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Full Length Research Paper

Evaluation of antiplasmodial activity of *Berlina grandiflora* leaf extract against *Plasmodium berghei* in mice

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The *in vivo* antiplasmodial activity of methanol leaf extract of *Berlina grandiflora* was investigated in *Plasmodium berghei* infected mice. Four day suppressive and curative effect against established infection models of antiplasmodial studies was used. The extract (100 to 400 mg/kg, p.o.) exhibited significant ($p < 0.05$) antiplasmodial activity in early and established infection tests with a considerable mean survival time comparable to that of chloroquine, 10 mg/kg. The leaf extract showed a significant ($p < 0.05$) activity against the parasite in the suppressive and curative tests. The finding supports the traditional use of the plant for the treatment of malaria.

Key words: *Berlina grandiflora*, medicinal plant, antimalarial activity, *Plasmodium berghei*.

INTRODUCTION

There are about 300 million acute cases of malaria each year globally, resulting in more than a million deaths annually (Muentener et al., 1999; Sachs and Malaney, 2002).

About 90% of these deaths occur in Africa, mostly in young children. Malaria is Africa's leading causes of under five mortality and constitutes 10% of the continents overall disease burden, it accounts for 40% of public health expenditure, 30 to 50% inpatient and up to 50% out-patient in areas with high malaria transmission. Despite the increase threat of malaria to lives especially in Africa, success in controlling the disease is possible (White et al., 2004). Different approaches are currently being advocated to achieve this which includes: exploring evidences of immunity, revisiting the abandoned vector control methods and investigation into traditionally used herbal medicines (Wright, 2005).

Berlina grandiflora Hutch and *Dalz (Leguminasae)* is a popular plant in Nigeria traditional medicine. The plant is used in the treatment of gastrointestinal disorders (Asuzu et al., 1993). In Ghana, the stem is used as chewing stick and in the preparation of enemas against constipation while the bark and fruits are used in South Africa to stupefy fish (Asuzu et al., 1993). The plant is widely distributed in Africa from Nigeria to Ghana, Congo Brazzaville, Central and Southern Africa (Dalziel, 1937). In an effort to validate the basis for the medicinal uses of *B. gradiflora*, (Enwerem et al., 2001a; Enwerem et al., 2001b) observed a remarkable antihelminthic activity of the plant. The plant also possesses analgesic and antimalarial properties. Although, the scientific proof of the efficacy of this plant extract as antimalria has not been documented. The present study is concerned with the preliminary in vivo antimalarial evaluation of the methanolic leaf extract of *B. gradiflora* using *Plasmodium*

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berghei in mice.

MATERIALS AND METHODS

Plant collection

The leaves of *B. grandiflora* were collected from Suleja, Niger, State, Nigeria in April, 2009. It was identified and authenticated by Mallam Ibrahim Muazzam and Mrs Grace Ugbabe of the Department of Medicinal plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria where Voucher specimen (No. 6400) was deposited at the herbarium unit of the institute for future reference.

Preparation of plant extract

The leaves were air-dried at room temperature and ground into powder using pestle and mortar. The powdered material (200 g) was macerated with 1.5 L of 70% methanol in water for 24 h with constant shaking; the resultant mixture was filtered using Whatman (No. 1) filter paper and the filtrate concentrated to dryness in Vacuum at 40°C using rotary evaporator. This gave a yield of 23 g (11.5% w/w).

Phytochemical test

The constituents of the extract were tested for the presence of alkaloids, tannins, saponins, terpenes, flavonoids, steroids, and carbohydrates using standard procedures (Trease and Evans, 1989).

Animals

Swiss albino mice (18 to 25) of either sex obtained from the Animal Facility Centre, National Institute for Pharmaceutical Research and Development (NIPRD) were used for the study. The animals were kept in plastic cages at room temperature and moisture, under naturally illuminated environment of 12:12 h dark/light cycle. They were fed on standard diet and had water *ad libitum* according to the NIH Guide for the care and use of laboratory Animals (NIH Publication No. 85 to 23, revised 1985).

Acute toxicity study

The safety of the extract was evaluated by determining its LD_{50} using the Locke's (1983) method. Dose levels used ranged from 10 to 5000 mg/kg. The animals were all kept under the same condition and observed for toxicity signs and mortality for 24 h.LD_{50} values were calculated as geometric mean of the dose that resulted in 100% lethality and that which caused no lethality at all.

Inoculum

Parasitized erythrocytes were obtained from a donor-infected mouse maintained at Animal Facility Centre, National Institute for Pharmaceutical Research and Development (NIPRD). Parasites are maintained by continuous reinfestation in mice. Animals were inoculated intraperitoneally with infected blood suspension (0.2 ml) containing 1×10^7 *P. berghei* parasitized red blood cells.

Suppressive study (4 day test)

Tests were performed in a 4 day suppressive standard test using the methods of (Peters and Anatoli, 1998; David et al., 2004). Thirty Swiss albino mice of either sex weighing (18 to 25 kg) were inoculated by intra-peritoneal (i.p) injection with 1×10^7 infected erythrocytes. The animals were divided into five groups of six per cage and treated during four consecutive days. Group 1 received normal saline (10ml/kg) daily, group 2, 3 and 4 received daily doses of the extract by oral route (100, 200 and 400 mg/kg), while group 5 received 10 mg/kg of chloroquine daily by intraperitoneal route. On day five of the study, thick and thin films were prepared with blood collected from the tail of each mouse. The films were fixed with methanol stained with Giemsa and parasitemia was determined by counting the number of infected and uninfected red blood cells in 10 different fields. The percentage suppression of parasitemia was calculated for each dose level by comparing the parasitemia in infected controls with those of treated mice.

