International Journal of Bioscience and Biochemistry
www.biosciencejournal.net
Online ISSN: 2664-6544; Print ISSN: 2664-6536; Impact Factor: RJIF 5.46
Received: 05-11-2019; Accepted: 07-12-2019; Published: 19-06-2020
Volume 2; Issue 1; 2020; Page No. 16-20

Antimalarial activities of alkaloid-rich fraction of stem bark extract of *Lannea acida* in mice infected with *Plasmodium berghei*

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Abstract
Malaria is a mosquito-borne disease that has plagued mankind for ages and continues to be most common and deadly parasitic disease in the world. The search for novel antimalarial compounds has been required by *Plasmodium falciparum* resistance to standard antimalarial drugs. Plants derivative are significant sources of biologically active metabolites which have potentials for new antimalarial drugs' discovery. Meanwhile a number of alkaloids have been successfully used for the treatment of malaria. In this study, alkaloid-rich fraction of stem bark extract of *Lannea acida* was evaluated for possible antiplasmodial activity, against *Plasmodium berghei*, a chloroquine-sensitive protozoan. Suppressive activity of the fraction was examined for five consecutive days with the extract doses and chloroquine as the control drug, curative experimental groups were infected for five days prior to extract treatment, while the prophylactic groups were pretreated daily for five days before inoculation with 1×10^7 chloroquine-sensitive *Plasmodium berghei* intraperitoneally. The control group was administered with 10 ml distilled water/kg; 200 and 400 mg extract/kg weight were administered to the experimental groups, and chloroquine 5 mg/kg body weight respectively. All doses of the fraction produced significant, dose-dependent, chemo suppressive activity against the parasite in the suppressive, curative and prophylactic tests compared with chloroquine treated mice. The extract treatment also casused an elongation in the mean survival time of treated mice compared to the untreated mice.

Keywords: *Lannea acida, Plasmodium berghei*, antimalarial

1. Introduction

Malaria remains one of the most deadly parasitic diseases in human population regions of the world, both in the tropics and subtropics, especially the African and Asian developing or under developed nations. In Africa, about 5 million become severely ill with malaria most of them are children under five years and pregnant women (WHO, 2008). Mortality, currently estimated at about 405,000 people per year (WHO, 2019). This is attributed to the resistance of the *Plasmodium* parasite to the synthetic antimalarial drugs currently in use. In addition to the consequences of malaria parasite on the human health impact, the disease has a measurable direct and indirect cost on the socio-economic development of the population (RBM, 2010) [14]. Artemisinin Combination Therapy (ACT) has been used as first-line of treatment for uncomplicated malaria throughout Nigeria and Africa at large. ACTs has been shown to exhibit high efficacy against *Plasmodium* parasites and several have been shown to be moderately effective against the early stage of infection and reduce transmission to mosquitoes. Artemether-lumefantrine (AL) is the most widely used ACT in Nigeria and the African continent. The dihydroartemisinin-piperaquine (DP) has also shown high efficiency in treatment of malaria. Its advantages include those of simpler dosage and prolonged prophylactic period (Bassat *et al*., 2009).

The reduction in parasite transmission is now a strategic component of global efforts to control and eliminate malaria disease (Alonso *et al*., 2011). The occurrence of drug resistance to malarial parasites in prevalent areas has posed a great threat to the use of cheapest antimalarial drugs; chloroquine (CQ) and sulphadoxine-pyrimethamine (SP). Currently, most malaria endemic areas in Africa including Nigeria have changed their first line antimalarial treatment from CQ or SP to artesunate/amodiaquine combination or artemether/lumefantrine combination. The ACTs used in most malaria prevalent countries have demonstrated high efficiency, protection against the development of resistance to each component and reduction of malaria transmission (Bloland *et al*., 2000; Sutherland *et al*., 2005). However, malaria is often referred to as the disease of low-economy and poverty-stricken people. Consequently, the relatively high costs, dosage complication and the limited knowledge of their uses in sub-Saharan Africa had affected the adequate deployment of these drug combinations (Bloland *et al*., 2003).

Alkaloids are one of the most important classes of natural products providing drugs since ancient times (Benoit-Vical, 2005). Numerous types of alkaloids had been effectively used for the treatment of parasitic infections. Quinoline-based antimalarials which include alkaloids consist of quinine from Cinchona and its derivatives are the most commonly used drugs against malaria (Kayse *et al, 2003*). A number of alkaloids have been successfully used for the treatment of malaria since ancient times.

