Suppressive, Curative and Prophylactic Potentials of *Morinda lucida* (Benth) against Erythrocytic Stage of Mice Infective Chloroquine Sensitive *Plasmodium berghei* NK-65

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Research Article

ABSTRACT

Malaria caused by plasmodium parasite is at the moment the highest killer disease in the tropics, killing mostly pregnant women and children under the age of five years. Efforts are on to developing more potent antimalarials from plants' sources that will be cheaper, without adverse effects, readily available and will be able to replace existing antimalarials that are already facing resistance by plasmodium. *Morinda lucida* is a medicinal plant used in many part of Nigeria for the treatment of malaria and other diseases. This work was set out to investigate the *in vivo* antiplasmodial effect of *M. lucida* in mice. The oral median lethal dose (LD_{50}) was calculated to be 6400 mg kg^{-1} body weight. The *in vivo* antiplasmodial activity of *M. lucida* against early infection, curative effect against established infection and prophylactic effect against residual infection were as well studied in chloroquine-sensitive *Plasmodium berghei* NK-65 –infected mice. The extract at all the doses (100, 200, 400, and 800 mg kg^{-1}, p.o) administered, produced significant ($p<0.05$), dose-dependent activity against the parasite in the suppressive, curative and prophylactic studies. The result of this study showed that *M. lucida* aqueous leaf extract posses potent antimalarial effects and may therefore offer a potential drug lead for development of a safe, effective and affordable antimalarial.

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1. INTRODUCTION

Malaria is undoubtedly one of the world's most deadly infections (Greenwood et al., 2005; Winter et al., 2006). It is a wide spread disease that continues to be associated with considerable morbidity and mortality with significant social and economic impact in developing countries. More than 2 billion people are at risk of malaria (Snow et al., 2005) which is endemic in 91 countries, predominantly in Africa, Asia, and Latin America.

The World Health Organization estimates that 3 million people, and that 41% of the total world population, live in areas with Malaria risk. More than 300 to 500 million clinical cases are reported annually resulting in at least 1.5 to 2.7 million deaths. Approximately one million deaths among children under 5 years old are attributed to malaria alone or in combination with other diseases (WHO, 2009).

In the late 1940s, Chloroquine was massively used and accepted worldwide, but resistance has spread to the vast majority of the malaria endemic regions like Africa, South East Asia and East Asia (Sanket and Sarita, 2009). A combination of the antifolate drugs, sulfadoxine pyrimethamine, soon became choiced antimalaria widely used because it was inexpensive. This drug also faced unacceptable levels of therapeutic failure in many countries in South America, Asia and more recently, Africa (Boland, 2001). Resistance to mefloquine has become an issue in Combodia, Myanmar, and some border areas of Thailand. Whereas in some areas like Brazil and South East Asia where quinine and tetracycline are used in combination for treating uncomplicated malaria, sensitivity to quinine is seriously diminishing (Fidock et al., 2004). Hence, the problem of resistance of plasmodium to antimalarials in the malaria endemic regions of the world has left this region with an unprecedented situation in which the few an affordable treatment options are rapidly losing therapeutic efficacy (Khozirah et al., 2011). Recently, there was a major breakthrough by Chinese researchers in the discovery of the antimalaria, artemisinin (qinghaosu), an endoperoxide sesquiterpene lactone as the active component of *Artemisia annua*, a herb remedy used in Chinese folk medicine for Over 2000 years (Ene et al., 2009). The uses of artemisinin derivatives have been negatively impacted by the observation that high parental doses of certain compounds can produce a limited, unique selective brain stem neuropathy in laboratory animals (Ridley, 2002). Although, clinically relevant artemisinin resistance has not been demonstrated, but it is likely to occur since artemisinin resistance has been obtained in laboratory models (Meshnik, 2002; Sanket and Sarita, 2009).

Discovering new antimalarial compound is more than ever a priority due to the alarming rate of resistance to available drugs and the limited number of effective antimalarials (Peter and Antoli, 1998). Plants are usually considered to be possible candidates as alternative and rich source of new drugs. Majority of the populations in many tropical countries depend on traditional medical remedies using herbs (WHO, 2002; Zirihi et al., 2005).

Different parts of *Morinda lucida* benth have been reported to posses medicinal properties. Oliver-Bever (1986) reported the use of a weak decoction of the stem bark to treat severe

jaundice. *Morinda lucida* leaf extract was also reported to posses hypoglycemic, and trypanocidal activity (Taofeeq et al., 2010).

