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Evaluation of *in vivo* Antiplasmodial Activity of Ethanolic Leaf Extract of *Lasianthera Africana*

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Abstract: The *in vivo* antiplasmodial activity of the ethanol leaf extract of *Lasianthera africana* grown particularly for the leaf in Niger Delta region of Nigeria was evaluated in *Plasmodium berghei* infected mice. *Lasianthera africana* (1000-3000 mg.kg.day^{-1}) exhibited significant ($p<0.05$) blood schizonticidal activity both in 4- day early infection test and in established infection with a considerable mean survival time though not comparable to that of the standard drug,chloroquine, 5 mg.kg.day^{-1}. The leaf extract possesses significant ($p<0.05$) antiplasmodial activity, which can be exploited in malarial chemotherapy.

Key words: Antiplasmodial activity, ethanol leaf extract, *Lasianthera africana*

INTRODUCTION

From time immemorial plants have served as food and medicine to man. Vegetables and leaves of some shrubs, domesticated or wild, are use by the Ibibios of Niger Delta region of southren Nigeria in the preparation of their soup daily. Some of these edible plants are equally medicinal and are use in the therapy of some diseases, majority of which have been reported to contain vital chemical compounds of medicinal importance.

Lasianthera africana (P. Beav.) is a perennial glabrous shrub of the family Icacinacea whose height may reach from 61 to 136 cm and is widely distributd in the tropical rain forest (Hutchinson and Dalziel, 1973). There are four ethnovarieties distinguish by their taste, leaf colour and ecological distribution. The leaves are consumed as vegetable in southren Nigeria. Ethnobotanically, *L. africana* is use as antacid, analgesic, antispasmodic, laxative, antipyretic, antiulcerogenic, antidiabetic and antimalarial. *L. africana* has been reported to be bacteriostatic (Itah, 1997) fungicidal (Itah, 1996) and antidiabetic (Ekanem, 2006). The aim of the present study, was to evaluate the nutraceutical potential of the dark green variety against *Plasmodium berghei* infection in mice.

MATERIALS AND METHODS

Plant materials: Fresh leaves of *Lasianthera africana* were collected in August, 2006 from a garden in Uruan, Akwa Ibom State, Nigeria. The plant was identified and authenticated by Dr. Margaret Bassey, a taxonomist in the Department of Botany, University of Uyo, Uyo. Nigeria. Hebarium specimen was deposited at Faculty of Pharmacy Hebarium. The fresh leaves (2kg) of the plant were dried on laboratory table for 2 weeks and reduced to powder. The powder 100g was macerated in 95% ethanol (300 mL) for 72 h. The liquid filtrate obtained was concentrated in vacuo at 40°C. The yield was 0. 98% w/w. The extract was stored in a refrigerator at 4°C until used for experiment reported in this study.

Phytochemical screening: Phytochemical screening of the extract was carried out employing standard procedures (Harbone, 1993; Trease and Evans, 1989).

Animals: Albino swiss mice (21-28g) of either sex were obtained from the University of Uyo animal house. They were maintained on standard animal pellets and water ad libitum. Permission and approval for animal studies were obtained from the College of Health Sciences Animal Ethics committee, University of Uyo

Parasite inoculation: The chloroquine-sensitive *Plasmodium berghei berghei* was obtained from National Institute of Medical Research, Lagos, Nigeria and maintained in mice. The inoculum consisted of $5x10^7$ *P. berghei berghei* parasitized erythrocytes per mL. This was prepared by determining both the

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percentage parasitaemia and the erythrocytes count of the donor mouse and diluting the blood with isotonic saline in proportions indicated by both determinations. Each mouse was inoculated on day 0 , intraperitoneally, with 0. 2 mL of infected blood containing about 1 x 107 *P. berghei beghei* parasitized red blood cells.

Determination of LD_{50}: The LD_{50} of the extract was determined using albino mice by Intraperitoneal (I.P.) route using the method of Lorke (1983).

