

IN VIVO ANTITRYPANOSOMAL EFFECTS OF SOME ETHNOMEDICINAL PLANTS FROM NUPELAND OF NORTH CENTRAL NIGERIA

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Abstract

Four medicinal plants *Acacia nilotica*, *Bombax buonopozense*, *Terminalia avicennioides* and *Zanthoxylum zanthoxyloides* traditionally used for treatment of sleeping sickness in Nupeland were investigated for *in vivo* antitrypanosomal activity. Methanol extracts of different parts of each plant (stem barks and fruits) were obtained and evaluated for their *in vivo* antitrypanosomal activities against *Trypanosoma brucei brucei*. Phytochemical screening of the methanol extracts of each plant were performed by standard procedures. Methanol extracts of *A. nilotica* (stem bark), *B. buonopozense* (stem bark), *T. avicennioides* (round fruit) and *Z. zanthoxyloides* (stem bark) were effective on trypanosomes. The extracts of *A. nilotica* and *B. buonopozense* exhibited antitrypanosomal effects at 200 and 300 mg/kg body weight respectively. Doses were able to clear the parasites from circulation within 6 and 7 days of treatment respectively with prolonging survival period of up to 30 days. While the extracts of *T. avicennioides* and *Z. zanthoxyloides* showed trypanostatic effects and could not clear the parasites completely. The methanol extracts of these plants contain metabolites that are associated with antitrypanosomal effects; therefore, these medicinal plants may be sources of new compounds that may be active against *T. b. brucei*. This study has also justified the claim that some medicinal plants of Nupeland possess antitrypanosomal activity and could be useful in the management of trypanosomiasis.

Key words: Antitrypanosomal activity, Ethnomedicinal plants, Nupeland, Sleeping sickness, Trypanosomiasis, Trypanostatic effect

Introduction

Nigeria's biodiversity is rich in medicinal plants. Over 25% of our common medicines contain at least some compounds obtained from plants (Farnsworth, 1988). The World Health Organization reported that 70–90% of the world's population relies chiefly on traditional medicine (WHO, 2004) and a major part of the traditional therapies involve the use of plant extracts or their active constituents. The local use of natural plants as primary health remedies is due to their pharmacological properties. Many plant extracts owe their potency to the presence of metabolites. These metabolites are usually found in various parts of the plants like roots, leaves, shoots and bark. Many plants have therefore become sources of important drugs and as such the pharmaceutical industries have exploited traditional medicine as a source of bioactive agents that can be used in the preparation of synthetic medicine (Kingham, 1994). Natural products play important roles in drug discovery and development process, particularly in the field of infectious diseases, where 75% of these drugs are of natural origin (Newman et al., 2003).

Trypanosomiasis, a disease of major importance in human and animals has continued to threaten human health and economical development (WHO, 1995; Kuzoe, 1993). *T. b. gambiense* and *T. b. rhodesiense* as the etiological agents of trypanosomiasis affect millions of people in sub-Saharan Africa and are responsible for the death of about half a million patients per year (Barrett, 1999).

Chemotherapy is the most widely used means of controlling the trypanosomiasis. The few registered trypanocides are often associated with severe side effects (Guttering, 1985) and require lengthy parenteral administration, lack efficacy and are unaffordable for most of the patients (Legros et al., 2002). There is urgent need to source for new, cheap and safe alternative chemotherapy against trypanosomiasis from natural origin.

In African countries where sleeping sickness is endemic, plants have traditionally been used for generations and are still widely used to treat this ailment with possible therapeutic activities, which have not been proved scientifically. However, Nupeland located on the coastal basins of Rivers Niger and Kaduna in North Central Nigeria is greatly influenced by the savannah-forest vegetations of the area. Naturally, this type of vegetation harbours tsetsefly which are the vector of trypanosomes, thus making the area highly endemic to sleeping sickness. Nupeland inhabited predominantly by Nupe tribe are agrarian in nature and practice farming with traditional medicine. Nupe tribe is traditionally noted for centuries of using medicinal plants in curing human

trypanosomal infections among the indigenous people of Nupeland (Mann et al., 2003). Natural products derived from plants offer novel possibilities to obtain new drugs that are active against trypanosomes (Hoet et al., 2004).

