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Research Article

Prophylactic Potentials of Extracts of *Alstonia boonei* Stem Bark on Chloroquine-Sensitive *P. berghei*- Induced Malaria in Mice

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Abstract

Malaria still remains the most important parasitic disease in the tropics. It is the world's dominant parasitic infection. The problems of drug resistance and cross resistance of antimalarials especially plasmodium constitute a great handicap to the eradication of this disease. Plant decoction that either treats or prevents parasite invasion is highly desirable in developing countries as it will be cost effective and also readily available for the treatment and prevention of malaria. It was in this regard that we investigated the prophylactic potentials of the various fractions of the stem bark of *Alstonia boonei* which is locally used for the treatment of malaria in South Western Nigeria. Male albino mice were pretreated separately daily with a single dose of Sulfadoxine-Pyrimethamine (SP) (5mg/kg body weight) while the other groups were pretreated separately with two different doses (200mg/kg body weight and 400mg/kg body weight) of methanol extracts (ME), n-hexane (HF), chloroform (CF), ethylacetate (EF) and aqueous fractions (AF) of *Alstonia boonei* stem bark for seven consecutive days. The animals were then challenged with inoculums of $1x10^7$ chloroquine- sensitive *P. berghei* infected erythrocyte intraperitoneally. The results showed a mutual delay in parasitemia with chloroform, n-hexane and aqueous fractions. The mean percentage suppression of the parasite using SP and the extracts and fractions of *Alstonia boonei* (ME, HF, CF, EF, AF) on the seventh day of the experiment were: 100±0.00, 40.22±0.00, 45.45±0.00, 50.00±0.01, 30.46±0.00, and 43.42±0.11 respectively for 200mg/kg body weight dose and 100±0.00, 67.50±0.11, 80.20±0.00, 96.40±0.01, 62.55±0.02 and 70.20±0.00 respectively for 400mg/kg body weight dose. These results showed that 200mg CF/kg body weight prevented parasitemia by half the capacity of SP while 400mg CF/kg body weight was almost as good as SP. The percentage parasitemia also decreased in this order. The packed cell volume (PCV) increased significantly ($p<0.05$) in the SP and the 200mg/kg body weight dose CF (48.50±0.02 and 35.01±01) respectively and increased for all the fractions used for 400mg/kg body weight dose compared with the control. The result showed that chloroform fraction of *Alstonia boonei* has promising anitimalarial potential which could be of future importance.

Keywords: *Malaria, parasitemia, Alstonia boonei, parasite clearance*

Introduction

There is an urgent need to improve malarial control strategies in areas with high risk of malaria-related morbidity and mortality (Snow, *et al*., 2005). In most parts of sub-saharan Africa, individual control measures are restricted to the reduction of transmission by the use of insecticide- treated bed nets (Lengeler, 2004) and treatment of malaria attacks (Breman, *et al*., 2004) often with an ineffective drug due to parasite drug resistance (May and Mayer, 2003). Therefore, prophylactic preventive treatment aims to inhibit the parasitization of the red blood cells and consequent multiplication of *Plasmodium falciparum* after infection (Grobush, *et al*., 2007).

The first effective treatment for malaria came from the bark of cinchona tree which contains quinine. This tree grows on the slopes of the Andes, mainly in Peru. The indigenous peoples of Peru made a tincture of cinchona to control malaria. In the 20th century, chloroquine replaced quinine as treatment of both uncomplicated and severe falciparum malaria until resistances in the 1970s occurred as an unexpected development. Artemisnins, discovered by Chinese scientists in the 1970s, are now recommended treatment for falciparum malaria, administered in combination with other antimalarial as well as in severe disease (Kaufman and Ruveda, 2005).

