

Antiplasmodial Activity of Aqueous leaf Extract of *Mucuna Pruriens* Linn in Mice Infected with *Plasmodium Berghei* (Nk-65 Strain)

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ABSTRACT

The antiplasmodial activity of the aqueous leaf extract of *Mucuna pruriens* (*M. pruriens*) was evaluated against *Plasmodium berghei* NK-65 strain in mice. The plant was selected based on their traditional claims for treatment of fever and other malaria related diseases in southeastern region of Nigeria. An aqueous leaf extract (90 – 270 mg/kg) was investigated for antiplasmodial activity against Chloroquine-sensitive *Plasmodium berghei* infections in mice. The antiplasmodial activity during early and established infections as well as prophylactic action of the plant in blood was investigated. Chloroquine (10 mg/kg) and pyrimethamine (1.2 mg/kg) were used as positive controls. The extract (90 – 270 mg/kg) dose dependently reduced parasitaemia induced by Chloroquine sensitive *Plasmodium berghei* infection in suppressive, prophylactic and curative models in mice. The extract at these doses caused 60.06 – 71.75% inhibition of parasitaemia in the suppressive test, 65.97 – 84.38% parasitaemia inhibition in prophylactic test and a mean survival time of 16 – 30 days representing 64.41– 89.71% inhibition of parasitaemia in the curative test. These reductions were statistically significant ($P < 0.05$) comparable to that of the standard drug used (Chloroquine and Pyrimethamine). These results show that the aqueous leaf extract of *M. pruriens* possesses significant ($P < 0.05$) antiplasmodial activity which confirms its use in folkloric medicine in the treatment of fever and other malaria-related disease.

INTRODUCTION

Malaria still remains one of the most deadly infectious diseases in tropical and subtropical regions of the world despite various control programmes. Each year, there are about 216 million episode of acute malaria illness, 655,000 mortality and 91% occurred in the African region (CDC, 2012). Nigeria alone accounts for a quarter of the malaria burden in Africa (Amajoh, 2012). Almost 100% of the population is at risk for malaria infection and approximately 50% of the population will experience at least one episode each year. The social and economic burdens of malaria are also significant: malaria reduces the GDP of Nigeria by approximately 1% annually and is the leading cause of absenteeism. The fight against malaria still depends on the chemotherapy and the reduction/prevention of human anopheles mosquito contacts through the use of insecticides treated bed nets

(ITN), insecticides and environmental care (Titanji *et al.*, 2008). Antimalarial drug resistance has emerged as one of the greatest challenges facing malaria control today. The situation is worsening with the discovery of new plasmodium specie that can infect human (Ollomo *et al.*, 2009). Thus it is important to search for new anti-malarial compounds that kills parasite. Plants have been found to provide useful clues for potential anti-parasitic compound (Ogbadoyi *et al.*, 2007). Nigeria is blessed with such medicinal plants; therefore it is important to scientifically exploit this gift of nature to the fullest for the benefit of mankind. *Mucuna pruriens* (Linn) Family leguminoseae is an annual climbing legume of the tropics, 3-18m high with long, thin branches and broadly ovate leaves. All parts of *Mucuna Pruriens* possess valuable medicinal properties (Caius, 1989). *M. pruriens* are used to treat various ailments such as diabetes, cancer, helminthiasis, diarrhea, elephantiasis, ulcers, impotency, snakebite and Parkinson's disease (Tripathi and Upadhyay, 2001; Rajeshwar *et al.*, 2005; Sathiyarayanan and Arulmozhi, 2007).

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M. pruriens locally known as agbala (Igbo), werepe (Yoruba) and Karara (Hausa), is locally used in southeastern part of Nigeria for the treatment of fever and other malaria-related diseases. The antiplasmodial activity of aqueous leaf extract of *M. pruriens* was designed to validate the claim of its use locally using mice infected with the rodent malaria parasite *Plasmodium berghei* NK-65 strain.

MATERIALS AND METHODS

Plant collection and identification

Fresh leaves of *M. pruriens* were collected in October from Nnewi town, Anambra State, Nigeria. The plant was identified by Mallam U S. Gallah, a herbarium keeper in the Department of Biological Science, Ahmadu Bello University Zaria, Nigeria, where a voucher specimen already exists (669).

Plant extraction

The leaves were cleaned, air-dried at room temperature and crushed into coarse powder using pestle and mortar. One hundred grams of the powdered material was macerated with distilled water (800 ml) for 24 h. The mixture was then sieved first with cheese cloth and then with filter paper (Watman № 1). The liquid filtrate obtained was concentrated to dryness in water bath at 45°C. The extract was stored at 4°C until use.