Though many, people living in rural part of Nigeria traditionally treat malaria by drinking aqueous leaf extract of *Morinda lucida*, there is still paucity of literature on its antimalarial activity. At the moment, there is also no report in literature regarding its suppressive, curative, and prophylactic potential on erythrocytic stage of mice infective chloroquine sensitive *Plasmodium berghei* NK-65.

Hence this study was undertaken to scientifically validate the claim or prove otherwise the folkloric believe on *Morinda lucida*, as well as to evaluate its suppressive, curative, and prophylactic efficacy against *P. berghei*.

2. MATERIALS AND METHODS

2.1 PLANT MATERIALS AND PREPARATION OF PLANT EXTRACT

The plant used in this study was collected at Anyigba, Nigeria, where it is being used by the local population to prepare antimalarial herbal teas which are taken orally for 1 week or longer to treat malaria fever.

The specimen was authenticated by Dr. Patrick Ekwendo, a taxonomist at the herbarium section of the Department of Biological Sciences, Kogi State University, Anyigba, Kogi State, Nigeria. Anyigba lies between longitude 7° 10'E and Latitude 7° 37'N.

The leaves of *M. lucida* were air dried under shade. Considering the fact that people in Nigeria usually use water to prepare the herbal remedy, aqueous infusions of the plant was prepared by cold maceration of 200 g of the pulverized plant material in 1000 mL of distilled water. It was kept in the shade for 48 hours after which it was filtered. The filtrate was collected in a beaker, the water was allowed to evaporate over a water bath to yield the extract concentrate (Balogun et al., 2009).

2.2 PHYTOCHEMICAL SCREENING

Preliminary qualitative and quantitative phytochemical analysis of aqueous extract of M. lucida was carried out using standard methods adopted in similar surveys (Harbone, 1983).

2.3 PARASITE

To test for the antimalarial activity of aqueous extract of M. lucida, Mouse-infective Chloroquine-sensitive strain of Plasmodium berghei NK-65 was obtained from the Laboratory of the World Bank sponsored malaria Vaccine development project, Department of Biochemistry, Ahmadu Bello University, Zaria. The parasites were kept alive by continuous intraperitoneal passage in mice every 4 days (Adzu and Haruna, 2007). These infected mice were used for the study. Before the study began, one of the infected mice was kept and observed to produce disease symptoms similar to human infection (English, 1996).

2.4 ACUTE TOXICITY TEST/LD$_{50}$ DETERMINATION

The extract was administered at different doses to the mice randomly divided into seven groups of six mice each according to the method of Aliu and Nwude, (1982). Administration of extract was done orally with a canula attached to a graduated syringe. The animals were observed for 24 h for signs of toxicity. The LD50 was then calculated (Aliu and Nwude, 1982).

2.5 ANTIPLASMODIAL STUDIES

2.5.1 SUPPRESSIVE TEST

The Peters' 4 day suppressive test was adopted against chloroquine sensitive Plasmodiun berghei NK-65 infection in mice (Peters, 1967). Swiss albino mice weighing 18-25 g were inoculated by intraperitoneal (I.P) injection with standard inoculum of plasmodium berghei with 107 parasitized erythrocytes. After parasitaemia was confirmed, the mice were then randomly divided into 6 groups made up of 6 mice per group and treated for 4 consecutive days using 100, 200, 400 and 800 mg extract per kg body weight. The extract were administered to the mice orally at a single dose per day.

Two control groups were used; the positive control group was treated daily with 5 mg chloroquine per kg body weight, while the negative control group was given an equivalent volume of sterile distilled water.

On day 5 of the experiment, blood was collected from the tail vein of each mouse and smeared on to a microscope slide in order to make a thin film (Saidu et al., 2000).

The blood films were fixed using methanol, it was stained with 10 % Giemsa at pH 7.2 for 10 minutes and parasitaemia examination was done using the microscope (X100 magnification). The percentage parasitaemia was calculated for each dose level by comparing parasitaemia in infected control (untreated) group with those of treated; the result was presented as a percentage.

2.5.2 EVALUATION OF SCHIZONTOCIDAL ACTIVITY OF *M. LUCIDA* ON ESTABLISHED INFECTION (CURATIVE OR RANE TEST)

The curative potential of *M. lucida* was done employing the method described by Ryley and Peters (1970). The mice were injected intraperitoneally with standard inoculum of 107 P. berghei NK 65 infected erythrocytes on the first day (day 0). After 72 hours, and following comfirmation of parasitaemia, the mice were divided into 6 groups of six mice per group. These groups were treated with the prepared leaf extract of *M. lucida* (100, 200, 400 and 800 mg/kg/day), chloroquine (5mg/kg/day) was given to the positive control and an equal volume of distilled water was given to the negative control group. The treatment lasted for 5 days at a single dose per day after which blood smears were collected and examined microscopically to monitor the parasitaemia level.