In natural medicine, *Alstonia boonei* is recognized as an effective treatment for many diseases.. *Alstonia boonei* de Wild (Apocynaceae) is a medicinal plant that is widely used across Africa for various ailments. The stem bark of *Alstonia boonei* has been reported to possess anti-inflammatory, analgesic and antipyretic activities (Olajide *et al*., 2000). An infusion of the bark is used as antivenom for snake bites. It is also used in treating painful micturation and rheumatic conditions. Decoctions made from the root, stem bark and leaves are used in various

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forms of treatment such as in asthma, treatment of impotence, tooth ache and child delivery The stem bark is commonly used in malaria, and it is listed in the African Pharmacopoeia as an antimalaria drug. Alkaloids, tannins, saponins, flavonoids and cardiac glycosides were among the phytochemicals detected together with the important vitamin, ascorbic acid (Akinmoladun *et al*., 2007).

Despite a clear need, no vaccine offering a high level of protection currently exists. Efforts to develop one are ongoing (Kilama and Ntoumi, 2009). A number of medications are also available to prevent malaria in travelers to malaria-endemic countries. Anitmalarial drugs taken for prophylaxis by travelers can delay the appearance of malaria symptoms by weeks or months long after the traveler has left the malaria-endemic area.

Several drugs, most of which are also used for treatment of malaria, can be taken preventively. Sulfadoxine-pyrimethamine may be used where the parasite is still sensitive (Barnes, 2005). However due to resistance one of three medications: mefloquine (Lariam), doxycycline and combination of atovaquone and proguanil hydrochloride (Malarone) is frequently needed. Doxycycline and the atovaquone and proguanil combination are the best tolerated with mefloquine associated with high rates of neurological and psychiatric symptoms. The prophylactic effect does not begin immediately upon starting the drugs, so people temporarily visiting malaria-endemic areas usually begin taking the drugs one or two weeks before arriving and must continue taking them for 4 weeks after leaving (with the exception of atovaquone, proguanil that only needs to be started 2 days prior and continue for seven days afterwards). Generally, these drugs are taken daily or weekly, at a lower dose that would be used for treatment of a person who had actually contracted the disease.

Use of prophylactic drugs is seldom practical for full-time residents of malaria-endemic areas, and their use is usually restricted to short-term visitors and travelers to malaria regions. This is due to the cost of purchasing the drugs, negative side effects from long term use, and because some effective antimalarial drugs are difficult to obtain outside of wealthy nations. The use of prophylactic drugs where malaria-bearing mosquitoes at present may encourage the development of partial immunity (Jacquerioz *et al*., 2009). Due to development of resistance of malaria parasites to conventional drugs, search for new drugs from cultural antimalarial phytomedicine compendium becomes necessary.

The beneficial properties of *Alstonia boonei* coupled with low manufacturing cost may constitute a locally viable alternative to the more expensive anti-malaria medication. If proven effective as a prophylactic, it may provide a much needed answer to the current upsurge in malaria cases in developing countries. We therefore report the potentials of various fractions of *Alstonia boonei* stem on malaria induced by chloroquine-sensitive *P. berghei.*

Materials and Methods

Plant materials: The freshly peeled stem bark of *Alstonia boonei* (De wild) samples were authenticated and identified at the Herbarium, Plant Science Department, Ekiti State University and a specimen (Voucher No.UHAE 013) was deposited in the Herbarium. The stem bark were air-dried for three weeks in the laboratory. The dried stem bark was blended to powder using an electric blender. Thereafter, 10kg of blended air-dried stem bark was soaked in 20 liters of methanol for 72 hours at room temperature. It was continually stirred after each 24 hours. After 72 hours, the mixture was filtered and the filtrate was concentrated using rotary evaporator at 40^0C. The crude methanol extract concentrate was heated over a water bath at 50^0 C to obtain a solvent free extract that weighed 100g (1.0% yield).

Solvent partitioning: About 500mls of distilled water was added to 35g of the dried methanol extract to form a slurry; this was poured inside a separating funnel and washed repeatedly with n-hexane until near exhaustion. The marc remaining was washed again with chloroform until near exhaustion. The remaining marc was finally washed with ethylacetate until exhaustion. The aqueous fraction was filtered to remove plant fibers. The obtained fractions: n-hexane fraction (HF), chloroform fraction (CF), ethylacetate fraction (EF) and aqueous fraction (AF) were concentrated under pressure using rotary evaporator and subsequently freeze dried at – 61^0 C.

