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Antimalarial Activity of Ethanolic Stem Bark Extract of *Alstonia boonei* in Mice

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ABSTRACT

Alstonia boonei De Wild is a medicinal plant used widely in Nigeria for the management of malaria and other ailments. The aim of the present study was to investigate *in vivo* antiplasmodial effect in mice. Oral acute toxicity of the ethanolic stem bark extract of *Alstonia boonei* was evaluated in mice using modified Lorke's method and 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 oral median lethal dose of the extract in mice was determined to be greater than 5000 mg kg^{-1} body weight. The extract at all the doses (100, 200 and 400 mg kg^{-1}, p.o.) used, produced significant ($p<0.05$), dose-dependent activity against the parasite in the suppressive, curative and prophylactic tests. These results showed that *Alstonia boonei* ethanolic stem bark extract possesses potent antimalarial effects and may therefore offer the potential for a safe, effective and affordable antimalarial phytomedicine.

Key words: *Alstonia boonei*, antimalarial activity, *Plasmodium berghei berghei*, mice, chloroquine

INTRODUCTION

Malaria is undoubtedly the single most destructive and dangerous infectious agent in the developing world (Greenwood *et al*., 2005; Winter *et al*., 2006). This vector-borne infectious disease is a classic example of one that affects the productivity of individuals, families and the whole society, since, it causes more energy loss, more debilitation, more loss of work capacity and more economic damage than any other human parasitic diseases (Sachs and Malaney, 2002). Malaria is commonly associated with poverty, but is also a cause of poverty and a major hindrance to economic development. There were an estimated 247 million malaria cases among 3.3 billion people at risk in 2006, causing nearly a million deaths, mostly of children under 5 years of age (WHO, 2008). It is widespread in tropical and subtropical regions, including parts of the Americas, Asia and Africa. A total of 109 countries were endemic for malaria in 2008, 45 within the WHO African region (WHO, 2008; Batista *et al*., 2009). In view of the problems associated with antimalarial drug resistance and prevalence of fake drugs in general circulation in Nigeria, new drugs or drug combinations are urgently required today for treatment of malaria.

Discovering new antimalarial compounds is more than ever a priority due to the alarming spread of resistance to available drugs and the limited number of effective antimalarial drugs still in store (Peter and Antoli, 1998b). Plants have always been considered to be a possible alternative and rich source of new drugs. Indeed most of the antimalarial drugs in use today such as quinine and artemisinin were either obtained directly from plants or were developed using chemical structures of plant-derived compounds as templates (Basco *et al*., 1994). Due to limited availability and/or affordability of pharmaceutical medicines in many tropical countries, the majority of the populations depend on traditional medical remedies (WHO, 2002; Zirihi *et al*., 2005). *Alstonia boonei* De wild is among the medicinal plants that have been widely used in recipes to treat malaria (Titanji *et al*., 2008; Idowu *et al*., 2010). It belongs to the family Apocynaceae which consists of about 50 species widely distributed in the continents of Africa, Asia and America. The stem bark of this plant has been found to be effective in the treatment of several diseases such as fever, painful micturition, insomnia, chronic diarrhoea and rheumatic pains (Ojewole, 1984; Asuzu and Anaga, 1991; Olajide *et al*., 2000; Odeku *et al*., 2008). Earlier studies have also suggested that *Alstonia boonei* and some other plants namely *S. latifolius*, *Petivera alliacea*, *Mangifera indica* and *Khaya grandifolia* have significant antimalarial properties (Awe *et al*., 1998; Agbedahunsi *et al*., 1998; Pedro and Antonio, 2001; Zirihi *et al*., 2005; Idowu *et al*., 2010). However, few reports exist in the literature on the antimalarial activity of ethanolic stem bark extracts of *A. boonei*. Olajide *et al*. (2000) reported that the stem bark of *A. boonei* has anti-inflammatory, antipyretic and analgesic properties. Taiwo *et al*. (1998) investigated the activity of stem bark of *A. boonei* de wild on human complement and polymorph nuclear leucocytes, Taiwo and Makinde (1996) reported on the effect of lyophilized aqueous extracts of *A. boonei* stem bark on guinea pig ileum and rat stomach strip. Oze *et al*. (2007) also investigated the nephrotoxicity caused by the extract of this plant in guinea pigs and (Raji *et al*., 2005) on reproductive functions of methanolic extract of *A. boonei* in male rats. Here, we report the antimalarial activity of ethanolic stem bark extract of *Alstonia boonei* using mice as our model organism.

