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Research Article

Evaluation of antiplasmodial effects of the ethanolic leaf extract of *Salacia lehmbachii* on *Plasmodium berghei* infected mice

A. D. Essien[1], G. A. Essiet[1], G. C. Akuodor[2], N. N. Nwobodo[2], J. L. Akpan[2], S. J. Utsalo[3]

[1] Department of Pharmacology, College of Medical Sciences, University of Calabar, Nigeria

[2] Department of Pharmacology and Therapeutics, Faculty of Medicine, Ebonyi State University, Abakaliki, Nigeria

[3] Department of Medical Laboratory Sciences, Faculty of Allied Medical Sciences, University of Calabar, Nigeria

* Corresponding author: A. D. Essien; Tel. +2348032691994; E-mail: augprogclinic@yahoo.com

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ABSTRACT

Salacia lehmbachii leaves are used in Nigerian traditional medicine for the treatment of malaria and other diseases. The ethanolic extract was tested for its activities against suppressive, prophylactic and established infections in *Plasmodium berghei* infected albino mice at dose levels of 100, 200 and 400 mg/kg; while chloroquine (10 mg/kg) was used as positive control. The extract exhibited significant dose-related antiplasmodial activities on parasites with the used-dose levels, showing significant mean survival time. The results, therefore, co-relate with claims by traditional users for the treatment of malaria and other feverish conditions; and could serve as source of potential new antimalarial agents.

Keywords: Malaria; *Salacia lehmbachii*; Mice; Suppressive; Prophylactic; Curative.

1. INTRODUCTION

Malaria is mosquito-borne plasmodial infection. It is a global killing parasitic disease, causing approximately up to 2 -3 million deaths annually, and still causing major setback to health in Sub-Sahara Africa and other endemic areas [1, 2]. The annual occurrence of 400-500 million clinically newly-manifested cases portrays the severity of the disease, making it a global burden [3, 4]. It is of necessity to circumvent this global burden by widening research into new potential potent compounds with antimalarial activity, that are not based on existing synthetic antimalarial agents [5]. Plants are widely accepted to contribute major parts of medications globally used by traditional healers. Some plants are notably known to be used against malaria [6], thus leading to increased scientific authentication of medicinal plants used in Nigeria as claimed by traditional users.

Salacia lehmbachii which belongs to Celastraceae family and genus of *Salacia* is one of such plants. It is a shrub-like to small tree of about three meters high, richly found in the tropical rain forest of Central, west and East Africa [7]. The leaves are seasonally evergreen, firm and difficult to slice. There are diverse therapeutic applications of

S. lehmbachii justifying its folkloric background; the leaf extract as an antipyretic [8] anti-diarrheal, antimotility and anti-ulcer properties [9], while the root extract exhibits analgesic/anti-inflammatory effect, anticholinergic property and anti-infertility in male [10-12]. The wet pad from the root is used in hemorrhoids [13].

2. MATERIALS AND METHODS

2.1. Collection and preparation of plant materials

The fresh leaves of *S. lehmbachii* Loes were collected in November, 2014 from fully grown plant in a local farm forest in Ukanafun, Akwa Ibom State, Nigeria. The plant materials were identified and authenticated by a taxonomist, Department of Botany, University of Calabar, where a voucher specimen (No. 688) is maintained. Internationally, the plant is indexed as "*Salacia lehmbachii* Loes. Bot. Jahrb.Syst.XLIV.(2-3) 173 (22/03/1910)". The leaves were cleaned, cut into smaller pieces, air-dried at room temperature (28-30^{o}C) for 15 days and pulverized to dry powder with the help of mortar and pestle.

2.2. Extraction of plant (leaf) material

Five hundred grams (500 g) of the dried-leaf powder was extracted in ethanol (BDH chemicals Ltd, England) using a Soxhlet extractor (Friedrich Polzine, England) and the filtrate were dried on a water bath at a controllable temperature. The yield was 12.5% w/w. The leave extract was subsequently reconstituted in normal saline for routine use during the study.