Evaluation of schizontocidal activity on early infection (4-day test): Schizontocidal activity of the extract was evaluated using the method described by Knight and Peters (1980). Each mouse was inoculated on the first day (day 0), intraperitoneally, with 0. 2 mL of infected blood containing about $1x10^7$ *P. berghei berghei* parasitized erythrocytes. The animals were divided into five groups of five mice each and orally administered, shortly after inoculation with 1000, 2000 and 3000 $mg.kg.day^{-1}$ doses of the *Lasianthera africana* extract, chloroquine 5 $mg.kg.day^{-1}$ and an equivalent volume of distilled dater (negative control) for four consecutive days, (day 0 to day 3). On the fifth day (day 4), thin films were made from the tail blood of each mouse and the parasitaemia level was determined by counting the number of parasitised erythrocytes out of 200 erythrocytes in random fields of the microscope. Average percentage chemosuppression was calculated as

$$100\left[\frac{(A-B)}{A}\right]$$

Where A is the average percentage parasitaemia in the negative control group and B, average percentage parasitaemia in the test group.

Evaluation of schizontocidal activity established infection (Curative or Rane test): Evaluation of curative potential of the extract was done using a method similar to that described by Ryley and Peters (1970). The mice were injected intraperitoneally with standard inoculum of $1x10^7$ *P. berghei berghei* infected erythrocytes on the first day (day 0). Seventy-two hours later, the mice were divided into five groups of five mice each. The groups were orally administered with *Lasianthera africana* leaf extract (1000, 2000, 3000 $mg.kg.day^{-1}$), chloroquine (5 mg kg^{-1}) was given to the positive control group and an equal volume of distilled water to the negative control group. The drug/extract was given once daily for 5 days. Thin films stained with Giemsa stain were prepared from tail blood of each mouse daily for 5 days to monitor the parasitaemia level. The mean survival time for each group was determined arithmetically by finding the average survival time (days) of the mice (post inoculation) in each group over a period of 28 days (day 0 to day 27).

Statistical analysis: Data obtained from the study were analyzed statistically using Student's test and values of $p < 0.05$ were considered significant.

RESULTS

Acute toxicity: The mice were treated intraperitoneally with a single dose of 1-5 g kg^{-1} of either *Lasianthera africana* leaf extract after being starved for 24h. The route was chosen because of its sensitivity and rapid results. *Lasianthera africana* (1-5 g kg^{-1}) produced no physical signs of toxicity in the animals 24 h after administration except writhing within the first minuter of administration.

Phytochemical screening: Phytochemical screening of the ethanolic leaf extract of *Lasianthera africana* revealed the presence of compounds like alkaloids, terpenes, flavonoids, anthraquinones, saponins, cardiac glycosides and phlobatannins.

4-day test: Ethanolic leaf extract of *Lasianthera africana* produced a dose dependent chemosuppressive effect at various doses employed in this study. The chemosuppression were 41. 15, 51. 14 and 60. 05 % for 1000, 2000 and 3000 $mg.kg.day^{-1}$ doses. The chemosupression produced by the extract were significant ($p<0.05$) compared to control and uncomparable to that of the standard drug (chloroquine 5 $mg.kg.day^{-1}$) with a chemosuppression of 88. 2% (Table 1).

Curative test: On established infection, it was observed that there was a daily increase in parasitaemia of the control group. However, there was a daily reduction in the parasitaemia levels of the extract treated group as well as that of positive control (chloroquine).

On day 7, the average percentage parasitaemia for the groups were 30, 29. 3, 26, 7. 0 and 81 % for 1000, 2000, 3000 $mg.kg.day^{-1}$ of the extract, chloroquine and control groups respectively (Fig. 1). The mean survival time (m. s. t) of the extract treated groups were significantly ($p<0.05$) longer than that of control and was uncomparable to that of the standard drug, chloroquine. The values are given in Table 2.