Some Nigerian medicinal plants have been evaluated for their *in vitro* trypanocidal activity (Adewunmi et al., 2001; Atawodi et al., 2003; Maikai et al., 2008; Ogbunugafor et al., 2007; Owolabi et al., 1990; Shuaibu et al., 2008; Wurochekke and Nok, 2004) and *in vivo* antitrypanosomal efficacies in mice (Abubakar et al., 2005; Asuzu and Chineme, 1990; Asuzu and Ugwuja 1989; Ibrahim et al., 2008; Mann et al., 2009; Ogbadoyi et al., 2007). The discovery of these potent antitrypanosomal extracts from plant has increased the great potentials of plant species to provide lead compounds for the development of new natural drugs for effective treatment of sleeping sickness. Therefore, the present study aimed to investigate the *in vivo* antitrypanosomal activity of Nupeland medicinal plants against *T. b. brucei*.

Materials and Methods

Plant materials

The plant materials (*Z. zanthoxyloides* (stem bark) *T. avicennioides* (round fruit) *A. nilotica* (stem bark) *B. buonopozense* (stem bark) with the following voucher Nos.: NIPRDH 68033, NIPRDH 5735, NIPRDH 02, NIPRDH 128 respectively were collected from a forest near Emitete village along Bida-Doko Road in Lavun local Government area of Niger State, Nigeria as described by the healers. They were botanically identified at the Herbarium Unit of the Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development, Garki – Abuja, Nigeria where voucher specimens were deposited.

Preparation of plant extract

The plant parts were air-dried to a constant weight. The dried parts were ground first into coarse form using a pestle and mortar and then reduced to a powdery form using a mill.

Preparation of crude plant extracts

The powdery plant materials (250 g) were separately soaked in 500 ml of 70% methanol each for 24 hr with constant stirring. The extracts were filtered through a clean muslin cloth (2 mm) and concentrated using a rotary evaporator. The concentrated extracts were dried under a ceiling fan at room temperature. The residue was weighed and used in the preparation of the stock extract for administration.

Phytochemical screening of extracts

Phytochemical screening of the plant extracts were carried out to assess the concentration of Alkaloids (Dragendorff's, Mayer's, and Wagner's reagents), Anthraquinones (Borntrager's test), Balsams (Ferric chloride solution test), Carbohydrate (Fehling's solution test), Cardiac glycosides (Salkowski's test and Keller-Kiliani's test), Flavonoids (Lead acetate test, Ferric chloride test, Shinoda's test), Resins (Acetic anhydride test + conc. HS_2O_4), Saponins (Froth test and Legal test), Tannins (Ferric chloride solution test, Bromine water test and Formaldehyde test), Terpenes (Liebermann-Burchard's test) in each plant sample using standard methods (Harborne, 1989; Sofowora, 1993; Trease and Evans, 1989).

Trypanosome stock

A stabilate of pleomorphic trypanosome bruce, strain (*Trypanosoma brucei brucei*) was obtained from the Nigerian Institute for Trypanosomiasis Research Vom, near Jos, Nigeria and maintained in the Laboratory of Department of Biochemistry by serial passage in mice.

Experimental animals

Mice used were purchased from the University of Ibadan and Ilorin and kept in well ventilated plastic cages. They were exposed to 12 hr light and dark cycles and allowed free access to growers feed and water. The experiment was conducted in compliance with international accepted principles for laboratory animal use. Animal care guidelines on animal use protocol review (CCAC, 1997). The animals were allowed a 7-day period of acclimatization before they were divided into four groups of four animals per cage.

Inoculation of donor mice

Blood was collected by cardiac puncture with an EDTA coated syringe from a heavily infected mouse and immediately diluted with physiological saline. The dilute infected blood contains 1 or 2 parasite per field (microscopic field) 0.1ml of this blood was inoculated into two clean healthy mice intraperitoneally to serve as the donor. Infection was monitored every morning by microscopic examination of blood samples taken from the tail of the two infected mice.

Infection of animals

Blood was collected by cardiac puncture with an EDTA coated syringe from the heavily infected mouse and immediately diluted with physiological saline to serve as the inoculum. Healthy mice were infected intraperitoneally with 0.01ml of the inoculum containing about 103 trypanosome cells. The extracts were administered every morning and parasitaemia was monitored every two days by microscope examination.