2.3. Phytochemical analysis

The phytochemical screening of ethanolic leaf extract of *S. lehmbachii* was carried out for various secondary metabolites such as tannins (ferric chloride test), alkaloids (Mayer's and Draggendorff reagents), saponins (Froth test), steroids (Liebermann-Burchard test), terpenoids (Salkowski test), flavonoids (ammonia and sulphuric acid test) and anthraquinones (Borntrager's test) [14, 15].

2.4. Animals

The albino mice (18-22 g) of both sexes were obtained from animal house, Department of Pharmacology, College of Medical Sciences, University of Calabar, Nigeria. The animals were housed in cages under standard laboratory conditions, with naturally illuminated environment of 12 hrs dark and 12 hrs light cycles. They were fed on standard pellet diet and had free access to water. Care according to recommendation by Helsinki Declaration was implemented. Approval for study was obtained from the Research and Ethical Committee of Faculty of Basic Medical Sciences of University of Calabar.

2.5. Acute toxicity study of the extract

The LD_{50} of the ethanolic extract of the leaf to authenticate the safety of the extract was determined as described by Lorke [16] method. All the doses were administered orally. During the two phases, the mice were observed for signs of toxicity for 48 hours. There was no overt evidence of toxicity at the dose above 5000 mg/kg.

2.6. Malaria parasites (donor)

The chloroquine sensitive *P. berghei* (NK65) was sourced from National Institute for Medical Research, Lagos Nigeria. Parasites are maintained in the Animal House of the Department of Pharmacology, College of Medical Science, University of Calabar by continuous re-inoculation of mice and affirmation of concentration of the parasites.

2.7. Inocula

Parasitized erythrocytes for this study were obtained from a donor infected mouse by cardiac puncture, and prepared according to methods of Akuodor et al. [17] and David-Oku et al. [18]. To each mouse was administered intraperitoneally with infected blood suspension (0.2 ml) containing $1x10^{7}$ *P. berghei* parasitized red blood cells.

2.8. Suppressive test

This study was carried out according to methods described by Akuodor et al. [19]. Thirty albino mice of both sexes were selected and passaged. After three hours, the infected mice were randomly divided into 5 groups, each cage containing 6 mice. Animals in each group were treated orally for four consecutive days (D_0-D_3) with 100, 200, and 400 mg/kg of the ethanolic leaf extract, chloroquine diphosphate (10 mg/kg) and Normal saline (20 ml/kg) for positive and negative controls respectively. On day five (D_4), the films were prepared from tail blood of each mouse, and parasite concentration examined microscopically, counting the parasitized red blood cells on 1000 red blood cells in 10 different fields.

2.9. Prophylactic study

This study was carried out according to the methods described by Peters et al. [20]. Thirty albino mice of both sexes selected for this study were grouped into 5 of 6 mice per cage. Groups 2-4 were treated orally with graded doses of 100, 200, and 400 mg/kg of ethanolic extract of the leaf for four days (D_0-D_3); whilst group 1 and 5 received 20 ml/kg of normal saline and 10 mg/kg of chloroquine diphosphate respectively. On the last day of treatment, mice in all groups were injected intraperitioneally with constituted *P. berghei* erythrocyte suspension. After 72 hours, films were prepared (as previously described) and examined microscopically.

2.10. Curative test

On the first day, thirty Swiss albino mice were inoculated with *P. berghei* infected erythrocytes. After 72 hrs, the mice were randomly grouped into 5 groups of 6 mice, and treated daily (groups 2-4) for four days with the extract (100, 200, and 400 mg/kg); while the animals in group 1 and 5, were given 20 ml/kg of normal saline and chloroquine diphosphate (10 mg/kg). Thereafter, films were made and viewed to determine the parasite density. Mortality was monitored daily for mean survival time (MST), and the number of days from the time of inoculation of the parasite up to death was recorded for each mouse in the extract treated and control groups throughout the follow up period (D_0-D_{29}) [21].

