African Journal of Biochemistry Research Vol. 5(7), pp. 214-219, July 2011
Available online at http://www.academicjournals.org/AJBR
ISSN 1996-0778 ©2011 Academic Journals

Full Length Research Paper

Anti-plasmodial activity of aqueous root extract of *Acacia nilotica*

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Accepted 25 May, 2011

***Acacia nilotica,* of family *Fabaceae,* is a thorny tree commonly used in Northern Nigeria for the treatment of cough, diabetes and malaria. Aqueous root extract of *A. nilotica w*as analyzed for antiplasmodial activity in mice. Acute toxicity of the extract was studied using Organization for Economic Cooperation and Development (OECD) guideline 423. Suppressive activity, curative and prophylactic effect was studied in chloroquine-sensitive *Plasmodium berghei berghei* NK 65 infected mice. Five groups, of five mice in each group were used. Group 1 or control, was administered with 10ml distilled water/kg body weight; groups 2, 3 and 4 were administered with 100, 200, and 400mg extract/kg body weight, respectively, while group 5 was administered with 5mg chloroquine/kg body weight. The doses were administered orally. All doses of the extract produced significant, dose-dependent, chemo suppressive activity against the parasite in the suppressive, curative and prophylactic tests. This is comparable to the group treated with chloroquine. The extract also prolonged the mean survival time of treated mice compared to the untreated group. The oral median lethal dose (LD_{50}) of the extract in mice was 5000mg/kg body weight. The results of this study showed that the aqueous root extract of *Acacia nilotica* is safe and has anti plasmodial activity.**

Key words: *Acacia nilotica*, antimalarial, *Plasmodium berghei berghei,* medicinal plant.

INTRODUCTION

Malaria remains one of the most dreaded human parasitic diseases in the tropics and subtropics, especially the African and Asian developing/under developed nations, because it is still a major cause of mortality in children (< 5 years) (WHO, 2008). Mortality, currently estimated at about 781,000 people per year (WHO, 2010), is attributed to resistance of the parasite to commonly used antimalarial drugs. In addition to its direct health impact, malaria imposes a huge economic burden on afflicted individuals and nations, through high health care cost, missed days at work or school, and reduced economic output and productivity (Sachs and Malaney, 2002).

Despite the success recorded with the Artemisinin Combination herapy (ACT), most malaria endemic Combination Therapy **(**ACT), most malaria endemic communities still rely on traditional herbal medicines, which are often more affordable and available (Etkin, 2003). In view of the problems associated with antimalarial drug resistance and the use of substandard ACT's, researchers are now focusing on other alternatives, including investigation of medicinal plants known to have antiplasmodial activity (Ajaiyeoba et al., 2006; Etkin, 1997). In Africa, up to 80 per cent of the population still rely on herbal medicine to treat malaria and other diseases (Agbedahunsi, 2000), because of their affordability and accessibility. One of such popularly used medicinal plants is *Acacia nilotica,* a scented thorny tree commonly found and used in Northern Nigeria as a traditional herbal remedy against malaria (Etkin, 1997). The fruits and root of the plant was reported to have antitubercular (Oladosu et al., 2007) and antidiabetic activities (Dalziel, 1997), while the bark is used in treatment of cough, diarrhea and as an aphrodisiac (Van Wyk, 2000).

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With this view, the present study was executed to analyse the antiplasmodial activity of the aqueous root extract of *Acacia nilotica* in *Plasmodium berghei berghei* infected mice in order to provide scientific evidence for its continuous use in ethno therapeutic management of malaria.

MATERIALS AND METHODS

Plant materials

A. nilotica roots were collected at Chaza village, Suleja, Niger State, Nigeria. It was identified and authenticated by Mrs. Grace Ugbabe, a taxonomist at the Department of Medicinal Plant Research and Traditional Medicine (MPR & TM) of National Institute of Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria. A voucher specimen (NIPRD/H/6401) was prepared and deposited at the NIPRD herbarium for future reference. The root samples were cleaned, air-dried and pounded into fine powder using mortar and pestle. The powder was stored in a dry air-tight container.

Extract preparation

400 g of powdered root was macerated in 1L of distilled water for 24 h. The mixture was filtered using muslin cloth, followed by Whatman filter paper (NO. 1) and freeze-dried using AMSCO/FINN-AQUA GT2 Freeze dryer (Germany). Aliquot portion of this crude extract were weighed and dissolved in distilled water for preparation of appropriate doses on each day of the experiment.

Phytochemical screening

The extract was screened for phytochemical constituents using standard procedures as described by Evans (2005).