Animals: Sixty male albino mice weighing between 18-20g were obtained from the Institute of Advanced Medical Research and Training (IAMRAT), College of Medicine, University of Ibadan, Nigeria. The animals were acclimatized for two weeks in the animals house of the Institute and were fed *ad libitum* on rat chow and water throughout the period of the experiment. The mice (n=5) were distributed into control, reference drug and five experimental groups for methanol extract (ME), n-hexane (HF), chloroform (CF), ethylacetate (EF) and aqueous (AF) fractions.

Transfection and pretreatment: The prophylactic potential of the various solvent extracts of the stem bark of *Alstonia boonei* was carried out according to the method of Peters (1967) with little modification. The animals were divided into twelve groups of five mice each and they were pretreated for seven days using two different doses (200mg and 400mg per kg body weight/day) for each of the fractions and methanol extracts. The SP group was equally pretreated once with 5mg/ kg body weight/ day of SP drug base for seven days, while the negative control group received the vehicle (5% v/v tween 80) only. After seven days, the mice were intra-peritoneally transfected with an inoculum size of $1x10^7$ of *Plasmodium berghei* infected erythrocytes obtained from IAMRAT. After seventy-two hours, blood samples were collected from the tails of the animals and smeared on to microscope slides to make both the thick and thin film. The blood film were stained with Giemsa prepared with buffered water (pH 7.2) and parasitemia was examined microscopically (using x 100 immersion oil objective). Slides were collected for seven consecutive days. The packed cell volume (PCV) was determined on day seven by the microhematocrit method.

Statistical analysis: Descriptive analysis, Duncan multiple range test and student t-test were used to analyze and compared the results at 95% confidence level.

Results

Table 1 showed the prophylactic effect of methanol extract of *Alstonia boonei* stem bark on *P. berghei* induced malaria in mice. The results obtained showed a delay in parasitemia in the methanol extract and SP treated groups compared with the untreated control. There was a significant ($p<0.05$) decrease in the percentage parasitemia at 400mg/kg body weight of the methanol extract than a 200mg/kg body weight dose. As the treatment progressed, there was no significant ($p<0.05$) difference between the values for percentage parasitemia and percentage clearance though percentage clearance increased. The percentage clearance of SP on the first day was significantly ($p<0.05$) higher than both the 200mg and 400mg dose of the methanol extract and zero parasitemia was achieved as from the 3rd day. Table 2 showed the prophylactic effect of n-hexane fraction of *Alstonia boonei* stem bark on *P. berghei*-induced malaria in mice. Again, the 400mg/kg body weight dose of the n-hexane fraction was significantly ($p<0.05$) potent than the 200mg dose but less that SP in activity. As the experiment progressed, the activity of n-hexane fraction became significantly ($p<0.05$) higher than that of the methanol extract. This was noticeable both in percentage parasitemia and percentage clearance. On the 7th day of the experiment, the 400mg/kg body weight of the n-hexane fraction cleared the parasite in two fold than the 200mg/kg body weight dose.

Table 3 showed the prophylactic effect of chloroform fraction of *Alstonia boonei* stem bark on *P. berghei*-induced malaria parasite in mice. The 5mg/kg body weight dose of SP has a significant ($p<0.05$) decrease in percentage parasitemia and a significant increase in percentage clearance throughout the experiment. The 400mg/kg body weight dose has a significant ($p<0.05$) increase in activity than the 200mg dose both in percentage clearance and in percentage parasitema. On day 7, 400mg/kg body weight chloroform fraction cleared the parasite more than the 200mg dose more than 2 folds.