DISCUSSION

Lasianthera africana, a vegetable use by the Ibibios of the Niger Delta Region of Ngeria, was evaluated for its antiplasmodial potentials in *Plasmodium berghei* infected mice. Phytochemical screening and acute toxicity test of the ethanolic leaf extract were carried out.

Table 1: Antiplasmodial activity of *Lasianthera africana* leaf extract during 4-day test

Drug/Extract	Dose (mg.kg.day^{-1})	Average (%) parasitaemia	Average (%) suppression
Lasianthera africana extract	1000	25. 66± 0. 94*	41. 15
	2000	21. 33± 1. 24*	51. 14
	3000	17. 33± 1. 25*	60. 05
Chloroquine (standard)	5	5. 13 ± 0. 38*	88. 2
Distilled water (control)	0. 2 mL	43. 6 ± 3. 16	-

Data are expressed as mean ± S. D for five animals per group. p<0. 05 when compared to control

Table 2: Mean survival time of mice receiving various doses of ethanolic leaf extract of *Lasianthera africana*

Drug/Extract	Dose (mg.kg.day^{-1})	Mean survival time (day)
Lasianthera africana extract	1000	12. 3± 1. 53*
	2000	14. 7± 3. 84*
	3000	18. 4 ± 0. 95*
Chloroquine (standard)	5	30. 0 ± 0. 00*
Distilled water (control)	0. 2 mL	9. 51 ± 2. 04

Data are expressed as mean ± S. D for five animals per group. p<0. 05 when compared to control

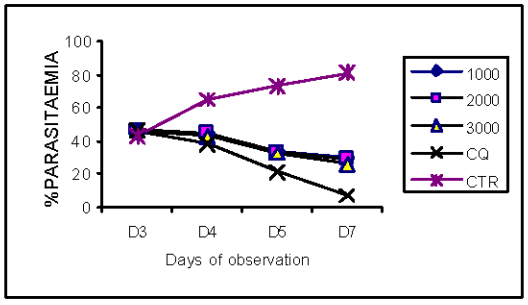

Fig. 1: Effect of *Lasianthera africana* leaf extract on established infection (curative test)

The leaf extract which had negligible acute toxicity contains alkaloids, terpenes, flavonoids, tannins, saponins and cardiac glycosides. Thus revealing an enormous medicinal value of this leaves. The leaf extract demonstrated a significant ($p<0.05$) antiplasmodial activity in both early and established infections, which can be attributable to the phytochemical components of the extract like alkaloids, flavonoids and terpenes which already had been implicated in antiplasmodial activity of various plants (Philipson and Wright, 1991; Christensen and Kharazmi, 2001). The antiplasmodial activity of the leaf extract was uncomparable to that of the standard drug, chloroquine, due to the crude nature of the extract. Despite this moderate antiplasmodial activity of the crude leaf extract, diets have been considered very important because plants consumed as food are ingested in relatively large amount and more regularly than that of same plant use in rituals and cosmetics (Etkin and Ross, 1991; Etkin, 1994). Accumulation of these medicinal constituents of these leaves in the body may culminate in a higher antiplasmodial activity. Moreso, plants compounds which merely slow down or temporary arrest the growth of the parasite (plasmodistatic)as well as those whish act as immune stimulant or helping to alleviate symptoms and reverse some pathological result of malaria infection are reported to potentiate malaria resistance and antiplasmodial activity in immune individuals living in endemic areas (Kirby, 1997). In this study, considerable antiplasmodial activity of the leaf extract was observed. Consumption of these leaves in medicinal and dietary context may have cummulative effect and may be responsible for the reported low parasitaemia and rates of malaria in adult malaria patients in some areas of the Niger Delta region of Nigeria (Ezedinachi *et al*., 1992; Okokon and Ezedinachi, 2002). Etkin (1997) had observed a similar case in a malaria endemic community in Northren Nigeria.

CONCLUSION

The results of this study demonstrated that the leaves of *Lasianthera africana* possess considerable antiplasmodial activity. Their consumption in diets can promote malaria resistance. Therefore, it would be interesting if the active principle is isolated, identified and characterised.

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