**In vivo Antimalarial Activity of Crude Extract of Aerial Part of Artemisia abyssinica Against Plasmodium berghei in Mice**

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**Abstract:** Antiplasmodial activity of the 80% methanolic extract of *Artemisia abyssinica* (*A. abyssinica*) aerial part against chloroquine sensitive *Plasmodium berghei* (*P. berghei*) in mice using the four day suppression test was conducted. A total of thirty mice assigned to 5 groups of 6 animals each were infected with chloroquine sensitive *P. berghei* (*1x10⁷* parasites each) intraperitoneally. The hydro-alcoholic extract (100, 200 and 400 mg/kg), standard drug (Chloroquine, 10mg/kg) and vehicle (distilled water) were administered orally daily for the treatment period. Percent Parasitemia was determined on the 5th day from Giemsa stained smears obtained from tail vein and percent parasitemia suppression was calculated. Daily measurement of rectal temperature was also taken while body weight and packed cell volume (PCV) were recorded on day 0 & 5. The extract produced a dose dependent reduction in parasite density compared to the control group. Percent parasitemia calculation revealed 20.59, 64.7 and 82.4% inhibition at 100, 200 and 400 mg/kg of the extract, respectively (*P*<0.01 at the latter two doses). The result obtained from the present work indicated that *A. abyssinica* has a promising antiplasmodial activity against chloroquine sensitive *P. berghei* in a dose dependent manner for which further research is needed to elucidate its active principles.

**Key words:** *Artemisia abyssinica* • Antiplasmodial Activity • Hydro-Alcoholic • *In vivo* • *Plasmodium berghei*

**INTRODUCTION**

Malaria is an infectious disease caused by the parasite *Plasmodia* and roughly 120 species of *Plasmodium* exist that can be found in the blood of mammals, reptiles and birds. Those parasites affecting humans are exclusively transmitted by the female *Anopheles mosquito*. Thus, it is one of the most serious and widespread diseases encountered by human being [1]. In rare cases transmission can be through accidents such as transfusion, inoculation of infected blood from one person to another, or transfer through the placenta from an infected mother to her unborn child [2]. At present, five members of *Plasmodium* species (*P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale* and *P. knowlesi*) have been shown to cause human malaria and all develop through the same general life cycle which alternates between the human host and the *Anopheline mosquito*. The cycle begins when a *Plasmodium* infected female *Anopheline* mosquito probes for a blood meal and injects the sporozoites into the dermis [3]. Although all the five species of malaria parasites can infect humans and cause illness, only *P. falciparum* is known to be potentially life threatening and some of infected persons die, usually as result of delayed treatment [4]. *P. vivax* is less dangerous but more widespread and the other three species found much less frequently [5].

The antimalarials in common use came from five classes of compounds: the quinolines and arylaminoalcohols, the antifols, artemisinin derivatives, hydroxynaphthaquinones and antibacterial agents [6, 7]. Options for the treatment of acute uncomplicated chloroquine-resistant *P. falciparum* infections in Africa include the use of amodiaquine (AQ) and...
sulfadoxine-pyrimethamine (SP). The choice of these drugs is not only based on their clinical efficacy but also on their affordability to the great majority of African patients, good tolerance and safety for young children and low toxicity risk [8]. *Plasmodium* parasite has extremely complex genome and case with which they can switch between the micro-environments in different hosts and the metabolic changes they require illustrates the difficulty in studying the exact modes of action of the antimalarial drugs on parasite metabolism. In general, resistance appears to occur through spontaneous mutations that confer reduced sensitivity to a given drug or class of drugs. Resistance also develops more quickly where a large population of parasites is exposed to drug pressure since it will remove sensitive parasites, while resistant parasite would survive. Resistance to antimalarials has been documented for *P. falciparum*, *P. malariae* and *P. vivax*. In *P. falciparum*, resistance has been observed in all currently used antimalarials (amodiaquine, chloroquine, mefloquine, quinine and SP) and, even in best known antimalarial compounds, artemisinin derivatives. *P. vivax* has developed resistance rapidly to SP in many areas although the geographical distributions and rates of spread have varied considerably [9, 10]. Malaria vaccines are being developed to achieve both protection of the vaccinated individual and the reduction of malaria transmission through the community. Four stages of the malaria parasite’s life cycle have been the targets of vaccine development efforts. The first two stages are often grouped as pre-erythrocytic stages: these are the sporozoites inoculated by the mosquito into the RBC (erythrocytic stage) and the gametocyte stage, when the parasites emerge from RBC and fuse to form a zygote inside the mosquito vector [11, 12]. There are medicinal plants that have been used to treat malaria for thousands of years. These plants are the source for the two main groups (quinine and artemisinin derivatives) of modern antimalarial drugs. With the problems of increasing levels of drug resistance and difficulties in poor areas of being able to afford and access effective antimalarial drugs, traditional medicines could be an important and sustainable source of treatment [13].

