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Evaluation of *in vivo* Antiplasmodial Activity of *Aspilia africana*

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Abstract: The antiplasmodial activity of ethanol leaf extract of *Aspilia africana* was evaluated in mice infected with *Plasmodium berghei berghei* during early and established infections. *Aspilia africana* extract (100-300 mg kg^{-1} day^{-1}) exhibited a significant ($p<0.05$) blood schizonticidal activity both in 4 day early infection and in established infection. The extract produced a significant ($p<0.05$) chemosuppression in both early and established infections tests though lower than that produced by chloroquine, 5 mg kg^{-1} day^{-1}. Also, significant mean survival time was recorded in extract treated groups compared to the control group during established infection. The leaf extract posseses a promising antiplasmodial activity which can be exploited in malaria therapy.

Key words: *Aspilia africana*, antimalarial, antiplasmodial, *Plasmodium berghei*, schizonticidal

INTRODUCTION

Malaria is endemic in most part of Nigeria and consistently ranks among the five most common causes of death for all ages, with mortality rate of 100,000 persons annually (Ogunbona *et al*., 1990). It scourge cannot be undermined because of the increasing mortality rate. The resistance of malaria parasite to the available antimalarials particularly chloroquine, the cheapest among them and the high cost of newer effective ones are major concerns. Consequently, a large proportion of the world's population relies on the use of plants as malarial remedy. In Nigeria, many plants preparations are used traditionally in the treatment of malaria with most of them without scientific evidence of efficacy. *Aspilia africana* (Pers.) C. D. Adams (Asteraceae), a perennial herb which grows on waste land region of savannah forest zones and distributed widely across tropical Africa (Daziel, 1937) is one of such plants used traditionally as malarial remedy. The plant has been reported to contain flavonoids, triterpenes, saponins, tannins and alkaloids (Adeniyi and Odufowora, 2000; Page *et al*., 1992; Nguelefack *et al*., 2005) essential oil (Kuiate *et al*., 2005). Studies have reported on the antimicrobial (Adeniyi and Odufowora, 2000) and *in vitro* antiplasmodial activity (Waako *et al*., 2004). Although, *in vitro* antiplasmodial activity of this plant has been reported, the present study was undertaken to evaluate the *in vivo* antimalarial potential of the ethanolic extract of the leaves of *A. africana* in *Plasmodium berghei berghei* infected mice both in early and established infections to ascertain the traditional claim and also correlate the findings with that already reported.

MATERIALS AND METHODS

Plants materials: Fresh leaves of *Aspilia africana* were collected in October, 2005 at University of Uyo farm area. The plant was identified and authenticated by Dr. Margaret Bassey, a taxonomist in the department of Botany, University of Uyo, Uyo, Nigeria. Herbarium specimen was deposited at Faculty of Pharmacy Herbarium, University of Uyo, Uyo with voucher no FPUU428.

The fresh leaves (2 kg) of the plant were dried on laboratory table for 2 weeks and reduced to powder. The powder (100 g) was macerated in 95% ethanol (300 mL) for 72 h. The liquid extract obtained was concentrated in vacuo at 40°C. The yield was 3.26% w/w The extract was stored in a refrigerator at 4°C until used for experiment reported in our study.

Animals: Swiss albino mice (21-30 g) of either sex were obtained from the University of Uyo Animal House. The

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were maintained on standard animal pellets and water *ad libitum*. Permission and approval for animal study were obtained from the College of Health Sciences Animal Ethics Committee, University of Uyo, Uyo.

Determination of LD_{50}: The LD_{50} of the extract was estimated using swiss albino mice by intraperitoneal (ip) route using the method of Lorke (1983). This method involved the administration of different doses of the extract to groups of five mice each. The animals were observed for manifestation of physical signs of toxicity and the number of death in each group within 24 h was recorded. The LD_{50} was calculated as the geometrical means of the maximum dose producing 0% mortality and the minimum dose producing 100% mortality.

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 5×10^7 *P. berghei berghei* parasitized red blood cells per mL. This was prepared by determining both the percentage parasitaemia and the red blood cell 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 1×10^7 *P. berghei berghei* parasitized red blood cell.

