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Evaluation of anti-*Plasmodium berghei* activity of crude and column fractions of extracts from *Withania somnifera*

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Abstract: Different crude methanolic and chloroform extracts of *Withania somnifera* leaves, and column fractions of the methanolic extract, were tested in vivo for antimalarial activity on Swiss albino male mice. Each mouse in the study was infected intraperitoneally with 0.2 mL of blood containing 10^6–10^7 infected erythrocytes taken from mice previously infected with chloroquine-sensitive *Plasmodium berghei*. The extracts were given to the infected mice intraperitoneally starting from 3 h following inoculation. Antimalarial activity was evaluated by taking blood smears on day 4. The results revealed that column fractions of methanolic extract obtained from leaves of *Withania somnifera* showed significantly improved suppression of parasitemia at lower doses compared to the crude extracts. Reduction of parasitemia by 44% and 57% was observed at doses of 200 and 300 mg/kg body weight, respectively, by column fractions of methanolic crude extract.

Key words: Antimalarial activity, in vivo, *Plasmodium berghei*, *Withania somnifera*

1. Introduction

Malaria is one of the parasitic infections that cause enormous medical, economic, and emotional burden in the world. It has been estimated that more than 300–500 million people are affected by malaria throughout the world (1). In Sub-Saharan Africa alone, an estimated 0.9–2.3 million annual child deaths are attributed to malaria (2). However, these estimates are changing, as a recent report by the World Health Organization indicated that promising malaria prevention and control measures, such as insecticide-treated mosquito nets and indoor spraying with residual insecticides, have led to a significant drop of the mortality rate by 25% since 2000 (3). On the other hand, resistance of the vector mosquitoes to the current insecticides and the emergence of resistance by *Plasmodium* species to widely used antimalarial drugs, such as chloroquine, have made malaria control and treatment much more difficult (4), leading to the use of traditional methods of treatment (5). Recently, the use of natural products in treating various ailments is gaining attention, as the synthetic drugs are causing undesirable side effects (6) and microbes are showing resistance to most of the currently available antibiotics (7).

It is estimated that more than 75% of the world's population relies on medicinal plants for treatment of various ailments. There are hundreds of clinically useful prescription drugs that are derived from plants, and about 74% of them came to the attention of the pharmaceutical industry because of their use in traditional medicine (8,9). Different parts of the studied plant, *Withania somnifera* (also called ashwagandha, Indian ginseng, and winter cherry), have been reported to have antimalarial activities both in vivo and in vitro (10). Therefore, the aim of the present study was to evaluate and improve the reported in vivo antimalarial activity of the plant through testing different column fractions of the crude extract from its leaves.

2. Materials and methods

2.1. Plant material and extraction procedure

Fresh leaves of *W. somnifera* were collected from Zeway (160 km south of Addis Ababa). Identity of the plant material was confirmed at the National Herbarium, Addis Ababa University Department of Biology. Fresh leaves of *W. somnifera* were ground using a grinding mill (Straub Model 4E, USA). The powdered materials obtained were extracted first with chloroform and then with methanol by shaking overnight on an orbital shaker (GFL Model 3020, Germany). The extracts were filtered through cotton and then through Whatman filter paper, and the

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organic solvents were removed from the filtrate by rotary evaporator (BUCHI RE 121, Switzerland). The obtained crude extracts were kept in deep freeze until used for the test.

2.2. Column separation of crude extracts

The methanolic crude extract was fractionated by means of column chromatography using silica gel (60–120 mesh) and eluted with chloroform, then subsequently eluted with chloroform containing increasing concentrations of methanol. Fractions were collected and monitored by thin layer chromatography (TLC), and fractions with similar TLC profiles were combined and tested for activity.

2.3. Antimalarial tests

In vivo antimalarial testing was carried out using the classic 4-day suppressive test against *P. berghei* (11). Five- to seven-week-old Swiss albino mice weighing 24–31 g were obtained for the study from the animal house of the Department of Biology of Addis Ababa University. The mice were fed with standard mice pellet and given water ad libitum. The test animals were put randomly into 5 groups, each containing 5 mice. Blood samples taken from donor mice previously infected with chloroquine-sensitive *P. berghei* with parasitemia of about 20% were diluted with physiological saline so that 0.2 mL contained 10^6–10^7 infected erythrocytes. Each mouse used in the study was infected intraperitoneally by using a hypodermic needle fitted to a 1-mL syringe. The test extracts in the proportion of 200 or 300 mg/kg were administered intraperitoneally. Mice in the first group of each test were the negative control (NC) and were given the vehicle (3% w/v Tween 80) (11).

To evaluate the safety of the extracts and rule out acute toxicity, both crude and column fractions were given at doses of 1000 and 500 mg/kg, respectively, to noninfected mice following the procedure of Tchoumbougnang et al. (12) with minor modifications. For the 4-day suppressive test, the extracts were given once daily continuously for 4 days starting from day 0 to day 3, and percentage of parasitemia was recorded on day 4 by collecting blood samples from the tail using the Giemsa-stained thin blood smears technique, following the procedure used by Satyavivad et al. (11). Each experiment was done in duplicate, and results obtained from the study are presented as mean plus or minus standard error of the mean (M ± SEM). Statistical significance was determined by one-way ANOVA and Scheffé's post hoc test using SPSS 13.0. Student's paired t-test was used to compare parameters within groups. For all the data obtained, statistical significance was set at $P = 0.05$. Percentage of parasitemia and percentage of suppression were calculated using the following formulas, proposed by Li et al. (13) and Devi et al., respectively (14).

$$\%Suppression = \frac{Parasitemia\ in\ control - Parasitemia\ in\ study\ group}{Parasitemia\ in\ control} \times 100$$

$$\%Parasitemia = \frac{Number\ of\ infected\ red\ blood\ cells}{Number\ of\ uninfected\ red\ blood\ cells - Number\ of\ infected\ red\ blood\ cells} \times 100$$

3. Results and discussion

Results from the safety tests of the extracts showed no visible toxic effects up to 1000 and 500 mg/kg for the crude extracts and column fractions, respectively. Examination of thin blood smears from mice of each group showed a lower percentage of parasitemia in all the mice administered with the extracts compared to the negative control groups.

