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Therapeutic Effects of Annona senegalensis Pers Stem Bark Extracts in Experimental African Trypanosomiasis

Abstract

Purpose: To evaluate the efficacy of the stem bark extracts in the treatment of model *Trypanosoma brucei brucei* infection in mice.
Methods: Powdered stem bark of *A. senegalensis* was sequentially extracted under reflux in hexane, methanol and water and the extracts were investigated for their efficacies in the treatment of albino mice infected with *T.b brucei*. Blood and cerebrospinal fluid infectivity (CSF) tests were done to confirm cure of infection.
Results: Hexane extract, at a dose of 400 mg/kg body weight and aqueous extract at a dose of 300 mg/kg body weight, cured the experimental infection in mice. Blood and CSF infectivity tests confirmed cure of infected animals.
Conclusion: The two different extracts of the same plant being efficacious brings hope that appropriate combinations of preparations from these extracts or preparations thereof may help in overcoming the problem of resistance, a major limitation of current chemotherapy, since the different extracts may be acting by different mechanisms.

Keywords: *Trypanosoma*, anti-trypanosome, chemotherapy, trypanocidal, sleeping sickness

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Introduction

African trypanosomiasis is a deadly disease if left untreated[1]. The disease is transmitted through the bite of tsetse fly which introduces the causative agent, trypanosome, into the blood[2]. Two forms of the disease exist, Human African trypanosomiasis (sleeping sickness), caused by *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense*, and Animal trypanosomiaisis, caused by *trypanosoma brucei brucei*.. Worldwide, approximately 40,000 new cases of both forms of the disease are reported each year. However, majority of cases are not reported due to lack of monitoring and infrastructure and it is likely that there are more than 100,000 new cases annually[3].

Chemotherapy of African trypanosomiasis is beset with problems that include among others, development of resistance to available drugs, long treatment protocols and toxic effects for users of the drugs. Presently, there is neither a vaccine nor drug available to prevent infection[4].

Annona senegalensis Pers (Annonaceae), commonly known as wild custard apple and locally called *Gwandar daji, Abo, Uburu ocha,* and *Ikpokpo* in Hausa, Yoruba, Ibo, among the Idomas of Nigeria, respectively, has been demonstrated to possess a high level of trypanocidal activity[5]. In furtherance of the search for trypanocidal agents, the stem bark of the same plant was evaluated for trypanocidal activity. We report here for the first time that hexane and aqueous extracts of the stem bark of *A. senegalensis* cure experimental infection in mice.

Methods

Plant Material

Annona senegalensis plant was collected from the bush in the suburb of Minna, Niger State, Nigeria between the months of April and May. The plant was identified in the Department of Crop Production, Federal University of Technology, Minna, Nigeria and also at the National Institute for Pharmaceutical Research and Development, Abuja (NIPRD/H/5868). The stem bark was removed, washed and dried at room temperature.

Animals

This study were conducted in compliance with the Canadian Council on Animal Care[6] to ensure humane treatment of all experimental animals. Albino mice of average weight (25 g) were purchased from the Biochemistry and Chemotherapy Division of the Nigerian Institute for Trypanosomiasis Research, Vom, Plateau State, Nigeria. The mice were acclamatized to laboratory condition for 7 days prior to use.

Blood from a mouse highly infected with *Trypanosoma brucei brucei* (obtained from stabilates maintained at the Nigerian Institute for Trypanosomiasis Research, Vom, Plateau State, Nigeria) was collected with EDTA-coated syringe and diluted with physiological saline to serve as inoculum. Healthy mice were infected intraperitoneally with 0.1 ml of the inoculum containing about 10^3 trypanosomes/ml and maintained in our laboratory by continuous passage of infected blood into mice.

Sample preparation and Extraction

The sample preparation and extraction was done using the method described by Ogbadoyi et al[5] with slight modification in which the duration of extraction was reduced from 4 to 2 hr. Briefly, the dried stem bark was reduced to small pieces and ground into a powdered form. Some sample (50 g) was sequentially extracted under reflux with 400 ml each of hexane, methanol and water (in this order) for 2 hr. The extracts were filtered hot using a muslin cloth and subsequently evaporated using a rotary evaporator. The dry extracts were weighed, placed in sterile sample bottles and stored in a refrigerator until required for use.