Animals

Both sexes of Wistar albino mice having body weight of 18 to 22 g, obtained from Animal Facility center, NIPRD, Abuja, were used as test animals for the study after receiving approval from NIPRD animal ethics committee on use of laboratory animals. They were housed in standard cages and maintained under standard laboratory conditions in accordance with the "NIH guideline for the care and use of Laboratory animals" (NIH Publication No. 85; rev. 1985).

Chemicals

The standard chloroquine used for the study was obtained from Sigma –Aldrich (C 6628-25G) representative in Nigeria (Zayo-sigma International ltd, Jos, Nigeria).

Acute toxicity test

The safety of the extract was assessed by determining its median lethal dose (LD_{50}) using the OECD Guideline 423 (2001).The extracts were administered at a stepwise doses of 300, 2000 and 5000 mg/kg orally to three groups of mice, comprising three females. The mice were observed for signs of toxicity after treatment for the first four (critical) hours, then over a period of 24 h, thereafter daily for 7days. Mortality occurring at a particular dose will indicate either to continue administration of subsequent higher dose or to estimate the LD_{50} by comparing the mortality to a fixed LD_{50} cut-off values provided in the guideline.

Rodent parasite

Chloroquine sensitive rodent *Plasmodium berghei berghei* NK 65 was obtained from National Institute for Medical Research (NIMR), Lagos, Nigeria and maintained alive in mice by continuous intraperitoneal passage in mice after every five days. The reinfected mice were kept at the Animal facility Center of NIPRD where the study was carried out. A standard inoculum of 1×10^7 of parasitized erythrocytes from a donor mouse in volumes of 0.2 ml was used to infect the experimental animals intra-peritoneally.

Antiplasmodial studies

Test on early malaria infection (4-day suppressive Test)

The Peter's 4-day suppressive test against chloroquine sensitive *Plasmodium berghei berghei* (NK 65) infection in mice was employed (Peters, 1965). Twenty five albino mice of both sexes were inoculated as described above. They were randomly grouped, having five mice in each group and administered extract daily for four (4) consecutive days. Treatment started immediately after the mice were infected with the parasite. Group 1 that served as control was administered with 10ml/kg body weight of distilled water. Groups 2, 3 and 4 were orally administered with 100, 200 and 400 mg extract/kg body weight daily respectively, while group 5 was administered with 5 mg chloroquine /kg body weight orally daily. On the fifth day (D_5), blood was collected from the tail of each mouse and spread on a microscope slide to make a thin film. The blood films were stained with Giemsa and examined microscopically following Cheesbrough (2004). The parasite count was recorded and the suppression of parasitemia was expressed as per cent for each dose, by comparing the parasitemia in the control group with the treated one.

Average suppression = APC – APT/APC X 100.
APC = Average parasitemia in the control.
APT = Average parasitemia in the test group.

Test on established infection (Rane test)

Evaluation of the curative potential of *A. nilotica* root extract against established infection was carried out as described by Ryley and Peters (1970). Twenty five mice were all inoculated as described above, and left untreated until the fourth day (D_4) post inoculation. The mice were weighed and randomized into five groups of five mice each. Group 1 was administered with 10ml/kg of distilled water; groups 2, 3 and 4 received graded extract doses of 100, 200 and 400 mg extract/kg body weight/day orally respectively, while group 5 was administered with 5 mg chloroquine /kg body weight /day orally for four days (D_4-D_7). On Day-7 each mouse was tail-bled and a thin blood film was made on a microscope slide. The films were stained with Giemsa stain and examined microscopically to monitor the parasitemia level. The mean survival time of the mice in each treatment group was monitored for 30 days (Saidu et al., 2000; Adzu and Salawu, 2009).

Repository test

The prophylactic activity of the extract was tested using the residual infection procedure described by Peters (1965). Twenty-five mice of

Table 1. Phytochemical constituent of aqueous root extract of *A. nilotica.*

Phytochemical	Tannin	Saponin	Flavonoid	Terpene	Sterol	Phenol	Alkaloid	Anthraquinone
Qualitative	++	++	+	+	+	+ +	++	+
Quantitative (%)	27.0	9.8	0.5	0.1	0.1	34.5	23.3	4.7

++ = moderately present, + = slightly present.

both sexes were weighed and randomized into five groups of five mice each. Group 1 was administered with 10ml/kg distilled water, group 2, 3 and 4 were administered with 100, 200 and 400 mg extract /kg body weight orally respectively, while group 5 was administered with 5mg chloroquine/kg body weight orally daily. Treatment continued daily for four days (D_1-D_4) and mice were all infected with the parasite on the fifth day (D_5). Thin blood films were prepared from each mouse 72hours (D_7) post treatment and mean parasitemia in each group determined microscopically. The mice were reweighed on seventh day and the differences between the pre- and post-treatment body weight recorded.