Evaluation of schizonticidal activity on early infection (4 day test): A method described by Knight and Peters (1980) was used. The animals were divided into five groups of five mice each and were orally administered with 100, 200 and 300 mg kg^{-1} day^{-1} of *Aspilia africana* leaf extract, chloroquine 5 mg kg^{-1} day^{-1} (positive control) and an equivalent volume of distilled water (negative control group) for four consecutive days (day 0 to day 3) between 8.00 and 9.00 am. On the fifth day (D4), 24 h after the administration of the last dose, thin blood films were made from the tail blood and stained with Giemsa stain and the percentage parasitaemia was determined by counting the number of parasitized erythrocytes out of 200 erythrocytes in random fields of the microscope. Average percentage chemosuppression was calculated as 100 [(A-B)/A)], where A is the average percentage parasitaemia in the negative control group and B, average parasitaemia in the test group.

Evaluation of schizonticidal activity in established infection (Rane test): A modified method similar to that described by Ryley and Peters (1970) was used. On the first day (day 0), standard inoculum of 1×10^7 *P. berghei berghei* infected erythrocytes was injected intraperitoneally into mice. Seventy-two hours later, the mice were divided into five groups of five mice each. Different doses of *Aspilia africana* extract (100, 200 and 300 mg kg^{-1} day^{-1}) were administered orally to these groups. Chloroquine (5 mg kg^{-1} day^{-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 30 days (D0 to D29). The parasitaemia level of the animals that survived after 30 days of monitoring were determined using thin blood film made from tail blood of each surviving animal.

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 extract (500-3000 mg kg^{-1}) produced physical signs of toxicity ranging from writhing, decreased motor activity, decrease body and limb tone, decreased respiration to death. The intensities of all these effects were proportional to the dose administered. The i.p. LD_{50} of the extract in mice was 1414.2 mg kg^{-1}.

4 day test: The leaf extract of *Aspilia africana* produced a dose dependent significant ($p<0.05$) reductions in percentage parasitaemia of the extract-treated mice. On day 5, the percentage parasitaemia of the group treated with the highest dose of the extract (300 mg kg^{-1} day^{-1}) was 8.33±2.05, this was significantly higher than that recorded in the groups treated with 100 and 200 mg kg^{-1} of the extract (Table 1). The percentage parasitaemia of the extract treated groups were significantly ($p< 0.05$) lower than that of the control group, but uncomparable to that of chloroquine group. Moreso,, the extract also produced a significant ($p<0.05$) dose dependent chemosuppression effect at the different doses of the extract employed. Doses of 100, 200 and 300 mg kg^{-1} of the extract administered orally caused chemosuppression of 56.1, 69.57 and 82.27% (Table 1), respectively, which was significant ($p<0.05$)when compared to control. The standard drug, chloroquine, caused 90.1% chemosuppression, which was higher than that of the extract treated group (Table 1).

Table 1: Blood schizonticidal activity of *Aspilia afriana* leaf extract during early infection

Drug/Extract	Dose (mg kg^{-1} day^{-1})	Average (%) parasitaemia	Average (%) suppression
Aspilia afriana	100	20.6±56.1*	56.10
extract	200	14.3±2.05*	69.57
	300	8.33±4.18*	82.27
Chloroquine	5	4.66±2.05*	90.08
Control (distilled water)	0.2 mL	47.0±4.0	-

Data are expressed as mean±SD for five animals per group (* $p<0.05$) when compared with control

Table 2: Mean survival time of mice receiving ethanolic leaf extract of *Aspilia africana*

Drug/Extract	Dose (mg kg^{-1} day^{-1})	Mean survival time (day)
Aspilia afriana	100	10.3±0.94*
extract	200	11.0±1.63*
	300	24.5±7.5*
Chloroquine	5	30.0±0.00*
Control (distilled water)	0.2 mL	8.0±2.16

Data are expressed as mean±SD for five animals per group (* $p<0.05$) when compared with control

