

In Vivo anti-malarial activities of Clerodendrum myricoides, Dodonea angustifolia and Aloe debrana against Plasmodium berghei

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Abstract

Background: Malaria caused by the parasite *Plasmodium falciparum* is an acute disease which kills an estimated 863,000 people per year according to the WHO report of 2009. The fight against malaria is faced with the occurrence of widespread resistance of *P. falciparum*. The search for plant-derived antimalarial drugs has great importance in this regard. Thus this study evaluates the toxicity and antimalarial activity of extracts of *Clerodendrum myricoides*, *Dodonia angustifolia* and *Aloe debrana*.

Method: Acute and sub acute toxicity studies of the extracts were carried out by giving up to 3000mg/kg to non-infected mice. Weight loss, change in general behavior and mortality were used as indicators of toxicity. Doses of 200, 400 & 600mg/kg/day of each extract of *C.myricoides*, *D. dodonia* and *A.debrana* were given orally to *Plasmodium berghei* infected mice following the four-day suppressive test procedure.

Results: None of the extracts caused symptoms of toxicity at the given doses. Each extract showed variable level of parasitaemia suppression in dose related manner. Methanol extract of *C. myricoides* leaves exerted 82.50% suppression at the dose of 600mg/kg. The methanol extract of the root of *D. angustifolia* showed the highest (84.52%) suppression of parasitaemia at the dose of 600mg/kg. Furthermore, methanol extract of *A. debrana* induced 73.95% suppression, whereas its water extract exerted 54.36% suppression of parasitaemia.

Conclusion: Crude extracts of *C. myricoides*, *D. angustifolia* and *A.debrana* caused strong activities against *P. berghei* indicating that they contain some chemical constituents that possibly lead to antimalarial drug development. [Ethiop. J. Health Dev. 2010; 24(1):25-29]

Introduction

Malaria remains one of the world's leading health problems with about 863,000 deaths annually (1). Most of these deaths are caused by *Plasmodium falciparum*, one of the four species of malaria causing parasites in humans. The burden of mortality in sub-Saharan Africa is accounted for 89% of the deaths where 5% of children die from the disease before reaching 5 years of age. One of the factors that contributed to this grim picture of malaria is the emergence and wide spread of *P. falciparum* resistance to the standard antimalarial drugs, which necessitates a continuous effort to search for new drugs (2).

In malaria endemic countries traditional medicinal plants are frequently used to treat malaria (3). The analysis of traditional medicines that are employed for the treatment of malaria represents a potential for discovery of lead molecules for development of antimalarial drugs (4). For example, quinine derivatives were modeled on the quinine molecule, found from the bark of Cinchona tree of South America (5). The recently formulated potent and effective artemisinin derivatives are isolated from *Artemisia annua*, a plant used for thousands of years to treat malaria by the Chinese people (6). The success in isolation of artemisinin has inspired many researchers to look for new antimalarial drugs from plants that are being used to treat malaria in traditional health care systems.

In Ethiopia it is estimated that about 80% of the Ethiopian population is still dependent on traditional medicine, which essentially involves the use of plants (7). Despite their wide use in the traditional health care, the work that has been done to evaluate the safety and efficacy of Ethiopian traditional medicinal plants is not extensive. Previous studies have shown the antimalarial activities of *Vernonia amygdalina* and *Withania somnifera* *in vitro* against *P. falciparum* (8) and activities of these plants against *P. berghei* in mice (9). Similarly, *Dodonia angustifolia* was reported to have activity against *P. falciparum* *in vitro* (10). Due to the aforementioned facts and the claimed use of these plants for malaria control, the objective of the this study is to test the antimalarial activities of *Clerodendrum myricoides*, *Dodonia angustifolia* and *Aloe debrana* *in vivo* system in mice.

Methods

Plant materials and extraction: Leaves of *C. myricoides*, *D. angustifolia* and *A. debrana* were collected from three different localities namely Wolisso (114 Km Southwest of Addis Ababa), Wondogenet (about 270 Km South of Addis Ababa) and Addis Ababa, during the months of November and December, 2006. The identification and authentication of the plant specimens was done at The National Herbarium, Department of Biology of the Addis Ababa University. These species were previously collected and voucher specimens deposited. The plant

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samples were air-dried at room temperature under shade, and ground into powder using an electric mill.