Statistical analysis

Graph pad prism version 5.02 was used to analyze the data obtained and these were expressed as mean ± standard error of mean. The differences between means were compared using one-way analysis of variance (ANOVA), followed by Dunnet's test. $p < 0.05$ was considered significant.

RESULTS

Phytochemical tests

The aqueous root extract of *A. nilotica* comprised of tannins, saponins, flavonoids, terpenes, sterols, phenols, alkaloids and anthraquinones (Table 1).

Acute toxicity test

The oral median lethal dose (LD_{50}) of the extract was estimated to be 5000 mg/kg in mice. There were no remarkable behavioral changes in the extract-administered mice (reaction to food supply, contact and noise), however activity was reduced in all the extract-administered groups within the first four hours. No mortality occurred within observation period of a week.

Evaluation of antiplasmodial activity

Test on early malaria infection (4-day suppressive test)

The administration of extract resulted in a significant dose-dependent decrease in parasite counts. The average parasite, percentage chemo suppression recorded was 68.8, 78.5 and 79.5% at 100, 200 and 400 mg extract/kg body weight, respectively, while administration of 5 mg chloroquine/kg body weight resulted into complete chemo suppression (Table 2).

Test on established infection (Curative test)

The administration of extract also resulted in significant and dose dependent decrease in parasite counts in the treated groups when compared to the control group (Table 3). The mean parasite count was 10, 7 and 6, at the doses of 100, 200 and 400 mg extract/ kg body weight, respectively, as compared with the mean parasite count of 44 in the control group. Chloroquine administration of 5 mg/kg chloroquine reduced the mean parasite density to 3. The dose of extract administered also affected the survival period of the mice. Mortality started in the control group from seventh day after extract administration, and completed on eleventh day (mean survival time was 9 days after extract administration). Mice administered with100 and 200 mg extract/kg body weight survived for 17 and 21days respectively, but those administered with 400 mg/kg survived for 18 days only. Chloroquine administered mice had longest survival of 28 days after administration (Table 3).

Test on residual infection (Repository test)

The *Plasmodium* count also reduced significantly with increase in the dose of extract when compared with control. The mean parasite counts at 100, 200 and 400 mg extract /kg body weight were 19.2, 12.8 and 10.0, compared with 26.4 for the control group. The mean parasite count for chloroquine administered group was 2.8. Significant reduction in body weight of the mice in the control and the mice administered with 100 and 400 mg/kg of the extract was observed (Table 4).

DISCUSSION

The results obtained from the present study showed that the aqueous root extract of *A. nilotica* possess significant suppressive effect against early *Plasmodium* infection, curative effect against established infection and prophylactic effect against residual infection in *P. berghei berghei* infected mice.

Survival of mice, after oral administration of 2500 mg/kg body weight of the extract, up to 7 days, indicates

Table 2. Suppressive activity of aqueous root extract of *Acacia nilotica* in *P. berghei berghei* infected mice.

Treatment	Dose (mg/kg) p. o.	Mean parasitaemia count D_5	Inhibition (%)
Control	-	26.5 ± 1.04	-
Extract	100	8.25 ± 0.48*	68.8
	200	5.5 ± 0.80*	78.5
	400	5.25 ± 1.11*	79.5
Chloroquine	5	0*	100

D_5 = Day five, *significantly different from the control at $p < 0.05$.

Table 3. Curative effect of aqueous root extract of *A. nilotica in P. berghei berghei* infected mice.

Treatment	Dose Mg/kg, p.o	Mean parasitemia count		Survival time (Days)
		Pre- (D_3)	Post- (D_7) - treatment	
Control	-	18.0 ± 0.73	44.0 ± 0.48	9 ± 0.19
Extract	100	16.0 ± 0.63	10.0 ± 0.58***	17 ± 0.48
	200	16.0 ± 0.80	7.0 ± 0.58***	21 ± 0.14
	400	17.0 ± 0.58	6.0 ± 0.68***	18 ± 0.46
Chloroquine	5	14.0 ± 0.82	3.0 ± 0.37***	28 ± 0.58

D_3 = Day three, D_7 = Day seven, *** significantly different at $p < 0.001$.

Table 4. Prophylactic effect of aqueous root extract of *A. nilotica in P. berghei berghei* infected mice.

