The present study was aimed to assess the antiplasmodial properties of crude extracts of *Capparis tomentosa* Lam. The plant materials were collected from their natural habitat and extracted using 80% methanol and non-polar solvents. A *Plasmodium berghei*, which is a parasite that infects mice, was kept up at the Akilu Lemma Institute of Pathobiology laboratory and it was injected into experimental mice. The mice were given 1x10<sup>5</sup> parasites intraperitoneally for initial infection. The extracts were given through oral gavage daily for four days, since the day when infection was commenced. The negative control groups were given the equivalent amount of solvent (vehicle) used to suspend each dose of the extract. For the positive control groups, Chloroquine was given orally as a standard drug. Data obtained from the experiment was analyzed using one way ANOVA. The results indicated that the plant extracts exhibited significant antimalarial activities. Extracts from the plant extract exhibited significant antimalarial activities and prevented body weight loss (P<0.05) but did not prevent PCV reduction in dose dependent manner and mean survival time was not prolonged. The hydro-alcoholic extract of *C. tomentosa* was found to be the most active with a suppression of 78.2% at the dose of 1000 mg/kg. Therefore, the results indicated the potential use of the claimed medicinal plant for the treatment of malaria in primary health care.

**Keywords:** Malaria, *Capparis tomentosa*, Traditional medicine, *Plasmodium berghei*, in vivo.

**INTRODUCTION**

Malaria is a main cause of anemia in infants and teens and pregnant women which results in undesirable birth outcomes such as unplanned abortions, death of fetus, early birth and under-weight births [1]. In many African countries, 30 percent or more of outpatients visit and hospital admissions are due to malaria [2]. Malaria exists in all parts of Ethiopia, except in the central highlands, and 56 million people are at risk [3]. The disease is one of the country’s leading health problems in terms of morbidity, mortality and impediment to socioeconomic development and top ranking in the list of common communicable diseases, consistently ranking in the ten leading disease causes of morbidity and mortality [4]. In Ethiopia, malaria is a major public health problem with an estimated 5-10 million cases and 70,000 deaths each year [5]. Moreover, it leads for approximately 17% of to visit hospitals for consultations, 15% of admission and 29% of serious diseases and deaths [6]. However, as 36% of the population does not have access to health facilities, these numbers could underestimate the real condition. The socio-economic impact of the disease in Ethiopia is serious as it results in low productivity since it keeps a large number of people from work as a result of illness [7]. But quantification of the impact is difficult since most of the victims reside largely in rural areas where social scientists and other researchers do not focus and reach them [6]. Ethiopia is a home of diversified flora which have been widely using as traditional medicine [8]. Traditional medicinal plants have become a vital part of the various traditions in Ethiopia as it has been used since long period of time [9]. On the other hand, 90% of the population in Ethiopia uses traditional medicine for primary health care [10]. Even though these plants are used extensively, a little has been done to evaluate their toxicity and efficacy in a scientific way as source of drugs [11]. *Capparis tomentosa* Lam. is among Ethiopian traditional medicinal plants which have been used to treat different diseases. These plants are traditionally used for antimalarial treatment in Ethiopia [12]. *C. tomentosa* is found widely in local pharmacopoeias throughout Africa [13]. It is traditionally used to cure mental illness, snakebite, headache, erectile dysfunction and infertility (in women). It is also used to cure fever, pneumonia, asthma and chest pains [14]. The plant is also used treat inflammation, convulsion, and also used as anti-rheumatism, anti-jaundice, and anti-malarial agent [15]. Therefore, this study was designed to substantiate the scientific relevance of the claimed medicinal plants, that is, roots extract of *Capparis tomentosa* as antimalarial treatments.
MATERIALS AND METHODS

Plant materials collection

The plant materials used in the present study was collected, from its natural habitat. The choice of plant was made based on the traditional usage of the plant by the local communities as antimalarial agent. The plant was identified and authenticated by a Botanist, at Addis Ababa University, Aklilu Lemma Institute of Pathobiology and a voucher specimen of plant samples were put at the National Herbarium of Addis Ababa University.