2.11. Statistical analysis

The obtained results were expressed as mean ± SEM. Data were analyzed using One-way ANOVA and differences between the means were considered significant at $P< 0.05$.

3. RESULTS

3.1. Phytochemical test

Phytochemical results of the screened ethanolic extract of the leaf of *S. lehmbachii* revealed the presence of alkaloids, saponins tannins, terpenoides, flavonoids, phenols, steroids and anthraquinones while resin is absent (Table 1).

Table 1. Phytochemical constituents of the ethanolic leaf extract of *Salacia lehmbachii.*

Alkaloids	++
Saponins	++
Tannins	+
Terpenoids	++
Flavonoids	+
Phenols	+
Steroids	+
Anthraquinones	+
Balsam	-

Key: (+) = presence, (−) = absence

3.2. Acute toxicity study of the extract

The acute toxicity test of the ethanolic leaf extract was negative. The LD_{50} was greater than 5000 mg/kg orally, in tested mice.

3.3. Suppressive effect

The ethanolic extract of the leaf showed a dose-related effect at different graded doses used. Doses of 200 and 400 mg/kg significantly ($P < 0.05$) produced 66.5% and 80.1% inhibition

of parasitaemia respectively, compared to 89.8% exhibited by 10 mg/kg of chloroquine (Table 2).

Table 2. Suppressive effect of ethanolic extract of the leaf of *S. lehmbachii* against *P. berghei* in mice.

Drug	Dose (mg/kg)	Mean parasitemia density	% suppression
Control	20 ml/kg	41.20±1.17	-
S. lehmbachii	100	25.00±1.12	39
	200	13.80±0.67*	67
	400	8.20±0.53*	81
Chloroquine	10	4.20±0.53*	90

Values represent the mean ± SEM (n=6), *significantly different from control at P< 0.05.

Table 3. Prophylactic effect of ethanolic extract of the leaf of *S. lehmbachii* against *P. berghei* in mice.

Drug	Dose (mg/kg)	Mean parasitemia density	% suppression
Control	20 ml/kg	40.40±1.18	-
S. lehmbachii	100	26.00±1.18	36
	200	11.40±0.47*	72
	400	5.80±0.53*	86
Chloroquine	10	4.00±0.50*	90

Values represent the mean ± SEM (n=6), *significantly different from control at P< 0.05

3.4. Prophylactic effect

The leaf extract exhibited a dose-related effect at different doses used. Doses of 200 and 400 mg/kg significantly (P< 0.05) prevented the replication of the invaded parasites by producing 71.1% and 82.1% inhibition of parasitemia respectively, compared 88.6% exhibited by 10 mg/kg of chloroquine (Table 3).

3.5. Curative effect

The extract exhibited a significant dose-dependent reduction in parasitemia density. The doses of 200 and 400 mg/kg produced significant (P< 0.05) effect comparable to the effect exhibited in chloroquine treated group; whilst in the negative group, there was a consistent increase in the blood parasites. The survival rate among the mice also reflected dose-dependent response and showed that the extract significantly (P< 0.05) destroyed the invaded parasites at (71.3 and 82.3 % for 200 and 400 mg/kg respectively) of the established infection (Table 4). In the negative control group, from the 9^{th} day mice started dying, and by the 12^{th} day, no mouse survived, whereas, in the positive control group (chloroquine treated), there was no death observed, Table 4. It is worthy to note that some mice which received 200 and 400 mg/kg survived the 30-day observation period. Photomicrographs of thin blood smears are on the Fig. 1.

4. DISCUSSION

In this study, suppressive, prophylactic and curative antiplasmodial activities of *S. lehmbachii* were investigated in albino mice infected by *P. berghei*, which produces disease similar to those of human plasmodium infections, for the prediction of treatment outcomes [22].

Table 4. Data on curative effect of ethanolic extract of the leaf of *S. lehmbachii* against *P. berghe*i in mice.

