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Antiplasmodial Activity of *Lantana camara* in Mice Infected with *Plasmodium berghei*

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: To investigate the antiplasmodial activity of *Lantana camara* L. (Verbenaceae) in mice infected with *Plasmodium berghei*.
Study Design: The plant extract was evaluated for its antiplasmodial activity in *P. berghei* infected mice model.
Place and Duration of Study: Department of Pharmaceutical Sciences, Saurashtra University, Rajkot, Gujarat, India, during June 2010 to August 2013.
Methodology: The schizonticidal potential of *L. camara* methanolic extract (LCME, 250 and 500 mg/kg, orally) was evaluated during the early stage (suppressive model), established infections (curative model) as well as the prophylactic model in Swiss albino mice. Chloroquine (CQ, 5 mg/kg) and pyrimethamine (PM, 1.2 mg/kg) were used as a reference standard drugs. The parasitemia levels were measured from thin smears made from tail blood of each mouse employed in the study. The curative activity of LCME was assessed from the mean survival time (MST). Rectal temperature measurement was used as a supportive parameter. Oxidant generation potential of the LCME was also evaluated to elucidate the probable mechanism of action.
Results: LCME exhibited significant antiplasmodial activity in both suppressive and prophylactic

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models. The effect was well-comparable to CQ and PM, respectively. The MST was found much longer in LCME-treated animals than the control ones. The rectal temperature was remained near to normal in these groups. The glutathione level was significantly lowered in LCME-treated groups.
Conclusion: In our study, the LCME exhibited a significant antiplasmodial activity in all three models (viz. suppressive, prophylactic and curative).

Keywords: Antiplasmodial; Lantana camara; four-day suppression; malaria; P. berghei.

1. INTRODUCTION

Malaria is a major public health problem in India though it is both a preventable and treatable disease. As per the WHO estimates 207 million cases of malaria occurred globally in 2012 (uncertain range 135-287 million) and 6,27,000 deaths (uncertain range 4,73,000- 7,89,000); about 80 per cent of these cases were found in African countries and 13 per cent in South East Asia Region (SEAR) countries [1]. India contributes to 61 per cent of malaria cases and 41 per cent of malaria deaths in SEAR countries [2]. About 91% of malaria cases and 99% of deaths due to malaria are reported from high disease burden states namely Northeastern (NE) States, Andhra Pradesh, Chhattisgarh, Gujarat, Jharkhand, Karnataka, Madhya Pradesh, Maharashtra, Orissa, Rajasthan and West Bengal [3].

Plants have invariably been a rich source for new drugs and some antimalarial drugs in use today (quinine and artemisinine) were either obtained from plants or developed using their chemical structures as templates. History shows that plants have been an important source of medicines against malaria with two of the major drugs used in malaria treatment, quinine and more recently artemisinin both derived from plants. These two drugs are now the mainstay of the treatment of severe malaria worldwide [4]. *Lantana camara* L. (Family: Verbenaceae) which is commonly known as wild or red sage. It is the most widespread species commonly found in tropical, sub-tropical and temperate regions [5]. The plant has been used in many parts of the world to treat a wide variety of disorders. Even though, the plant has toxic components, it also found important applications in various illnesses. *L. camara* found use in folk remedies for cancers and tumours. Decoctions were applied externally for leprosy and scabies. It has been claimed that lancamarone, a steroid from the leaves, exhibited potent cardiotonic properties. Triterpenoids isolated from the plants exhibited antibacterial activity [6]. Extracts of the leaves, stems and roots showed larvicidal activity [7]. The flavonoid, lantanoside isolated from the aerial parts of the plant exhibited the antimycobacterial activity [8]. Lantamine, an alkaloid from the stem bark and roots, showed antipyretic and antispasmodic properties comparable to those of quinine [9] but the validity of these claims has not been confirmed.

In this context, we scientifically evaluated the *L. camara* methanolic extract of leaves *in-vivo* against *P. berghei*-infected mice for validating its traditional claim to possess an antimalarial action.

2. MATERIALS AND METHODS

2.1 Drugs and Chemicals

Chloroquine (CQ) and Pyrimethamine (PM) were obtained as a gift sample from the Nectar Drugs, India. All other chemicals and reagents used were of analytical grade.