Phytochemical screening

The aqueous leaf extract of *M. pruriens* was subjected to qualitative and quantitative phytochemical investigation according to standard procedures (Trease and Evans, 1989).

Experimental animals

Swiss albino mice of both sexes were used for these experiments. They were obtained from the animal house, faculty of pharmaceutical science, Ahmadu Bello University Zaria. The animals were housed in a well-ventilated room and fed with standard diet (Vital feed) and water ad libitum.

Acute toxicity studies

The minimum lethal dose (LD₅₀) of the extract was determined via oral route (p.o) using Lorke's (1983) Method. The extract was administered with dose levels ranging from 10 – 5000 mg/kg. Toxicity signs such as death, behavioral changes were observed and recorded within 48 hours after administration of the plant extract.

Parasites strain and inoculums

Chloroquine-sensitive *Plasmodium berghei* (NK-65) was a gift from Dr. T.O Olurise, Department of Pharmacology and therapeutics, Faculty of Pharmaceutical science, Ahmadu Bello University, Zaria and maintained in mice through weekly passage. For the study, a donor mouse with a rising parasitaemia of 20 - 30% was sacrificed and its blood was collected from the jugular vein. The blood was diluted with 3.8% sodium citrate solution so

that each 0.2 ml contained approximately 10⁷ infected red cells (Peter and Anatoli, 1998; David *et al.*, 2004; Dikasso *et al.*, 2006). Each animal received inoculums of about 1 × 10⁷ *P. berghei* parasitized erythrocytes per body weight, which is expected to produce a steadily rising infection in mice.

Monitoring of infection

Thin films stained with Giemsa stain were prepared from the tail blood of each mouse. The parasitaemia level was determined by counting the number of parasitized erythrocytes out of 500 erythrocytes in random fields of the microscope.

EVALUATION OF IN VIVO ANTIPLASMODIAL ACTIVITY OF THE EXTRACTS

Evaluation of suppressive activity of the extract (4-day test)

The schizonticidal activity of the extract and chloroquine against early *P. berghei* infection in mice was determined using the method described by Peters *et al.*, (1993). Twenty mice were selected and inoculated intraperitoneally with infected blood suspension (0.2 mL) containing 1 × 10⁷ infected erythrocytes. The mice were randomly divided into five groups (I-V) of four mice each; 2 hours later the animals were administered via p.o with 90, 180, 270 mg/kg/day of the extract, 10 mg/kg/day chloroquine (positive control) and 8 mL/kg normal saline respectively. All the administration continued for four consecutive days (D0-D3). On the fifth day (D4), the parasitaemia level was assessed by blood smears. The percentage suppression of the parasitaemia was calculated by the following formula:

$$\% \text{ Suppression} = \frac{\text{Parasitaemia in negative control} - \text{Parasitaemia in study group}}{\text{Parasitaemia in negative control}} \times 100$$

Evaluation of prophylactic (repository) activity of the extract

Repository activity was determined using the method described by Peters (1965). In this procedure, mice were divided into five groups (I-V) of four mice each and the animals were administered 90, 180, 270 mg/kg/day dose of the extract, 1.2 mg/kg/day of Pyrimethamine (positive control) and 8 mL/kg/day normal saline (negative control) for 4 days (D0-D3). On the fifth day (D4), animals were inoculated with *Plasmodium berghei* NK-65. The parasitaemia level was assessed by blood smears seventy two hours later.

Evaluation of curative activity of the extract in established infection (Rane's test)

The curative activity of the extract against established *P. berghei* infection in mice was determined using the method described by Peters *et al.*, (1993). *P. berghei* was injected intraperitoneally into 20 mice on the first day (D0). Seventy two hours later (D3), the mice were divided randomly into five groups of four mice each. The mice groups (I-V) were administered via p.o with 90, 180, 270 mg/kg of the extract, 10 mg/kg chloroquine (positive control) and 8 mL/kg normal saline respectively; twice a

day for 5 consecutive days. Parasitaemia levels were monitored for each mouse per group until thirty days (30days). The mean survival time for each group was determined arithmetically by finding the average survival time (days) of the mice (post inoculation) in each group (Okokon *et al.*, 2008).

Statistical analysis

Data obtained were analyzed using student's t-test and ANOVA (one – or two – way). Differences between means were considered significant at 5% level of significant i.e $p \leq 0.05$. The results were expressed as Mean \pm SEM.