Preparation of crude plant extracts

The plant materials were unsoiled, air dried under shade at room temperature and chopped up to small size using a crushing mill (IEC, 158 VDE 066, Germany). The powdered plant parts were weighed up by electronic digital weighing balance (Scietentec balance) and extracted in 80% alcohol and alcoholic solvents in Erlenmeyer flask. The crushed plant materials were soaked separately in alcoholic (non-polar) and 80% methanol (hydro-alcoholic) for 72 hours by shaking using an orbital shaker at 130 rpm. After 72 hours, the extract was separated from the marc by filtration (Whatman filter paper number 1 with pore size 0.7µm). This procedure was done in triplicate. In the non-polar extracts, the solvents were removed by evaporation using rotary evaporator (Buchi Rota vapor, TRE 121, Switzerland) in distillation flask at 45 rpm and temperature 45°C to get the crude plant extracts. The obtained extract was made more concentrated to dryness in a water bath. While the hydro-alcoholic extracts remains was solidified in refrigerator overnight and it was further frozen and dried out in a lyophilizer (CHRIST, 3660 Osterode/harz/ France) at -40°C and vacuum pressure to obtain a freeze dried product. Lastly, the semi-solid crude extracts were then stored in a refrigerator at 4°C until used for the experiment.

Experimental animals and the Plasmodium parasite strain

Experimental Animals

Swiss albino mice (25-38 grams), 6-8 weeks of age acquired in Aklilu Lemma Institute of Pathobiology (ALIPB), were used for the study. They were given a standard diet and tap water ad libitum.

Parasite Inoculation

In evaluating the efficacy of the plant extract, Plasmodium berghei which is chloroquine sensitive strain was used. Plasmodium berghei was obtained from Aklilu Lemma Institute of Pathobiology (ALIPB), Addis Ababa University. Donor mice was prepared by formerly infect with P. berghei having variable parasitaemia. In each experiment blood sample infected with about 1 ml P. berghei was taken from the heart by puncturing it; ethyl ether was used as anesthesia while sacrificing the donor mouse with growing parasitaemia of about 20-30% in such a way that 1 ml blood contains 5x10^7 infected erythrocytes [16]. This was set by calculating the percentage of parasitaemia and diluting 1ml of blood in 4 ml of physiological saline (0.9% NaCl). Each mouse was given intraperitoneally 0.2 ml of this diluted blood which contains 1x10^7 P. berghei infected erythrocytes. To avert inconsistency in parasitaemia, the blood taken from all donor mice was pooled together. The parasites were maintained by serial passage of blood from infected mice to non-infected ones on weekly basis.

Determination of Lethal Dose 50 (LD50)

The acute toxicity studies were carried out as per the OECD guidelines. Acute oral toxicity of each of the crude extract was tested in non-infected mice aged of 6–8 weeks weighing 23-25 grams. Five mice in each group were fasted over night and orally given a dose of up to 5000 mg/kg of the crude extract. The mice were observed for indicators of toxicity such as physical and behavioral change vis-à-vis reduction in their motor, loss of appetite and death for four hours and followed for 24 hours and/or 14 days. The Lethal Dose 50 (LD50) was done between 72 h and 14 days [17, 18].