2.2 Plant Material

Fresh leaves of the plant were collected from the surrounding areas of Girnar, Junagadh, during the month of September and October. The plant leaves were then identified and authenticated by a taxonomist Dr. A. S. Reddy, Department of Bioscience, Sardar Patel University, Vallabh Vidhyanagar, Gujarat, India. A voucher specimen (SU/DPS/Herb/22) of the plant was deposited at the herbarium of the Department of Pharmaceutical Sciences, Saurashtra University, Rajkot. The Leaves were thoroughly washed with distilled water and then shade dried at room temperature, powdered and then passed through sieve (# 40) to get uniform powder. The powdered material (500 g) was then successively extracted with petroleum ether (40-60°C) and methanol by using Soxhlet apparatus. After the residue extraction, solvent was distilled off and excess solvent was completely removed by using a rotatory flash evaporator to get concentrated, then completely dried in freeze drier and stored in airtight container under refrigeration. The

obtained extract (43 g, percentage yield-8.6%) was used for antiplasmodial activity.

2.3 Phytochemical Screening

Phytochemical screening of the crude extract was carried out employing standard procedures and tests to reveal the presence of chemical constituents such as mono and sesquiterpenes, triterpenes, iridoid glycosides, flavonoids, among others [10].

2.4 Animals

The Swiss albino mice were obtained from the Central Animal House of the Department of Pharmaceutical Sciences, Saurashtra University, Rajkot, India. The animals were housed at room temperature (25±1°C) with 50-55% relative humidity and given standard laboratory feed (Pranav Agro, Maharashtra) and water *ad libitum*. The study protocol was approved by the Institutional Animal Ethics Committee of the Department (IAEC/DPS/ SU/1002) and conducted as per the guidelines laid down by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment & Forest, Government of India.

2.5 Parasite Inoculation

All the mice used in the experiment were inoculated intraperitoneally with 0.2 ml of infected blood containing about 1×10^7 *P. berghei* parasitized erythrocytes. This was prepared by determining both the percentage parasitemia and the erythrocyte count of a donor mouse and further by diluting the blood with isotonic saline [11].

2.6 Evaluation of Suppressive Activity on Early Infection (Four-day Suppressive Test)

Suppressive activities of different doses of *L. camara* methanolic extract (LCME) were evaluated by using the method described by Knight and Peters [12]. The mice were inoculated, intraperitoneally, with 0.2 ml of infected blood containing about 1×10^7 *P. berghei* parasitized erythrocytes on day 0 (D0). After parasite inoculum, the animals were randomly divided in to four groups of six mice each. The first group received equivalent amount of distilled water (0.2 ml/kg, orally, negative control). The second and third group received 250 and 500 mg/kg, orally, of LCME respectively. The group four received CQ (5 mg/kg, orally), and served as reference standard group. All the above treatments were carried out daily for four consecutive days (D0-D3). On the fifth day (D4), thin blood smears on slides were made from tail blood of each mouse. The slides were then fixed with methanol and stained with JSB (Jaswant-Singh-Bhattacherji) stain to reveal parasitized erythrocytes. The percentage parasitemia was determined by counting the number of parasitized red blood cells (RBC) out of 200 erythrocytes in random fields under the microscope (40X).

$$\%\ parasitemia = \frac{No.\ of\ parasitized\ RBC}{Total\ no.\ of\ RBC\ counted} \times 100$$

Average percentage chemosuppression was calculated by using the equation; $100\left[\frac{A-B}{A}\right]$ where *A* is the average percentage parasitemia in the negative control group and *B* is the average percentage parasitemia in the test group.

2.7 Evaluation of Prophylactic Activity

The prophylactic activity of LCME was assessed using the method described by Peters [13]. Accordingly, mice were randomly divided into four groups of six mice each. The first group received equivalent amount of distilled water (0.2 ml/kg, orally, negative control). The second and third group received 250 and 500 mg/kg, orally, of LCME respectively. The group four received PM (1.2 mg/kg, orally), and served as reference standard group. All above treatments were carried out for three consecutive days (D0-D2). On the fourth day (D3), the mice were inoculated with *P. berghei*. After 72 hours, the parasitemia levels were assessed in thin blood smears made from tail blood of each mouse.

2.8 Evaluation for Curative Activity

For the evaluation of schizonticidal activity of LCME during established infection, the method described by Ryley and Peters [14] was used. Briefly, twenty-four mice were injected intraperitoneally with standard inoculum of 1×10^7 *P. berghei* infected erythrocytes on the first day (D0). Seventy-two hours later, the mice were randomly divided into four groups of six mice each. The groups were orally administered with distilled water (0.2 ml/kg) to negative control

group, 250 and 500 mg/kg of LCME respectively to treatment groups and CQ (5 mg/kg) to the reference standard group. All above treatments were given to the mice once daily for five days (D0-D4). Thin blood smears stained with JSB stain were prepared from tail blood of each mouse daily for five days to monitor the parasitemia level. The mean survival time (MST) for each group were determined arithmetically by finding the average time (days) of the mice (post inoculation) in each group over a period of 30 days (D0-D29).