Lannea acida belongs to the family *Anacardiaceae* commonly called, awerekagun in Akoko area of Ondo state, akogun in Ondo town. It is widely used as herbal medicine in West Africa. *Lannea acida* is one of the most widely distributed species of the *Lannea* family found in the hot and dry savannahs of sub-Saharan Africa. It has a rich history of ethnobotanical and ethno pharmacological usage in the treatment of a wide range of illnesses including

malaria, rheumatism, dysentery and hemorrhoids. Extract of *L. acida bark* is traditionally used in Nigeria as anti-abortifacient, vermifuge and to treat anal hemorrhoids, diarrhoea, dysentery, malnutrition, and debility while the leaf is used to treat rheumatism. Information provided by the traditional healer in Akoko area of Ondo state revealed that the bark aqueous or alcoholic extract is used in treatment of many infectious diseases. Even though *Lannea acida* demonstrated biological activity that validated its medicinal roles, no phyto-chemical study was performed to characterize the chemical constituents responsible for the observed activity. Consequently, the present study was carried out to evaluate the antiplasmodial potentials of alkaloid-rich fraction from *Lannea acida* stem bark in order to provide scientific evidence for its continuous usage in ethno therapeutic management of infectious diseases.

2. Materials and methods

Plant Material

The stem bark of *Lannea acida* was collected from Ugbe town from a location ($7^0$15′42.9″N $5^0$15′01.9″E) of Ondo state and was authenticated at the Department of Plant Science and Biotechnolgy, Adekunle Ajasin University, and a sample specimen deposited at the herbarium for future reference.

2.1 Extract preparation

The fraction was prepared by weighed 20 g of the methanolic extract into a beaker containing 300 ml of warm distilled water (37ºC). The crude extract was allowed to dissolve before transferring it into a separatory funnel. Few drops of conc. H_2SO_4 were then added to make the solution acidic (the solution was tested with litmus paper). After this, it was decanted, and the residue was dissolved in water to test for acidity. The solution was decanted again and this step was repeated several times to concentrate the alkaloids extracted. Ammonia was then added until alkalinity level was achieved. The solution was tested with litmus paper, and then addition of chloroform until the complete extraction of alkaloids was obtained. After each extraction with chloroform, the test for alkaloids was carried out, in line with the method of Manuwa *et al*. (2015) [10].

2.2 Animals

Both sexes of wister mice (17-20 g), bred at the animal house of the Institute of Advanced Medical Research and Training (IAMRAT), College of Medicine, University of Ibadan, Ibadan, Oyo state, Nigeria were used for the study after receiving approval from animal ethics committee of Adekunle Ajasin University Akungba Akoko, Ondo State, Nigeria on the use of laboratory animals.

2.3 Rodent parasite

Chloroquine-sensitive rodent *Plasmodium berghei* NK 65 was obtained from Institute of Advanced Medical Research and Training (IAMRAT), College of Medicine, University of Ibadan, Nigeria, Oyo State, Nigeria. A standard inoculum of 1×10^7 of parasitized erythrocytes from a donor mouse in volumes of 0.2 mL was used to infect the experimental animals.

2.4 Test on early malaria infection (4-day suppressive Test)

The Peter's 4-day suppressive test against chloroquine-sensitive *Plasmodium berghei* (NK 65) infection in mice was employed (Peters, 1965) [14]. Twelve mice of both sexes were inoculated as described above. They were randomly selected into four groups of three mice each. Treatment started immediately after the rats were infected with the parasite, and extract administered daily for four (4) consecutive days. Group 1 that served as control was given 10 ml/kg body weight of distilled water. Groups 2, 3 and 4 mice were orally administered with 200, 400 mg extract/kg body weight and 5 mg chloroquine /kg body weight respectively daily. On the fifth day, blood was collected from the tail of each rat and spread on a microscope slide to make a thin film. The blood films were stained with Giemsa and examined microscopically according to the method of Cheesbrough (2004). The parasite count was recorded and the suppression of parasitemia was expressed as percentage for each dose, by comparing the parasitemia in the control group with the treated ones.

Average suppression = APC – APT/APC X 100.

APC = Average parasitemia in the control.

APT = Average parasitemia in the test group.

2.5 Test on established infection (Rane test)

Evaluation of the curative potential of alkaloid-rich extract of stem bark extract of *Lannea acida* against established infection was carried out as described by Ryley and Peters (1970) [15]. Twelve mice were all inoculated as described above, and left untreated until the fourth day (D_4) post inoculation. The mice were weighed and randomly picked into four groups of three mice each. Group 1 mice were given 10 ml/kg of distilled water while Groups 2,3 and 4 received alkaloid-rich extract doses of 200, 400 mg extract/kg body weight and 5 mg chloroquine /kg body weight daily orally respectively, for four days (D_4-D_7). On Day-7 each rat was tail-bleeded and a thin blood film was made on a microscope slide. The films were stained with Giemsa dye and examined microscopically to monitor the parasitemia level. The mean survival time of the rats in each treatment group was monitored for 30 days.

