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IN VIVO ACTIVITY OF ETHANOLIC EXTRACT OF ALSTONIA BOONEI LEAVES AGAINST PLASMODIUM BERGHEI IN MICE

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Abstract

This study evaluates the toxicity and antimalarial effect of Alstonia boonei leaves. Qualitative phytochemical analyses of the ethanolic *extract which included saponins, tannins, phlobatannins, flavonoids, steroids, cardiac glycosides, anthraquinones, alkaloids and reducing sugar were carried out. Acute toxicity of the extract was investigated at 300mg/kg, 2000mg/kg and 5000mg/kg concentrations in non-infected mice and their effects were evaluated from change in behavioural patterns of the mice. Also dose extract of 200,400 and 800mg/kg/day of Alstonia boonei leave extract were given orally to plasmodium berghei infected mice following the four-day suppressive test procedure. Tannin, phlobatannin, alkaloid, cardiac glycosides, steroid, reducing sugar, saponin and anthraquinone were present in the extract. None of the doses were toxic but mild weakness was observed at 5000mg/kg. Furthermore, ethanolic extract of Alstonia boonei leaves exerted much suppression of parasitaemia (43.1%) at the dose of 400mg/kg. Crude extract of Alstonia boonei possess strong activity against P.berghei indicating presence of chemical compositions that may be useful in preparing antimalarial drugs.*

Keywords*:* malaria, antimalarial, phytochemical, acute toxicity, plasmodium berghei, Alstonia boonei, suppressive test

1. Introduction

Malaria causes more energy loss, debilitation, loss of work capacity and economic damage than any other human parasitic diseases (Sachs and Malaney, 2002). A total of 109 countries were endemic for malaria in 2008, with 45 in the African region (WHO, 2008*; Batista et al., 2009*). Malaria is endemic in most African countries and one of the major causes of sickness and death in sub-Saharan Africa, and it continues to be a major public-health challenge. The alarming number of deaths caused by malaria is on the increase due to mosquito resistance to chemical insecticides, thus it is a huge challenge to mankind (Kamalinder et al., 2008).

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In view of the problems associated with antimalarial drug resistance, new drugs or drug combinations are urgently required today for treatment of malaria. 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 or plants-derived compounds as templates (Basco et al., 1994). Therefore *Alstonia boonei* stem bark has been found to be effective in the treatment of several diseases such as fever, painful micturition, insomnia, chronic diarrhea and rheumatic pains (Ojewole, *1984;* Asuzu and Anaga, 1991*;* Olajide *et al.*, 2000; Odeku *et al.*, 2008). Furthermore, Olajide *et al. (*2000) also reported that the stem bark of *A.boonei* has anti-inflammatory properties. An infusion of root and stem bark serves as remedy for asthma and a liquid made from the stem bark and fruit taken daily was effective in the treatment of impotence. It is documented that a decoction of the bark given after childbirth helped the delivery of the placenta (Orwa et al., 2009*).*

Research has also shown *Alstonia boonei* to be a very economical plant which serves as good shade for coffee, tea and banana plantations while it has a local potential for stools, carvings, domestic utensils, toys, masks, canoes, and the latex gives an inferior resinous coagulate, which has been used to adulterate better rubbers (Orwa et al.2009*). A. boonei* as an antimalarial drug is the focus of this research in mice.

Malaria continues to be the single most destructive and dangerous infectious disease in the developing countries (Winter et al., 2006). Due to the widespread resistance of P. falciparum, the search for plant with anti-malarial effect is relevant the aim of the study is to evaluate the effect of ethanolic extract of *Alstonia boonei* leaves against *plasmodium berghei*.

2. **Methodology *2.1.*Plant material and Extract**

Leaves of *Alstonia boonei* were obtained from the botanical garden of the University of Lagos, Akoka, Lagos, Nigeria. Identification and authentication (LUH 6309) was done at the Herbarium, Faculty of Science, and Department of Botany of the University of Lagos. The leaves were air-dried at room temperature under shade, and ground into powder using an electric mill.

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The crude extracts were prepared by cold maceration technique. The extraction was performed by soaking 100g of leave in 1000ml of 70% ethanol for 48 hours. Afterwards, the solvent was evaporated from the mixture in a water bath at 40 ^{o}C.

*2.2.*Phytochemicals analysis

Chemical tests were carried out on the ethanolic extract to determine the phytochemical constituents.