2.9 Red Blood Cell Oxidation Studies

Oxidant generation by the different doses of LCME was evaluated by monitoring the generation of reduced glutathione (GSH). GSH was measured by the method described by Ellman [15].

2.10 Measurement of Rectal Temperature

All the experimental animals were checked daily for a reduction in body temperature using rectal thermometers conducted daily for six consecutive days starting from the day prior to the parasite inoculation [16].

2.11 Statistical Analysis

All the data are presented as mean ± S.E.M. The significance of difference in means between control and treated animals for different parameters was determined by using One-way Analysis of Variance (ANOVA) followed by multiple comparisons Dunnett test. A *P*-value of < .05 was considered statistically significant.

3. RESULTS

3.1 Phytochemical Screening

The phytochemical investigation revealed the presence of various classes of chemical constituents such as mono and sesquiterpenes, triterpenes, iridoid glycosides and flavonoids.

3.2 Four-day Suppressive Test

The LCME exhibited a dose-dependent chemotherapeutic effect at different doses employed in our study. The % chemosuppression were 60.04 and 72.66% for 250 and 500 mg/kg of body weight of LCME, respectively. The effects produced by the various doses of LCME were statistically significant (P < .001) compared to control group whereas, it is comparable to that of standard drug (CQ) with a chemosuppression of 83.13% (Table 1).

3.3 Four-day Prophylactic Test

The LCME showed a dose-dependent prophylactic activity for both doses used in the study. There was a significant reduction (P < .001) in the % parasitemia in the LCME-treated group when compared to control group. The % chemosuppression were 62.61 and 74.42% for the corresponding doses 250 and 500 mg/kg of body weight respectively. The standard drug, PM also exerted the significant (P < .001) reduction in % parasitemia of 8.08% with chemosuppression of 81.49% (Table 2).

Table 1. Suppressive activity of *L. camara* methanolic extracts against *P. berghei*-infected mice (4-day test)

Drug/extract	Dose	% parasitemia	% chemosuppression
Control	0.2 ml	48.17 ± 1.30	--
LCME	250 mg/kg	19.25 ± 0.95▲	60.04
	500 mg/kg	13.17 ± 0.88▲	72.66
Chloroquine	5 mg/kg	8.08 ± 0.65▲	83.13

Values are expressed as mean ± S.E.M. ▲P < 0.001 vs. control, n=6, LCME: L. camara methanolic extract.

Table 2. Prophylactic activity of *L. camara* methanolic extracts against *P. berghei*-infected mice (4-day test)

Drug/extract	Dose	% parasitemia	% chemosuppression
Control	0.2 ml	43.67 ± 1.17	--
LCME	250 mg/kg	16.33 ± 0.97▲	62.61
	500 mg/kg	11.17 ± 0.54▲	74.42
Pyrimethamine	1.2 mg/kg	8.08 ± 0.52▲	81.49

Values are expressed as mean ± S.E.M. ▲P < 0.001 vs. control, n = 6, LCME: L. camara methanolic extract.

Table 3. % parasitemia in curative test

Treatment	Days of observation				
	D3	D4	D5	D6	D7
Control	45.17 ± 0.53	50.33 ± 0.80	53.25 ± 0.62	59.08 ± 0.60	66.50 ± 1.13
LCME 250	44.92 ± 0.44	43.67 ± 036	42.58 ± 0.33	41.50 ± 0.29	40.25 ± 0.38
LCME 500	44.58 ± 0.33	40.67 ± 0.67	37.75 ± 0.38	32.58 ± 0.44	26.08 ± 0.65
CQ	44.83 ± 0.28	37.25 ± 0.42	28.92 ± 0.51	21.33 ± 0.44	12.08 ± 0.65

Values are expressed as mean ± S.E.M., n = 6, LCME: L. camara methanolic extract; CQ: Chloroquine

3.4 Curative Test

As shown in Fig. 1, LCME-treated animals showed a dose-dependent reduction in parasitemia levels. Furthermore, CQ-treated group also showed a significant reduction ($P < .001$) in parasitemia level. Paradoxically, the control group showed daily increase in parasitemia level. On day 7, the average parasitemia levels were 40.25, 26.08, 12.08 and 66.5% for LCME 250 mg/kg, LCME 500 mg/kg, CQ and control groups, respectively (Table 3).