2.6 Repository test (prophylactic activity)

The prophylactic activity of the extract was tested using the residual infection procedure described by Peters (1965) [14]. Twelve mice of both sexes were weighed and randomly distributed into four groups of three mice each. Group 1 mice were administered with 10 ml/kg body weight of distilled water. Groups 2 and 3 mice were administered with 200 and 400 mg extract /kg body weight orally respectively, while group 4 animals were treated with 5 mg chloroquine/kg body weight orally daily. Treatment continued daily for four days (D_1-D_4) and mice were all infected with the parasite on the fifth day (D_5). Thin blood films were prepared from each mice 72 hours (D_7) post-treatment and mean parasitemia in each group was determined microscopically. The mice were re-weighed on the seventh day and the differences between the pre- and post-treatment body weight were recorded.

2.7 Statistical analysis

Graph pad prism version 7 was used to analyze the 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 test. $p \leq 0.05$ level was considered significant.

3. Results

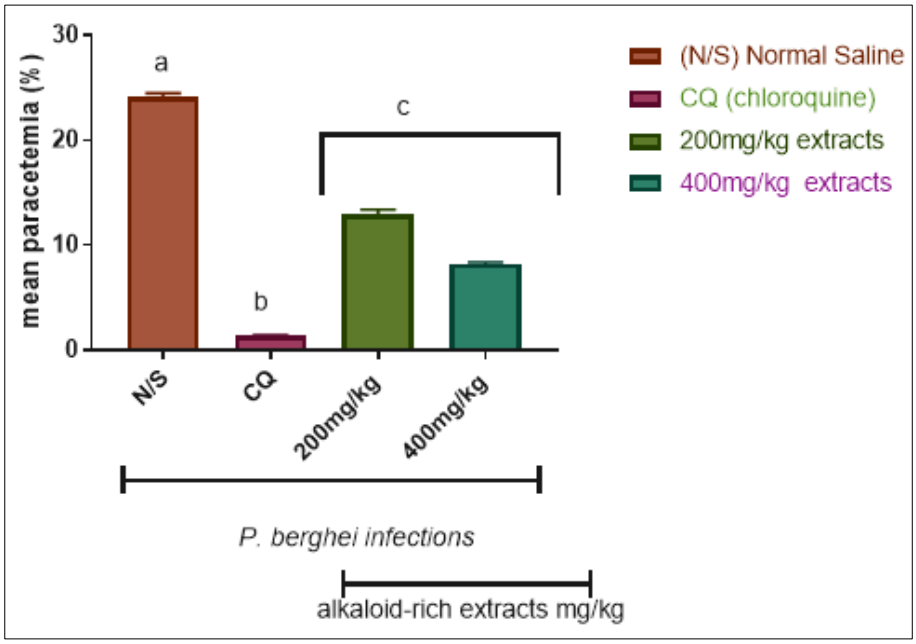

Fig 1: Effect of alkaloid rich extract on suppressive activity of *Lannea acida* plant in *P. berghei* infected mice. a-c significantly different compared to the control at $p < 0.05$.

Table 1: Effect of alkaloid-rich extract on curative activity of *Lannea acida* plant in *P. berghei* infected mice.

Mean parasitaemia count				Body Weight (g)			
Treatment	**Dose (mg/kg**	**Pre-(D4) Treatment**	**Post-(D7)-Treatment**	**D7-% Inhibition**	**Survival Time (Days)**	**D1**	**D7**
Untreated	N/S	17.33 ± 0.11	36 ± 0.5^{d}	0	6.8 ± 0.60	13.6 ± 0.10	12.3 ± 0.04
Extract	200	20.33 ± 0.46	16.6 ± 0.2^{c}	50.14	20 ± 2.22	18.4 ± 0.09	17.4 ± 0.04
	400	13.66 ± 0.11	11.0 ± 0.4^{b}	69.037	25.2 ± 1.8	16.4 ± 0.07	15.9 ± 0.04
Chloroquine	5	14.66 ± 0.11	1.66 ± 0.1^{a}	95.370	29.8 ± 0.8	14.5 ± 0.07	13.4 ± 0.07

D_5 = Day five, **significantly different compared to the control at $p < 0.05$.

Dose-dependent percentage inhibition of parasitaemia in mice placed on curative treatment with Alkaloid-rich extract and chloroquine ($p < 0.05$). Each point is an average count from five infected mice (±SEM).

