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The Antiplasmodial Activity of Methanol Root Bark Extract of Alstonia Boonei against *Plasmodium Berghei Berghei Infection* in Mice

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Abstract: ***This study was conducted to evaluate the antiplasmodial activity of methanol root bark extract of Alstonia boonei on chloroquine-sensitive Plasmodium berghei berghei infection in mice in order to justify its use as antimalarial remedy in Nigeria folk medicine. The plant extract was screened for blood schizontocidal activity against chloroquine-sensitive plasmodium berghei infection in the mice. The schizontocidal activity was monitored at stages of early and established infection. The prophylactic activity was also investigated. Results showed a dose dependent blood schizontocidal activity at all the stages of malarial infection studied. For instance when the extract dose increased from 400 to 800 mg/kg/day, chemosupressive activity of the extract increased from 62.2% to 79.5%. There was also an increase from 58.8% to 72.2% and from 66.4% to 82.9% for the repository and curative activities respectively. These schizontocidal activities observed were however, comparable to that observed for the standard drug (chloroquine phosphate). It was thus concluded that the herbal extract possesses significant antimalarial potency which could be exploited in the formulation of standard antimalarial drugs.***

Keywords: Antiplasmodial, *Alstonia boonei*, *Plasmodium berghei*, Mice.

1. Introduction

Malaria is a disease caused in humans by parasites of the plasmodium species through the bite of an infected female anopheles mosquito. About 3.3. Billion people, half of the world's populations are at risk of malaria. Every day, this leads to about 250 million malaria cases and nearly one million deaths (AMRF 2012, Soniran et al, 2012). World Health Organization recently listed Nigeria among high burden countries with limited evidence of decrease in malaria cases (WHO, 1977, Soniran et al , 2012). A lot of effort has been made by man to tackle the scourge of malaria. These have covered both the use of standard orthodox medicines and the less formal use of medicinal plants.

Alstonia boonei is a widespread genus of evergreen trees and shrubs from the dog-bane family (*Apocynaceae*). It is commonly known as cheesewood or stoolwood. It is a large deciduous tree that grows up to 45m tall and 1.2m in diameter. In Nigeria, it occurs in moist lowland forests. The plant has many practical and divers uses. The stem bark has been reported to exhibit diuretic, spasmolytic and hypotensive properties (Oliver, 1986). The plant has also demonstrated remarkable activity against conditions like fever, insomnia, chronic diarrhea and rheumatic pains (Olajide et al, 2000).*A boonei* is used as a decoction to cleanse suppurating sores and ulcers and also as a remedy for snake bite and arrow poison (Oliver, 1986). A decoction of the stem bark is drunk by the Ikeduru people of Imo State, South-East Nigeria as a strong remedy for malaria. In the present study, the blood schizontocidal activity of root bark extract of *Alstonia boonei* against *Plasmodium berghei berghei* infection in Swiss albino mice was evaluated as a justification for its application in the treatment of malaria in Nigeria folk medicine.

2. Materials and Methods

2.1 Plant

Fresh leaves and root bark of *Alstonia boonei* were collected from Umuri-Amaimo in Ikeduru area of Imo State. These were identified by a taxonomist in the department of Biotechnology, Federal University of Technology Owerri.

2.2 Preparation of Extract

The fresh root bark of *A. boonei* was sorted to get rid of dead matter and other unwanted materials. They were subsequently cleaned, cut into pieces and air dried under shed for two weeks. After drying the root barks were ground in a mortar to get a coarse powder. This was subsequently milled to a fine powder using mechanical blender. 500g of the powdered material was macerated in 1500ml of 95% methanol for 72 hours. The liquid extract was concentrated by rotary evaporation at temperature of 45-50^{0}C.

2.3 Animals

Swiss Albino mice of both sexes of average weight 29g were obtained from the animal house of department of Biochemistry University of Port Harcourt. The animals were kept in cages and allowed to acclimatize for a period of one week before the commencement of the study. They were allowed a liberal access to feed and water throughout the test period.

2.4 Inoculation of *P. berghei* Parasite

Albino mice previously infected with *Plasmodium berghei* having different levels of parasitaemia were used as

donors. The parasitaemia levels of donor mice were first determined by cutting the tails of the animals with a sterile pair of scissors, and the blood collected into 0.5ml normal saline. A drop of the diluted blood was placed on a microscope slide and observed under the light microscope using 40 x magnifications. The stained blood showed the presence of parasite. The donor mice were then sacrificed and blood collected by cardiac puncture. The parasitized blood was subsequently diluted with normal saline. 0.05ml donor blood was added to 9.95% normal physiological saline. 0.2ml of the diluted blood which contained about 1 x 10^7 *Plasmodium berghei* infected erythrocytes was administered intraperitoneally to each animal.

2.5 Drug/Extract Administration

Both the extract and drug used were administered orally using sterile orogastric tubes.

2.6 Evaluation of Schizontocidal Activity on Early Infection (Chemosupressive Test)

The chemosupressive test was done using Peter's 4-day suppressive test against *P. berghei* infection in mice (Peters, 1970; Knight and Peters, 1980). About one hour after parasite inoculation, the animals were divided into five groups of five mice each and were administered 400, 600 and 800 mg/kg/day doses of the plant extract, chloroquine phosphate 5mg/kg/day and equivalent volume of normal saline (control group) for four consecutive days. One the fifth day, thick blood films were made from the tail blood and fixed with methanol. The films were subsequently stained with Giemsma stain and the parasitaemia levels were determined by counting the number of parasitized erythrocytes out of 500 red blood cells in random fields (x100 objective) of the light microscope.