A number of studies have been conducted on the *in vitro* evaluation of the antimalarial activity of Ethiopian traditional medicinal plants. It has been reported that extracts from plants such as *Hagenia abyssinica*, *Bersema abyssinica*, *Artemesia afr*, *Artemesia rehan* and *Ajugaremo*, as well as with *Aniaso menifer* and have significant antimalarial activity against *P. falciparum*. An *in vitro* study previously conducted on extracts from the root of *Asparagus africanus* has shown a counter activity against four different malaria schizont strains. Hence, it was deemed prudent to scientifically investigate the antimalarial activity of these medicinal plants. The genus *Artemisia*, belonging to the *Asteraceae* family, contains a large number of aromatic plants. The genus contains more than 400 species and most of its known species are found predominantly in Asia, Europe and North America [14]. The genus is known for the production of various types of sesquiterpene lactones, including artemisinin which is the best known antimalarial compound [15]. Studies on the phytochemical compositions of the whole plant have shown the presence of alkaloids, flavonoids, triterpenes, tannins and volatile oil [16]. Also 4-hydroxycyclohexanemethanol and [alpha]-terpinolene were reported to be the main components of essential oil of the plant [17]. On the other hand, octa-3,5-diene-2,7-dione, 4,5-dihydroxyl, 1-tetrasacol and butanoic acid, 6-ethyl-3-octyl ester, were identified and most likely responsible for the biological activity of the plant [18]. Many *Artemisia* species have a high economic value in several fields, as food plants and as antihelmintic and antimalaria in medicine. The *Artemisia* is known to contain many bioactive compounds; artemisinin exerts not only antimalarial activity but also profound cytotoxicity against tumor cells [19] and arglabin is employed for treating certain types of cancer in Russia [20]. Over the past decade *Artemisia* species have been used traditionally in various populations [21] and are one of the many traditional medicinal plants of Ethiopia used for the treatment of infectious and non-infectious health problems [18]. Indigenously the traditional use includes rabies, tonsillitis, gonorrhea, cough, syphilis and leprosy [22, 23]. The fresh root in the form of juice is also employed for treatment of epilepsy in domestic animals [24]. It was also reported as being used as a remedy (leaves boiled with milk) for heart troubles and as cough and leave mix with other plants for malaria [25]. In spite of tremendous advances in modern medicine, a large number of medicinal preparations are recommended for the treatment of malaria and quite often claimed to offer significant relief. Attempts are being made globally to get scientific evidences for these traditionally reported herbal drugs. Therefore, the objective of the study include evaluating the *in vivo* antiplasmodial activity of the crude extract of *A. abyssinica* on mice by using 4 day suppressive test.
MATERIALS AND METHODS

Collection and Extraction of Plant Material: After the aerial part of *A. abyssinica* was collected from its natural habitat, it was authenticated by a taxonomist and a voucher specimen (AG001) was deposited at the National Herbarium, Addis Ababa University, Addis Ababa, Ethiopia. The air-dried and powdered plant materials (100g) was extracted by cold maceration technique as described by O’Neill et al. [26] with 80% methanol for three consecutive days at room temperature. The resulting liquid extract was then filtered with filter paper (Whatman No. 3, Whatman Ltd. England) and the organic solvent was removed by evaporation using oven set at 40°C until dried. This procedure was repeated two times. The dried extracts were then transferred into vials and kept in a refrigerator until further use.

