In vivo Anti-malarial Activity of Cleome viscosa L. Whole Plant

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ABSTRACT
This study aimed to investigate in vivo anti-malarial effect of methanolic extract of Cleome viscosa whole plant and fractions (n-hexane and ethyl acetate) against early, established and residual malaria infections. The in vivo anti-malarial effect against early infection, curative effect against established infection and prophylactic effect against residual infection were studied in chloroquine-sensitive Plasmodium berghei NK-65 strain infected mice. The results showed that the methanol extract of Cleome viscosa whole plant and partitioned fractions demonstrated significant (p<0.05) dose-dependent anti-malarial activity in all three antimalarial evaluation models thus justifying the inclusion of this plant in recipes for treating malaria.

Key words: Cleome viscosa, Plasmodium berghei, antimalarial activity

INTRODUCTION
Malaria is still one of the most serious health problems facing the developing world today. In Africa, malaria is the 2nd leading cause of death from infectious diseases after HIV/AIDS (WHO., 2010). It is the 3rd leading cause of death for children under five years worldwide, after pneumonia and diarrheal disease (WHO., 2010). In Nigeria, malaria is a major public health problem where it accounts for more cases and deaths than any other country in the world. It is a risk for 97% of Nigeria’s population (MIS., 2010).

The impact of malaria infection is great. Cerebral malaria is one of the leading cause of neurological disabilities in African children (Idro et al., 2010). Cognitive functions before and after treatment for severe malarial illness has been reported to significantly reduce (Fernando et al., 2010). This means the socio-economic consequences of severe and cerebral malaria are far reaching and extend beyond the immediate effects of the disease (Ricci, 2012).

Currently, malarial control efforts are targeted at combating multi drug resistance (Taylor et al., 2009). This has led to attempts at discovering other anti-malarial agents, mainly from plant sources. Traditional herbal medicines have been used to treat malaria for thousands of years in various parts of the world and the first antimalarial drug, the alkaloid quinine was extracted from the bark of the Cinchona species (Saxena et al., 2003). Another ancient medicinal plant is Artemisia annua was rediscovered in China in the seventies as an important source of the antimalarial artemisinin (Klayman, 1985). Artemisinin Combined Therapies (ACT) were formally adopted as first-line treatment of uncomplicated malaria in Nigeria in 2005 (Mokuolu et al., 2007).

Thus, medicinal plants may provide useful drugs or they may supply template molecules on which to base further new structures by organic synthesis (Valli et al., 2012).
Cleome viscosa Linn. (Cleomaceae), a sticky herb with strong penetrating odor, is an annual weed growing in the tropics (Gupta and Rao, 2012). The plant found usefulness in Ayurvedic medicine for the treatment of liver diseases, bronchitis, diarrhea, inflammations and fever (Chatterjee and Prakashi, 1991). The fresh juice of the crush seed is reported for the treatment of infantile convulsions and in mental disorder (Shah et al., 1983). Juice from the plant, diluted in water and given in small quantities is useful for treating fever and leaves for ulcer and wound (Nadkarni, 1982; Kirtikar and Basu, 1984).

Reported biological activities of the plant include larvicidal (Saxena et al., 2000), anti-diarrheal (Parimaladevi et al., 2003), analgesic and antipyretic (Devi et al., 2003) and antimicrobial (Mahady et al., 2006; Sudhakar et al., 2006). The psycho-pharmacological effect (Parimala Devi et al., 2004) and wound healing (Panduraju et al., 2011) has also been reported.

The present studies investigated the in vivo antimalarial activity of methanolic extract and partitioned fraction of the whole plant against early, established and residual malaria infections.

MATERIALS AND METHODS

Plant materials: The plant Cleome viscosa Linn. (Cleomaceae), whole plant was obtained from Jeje area of Ibadan, Oyo State in May 2013. It was authenticated at Forestry Herbarium Ibadan where voucher specimen (FHI number 109669) was deposited. It was air dried for 2 weeks and milled. Two kilogram of the plant material was extracted with 100% methanol for 48 h. The extract was concentrated in vacuo. The dry extract was stored in a refrigerator at 4°C until required. Sixty seven gram of the methanolic extract was partitioned into n-hexane and ethyl acetate.