The crude extracts were prepared by cold maceration technique (11). Aqueous and/or methanol (98%) extract of each specimen was prepared. The extraction was done by refluxing 50 g of plant material in 300 ml of methanol (98%) or distilled water and placing the mixture on orbital shaker (at 160rpm) for 24 hrs. The mixture was first filtered using cotton and then the filtrate was passed through Whatman filter paper (No.3, 15cm size with retention down to 0.1µm in liquids). The methanol (98%) extracts were concentrated in a rotary evaporator (Buchi type TRE121, Switzerland) at a temperature of 45°C; whereas the water extract freeze-dried using centrifugal freeze drier (model 5 PS, England). All the extracts were stored in screw cap vials at -20°C until they were applied in the experiment. The water and methanol (98%) extracts were dissolved in 10ml of distilled water and Tween 80 (3 %) respectively for the experiment.

Acute toxicity study: The crude extracts were evaluated for their toxicity in *P. berghei* non-infected male Swiss albino mice aged six to eight weeks and weighing 27-34g. For the test of each extract, 15 mice were randomly divided into three groups of five animals per cage. Before oral administration of a single dose of each extract, the mice were fasted for one to two hrs (12, 13). Then, the mice in Group 1 were given orally 0.2ml of methanol and/or water extracts of *C. myricoides* and *A. debrana* leaves and methanol extract of *D. angustifolia* roots at a single dose of 3,000 mg/kg, and the mice in Group 2 were given 0.2ml of each extract at dose of 1500mg/kg of body weight. The mice in control group received 0.5ml of respective vehicle of each extract (dH₂O or 3% Tween 80). Then, the mice were observed continuously for one hr after the treatment; intermittently for four hrs, and thereafter over a period of 24 hrs (14). The mice were observed for gross behavioral changes such as feeding, hair erection, lacrimation, mortality and other signs of toxicity manifestation (15).

The parasite and infection: For *in vivo* antimalarial assays of plant extracts, Chloroquine (CQ) sensitive strain of *P. berghei* maintained at the animal house of the Biology Department, Addis Ababa University was used. The parasites were maintained by serial passage of blood from infected mice to non-infected ones on weekly basis.

To infect the mice, blood sample was collected from auxiliary vessels of a donor mouse with a rising parasitaemia of about 30-37%. Then, the blood was diluted in normal saline so that each 0.2ml contained approximately 10⁶ infected red blood cells. Each animal received inoculums of about 10⁶ parasites via intraperitoneal route (IP). The inoculated mice were then randomized into five mice per cage and maintained in the animal house on a commercial diet and water *ad libitum*.

Antimalarial activity of plant extracts: In screening of the plant extracts, the standard four-day suppressive

method was used (16). Male Swiss albino mice weighing 27-32g were infected with 10⁶ *P. berghei* and randomly divided into five groups of five mice per cage. The infected mice were randomly divided into three test groups and two control groups (each for CQ as a standard drug and dH₂O or 3% Tween 80 as a negative control). The test extracts were prepared in three different doses (200mg/kg, 400mg/kg, and 600mg/kg of body weight) and CQ at 25mg/kg in a volume of 0.2ml and vehicles at 0.5ml/mouse. Each extract was administered as a single dose per day. All the extracts and the drug were given through intragastric route by using standard intragastric tube to insure safe ingestion of the extracts and the drug. Treatment was started after 3 hrs of infection on day 0 and was then continued daily for four days (i.e. from day 0 to day 3). On the fifth day (D4) blood sample was collected from tail snip of each mouse. Thin smears were prepared and stained with 10% Geimsa solution. Then, each stained slide was examined under the microscope with an oil immersion objective of 100x magnification power to evaluate the percent suppression of each extract with respect to the control groups. The experiments were done by one of the authors, the parasite count was performed by an experienced technician (double blinded) and the animal keeper looked after the experimental mice during the course of the study.

Data analysis: Results of the study were expressed as mean ± standard error of mean (M ± SEM). Comparison of parasitaemia and statistical significance was determined by one-way ANOVA (repeated measure of analysis of variance) and post hoc Scheffe's test using SPSS for window (Version 13.0) statistical package. All data were analyzed at a 95% confidence interval (P = 0.05). Percent parasitaemia and percent suppression were also calculated (17, 18).