Table 4 depicts mean survival time in different groups. LCME-treated groups showed significantly ($P < .001$) longer MST than that of control. CQ-treated group showed the highest MST value of 24.17 days.

3.5 Glutathione Oxidation

The results presented in the Table 5, depicts the values of glutathione oxidation exhibited by LCME (250 and 500 mg/kg doses) and the reference standard drug (CQ). There was a significant reduction ($P < .01$, at 60 min) in the glutathione level observed in LCME-treated group when compared to control group.

3.6 Measurement of Rectal Temperature

The rectal temperature was measured in all experimental animals for five consecutive days and presented in Fig. 2. The rectal temperature was decreased consecutively for all five days (D0-D4) in control group, whereas the rectal temperature was remained near normal in LCME-treated group as well as in CQ-treated group.

Table 4. Mean survival time (MST) of mice received various doses of *L. camara* methanolic extracts against *P. berghei*-infected mice

Drug/extract	Dose	MST (days)
Control	0.2 ml	9.67 ± 0.89
LCME	250 mg/kg	14.67 ± 0.99▲
	500 mg/kg	19.17 ± 0.48▲
Chloroquine	5 mg/kg	24.17 ± 0.79▲

Values are expressed as mean ± S.E.M. ▲P < 0.001 vs. control, n = 6, LCME: L. camara methanolic extract

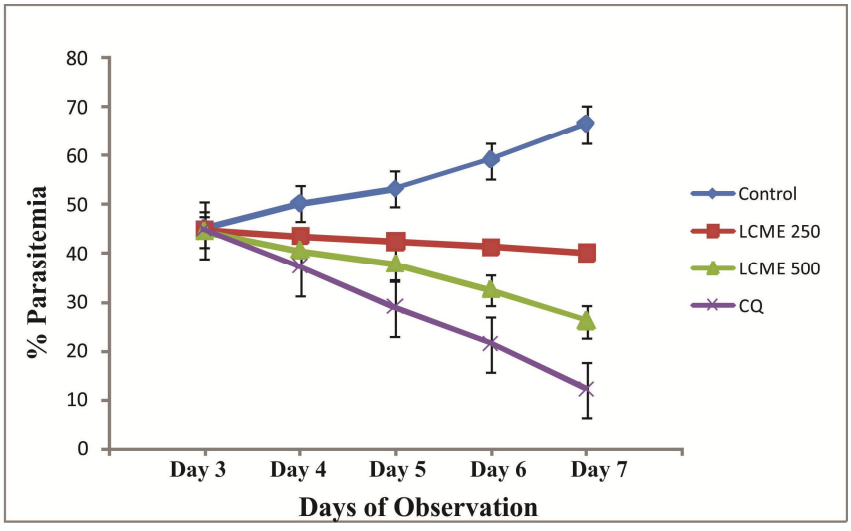

Fig. 1. Antiplasmodial effect of *L. camara* extract on established infection
LCME: L. camara methanolic extract, CQ- Chloroquine

Table 5. Estimation of glutathione levels (nM)

Drug/extract	Dose	0 Min	30 Min	60 Min
Control	0.2 ml	9.01 ± 1.04	8.99 ± 0.98	8.23 ± 0.81
LCME	250 mg/kg	6.70 ± 0.41	5.77 ± 0.43$^{\Delta}$	5.36 ± 0.32$^{\Delta}$
LCME	500 mg/kg	6.07 ± 0.39$^{\blacksquare}$	5.34 ± 0.29$^{\Delta}$	4.89 ± 0.32$^{\Delta}$

Values are expressed as mean ± S.E.M. $^{\blacksquare}P < 0.05$, $^{\Delta}P < 0.01$ vs. control, n = 6, LCME: L. camara methanolic extract

