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Archives of Applied Science Research, 2010, 2 (5):261-268

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ISSN 0975-508X
CODEN (USA) AASRC9

Anti-malarial activity of ethanolic stem bark extract of Faidherbia Albida (Del) a. Chev (Mimosoidae) in mice

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ABSTRACT

Malaria is an increasing worldwide threat, with more than three hundred million infections and one million deaths every year. In Africa and elsewhere, medicinal plants including Faidherbia albida Del. are still widely used in the treatment of malaria and other ailments. Previous studies in our laboratory have shown that the ethanolic stem bark extract of Faidherbia albida possesses antipyretic, anti-diarrhoeal and anti-inflammatory properties. The present study was carried out to investigate its in vivo antiplasmodial effect in mice. The in vivo anti- plasmodial effect against early infection, curative effect against established infection and prophylactic effect against residual infection were studied in chloroquine - sensitive Plasmodium berghei berghei NK65-infected mice. The extract at all the doses (100, 200 and 400 mg/kg, p.o.) used, produced significant ($P<0.05$), dose - dependent activity against the parasite in the suppressive, curative and prophylactic tests. The result suggests that Faidherbia albida ethanolic stem bark extract possesses potent antimalaria effect.

Keywords: Malaria, *Faidherbia albida, Plasmodium berghei berghei NK65.*

INTRODUCTION

Malaria is an increasing worldwide threat, with more than three hundred million infections and one million deaths every year [1]. The majority of those who die from malaria are infants and children living in sub-Saharan Africa. In many of the affected communities, average yearly per

capita income is the equivalent of 200–600 US$ and government expenditure on health is low (not more than 1–2% of the gross domestic product). The result is poor access to effective malaria treatment [2]. This situation has in recent times become aggravated by the progressive spread of *Plasmodium falciparum* resistant to the most commonly used and affordable antimalarial drugs such as chloroquine. The world's poorest are the worst affected, and many treat themselves with traditional herbal medicines which are often more available, affordable, and sometimes perceived as being more effective than conventional antimalarial drugs including artemisinin combination therapy (ACT). In view of the problems associated with antimalarial drug resistance, new drugs or drug combinations are urgently required today for treatment of malaria. Preferably, the new drugs should have novel modes of action or be chemically different from the drugs in current use [3] . Plants have always been considered to be a possible alternative and rich source of new drugs and most of the antimalarial drugs in use today such as quinine and artemisinin were either obtained directly from plants or developed using chemical structures of plant-derived compounds as templates [4] . Due to limited availability and/or affordability of pharmaceutical medicines in many tropical countries, the majority of the populations depend on traditional medical remedies [5], mainly from plants. In Africa and elsewhere, plant extracts are still widely used in the treatment of malaria and other ailments, and up to 80% of the African population use traditional medicines for primary health care [6]. Since little scientific data exist to validate antimalarial properties of these medicinal plants, it is important that their claimed antimalarial properties are investigated, in order to establish their efficacy and determine their potential as sources of new antimalarial drugs (such as, artemisinin isolated from *Artemisia annua*). *Faidherbia albida* otherwise known as *Acacia albida* (Del) is of the family Mimosoideae. It is native to Southwest Africa, through West, North Africa to Egypt and East Africa. Common names include winter thorn and apple-ring acacia. The Hausa people of northern Nigeria call it 'Gawo' while in Fulfuldes it is called 'Chayski'. Phytochemical studies reveal that plants in this family contain tannins [6], which account for their use in making of dyes. In folkloric medicine, it is used in fevers by the Masai people of Kenya as well as for diarrhoea in Tanganyika [7]. A liniment, made by steeping the bark, is used for bathing and massage in pneumonia. The bark infusion is used for difficult delivery, and is used as a febrifuge for cough [7]. In northern Nigeria, especially among the cattle rearing nomads, a decoction of the stem bark is taken orally for the management of the sleeping sickness and malaria. Previous studies in our laboratory has demonstrated that the plant possess anti-pyretic, anti-inflammatory, anti-diarrhoea [6] and anti-trypanosomiasis [7] effects in experimental rats. The aim of the current study was to evaluate the anti-malarial activity of the ethanolic stem bark extract of *Faidherbia albida* against *Plasmodium berghei berghei* infected mice in order to scientifically ascertain the folkloric use of the plant in the management of malaria

MATERIALS AND METHODS

Chemicals and test agents

The ethanol used for the extraction, was of analar grade and was purchased from Sigma-Aldrich representative in Nigeria (Zayo International Ltd, Jos, Nigeria). *Faidherbia albida* stem bark extract was prepared as aqueous tragacanth (a biologically inert surfactant) and were freshly prepared prior to each experiment.