A. Test for saponins

The powdered sample (2g) was boiled in 20mls of distilled water in a waterbath and filtered. 10mls of the filtrate was mixed with 5ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

B. Test for tannins

The powdered sample (0.5g) was boiled in 20mls of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration.

C. Test for phlobatannins

Deposition of a red precipitate when an aqueous extract of each plant sample was boiled with 1% aqueous hydrochloric acid, taken as evidence for the presence of phlobatannins.

D. **Test for flavonoids**

A portion of the powdered plant sample was heated with ethyl acetate over a steam bath for 3 minutes. The mixture was filtered and 4ml of the filtrate was shaken with 1ml of dilute ammonia solution. A yellow coloration was observed indicating a positive test for flavonoids.

E. Test for steroids

Acetic anhydride (2mls) was added to 0.5g of ethanolic extract with 2mls sulphuric acid (H_2SO_4).

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The color changed from violet to blue or green in some samples indicating the presence of steroids.

F. Test for cardiac glycosides

The extract (5mls) was treated with 2mls of glacial acetic acid containing 1 drop of ferric chloride solution (0.1%). A brown ring of the interface indicates deoxysugar characteristics of cardenolides.

G. Test for anthraquinones

The powdered extract (0.2g) was boiled with about 5mls of dilute sulphuric acid for 5 minutes, centrifuged while hot and supernatant pipetted off. This was shaken with equal volume of carbon tetrachloride. The carbon tetrachloride was separated and shaken with half its volume of dilute ammonia solution. A rose pink to red colour shows presence of anthraquinone derivatives.

H. Test for alkaloids

Picric acid was added to 2mls of the extract. An orange coloration was taken as evidence for the presence of alkaloids.

I. Reducing sugar

The extract (0.1g) was shaken vigorously with 5ml of distilled water and filtered. To 1ml portion of the filtrate was added 2mls of Benedict's reagent. The mixture was shaken, heated on a water bath for 5 minutes. A rusty brown precipitate indicates the presence of reducing sugar.

J. Acute toxicity test

The crude extract was evaluated for toxicity in P. *berghei* non-infected female mice with mean weight of 16-28g. 15 mice were randomly divided into three groups of five animals per cage. Before oral administration of a single dose of the extract, the mice were fasted for two hours (WHO 2000; OECD 2001). Then, 0.2ml of the extract at dose of 300mg/kg, 2000mg/kg, and 5000mg/kg body weight was administered (CDER 1996).

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K. Preparation of parasites and the innoculum.

The NK65 strain of *Plasmodium berghei* which is sensitive to chloroquine *used* for this study was obtained from Dr Aina's Laboratory, Biochemistry Department, Nigerian Institute of Medical Research, Yaba, Lagos (NIMR). An infected donor mouse with the *Plasmodium berghei* strain of the rodent malaria Plasmodium spp was used for parasites inoculums preparation. Each mouse was passaged with 0.1ml of the infected blood containing about 1×10^6 *Plasmodium berghei* parasitized red blood cells intraperitoneally.

L. Suppressive treatment

A four-day suppressive test according to Peter *et al.* (1975) was adopted and used for this study. A total number of 25 mice with mean weight of (16-28g) were divided into groups of five (5) with each group having five (5) animals in a cage. Group A served as a negative control and was given distilled water while groups B, C, and D were administered the extracts at 200mg/kg, 400mg/kg and 800mg/kg respectively, and the last group E was given chloroquine, the chloroquine was used as a positive control. The animals were administered with the extract 2 hours after the parasite was inoculated on day 0 (D0) and everyday till D3 using an oral cannular. Plant extracts were administered orally. On the fourth day, a thin blood film was made from the tail of each mouse. The smear was prepared by spreading the blood on a clean slide over an area of 1.5 cm × 2.5 cm, allowed to dry and fixed with methanol, stained with 3mls of 3% Giemsa stain for 45 minutes and examined with microscope under the oil immersion objective to determine the parasite density microscopically (Olympus CX, Japan) at 100x magnification. The suppression of parasitaemia in relation to the control was assessed using the standard expression.

$$\text{Average (Av) \% suppression} = \frac{\text{Av\% parasitaemia in negative control} - \text{Av\% parasitaemia in test group}}{\text{Av\% parasitaemia in negative control}} \times 100$$

Data analysis: Results of the study were expressed as mean ± standard error of mean (M ± SEM).