Drug	Dose (mg/kg)	Mean parasitemia density		% suppression
		Pre-treatment	Post-treatment	
Control	20 ml/kg	38.60±0.62	41.40±1.18	-
S. lehmbachii	100	40.20±1.86	25.53±0.37	37
	200	40.60±1.84	11.65±0.62*	71
	400	39.40±0.94	6.97±0.88*	82
Chloroquine	10	39.00±1.04	2.26±0.34*	94

Values represent the mean ± SEM (n=6), *significantly different from control at P< 0.05.

These parameters are accepted scientific methods for evaluating and identifying new potential antiplasmodial agents [5, 18]. This usually determines the level of destruction of parasites in blood, hence a mean parasitaemia levels of about ninety percent mock-treated control animals indicates that the test agent is potent in standard screening studies [23]. Therefore, the result could be used to show that *S. lehmbachii* leaf extract is capable of destroying or suppressing plasmodial growth to near non-detectable levels in the infected erythrocytes.

Table 5. Mean survival time (days) of ethanolic extract of the leaf of *S. lehmbachii* against *P. berghei* in mice during curative study.

Drug	Dose (mg/kg)	Mean survival time (days)
Control	20 ml/kg	11.00±0.96
S. lehmbachii	100	20.80±1.65
	200	27.60±0.98*
	400	29.80±0.18*
Chloroquine	10	30.00±0.00*

Values represent the mean ± SEM (n=6), *significantly different from control at P< 0.05.

