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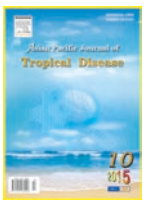

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In vivo trypanocidal activity of *Nymphaea lotus* Linn. methanol extract against *Trypanosoma brucei brucei*

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ABSTRACT

Objective: To evaluate the antitrypanosomal potentials of methanol extract of *Nymphaea lotus* Linn. (*N. lotus*) with the aim of obtaining a new lead for formulating safe, inexpensive, non-toxic and readily available trypanocidal drugs.

Methods: Seventy percent (v/v) (methanol/water) crude extract of *N. lotus* was evaluated for antitrypanosomal activity in experimental trypanosomiasis using *Trypanosoma brucei brucei*-infected mice. Infected mice in different groups were administered intraperitoneally 100, 200, 300 and 400 mg/kg body weight/day of the crude for two weeks, while a positive control group was treated with standard drug, berenil.

Results: The crude extract at a dose of 100 mg/kg body weight/day was more effective than the higher doses in completely clearing parasites from the blood of mice infected with *Trypanosoma brucei brucei*. Pre-treatment of healthy mice with the crude extract for 5 days before infection did not prevent the establishment of the infection, indicating that the extract had no prophylactic activity. Subinoculation of the blood and cerebrospinal fluid drawn from the cured mice into healthy mice failed to produce any infection within 50 days post inoculation. Administration of 1 000 mg/kg body weight of the crude extract led to the death of 50% of the experimental animals indicating a high level of toxicity of the extract at higher doses.

Conclusions: This study has demonstrated the potency of the crude extract of *N. lotus* in treating experimental trypanosomiasis at lower doses.

1. Introduction

African trypanosomes cause diseases in humans (sleeping sickness) and domestic animals. It is estimated that over 60 million people and 50-70 million animals are exposed to the infection[1-3]. The chemotherapy of African trypanosomiasis still remains far from being satisfactory. There is growing resistance to the drugs currently available[4,5]. Relapse met with melarsoprol in Northern Uganda, Southern Sudan and Northern Angola is as much as 30%[6]. Most of the available drugs are highly toxic and about 5% of the patients treated with melarsoprol have died as a result of the high toxicity of the drug[7]. The drugs available in the market are not even accessible to the rural African patients who bear most of the burden of the disease[1]. Without treatment, the disease is 100% fatal but when treated early, the cure rate is over 90%[8-10].

In many African countries including Nigeria, traditional medicinal plants are commonly used and is an acceptable practice. These plants provide very useful clues for potential anti-parasitic compounds[10]. It is estimated that 66%-85% or four billion people of the world population depend directly on plants as medicine[11]. Plants have a long history in medicine with a number of recorded successes. The most recent one being the antimalarial artemisin is obtained from *Artemisia annua*[12]. Nigeria is blessed with abundant medicinal plants and traditional medicine is officially recognised as an integral part or complimented to the nation's health care delivery system. It is therefore more than ever before

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very important to scientifically exploit these gifts of nature to the fullest for the good of the humanity[12].

Nymphaea lotus (Nymphaeaceae) (*N. lotus*) commonly known as water lilly and locally called "Bado" in Hausa and "Osibata" in Yoruba[11], is commonly found floating in stagnant or slow-moving streams and rivers in both Southern and Northern Nigeria. The whole plant decoction has been reported to be used traditionally in the treatment of rheumatic pains, anti-tumour and as antiseptic in the South-western Nigeria and in the treatment of Guinea worm infection in Northern Nigeria.

Taking into account the wide ethnomedicinal application of this plant, it will not be out of place to subject it to further validation on its efficacy as an antitumour and antiparasitic agent (particularly its antitrypanosomal activity). Despite spectacular advaces made in the drug research and development against other diseases such cancer and AIDS, little attention is focused on parasitic diseases in general and trypanosomiasis in particular, probably due to their nature of being diseases of the poorest of the poor. As pharmaceutical companies have merged and evolved into ever-larger multinational conglomerates, investment has declined in drug development for tropical diseases and other less profitable "orphaned diseases"[13-15].

This major aim of this study was to evaluate the methanol extracts of *N. lotus* for antitrypanosomal potentials with a view to obtaining potent ethno-medicine for the treatment of African trypanosomiasis.