Extract administration

In order to determine the effective dose, seven groups of three mice each were distributed into cages. Four groups (I-IV) of mice were intraperitoneally administered extract at doses of 100, 200, 300 and 500 mg/kg body weight per day. Group V was administered with Diminazene aceturate, Berenil (standard drug) at 3.5 mg/kg body weight per day. Group VI consist of the negative control which were infected with the parasite but were not treated with the extract. Group VII which were made up of Positive control were neither infected nor treated with the extract. Treatment was daily for 5 consecutive days. Parasitaemia was monitored every two days under the microscope.

Therapeutic monitoring of extract

Development of parasitaemia in these rats was checked daily by wet blood film prepared from tail blood at x40 magnification. The number of parasite seen per field under the microscope was counted as described by Herbert and Lumsden (1976).

Results

The plant extracts were percolated under laboratory conditions, and concentrated *in vacuo* with a rotary evaporator. Saponins, tannins and terpenes have been identified in all plant parts tested in all the four species. Alkaloids were found in only *B. buonopozense*. Flavonoids were also found in the extracts of *A. nilotica* (stem bark), *T. avicennioides* (round fruit) and *Z. zanthoxyloides* (stem bark) (Table 1).

Table 1: Phytochemical analysis of six crude plant extracts

Test	<i>A. nilotica</i>	<i>B. buonopozense</i>	<i>T. avicennioides</i>	<i>Z. zanthoxyloides</i>
Alkaloids	-	+	+	-
Tannins	+	+	+	+
Glycosides	+	-	+	+
Saponins	+	+	+	+
Terpenes	+	+	+	+
Sterols	+	-	+	-
Resins	-	+	-	+
Carbohydrates	+	-	+	-
Balsam	-	+	-	+
Flavonoids	+	-	+	+
Anthraquinones	+	-	-	-

Key + = present, - = absent

Antitrypanosomal activity of crude plant extracts and their acute toxicity

The acute toxicity of the extracts were determined and the appropriate dosage used for the trypanocidal efficacy. Those groups that were administered doses of 500mg/kg body weight served as controls to check death resulting from the acute toxicity. For instance, the group administered with *Z. zanthoxyloides* at doses of 500mg/kg body weight died on the fifth day of treatment. This shows that the extract was acutely toxic, because of the toxicity; the only dose that could suppress the activity of the parasite was 100mg/kg body weight because they stayed six days after the death of the control. Those treated with 200mg/kg body weight stayed four days after the death of the control as shown in table 2. The group treated with the standard drug, Diminazene aceturate

(Berenil) survived. The drug was able to clear the parasite from circulation within three days of treatment. The effects of medicinal plant extracts on parasite count in mice infected with *T. b. brucei* are as shown in Tables II-V.

Table 2: Effect of *A. nilotica* on parasite count in mice infected with *T. b. brucei*

Post treatment days	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII
2	0	0	0	0	0	0	0
4	8	5	0	D	D	0	5
6	100	0	D			0	100
8	50	0				0	150
10	50	0				D	200
12	40	0					D
14	20	0					

Key:

D = Death of mice

Group I - Infected but treated with 100mg/kg of the extract; Group II – Infected but treated with 200mg/kg of the extract; Group III – Infected but treated with 300mg/kg of the extract; Group IV – Infected but treated with 500mg/kg of the extract; Group V – Not infected but treated with 500mg/kg of the extract; Group VI – Infected but treated with 3.5mg/kg of Berenil; Group VII – Infected but not treated.

Table 3: Effect of *B. buonopozense* on parasite count in mice infected with *T. b. brucei*

Post treatment days	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII
2	10	5	0	5	0	0	20
4	30	25	10	10	0	5	50
6	50	15	5	10	0	10	100
8	D	10	3	5	0	0	D
10		D	0	D	0	0	
12			0		0	0	
14			0		0	0	

Key:

D = Death of mice

Group I - Infected but treated with 100mg/kg of the extract; Group II – Infected but treated with 200mg/kg of the extract; Group III – Infected but treated with 300mg/kg of the extract; Group IV – Infected but treated with 500mg/kg of the extract; Group V – Not infected but treated with 500mg/kg of the extract; Group VI – Infected but treated with 3.5mg/kg of Berenil; Group VII – Infected but not treated.