Screening of extracts for trypanocidal activity

To determine the effective doses of the extracts, 7 groups, consisting of 2 mice each, were set up for each of the extracts (hexane, methanol and water). Groups A – E were infected with *T. b. brucei* and subsequently administered extracts at doses of 100, 200, 300, 400 and 500 mg/kg body weight per day when parasitemia was established. Group F was not infected but administered the highest dose of 500 mg/kg body weight to act as a check for acute toxicity (this is not toxicity studies but only a control group), while Group G was infected and treated with physiological saline or DMSO as appropriate.

Effective doses from the initial screening were used for the second stage of screening. Five groups, consisting of 3 mice each, were infected with *Trypanasoma brucie brucei*. Groups A and B were administered doses of 100 and 400mg/kg body weight per day of the hexane extract, group C was administered the dose of 300mg/kg bodyweight of the aqueous extract, group D was treated with the standard drug, 3.5mg/kg body weight per day of berenil (a standard drug for the treatment of African trypanosomiasis), and group E was infected and left untreated. Parasitemia was monitored thrice a week under a microscope as described by Herbert and Lumsden[7].

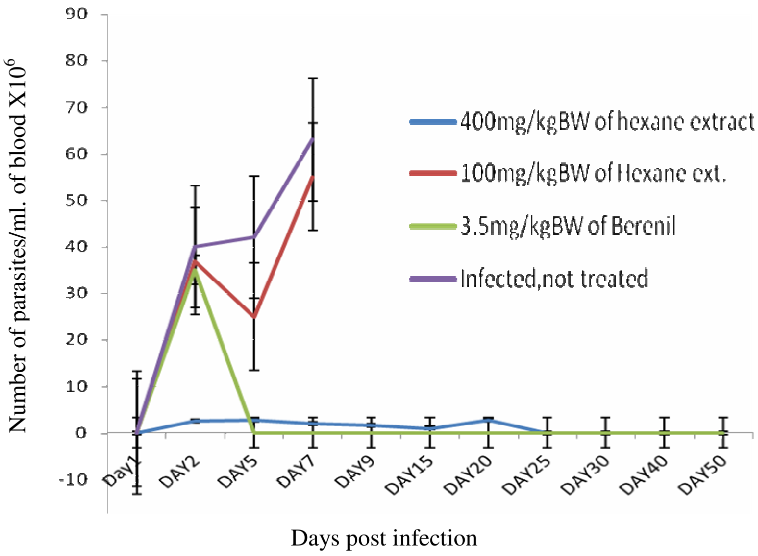

Figure 1: Course of parasitemia in infected mice treated with hexane extract and berenil

Administration of Extracts

Solutions of the crude extracts were prepared by dissolving in physiological saline or DMSO and administered appropriately to the animals via intraperitoneal route when parasites were detected in the infected mice.

Blood and CSF infectivity tests

Blood and cerebrospinal fluid (CSF) withdrawn from cured mice were used to inoculate two groups, consisting of 2 mice each, in order to test for infectivity. Parasitemia was monitored as above for the next two months. Doses used were chosen based on our previous experience (data not the preliminary experiments

Results

Trypanocidal Effects of Extracts

The administration of two of the doses with significant antitrypanosomal activity obtained from the preliminary screening was found to clear parasites from circulation after three weeks of treatment. The effective doses were 400mg/kg body weight of hexane extract and 300mg/kg body weight of aqueous extract of the stem bark of *Annona senegalensis* (Figures 1 and 2) The cured mice survived for four months post treatment and were used for blood and CSF infectivity tests.

Figure 1 shows the course of parasitemia in infected mice treated with hexane extracts. At a dose of 400mg/kg bodyweight per day for three weeks, the number of parasites in circulation was reduced to zero. The clearance was effected on the seventh day of treatment and the animals remained aparasitemic until used for blood and CSF infectivity tests. The group treated with berenil had parasites cleared on the 5th day post infection, while the untreated group died on day 7 post infection.