MATERIALS AND METHODS

Plant collection and authentication: Fresh stem bark of *Alstonea boonei* was collected in Shagari Village, Akure South Local Government, Ondo State of Nigeria. It was identified and authenticated by Mal. Ibrahim Muazzam of the Medicinal Plant Research and Traditional Medicine (MPR and TM) Department, National Institute Pharmaceutical Research Development (NIPRD), Idu, Abuja in 2009.

Extraction of plant material: The fresh stem bark was cleaned, cut into pieces and air dried under shade in the laboratory for 2 weeks and reduced to powder. The dry stem bark was ground to coarse powder in a mortar. Extraction was carried out by dispersing 500 g of the ground plant material in 2.5 L of 70% alcohol and shaking was done with a GFL shaker (No. 3017 MBH, Germany) for 72 h. This was followed by vacuum filtration and extract concentration using a rotary evaporator at a temperature not exceeding 400°C. The concentrate was heated over a water bath to obtain a solvent free extract, which was stored in a refrigerator, at 40°C.

Animals: The animals used in the study were 4 week-old-albino mice weighing 18-22 g obtained from the Animal Facility Centre of National Institute for Pharmaceutical Research and

Development (NIPRD), Idu, Abuja. They were housed in plastic cages with saw dust as beddings and given food and water *ad libitum*. The mice were used in accordance with NIH Guide for the care and use of laboratory animals; NIH Publication (No. 83-23) revised (1985).

Acute toxicity test (LD50): The oral acute toxicity study of *Alstonia boonei* ethanolic stem bark extract was carried out in mice using modified Lorke (1983). The study was carried out in two phases. In the phase one of the study, nine mice were randomized into three groups of three mice each and were given 10, 100 and 1000 mg kg^{-1} b.wt. of the extract orally. The mice were observed for paw licking, salivation, stretching of the entire body, weakness, sleep, respiratory distress, coma and death in the first 4 h and subsequently daily for 7 days. In the second phase of the study another fresh set of nine mice were randomized into three groups of three mice each and were given 1600, 2900 and 5000 mg kg^{-1} b.wt. of the extract orally based on the result of the first phase. These were observed for signs of toxicity and mortality for the first critical 4 h and thereafter daily for 7 days. The LD50 was then calculated as the square root of the product of the lowest lethal dose and highest non-lethal dose, i.e., the geometric mean of the consecutive doses for which 0 and 100% survival rates were recorded in the second phase. The oral median lethal dose was calculated using the formula:

$$LD50 = \surd \text{Minimum toxic dose} \times \text{Maximum tolerated dose}$$

Rodent parasite (*Plasmodium berghei berghei*): The rodent parasite *Plasmodium berghei berghei* NK 65 was sourced from National Institute for Medical Research (NIMR), Lagos, Nigeria and kept at Animal Facility Centre, NIPRD, Idu, Abuja, Nigeria. The parasites were kept alive by continuous intraperitoneal passage in mice (Adzu and Haruna, 2007) every 4 days. These infected mice were used for the study. Prior to the beginning of the study, one of the infected mice was kept and observed to reproduce disease symptoms similar to human infection (English, 1996).

Anti-plasmodial studies

Suppressive test: The Peter's 4 day suppressive test against chloroquine sensitive *Plasmodium berghei berghei* NK 65 infection in mice was employed (Peters, 1967). Adult Swiss albino mice weighing 18-22 g were inoculated by intraperitoneal (I.P) injection with standard inoculum of Plasmodium berghei berghei with 1×10^7 infected erythrocytes. The mice were randomly divided into 5 groups of 6 mice per group and treated for 4 consecutive days with 100, 200 and 400 mg extract kg^{-1} b.wt. orally daily, respectively. Two control groups were used; the positive control was treated daily with 5 mg chloroquine kg^{-1} while the negative control group was given 5 mL kg^{-1} normal saline. On day 5 of the experiment, blood was collected from the tail of each mouse and smear on to a microscope slide to make a film (Saidu *et al*., 2000). The blood films were fixed with methanol, stained with 10% Giemsa at pH 7.2 for 10 min and parasitaemia examined microscopically. The percentage suppression of parasitaemia was calculated for each dose level by comparing the parasitaemia in infected controls with those of treated mice.

Evaluation of schizontocidal activity of *A. boonei* on established infection (curative or rane test): Evaluation of the curative potential of *A. boonei* stem bark extract was carried out according to the method described by Ryley and Peters (1970). The mice were injected intraperitoneally with standard inoculum of 1×10^7 *P. berghei berghei* NK 65 infected erythrocytes

on the first day (day 0). Seventy two hours later, the mice were divided into 5 groups of six mice each. The groups were orally treated with stem bark extract of *A. boonei* (100, 200 and 400 mg/kg/day), chloroquine (5 mg/kg/day) was given to the positive control and an equal volume of distilled water was given to the negative control group. The treatment was carried out once daily for 5 days and blood smears were collected and examined microscopically to monitor the parasitaemia level.