Table 4: Effect of *T. avicennioides* on parasite count in mice infected with *T. b. brucei*

Post treatment days	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII
2	0	0	0	0	0	0	0
4	0	0	10	25	0	0	10
6	10	50	60	60	0	5	150
8	50	D	100	90	0	0	D
10	100		100	90	0	0	
12	D		150	D	0	0	
14			D		D	0	

Key:

D = Death of mice

Group I - Infected but treated with 100mg/kg of the extract; Group II – Infected but treated with 200mg/kg of the extract; Group III – Infected but treated with 300mg/kg of the extract; Group IV – Infected but treated with 500mg/kg of the extract; Group V – Not infected but treated with 500mg/kg of the extract; Group VI – Infected but treated with 3.5mg/kg of Berenil; Group VII – Infected but not treated.

Discussion

Investigation of antitrypanosomal activity of traditionally used plants has been a major area of contemporary research focus. Natural products such as alkaloids, terpenes, quinones, and polyphenols found in these extracts 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). The antitrypanosomal activities of alkaloids like actinodaphine, dicentine, cassythine isolated from *Cassipoupa filiformis* (Hoet et al., 2004a) are also found in *B. buonopozense* and several other alkaloids (Merschjohann et al., 2001) displayed significant *in vitro* antitrypanosomal activity. The DNA intercalation in combination with portion

biosynthesis inhibition is reported to be the mechanism of action responsible for the observed anti-trypanosomal effect of the active alkaloids (Merschjohann et al., 2001). The trypanocidal activity of several flavonoids such as quercetagenin (Hoet et al., 2004b); hispidulin and santin (Sülßen et al., 2007) has been previously reported. An azaanthraquinone early reported in *A. nilotica* was associated with the observed antitrypanosomal effects (Nok, 2002).

Table 5: Effect of *Z. zanthoxyloides* on parasite count in mice infected with *T. b. brucei*

Post treatment days	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII
2	0	0	0	0	0	0	0
4	0	0	0	0	0	0	80
6	0	0	0	D	D	8	90
8	80	80	65			0	100
10	100	100	100			0	D
12	100	110	100			0	
14	120	D	D			0	

Key:

D = Death of mice

Group I - Infected but treated with 100mg/kg of the extract; Group II – Infected but treated with 200mg/kg of the extract; Group III – Infected but treated with 300mg/kg of the extract; Group IV – Infected but treated with 500mg/kg of the extract; Group V – Not infected but treated with 500mg/kg of the extract; Group VI – Infected but treated with 3.5mg/kg of Berenil; Group VII – Infected but not treated.

Antitrypanosomal activity of crude plant extracts tested *in vivo*

The crude extract of the stem bark of *Z. zanthoxyloides* shows no trypanocidal activity. However, it was found to be trypanostatic since parasite were undetected between day one of infection and day five of treatment. The present result showed that 100mg/kg of the doses administered is trypanostatic because the parasitaemia was kept relatively low when compared to the untreated. The group infected but not treated died between the 7th day and 8th day of infection while those infected and treated died days after.

In our present studies, we focused on *in vivo* screening of *Z. zanthoxyloides* (stem bark) *T. avicennioides* (round fruit), *A. nilotica* (stem bark) and *B. buonopozense* (stem bark) which are the plant parts prominently used in Nupe Ethnomedicine (Mann et al., 2003). Therefore, the antitrypanosomal activity of four crude methanol extracts of medicinal plants from Nupeland was determined *in vivo* using *T. b. brucei* as shown in Tables II-IV. The crude extract of the stem bark of *Z. zanthoxyloides* had no trypanocidal effect, even though its root bark extract was reported to be active (Igweh and Onabanjo, 1989). The failure of the crude extract of the stem bark of *Z. zanthoxyloides* to show any trypanocidal action depicts that the anti-trypanosomes are lacking in the stem bark as opposed to the claims by the healers which according to the existing literature is used in Nupe ethnomedicine in the management of sleeping sickness in Nupeland (Mann et al., 2003) However, we have also observed that the crude extract of the stem bark of *Z. zanthoxyloides* was found to exhibit trypanostatic activity. Similar to the early reports of the trypanostatic effect of ethanolic extract of the stem bark of *F. albida* and it is also effective in the management of anaemia induced by *T. b. brucei* in rats (Tijani et al., 2009). Trypanostatic effect is said to suppress the activity of the parasite there by sustaining the life of the mice when compared to the control group. It is worthy to mention that some plants have already been investigated for their antitrypanosomal activity in other studies. For instance; water, methanol and dichloromethane extracts of the leaves of *T. avicennioides* tested, but only methanolic extracts were active on *T. b. brucei* (Bizimana et al., 2006). Stem bark extracts of the same plant species has shown *in vitro* effect against *T. b. brucei* (Shuaibu et al., 2008).

