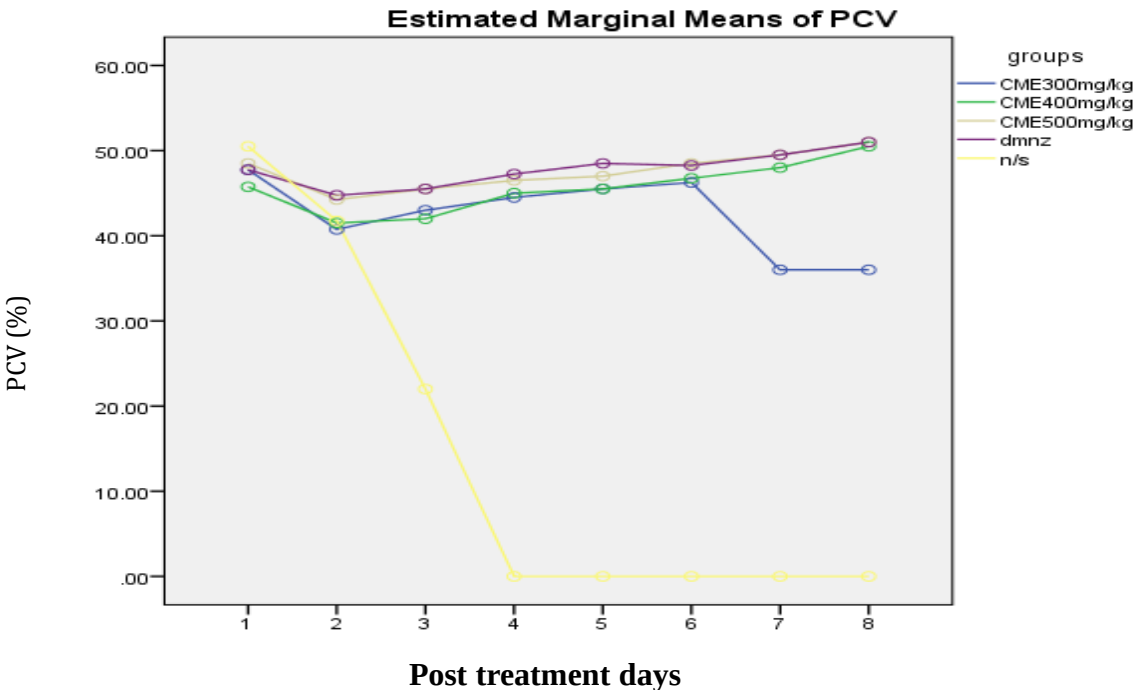

Figure 2: Average parked cell volume of groups of rats (1-5) treated with CME of *A. nilotica* stem bark extract. Key: Grp= group, CME= crude methanol extract, DMZ= diminazene, n/s= normal saline

4.0 Discussion

The present study evaluated the antitrypanosomal activities of *Acacia nilotica* stem bark methanol extracts in Wistar rats infected with *Trypanosoma brucei brucei*. The result of the phytochemical analysis of the crude methanol stem bark extract of *A. nilotica* reveals the presence of carbohydrates, flavonoids, tannins, saponins, alkaloids, cardiac glycosides, anthraquinones, steroids and triterpenes (Table 1). This data supports several other studies that reported similar phytochemical constituents from this plant [17, 18].

The report of Abdulhamid and Amar [9] on phytochemical analysis of this plant's leaves also agreed with our findings. Moreover, Jatau and Tsok-Nwok, [14] reported that fruit pods of *A. nilotica* contain the same compounds similar to our result. Anyam et al., [19] reported a similar result from the root methanol extract of this plant. Thus, this proves that different parts (stem, leaves, and fruits) of the plants tend to have similar phytochemical constituents. These phytochemicals are physiologically active compounds possessing great potential for several therapeutic and prophylactic uses/applications [20].

The median lethal dose (LD50) determination/Acute toxicity test assesses the adverse effects that occur within a short time after administration of a single dose of a test substance. The acute toxicity study carried out in this research did not show any toxicity signs and symptoms at 600, 800, 1000 and 1200 mg kg-1bw. No morbidity or mortality was observed in the treated groups for each dose during the acute toxicity study. As a result, the LD50 of the extract could be greater than 1200 mg/kg body weight (Table 2). The present results from the acute toxicity study agreed with the study reported by [14] who reported that methanol fruit pod extract of *Acacia nilotica* was relatively safe at 5000 mg/kg. Therefore, 80% methanol extract may be considered relatively safe on acute exposure.

The compounds obtained from phytochemical screening are known to be biologically active and thus may have contributed to the observed antitrypanosomal activities in this plant stem bark methanol extract [22]. The stem bark methanol extract at 400 and 500mg/kg doses showed a very similar effect as the standard antitrypanosomal agent (diminazene) and cleared all parasites in the infected rats after 5 days of treatment and these rats remain aparasitic for up to 20 days while the Parked Cell Volumes (PCV) mean values remain within the normal range (43% - 53%). This indicates the absence of anaemia which is the major pathological feature of trypanosomiasis which is considered as a pointer of the acute nature of the infection [21]. The PCVof the

"infected but not treated" control group dropped faster (48% to 22%) compared to the gradual decrease observed in the treated groups. There was a significant statistical difference ($p<.05$). between the crude methanol extract treated groups and control group (infected but not treated) This agrees with the report of Ogbadoyi et al. [13[who reported that methanol crude extract of Acacia stem bark at 400 and 500 mg/kg doses cleared *T. brucei brucei* in mice after 3 days of treatment. Findings from Jatau and Tsok-Nwok, [14], revealed that the methanol extract of fruit pods of this plant exerted a curative effect on Trypanosoma infected rats, after 5 days' treatments. Crude methanolic root extracts of *A. nilotica* demonstrate significant activity against chloroquine-sensitive protozoa strain of Plasmodium berghei in mice [7].

Moreover, the plants exhibited dose and time-dependent activities, this suggests that the antitrypanosomal effect of the extract is dose-dependent, as higher dosage (evidencing a higher PCV value) may mean a higher concentration of the active phytochemical components, which could be responsible for the survival of the rats treated with 400 and 500 mg/kg/day for 10 days after the experiment was concluded. More specifically, the activities reported on this plant against *T. brucei brucei* may be due to the presence of triterpenes and tannins which were reported to have antiprotozoal activities [6, 23]. Flavonoids and alkaloid compounds were also reported to have antitrypanosomal [24].

5.0 Conclusion

The crude methanol extract of *Acacia nilotica* stem bark was evidenced to entail several bioactive compounds and have shown a very promising and positive anti-trypanosomal effect on *T. brucei brucei* in the experimental rats. Further research on these plant stem back fractions is in progress.

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Conflicts of Interest

The authors declared no conflict of interest.

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