Figure 2 shows the course of parasitemia in infected mice treated with the aqueous extract at 300mg/kg bodyweight per day. The number of parasites in circulation was reduced to zero after 15 days of treatment and the animal continued to survive for over two months until it was used for blood and CSF infectivity tests. The untreated group did not survive beyond the 6th day post infection.

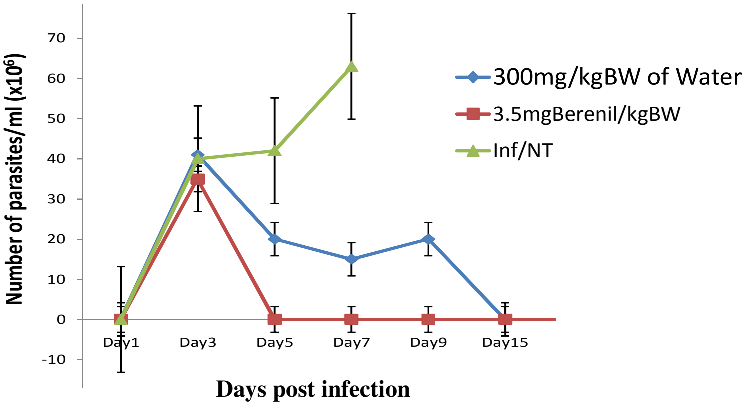

Figure 2: Course of parasitemia in infected mice treated with aqueous extract of A. senegalensis stem bark and berenil

Blood and CSF infectivity test

In order to ascertain that parasites had been cleared completely from the infected but cured animals, blood and cerebrospinal fluid (CSF) from them were used to inoculate healthy animals. The animals remained aparasitemic 2 months after inoculation.

Discussion

Different parts of *Annona senegalensis* have been reported to possess trypanocidal activities. The aqueous extract of the leaves[5], the whole root[8], and an unspecified part[9] have been shown to demonstrate *in vivo* trypanocidal activity. It is interesting to note that we have demonstrated the antitrypanosomal activity of hexane and aqueous extracts of the stem bark, contrary to the result obtained by Ogbadoyi *et.al*[5], where the stem bark extracts were found to be inactive against *T. b. brucei*. Two reasons can be advanced to explain this. It is most likely that the relatively prolonged period of hot extraction (4 hr) used in the earlier study might have inactivated some bioactive constituent(s) which remained intact and active in the present study in which the extraction period has been reduced to 2 hr. It may well be due to the dependence of the efficacy of medicinal plants in part, on factors like season and time or period of harvest, and the location of the plant. Although the later is most unlikely in this case, it raises the challenge of the necessity to carry out all-year round pharmacological screening of a plant before conclusions can be drawn as to the efficacy or otherwise.

We have demonstrated that different solvent extracts of the same organ of a plant may exhibit pharmacological activity against a parasite, just as extracts of different parts of the same plant[8]. Therefore, the statement that a plant extract is efficacious or not should be taken in the context of the solvent used, the part investigated, the season of harvest and the geographical location of the plant.

In Nigeria, different parts of the Annona plant are used locally to treat ailments such as fever, sexually transmitted diseases[5] and cancer[10]. The ripe fruit is a delicacy among the Hausas of Northern Nigeria. The demonstration of the antitrypanosomal activity of the stem bark is therefore encouraging and the need to exploit the plant to the fullest cannot be over emphasized bearing in mind the global interest in the sourcing of trypanocides from natural products[11].

The most thrilling aspect of the results obtained is the fact that two different extracts of the same plant portion are trypanocidal. It is of particular interest because of the potential in overcoming resistance problem, a major limitation, in trypanomiasis chemotherapy. This is so because the two extracts are likely to be acting at different points on the parasite. Therefore herbal preparations comprising these extracts and those of the leaves administered as combination therapy may provide the much needed solution to the resistance problem associated with trypanosomiasis chemotherapy.

Acknowledgments

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