Table 1:
Prophylactic effects of methanol extract of *Alstonia boonei* stem bark on P. *berghei*-induced malaria in mice

DAYS	**1**		**3**		**5**		**7**	
	%P	**%C**	**%P**	**%C**	**%P**	**%C**	**%P**	**%C**
200mg	2.25^{b}±0.09	12.46^{c}±0.02	1.33^{b}±0.01	28.15^{c}±0.02	1.49^{b}±0.01	31.11^{c}±0.02	1.40^{c}±0.10	40.22^{c}±0.00
400mg	2.25^{b}±0.09	40.90^{b}±0.00	1.31^{c}±0.00	46.20^{b}±0.00	1.42^{c}±0.01	58.00^{b}±0.00	0.61^{b}±0.05	67.50^{b}±0.11
SP	0.39^{c}±0.00	76.50^{a}±0.00	0.00±0.00	100^{a}±0.00	0.00±0.00	100^{a}±0.00	0.00±0.00	100^{a}±0.00
Ctrl	2.48^{a}±0.11	-	2.57^{a}±0.01	-	4.08^{a}±0.02	-	12.01^{a}±0.00	-

200mg=200mg/kilogramme body weight dose; 400mg=400mg/kg body weight dose; SP =5mg/kg body weight dose of sulfadoxine pyrimethamine; Ctrl=control; %P=percentage parasitemia; %C=percentage clearance

Table 2:
Prophylactic effects of n-hexane fractions of *Alstonia boonei* stem bark on P. *berghei*-induced malaria in mice

DAYS	1		3		5		7	
	%P	%C	%P	%C	%P	%C	%P	%C
200mg	2.14^{b}±0.01	3.33^{c}±0.01	2.02^{a}±0.01	20.27^{c}±0.03	2.31^{b}±0.00	24.24^{c}±0.05	2.31^{b}±0.01	45.45^{c}±0.00
400mg	0.61^{c}±0.00	62.00^{b}±0.01	0.49^{c}±0.00	70.80^{b}±0.00	0.25^{c}±0.00	72.60^{b}±0.05	0.20^{c}±0.00	80.20^{b}±0.00
SP	0.39^{c}±0.00	76.50^{a}±0.00	0.00±0.00	100^{a}±0.00	0.00±0.00	100^{a}±0.00	0.00±0.00	100^{a}±0.00
Ctrl	2.48^{a}±0.11	-	2.57^{a}±0.01	-	4.08^{a}±0.02	-	12.01^{a}±0.00	-

Table 3:
Prophylactic effects of chloroform fractions of *Alstonia boonei* stem bark on P. *berghei*-induced malaria in mice

DAYS	**1**		**3**		**5**		**7**	
	%P	**%C**	**%P**	**%C**	**%P**	**%C**	**%P**	**%C**
200mg	2.00^{b}±0.02	6.67^{c}±0.00	1.79^{b}±0.12	30.77^{c}±0.10	1.46^{b}±0.11	48.24^{c}±0.00	1.07^{b}±0.00	50.00^{c}±0.01
400mg	1.46^{c}±0.00	50.80^{b}±0.00	0.40^{c}±0.10	82.70^{b}±0.10	0.10^{c}±0.11	90.58^{b}±0.00	0.10^{c}±0.04	96.40^{b}±0.01
SP	0.39^{c}±0.00	76.50^{a}±0.00	0.00±0.00	100^{a}±0.00	0.00±0.00	100^{a}±0.00	0.00±0.00	100^{a}±0.00
Ctrl	2.48^{a}±0.11	-	2.57^{a}±0.01	-	4.08^{a}±0.02	-	12.01^{a}±0.00	-

Table 4:
Prophylactic effects of ethylacetate fractions of *Alstonia boonei* stem bark on P. *berghei*-induced malaria in mice