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3. Results

Phytochemical qualitative analysis showed that tannin, phlobatannin, alkaloid, cardiac glycoside, steroid, reducing sugar, saponin and anthraqinione are present in the leave extract of *Alstonia boonei* while flavonoid was absent as displayed below.

Table 1 Phytochemical constituents of ethanolic extract of *Alstonia boonei le*aves

CONSTITUENTS	*Alstonia boonei*
Flavonoid	–
Tannin	+
Phlobatannin	+
Alkaloid	+
Cardiac glycosides	+
Steroid	+
Reducing sugar	+
Saponin	+
Anthraquinone	+

The phytochemical analysis was carried out according to Trease and Evans 2002 **Table 4.1:** Table showing the presence/absence of phytochemicals in *Alstonia boonei* leaves; **+** = presence of compound**;** **-** = absence of compound.

*3.1.*Acute toxicity

The acute toxicity study indicated that none of the three doses caused mortality of mice within 24 hrs. The behavioural and physical observation of the experimental mice revealed no sign of acute toxicity the skin fur, lethargy, salivation and eyes were all normal but slight weakness was observed at 5000mg/kg for some minutes**.**

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*3.2.*Suppressive test

The ethanolic extract of *Alstonia boonei* leaves showed the highest 43.1% suppression of parasitaemia at 400mg/kg body weight in mice (Table 2). Percentage suppression was observed to indicate the exact increase in concentration; after four days treatment with different doses, the mean parasitaemia count of the test groups ranged from 5.1 ± 3.30 to 3.4 ± 1.62 to 5.2 ± 3.45 while the corresponding values of the negative control group were 6.6 ± 2.35. The mice treated with CQ were completely free from the parasites on day four.

Table 2 Activity of ethanolic extract of *Alstonia boonei* leaves against P. berghei in mice

Extract	Dose (mg/kg/day)	Antimalarial activity	
		% Parasites Count	% Suppression
Alstonia boonei	*NC*	6.6 ± 2.35	0
	200	5.1 ± 3.30	23.1 %
	400	3.4 ± 1.62	43.1%
	800	5.2 ± 3.45	20.0%
	CQ	0	100%

Values are Mean ±SEM; n=5, NC: Negative control, CQ: chloroquine

4. Discussion

Ethanol extracts of *A. boonei* leaves possess intrinsic anti-plasmodial activity, evident from the suppression they produced during the 4- day suppressive test against Swiss albino mice. The use of *Alstonia boonei* in the treatment of malaria attack in traditional African settings can be partially satisfied with these results. The suppression study implies that the extracts can suppress parasite growth. From the results, *Alstonia boonei* has high quantities of alkaloids tannins, saponins and steroids which are the core anti-plasmodial agents (Watt *et al*., 1981; Switch and Jarilla 2004; Oigiangbe *et al*., 2010).

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Many alkaloids are known to have effect on the central nervous system. Previous reports indicates that alkaloids could be highly responsible for the antimalarial activity of plants (Oigiangbe *et al*., 2010). In this study, the acute toxicity test extracts were given to animal model at different doses, after 24hours, they showed no sign of toxicity or weakness or death at the various doses employed (300mg/kg, 2000mg/kg and 5000 mg/kg). This means that the extract is safe and the lethal dose (LD50) could be >5000mg/kg. The observed mild weakness at 500mg/kg of the treated mice showed that the extract possesses central depressant effects, consistent with previous studies (Gbadamosi *et al*., 2011).This method of 4-day suppressive testing for anti-malarial activity has become popular during scientific evaluation of potential phyto-medicines for treatment of experimental malaria (Peters *et al*., 1975; Bello *et al*., 2009). Average % parasite count of leaves extract gave 5.1±3.30, 3.4±1.62 and 5.2±3.45 respectively, and average % suppression of leaves extract at various doses (200, 400 and 800mg/kg) gave 23.1%, 43.1% and 20.0% suppression respectively (as shown in table 2). The standard drug (chloroquine 5mg/kg) average % parasite count for leaves groups gave 6.6±2.35 leaves extract of *Alstonia boonei* at 400mg/kg, producing the best parasitaemia reduction compared to the negative control.

5. Conclusion

The study established the antiplasmodial properties present in ethanolic extract of *A. boonei against* P. *berghei*. However, the crude extract should be further tested against P. *falciparum* and P. *vivax* in the development of antimalarial drugs for human malaria.

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