Plant material

Fresh leaves and stem bark of *F. albida* were collected from Gyamso ward in Toro Local Government Council of Bauchi State, Nigeria. They were identified and authenticated by Mrs. Jemilat Ibrahim of the Department of Medicinal Plant Research and Traditional Medicine (MPR&TM) of National Institute for Pharmaceutical Research and Development (NIPRD). A voucher specimen (number NIPRD/H/6151) was prepared and deposited for future reference at NIPRD herbarium. The stem bark were cleaned, air-dried and pounded into fine powder using mortar and pestle. The powder was stored in an airtight container and kept in a cool, dry place.

Extract preparation

Two hundred grams (200g) of powdered stem bark were weighed and macerated in 2 L of 50% ethanol for 48 h. The mixture was filtered using muslin cloth followed by Whatman filter paper (No. 1). The filtrate was heated to dryness over a water bath to give a dark brown solvent free extract which was stored at -4°C until required for use. Aliquot portions of the crude extract residue were weighed and suspended with 2.5% tragacanth in distilled water for use on each day of the experiment.

Animals

Wistar albino Mice (18 - 22 g) of both sexes obtained from Animal Facility Centre, National Institute for Pharmaceutical Research and Development, Abuja, Nigeria were used for the study. They were housed in polypropylene cages, and given standard laboratory diet and water *ad libitum* and maintained under laboratory conditions of temperature (22 ± 1°C), relative humidity (14 ± 1%) and 12 h light and 12 h dark cycle. The antimalarial study was carried out to the "Principles of Laboratory Animal Care" (NIH Publication No. 85; rev. 1985) and NIPRD Standard Operating Procedures (SOPs) on antimalarial study of the department of Pharmacology and Toxicology (P&T), NIPRD.

Rodent parasite (*Plasmodium berghei berghei NK65*)

The rodent parasite was sourced from National Institute for Medical Research (NIMR), Lagos, Nigeria and maintained alive in mice by continuous intraperitoneal passage in mice [8] after every 5 days. The reinfected mice were moved to the Animal Facility Center (AFC) of National Institute for Pharmaceutical Research and Development (NIPRD) where the study was carried out. Prior to the start of the study, one of the infected mice was kept and observed to reproduce signs of diseases similar to human malarial infection [9].

Parasite Inoculation

The inoculums consisted of $5x10^{7}$ of P. berghei berghei parasitized erythrocytes per ml. This was carried out by determining both the percentage parasitaemia and erythrocytes count of the donor mouse and diluting the blood with phosphate buffer saline in proportions indicated by both determinations. Each mouse was inoculated on day 0 with 0.2 ml of infected blood containing $0.1 x10^{7}$ P. berghei berghei parasitized red blood cells.

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 [10]. Adult Swiss albino mice of both sexes were inoculated as described above. These mice were randomly divided into 5 groups of 6 mice per group and treated for 4 consecutive days. Group I mice were given 10ml normal saline/kg body weight orally daily. Groups II, III and IV were given 100, 200 and 400mg extract/kg body weight orally daily respectively, while group V mice received 5mg chloroquine/kg body weight orally daily. On day 5 of the experiment, blood was collected from the tail of each mouse and smeared on to a microscope slide to make a thick film [8]. The blood films were stained with10% Giemsa at pH 7.2 for 10 minutes and [9] examined microscopically.The number of parasites per field were counted for ten fields on each slide. The percentage suppression of parasitaemia was calculated for each dose level by comparing the parasitaemia in infected controls with those of treated mice and the results multiplied by 100.

Test on established infection (Rane test)

Evaluation of the curative potential of *Faidherbia albida* stem bark extract was carried out as described by [13]. Thirty adult mice were inoculated as described above and left untreated. Three days later, the mice were randomized into five groups of six mice each. Group I mice were given 10ml normal saline/kg body weight orally. Groups II, III and IV were treated with 100, 200 and 400 mg extract/kg/day orally respectively, while group V mice received 5 mg chloroquine /kg daily orally for 5 days. On each day, about 3 drops of blood were collected from the tail of each mouse smeared unto a microscope slide to make a thick film, stained with 10% Giemsa stain and examined microscopically to monitor the parasitaemia level. The mean survival time (MST) of the mice in each treatment group was determined over a period of 29 days (D0–D28).