Animals: The animals (mice), both male and female, 6-7 weeks old (18-27 g) which were used for the experiments were obtained from the Animal House, Institute for Advance Medical Research and Training (IAMRAT), University College Hospital, University of Ibadan, Ibadan. The mice were housed under standard conditions and fed on standard diet (Pfizer Company, Nigeria) ad libitum throughout the study period. They were allowed to acclimatize for fourteen days before the test was commenced, in accordance with the internationally accepted principles for laboratory animals' use and care. All experimental protocols were in compliance with University of Ibadan Ethics Committee Guidelines as well as internationally accepted principles for laboratory animal use and care as found in the US guidelines (NIH., 1985).

Parasite inoculation: Donor mouse blood infected with the P. berghei NK-65 strain was used for inoculum preparation. The desired blood volume was drawn from the donor mouse by cardiac puncture and diluted serially in normal saline. The final suspension would contain about 1×10^6 infected RBC’s in every 0.2 mL suspension. This 0.2 mL suspension was injected into mice intraperitoneally to initiate infection (Ishih et al., 2004). The inoculated animals were then randomized into five mice per group for the experiment.

In vivo antimalarial assays: A series of in vivo antimalarial assays were carried out to evaluate the anti-malarial activities of the methanolic extract of Cleome viscosa whole plant at 100, 200, 400 and 800 mg kg\(^{-1}\) doses as compared to control groups treated with distilled water containing 1% DMSO and reference groups treated with standard drugs (chloroquine 20 mg kg\(^{-1}\) or pyrimethamine 1.2 mg kg\(^{-1}\)). Malaria infection was first established in mice by the intraperitoneal
(i.p.) administration of donor mouse blood containing $1 \times 10^6$ parasites. The percentage parasitaemia was determined by counting the parasitized red blood cells out of 9000 RBC’s in random fields of the microscope and calculated as:

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

Average percentage of chemo-suppression was calculated as:

$$\text{Average percentage of chemo-suppression} = \frac{A-B}{A} \times 100$$

where, A is the mean percentage of parasitaemia in the negative control group and B is the mean percentage of parasitaemia in the test group.

**Four day suppressive activity (early malaria infection):** Suppressive activity of the extract was assessed using the method described by Peters and Robinson (1992). Thirty female/male mice were first inoculated intraperitoneally with 0.2 mL suspension containing $1 \times 10^6$ *P. berghei* NK-65 strain on the first day (D0). After 2-4 h post-infection, the experimental groups were treated orally with 100, 200, 400 and 800 mg kg$^{-1}$ day$^{-1}$ doses of the extract. The reference drug group was treated with chloroquine (20 mg kg$^{-1}$) and the control group received distilled water 0.2 mL kg$^{-1}$ containing 1% DMSO. All the treatments were repeated for the next three days (D1-D3). On the fifth day (D4), thin blood smears were prepared from each mouse and stained with Giemsa’s stain.

**Curative activity (established malaria infection):** Thirty mice were selected and inoculated with 0.2 mL of $1 \times 10^6$ *P. berghei* NK-65 strain intra peritoneally. The mice were then regrouped into six groups of five mice each. Seventy two hours after inoculation, the experimental groups were treated orally with 100, 200, 400 and 800 mg kg$^{-1}$ day$^{-1}$ doses of the extract. The reference drug group was treated with chloroquine (20 mg kg$^{-1}$) and the control group received distilled water 0.2 mL containing 1% DMSO. The treatments were continued daily until D7. Thin smears were made daily from blood collected from tail of mice, stained with Giemsa’s stain and examined microscopically for determination of parasitaemia level. On the sixth day of the infection, the mean percentage suppression of parasitaemia was calculated according to the procedure described by Ryley and Peters (1970) and Saidu et al. (2000). The mean survival time (days) for each group was determined over a period of 30 days post-infection.