2.5.3 EVALUATION OF PROPHYLACTIC ACTIVITY OF *M. LUCIDA* (REPOSITORY TEST)

The method of Peters (1967) was adopted in the evaluation of the prophylactic potential of M. lucida. The mice were randomly divided into 6 groups with 6 mice in each group. Negative control group were given an equivalent volume of distilled water kg-1 body weight orally. While the positive group was given 5 mg chloroquine per kg body weight intraperitoneally, the experimental groups were administered 100, 200, 400 and 800 mg extract kg-1 body weight.

All treatments were initiated on day 0 and continued until day 4; the mice were all infected with the parasite. Blood smears were then made from each mouse 72 hours after treatment. Increase or decrease in parasitaemia was then determined.

2.6 STATISTICAL ANALYSIS

Values were expressed as Mean ± SEM. The one way ANOVA test was used to analyze and compared the results at a 95% confidence level. Values of $p < 0.05$ were considered significant.

3. RESULTS AND DISCUSSION

3.1 DETERMINATION OF MEDIAN LETHAL DOSE (LD$_{50}$)

In the acute oral toxicity study, the LD50 of the leaves of Morinda lucida was found to be 6400mg/kg. The only observed signs of toxicity of the extract were paw licking, watery stool, and depression.

3.2 PHYTOCHEMICAL SCREENING OF PLANT EXTRACT

Result of phytochemical screening showed predominance of Alkaloids and flavoniods (12.00 and 9.40 mg/g) among the phytochemicals tested.

3.3 EVALUATION OF ANTIPLASMODIAL ACTIVITY

The in vivo antiplasmodial activity of aqueous leaf extract of *M. lucida* was carried out in Plasmodium berghei NK-65 parasitized mice.

The extract showed a significant chemosuppression of up to 85.05 % (Table 1) following a 4 day treatment with 800 mg/kg extract compared with the infected untreated group. This chemosuppressive effect was in a dose dependent order. Chloroquine gave the highest percent chemosuppression of 95.35 %. In all the groups that received treatment, parasitemia decreased with increase in chemosuppresion.

Table 1. Suppressive effect of aqueous leaf extract of *M. lucida* on parasitaemia in mice

Animal groups	Treatment	Parasite count	% suppression
A	Sterlie distilled water	6.02 ± 0.32^d	-
B	Extract 100 mg kg^{-1}	4.11 ± 0.19^c	31.73
C	Extract 200 mg kg^{-1}	3.00 ± 0.27^c	50.17
D	Extract 400 mg kg^{-1}	1.05 ± 0.10^b	82.56
E	Extract 800 mg kg^{-1}	0.90 ± 0.08^b	85.05
F	Chloroquine 5 mg kg^{-1}	0.28 ± 0.07^a	95.35

Values are expressed as mean ± SEM, n = 6
Values with different superscript are statistically significant (p<0.05)

The result for the curative and prophylactic study also gave a result similar to that noticed during the suppressive test (Table 2 and 3 respectively). There was no significant difference (P<0.05) in chemosuppressive potentials of the extract at 400 and 800 mg/kg body weight (Table 2). There was also no significant difference between doses of 100 and 200 mg/kg body weight (Table 2).

Table 2. Curative effect of aqueous leaf extract of *M. lucida* on parasitaemia in mice

Animal groups	Treatment	Parasite count	% cure
A	Sterlie distilled water	12.02 ± 0.57^d	-
B	Extract 100 mg kg^{-1}	5.31 ± 0.80^c	55.97
C	Extract 200 mg kg^{-1}	4.02 ± 0.64^c	66.67
D	Extract 400 mg kg^{-1}	2.98 ± 0.22^b	75.29
E	Extract 800 mg kg^{-1}	2.00 ± 0.12^b	83.42
F	Chloroquine 5 mg kg^{-1}	0.26 ± 0.07^a	97.84

Values are expressed as mean ± SEM, n = 6
Values with different superscript are statistically significant (p<0.05)

In the prophylactic test, no significant difference was observed between doses of 100 and 200 mg/kg body weight (Table 3). But it appeared from the result of this experiment that Morinda lucida leaves have more curative potential than its prophylactic ability (Table 2 and 3).

Result of the oral acute toxicity study (LD50) of *M. lucida* aqueous leaf extract revealed that *M. lucida* is safe for consumption considering the very high LD50 recorded in this work. The death of 2 mice observed in the negative control group during parasitaemia study would have been due to the parasite and not the extract.