2. Materials and methods

2.1. Plant collection and preparation

Fresh plant sample of *N. lotus* was collected in the month of December, 2013 from Monnai, a riverine village, few kilometres east of New Bussa, the headquarters of Borgu Local Government Area of Niger State, Nigeria. The plant sample was dried at room temperature to a constant weight. Dried sample was kept in polythene bag until required for the preparation of extract. The plant sample was deposited at Herbarium of the Limnology section of National Institute of Freshwater Fisheries Research, Headquaters, New Bussa, Nigeria (v/n: NIFFR 1308).

2.2. Trypanosoma brucei brucei (T. b. brucei)

A stabilate of pleomorphic *T. b. brucei*, strain 8/18 was obtained from the Nigerian Institute for Trypanosomiasis Research, Vom, Jos, Nigeria.

2.3. Mice

Albino mice were purchased from the Department of Pharmacology, Ahmadu Bello University, Zaria, Nigeria. The experiment was conducted in compliance with the internationally accepted principle for animals care as contained in the Canadian Council on Animal Care guidelines on animal use protocol review (1997)[16].

2.4. Preparation of crude extract

The crude extract was prepared by slight modification of the previously reported method[17,18]. Briefly, 50g of the dried sample was pulverised to powdered form and extracted under reflux in 400 mL of 70% v/v (methanol/water mixture). Extraction lasted for 4 h. Extract was filtered using muslin cloth; solvent was recovered using rotary evaporator, and the extract was transferred into sterile universal bottle and stored until required for use. The yield of the extract was 8.37 g/100 g of sample.

2.5. Infection of animals

Blood was collected by cardiac puncture with ethylenediaminetetraacetic acid-coated syringe from a heavily infected mouse and immediately diluted with physiological saline to serve as the inoculums. Healthy mice were infected intraperitoneally (*i.p.*) with 0.02 mL of the diluted blood containing 1×10^6 trypanosomes. Monitoring of parasitaemia was done every 48 h by microscopic examination of blood sample taken from the tail of infected mouse pre-sterilised with methylated spirit.

2.6. Antitrypanosomal activity of crude extract

Four groups (A, B, C, D) each containing three mice were administered extract at doses of 100, 200, 300 and 400 mg/kg body weight/day (*i.p.*). Three uninfected mice to which 400 mg/kg body weight per day was administered were in the fifth group (E) and served to determine toxicity at the highest dose of the extract. Another group (F) of three mice was infected but not treated with the extract serving as the negative control. For reference, a group (G) of three mice was infected and treated with the standard drug (445 mg diminazine diaceturate + 555 mg phenazone/g, Eagle Chemical Company LTD, Ikeja, Nigeria) a commercial trypanocidal drug.

2.7. Blood and cerebrospinal fluid (CSF) infectivity test

One of the two mice that survived after the treatment with the crude extract was sacrificed six weeks post treatment and 0.02 mL of blood was drawn from the heart and sub inoculated into two clean parasite-free mice and parasitaemia was monitored daily over a six weeks period.

Inoculation of mice with CSF obtained from the second surviving mouse was done as per reported method[19,20]. Two clean, parasite-free mice were each sub-inoculated with 0.02 mL of the CSF, and parasitaemia was monitored daily for six weeks.

2.8. Haematocrit determination

A small volume of blood was collected from the tail (pre-sterilised with methylated spirit) of the experimental animals into a heparinised capillary tube, one end of which was sealed with plasticine and then spun for 5 min in a Micro-haematocrit centrifuge (Hawksley & Sons Ltd, UK). The packed cell volume (PCV) was determined with the aid of Hawksley Micro haematocrit reader which gave reading in percentage.

2.9. Prophylaxis test

The test for prophylactic activity was done as described by Li *et*

al.[21]. Three mice were each treated with the highest dose of the extract (400 mg/kg body weight) for five consecutive days before being infected with 1×10^6 trypanosomes cells. They were then routinely monitored for establishment of parasites.

2.10. Acute toxicity studies

This was done as reported earlier by intraperitoneal administration of higher doses of the crude extract (*i.e.* 300, 400, 600, 800 and 1 000 mg/kg body weight) to five different groups of healthy mice, each containing four mice[22,23].

