Antiplasmodial activity of *Ajuga bracteosa* against *Plasmodium berghei* infected BALB/c mice

S. Chandel & U. Bagai

*Department of Zoology, Panjab University, Chandigarh, India*

Received August 14, 2008

**Background & objectives:** The present work was undertaken to evaluate antiplasmodial activity of ethanolic leaves extract of traditional medicinal plant *Ajuga bracteosa* in *Plasmodium berghei* infected BALB/c mice along with its phytochemical screening and acute toxicity test to support its traditional use as a remedy for malaria.

**Methods:** Plant extract (ethanolic) 250, 500, 750 mg/kg/day was evaluated in the early and established infection along with repository activity in *P. berghei* infected BALB/c mice through suppressive, curative and preventive test. The phytochemical screening was carried out by employing standard procedures. The acute toxicity was checked through limit test.

**Results:** The ethanolic leaves extract of *A. bracteosa* (250, 500 and 750 mg/kg/day) demonstrated a dose-dependent chemosuppression during early and in established infections, along with significant (*P*<0.05) repository activity. At a concentration of 750 mg/kg/day maximum 77.7 per cent chemosuppression during early infection and 68.8 per cent chemosuppression in repository activity were found. This dose enhanced significant mean survival period up to 27.4 ± 0.46 days in established infection. ELEAB was found to be safe up to 5 g/kg weight when administrated orally in the female BALB/c mice, which is upper limit for oral administration of the test material to rodents. ED$_{50}$ of ELEAB was 300 mg/kg body weight of mice.

**Interpretation & conclusion:** ELEAB inhibited parasitaemia and enhanced mean survival time in a dose-dependent manner upto 750 mg/kg/day dose in treated mice. Further studies need to be done to isolate and characterize active constituents of extract and to study their mechanism of action.

**Key words** ELEAB - *in vivo* - medicinal plant - *Plasmodium berghei*

About 300-500 million clinical cases and 1.2–2.8 million deaths due to malaria occur each year$^{1,2}$. The rising problem of resistance to the classical drugs (chloroquine and sulphadoxine pyrimethamine)$^{3,4}$ and the problem of recrudescence of artemisinin stress the need to look for new antimalarial agents$^5$. Plants and plant products have always been used for the treatment of various ailments$^6$. Quinine and artemisinin both are plant derivatives and have been obtained from *Cinchona* species$^{7,8}$ and *Artemisia annua*$^{9,11}$ respectively.
**Ajuga bracteosa** Wall ex Benth. Lamiaceae (Syn. *A. remota*) is a perennial herb growing wild from Kashmir to Nepal in western Himalaya at an altitude of 1300 m. Leaves of this plant are used as stimulant, diuretic and in the treatment of various diseases like rheumatism, gout, palsy and amenorrhoea12. Its clinical efficacy as an antimalarial has not been established yet. The present study was undertaken to evaluate antiplasmodial activity of ethanolic leaves extract of *Ajuga bracteosa* against *Plasmodium berghei* infected BALB/c mice during early and established infection. Repository activity has also been checked to support its traditional use as a malaria remedy.

**Material & Methods**

**Plant materials:** Present study was undertaken in Parasitology Laboratory, Department of Zoology, Panjab University, Chandigarh. The leaves of *A. bracteosa* were collected in the month of September 2007 from the Mandi district of Himachal Pradesh, India. Voucher specimen (Voucher No. 8895) was deposited in the herbarium of Horticulture University, Solan, where identification of plant was confirmed by Dr N.S. Chauhan (a Taxonomist in the Department of Horticulture and Forestry, Nauni University, Solan).

The dried and powdered leaves of *A. bracteosa* (350 g) were subjected to Soxhlet extraction to prepare ethanolic extract. The solvent extract was evaporated to dryness in Rota evaporator. The dried residue (42 g) was stored in screw capped vials at −4°C. Phytochemical screening of the extract was carried out employing standard procedures13,14.

**Animals:** Swiss albino mice (25–30 g) of either sex were obtained from the Central Animal House, Panjab University, Chandigarh, India. The animals were housed in standard plastic cages and acclimatized for a period of 30 days. The mice were maintained on standard feed and water *ad libitum*. Approval for the study was obtained from the Animal Ethics Committee (CPCSEA/45/1999), Panjab University, Chandigarh.

**Parasite inoculation:** The chloroquine sensitive *Plasmodium berghei* NK-65 strain was maintained *in vivo* in BALB/c mice in our laboratory by weekly inoculation of $1 \times 10^7$ infected red blood cells in naive mice. Experimental mice were inoculated on day 0, intraperitoneally, with 0.2 ml of infected blood in citrate saline having $1 \times 10^7$ *P. berghei* parasitized red blood cells.

