In vivo antimalarial activity of *Vernonia amygdalina*

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ABSTRACT

Extracts from the leaves and root bark of *Vernonia amygdalina* are assessed for antimalarial activity against drug-sensitive *Plasmodium berghei* in mice. A standard inoculum of 1 x 10^7 infected erythrocytes is used, and leaf and root-bark extracts of 500 mg/kg, 250 mg/kg or 125 mg/kg are used in a four-day suppression test and a Rane test of established infection. Leaf extract produced 67% suppression of parasitaemia in the four-day test, while root-bark extract produced 53.5% suppression. These results are significant when compared to a placebo ($P<0.001$).

KEY WORDS: Antimalarials. Medicine, herbal. Plants, medicinal. Plasmodium berghei. Plasmodium falciparum. Vernonia amygdalina.

Introduction

In sub-Saharan Africa, malaria is responsible for approximately a million infant deaths a year, predominantly among the poor who have little or no access to modern medicine. This group represent some 75% of the world's population that relies on herbal remedies.[1]

Malaria is becoming more resistant to a number of current drugs and is on the increase because of the global warming process;[2] thus, many communities who live in endemic areas have started to look for malaria remedies in plants in their local environments.[3] Therefore, scientific investigation of the plants used in traditional herbal remedies for the disease will contribute to both knowledge and health.

Vernonia amygdalina, commonly known as bitter leaf, is a popular African vegetable – once the bitterness has been removed by soaking in several changes of water or by boiling – and it often appears in traditional medicines. Broad-spectrum antimicrobial activity of the leaf extract has been reported[4] and it has been used to treat malaria[5] and gastrointestinal ailments,[6] and aqueous leaf extract has been shown to reduce blood sugar levels in rabbits.[7]

This study describes the *in vivo* antimalarial activity of *V. amygdalina* leaf and root-bark extracts against the rodent malarial parasite *Plasmodium berghei*.

Materials and methods

Extracts
Leaves (33.9 g) and root bark (116.4 g) of *V. amygdalina* were collected from Maun, near the Okavango delta in Botswana in August 2000. They were sun-dried separately, powdered and then soaked in 95% ethanol. Every day for five days, the soaked leaf and root-bark powders were filtered, the extracts evaporated using a rotary evaporator and the recovered ethanol reused for soaking. Leaves and root bark yielded 2 g and 2.2 g of dried extract, respectively.

Prior to use, 1 g/mL of each extract was prepared in ethanol and subsequently 500, 250 and 125 mg/kg doses were prepared in distilled water.

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Mouse strain
National Medical Research Institute (NMRI) albino mice were bred in the animal house of the Department of Biology, University of Botswana. At four weeks they were an average weight of 20 ± 2 g. The mice were kept in plastic cages at room temperature and supplied with adequate drinking water and livestock feed.

Standard inoculum
Donor NMRI albino mice previously infected with chloroquine-sensitive *P. berghei* (obtained from the University of Witswatersrand, South Africa) and with a rising parasitaemia of 20% had blood samples taken, which were diluted with phosphate-buffered saline such that 0.2 mL contained 1 x 10^7 infected erythrocytes. To avoid variability in parasitaemia, all the animals used in the study were infected from the same mouse.

Evaluation of blood schizontocidal activity on early infection (four-day suppression test)
The method of Knight and Peters[8] was followed. Twenty-five albino mice were used to assess the effect of each plant extract. Each mouse was inoculated intravenously with the standard inoculum on day zero (D+0).

The mice were then divided into groups of five per cage. Three of the groups were given 500 mg/kg, 250 mg/kg or 125 mg/kg of the plant extract per day, beginning on the day of inoculation. The other two groups received either 5 mg/kg chloroquine per day (positive control group) or saline (placebo). Each day, for five days, thin blood films were made from the tail blood of each mouse, stained with Giemsa and examined microscopically to assess the level of parasitaemia.

Average percentage suppression of parasitaemia was calculated using the formula: A = ([B –C] /B) x100, where A = average percentage suppression, B = average percentage

parasitaemia in the placebo group, and C = average percentage parasitaemia in the test group.