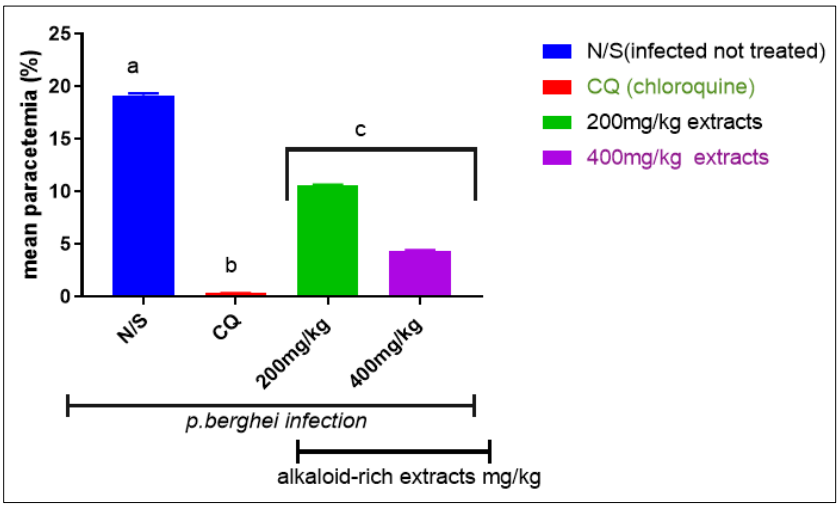

Fig 2: Effect of alkaloid-rich extract on prophylactic effects of *Lannea acida* bark extract in *P. berghei* infected mice. a-c showed significant difference from the control at $p < 0.05$.

4. Discussion

The current evaluation from the study demonstrated that the alkaloid-rich extract from the stem bark of *L. acida* had a potent suppressive effect against early *Plasmodium* infection, curative impact against conventional infection and prophylactic impact against residual infection in *P. berghei infected* mice. Although rodent models do not produce the same signs and symptoms as observed in the human *Plasmodium* infection, they have been reported (Pedroni *et al*., 2006) [12]. to produce disease features similar to those of human *Plasmodium* infection, when infected with *P. berghei* (Thomas *et al*., 1998) [17]. Moreover, several studies (Agbedahunsi, 2000 [1]; Adzu and Salawu 2009) have employed *P. berghei* in predicting treatment assumptions of suspected antimalarial agents, because of its high sensitivity to chloroquine, which has also been confirmed with this present study. Substances that reduce parasite multiplication (antiplasmodial effect) in the host were considered to possess antimalarial activity (Ryley and Peters, 1970) [15]. The 4-day suppressive test is a standardized test commonly used for antimalarial screening (Peters, 1965) [15]. The extracts produced significant ($p \leq 0.05$) dose-dependent chemo-suppression in all the treated groups with the highest chemo-suppression (38.66 and 73.33 %) observed in the group treated with (200 and 400) mg extract/kg respectively. The higher survival time in the 400 mg extract/kg dose suggests that the dose may be the optimal therapeutic dose in mice. The extracts also demonstrated a significant dose-related reduction in parasite count in both established (curative) and residual (repository) infection, comparable to the effect of chloroquine, which in this study was used as standard control drug (Table 1 and Figure 2). The reduction of parasite counts in the curative test is similar to the values recorded for the reduction in parasite count in the suppressive test. The significant reduction in body weight in the group administered with the extract as well as the control group may be due to the combined effects of plasmodial infection (Thomas *et al*., 1998) [17], and possible catabolic effects of the high dose of the extract on lipid components. These observations showed that the extract is active against the malaria parasite used in this study and is consistent with the ethnomedicinal use of *L. acida* as antimalarial agent in some parts of Nigeria (Etkin, 1997 [7]; Maroyi, 2018) [11]. The mechanism of antiplasmodial action of this extract has not been elucidated. However, antiplasmodial effects of natural plant products have been credited to some of their active secondary metabolites (Sofowora, 1980 [16]; Ayoola *et al*., 2008 [2]; Maroyi, 2018) [11]. Some of these secondary metabolites such as alkaloids and flavonoids (detected in *L acida*) were reported to have antiplasmodial activity (Christensen and Kharazmi, 2001 [6]; Go, 2003 [8], Maroyi, 2018) [11].

5. Conclusion

The results of this study show that the alkaloid-rich fraction of stem bark of *L. acida* A. Rich possesses potent antimalarial activity in mice. These results have established the scientific basis for the traditional use of the *L. acida* in the treatment and management of malaria.

6. Conflict of Interests

The authors have not declared any conflict of interests.

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