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 > 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.

**Materials and Methods**

**Reagents and chemicals**

Chloroquine phosphate, phosphate buffer saline (PBS), Giemsa’s stain, immersion oil was 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 housed 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 animals. 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).
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) 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 ×100. The total numbers of parasitized erythrocytes were carefully counted as the total number of erythrocytes. The percentage parasitemia was obtained by the mathematical formula:

\[
\text{Percentage parasitemia} = \left( \frac{\text{Number of parasitized erythrocytes}}{\text{Total number of erythrocytes}} \right) \times 100
\]

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}

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

<table>
<thead>
<tr>
<th>Tests</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>Present</td>
</tr>
<tr>
<td>Phenolic acids</td>
<td>Present</td>
</tr>
<tr>
<td>Tannins</td>
<td>Present</td>
</tr>
<tr>
<td>Saponins</td>
<td>Present</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Present</td>
</tr>
<tr>
<td>Anthraquinone</td>
<td>Absent</td>
</tr>
<tr>
<td>Cardiac glycoside</td>
<td>Present</td>
</tr>
</tbody>
</table>

### 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.

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Dose</th>
<th>% Parasitemia</th>
<th>% Suppression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10 mL/kg</td>
<td>3.16±1.40</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>250 mg/kg</td>
<td>2.73±0.98</td>
<td>13.61</td>
</tr>
<tr>
<td>2</td>
<td>500 mg/kg</td>
<td>2.78±0.57</td>
<td>12.03</td>
</tr>
<tr>
<td>3</td>
<td>1,000 mg/kg</td>
<td>2.43±0.64</td>
<td>23.10*β</td>
</tr>
<tr>
<td>4</td>
<td>5 mg/kg</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

Values are expressed as Mean±SD (n=5). *No significant difference compared with control (P>0.05). β Significant difference compared with chloroquine (P<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.

<table>
<thead>
<tr>
<th>D</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>6</th>
<th>9</th>
<th>14</th>
<th>17</th>
<th>19</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>2.04±1.67</td>
<td>2.18±1.91</td>
<td>5.06±2.31</td>
<td>8.10±2.95</td>
<td>13.16±7.47</td>
<td>27.51±6.31</td>
<td>41.48±5.15</td>
<td>54.41±5.84</td>
<td>65.35±4.71</td>
<td>--</td>
</tr>
<tr>
<td>1</td>
<td>5.30±1.80</td>
<td>3.76±2.54</td>
<td>8.74±2.68</td>
<td>6.72±1.36</td>
<td>18.88±5.32</td>
<td>37.54±2.36</td>
<td>48.89±2.36</td>
<td>59.85±2.04</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>2</td>
<td>4.54±0.84</td>
<td>3.80±1.09</td>
<td>6.59±0.92</td>
<td>6.98±1.90</td>
<td>11.24±2.16</td>
<td>30.89±3.84</td>
<td>44.29±4.57</td>
<td>53.48±3.99</td>
<td>12.10±27.07</td>
<td>--</td>
</tr>
<tr>
<td>3</td>
<td>6.21±6.27</td>
<td>7.18±6.27</td>
<td>7.57±2.15</td>
<td>8.72±1.74</td>
<td>17.63±12.41</td>
<td>37.44±14.56</td>
<td>50.04±10.48</td>
<td>58.95±4.87</td>
<td>16.09±32.1</td>
<td>--</td>
</tr>
<tr>
<td>4</td>
<td>2.48±0.49</td>
<td>0.51±0.29</td>
<td>0.16±0.10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

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

### 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.

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### 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<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±3.21; 12.10±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.

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

### Statistical analysis
Results are presented as mean ±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.
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.²⁷ 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.¹⁹,²⁰

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<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.

References