Repository test

The prophylactic activity of the extract was tested using the residual infection procedure described by [11]. Adult mice of both sexes were weighed and randomized into five groups of six mice each. Group I mice were given 10ml normal saline/kg body weight. Groups II, III and IV were given 100, 200 and 400 mg extract/kg body weight orally respectively while group V mice received 5mg chloroquine/kg body weight orally daily for 5 days. On the fifth day, all the mice were inoculated with standard inoculum of 0.1×10^7 *P. berghei berghei* NK 65 infected erythrocytes. Thick film of blood smears were then made from each mouse 72 h after treatment [12] and examined microscopically for parasitaemia level. The mice were weighed and changes in body weight noted.

Statistical Analysis

Graph pad prism version 5.02 was used to analyze 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 post hoc test. $P \leq 0.05$ were considered significant.

RESULTS

Anti-Plasmodial Effect of Ethanolic Stem Bark Extract of Faidherbia Albida

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

The extract produced a significant ($P \leq 0.05$) and dose- dependent decrease in parasite counts. The average percentage chemo suppression produced was 24, 72.93 and 89.50% at 100, 200 and

400 mg extract/kg body weight respectively while, 5mg Chloroquine /kg body weight produced 98.34% chemo suppression (Table 1).

Table 1: Effect of ethanolic stem bark extract of *Faidherbia albida* on early malaria infection

Treatment	Parasite Count	% Suppression
Normal saline 5mg/kg (control)	3.62 ± 0.20	-
100mg Extract /kg	2.72 ± 0.20*	24.00
200mg Extract /kg	0.98 ± 0.09**	72.93
400mg Extract /kg	0.38 ± 0.07**	89.50
5mg Chloroquine /kg	0.06 ± 0.03**	98.34

****** Significantly different from the control at P<0.05 and ** P<0.01*

Test on established infection (Rane test)

The extract produced significant (P≤0.05) and dose- dependent decrease in parasite counts. The mean percentage suppression of parasitaemia were 41, 86.67 and 88.33 at the doses of 100, 200 and 400 mg extract/kg body weight respectively. At 5 mg/kg, Chloroquine produced 99.44% chemo suppression. The extract produced significant (P<0.05,0.01) dose-dependently prolonged the survival time of mice while, chloroquine at 5mg/kg body weight significantly(P<0.001) prolonged the survival time of treated mice (Table 2).

Table 2: Effect of ethanolic stem bark extract of *Faidherbia albida* on established infection

Treatment	Parasite Count	% Suppression	Mean survival time (day)
Normal saline 5ml/kg(control)	3.60 ± 0.28	-	11.30 ± 0.47
100mg Extract /kg	2.10 ± 0.22*	41.67	23.50 ± 1.63**
200mg Extract /kg	0.48 ± 0.09**	86.67	28.00 ± 0.00**
400mg Extract /kg	0.42 ± 0.10**	88.33	28.00 ± 0.00**
5mg Chloroquine /kg	0.02±0.00***	99.44	28.23 ± 1.32

****** Significantly different from the control at P<0.05 and ** at P<0.01, *** at P<0.001*

Test on residual infection (repository test)

The extract produced significant (P≤0.05) and dose dependent decrease in parasite counts. The mean percentage chemo-suppresion produced were 25, 32.14 and 51.78 at 100, 200 and 400mg extract/kg body weight respectively. At 5mg/kg, Chloroquine produced 71.42% chemo-suppression. The extract at the doses used and chloroquine at 5 mg/kg body weight respectively significantly (P<0.05) increased the body weight of mice when compared to the control (Table 4).

Table 4a: Prophylactic effect of Faidherbia albida and chloroquine against *P. berghei berghei infected mice*

Treatment	Parasite Count	% Suppression	Body weight(g) Day 0	Body weight(g) Day 7
Normal saline 5ml/kg(control)	5.6 ± 0.68	-	19.22±0.12	13.02±0.32
Extract 100mg/kg	4.2 ± 0.48	25.00	18.85±0.01	21.15±0.21*
Extract 200mg/kg	3.8 ± 0.37*	32.14	19.45±0.25	22.40±0.15*
Extract 400mg/kg	2.7 ± 0.32**	51.78	18.92±0.54	23.42±0.14*
CQ 5mg/kg	1.6 ± 0.19**	71.42	19.25±0.32	24.52± 0.22*