Experimental Animals and Parasite Preparation: Swiss albino mice (8-12 weeks, weighing 25-37 grams) of either sex bred at the animal house of Aklilu Lemma Institute of Pathobiology, Addis Ababa University were used. All animals were housed in an air-conditioned room and were allowed to acclimatize for one week before study. They had free access to pellet diet and water *ad-libitum*. The animals were kept at room temperature and were exposed to a 12-h light/dark cycle. All the experiments were conducted in accordance with the internationally accepted laboratory animal use, care and guideline [27]. For in vivo anti-malarial assays of plant extracts, the mouse-infective chloroquine sensitive strain of *P. berghei* maintained in the Aklilu Lemma Institute of Pathobiology, Addis Ababa University were used. The parasites were maintained by serial passage of blood from infected mice to non-infected ones on weekly basis [28].

Animal Management and Grouping: A slightly modified method described by Peters and Robinson, 1992 was used for this test. The mice were divided into five groups of six each and which were all infected with malaria parasites. The negative control was treated with the vehicle (distilled water) used for reconstitution, whereas positive control was treated with chloroquine, 10 mg/kg (CQ10) (the standard antimalarial drug). The other groups were made to receive three different doses of (100 mg/kg, 200 mg/kg, 400 mg/kg) *A. abyssinica* (AA) extract. The vehicle, the plant extract and the standard drug were always administered orally (by gavage). The dose level of the extract were selected to be 100 mg/kg, 200 mg/kg and 400 mg/kg for mice after having considered the safety of the plant based on the information obtained from the toxicity study [29].

Inoculation of Mice: Chloroquine sensitive strain of *Plasmodium berghei* (*P. berghei*) was obtained from the Aklilu Lemma Institute of Pathobiology, Addis Ababa University, Ethiopia. Albino mice previously infected with *P. berghei* having variable parasitaemia were used as donor. The parasitemia of the donor mice was first determined [28]. The donor mice were then sacrificed by head blow and blood was collected by cardiac puncture into heparinized vacutainer tube having 0.5% trisodium citrate (TSC) added as anticoagulant. The blood was then diluted with physiological saline (0.9%). The dilution was made based on the parasitemia of the donor mice and the RBC count of normal mice (4.5x10^6 RBC/ml) [30, 31] in such a way that 1ml blood contains 5x10^7 infected erythrocytes. Each mouse was administered intraperitoneally with 0.2 ml of this diluted blood which contains 1x10^7 *P. berghei* infected erythrocytes. To avoid variability in parasitemia, the blood collected from all donor mice was pooled together. The parasite was maintained by weekly passage to other mice.

Suppressive Test (4 Day): The method described by Trager and Jensen [32] with slight modification was used. Briefly, three hours after inoculation of the parasite, the mice in the three treatment groups were administered with the extracts in doses of AA100, AA200 and AA400 mg/kg for four consecutive days by dissolving the extract in distilled water and tween 80 for each. Two control groups were used in the experiment, the negative control treated with distilled water for four consecutive days while the positive control administered chloroquine phosphate (BNo.6215C1RJB, Ipca laboratory), a standard anti-malarial drug at 10 mg/kg/day. The drug, the vehicle and the extracts used in this study were administered by oral route with the aid of an oral gavage. Treatment was continued for 4 days: parameters rectal temperature was measured daily starting from day 0 (before infection and also after infection) till day 4 while body weight and PCV were taken at day 0 and day 5 using Wintrobe’s method [33]. Then parasitemia was measured on the 5th day.

Packed Cell Volume (PCV) Measurement: PCV was measured to predict the effectiveness of the test extracts in preventing haemolysis resulting from increasing parasitemia associated with malaria, using Wintrobe method described by Gilmour and Sykes [33].
Parasitemia Measurement: Blood smears from tail were applied on Menzel-Glaser microscope slides (Germany), fixed with absolute methanol and stained with 10% Giemsa stain at pH 7.2 for 15 minutes as described by David et al. [28]. The slides were taken out, washed with gentle passage of tap water and dried with the room temperature. The number of parasitized red blood cells (PRBC) were counted using Olympus microscope (CHK2-F-GS, Taiwan) with an oil immersion nose piece of 100x magnification power. Two fields were counted for each slide, average was taken and percentage parasitemia was determined by using the formula described by David et al. and Sanni et al. [28, 34].