**Acute toxicity:** The LD$_{50}$ of the extract was determined by administration of different concentration of extract by oral route in BALB/c mice using the limit test of Lorke15. Extract, (5g/kg body weight/mouse) was administered orally to 5 female mice after a 4 h fast. This concentration is considered as the highest dose to be administered in rodents for the evaluation of acute toxicity of any drug15. Test animals were observed for 14 days for mortality including various sign of toxicity. Histopathological examination of organs (liver, spleen and kidney) was done at the end of study.

**Evaluation of schizontocidal activity on early infection (4-day test):** Knight & Peters 4-day test was employed to evaluate schizontocidal activity in early infection16. Four groups of mice (6 mice in each group) were orally administered 250, 500, 750 and 1000 mg/kg/day of ethanolic leave extract of *A. bracteosa* (ELEAB) in each group respectively and two groups of control mice, were administered chloroquine (5 mg/kg/day) as positive control in one group and equivalent volume of distilled water (0.2ml/mouse/day) as negative control in another group for 4 consecutive days; 24 h after the administration of the last dose, thin blood films were made from the tail of each mouse on fifth day. Smears were fixed in methanol and stained with Giemsa stain. The percentage of parasitaemia suppression was determined by counting the number of parasitized erythrocytes out of 500 red blood cells in random fields under light microscope. Average percentage of parasitaemia suppression was calculated as $100[(A−B)/A]$, where $A$ is the average percentage parasitaemia in the negative control group and $B$ is the average parasitaemia in the test group17.

**Evaluation of the repository activity:** Peters18 method was used to assess the repository activity. Five groups of mice (6 mice in each group), were administered orally with 250, 500 and 750 mg/kg/day doses of the extract and 1.2 mg/kg/day pyrimethamine (positive control) and 0.2ml/mouse/day distilled water (negative control) for 4 consecutive days (D0–D3) respectively. On day 5 (D4), the mice were inoculated with *P. berghei* infected red blood cells. Seventy two hours later, the parasitaemia level was assessed by studying Giemsa stained blood smears.

**Evaluation of schizontocidal activity in established infection (Rane test):** Rane test was employed to evaluate schizontocidal activity of extract in established infection19. On the first day (D0), standard inoculum of $1 \times 10^7$ *P. berghei* infected erythrocytes were injected intraperitoneally into mice. Seventy two hours later, the mice were divided into five groups of six mice each. Different doses of ELEAB (250, 500 and 750 mg/kg/day)
were administered orally to these groups. Chloroquine (5 mg/kg/day) was given to the positive control group and an equal volume of distilled water to the negative control group. The drug/extract was given once daily for 5 days. Thin blood smears were prepared from tail of each mouse for 5 days, to monitor the parasitaemia level. The mean survival time for each group was determined arithmetically by finding the average survival time (days) of the mice (post-inoculation) in each group over a period of 30 days (D0–D29).

Statistical analysis: Data were statistically analyzed using Student’s-test and P<0.05 was considered significant.

Results & Discussion

Plants have remained the ultimate source for the treatment of various ailments since ever20. ELEAB was found to exert significant in vitro antiplasmodial activity with an IC_{50} value of 10µg/ml against chloroquine sensitive *P. berghei* isolates, during schizont maturation inhibition assay as reported in our previous study. Therefore, in vivo antiplasmodial study along with phytochemical screening was undertaken to show its antimalarial efficacy in present study.

Phytochemical screening of ELEAB has shown the presence of alkaloids, flavonoids, steroids, triterpenoids, saponins and tannins like phenolic compounds. Although mechanism of action of these secondary metabolites has not been evaluated in the present study, some of these metabolites have been found to exert their antiplasmodial effect either by elevating red blood cell oxidation or by inhibiting protein synthesis21. The phytochemical investigation and isolation of various compounds from *A. remota* has led to the identification of various compounds like ajugarin I, II an III along with ajugasterone C, ajugalactone, cyasterone, β ecdysone and ergosterol 5, 8-peroxide. Among these compounds ergosterol 5, 8-peroxide has been found to exert significant IC_{50} (8.2 ± 1.1µM) value against chloroquine sensitive (FCA20/GHA) strain of *P. falciparum*22. Further, triterpenoid ergosterol 5, 8-peroxide acts as an antimicrobacterial compound23. This compound has been found to inhibit the growth of protozoan parasite of Trypanosomatidae family such as *Trypanosoma cruzi* and various *Leshmania* species by interfering with the integrity of the cell membrane24. The active involvement of this compound in the plant extract to inhibit plasmodial parasite growth cannot be denied.

The LD_{50} of ELEAB was found to be more than that 5g/kg body weight of naive mice, which proves the clinical safety of extract. Histopathological examination of liver, spleen and kidney did not reveal significant pathological changes. No hepatomegaly/splenomegaly was observed. Size and colour of organs was also comparable to normal tissues. The concentration of 5g/kg in mouse is prescribed upper limit for administration of test material in one dose in rodents according to limit test of Lorke15.