Results

The acute toxicity study indicated that none of the four extracts caused mortality of mice within 24 hrs up to 3000mg/kg. Gross physical and behavioral observation of the experimental mice also revealed no visible signs of acute toxicity like lacrimation, hair erection, and reduction in their motor and feeding activities. They were physically active.

The methanol extract of *C. myricoides* showed 54% suppression of parasitaemia at the dose of 200mg/kg (Table 1). The highest suppression of parasitaemia was observed at the dose of 600mg/kg body weight of mice. Percentage suppression was observed to increase as extract concentration increased. After four days treatment with the different extract doses, the mean parasitaemia of the test groups ranged from 2.7 ± 0.14% to 7.18 ± 0.32% while the corresponding value of the negative control group being 15.62 ± 0.68%. The mice treated with CQ were completely free from the parasites on day four.

Table 1: Activity of methanol extracts of *C. myricoides* leaves and *D. angustifolia* against *P. berghei* in mice

Extract	Dose (mg/kg/day)	Antimalarial activity	
		% Parasitemia \pm SEM	% Suppression
<i>C. myricoides</i>	NC	15.62 \pm 0.68 ^a	00.00 ^a
	200	7.18 \pm 0.32 ^b	54.14 ^b
	400	5.32 \pm 0.13 ^c	65.95 ^c
	600	2.74 \pm 0.14 ^d	82.50 ^d
<i>D. angustifolia</i>	NC	11.92 \pm 0.55 ^a	00.00 ^a
	200	5.37 \pm 0.28 ^b	57.74 ^b
	400	3.38 \pm 0.05 ^c	71.51 ^c
	600	1.84 \pm 0.05 ^d	84.52 ^d

Values are Mean \pm SEM; n=5, NC: Negative control (0.5ml vehicle); a,b,c,d = Values in the same column followed by the same letter do not differ significantly (P>0.05)

Significant reduction of parasitaemia (P < 0.05) was also observed in all groups of mice treated with methanol extract of the roots of *D. angustifolia* compared to the negative control. The percent parasitaemia of the mice treated with 200mg/kg of the extract was 5.37 \pm 0.28 and the mice that received 600mg/kg was 1.84 \pm 0.06. Whereas, the parasitaemia of the control mice (treated with dH₂O) was 11.92 \pm 0.95. Its parasitaemia suppression ranged from 57.74% to 84.52%. The highest

suppression was induced in the group treated with the highest dose (600mg/kg).

Methanol extract of *A. debrana* leaves induced 73.95% parasitaemia suppression at the highest dose (600mg/kg). The parasitaemia of the mice treated with the extract ranged from 2.89 \pm 0.04 to 11.15 \pm 0.52. Reduction of the parasitaemia was dose dependent (Table 2).

Table 2: Activity of methanol and water extracts of leaves of *A. debrana* against *P. berghei* in mice

Extract	Dose (mg/kg/day)	Antimalarial activity	
		% Parasitemia \pm SEM	% Suppression
<i>A. debrana</i> methanol extract	NC	11.15 \pm 0.52 ^a	00.00 ^a
	200	7.78 \pm 0.27 ^b	30.21 ^b
	400	6.22 \pm 0.17 ^c	44.15 ^c
	600	2.89 \pm 0.04 ^d	73.95 ^d
<i>A. debrana</i> water extract	NC	14.16 \pm 0.34 ^a	00.00 ^a
	200	10.82 \pm 0.14 ^b	23.53 ^b
	400	9.45 \pm 0.24 ^c	33.22 ^c
	600	0.46 \pm 0.11 ^d	54.36 ^d

Values are Mean \pm SEM; n=5, NC: Negative control (0.5ml vehicle); a,b,c,d = Values in the same column followed by the same letter do not differ significantly (P>0.05)

The aqueous extract of the leaves of *A. debrana*, mean parasitaemia in *P. berghei* infected mice ranged from 6.46 \pm 0.11 to 10.82 \pm 0.14, whereas the corresponding figure in the control group (treated with 0.2ml dH₂O) was 14.16 \pm 0.34. The extract induced statistically significant inhibition of parasitaemia in all the doses tested compared to the negative control (P < 0.05). Treatment with this extract at 600mg/kg exerted 54.36% parasite suppression.