Chloroform extracts of leaves of *W. somnifera* did not induce significant in vivo suppression on *P. berghei* in mice at different tested doses (Table 1) and therefore were not chromatographed. However, this result is in contradiction with the in vitro results reported by Bogale and Petros (15) on *Plasmodium falciparum*, which showed significant antimalarial activity at IC_{50} = 2.04 µg/kg in vitro. This could be because of the fact that antiplasmodial activity of plants in vitro may not necessarily imply the same activity in vivo; rather, compounds may either act as prodrugs, which must undergo metabolic activity, or as febrifuges or immunomodulators (16).

On the other hand, results obtained from in vivo antimalarial activity of methanolic extract of the leaves of *W. somnifera* were in agreement with the reported antimalarial activities of the plant in vitro (15). In addition, the observed antimalarial activity of methanolic crude extract of leaves of *W. somnifera* was improved through fractionation; the highest suppressive effect shown was 57% by fraction F3 (Table 2). This observation was in accordance with the activity reported in vitro (15).

The 2 fractions (F2 and F3) showed significant suppressive effect ($P < 0.01$ and $P < 0.001$, respectively) at a dose of 200 mg/kg. On the other hand, the first fraction of the extract (F1) showed no significant effect on the parasitemia level of the treated mice as compared to the negative control mice (Table 2). In all the groups treated with the fractions, a drop in packed cell volume and differences in body weight were observed. However, the suppression seen by F2 and F3 significantly improved the activities observed from crude methanolic and chloroform extracts. The level of suppression induced by the third fraction (F3) at a dose of 300 mg/kg (Table 2) is within the limits of what may be considered good antimalarial activity

Table 1. Activity of extracts from leaves of *W. somnifera* against *P. berghei* in mice.

Extract	Daily dose (mg/kg)	Antimalarial activity	
		% Parasitemia ± SEM	% Suppression
Methanol extract	NC	9.88 ± 0.82	00.00
	500	8.83 ± 1.05	10.63
	750	5.61 ± 0.43 *	43.20
	900	4.61 ± 0.24 **	53.34
Chloroform extract	NC	10.0 ± 0.71	00.00
	500	8.06 ± 0.86	18.42
	750	7.90 ± 0.52	17.63
	900	8.65 ± 1.16	14.00

Key: * = P < 0.01; ** = P < 0.001. All tests were compared against the negative control (NC).

Table 2. Activity of column fractions of methanolic crude extracts obtained from leaves of *W. somnifera* against *P. berghei* in mice.

Fractions	Daily dose (mg/kg/day)	Antimalarial activity	
		% Parasitemia ± SEM	% Suppression
F1	NC	9.75 ± 0.82	00.00
	200	9.37 ± 1.05	04.45
	300	nt	nt
F2	200	7.12 ± 0.31 *	28.14
	300	6.88 ± 0.1.5 *	29.00
F3	200	8.06 ± 0.86	44.33
	300	4.25 ± 0.30 **	57.00

Key: * = P < 0.01; ** = P < 0.001; nt = not tested. All tests were compared against the negative control (NC).

for a medicinal plant extract, which is a suppression of ≥50% at a dose of 250 mg/kg (17). This suppression is also comparable to the reported antimalarial activity of *Cassia occidentalis* root bark and *Phyllanthus niruri*, which produced 60% suppression of parasitemia in vivo (18), and that of the essential oil of *Cymbopogon citratus*, which produced 62.1% suppression at 200 mg/kg in vivo (12). The antimalarial activity observed for the fractions may be an indication of a nonsynergistic effect of the compounds present in the crude extracts. Therefore, the suppression induced by the extracts might be associated with the presence of chemical ingredients that have antimalarial properties. Biologically active chemical constituents, such as alkaloids, have been reported from different parts of the plant (19). Therefore, the observed antimalarial activity could be associated with some of the alkaloids reported from the plant, as alkaloids such as quinine are known for their antimalarial activities. The therapeutic value of the plant has also been extensively reported. Recently, this plant was demonstrated to possess strong antifungal activity and was found to be effective against murine aspergillosis, and it possesses strong antibacterial properties against various pathogens, including *Salmonella typhimurium* (20).

In conclusion, the present study has demonstrated that the antimalarial activity of the plant could be significantly improved through fractionation of the methanolic crude extract of its leaves. The third fraction obtained from the methanolic crude extract showed 57% suppression of parasitemia in *P. berghei*-infected mice at a dose of 300 mg/kg/day. The plant may contain an undiscovered antimalarial active ingredient that could serve as a template for the production of relatively inexpensive antimalarial drugs. Therefore, it is worth conducting a bioassay-guided fractionation and isolation of the active compound(s) and a detailed study of their activities both in vivo and in vitro.

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