**Prophylactic activity (residual malaria infection):** The prophylactic activity of the extracts was assessed using the method described by Peters (1965). The experimental mice were randomly divided into six groups of five mice each. The mice were administered orally with 100, 200, 400 and 800 mg kg$^{-1}$ day$^{-1}$ of the extract, pyrimethamine 1.2 mg kg$^{-1}$ day$^{-1}$ was administered to the reference drug group and distilled water 0.2 mL containing 1% DMSO to the control group. The treatment was given for 3 consecutive days (D0-D2). On the fourth day (D3), all mice were infected with $1 \times 10^6$ *P. berghei* NK-65 strain and kept for the next 3 days. On D7, blood smears were prepared from the tail blood. The percentage of suppression of parasitaemia was then calculated.
Statistical analysis: The statistical analysis were carried out using Statistical Package for Social Sciences (SPSS-17 computer package) and ANOVA (one-way) followed by Duncan’s multiple comparison test. All data was expressed as Mean±SD of triplicate parallel measurements. Differences between means at 5% level (p<0.05) were considered significant.

RESULTS AND DISCUSSION
The *in vivo* anti-malarial activity of four different doses (100, 200, 400 and 800 mg kg\(^{-1}\)) of the methanolic extract of *Cleome viscosa* whole plant administered orally in the early malaria infection test showed a dose-dependent chemo-suppressive activity which ranged from 67.55-86.70% in all group of mice (Fig. 1). A considerably high degree of chemo-suppression was shown by the 400 and 800 mg kg\(^{-1}\) doses which significantly decreased the parasitaemia of the infected mice when compared to control (p<0.05). The suppression of parasitaemia by chloroquine at 20 mg kg\(^{-1}\) day\(^{-1}\) was in agreement with a previous study by Muregi *et al.* (2007).

Also in the established malaria infection test, the chemo-suppression effects for the treated groups were dose dependent ranging from 63.47-86.77% for the various doses tested (Fig. 2). These treated groups of mice also had longer survival period which ranged between 16.00±4.96 and 21.6±4.92 days as compared to the control with survival period of 13.2±1.29 days. The chloroquine-treated group had a mean survival time of 29.0±1.41 days (Table 1). This shows that

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**Fig. 1:** Antimalarial activity of *Cleome viscosa* extract in early malaria infection test

**Fig. 2:** Antimalarial activity of *Cleome viscosa* in established malaria infection test
Fig. 3: Effects on *Cleome viscosa* extract on PCV of mice in established malaria infection test, PCV1: Normal PCV of animal before inoculation, PCV2: PCV of animal after infection, PCV3: PCV of animal on day 7 after treatment, p<0.05 (ANOVA)

Table 1: Mean survival period of mice treated with extract of *Cleome viscosa* in established malaria infection test

| Extract/drug and dose | Survival time (days) | Significance (ANOVA table) |
|-----------------------|----------------------|----------------------------|
| Control (distilled water+1% DMSO) 0.2 mL | 13.20±1.29* |  |
| MCV (mg kg\textsuperscript{-1}) | | |
| 100 | 16.00±4.96* | p<0.05 |
| 200 | 18.75±5.90* | p<0.05 |
| 400 | 19.20±5.16* | p<0.05 |
| 800 | 21.60±4.92 | p<0.05 |
| Chloroquine (mg kg\textsuperscript{-1}) 20 | 29.00±1.41 | p<0.05 |
| MCV: Methanolic extract of *Cleome viscosa* |

Table 2: Effect on *Cleome viscosa* extract on body weights of mice in established malaria infection test

| Extract/drug and dose | W1 (g) | W2 (g) | W3 (g) | W4 (g) | W5 (g) |
|-----------------------|--------|--------|--------|--------|--------|
| Control (distilled water+1% DMSO) 0.2 mL | 18.80±1.30* | 18.20±1.64* | 18.00±2.12* | 16.80±1.64* | 16.40±1.51* |
| MCV (mg kg\textsuperscript{-1}) | | | | | |
| 100 | 19.80±1.92* | 19.00±2.34* | 20.00±1.22* | 20.00±1.87* | 19.60±1.94* |
| 200 | 18.75±0.95* | 18.25±0.95* | 18.00±1.41* | 17.75±1.89* | 18.33±3.05* |
| 400 | 20.40±1.14* | 20.60±1.14* | 20.40±1.51* | 20.20±1.78* | 21.00±3.08* |
| 800 | 21.20±1.30* | 22.20±1.30* | 22.40±1.67* | 22.40±1.14* | 23.80±1.78* |
| Chloroquine (mg kg\textsuperscript{-1}) 20 | 19.25±1.50* | 19.00±0.81* | 20.50±1.29* | 20.25±0.95* | 20.00±1.63* |
| MCV: Methanolic extract of *Cleome viscosa*, W1-W5: Weight of animals treated and untreated in established malaria infection, W1: ns, W2-W5: p<0.05 (ANOVA) |