DAYS	**1**		**3**		**5**		**7**	
	%P	**%C**	**%P**	**%C**	**%P**	**%C**	**%P**	**%C**
200mg	1.51^{b}±0.01	13.33^{c}±0.01	1.04^{b}±0.11	16.22^{c}±0.11	0.85^{b}±0.00	20.52^{c}±0.00	0.77^{b}±0.03	30.46^{c}±0.00
400mg	0.85^{c}±0.00	50.70^{b}±0.00	0.70^{c}±0.10	58.20^{b}±0.11	0.67^{c}±0.04	60.00^{b}±0.03	0.58^{c}±0.07	62.55^{b}±0.02
SP	0.39^{c}±0.00	76.50^{a}±0.00	0.00±0.00	100^{a}±0.00	0.00±0.00	100^{a}±0.00	0.00±0.00	100^{a}±0.00
Ctrl	2.48^{a}±0.11	-	2.57^{a}±0.01	-	4.08^{a}±0.02	-	12.01^{a}±0.00	-

Table 5:
Prophylactic effects of aqueous fractions of *Alstonia boonei* stem bark on P. *berghei*-induced malaria in mice

DAYS	**1**		**3**		**5**		**7**	
	%P	**%C**	**%P**	**%C**	**%P**	**%C**	**%P**	**%C**
200mg	2.40^{b}±0.00	3.33^{c}±0.00	2.31^{b}±0.01	26.33^{c}±0.11	2.26^{c}±0.10	40.15^{c}±0.03	2.20^{b}±0.00	43.42^{c}±0.11
400mg	0.67^{c}±0.08	52.00^{b}±0.00	0.36^{c}±0.01	65.80^{b}±0.15	0.30^{b}±0.10	69.40^{b}±0.00	0.21^{c}±0.00	70.20^{b}±0.00
SP	0.39^{c}±0.00	76.50^{a}±0.00	0.00±0.00	100^{a}±0.00	0.00±0.00	100^{a}±0.00	0.00±0.00	100^{a}±0.00
Ctrl	2.48^{a}±0.11	-	2.57^{a}±0.01	-	4.08^{a}±0.02	-	12.01^{a}±0.00	-

Table 6:
Packed Cell Volume (PCV in percentage) of the prophylactic treated groups

TREATMENT	MF	HF	CF	EF	AF	*Control
200mg	14.00±0.00	13.00±0.00	35.01±0.01	14.00±0.00	17.00±0.00	10.00±0.00
400mg	17.50±0.00	35.00±0.00	36.01[c]±0.01	13.00±0.13	40.00±0.11	9.50±0.00
Sulfadoxine-pyrimethamine 5mg/kgbm/day = 48.50±0.10						

**P.berghei infected mice which did not receive any treatment except the vehicle. Each value is a mean of several determinations±SD after seven days of exposure to treatment*

Table 4 shows the propylactic effects of the ethylacetate fraction. This fraction had the least activity in percentage clearance of all the fractions used. The activity here also was dose dependent as the 400mg dose is potent than the 200mg dose. Table 5 shows the prophylactic effect of the aqueous fraction. This fraction is nearly as good as the chloroform fraction. There was a significant ($p<0.05$) decrease percentage parasitemia on day 7 when compared with other fractions used.

Table 6 showed the PCV of the prophylactic groups. The PCV improved in a dose dependent manner. The SP group has the highest value compared with all other treated groups and the negative control. Chloroform fraction had the highest value for PCV and differed insignificantly from other fractions used. The ethylacetate fraction had the lowest PCV both at 200mg and 400mg/kg body weight.

Discussion

Malaria has continued to be a major global public health concern in most African countries. It is thought that malaria is by far the most tropical disease causing one to two million deaths per year in Africa (Saidu *et al*., 2000). It is estimated malaria kills more people than AIDS. Malaria kills in one year what AIDs kills in 15years. For every death due to HIV/AIDS, there are about 50 deaths due to malaria. The worsening economic situation of the sub-sahara African countries makes it difficult to expand modern health services hence effective low-cost delivery medical system is urgently needed (Roestenberg *et al*., 2009). Since there is no safer, effective and cheaper anti-malaria remedies than chloroquine (Milken, 1997) in the treatment of malaria, development of new anti-malaria drugs especially from plant sources may be the way forward in dealing with global drug resistant problems of malaria.