Evaluation of blood schizontocidal activity of crude extracts on established infection (Rane test)

The Rane test[9] relies on the ability of a standard inoculum of *P. berghei* to kill the recipient mouse within six days of inoculation. Extension of survival beyond 12 days is regarded as activity.

Twenty-five mice were divided into groups of five per cage. Each mouse received a standard inoculum intravenously and treatment was withheld for 72 h to allow parasitaemia to establish. Three of the groups were then given 500 mg/kg, 250 mg/kg or 125 mg/kg of the plant extracts subcutaneously for three consecutive days. The positive control group was given 5 mg/kg chloroquine for three days and the placebo group received sterile saline.

Blood smears were made from the tail blood of each mouse on five consecutive days, starting from the day of treatment. Average percentage parasitaemia was assessed. The number of deaths was also recorded for 28 days and the mean survival time (MST) for each plant extract was obtained.

Statistical analysis

Results are presented as mean and standard error of the mean. Student's *t*-test was used to compare the differences in the results between the groups. $P<0.001$ was regarded as significant.

Results

Four-day test

In the four-day test, each of the three dose levels of *V. amygdalina* leaf and root-bark extract produced a reduction in parasitaemia, which was significantly lower than that achieved with the placebo ($P< 0.001$). Average percentage suppression was greater at higher concentration, and chemosuppression was also statistically significant ($P<0.001$). Results are summarised in Table 1.

Rane test

In the Rane test, the standard inoculum produced a mean parasitaemia of 8.0% in 72 h, and this increased in all groups despite treatment. From the third day of treatment, a gradual decline in parasitaemia was observed in all groups, with complete clearance observed in the chloroquine-treated group.

Table 1. Effect of *V. amygdalina* leaf and root extracts on *P. berghei* in mice

	Dose mg/kg	Average % parasitaemia (± SE)	% chemosuppression
Leaf	500	13.2 ± 1.8	67.0
	250	20.2 ± 1.6	49.5
	125	23.4 ± 1.5	41.5
Root bark	500	18.6 ± 1.3	53.5
	250	22.4 ± 1.6	43.0
	125	25.0 ± 0.8	38.5
Chloroquine	5	1.8 ± 0.4	80.4
Saline	0	40.0 ± 0.9	0

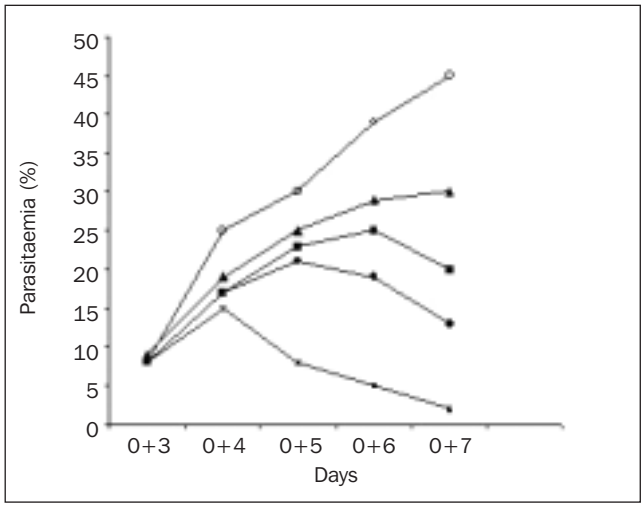

Fig. 1. Changes in parasitaemia following daily treatment with (●) 500 mg/kg (■) 250 mg/kg or (▲) 125 mg/kg *Vernonia amygdalina* leaf extract, (✳) 5 mg/kg chloroquine or (○) saline in the Rane test.