Rane (Curative test)

Evaluation of curative potential of *Berlina grandiflora* leaf extract was done adopting the method described by (Ryley and Peters, 1970) with slight modification. Thirty mice were selected and intra-peritoneally injected with 1×10^7 *plasmodium berghei* infected erythrocyte on the first day. Seventy two hours after, the animals were divided into five groups of six per cage. Group 1 received normal saline (10 ml/kg) daily, group 2, 3 and 4 received daily doses of the extract by oral route (100, 200 and 400 mg mg/kg), while group 5 received 10 mg/kg of chloroqine daily by intra-peritoneal route. Treatment continued until the seventh day when thick and thin films were prepared with blood collected from the tail of each mouse. The films were fixed with methanol, stained with Giemsa and parasitemia was determined by microscopic examination in 10 different fields. The mean survival time for each group was determined by finding the average survival time (days) of the mice in each group over a period of 30 days.

Statistical analysis

Results obtained were expressed as mean ± S.E.M. The data was analyzed using students t-test. $P < 0.05$ was considered significant.

RESULTS

Phytochemical test

The crude extract of *Berlina grandiflora* gave positive test for flavonoids, tannins, saponins, steroids, terpenes and carbohydrates.

Acute toxicity test

There was no mortality recorded in the mice upon oral administration even at doses as high as 5000 mg/kg. This indicates that the experimental doses used are relatively safe.

Suppressive effect

The methanol leaf extract of *Berlina grandiflora* at 100

Table 1. Suppressive effect of methanol leaf extract of *B. grandiflora* against *P. berghei* in mice.

Treatment	Dose (mg/kg)	Parasitemia count	% Inhibition
Saline	10 ml/kg	36.13 ± 0.80	
Berlina grandiflora	100	5.97 ± 0.37	83
Berlina grandiflora	200	3.97 ± 0.52	89
Berlina grandiflora	400	2.1 ± 0.06	94
Chloroquine	10	1.73 ± 0.30	95

Results are mean count + S.E.M. (n = 6). * significantly different from control at P < 0.05.

Table 2. Curative effect of methanol leaf extract of B. grandiflora against P. berghei in mice.

Treatment	Dose (mg/kg)	Parasitemia count	Mean survival time (days)
Saline	10 ml/kg	38.43 ± 0.46	9.5 ± 0.72
Berlina grandiflora	100	13.35 ± 1.30	17.0 ± 1.15
Berlina grandiflora	200	12.42 ± 0.64	22.17 ± 0.40
Berlina grandiflora	400	8.17 ± 0.72	28.5 ± 0.56
Chloroquine	10	2.28 ± 0.28	30.0 ± 0.00

Results are mean count + S.E.M. (n = 6). * significantly different from control at P < 0.05.

and 200 mg/kg gave 83 and 89% suppression of parasitemia and at 400 mg/kg, induced the highest chemosuppression of parasitemia (94%). The chemosuppression produced by the extract was significant (P < 0.05) comparable to chloroquine group which had a chemosuppression of 95% (Table 1).

Curative effect

The methanol leaf extract caused a dose-dependent reduction in parasitemia with the extract, similar to the chloroquine-treated group, while the control group showed a daily increase in parasitemia. Chloroquin at 10mg/kg gave a mean survival time of 30.0 ± 0.00 days as compared to 17.0 ± 1.15, 22.17 ± 0.40 and 28.5 ± 0.50 days observed with 100,200 and 400 mg/kg of the plant extract, respectively. However some of the mice in the 400 mg/kg group survived the 30 days observation period, and the group treated with chloroquine recorded no death at all. The mean survival values showed that the plant extract significantly (p < 0.05) suppressed established infection at the doses employed compared to the control group, they were lower than the group treated with chloroquine, the reference drug (Table 2).

DISCUSSION

The rodent parasite, *P. berghei* have been used in studying the activity of potential antimalarials in mice (Thomas et al., 1998) and in rats (Pedroni et al., 2006). Rodent models of antimalarial study have been validated through the identification of several conventional anti malarials especially with the success of quinine and more recently artemisinin derivatives (David et al., 2004).

The results indicate that the leave extract of *B. grandoflora* possessed significant antiplasmodial activity as evident from the chemosuppression obtained during the 4 day early infection test. However, on established infection the plant extract also exhibited significant activity. Agents with suppressive activity against *plasmodium berghei* were known for antimalarial activity, (Calvalho et al., 1991). It is noteworthy that the antiplasmodial activity of the extract at all doses during early and established infections was comparable to that of the standard drug, chloroquine. There was a dose-dependent chemosuppression of parasitemia seen with the extract. This was confirmed by the mean survival time values particularly in the group administered 400 mg/kg of the extract. In untreated mice, the parasite count increased daily until the death of the animals, which was also observed in our previous studies (Akuodor et al., 2010).

It is evident based on these findings that *Berlina grandiflora* leaf extract is a potential antiplasmodial agent. However, the active principle known to give these observed activity needs to be identified. The plant might be acting mainly by causing elevation of red blood cell oxidation (Etkin, 1997) or by inhibiting protein synthesis (Kirby et al., 1989) the activity may be attributed to terpenes and flavonoids present in the extract. Antiplasmodial screening of plant substances has implicated terpenes, flavonoids and alkaloids (Philpson and Wright, 1990; Milliken, 1997; Christensen and Kharazmi, 2001).

These compounds may be acting singly or in synergy with one another to exert antiplasmodial activity observed in the study.

It is hope that the screening of the locally used medicinal plants for antimalaria properties can fully be investigated with a view to establishing their efficacy and to determine their potentials as sources of new antimalarial agent.

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