Anti-malarial Activity Studies

Evaluation of suppressive activities of the plant extracts

In evaluating the crude extracts for its anti-malarial activity, a standard Peter’s four-day suppressive test was employed against CQ sensitive P. berghei ANKA strain in mice [19]. Male Swiss albino mice weighing 25-38 were infected with 10^7 P. berghei and arbitrarily divided into five groups of five mice per cage. Mice infected with P. berghei were divided into three experimental groups and two controlled groups in case of hydro-alcoholic (both in lower and higher doses) and were grouped into six test groups and two control groups (each for chloroquine as a standard drug and dH2O/20% DEMSO as a negative control). The test extracts were prepared in six different doses 100 mg/kg, 200 mg/kg, 400 mg/kg, 600 mg/kg, 800 mg/kg, 1000 mg/kg and Chloroquine at 10 mg/kg in a volume of 0.2 ml and vehicles at 0.2 ml/mouse. Each extract was given as a single dose on daily basis. Extracts and the drug were given via oral route using standard gavage to make sure of safe ingestion of the given extracts and the standard drug [17]. Treating with the plant extract to the experimental mice was commenced after 3 hours of infection on day 0 and resumed daily for four days (i.e. from day 0 to day 3). On the fifth day (Day-4), 24 h after the last dose (i.e. 96 h post-infection), blood sample were taken from tail snip of each mouse [20]. Thin smears were prepared fixed with methanol for 30 seconds and stained with 10% Giemsa solution for 25 minutes. Each stained slide, mainly the thin smear, was examined under the microscope with an oil immersion objective of 100x magnification power to assess the percent suppression of each extract in comparison to the control groups. The microscope had an Ehrlich’s eyepiece showing about 100 red blood cells per field [21]. The parasitaemia level was evaluated by counting minimum of ten fields per slide with 100 RBC in random field of the microscope. Percent parasitaemia and percentage suppression was determined using the following formula [22].

\[
\% \text{ Suppression} = \frac{\text{Parasitaemia in negative control} - \text{parasitaemia in treated group}}{\text{parasitaemia in negative control}} \times 100
\]

Determination of Packed cell Volume

The packed cell volume (PCV) of each mouse was assessed before infection and on day 4 after infection. To this end, blood was taken from tail of each mouse in heparinized microhaematocrit capillary tubes up to 3/4th of their length. The tubes were closed by crystal seal and placed in a microhematocrit centrifuge (Hettich haematokrit) with the closed ends outwards. The blood was centrifuged at 12,000 rpm for 5 minutes. The volume of the total blood and the volume of red blood cells were measured and PCV was determined as follows:

\[
\text{PCV} = \frac{\text{Volume of erythrocytes in a given volume of blood}}{\text{Total blood volume}} \times 100
\]

Determination of Mean Survival Time

Death was recorded every day and the number of days since the time of infection of the mice with the parasite until death was marked for each extract treated mouse in the experiment group and control groups during the follow up period. The mean survival time (MST) for each group was determined using the following formula [23].

\[
\text{MST} = \frac{\text{Sum of survival time (days) of all mice in a group}}{\text{Total number of mice in that group}}
\]

Determination of Body weight Change
The body weight of each mice in all the groups was measured prior to parasite inoculation (day 0) and on day 4 in case of treatment, it was assessed before and after the different doses were given by a sensitive digital weighing balance (Scientech balance).

\[
\text{Mean body weight} = \frac{\text{Total weight of mice in a group}}{\text{Total number of mice in that group}}
\]

Data analysis

Statistical analysis was carried out as mean of variance ± SEM (n = 5) followed by ANOVA using SPSS version 20 for windows software. The data obtained from suppressive studies, mean PCV and body weight prior to and post infection were analyzed among different groups corresponding to each dose levels and vehicle control group at fixed time and overtime (D0 and D4). Mean PCV and body weight prior to and post infection and extract treated were compared by two-tailed paired t-test. To observe any significance differences in the parameters across the two time periods, the average of both parameters was calculated and compared using one way ANOVA followed by Tukey-multiple comparison test. The result was considered statistically significant at 95% confidence level (P-value <0.05).

RESULTS

Table 1: Effect of crude extract of C. tomentosa on PCV and body weight of P. berghei infected

