

Evaluation of the antimalarial activity of the aqueous leaf extract of *Gossypium barbadense* (Malvaceae) in mice

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Abstract

Some medicinal plants have been shown to have antimalarial activity when used as combination therapy. *Gossypium barbadense* has been used by herbal medicine practitioners in combination with other herbs, and as a monotherapy in the treatment of malarial infection. The study was, therefore, aimed at evaluating the antimalarial effect of the aqueous leaf extract of *G. barbadense* using mice infected with *P. berghei*. The suppressive effect was evaluated by administering 25 mice divided into five groups with 250, 500, and 1,000 mg/kg of aqueous leaf extract of *G. barbadense*, 5 mg/kg of chloroquine, and 10 mL/kg of distilled water, respectively, starting from the day of inoculation with *P. berghei* for four days. The curative effect was evaluated by administering 25 mice divided into five groups as above with treatment starting 72 h post inoculation with *P. berghei*. The results indicate that the aqueous leaf extract of *G. barbadense*, when used alone as monotherapy, has a non-significant ($P \geq 0.05$) but slight suppressive antimalarial activity (23%) when compared with that of chloroquine (100%). The curative model also revealed that aqueous leaf extract of *G. barbadense* showed no significant antimalarial activity. It can be concluded that the use of aqueous leaf extract of *G. barbadense* as monotherapy for malaria has no significant therapeutic effect. Therefore, it is not recommended to be used alone to manage malaria infection as practiced by some herbal medicine practitioners.

Introduction

Malarial infection is still a threat to global health. It is as old as Man and is the most deadly disease in the tropics.^{1,2} All efforts by the World Health Organization (WHO), governmental bodies, and scientists and researchers of all kinds to reduce the economic burden malaria imposes on people, as well as its morbidity and mortality, has not produced many

results. The consequence of malaria is greater among children (under five years of age), and pregnant women and their newborn babies.³

Over the years, various chemical agents have been used in the treatment and management of malaria infection with varying results. However, one major challenge in malarial chemotherapy is the emergence of resistant strains.⁴

About 70% of the population in developing countries rely on traditional medicine for their primary health care needs.⁵ Thus, the use of medicinal plants for the treatment and management of disease is a significant alternative to using orthodox medicines which are sometimes expensive and can lead to unwanted effects.^{6,7} This has led researchers to investigate the antimalarial activity of different herbs.

Gossypium barbadense is a plant well known for the cotton it produces. It also has some medicinal applications in emetics, venereal diseases, tumors, paralysis, epilepsy, convulsions, spasm, and cutaneous and subcutaneous parasitic infection.⁸ It has antifungal properties and contains the chemical gossypol, making it less susceptible to insect damage.^{9,10} It is also sometimes used as a male anti-fertility drug.¹¹ In Suriname's traditional medicine, the leaves of *G. barbadense* are used to treat hypertension and delayed or irregular menstruation.¹² It is known to have antimalarial effect. In a preliminary report,¹³ gossypol which is an active constituent of *G. barbadense*, was reported to have an *in vitro* antimalarial activity against the human pathogen *Plasmodium falciparum*.

Materials and Methods

Reagents and chemicals

Chloroquine phosphate, phosphate buffer saline (PBS), Giemsa's stain, immersion oil and distilled water were used in the experiment.

Laboratory animals

Adult albino mice (male and female), average weight 18-30 g, were obtained from the Laboratory Animal Centre of College of Medicine, University of Lagos, Nigeria. The animals were authenticated in the Zoology Department at the Faculty of Science, University of Lagos, Nigeria. They were allowed to acclimatize for two weeks before starting the experiment. The animals were fed on Pfizer Animal Feed cubes and water *ad libitum*. The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication N. 85-23, revised 1996) for studies involving experimental ani-

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mals. Ethical permission for use of animals for this research was obtained from the College of Medicine University of Lagos research ethics committee.

Plant raw material

Gossypium barbadense leaves were collected from the wild bush in Sagamu of Ogun state. The plant sample was identified and authenticated by Mr TK Odewo of the Botany Department, University of Lagos, with voucher specimen number LUH 2776. A specimen of the plant was preserved in the herbarium.

Preparation of aqueous leaf-extract of *Gossypium barbadense*

The *Gossypium barbadense* leaves were washed and chopped into pieces. These were air-dried for two weeks and then blended into tiny grains. The chemical constituents were extracted in distilled water with the aid of a Soxhlet extractor. The aqueous extract collected was then dried in an oven at 40°C until a consistent gel was formed.