***** *Significantly different from the control at P<0.05 and **P<0.01*

DISCUSSION

The results obtained from this study showed that the ethanolic stem bark extract of *Faidherbia albida* possess significant suppressive effect against early infection, curative effect against established infection and prophylactic effect against residual infection of the parasite at safe doses. Previous studies on the safety assessment of *Faidherbia albida* stem bark extract showed that its oral median lethal dose was greater than 5000 mg/kg body weight[15], which suggests that orally administered stem bark extract of *F. albida* is practically non-toxic [15]. This high safety profile may have been responsible for its wide spread use in different ethno-therapeutic interventions. Although primate models provide a better prediction of anti malarial efficacy in human than the rodent models, the latter have also been validated through the identification of several conventional antimalarials, such as chloroquine, halofantrine, mefloquine and more recently artemisinin derivatives [13]. *Plasmodium berghei berghei parasite* is used in predicting treatment outcomes of any suspected antimalaria agent due to its high sensitivity to chloroquine making it the appropriate parasite for this study [16,17].

The 4-day suppressive test is a standard test commonly used for antimalarial screening. The determination of percentage inhibition of parasitemia is the most reliable parameter for assessment of antimalarial effect of a test compound. A mean group parasitemia level of less than or equal to 90% of the mock-treated control animals usually indicates that the test compound is active in standard screening studies [14]. The extract produced remarkable dose related chemo suppression in the various models used for the study comparable with chloroquine used in the study, especially in the suppressive test. This observation further demonstrated that the extract is active against the malaria parasite used in the study. The significant chemo suppression produced by the extract on day 4 is consistent with the traditional use of the plant as a herbal medication against malaria in Northern part of Nigeria. This observation may be due to the interplay of phytochemicals in the extract which are not all selective against the malaria parasite. The decrease in parasitaemia produced by the extract in the established infection test was higher than the suppressive test probably due to non-selectivity of the extract against the

proliferative processes of the parasite. The presence of the parasite alone in the blood does not induce disorder, but the response of the host immune system against foreign pathogenic organism via free radical generation, activation of phospholipase cascade series and generation of prostaglandins and other haemolytic principles such as free fatty acids does. The pronounced anti-malarial activity of the extract observed in the established infection test may be due inhibitory effect of the extract on generation of free radicals and haemolytic principles such as free fatty acids resulting from high parasitaemia level [8]. It may also be due to the direct plasmocidal effect of the extract as shown by the decrease in parasite count produced by the extract. The low parasitaemia level observed in the established infection which translated into longer survival time is an additional evidence of antimalarial efficacy of the plant's extract. The low activity of the extract observed in the repository test when compared to the effect against early and established infections may be due to rapid hepatic clearance of the active component, notwithstanding the mice showed remarkable increase in body weight when compared with the infected untreated control, implying that other than direct parasiticidal effects, plants may possess other pharmacological benefits to the hosts, such as acting as analgesics, antipyretics or as immune stimulators [21]. The presence of pharmacologically active phytochemicals such as saponins, tannins and alkaloids in ethanolic stem bark extract of *Faidherbia albida* have been reported by [8]. Although, the exact mechanism of action of this extract has not been elucidated, antiplasmodal effects of natural plant products have been shown to depend on their constituent active phytochemicals [18]. Saponin, flavonoids and tannins have been suggested to act as primary antioxidant or free radicals scavengers that can counteract the oxidative damage induced by the malaria parasite [16, 19]. Furthermore, studies have shown that antiplasmodal effect of natural products of plant origin may be mediated via inhibition of protein synthesis [17]. The antiplasmodal effect demonstrated by the ethanolic stem bark extract of *Faidherbia albida* may be due to the presence of saponins, tannins and the alkaloidal constituent acting through either of the two mechanisms mentioned above or synergistically through a combination of the mechanisms suggested or another presently unknown mechanism. Furthermore this phytochemicals may be acting singly or in synergy with one another to produce the observed anti-malarial activity in this study. In addition, our earlier studies have shown that crude ethanolic extract of faidherbia albida stem bark possess anti-pyretic and anti-inflammatory effects [6]. Agents with such activity were reported to provide relief to malaria patients [20]. This study therefore showed that the extract possess greater potential to be explored for development of antimalarial phytomedicine and further established scientifically the basis for its continuous use in folk medicine for the management of malaria. Currently, further works are ongoing in our laboratories to isolate, characterize and establish the exact mechanisms of its antiplasmodal effect.

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