Phytochemical Screening: The 80% methanol extract of the plant was screened for the presence of secondary metabolites to relate the antimalarial activity of the plant with the presence or absence of these constituents. Thus, tests for alkaloids, flavonoids, terpenoids, polyphenols and tannins were performed according to procedures described by Sofowara and WHO [35, 36]. The procedures are included in the following methods:

Identification Test for Alkaloids: Two grams of thoroughly ground material was treated in a test tube with 10 ml of 1% Hcl for 30 minutes in a water bath. The suspension was filtered through cotton into test tube and was divided into two parts and, to one part of the solution five drops of Dragendorff’s reagent and to the other part five drops of Mayer’s reagent were added. If the alkaloids are present the test with Dragendorff’s reagent should form a yellowish orange precipitate or a whitish opalescence with the Mayer’s reagent.

Test for Flavonoids: 5ml of dilute NH₃ solution was added to 3ml aqueous filtrate of the sample followed by addition of 2ml concentrated H₂SO₄ and formation of yellow color indicates presence of flavonoids.

Test for Terpenoids: Five ml of extract dissolved in distilled water was mixed in 2 ml of chloroform and 3ml concentrated H₂SO₄ was carefully added to form a layer. A reddish brown coloration of the interface was formed to show positive result for the presence of terpenoids.

Test for Polyphenols: A mixture of one ml 1%FeCl₃ and one ml 1%K3Fe(CN)₆ was prepared immediately before this test. Then, to two ml of filtered solution of aqueous macerate of plant material, three drops of the mixture of the 1%FeCl₃ and 1%K3Fe(CN)₆ was added. The final solution should form a green blue color if it contains phenolic compounds.

Test for Tannins: 2ml of water diluted sample was treated with 3 drops of 10% ferric chloride and formation of bluish-black color indicates presence of tannins.

Statistical Analysis: The values were expressed as mean ± SEM and \( P<0.05 \) was considered significant. The statistical significance was assessed by one-way analysis of variance (ANOVA) followed by Tukey’s comparison test for determining significance using computerized Graphpad Prism version 4.0, Graph pad software, U.S.A. The analysis was performed with 95% confidence interval.

RESULTS

The crude extract of A. abyssinica displayed a very good activity against P. berghei (Table 1). The comparison analysis indicated that only two dose levels (200 mg/kg and 400 mg/kg) of the extract showed statistically significant \( (P<0.001) \) difference on the fifth day parasitemia compared to the negative control. The 100 mg/kg dose level, however, showed a statistically non-significant reduction. Percentage inhibition analysis indicated that inhibition was dose dependent with 20.6%, 64.7 and 82.4% inhibition by A. abyssinica 100mg (AA100), AA200 and AA400 of the extracts, respectively, for the 4 day suppressive test \( (P<0.001 \text{ in the latter two doses}) \) when compared the negative control. When compared amongst themselves, AA 200 and AA 400 showed a significant \( (P <0.001) \) percent parasitemia suppression than AA100. CQ10 had shown a statistically significant \( (P <0.001) \) inhibition compared to controls as well as AA100 and AA200. However, no significant difference was observed with AA400.

Table 1: Percentage parasitemia and percentage inhibition of P.berghei infected mice on the fifth day, for the 4 day suppressive test.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% parasitemia</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle (control)</td>
<td>34±2.113</td>
<td>0</td>
</tr>
<tr>
<td>CQ 10</td>
<td>0.0</td>
<td>100(^{10})</td>
</tr>
<tr>
<td>AA 100</td>
<td>27.17±2.52</td>
<td>20.59(^{11})</td>
</tr>
<tr>
<td>AA 200</td>
<td>12±1.211(^{12})</td>
<td>64.7(^{11})</td>
</tr>
<tr>
<td>AA 400</td>
<td>6±1.16(^{12})</td>
<td>82.4(^{11})</td>
</tr>
</tbody>
</table>