The ability of the extract to clear the malaria infection as indicated in the percentage chemo suppression has significant effect on the rate of survival of the animals. There was also statistically significant (p<0.05) gain in weight (W2-W5) in the treated mice. However, the control showed a significant loss in weight when compared to the treated group (Table 2).

The packed cell volume (PCV) of animals in all the groups dropped significantly following infection for the 72 h and prior to treatment. However, both chloroquine and plant extract treated groups subsequently recorded an increase in PCV after treatment as opposed to the control group. There was a significant (p<0.05) increase in the PCV in mice treated with 100-800 mg kg\textsuperscript{-1} when compared to the control. Constant decrease in packed cell volume observed in the untreated group of mice could be as a result of increased the level of parasitaemia giving rise to a decrease in red blood cells and hemoglobin (Fig. 3).
The methanol extract of *Cleome viscosa* whole plant showed a dose-dependent prophylactic activity at the different doses given resulting in significant (p<0.05) reduction of parasitaemia in extract treated groups when compared to control. The chemo-suppression shown by the highest dose of the extract (800 mg kg$^{-1}$) was comparable to that of the standard drug, pyrimethamine (1.2 mg kg$^{-1}$) with chemo-suppression of 83.06% (Fig. 4).

For the various fractions tested, the *in vivo* anti-malarial activity of the three different doses tested showed significant dose-dependent chemo suppressive action (Fig. 5 and 6). The ethyl acetate fraction however had better chemo suppressive activity ranging from 68.44-88.44% for dose range of 50-200 mg kg$^{-1}$.

Several compounds have been associated with plants having antimalarial activity. *Sida acuta*, *Pavetta crassipes* or *Mytragyna inermis* were found to exhibit anti-malarial activity based on their alkaloid contents (Karou et al., 2003; Sanon et al., 2003a, b). Azadirachtin isolated from *Azadirachta indica* (Khalid et al., 1989) and the most efficient anti-malarial drug today artemisinin from *Artemisia annua* are terpenoid compounds. Also, flavonoids revealed significant anti-parasitic activities against different strains of malaria, trypanosome and leishmania (Kim et al., 2004; De Monbrison et al., 2006; Tasdemir et al., 2006). *Cleome viscosa* has been shown to contain alkaloids, flavonoids and terpenoid (Elufioye and Onoja, 2015). These chemical compounds may be acting singly or in synergy with one another to exert the anti-malarial effect observed in the plant.
The antiplasmodial activity of other species, *Cleome rutidosperma* has been reported. Moderate activity was reported for the methanol extract of the plant against chloroquine sensitive D10 strain of *P. falciparum* (IC$_{50}$ value of 34.4 μg mL$^{-1}$) with the diethyl ether fraction found to be the most potent (IC$_{50}$ value of 8.1 μg mL$^{-1}$) (Bose *et al*., 2010; Upadhyay, 2015). However, the extract of *C. viscosa* exhibited larvicidal activity against the 2nd and 4th instar larvae of *Anopheles stephensi*, a malaria vector (Saxena *et al*., 2000). Other activities reported for *C. viscosa* which has some correlation with malarial symptoms include analgesic and antipyretic. Fixed oil from the seeds had analgesic activity on acetic acid induced writhing in mice (Ahmed *et al*., 2011), while the methanol extract showed dose dependent antipyretic activity with a significant reduction in normal body temperature and yeast provoked elevated temperature in albino rats (Devi *et al*., 2002).

**CONCLUSION**

This study provides a justification for the inclusion of *Cleome viscosa* in preparations used in ethnomedicine for the management of malaria. Both the methanol extract and the fractions had significant chemo suppressive effects in the three antimalarial models used in the study.

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