2.11. Phytochemical screening

The crude extract used (obtained by using seventy percent methanol as solvent) was screened for the presence of tannins, saponins, alkaloids, phlobatanins, cardiac glycosides *etc*. as described using simple chemical tests[24].

3. Results

3.1. Trypanocidal activity of 70% methanol extract

The minimum dose that effectively cleared parasites from circulation was found to be 100 mg/kg body weight administered *i.p.* per day (Figure 1). Administration of this dose of the extract to mice infected with *T. b. brucei* completely cleared the parasite from circulation within nine days of continued administration. Three mice infected but not treated died before the ninth day post infection. Two of the mice in the 100 mg/kg body weight group survived up to sixty days post treatment.

The mice administered 200, 300, and 400 mg/kg body weight also died (but not due to parasitaemia). Also the group treated with the maximum dose (400 mg/kg body weight) but not infected also died.

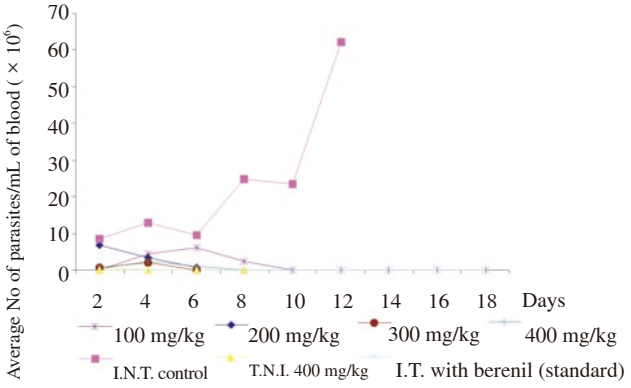

Figure 1. Trypanocidal activity of various doses of the extract.
I.N.T.: Infected not treated; T.N.I : Treated not infected; I.T.: Infected and treated.

3.2. Percentage PCV

The result obtained for percentage PCV revealed a drop during the first seven days of the treatment but this was reversed in the subsequent days, except in the negative control group (Figure 2).

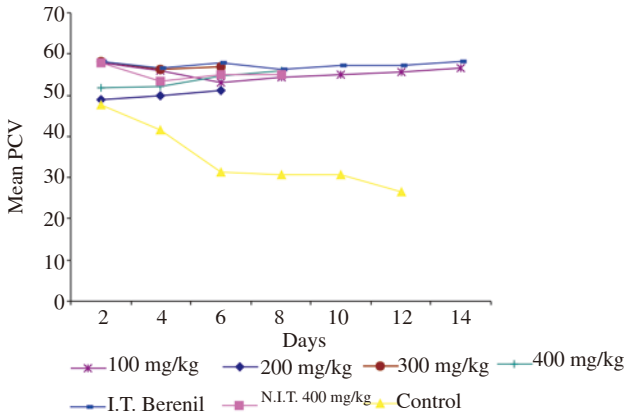

Figure 2. Mean PCV in group of mice treated with various doses of the extract and standard drug (Beneril).
I.T.: Infected and treated; N.I.T.: Not infected but treated.

3.3. Blood and CSF infectivity test

The blood and the CSF drawn from the cured mice and inoculated into the healthy mice did not induce/cause the development of infection six weeks after the sub inoculation.

3.4. Prophylactic activity of extract

The animals administered the effective dose of 400 mg/kg body weight for five consecutive days prior to infection were observed to develop infection 72 h post infection (Figure 3). This indicated the inability of the extract to protect mice against infection.

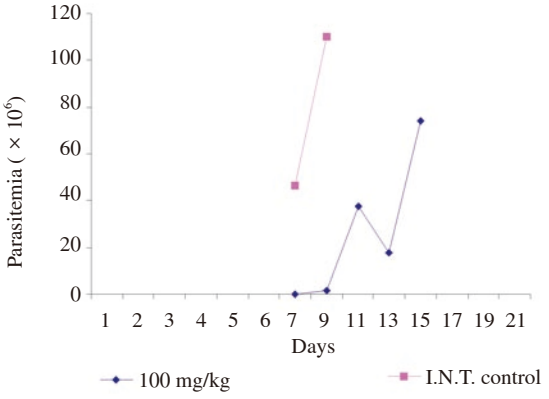

Figure 3. Prophylactic activity of the extract.
I.N.T.: Infected not treated.