Values are mean ± SEM; n=6 animals in each group; \(^{10}\) against control, \(^{11}\) against AA100, \(^{12}\) against AA200, \(^{10} P<0.001\). (One way ANOVA followed by Tukey’s multiple comparison tests).
A body weight change was observed between days 0 and 4 in all groups of mice treated with crude extract of *A. abyssinica*. Treatment with crude extract of *A. abyssinica* prevented loss of weight associated with the increase in parasitemia level at all the 3 dose levels compared to the negative controls. However, the increase in body weight was not found to be dose dependent, the highest increment being caused by AA100 followed by AA400 and the least was by AA200. There were no detectable differences in preventing weight reduction associated with increasing parasitemia between different doses of the extract as well as between the extract and standard (Table 2).

The effect of the test extracts on body temperature of *P. berghei* infected mice was also observed (Table 3). Only the AA100 and AA200 dose level showed a statistically significant difference on body temperature change when compared to negative control (*P* < 0.01) after the four day suppressive test. The analysis revealed that at AA200 and AA400 doses, the CQ10 and the negative control there was a reduction in rectal temperature. In all these groups there was a relatively comparable non-preventive effect when compared to each other although the result was not statistically significant (Table 3). In the AA100 however the rectal temperature seem to be maintained with only a slight rise at day 4 when compared to the day before infection. There was no statistically significant difference in the mean PCV on days 0 and 4 indicating that the extracts prevented significant PCV reduction (Table 4). The preliminary phytochemical screening of the methanolic extract of the aerial part of *A. abyssinica* revealed the presence of alkaloids, polyphenols, flavonoids, triterpenoids and tannins (Table 5).

**DISCUSSION**

Statistical comparison of the effect of the extract on the study parameters (weight, PCV and rectal body temperature) among groups at fixed time and over time,
have shown that extract of the plant administration did not cause significant change on any of these parameters. The present determination of anti-malarial effect of the A. abyssinica aerial part extract is additional proof of its medicinal values and it can, thus, be assumed that, the extract can be safe at the dose levels used in the study [22-25]. As revealed by phytochemical screening, the hydro alcoholic extract the plant contains different classes of secondary metabolites that have been reported to have antiplasmodial activity in other plants [4, 37]. Tannins [38], alkaloids [39], terpenoids and polyphenols [40] and flavonoids [41] which have been implicated in antiplasmodial activity of other plants were also detected in these plant extracts. This is in agreement with the work of Mossa [16] in which these and several other metabolite where identified. On the other hand, octa-3,5-diene-2,7-dione, 4,5-dihydroxy, 1-tetracosanol and butanoic acid, 6-ethyl-3-octyl ester, were identified to be most likely responsible for the biological activity of the plant [18]. Therefore, the antiplasmodial activity observed in many plants [4, 42] and also in this study could have resulted from single or combined action of these metabolites.

According to Saxena et al. [39] and Ramazan et al. [41] several classes of secondary metabolites are responsible for antimalarial activity, but the most important and diverse bio potency has been observed in alkaloids, quassinoids and sesquiterpene lactones. Alkaloids are one of the major classes of compounds possessing antimalarial activity. One of the oldest and most important antimalarial drugs, quinine, belongs to this class of compounds [43]. The antiplasmodial activity of A. abyssinica might also be attributed to the presence of alkaloids that have also been detected in this plant. The polyphenols present in this plant which have antioxidant effect [44, 45] may also contribute to the antimalarial activity.

The presence of natural antioxidants (flavonoids, phenolic compounds, terpenes and alkaloids) in the aerial part of A. abyssinica partly elaborates the observed effect of plant extract. Further studies are required to establish the phytoconstituents responsible for the antioxidant and anti-malarial activity of the plant and study indicated that antioxidative activity can inhibit haem polymerization as haem has to be oxidized before polymerization and the unpolymerised haem is very toxic for the intraerythrocytic plasmodia [46]. The anti-malarial activities exhibited by this extract may also be due to the presence of other active compounds. These plant contains tannin, terpenoids and flavonoids, which are metabolites that have been proved to possess potential immunomodulatory effects in other plants [43, 47], which as a consequence might have some impact on the host-parasite interrelationship.