Phytochemical screening

The aqueous leaf extract of *G. barbadense* was subjected to phytochemical screening for the presence of alkaloids, tannins, saponins, resins and secondary metabolites according to the methods described by Trease and Evans (Table 1).¹⁴

Acute toxicity

Acute toxicity was evaluated by injecting 5 mice with extract at the dose of 2,000 mg/kg. The mice were observed for 24 h for mortality.

Experimental models

Two experimental models were used to investigate the antimalarial activity of *Gossypium barbadense* leaves: suppressive and curative models.

Suppressive model

A suppressive model was made to investigate the suppression of *P. berghei* parasites by extracts acting on sexual erythrocytic schizogony and thereby preventing clinical attack. A total of 25 albino mice weighing between 18 and 30 g were divided into 5 groups, 5 mice in each group, as follows: group 1: aqueous leaf extract of *G. barbadense* at 250 mg/kg dose daily; group 2: aqueous leaf extract of *G. barbadense* at 500 mg/kg dose daily; group 3: aqueous leaf extract of *G. barbadense* at 1,000 mg/kg dose daily; group 4: chloroquine phosphate at 5 mg/kg (standard) daily; group 5: distilled water at 10 ml/kg (control) daily. Each mouse was inoculated intraperitoneally with *P. berghei* (about 1×10^6 parasitized erythrocytes) on day 0. Beginning on the day of inoculation, the mice received treatment in the above order for four days (Table 2). The mice were then observed and blood smears obtained on the fourth day to monitor the level of parasitemia.^{15,16}

Curative model

The curative model was used to investigate

complete elimination of *P. berghei* parasites from the blood through continued therapy with aqueous leaf-extract of *G. barbadense*. A total of 25 albino mice weighing between 18-30 g were divided into 5 groups, 5 mice in each group. Each mouse was inoculated intraperitoneally with *P. berghei* (about 1×10^6 parasitized erythrocytes) and treatment withheld for 72 h so as to establish parasitemia. The mice were then treated with extract and standard drug as in the previous model and parasitemia levels were monitored (Table 3).^{15,16}

Preparation of blood smear

Blood was obtained from the mice for observation through tail bleeding. The first blood droplet was wiped off using clean cotton wool so as to give an accurate result since the first droplet might not contain the malaria parasite. The subsequent blood was allowed to drop onto a clean microscope slide, a thin film of blood smear was made, and then fixed with methanol. The slide was stained with Giemsa's stain for 10 min. It was then rinsed and allowed to dry in the open air and was thus ready to be viewed under the microscope with proper labeling on the various slides.

Counting of parasitized erythrocytes

The stained blood smear was mounted on a microscope using immersion oil and the oil objective lens was used to view the slide at a magnification of $\times 100$. The total numbers of parasitized erythrocytes were carefully counted as were the total number of erythrocytes. The percentage parasitemia was obtained by the mathematical formula:

$$\frac{\text{Parasitized erythrocytes}}{\text{Total erythrocytes}} \times 100$$

The average percentage parasitemia was taken from five fields counted per slide.

Statistical analysis

Results are presented as mean \pm S.E.M. Statistical significance between the control groups and the test groups were analyzed by means of Student's t-test. $P < 0.05$ were considered significant.

Results

There were no deaths in the acute toxicity experiment when extracts were administered up to 2,000 mg/kg for mice. This suggests that the oral LD₅₀ of aqueous leaf extract of *G. barbadense* is greater than 2,000 mg/kg for mice. Oral administration of aqueous leaf extract of *G. barbadense* to mice in a 4-day suppressive test showed that the extract had slight suppressive effects on the parasitemia (23.10%) at 1,000 mg/kg compared ($P \geq 0.05$) to chloroquine (100%). Oral administration of aqueous leaf extract of *G. barbadense* to mice for curative testing showed progressive increase in the parasitemia for all the doses until Day 17 when a decline in parasitemia was observed 16.09 \pm 32.1; 12.10 \pm 27.07 and 0.0 for 1,000 mg/kg, 500 mg/kg and 250 mg/kg, respectively. However, there was complete eradication of parasitemia on Day 3 for chloroquine 5 mg/kg. There was no significant difference in results for the extract compared with the control group.

Table 1. Phytochemical composition of aqueous leaf extract of *G. barbadense*.

Tests	Inference
Alkaloids	Present
Phlobatannins	Present
Tannins	Present
Saponins	Present
Flavonoids	Present
Anthraquinone	Absent
Cardiac glycoside	Present

Table 2. Suppressive effect of aqueous leaf extract of *G. barbadense* (GB), chloroquine (CQ) and distilled water (DW) on percentage parasitemia due to *P. berghei* infection in mice for a 4-day period.