Evaluation of prophylactic activity of *A. boonei* (repository test): Evaluation of the prophylactic potential of *A. boonei* stem bark extract was carried out according to the method of Peters (1967). Adult mice were randomized into 5 groups of six mice each. Group I mice were given 10 mL distilled water kg^{-1} b.wt. orally. Groups II, III and IV were given 100, 200 and 400 mg extract kg^{-1} b.wt. orally, respectively. Group V was however given 5 mg chloroquine kg^{-1} b.wt. intraperitoneally. Treatments were initiated on day 0 and continued till day 4 when, the mice were all infected with the parasite. Blood smears were then made from each mouse 72 h after treatment (Abatan and Makinde, 1986) and increase or decrease in parasitaemia determined.

Statistical analysis: The one way ANOVA test was used to analyze and compare the results at a 95% confidence level. Values of $p \geq 0.05$ were considered significant. Results were expressed as Mean±SE of mean.

RESULTS

Acute toxicity test: Behavioural signs of toxicity observed in mice given 1000 mg extract kg^{-1} b.wt. and above include; paw licking, salivation, stretching and reduced activity. There was however, no mortality at all the dose levels used. The median lethal dose LD50 was estimated to be ≥5000 mg kg^{-1} b.wt.

Suppressive effect: The ethanolic extract stem bark of *A. boonei* exerted dose dependent chemosuppressive effect against *Plasmodium berghei berghei* malaria parasite. The extract caused a significant ($p<0.05$) chemo suppression of 46.43, 57.14 and 75.00%, respectively, when compared to the control. The standard drug, chloroquine caused chemosuppression of 95.71%, which was higher than those of the extract treated groups (Table 1).

Curative effect: The ethanolic stem bark extract of *A. boonei* produced significant ($p<0.05$) dose dependent reduction in parasitaemia levels in the extract treated groups, with a similar reduction in the chloroquine treated group (positive control). The average percentage suppression of parasitaemia of the extract treated groups on day 7 were 61.02, 67.80 and 81.36% for the 100, 200

Table 1: Suppressive Effect of *A. boonei* ethanolic stem bark extract and chloroquine against *P. berghei berghei* infection in mice

Treatments	Parasite count	Suppression (%)
Normal saline 5 mL kg^{-1} (control)	5.60±1.52	-
Extract 100 mg kg^{-1}	3.00±1.00*	46.43
Extract 200 mg kg^{-1}	2.40±0.55*	57.14
Extract 400 mg kg^{-1}	1.40±0.55**	75.00
CQ 5 mg kg^{-1}	0.24±0.54**	95.71

*Significantly different from the control at $p \leq 0.05$ and **at $p \leq 0.01$

Table 2: Curative effect of *A. boonei* ethanolic stem bark extract and chloroquine against *P. berghei berghei* infection in mice

Treatments	Parasite count	Suppression (%)
Normal saline 5 mL kg^{-1} (control)	11.80±3.64	-
Extract 100 mg kg^{-1}	4.60±1.52*	61.02
Extract 200 mg kg^{-1}	3.80±0.84*	67.80
Extract 400 mg kg^{-1}	2.22±0.45**	81.36
CQ 5 mg kg^{-1}	0.08±0.71**	99.32

*Significantly different from the control at $p \leq 0.05$ and **at $p \leq 0.01$

Table 3: Prophylactic Effect of *A. boonei* ethanolic stem bark extract against *P. berghei berghei* infection in mice

Treatments	Parasite count	Suppression (%)
Normal saline 5 mL kg^{-1} (control)	8.90±1.16	-
Extract 100 mg kg^{-1}	5.80±1.20*	34.83%
Extract 200 mg kg^{-1}	4.92±0.18*	44.72%
Extract 400 mg kg^{-1}	3.50±0.28**	60.67%
CQ 5 mg kg^{-1}	0.85±0.018**	90.45%

*Significantly different from the control at $p \leq 0.05$ and **at $p \leq 0.01$

and 400 mg/kg/day of the extract, respectively. While 5 mg chloroquine kg^{-1} b.wt. exerted 99.32% decrease in parasite count (Table 2).

Prophylactic effect: The ethanolic stem bark extracts of *A. boonei* produced significant ($p<0.05$) dose dependent reduction in level of parasiteamia of 34.83, 44.72, 60.67% while, 5 mg chloroquine kg^{-1} b.wt. caused 90.45% reduction in parasite count (Table 3).