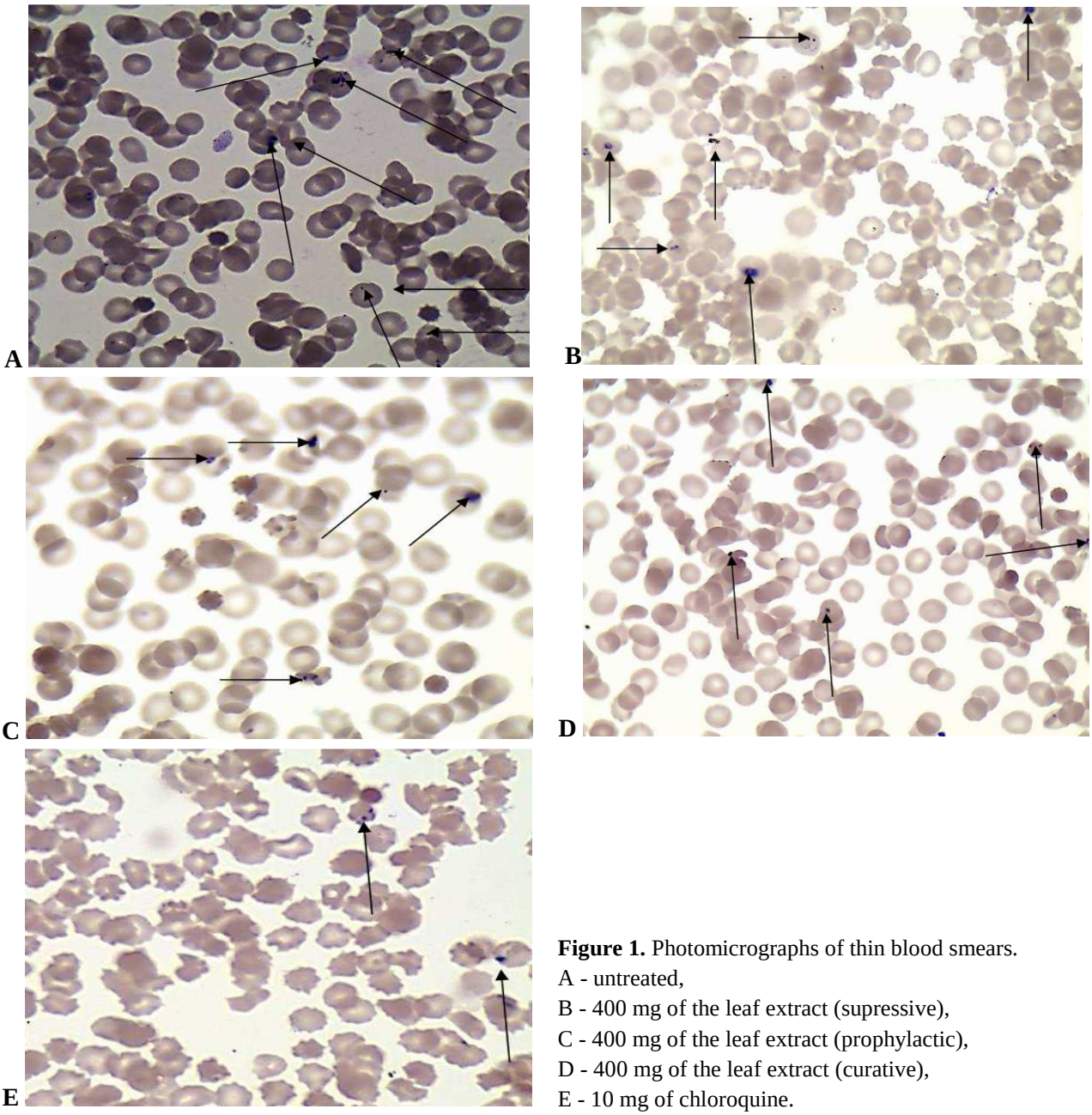

Figure 1. Photomicrographs of thin blood smears.
A - untreated,
B - 400 mg of the leaf extract (supressive),
C - 400 mg of the leaf extract (prophylactic),
D - 400 mg of the leaf extract (curative),
E - 10 mg of chloroquine.

The leaf extract, apart from exhibiting suppressive and prophylactic effects, also exerted significant curative activity during established infections. This could be seen in high percentage inhibition of parasites in blood as well as mean survival time especially in 200 and 400 mg/kg extract treated groups. It was recorded that some mice in these groups survived the 30 days of observation. However, the traditional use of *S. lehmbachii* by herbalists could be attributed to the presence of certain phytochemicals identified in the leaf extract. Most medicinal plants possess a wide variety of important phytoconstituents such as: anthraquinones, flavonoids, alkaloids and terpenoids as their bioactive compounds. The activities of these compounds have been proved against plasmodial infections [24-26]. Thus, the exhibited antimalarial activities of *S. lehmbachii* leaf extract might be due to the presence of these compounds. *S. lehmbachii* leaf also possess phenols known for their anti-oxidant and other diverse physiological properties: anti-carcinogenic, anti-inflammatory and anti-parasitic activities [27].

A standard antimalarial drug suppresses parasitemia significantly [28] which is in agreement with the effect of chloroquine in this study. Chloroquine is both clinically used for suppressive, prophylactic and curative, treatment of malaria, except for resistant strains of *Plasmodium falciparum* [29]. It destroys plasmodia by preventing the digestion of hemoglobin, and blocking the parasites source of amino acids, or by inhibiting hem polymerase to prevent the production of hemozoin, a protective medium against autolysis.

5. CONCLUSION

The results obtained from the present work have scientifically justified the reasons for the folkloric use of this local plant in the treatment of malaria attack in Nigerian traditional herbal practice. *S. lehmbachii* leaf extract has also proved to be a potential source of lead molecule(s) for the development of a new antimalarial agent. Further studies are however recommended to isolate and characterize the active ingredients responsible for the observed antimalarial activities.

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AUTHORS' CONTRIBUTION

EAD and AGC designed and carried out the experiments, EGA wrote the first draft of the manuscript, NNN did extensive literature review, and AJL performed the statistical analysis, while USJ supervised the study. All authors read and approved the final manuscript.

TRANSPARENCY DECLARATION

The author declares no conflicts of interest.

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