



Short communication

Afr. J. Traditional,
Complementary and
Alternative Medicines
www.africanethnomedicines.net

ISSN 0189-6016©2005

IN VIVO ANTIMALARIAL AND CYTOTOXIC PROPERTIES OF *ANNONA SENEGALENSIS* EXTRACT

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Abstract

The *in vivo* animal antimalarial and *in vitro* cytotoxic activities of the methanol extract of *Annona senegalensis* Pers. (Annonaceae) was investigated in this study. The *in vivo* antimalarial activity of the methanol extract against *Plasmodium berghei* was assessed using the 4-day suppressive test procedure. The extract of *A. senegalensis* had intrinsic antimalarial property that were dose – dependent. At doses of 100mg/kg weight of mice, it produced significant chemosuppression of parasitemia (> 57%) when administered orally. It had the highest activity at 800mg/kg weight of mice (91.1%) compared to Chloroquine disphosphate, the standard reference drug which had a chemosuppression of 96.2%. The *in vitro* cytotoxicity evaluations were performed using A2780 ovarian cancer cells in the drug sensitivity assay. Extract of *A. senegalensis* exhibited low cytotoxicity with an IC₅₀ of 28.8µg/ml. Preliminary phytochemical screening of the plant extract indicated the presence of alkaloids, saponins, tannins and cardiac glycosides. This finding supports the traditional use of the plant for the treatment of malaria.

Key words: *Annona senegalensis*, Mice Antimalarial, Cytotoxicity, *Plasmodium berghei*

Introduction

Malaria is one of the most important parasitic diseases in the world. It remains a major public health problem in Africa responsible for the annual death of over one million children below the age of five years (Butler, 1997; Geoffrey, 1998). *Plasmodium falciparum*, the most widespread etiological agent for human malaria, is becoming increasingly resistant to standard antimalarial drugs which necessitate a continuous effort to search for new drugs, particularly with novel modes of action (Muregi et al., 2003).

Plants have invariably been a rich source for new drugs and some antimalarial drugs in use today (quinine and artemisinin) were either obtained from plants or developed using their chemical structures as templates (Gessler et al., 1994). It is already estimated that 122 drugs from 94 plant species have been discovered through ethnobotanical leads (Fabricant and Farnsworth, 2001). Plants commonly used in traditional medicine are assumed to be safe due to their long usage in the treatment of diseases according to knowledge accumulated over centuries. However, recent scientific findings has shown that many plants used as food or in traditional medicine are potentially toxic, mutagenic and carcinogenic (Schimmer et al., 1994; De Sã Ferrira and Ferrão Vargas, 1999). A number of studies have been undertaken to evaluate the inhibitory effects of various plant extracts on *P. falciparum* (Le Tran et al., 2003; Muregi et al., 2003). Similarly, the *in vivo* antimalarial properties of several plant extracts have been studied in mice (Agbaje and Onabanjo, 1991; Perez et al., 1994; Andrade-Neto et al., 2003). Following this trend, this study presents the results obtained from the evaluation of the *in vivo* antiplasmodial activity of *A. senegalensis*, a plant commonly used in Nigerian folk medicine against malaria and its *in vitro* cytotoxicity evaluation using Human A2780 ovarian cancer cells.

Materials and Methods

Plant Preparation

The fresh leaves of *A. senegalensis* (Pers)(Annonaceae) were collected in Oyo, Oyo State of Nigeria. The identification and authentication was done by Mr. T. K. Odewo of Forestry Research Institute of Nigeria (FRIN), Ibadan, where a voucher specimen was deposited with FHI number 106411.

The leaves of the plant were carefully sun-dried for three days and samples were pulverized to a coarse powder. 650g of the leaves were extracted exhaustively using methanol by maceration for 72 hours. The extracts were filtered and the filtrate concentrated over a water bath to dryness. Appropriate concentrations of the extract were made in water and used in the experiments.

Phytochemical screening

Standard screening tests of the extract were carried out for various plant constituents. The methanol extract was screened for the presence of alkaloids, flavonoids, saponins glycosides and tannins using standard procedures (Sofowora, 1993).

Malaria Parasites

The chloroquine-sensitive strain of *Plasmodium berghei* (NK-65) was used to test for antimalarial activity of the methanol extracts of *A. senegalensis* leaves. Parasite was obtained from the Malaria Research Laboratories, Institute for Advanced Medical Research and Training (IAMRAT), College of Medicine, University of Ibadan. Parasites are maintained through weekly blood passage in mice.