Although primate models provide a better prediction of efficacy in human than the rodent models, the latter have also been validated through the identification of several conventional antimalarials, such as chloroquine, halofantrine, mefloquine and more recently artemisinin derivatives [28]. The 4 day suppressive test is one of the main methods of the rodent model which are widely utilized to screen for antimalarial activities [48, 49]. The 4-day test using the P. berghei infected mice model is widely used as a test for the in vivo antiplasmodial activity of potential antimalarial agents, as it provides a preclinical indication of potential bioactivity of the test sample [50]. As the 4 day suppressive test from the results of the in vivo antiplasmodial study would seem to suggest that the extract exhibited suppressive activity clearly in a dose dependent manner. It can be inferred from this result that in P.berghei infected mice the percentage parasitemia measured changed significantly from those in the negative control group. The percentage parasitemia suppression was 20.59, 64.7 and 82.4% for AA100, AA200 and AA400 (P<0.001 in the latter two cases) of the extracts, respectively. A compound is considered active when reduction in parasitemia is ≥ 50% [51] supports the result of parasite inhibition in the present study.

An in vivo antiplasmodial activity can be classified as moderate, good and very good if an extract displayed a percent parasite suppression equal to or greater than 50% at a dose of 500, 250 and 100mg/kg body weight per day, respectively [51, 52]. Based on this classification, the hydromethanolic aerial part extract of A. abyssinica exhibited a good antipalmodial activity. CQ10 inhibited parasitemia to undetectable point and the result is in agreement with other in vivo studies [47]. Aneamia, hypoglycemia, body weight loss and body temperature reduction are the general features of malaria-infected mice [37]. Body weight loss is one feature of rodent malaria infections [53]. The result of the present work, however, showed only a statistically non-significant slight gain in body weight of crude extract administered of P. berghei infected mice. This could possibly be due to depressing effect of the crude extract on feed intake/appetite. In a similar study by Chinchilla et al. [54], mice treated with crude extracts of some plants showed a lower body weight pattern as compared with the non-treated ones which is in agreement with this result.
According to Taylor and Hurd [55] the effect of rodent malaria on PCV as measured by hematocrit was parasite-induced fall down to 43-44%, which occurred approximately 48 hours post-infection. *P. berghei* infected mice suffer from anaemia because of RBC destruction, either by parasite multiplication or by spleen reticuloendothelial cell action as the presence of many abnormal RBC stimulates the spleen to produce many phagocytes [54]. The significant decrease in PCV could be attributed to the presence of antinutritive factors in the crude extract. Plasmodium infection is correlated with the incidence of high destruction of red blood cells, hence anemia which could be life threatening [47]. In this study, the extract of plant prevented significant PCV reduction in a dose dependent manner. A decrease in the metabolic rate of infected mice occurred just before death and was accompanied by a corresponding decrease in internal body temperature [56]. In this study, however, the extract of plant prevented significant body temperature reduction in a dose dependent manner. This could be attributed to the effect of the extract as it may have hypothermic effect on the treated mice.

**CONCLUSION**

The present study demonstrated the antiplasmodial effect of the 80% methanolic extract of *A. abyssinica* aerial part *in vivo* suggesting its ethnopharmacological usefulness. The finding revealed the extract to have a promising antiplasmodial activity against chloroquine sensitive *P.berghei* in a dose dependent manner. Phytochemical screening of *A. abyssinica* showed the presence of flavonoids, alkaloids, tannins, triterpenes and polyphenolic compounds. The presence of those different secondary active metabolites in single or in combination with other components present in the extracts might be responsible for the reduction of the different parameters such as weight, PCV and rectal temperature which are associated with the increasing parasitemia, although it fails to attain significant reduction. Since the present work holds up the traditional use of the plant for antiplasmodial activity, further fractionation of the extract to identify its active principles that are responsible for the aforementioned activities along with their mechanism of actions at molecular level is recommended.

**REFERENCES**