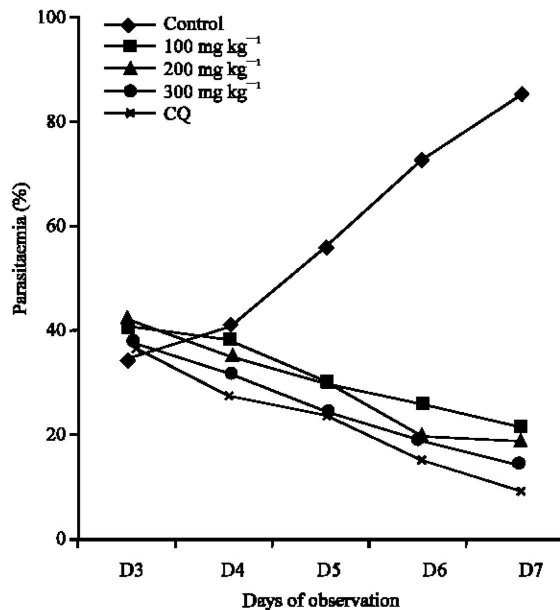

Fig. 1: Effect of *Aspilia africana* extract on established infection

Schizonticidal activity during established infection (curative test): There was a dose dependent reduction in parasitaemia of the extract treated grouped, while the control group showed a daily increase in parasitaemia chloroquine (5 mg kg^{-1} day^{-1}) also produced a daily reduction in parasitaemia. The highest dose of the extract (300 mg kg^{-1})produced the most significant ($p< 0.05$) reduction in percentage parasitaemia in all the extract treated groups (Fig. 1) compared to control. The percentage parasitaemia of the extract treated groups on day 7 were 21, 18 and 14% for 100, 200 and 300 mg kg^{-1}/day of the extract (Fig. 1), respectively, while that of control and chloroquine treated groups respectively were 82 and 9%. Also mice treated with the highest dose of the extract survived longer than those treated with the lower two doses of the extract (Table 2). The mean survival time (m.s.t.) of the mice in various groups were 10.3±0.94, 11.0±1.63, 24.5±7.5, 30.00±0.00 and 8.0±2.16 days for 20, 40 and 60 mg kg^{-1} day^{-1} of extract, chloroquine and control groups, respectively (Table 2). The animals that survived in the chloroquine treated group were found to be parasite free.

DISCUSSION

In the present study, the acute toxicity evaluation of the extract revealed that doses of 2000 mg kg^{-1} and above were lethal to the animals and the determined LD_{50} of the extract, 1414.2 mg kg^{-1} shows that the extract is moderately toxic (Homburger, 1989). *In vivo* evaluation of antimalarial activity of ethanolic leaf extract of *A. africana* revealed that the leaf extract possessed significant ($p<0.05$) blood schizonticidal activity as evident from the chemosuppression obtained during early and established infections as well as by the significant high m.s.t values recorded for extract treated groups compared to control. This likely to be due to the suppression of schizont development and maturity by the extract. Antiplasmodial screening of plants have implicated alkaloids, terpenes and flavonoids in this activity (Philipson and Wright, 1990; Christensen and Kharazmi, 2001). These compounds were found to be present in the extract studied and may be responsible for the observed antiplasmodial activity of the extract, though the active principle is yet to be identified. The results indicate that the leaf extract possess blood schizonticidal activity as evident from the chemosuppression obtained during the 4 day early infection test. A significant ($p<0.05$) activity was also recorded during established infection, which was comparable to the standard drug (Chloroquine, 5 mg kg^{-1} day^{-1}). The highest dose of the extract (300 mg kg^{-1} day^{-1}) was observed to sustain the mice for only 24.5 days out of the 30 days period of study lower than that of the standard drug, Chloroquine. Thus demonstrating a considerable antiplasmodial activity. Besides, this lower activity could have resulted from the crude nature of the extract which can be improved by further purification of the extract.

Although the mechanism of action of this extract has not been elucidated, some plants are known to exert antiplasmodial action either by causing elevation

of red blood cell oxidation (Etkin, 1997) or by inhibiting protein synthesis (Kirby *et al.*, 1989). The extract could have elicited its action through either of the two mechanism mentioned above or by some other unknown mechanism.

CONCLUSIONS

The results of this study correlate well with the *in vitro* study reported by Waako *et al.* (2005) and also justify and confirm the usage of this plant in folk medicine as malarial remedy. Further work is suggested to isolate, identify and characterize the active principle from this plant.

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