During early infection, oral administration of 250, 500 and 750mg/kg/day concentration of extract caused chemosuppression of 42.4, 63.2 and 77.7 per cent respectively, which was statistically significant (P<0.001) as compared to negative control. The standard drug chloroquine (5mg/kg/day) caused 86.2 per cent chemosuppression which was more significant as compared to extract treated groups (Table). Chloroquine has been used as a standard schizontocidal drug in early and established infection, as it interrupts with

<table>
<thead>
<tr>
<th>Drug/extract</th>
<th>Dose (mg/kg/day)</th>
<th>Schizontocidal activity</th>
<th>Repository test</th>
<th>Mean Survival Time (in days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Average % parasitaemia</td>
<td>Average % suppression</td>
<td>Average % parasitaemia</td>
</tr>
<tr>
<td>ELEAB</td>
<td>250</td>
<td>31.3 ± 0.44**</td>
<td>42.4</td>
<td>12.1 ± 2.19</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>20.0 ± 0.76**</td>
<td>63.2</td>
<td>7.1 ± 2.05*</td>
</tr>
<tr>
<td></td>
<td>750</td>
<td>12.1 ± 2.80**</td>
<td>77.7</td>
<td>5.6 ± 1.17*</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>7.2 ± 0.36*</td>
<td>86.6</td>
<td>-</td>
</tr>
<tr>
<td>Chloroquine</td>
<td>5</td>
<td>7.5 ± 0.44**</td>
<td>86.2</td>
<td>-</td>
</tr>
<tr>
<td>Pyrimethamine</td>
<td>1.2</td>
<td>-</td>
<td>-</td>
<td>4.3 ± 1.55*</td>
</tr>
<tr>
<td>Control (distilled water)</td>
<td>0.2ml</td>
<td>54.4 ± 0.73</td>
<td>-</td>
<td>18 ± 3.35</td>
</tr>
</tbody>
</table>

Data are expressed as standard mean error (SEM) ± for six mice per group when compared with control. P<0.05, **<0.001 compared to control; ELEAB-etanolic leaves extract of *Ajuga bracteosa*
the heme polymerization by forming FP- chloroquine complex. This complex is highly toxic to the cell, thus disrupts membrane function which ultimately leads to parasite cell autodigestion\(^\text{25}\). The highest concentration of extract used (1000 mg/kg/day) showed 86.6 per cent chemosuppression which was similar to that of standard drug chloroquine (5 mg/kg/day), however, 60 per cent mortality was observed with this concentration on day 7. As all natural plant product work in synergistic manner with immune system of host, the higher extract concentration might be downmodulating the natural immune resistance of host to malaria\(^\text{26}\). This concentration was not tested further for repository and curative activity of ELEAB.

ELEAB produced a dose dependent repository activity as 250, 500 and 750 mg/kg/day concentrations and exhibited 32.7, 60.5 and 68.8 per cent chemosuppression respectively, significantly \((P<0.05)\) compared to negative control group. However, standard drug pyrimethamine (1.2 mg/kg/day) was found to exert a considerable higher (76.2\%) chemosuppression \((P<0.001)\) as compared to extract treated groups (Table). For the evaluation of repository activity, the standard drug pyrimethamine was used as reference drug as it prevents DNA replication of parasite by binding to the dihydrofolate reductase (DHFR), which interferes with folic acid mechanism necessary for DNA and RNA synthesis leading to parasite death\(^\text{27}\).

In established infection, the percentage of parasitaemia on day 7 was 15.0, 11.0 and 9.1 per cent for 250, 500 and 750 mg/kg/day concentrations of the ELEAB treated groups respectively, while 55.1 per cent in control and 6.8 per cent in chloroquine treated groups (Fig.). The mean survival time (MST) of the mice in various groups was 15.2 ± 0.19, 21.6 ± 0.48, 27.4 ± 0.46, 29.6 ± 0.14 and 7.2 ± 0.19 days for 250, 500 and 750 mg/kg/day of extract, chloroquine and control groups respectively (Table).

In conclusion, our results showed that ELEAB was not only found to inhibit parasitaemia in dose dependent manner but also enhanced the mean survival time period of treated mice. These findings support the traditional use of plant for the treatment of malaria. It would be worthwhile to isolate its active constituents and characterize their exact mode of action which can be exploited for the treatment of malaria.

**Acknowledgment**

The first author (SC) is thankful to University Grants Commission (CAS programme) for providing fellowship. Authors thank Prof. Karan Vashist and Dr. Maninder Vashist of U.I.P.S, Panjab University, Chandigarh for preparing extract of plant and phytochemical analysis.

**References**


Reprint requests: Dr Upma Bagai, Department of Zoology, Panjab University, Chandigarh 160 014, India
e-mail: upmabagai@yahoo.co.in