***In vivo* antimalarial test**

The Peters' 4-day suppressive test against *P. berghei* infection in mice was employed (Peters, 1965). Briefly, adult Swiss male albino mice weighing 18-20g were inoculated by intra-peritoneal (i.p.) injection with 1×10^7 infected erythrocytes. The mice were randomly divided into groups of five per cage and treated during 4 consecutive days with daily doses of the extracts by oral route (800, 600, 400, 200 and 100mg/kg). Two control groups were used in each experiment, one treated with chloroquine at total dose of 25 mg/kg while the other group was kept untreated given normal saline as placebo. All experiments were done in triplicate. On day 5 of the test, thin blood smears were prepared and blood films were fixed with methanol. The blood films were stained with Giemsa, and then microscopically examined (1000 x magnification). The percentage suppression of parasitaemia was calculated for each dose level by comparing the parasitaemia in infected controls with those of treated mice. Chloroquine disphosphate was used as positive control while normal saline was used as a negative control.

***In vitro* cytotoxicity assay**

Cytotoxicity against A2780 human ovarian cancer cells was performed at Virginia Polytechnic Institute and State University (USA) as previously reported (McBrien et al., 1995). Briefly, growth inhibition was determined using a micro plate assay in which the A2780 cells were seeded in RPMI 1640 media plus L-glutamine (Gibco) and 100% fetal bovine serum (Gibco) at a density of 2.7×10^5 cells/ml. Samples were dissolved in 50% DMSO and transferred to 96-well microtiter plates in a 1:50 dilution, for a final testing concentration of 20 μ g/ml. Microtiter plates were incubated at 37 °C in 5% CO₂ for 48 hours. The medium was then replaced with RPMI 1640 plus 1% Alamar Blue (Bioresource International). After a further 4 hours of incubation, fluorescent Alamar Blue was measured using a microplate fluorometer (Cytofluor, Millipore) at an emission of 530 nm, an excitation of 590 nm and a gain of 40. Percentage fluorescence is directly proportional to percentage inhibition and growth inhibition was elucidated using a linear regression analysis of the dose response curve. Activity is reported as IC₅₀, which is the concentration (μ g/mL) necessary to produce 50% inhibition. Actinomycin (IC₅₀, 2 ng/ mL) was used as a positive control.

Results

The suppressive activity of the methanol extract of *A. senegalensis* against *P. berghei* in mice is shown in Table 1. The extract at 100mg/kg weight of mice gave 57.1% suppression of parasitaemia. At doses of 800mg/kg weight of mice, it induced the highest chemosuppression of parasitaemia (91.1%) compared to Chloroquine control group, which had a chemosuppression of 96.2%. Percentage chemosuppression was observed to increase as extract concentration increased. After 4 days treatment with extracts, the results showed mean parasitaemia in mice from the ranges of $0.33\% \pm 0.12\%$ to $2.04\% \pm 0.34\%$.

The mean parasitaemia in chloroquine control group was $0.17\% \pm 0.08\%$, while mean parasitaemia in the untreated control was $4.50 \pm 0.17\%$.

A. senegalensis was tested for cytotoxicity against human A2780 ovarian cancer cells. It was found to have an IC_{50} of $28.8\mu\text{g/ml}$.

Table I. Antimalarial activity of *Annona senegalensis* methanolic extracts and chloroquine in mice infected with *Plasmodium berghei*.

Extract/Drug dose ^a (mg/kg/day)	Activity against <i>P. berghei</i> in mice (%) ^b	
	Mean % Chemosuppression	Parasitaemia (%)
100	57.1	2.04 ± 0.34
200	59.3	1.83 ± 0.14
400	76.3	1.07 ± 0.08
600	89.8	0.46 ± 0.33
800	91.1	0.33 ± 0.12
CQ	96.2	0.17 ± 0.08
N.S	-	4.50 ± 0.17

^aCQ, Chloroquine diphosphate (25 mg/kg/day); N.S (Untreated control), normal saline.

^b Values are Parasite density \pm Standard deviation (PD \pm SD).

Discussion

Annona senegalensis, a plant commonly used in Nigerian folk medicine against malaria was observed to show some intrinsic antimalarial activity judging by its percentage chemosuppression in comparison with that of chloroquine in the 4 - day suppressive test (Peters, 1965). Treatment of mice infected with *P. berghei* with methanolic extracts of *A. senegalensis* showed a dose-dependent chemosuppression in comparison with chloroquine treated controls with the 800mg/kg treated group of mice showing the highest percent chemosuppression. The activity might be attributed to the presence of alkaloids that have been shown to be the major constituents identified in *Annona* species (Gbeassor et al, 1990; Rupprecht et al., 1990). However, the active compound(s) known to give this observed activity need to be identified. In this regard, efforts are presently directed towards biologically guided fractionation of this plant in order to isolate and identify the active compound(s) and also test for cytotoxic activity. The extract of *A. senegalensis* exhibited low cytotoxicity with an IC_{50} of $28.8\mu\text{g/ml}$. Although plants used medicinally are widely assumed to be safe, many are potentially toxic. This study has however, established the rationale for the traditional use of this plant in Nigeria and like many others, showed that

medicinal plants, which have folklore reputations for antimalarial properties, can be investigated, in order to establish their efficacy and to determine their potentials as sources of new antimalarial drugs.