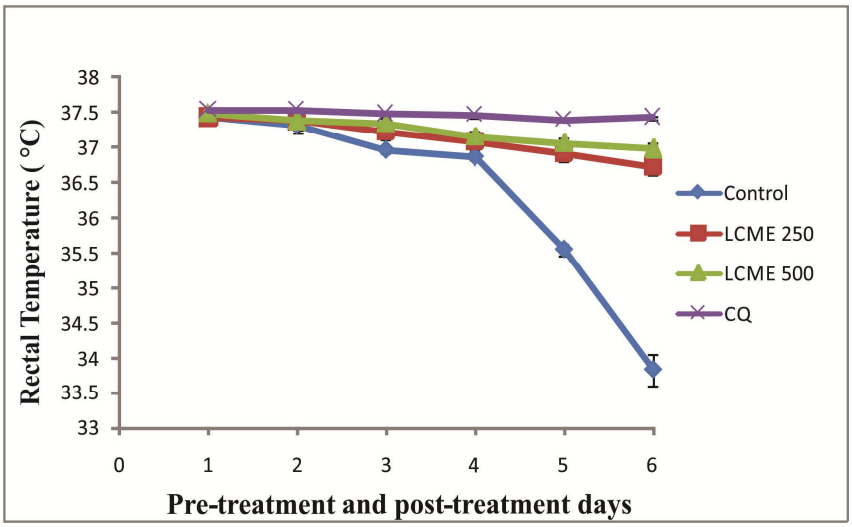

Fig. 2. Rectal temperature measurement for LCME against *P. berghei*-infected mice
LCME: L. camara methanolic extract, CQ- Chloroquine

4. DISCUSSION

Despite over a century of effort and early optimism, a malarial-free world remains as much a distant vision as ever. Multi-drug resistance is one of the most important problems in malaria control over the years. This has led scientists across the world to find other solutions, one of which is investigation of medicinal plants. Sourcing of artemisinine from *Artemisia annua* has further encouraged these scientists to revisit the medicinal plants frequently used in the traditional management of the disease [17].

The Indian traditional medicinal system is the oldest and widely accepted throughout the world. Many plants has been traditionally claimed and currently being used to treat malaria by the traditional peoples. Amongst them, we have identified *L. camara* and employed in our study to evaluate its *in vivo* antimalarial potential against *P. berghei*-infected mice. Many researchers have used this plant to evaluate its *in vitro* antiplasmodial activity. Dichloromethane and methanol extracts of *L. camara* have been reported to exhibit *in vitro* antimalarial activity against the 3D7 (IC_{50} = 5.7 µg/ml) and W2 (IC_{50} = 14.1 µg/ml) strains of *P. falciparum* [18]. The leaves ethanolic extracts of *Lantana* species have been reported to display good activity (IC_{50} < 17.5 µg/ml) against *P. falciparum* [19]. Flavonoids (Linaroside and lantanoside) isolated from *L. camara* were found to be active against *Mycobacterium tuberculosis* [8].

In accordance with these studies, we evaluated *in vivo* antiplasmodial activity of leaves methanolic extract of *L. camara* in mice infected with *P. berghei*. The results of the conventional four-day suppressive test showed that LCME exerted a significant suppressive activity compared to control group in dose-dependent manner. The effect was comparable to that of standard drug CQ. This test is the standard test commonly used for the screening of antimalarial compounds, and the determination of % parasitemia is the most reliable parameter [11]. In another study model, the LCME exhibited significant prophylactic activity when compared to control group. In this group, the % parasitemia was significantly decreased whereas, chemosuppresion was significantly increased

compared to control group. These effects were also comparable to that of standard drug PM.

Similarly, in a curative model, LCME showed a dose-dependent significant reduction in the % parasitemia compared to control group. The MST of LCME-treated animals was significantly increased whereas, decreased in case of control animals. CQ-treated animals also exhibited longer MST. These results are in accordance with the earlier report [11]. Additionally, a daily rectal temperature was measured to predict the effectiveness of the test extract. It is well-known that the body temperature of the mouse decreases in a rapid manner with increasing parasitemia, contrary to the situation in human subjects [16]. We observed the similar effects in our study. It is well-known that plant showing tonic inhibitory action in experimental malaria also possesses potent glutathione reduction activity. In our study also, we performed glutathione assay for the possible mode of action for antiplasmodial action of LCME [11]. The result of this assay revealed that the LCME treatment significantly reduced the glutathione levels as compared to the control group. These results are in accordance with the previous studies and support the traditional claim of *L. camara* as an antimalarial drug.

5. CONCLUSION

In conclusion, the present study shows antiplasmodial potential of *L. camara* plant extract, which justified its traditional claim for the treatment of malaria. Further studies are required to exploit the full therapeutic potential of the plant and to identify an interesting lead compounds along with their molecular mechanism(s).

CONSENT

It is not applicable.

ETHICAL APPROVAL

Authors declared that the whole study protocol was approved by the ethical committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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