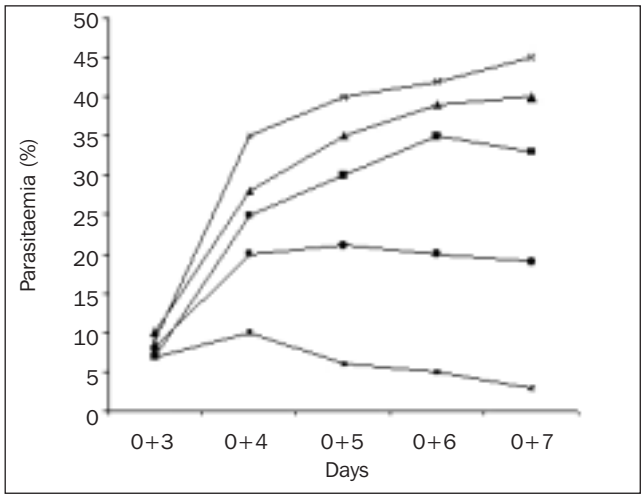

Fig. 2. Changes in parasitaemia following treatment with (●) 500 mg/kg, (■) 250 mg/kg or (▲) 125 mg/kg *V. amygdalina* root extract, (✳) 5 mg/kg chloroquine or (×) saline in the Rane test.

Figures 1 and 2 illustrate the parasitaemia changes following treatment with different doses of leaf and root-bark extracts, respectively. Table 2 summarises the survival times of the mice in the Rane test.

The first death occurred in the placebo group on day 7 following infection, while the last mouse in the extract-treated group died on day 18. All mice in the chloroquine-treated group survived the 28-day observation period.

Discussion

V. amygdalina suppressed parasitaemia during early infection when the parasites had not taken a strong hold. Leaf and root-bark extracts produced large decreases in parasitaemia,

Table 2. Mean survival times of mice treated with leaf and root extracts of *V. amygdalina* in the Rane test

	Dose mg/kg	Number survived by days								MST (days)
		+8	+10	+12	+14	+16	+18	+20	+28	
Leaf	500	5/5	5/5	4/5	3/5	3/5	0/5	-	-	15.0 +1.1
	250	5/5	4/5	3/5	2/5	1/5	0/5	-	-	13.0+1.3
	125	4/5	3/5	0/5	-	-	-	-	-	9.8 +0.7
Root	500	5/5	5/5	3/5	1/5	0/5	-	-	-	12.8 +0.7
	250	4/5	3/5	2/5	0/5	-	-	-	-	10.6+1.0
	125	3/5	1/5	0/5	-	-	-	-	-	8.6+0.7
Saline	-	1/5	0/5	-	-	-	-	-	-	7.4 + 0.4
Chloroquine	5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	> 28 .0

MST: mean survival time

resulting in chemosuppression ranging between 41.5% and 67.0% for the leaf extract and 38.5% and 53.5% for the root-bark extract. These are statistically significant ($P<0.001$) when compared to the placebo.

High levels of chemosuppression were produced at high doses of the leaf and root-bark extracts, indicating a dose-dependent effect. At 125 mg/kg per day, the leaf extract reduced parasitaemia by approximately 50% (80.4% with chloroquine), which indicates that the minimum effective dose (ED_{50}) of this extract is approximately 125 mg/kg per day. An ED_{50} of 250 mg/kg per day was seen with the root-bark extract. This suggests that the concentration of active compounds is higher in the leaf than in the root. However, in both cases, chemosuppression was less than that produced by chloroquine.

Most herbal remedies are consumed in relatively large quantities. Continuous consumption of the extract could reduce parasitaemia or retard multiplication of parasites until the body's immune system intervenes. *V. amygdalina* is a popular and non-toxic plant, and those who use the leaves regularly as a vegetable, and those who use either stem or root as a chewing stick,[4] may be protected against the effects of the malaria parasite.

Tona *et al*.[10] demonstrated *in vitro* antimalarial activity of ethanol and chloroform extracts of the *V. amygdalina* leaf, while Masaba[11] showed *in vitro* activity of an acetone-water extract.

Results of the present study show that *in vivo* administration of an ethanol extract of *V. amygdalina* is capable of suppressing parasitaemia, especially during early infection; the antimalarial action being attributed to sesquiterpene lactones.[12,13] However, under the experimental conditions employed, the extracts failed to eliminate *P. berghei* parasites completely. □

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