Treatment groups	Dose	% Parasitemia	% Suppression
Control	10 mL/kg	3.16 \pm 1.40	0
1	250 mg/kg	2.73 \pm 0.98	13.61
2	500 mg/kg	2.78 \pm 0.57	12.03
3	1,000 mg/kg	2.43 \pm 0.64	23.10* β
4	5 mg/kg	0	100

Values are expressed as Mean \pm SD (n=5). *No significant difference compared with control ($P \geq 0.05$). β Significant difference compared with chloroquine ($P \geq 0.05$).

Table 3. Curative effect of varying doses of aqueous leaf extract of *G. barbadense* (GB), Chloroquine (CQ), and distilled water (DW) on parasitemia and survival time due to *P. berghei* infection in mice for a 20-day period.

D	0	1	2	3	4	6	9	14	17	19
C	2.04 \pm 1.67	2.18 \pm 1.91	5.06 \pm 2.31	8.10 \pm 2.95	13.16 \pm 7.47	27.51 \pm 6.31	41.48 \pm 5.15	54.41 \pm 5.48	25.35 \pm 4.71	--
1	5.30 \pm 1.80	3.76 \pm 3.54	8.74 \pm 2.68	6.72 \pm 1.36	18.88 \pm 5.32	37.54 \pm 2.36	48.69 \pm 2.36	59.85 \pm 2.04	--	--
2	4.54 \pm 0.84	3.80 \pm 1.09	6.59 \pm 0.92	6.98 \pm 1.90	11.24 \pm 2.16	30.89 \pm 3.84	44.29 \pm 4.57	53.48 \pm 3.99	12.10 \pm 27.07	--
3	6.21 \pm 6.27	7.18 \pm 6.27	7.57 \pm 2.15	8.72 \pm 1.74	17.63 \pm 12.41	37.44 \pm 14.56	50.04 \pm 10.48	58.95 \pm 4.87	16.09 \pm 32.1	--
4	2.48 \pm 0.49	0.51 \pm 0.29	0.16 \pm 0.10	0	0	0	0	0	0	0

Values are expressed as Mean \pm SD (n=5). D, days of treatment; C, control.

Discussion

The results of the acute oral toxicity (LD₅₀) study of aqueous leaf extract of *G. barbadense* showed no mortality at the maximum dose of 2,000 mg/kg/body weight. The results may indicate that aqueous leaf extract of *G. barbadense* is safe orally (non-lethal) during acute administration as 2 g/kg dose was reported to be the ceiling point for medicinal plant toxicity when administered orally in an acute toxicity study.¹⁷ However, this safety assertion may not be applicable to medicinal plants over a long period. Slight dullness was observed in the animals at above 1,600 mg/kg acute dose administration. This observation is in agreement with the work of Sullivans *et al.* and Wang *et al.* who showed slight toxic effect due to the presence of gossypol.^{18,19}

Gossypium barbadense leaves have been used in combination therapy with *Citrus aurantium* (fruit), also with or without *Ocimum gratissimum* (leaves), in the treatment of malaria in traditional medicine.²⁰

It has also been reported that *G. barbadense* possesses *in vitro* antimalarial activity against *P. falciparum* in combination with *Momordica charantia*.²¹

However, from the present study, we can conclude that when *G. barbadense* is used alone (monotherapy) on mice infected with *P. berghei*, there was only a slight antimalarial activity ($P \geq 0.05$) for the 4-day suppressive test. On the other hand, there was no antimalarial activity for the curative test.

Administration of aqueous leaf extract of *G. barbadense* as monotherapy in mice resulted in only a slight suppressive effect, even at the highest dose of 1,000 mg/kg. It is suggested that *G. barbadense* probably acts synergistically with some components of the other herbs: *Citrus aurantium* (fruit), *Momordica charantia* in the treatment of malaria by traditional healers as mentioned earlier.^{20,21} The mechanism of action of *G. barbadense* is not clear. Therefore, more studies should be carried out to clarify the mechanism of action of *G. barbadense* in combination with other medicinal plants used in the treatment of malaria.

Conclusions

It can be concluded that the use of aqueous leaf extract of *Gossypium barbadense* as monotherapy for malaria has no significant therapeutic effect. Therefore, it is not recommended for use alone in the management of malaria infection as practised by some herbal medicine practitioners.

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