The % suppression of parasiteamia was then calculated in comparism with the negative control.

2.7 Evaluation of Prophylactic (Repository) Activity

The repository activity was assessed using the method described by Peter (1965). The mice were divided into five groups of five animals each, and administered 400, 600 and 800 mg/kg/day extract, 5mg/kg/day of chloroquine phosphate and 5ml/kg/day of normal saline (control group) for four consecutive days. On the fifth day, the mice were inoculated with the *P. berghei* parasite. 72 hours later, the levels of parasitaemia were assessed by thick blood smears.

2.8 Evaluation of Schizontocidal Activity in Established Infection (Curative Test)

A modified method similar to that of Riley and Peters (1970) was used. On the first day, standard inocular of 1 x 10^7 infected red blood cells were injected in the mice, intraperitoneally. 72hours later, the animals were distributed into five groups of six animals each and the parasitaemia levels were determined. Subsequently different doses of the extract (400, 600 and 800mg/kg/day), chloroquine phosphate (5mg/kg/day) and normal saline (5ml/kg/day) were given to the respective groups. These treatments were administered once daily for five consecutive days. For each animal, thick blood films stained with Giemsa stain was prepared daily for five consecutive days to monitor the levels of parasitaemia.

3. Results

The results of this work are summarized in tables 1-3. Basically they cover the findings relating to the chemosupressive test, Prophylactic test and curative test as follows:

Table 1: Chemosupressive effect of methanol extract of *A. boonei* against *P. berghei* infection in Mice

Treatment	*Dose*	*Chemosupressive activity (% suppression of parasitaemia)*
Extract	400mg/kg/day	62.2
Extract	600mg/kg/day	70.1
Extract	800mg/kg/day	79.5
Chloroquine	5mg/kg/day	87.1
Normal saline	5ml/kg/day	0.0

Table 2: Repository activity of methanol extract of *A. boonei* against *P. berghei* infection in mice

Treatment	*Dose*	*Prophylactic (Repository) activity (% suppression of parasitaemia)*
Extract	400mg/kg/day	58.8
Extract	600mg/kg/day	69.1
Extract	800mg/kg/day	72.2
Chloroquine	5mg/kg/day	85.6
Normal saline	5ml/kg/day	0.0

Table 3: Curative effect of methanol extract of *A. boonei* against *P. berghei* infection in mice

Treatment	*Dose*	*Curative activity (% suppression of parasitaemia)*
Extract	400mg/kg/day	66.4
Extract	600mg/kg/day	73.7
Extract	800mg/kg/day	82.9
Chloroquine	5mg/kg/day	91.7
Normal saline	5ml/kg/day	–

4. Discussion

The results obtained in this work have shown that the methanol extract of *Alstonia boonei* exhibits blood schizontocidal activity at all stages of malarial infection. The result of the chemosupressive activity of the extract is shown in table 1. The chemosupressive effect of the extract occurred in a dose dependent manner. The highest suppressive effect (87.1%) was observed with the standard drug chloroquine. The value was however similar to that observed when the extract dose was increased to 800mg/kg/day which gave 79.5% suppression. The repository effect of the extract also occurred in a dose dependent manner, for instance when the extract dose increased from 400mg/kg/day, to 800mg/kg/day (the highest dose used), repository activity increased from 58.8% to 72.2% which is also similar to the value (85.6%) obtained with the standard drug (chloroquine). The result

of the curative effect of the plant extract equally showed a concentration-dependent activity. The highest curative effect was observed for the plant extract when the highest dose (800mg/kg/day) was administered. This gave 82.9% curative effect compared with 91.7% obtained for chloroquine phosphate. The better performance observed for chloroquine compared with the extract in this study may be as a result of the inability of the parasite to develop as much resistance to the standard drug as it did to the herbal extract. The mechanism behind the antiplasmodial activity displayed by the plant extract is yet to be demonstrated. However, some plants have been shown to elicit antiplasmodial effects either by inducing an elevation of erythrocyte oxidation or by inhibiting the synthesis of proteins (Riley and Peters, 1970; Kirby et al, 1989). *Alstonia boonei* may owe its antiplasmodial potency to any of the above mentioned mechanisms or any other one yet to be demonstrated.

5. Conclusion

From the results of this study, it could be concluded that the methanol extract of the root bark of *A. boonei* has antiplasmodial/antimalarial activity as it has been shown to suppress the development of *Plasmodium berghei* parasite infection in mice at the different stage of malaria infection. This observation therefore justifies the use of the plant extract in the treatment of malaria in Nigerian folk medicine.

6. Recommendation

It is hereby recommended that a combination therapy be tried by combining the extract from *A. boonei* and that of any other established antimalarial herb to see if an antimalarial preparation of higher potency could be arrived at. Furthermore, the active principle in the plant should be isolated, identified and characterized. This could serve as a component in the formulation of a standard antimalarial drug.

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