<table>
<thead>
<tr>
<th>Dose mg/kg of bwt/day</th>
<th>PCV Day-0</th>
<th>Day-4</th>
<th>Mean % change</th>
<th>Body weight Day-0</th>
<th>Day-4</th>
<th>Mean % change</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>53.37±0.75</td>
<td>46.71±1.79*</td>
<td>-12.55±2.21ab</td>
<td>28.7±3.24</td>
<td>27.6±3.88</td>
<td>-3.83±2.50ab</td>
</tr>
<tr>
<td>100</td>
<td>57.67±1.38</td>
<td>50.89±1.30*</td>
<td>-11.75±6.23c</td>
<td>25.95±2.25</td>
<td>30.36±2.28</td>
<td>16.99±6.95c</td>
</tr>
<tr>
<td>200</td>
<td>54.09±2.83</td>
<td>50.09±0.95</td>
<td>-7.39±9.69b</td>
<td>31.62±1.83</td>
<td>29.25±1.24</td>
<td>-7.49±11.32a</td>
</tr>
<tr>
<td>400</td>
<td>51.85±1.59</td>
<td>45.27±1.95</td>
<td>-12.69±8.58b</td>
<td>35.51±1.41</td>
<td>35.29±2.53</td>
<td>-0.61±7.85b</td>
</tr>
<tr>
<td>600</td>
<td>52.78±0.96</td>
<td>53.29±0.29</td>
<td>0.9±6.6c</td>
<td>23.56±1.41</td>
<td>26.22±1.4</td>
<td>11.29±509c</td>
</tr>
<tr>
<td>800</td>
<td>55.19±1.09</td>
<td>56.26±2.2</td>
<td>1.93±8.4b</td>
<td>29.1±2.58</td>
<td>34.09±2.16</td>
<td>17.14±6.06c</td>
</tr>
<tr>
<td>1000</td>
<td>58.62±1.90</td>
<td>50.42±3.55*</td>
<td>-14.06±3.5b</td>
<td>26.5±3.0</td>
<td>27.35±1.86</td>
<td>3.20±9.1ab</td>
</tr>
<tr>
<td>QC</td>
<td>53.37±0.75</td>
<td>54.27±1.83</td>
<td>1.68±6.1a</td>
<td>35.3±1.5</td>
<td>37.93±1.03</td>
<td>7.45±1.22ab</td>
</tr>
</tbody>
</table>

* there was significant change between day 0 and day 4 (P<0.05)
Means in a column followed by the same letter do not differ significantly (P>0.05).
Key=Values are presented as M±SEM; n=5; CQ= Chloroquine Phosphate; NC= negative control (0.2ml of dH2O).

Table 2: Antimalarial activities of crude extract of C. tomentosa and mean survival time

<table>
<thead>
<tr>
<th>Doses mg/kg of bwt/day</th>
<th>% Parasitaemia ± SEM</th>
<th>% Suppression± SEM</th>
<th>M ST</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>41.66 ±4.33a</td>
<td>0.00c</td>
<td>7±0.3c</td>
</tr>
<tr>
<td>100</td>
<td>34±4.29ab</td>
<td>17.74±1.96b</td>
<td>9.4±0.60b</td>
</tr>
<tr>
<td>200</td>
<td>31.66±1.66ab</td>
<td>23.4±1.84b</td>
<td>9±0.54b</td>
</tr>
<tr>
<td>400</td>
<td>24.33±2.06bc</td>
<td>41.13±1.95c</td>
<td>9±0.54b</td>
</tr>
<tr>
<td>600</td>
<td>18.7±1.75cd</td>
<td>56.09±3.01d</td>
<td>8.4±0.24c</td>
</tr>
<tr>
<td>800</td>
<td>14.5±2.17cd</td>
<td>64.92±1.45c</td>
<td>8.2±0.24c</td>
</tr>
<tr>
<td>1000</td>
<td>9±0.31de</td>
<td>78.22±0.89f</td>
<td>8±0.31f</td>
</tr>
<tr>
<td>QC(PC)</td>
<td>0.00e</td>
<td>100g</td>
<td>30(+)c</td>
</tr>
</tbody>
</table>

Means in a column followed by the same letter do not differ significantly (P=0.05).
Key: Values are presented as M±SEM; n=5; CQ= Chloroquine Phosphate; PC= Positive control; NC=negative control (0.2ml of dH2O); += maximum days for follow-up

The crude extract of C. tomentosa showed significant suppression in the doses of 100, 200, 400 and 600 mg/kg (P<0.05). Moreover, it exhibited a highly significant (P<0.01) suppression of the parasitaemia at the higher doses (800 and 1000 mg/kg). Paradoxically, the given extract
could not significantly (P>0.05) improve the mean survival of the higher doses extract treated mice (Table 2).