Treatment	Dose (mg/kg, p.o)	Mean parasitemia count D_7	Body weights (g)	
			D_1	D_7
Control	_	26.4 ± 1.0	18.6 ± 0.68	15.5 ± 0.50*
Extract	100	19.2 ± 0.86*	18.2 ± 0.66	16.0 ± 0.98*
	200	12.8 ± 0.37**	19.2 ± 0.86	19.0 ± 0.01
	400	10.0 ± 0.71**	22.0 ± 0.68	18.5 ± 0.50*
Chloroquine	5	2.8 ± 0.37***	18.8 ± 0.67	18.3 ± 0.33

D_1 = Day one, D_7 = Day seven, *, ** and *** = significantly different at $P < 0.05$, 0.01 and 0.001, respectively.

that the estimated oral median lethal dose (LD_{50}) of the extract at 5000 mg/kg body weight (OECD, 2001) is non-toxic. This suggests that acute oral administration of the extract is safe, and also explains the reason why the root portion of the plant is widely used in traditional treatment of malaria.

Although rodent models do not produce exactly the same signs and symptoms observed in the human plasmodial infection but they have been reported (Pedroni et al., 2006; Pierrot et al., 2003) to produce disease features similar to those of human plasmodial infection, when infected with *P. berghei berghei* (Thomas et al., 1998). Moreover, several studies (Calvalho et al., 1991; Agbedahunsi, 2000; Adzu and Salawu 2009) have employed *P. berghei berghei* in predicting treatment outcome of suspected antimalarial agents, because of its high sensitivity to chloroquine, making it appropriate for this study. Substances that reduce parasite multiplication (anti plasmodial effect) in the host were considered to possess antimalarial activity (Ryley and Peters, 1970).

The 4 day suppressive test is a standard test commonly used for antimalarial screening (Peters, 1965). The extract produced significant dose related chemo suppression in all the treated groups with the highest chemo suppression (79.5%) observed in the group treated with 400 mg extract/kg. The closeness of the chemo-suppression

value of 78.5 and 79.5% in 200 and 400 mg/kg body weight administered group respectively, and the higher survival time in the former group suggests that the 200 mg extract/kg dose may be the optimal therapeutic dose in mice. The higher doses of extract may not possess significantly more beneficial antiplasmodial effect. The aqueous root extract also demonstrated significant dose related reduction in parasite count in both established (curative) and residual (repository) infection, comparable to the effect of chloroquine, which in this study was used as standard control drug.

The reduction of parasite count in the curative test is similar to the values recorded for reduction in parasite count in the suppressive test, but lower than the mean parasite count in the repository test. This may probably be due to rapid parasite clearance by the extract in early and established infection, as against a situation where the extract was initially administered for days (Prophylactic) before inoculation with parasite. The high parasite count in the repository test may be attributed to rapid metabolism of administered extract (before inoculation) to inactive products (Dahanukar et al., 2000).

The significant reduction in body weight in the group administered with 400 mg/kg body weight as well as the control group may be due to combined effect of plasmadial infection (Thomas et al., 1998) and possible catabolic effect of high dose of the extract on stored lipids.

These observations showed that the extract is active against the malaria parasite used in this study and is consistent with the ethnomedicinal use of *A. nilotica* roots, as anti malaria in Northern part of Nigeria (Etkin, 1997).

The mechanism of anti plasmodial action of this extract has not been elucidated, however, anti plasmodial effects of natural plant products have been attributed to some of their active phytochemical components (Ayoola et al., 2008; Sofowora, 1980). Some of these phytochemicals such as terpenes and flavonoids (detected in *A. nilotica*) were reported to have antiplasmodial activity (Phillipson and Wright, 1990; Christensen and Kharazmi, 2001; Go, 2003). Earlier studies by Etkin (1997), reported the oxidant generation potential of *A. nilotica* extract, based on the ability of the extract to increase conversion of reduced glutathione (GSH) to oxidized glutathione (GSSG). Increased oxidation has also been shown to create an intracellular environment that is unfavourable to plasmodial growth (Borris and Schaeffer 1992; Levander and Ager, 1993). The mechanism of action of artemisinin, which depends on oxidant action for its potent antimalarial activity, validates this (Etkin, 2003). However, lack of oxidizing action in some plants does not rule out anti plasmodial activity since they may be active through other biochemical mechanisms.

The anti plasmodial effect of aqueous root extract of *A. nilotica* may therefore be due to the phytochemical components (alkaloids, flavonoids and terpenes) or the oxidant generation potential or a combination of these mechanisms.

It can therefore be concluded that aqueous root extract of *A. nilotica* possess good potential as antimalarial phytomedicine and there is a scientific basis for its continuous use in traditional medicine for the management of malaria.

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