Table 3. Prophylactic effect of aqueous leaf extract of *M. lucida* on parasitaemia in mice.

Animal groups	Treatment	Parasite count	% Prophylaxis
A	Sterlie distilled water	9.02 ± 0.80^d	-
B	Extract 100 mg kg^{-1}	6.03 ± 0.66^c	33.15
C	Extract 200 mg kg^{-1}	5.20 ± 0.24^c	42.35
D	Extract 400 mg kg^{-1}	3.90 ± 0.30^{bc}	56.76
E	Extract 800 mg kg^{-1}	2.69 ± 0.11^b	70.18
F	Chloroquine 5 mg kg^{-1}	0.98 ± 0.09^a	89.14

Values are expressed as mean ± SEM, n = 6
Values with different superscript are statistically significant (p<0.05)

In an acute oral toxicity study, *Morinda lucida* leaf extract was documented to be non-lethal in rats at 2000 mg/kg body weight (Adeneye and Agbaje, 2008).

With reference to the current LD_{50} values based on acute oral toxicity recommended by the Globally Harmonized system of classification and Labelling of chemical (Anonymous, 2003), $LD_{50} > 5000$ mg/kg was not classified, had no specified label and said not to be harmful when swallowed. Hence, the LD_{50} of 6400 mg/kg is an indication that the extract may be safe for human consumption, therefore, confirming the folkloric claim that *Morinda lucida* leaf is not harmful. Also the high safety profile may have been responsible for its wide spread use in different ethno-therapeutic interventions.

The phytochemical screening of the aqeous leaf extract of M. lucida revealed the presence of alkaloids and flavonoid as the predominant secondary metabolite. Therefore, the observed antimalaria activity in the extract treated group may be attributed to its high alkaloid and flovonoid contents. Previous works have also shown the antimalaria activity of alkaloids and flavonoids in plants (Balogun et al., 2009; Okokon et al., 2005). The antimalarial activity observed in this study could be due to single or combined effect of these compounds although no active principle has been identified.

Flavonoids are well known for their diverse physiological properties, which include anti-inflammatory, anti-carcinogenic and anti-parasitic properties (Hilou et al., 2006). Plasmodium berghei NK 65 is used in predicting treatment outcomes of any suspected antimalarial plant due to its high sensitivity to chloroquine making it the appropriate parasite for this study (Peter and Anatoli, 1998).

The significant chemosuppression noticed in the extract treated groups (groups D and E in particular) on day 5 (Table 1) is in agreement with the traditional use of the plant as a herbal medication against malaria in many parts of Nigeria.

The aqueous leaf extract of *M. lucida* exerted a dose dependent chemosuppressive effect against plasmodium berghei. The extract gave a significant (P< 0.05) chemo suppression of 31.73% for the low dose of 100 mg/kg and 85.05% for the highest dose of 800 mg/kg when compared to the control. Although there was no significant difference (P<0.05) in chemosuppression between the group that received 400 mg/kg and 800 mg/kg. A dose of 400 mg/kg might as well be enough to bring about the desired effect. The observed higher

efficacy of the standard drug, chloroquine (95.35%) which was higher than extract treated groups may in part be due to non selectivity of the extract or slow absorption and poor bioavailability of the crude extract. Adzu and Haruna, (2007) also made similar observation. In the curative study, the aqueous extract of *M. lucida* leaves produced a dose dependent reduction in parasitaemia levels in the extract treated groups; there was also a similar reduction in the chloroquine treated group (positive control). This finding is sufficient to say that M. lucida leaf extract has therapeutic efficacy against established malaria parasite. Present findings are consistent with earlier reports by Odeku et al. (2008), and Idowu et al. (2010) who demonstrated the curative potentials of Nigeria medicinal plants against established plasmodial infections.

In the prophylactic study, the aqueous leaf extract of *M. Lucida* significantly (P< 0.05) exerted a dose dependent reduction in level of parasitaemia in the extract treated groups, while the standard drug chloroquine gave the highest effect. This slight difference noticed between the chloroquine treated group and extract treated groups would also have been due to the crude nature of the extract. The results also indicates that the leaf extract of M. lucida possess blood schizonticidal activity as evident from the chemosuppression obtained during the 4 day early infection test.

4. CONCLUSION

The results presented herein suggest that the aqueous leaf extract of M. lucida is safe and possesses potent anti-malarial activity which justifies its continuous use in traditional medicine as an antimalarial remedy.

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