Acknowledgements

This study received partial funding from UNDP/WHO/TDR/MIM African grant ID 980046. We are grateful to Jennifer Schilling, Prof. DGI Kingston Research Group, VT, Blacksburg, USA for cytotoxicity assay.

References

1. Andrade-Neto, V. F., Brandao, M.G. L., Stehmann, J. R., Oliveira, L. A. and Krettli, A. U. (2003). Antimalarial activity of *Cinchona*- like plants used to treat fever and malaria in Brazil. *J. Ethnopharmacol.* **87**: 253-256.
2. Agbaje, E.O. and Onabanjo, A.O. (1991). The Effects of extracts of *Enantia chlorantha* in malaria. *Ann. Trop. Med. Parasitol.* **85**:585-590.
3. Butler, D. (1997). Time to put malaria control on the global agenda. *Nature* **386**: 535 -536.
4. De Sã Ferrira, I. C. F. and Ferrão Vargas, V. M. (1999). Mutagenicity of medicinal plant extracts in salmonella/microsome assay. *Phytother. Res.* **13**: 397 – 400.
5. Fabricant, D. S. and Farnsworth, N. R. (2001). The value of plants used in traditional medicine for drug discovery. *Environmental Health Perspectives* **109**: 69 -75.
6. Gbeassor, M., Kedjagni, A.Y., Koumaglo, de Souza, C., Agbo, K., Aklikokou, K. and Amegbo, D. A. (1990). *In vitro* antimalarial activity of six medicinal plants. *Phytother. Res.* **4** (3): 115-117.
7. Geoffrey, P. (1998). The treatment of Falciparum malaria in African children. *African Health* **20**: 19-20.
8. Gessler, M. C., Nkunya, M. H. H., Mwasunmbi, L. B., Heinrich, M. and Tanner, M. (1994). Screening Tanzanian medicinal plants for antimalarial activity. *Acta Trop.* **56**:65-77.
9. Le Tran, Q., Tezuka, Y., Ueda, J., Nguyen, N. T., Maruyama, Y., Begum, K., Kim, H.S., Wataya, Y., Tran, Q.K. and Kadota, S. (2003). *In vitro* antiplasmodial activity of antimalarial medicinal plants used in Vietnamese traditional medicine. *J. Ethnopharmacol.* **86**: 249-252.
10. McBrien, K. D., Bery, R. L., Lewes, S.E., Nedderman, K. M., Bursuker, I., Huang, S., Wehr, S. E. and Leet, J. E. (1995). Rakicidins, a new cytotoxic lipopeptide from *Micromonospora sp.* fermentation, isolation and characterization. *J. Antibiot.* **48**: 1446-1452.
11. Muregi, F. W., Chhabra, S. C., Njagi, E. N. M., Lang'at-Thoruwa, C. C., Njue, W. M., Orago, A. S. S., Omar, S. A. and Ndiege, I. O. (2003). *In vitro* antiplasmodial activity of some plants used in Kisii, Kenya against malaria and their chloroquine potentiation effects. *J. Ethnopharmacol.* **84**: 235-239.
12. Perez, H. A., De la Rosa, M. and Apitz, R. (1994). *In vivo* activity of ajoene against rodent malaria. *Antimicrob. Agents Chemother.* **38**: 337-339.
13. Peters, W., 1965 Drug resistance in *Plasmodium berghei* I. Chloroquine resistance. *Exptl. Parasitol.* **17**: 80-89.
14. Rupprecht, J. K, Hui, Y. H. and McLaughlin, J. L. (1990). Annonaceous acetogenins: a review. *Journal of Natural Products* **53**(2): 237-278.
15. Schimmer, O., Kruger, A., Paulini, H. and Haefele, F. (1994). An evaluation of 55 commercial plant extracts in the Ames mutagenicity test. *Pharmazie* **49**: 448 – 451.
16. Sofowora, E. A (1993). Medicinal plants and traditional medicine in Africa, 2nd edition, P. 85,174, 195-198.