The mice treated with CQ were completely free from the parasites on day four in all the experiments using the methanol extracts of *C. myricoides* leaves, *D. angustifolia* roots and methanol and water extract of the leaves of *A. Debrana*.

Discussion

Methanol extracts of the leaves of *C. myricoides*, *D. angustifolia*, *A. debrana* and water extract of the leaves of *A. debrana* were tested for their toxicity against Swiss albino mice and for their antimalarial activity against *P. berghei* in mice. None of the test mouse died or showed signs of acute toxicity within 24 hours of treatment with

the test extracts. According to CDER (14), acute toxicity is a toxicity produced by a pharmaceutical when administered in one or more doses within a period not exceeding 24 hours. Accordingly, the extracts were non-toxic to test mice, as they did not show signs of acute toxicity within 24 hours at the doses of 1500mg/kg and 3000mg/kg. Changes in general behaviors, variations in body weight and mortality are critical for the evaluation of the effect of a compound on test animals, since such changes are often the first signs of toxicity (19). Similarly, sub acute toxicity study of the extracts revealed no signs of toxicity manifestations within 14 days at the dose levels tested. This shows that, all the test extracts were non-toxic to the test mice.

Methanol extract of the leaves of *C. myricoides* significantly (p< 0.05) inhibited parasitaemia of *P. berghei* in mice. This parasitaemia suppression effect of the extract may be attributed to the presence of alkaloids that have been reported to be the major constituents of the *Clerodendrum* species (20). However, the active compound(s) known to give the observed activity need to be identified and quantified. The leaves of *Clerodendrum*

species were reported to cause 90.13% *in vivo* suppression (21) and strong *in vitro* activity with IC₅₀ 9.51-10.56µg/ml in *P. falciparum* (22). In addition, it is reported to have strong *in vitro* activity with IC₅₀ of less than 30µg/ml in combination with CQ against both CQ sensitive and CQ resistant *P. falciparum*. Even though the rodent malaria model, *P. berghei*, is not exactly similar to that of the human *Plasmodium* parasites, it is the first step to screen most of the *in vivo* antimalarial activities of new molecules and new therapeutics (23). Therefore, this study, together with the aforementioned reports, shows that the plant may serve as a potential source of different antiplasmodial compounds.

The result obtained from the methanol extract of root of *D. angustifolia* was more homogeneous, and all treated groups with the extract showed a significant reduction in parasitaemia compared to the negative control. The suppression activity of this extract was observed to be dose dependent, increasing with increase in the concentration of the extract. Previously *in vitro* activity of *D. angustifolia* extract of seeds of the plant against *P. falciparum* was reported (10); suggesting that the plant may possess antimalarial compounds.

Different studies indicated that *D. angustifolia* has several secondary metabolites like quinines, saponins, flavonoides, alkaloids, terpenoids, diterpenoids, and essential oils (24, 25). Although the active compound is not yet identified, the antiplasmodial activity observed in this study could probably have resulted from a single or combined effect of these compounds. The plant part is reported to be a remedy for stomach disturbances and diarrhea in humans (26); cestodes infection (27) and malaria (28, 29) in humans. The result of this study is therefore, in agreement with the traditional use of this plant for antimalarial therapy.

In this study, both the methanol and water extracts of *A. debrana* showed considerable antiplasmodial properties. The suppression activities of both extracts were increased with increase in the concentration of the extracts. The parasitaemia suppression effect of *A. debrana* extracts may be explained in light of the presence of high concentration of anthraquinones and other quinoid compounds that are the characteristic constituent of the genus *Aloe* (30). Comparatively the water extract of the leaves of *A. debrana* showed lower antimalarial activities than its methanol counterpart. This might indicate that the active compounds for the observed antimalarial activities of the two extracts are different. Hence, bioactivity guided isolation of the active ingredient that exhibit this activity is important.

In conclusion, crude extracts of *C. myricoides*, *D. angustifolia* and *A. debrana* caused strong activities against *P. berghei* indicating that these plants contain some lead antiplasmodial compounds. However, the crude extracts should be further fractionated and tested

for their activity against *P. falciparum* and *P. vivax* in order to consider them as potential sources for antimalarial drug development for human malaria.

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