3.5. Acute toxicity of extract

The mice in each group administered the higher doses of the extract displayed varied reaction to the administered dose. The death of half the number of animals under investigation in the group administered 1 000 mg/kg body weight indicated the LD_{50} of the extract to be 1 000 mg/kg body weight or thereabout.

3.6. Phytochemical composition of extract

Phytochemical analysis of the 70% crude extract revealed the presence of saponins, tannins, cardiac glycosides, and phlobatannins.

4. Discussion

The results obtained in this study have demonstrated the ability of 70% methanol extract of *N. lotus* to clear trypanosomes from the blood of *T. b. brucei*-infected mice. Infected mice treated with 100 mg/kg body weight of the extract for two weeks had parasites cleared from circulation nine days into treatment and survived more than sixty days post-infection. Higher doses of the extract cleared parasites from circulation but the animals died afterwards, thus indicating the likelihood of toxicity of the extract at these doses. This becomes clear due to the fact that the group treated with a dose of 400 mg/kg body weight but not infected also died.

There have been no previous reports on the trypanocidal activity of the whole plant extract of *N. lotus*. But traditionally, the whole plant aqueous decoction is used by the 'Hausas' in Northern Nigeria for the treatment of Guinea worm infection and by the 'Yoruba' in South Western Nigeria for the treatment of rheumatic pains and as an antitumor agent[12].

An interesting aspect of the result obtained in this screening is the non-appearance of the parasites in the blood of the healthy mice sub-inoculated with the blood and CSF drawn from the treated mice (42 days post clearance). This is an indication that the extract was able to completely clear parasites from circulation without residual parasites that could infect healthy mice. However, the result obtained in this study may not be used to infer that the extract had the ability to cross into tissues like the brain because treatment commenced at the acute stage of infection and not the chronic stage when parasites would have crossed into the brain.

The mechanism by which this plant extract exerted its trypanocidal activity is unknown for now since the active ingredients were not isolated in this study. However, previous studies have indicated that a number of plants contain constituents that have been demonstrated to be clinically effective against many protozoan diseases[25-30]. The existing trypanocidal drugs are known to exert their therapeutic actions through a variety of mechanisms depending on their chemical nature. Thus, while arsenic compounds-based drugs poison the cell by their action on glucose catabolism through glutathione oxidation, suramin targets glycolytic enzymes in the glycosomes. Pentamidine and other diamidines disrupt the kinetoplast and may also interfere with polyamine synthesis. Yet others, for example eflornithine, are selective inhibitors of ornithine decarboxylase, thereby depleting the biosynthesis of polyamines such as spermidine, a precursor of trypanothione[31,32]. That the active extract may be of peculiar polarity (considering the physicochemical property of the methanol solvent used for the extraction) is an indication that the bioactive constituents of the extract may belong to a variety of phytochemicals that will exert their trypanocidal action by one or more of the already identified mechanisms of action for trypanocidals. This is consistent with earlier reports which attributed the trypanocidal activity of certain plant extracts to the highly aromatic planar quaternary alkaloids, berberine, and harmine whose antiprotozoal action is through intercalation with DNA[33,34].

Anaemia is one of the most established major pathological features of African trypanosomiasis[35-37]. This result from the lysis of the cell is due to the lashing action of the flagellum of the parasites and also the action of an enzyme neuramidase secreted by the parasite. It is therefore imperative to incorporate the control of this pathological sign in the management of trypanosomiasis. Measurement of PCV gives a clue to the level of anaemia in infected and treated animals. It is clear from the profile in Figure 2 that administration of the extract leads to a remarkable improvement in the PCV of the treated animals compared to the infected and untreated ones. This ability of the extract to reverse the decrease in PCV in treated animals confers an added advantage on the extract. The presence of more alkaloids than saponins in the extract may be responsible for its ability to reverse haemolysis[38,39].

N. lotus is used in folk medicine as an antitumour agent and against rheumatic pains[12]. Further research on the plant to verify its anti-tumour potential in addition to its anti-trypanosome activity will definitely elicit interest from western drug companies because cancer is a great problem in the western world[40-43].

In conclusion, the trypanocidal activity of this plant is promising and needs to be exploited further to unravel its hidden potential.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

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