DISCUSSION

The highest suppression in the plant extracts was shown at the maximum dose given i.e. at 1000 mg/kg. This could be due to the reason that the active compounds, vital for the antimalarial effect, mostly occur in small amounts in natural products and effect may not be recognized in lower doses [24]. It is interesting to note that the antimalarial activities observed in both plant extracts are from hydrophilic extracts as these extracts are similar in composition to the water preparations usually applied by traditional healers [25]. Furthermore, in vitro study of dichloromethane extract of C. tomentosa was reported to have lower activity against *P. falciparum* with respect to its leaf, stem and root [26]. The observed differences with the present study might be due to the variation in method of preparation such as solvents used for extraction of the plant material since the secondary metabolites/compounds extracted probably be different. In addition, discrepancies in the *in vitro* and *in vivo* antimalarial effect of some plants as shown in the present study also occurred in other plants. Traditionally renown antimalarial plants like Kigelia africana and Strychnos usambarensis were found to have less activity in vitro test[25]. The possible reason for this phenomenon might be the loss of active compounds in the time storage or the presence of prodrugs that undergo enzymatic transformations in vivo to produce active antimalarial compounds [27] or the antimalarial action of the extract might be due to immunomodulation or intervention in attacking of new erythrocytes by parasites [28]. In addition to the suppression test, different parameters are used when evaluating antimalarial activity of a given plant extract in animal model. In the present study, the crude extracts of *C. tomentosa* prevented PCV reduction but it was not in a dose dependent manner. Similar result was also obtained in other studies [21] in which the extract did not prevent the PCV reduction consistently in dose related fashion. Although the suppressive activity of *C. tomentosa* was good the mean survival time of the treatment group was not prolonged significantly. The possible reason for this might be that the half life of the plant extract in the plasma is relatively shorter in case of *C. tomentosa* [29] in that the compounds responsible for the antimalarial activity are fast acting and rapidly metabolized. Plants posses different classes of secondary metabolites that are essential for antimalarial effect but mainly and various biopotency has been found in alkaloids, quassinoids and trychnoids and *C. tomentosa* was found to have flavonoids, fatty acids, lipids, alkaloids and glucosinolates [13,31] particularly the antimalarial activities of *C. tomentosa* could possibly be due to the presence of phytochemicals in the plant: alkaloids, anthranoids, flavonoids, glycosides, polyphenols, saponins and steroids[32]. These compounds present in this plant extracts may have contributed to the antimalarial activity of these extracts [33]. Biologically the effect of the phytochemical components can be due to the complicated concert of synergistic or antagonistic activities[34]. Moreover, the antimalarial activities exhibited by these extracts may also be due to the presence of other active compounds such as phytosteroids and flavonoids, which are metabolites that have been found to have immunomodulatory activities in other plants [35] which as a result might have some impact on the host-parasite interrelationship. This explanation can be the possible mechanism of action for *C. tomentosa* as its extract, in other study [36] was found to have effect on lymphocytes since immunomodulating agents that is directly enhance activation of lymphocytes and slight increase in the production of multi-potent cytokine IFN-γ. In the oral acute toxicological effect of the crude extract of plant, the lethal dose 50 (LD₅₀) was found to be greater than 5000 mg/kg. Similar result with the present study was also reported in a study elsewhere [36].

CONCLUSION

**Crude extracts of C. tomentosa exhibited antimalarial activity as seen in their capacity to repress P. berghei infection in mice in dose dependent manner. Antimalarial activities found in the present study may support the usage of the plant by traditional healers in the treatment of malaria disease and suggest its ethnopharmacological usefulness as antimalarials. Therefore, the active ingredients responsible for the activity should further be isolated by different extraction methods.**

Acknowledgments

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