In our present studies, the round fruit extract of *T. avicennioides* tested for its *in vivo* antitrypanosomal activity gave a result that are efficaciously comparable to those of previous investigators who showed that extracts of *T. avicennioides* were active *in vitro* on *T. b. brucei* (Bizimana et al., 2006; Shuaibu et al., 2008). *T. avicennioides* (round fruit) and *Z. zanthoxyloides* (stem bark) were effective on trypanosomes trypanostatically only.

The most interesting results are those of *A. nilotica* and *B. buonopozense* extracts which showed the antitrypanosomal effects at 200 and 300 mg/kg body weight respectively representing the first time *in vivo* activity of *B. buonopozense* and *Z. zanthoxyloides* on trypanosome, *T. b. brucei* is being reported. Doses were able to clear the parasites from circulation within 6 and 7 days of treatment respectively with prolonging survival period of up to 30 days. While the extracts of *T. avicennioides* and *Z. zanthoxyloides* showed trypanostatic effects and could not clear the parasites completely.

From the phytochemical studies, the type of compounds which could be responsible for the antitrypanosomal activities are associated with the metabolites and the results obtained showed that the crude extract of *Z. zanthoxyloides* contain some metabolites that suppresses the activity of the trypanosomes rather than killing the parasites completely.

This study has provided evidence that *T. avicennioides* and *Z. zanthoxyloides* extracts exhibits trypanostatic effect which is often associated with reduction in anemia and promote weight gain in experimental African trypanosomiasis (Ogbadoyi et al., 1999). Anaemia is the most outstanding clinical and laboratory feature of African trypanosomiasis (Bizimana et al., 2006) and also the primary cause of death (Mamo and Holmes, 1975). The stem bark of *Z. zanthoxyloides* is used in treating sickle cell anaemia and analgesic (Mann et al., 2003). Trypanostatic effect of the plant extracts were explained with corresponding increase in PCV

which prolong the lifespan of treated animals by reducing the parasite load or neutralizing the toxic metabolites produced by trypanosomes (Abubakar et al., 2005). Based on these results and the fact that plant part extracts are traditionally used in the treatment of African Trypanosomiasis, we analyzed in greater detail the antitrypanosomal activity of the stem bark methanol extracts of the four plants. Nevertheless, the activity values for the active extracts were high compared to the values obtained for commonly used trypanocidal drugs diminazene aceturate. However, since the crude extracts have a very complex composition, purification might lead to pure compounds with highly increased activity. Such differences between our results and those of other authors may be due to the known variation in the chemical composition of plants according to the geographical area and the time or season of collection (Shuaibu et al., 2008).

In our *in vivo* studies, with mice infected with *T. b. brucei*; the methanol extracts of round fruit of *T. avicennioides* and stem barks of *Z. zanthoxyloides* and *A. nilotica* and *B. buonopozense* were investigated, but only *A. nilotica* and *B. buonopozense* were found to be significantly active. The antitrypanosomal activity of four crude methanol extracts of medicinal plants from Nupeland determined by *in vivo* using *T. b. brucei*, only *A. nilotica* and *B. buonopozense* were the most promising ones. This is the first report of *in vivo* antitrypanosomal activity of these two plants. The results of the present study confirmed that the use of medicinal plants in folk medicine contributes significantly to primary health care, and that natural products are potential sources of new drugs for the treatment of important tropical diseases caused by trypanosomes. The high activity values obtained for these plants render them candidates for the isolation of anti-trypanosomal compounds which could be developed into new lead structures for drug development. Therefore, the trypanocidal effects of extracts will require further experimentation after fractionation and characterization using chromatographic and spectroscopic techniques, a work that is currently in progress.

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