DISCUSSION AND CONCLUSIONS

Currently no single drug is effective for treating multi-drug resistant malaria and effective combination therapy includes artemisinin derivatives such as artesunate (David *et al.*, 2004), or mixtures with older drugs such as the atovaquone (Deprez-Poulain and Melnyk, 2005)-proguanil (Jones and Good, 2006) combination Malarone® (Winter *et al.*, 2006; Taylor and White, 2004). Unfortunately first reports on drug resistance to artemisinin-derivatives (Jambou *et al.*, 2005) and to drug combination therapies (Wichmann *et al.*, 2004) have already appeared. So, in the absence of a functional, safe and widely available malaria vaccine, efforts to develop new antimalarial drugs continue.

There is a consensus among the scientific community that natural products have been playing a dominant role in the discovery of leads for the development of drugs for the treatment of human diseases (Newman *et al.*, 2003). Indeed, the vast majority of the existing anti-malarial chemotherapeutic agents are based on natural products and this fact anticipates that new leads may certainly emerge from the tropical plant sources, since, biological chemodiversity continues to be an important source of molecular templates in the search for antimalarial drugs (Portet *et al.*, 2007).

In this study, we evaluated the antimalarial activity of ethanolic stem bark of *Alstonia boonei* in mice. The acute behavioural signs of toxicity observed in mice given 1000 mg extract kg^{-1} b.wt. and above were paw licking, salivation, stretching and reduced activity. There was however no mortality at all the dose levels used. The oral median lethal dose (LD50) was estimated to be $\geq$5000 mg kg^{-1} b.wt. The observed reduced activity of the treated mice showed that the extract

possess central depressant effect. The absence of death following oral administration of *Alstonia boonei* stem bark ethanolic extract at 5000 mg extract kg^{-1} b.wt. observed in the rats suggests that the extract is practically non-toxic acutely (Salawu *et al*., 2009). 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 (Ryley and Peters, 1970). *Plasmodium berghei berghei* parasite is used in predicting treatment outcomes of any suspected antimalarial agent due to its high sensitivity to chloroquine making it the appropriate parasite for this study (Peter and Anatoli, 1998a).

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 Southern part of Nigeria.

With respect to suppressive effect, the ethanolic extract stem bark of *A. boonei* exerted a dose dependent chemosuppressive effect against *Plasmodium berghei berghei* malaria parasite. The extract caused a significant ($p<0.05$) chemosuppression of 46.43, 57.14 and 75.00%, respectively, when compared to the control. The standard drug, chloroquine caused chemo suppression of 95.71%, which was higher than those of the extract treated groups. The observed higher efficacy of chloroquine may in part be due to non selectivity of the extract or slow absorption and poor bioavailability of the crude extract. Similar observation was reported with the use of medicinal plant extract by Adzu and Haruna (2007).

In a curative study, we observed that the ethanolic stem bark extract of *A. boonei* produced a dose dependent reduction in parasitaemia levels in the extract treated groups, with a similar reduction in the chloroquine treated group (positive control). While, we observed a daily increase in parasitaemia in the negative control group, the average percentage suppression of parasitaemia of the extract treated groups on day 7 were 61.02, 67.80 and 81.36% for the 100, 200 and 400 mg/kg/day of the extract, respectively and 5 mg chloroquine kg^{-1} b.wt. exerted 99.32% decrease in parasite count. Present findings are consistent with earlier reports by Tantchou *et al*. (1986), Adjanohoun *et al*. (1996), Odeku *et al*. (2008), Titanji *et al*. (2008) and Idowu *et al*. (2010) who demonstrated that *A. boonei* has antimalarial activity and Agbedahunsi *et al*. (1998), who used *Khaya senegalensis*. This is therefore, common to natural product of plant origin due partly to the crude nature of the extract.

However, present findings seem to deviate from previous study by Oze *et al*. (2007), who demonstated that this plant may be nephrotoxic when administered at higher doses.

In the prophylactic study, the ethanolic stem bark extracts of *A. boonei* exerted significant ($p<0.05$) dose dependent reduction in level of parasitemia of 34.83, 44.72, 60.67% while 5 mg chloroquine kg^{-1} b.wt. caused 90.45% reduction in parasite count. The results indicates that the stem bark extract possess blood schizonticidal activity as evident from the chemosuppression obtained during the 4 day early infection test. A significant ($p<0.05$) activity was also recorded during established infection, which was comparable to the standard drug (Chloroquine, 5 mg/kg/day).

The results presented herein suggest that the extract of *A. boonei* is safe and possesses potent anti-malarial activity which justifies its continuous use in folk medicine as an anti-malarial remedy. Further works are on-going in our laboratories to isolate, identify and characterize the active ingredients from this plant.

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