Recent improvement in malaria prevention strategies have further enhanced its effectiveness in combating areas highly infected with the malaria parasite. Plasmodia infect the liver for about seven days before subsequently infecting the blood. Present prophylaxis against *Plasmodium falciparum* malaria employs agents that primarily kill blood stages and must be continued for 28days after the last exposure (Khalid and ElKamali, 1996). Casual chemo-prophylactics target not only the blood stages of malaria, but the initial liver stage as well. This means that the user can stop taking the drug seven days after leaving the area of risk. Prophylaxis against *Plasmodium vivax* requires different approach given the long liver stages of this parasite (Gessler, 1995).

In this prophylactic study, the various solvent fractions of the stem bark of *Alstonia boonei* used exerted significant ($p<0.05$) dose dependent reduction in percentage parasitemia level of 87.50, 90.20, 96.40, 62.55, 90.20% for ME, HF, CF, EF and AF respectively all at 400mg/kg body weight while SP recorded a zero parasitemia as from day 3. The results of this study showed that there was a significant ($p<0.05$) delay in parasitemia in virtually all the fractions used. The results indicate that the fractions used possess blood schizonticidal activity, meaning that the traditional medicinal practices is dynamic where this plant is used especially in the south western part of Nigeria.

All the extracts used in this study produced decrease in percentage parasitemia and increase clearance. Pretreatment of all groups increased the antiplasmodial activity of the extract used possibly via a build up in the putative compounds in the animals prior to transfection, thus producing higher activity than when the animals were treated after transfection. Where this herb is commonly used, it is usually taken as antimalarial herbal remedies as prophylactics and the frequency of consumption depends on the severity of infection (Berman *et al*., 2001).

Some herbal remedies may produce low rates of parasite clearance but higher rates of adequate clinical response. For example, in one study, parasitemia declined to insignificant levels of treatment with a decoration of *Terraplis interretis* (Schwartz *et al*., 2000), in another study, the Ugandan herbal remedy "AM" cleared parasites in only 8% of patients, but parastemia declined to lower levels, and 55% of the patients had an adequate clinical response (Idowu *et al*., 2010). So also, malaria parasites were not cleared completely, but there was s good clinical response which was sustained for the 7 days experiment, though the experiment was not followed thereafter. In the trial of *Cochlosspermum tinctorium,* patients were only followed for five days and clinical outcomes were not used, so it is not possible to comment on long term efficacy (Wilcox *et al*., 2004). The apparent effectiveness of the various fractions of *Alstonia boonei* stem bark used can also be explained by the antipyretic effect of some substances which may be found in the extracts. The standard antimalarial quinine is known to exert analgesic, antipyretic and muscle relevant activities

The PCV values obtained in the result showed an improvement over therapeutic treatment. SP group has the highest value of 48.50% while chloroform fraction has the highest value of 36% among the fractions used at 400mg/kg body weight. The normal PCV range in healthy mice is 39-49%. Further purification of the putative chloroform fraction could make it a promising candidate both in the antiplasmodial activity and a better PCV booster in malarial treatment. Among malaria patients, an inverse relationship exists between PCV and density of malaria (Guinovart *et al*., 2008). The decrease of parasitemia density and the increase relationship exists between PCV reflect the development of naturally acquired immunity against malaria. The mean PCV difference between treated and untreated groups suggests that malaria is a greater

contributor to anemia (Aponte *et al*., 2007). This is also in agreement with chemoprophylaxis and bed net studies, where it was also observed that efficacy against anemia was higher in younger infants (Geerlings *et al*., 2003, Ter Kuile *et al*., 2003). Promising prophylactic potentials of the various solvent extracts of the stem bark of *Alstonia boonei* on *P.berghei*-induced malaria in mice may establish its age long usage in traditional herbal medicine.

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