RESULTS AND DISCUSSION

Traditionally, the leaves of *M. pruriens* have been reported to be used locally in the treatment of fever and other malaria-related disease in the southeastern region of Nigeria. These prompted the need to evaluate the *in vivo* antiplasmodial potentials of the crude extract of *M. pruriens* to confirm its antiplasmodial activity. The crude extract of *M. pruriens* orally administered into mice, even at doses as high as 5000 mg/kg did not lead to mortality. The mice were monitored for over 48 h but no sign of toxicity was observed, except initial weakness. This signifies that the oral LD₅₀ was greater than 5000mg/kg.

The antiplasmodial properties of the extract were investigated using standard models: chemosuppressive, prophylactic and curative models (Akpan *et al.*, 2012).

The Chemosuppressive activity of aqueous leaf extract of *M. pruriens* exhibited a dose dependent chemotherapeutic effect at the different doses employed in the study. The chemosuppressions were 60.06, 65.91 and 71.75% for the corresponding dose of extract (90, 180 and 270 mg/kg/day). The effects produced by the extract were statistically significant ($P < 0.05$) relative to control. The effect of the highest dose (270 mg/kg) exerted a chemosuppression (71.5%) comparable to that of standard drug (Chloroquine 10 mg/kg) with a chemosuppression of 83.12% (see Table 1).

Table. 1: Antiplasmodial activity of *M. pruriens* during chemosuppressive test.

Treatments	Dose (mg/kg)	Parasitaemia Count	% Inhibition
Normal saline	8 mL/kg	30.8 \pm 0.58	–
<i>M. pruriens</i>	90	12.3 \pm 0.28	60.06
	180	10.5 \pm 0.21	65.91
	270	8.7 \pm 0.26	71.75
Chloroquine	10	5.2 \pm 0.17	83.12

Data are expressed as Means \pm SEM

* $P < 0.05$ when compared with normal control

The prophylactic activity of aqueous leaf extract of *M. pruriens* at the different doses employed results in significant reduction of parasitaemia in extract treated groups when compared to control. Chemotherapeutic effects of 65.97, 71.18 and 84.38% were respectively recorded for the corresponding dose of extract (90, 180 and 270 mg/kg/day). The effects produced by the extract were statistically significant ($P < 0.05$) relative to control. The chemosuppression exerted by the highest dose of the extract was

comparable to that of standard drug (pyrimethamine 1.2 mg/kg) with chemosuppression of 92.71% (see Table 2).

Table. 2: Antiplasmodial activity of *M. pruriens* during prophylactic test.

Treatments	Dose(mg/kg)	Parasitaemia Count	% Inhibition
Normal saline	8 mL/kg	28.8 \pm 0.31	–
<i>M. pruriens</i>	90	9.8 \pm 0.23	65.97
	180	8.3 \pm 0.29	71.18
	270	4.5 \pm 0.18	84.38
Pyrimethamine	1.2	2.1 \pm 0.24	92.71

Data are expressed as Means \pm SEM

* $P < 0.05$ when compared with normal control

The curative activity of aqueous leaf extract of *M. pruriens* showed a dose dependent schizonticidal effect on the parasitaemia similar to chloroquine treated group. These effects were statistically significant ($P < 0.05$) compared to the control. The negative control group (normal saline treated group) showed daily increase in parasitaemia. The result in Table 3 also showed that the mean survival time (MST) of the extract treated groups were significantly ($P < 0.05$) longer than of the control. Both Chloroquine (10 mg/kg) and the highest dose of the extract at 270 mg/kg gave a mean survival time of 30 days as compared to 24.0 \pm 0.58 and 16.8 \pm 0.48 days observed with 180 and 90 mg/kg of the extract (see Table 3).

Table. 3: Antiplasmodial activity of *M. pruriens* during curative test.

Treatments	Dose (mg/kg)	Parasitaemia Count	Mean survival time (day)
Normal Saline	8 mL/kg	34.00 \pm 0.89	8.0 \pm 0.41
<i>M. pruriens</i>	90	12.10 \pm 0.38	16.8 \pm 0.48
	180	5.85 \pm 0.26	24.0 \pm 0.58
	270	3.50 \pm 0.17	30 \pm 0.00
Chloroquine	10	2.25 \pm 0.22	30 \pm 0.00

Data are expressed as Means \pm SEM

* $P < 0.05$ when compared with normal control

Table. 4: The qualitative and quantitative phytochemical analysis of *M. pruriens*.

Parameters	Qualitative Analysis	Quantitative Analysis (mg/100 g)
Alkaloids	+	0.05 \pm 0.001
Flavonoids	+	0.02 \pm 0.001
Saponins	+	0.02 \pm 0.002
Tannins	+	0.01 \pm 0.001
Glycoside	+	0.01 \pm 0.002
Cardiac Glycosides	+	0.01 \pm 0.001

Mean value of triplicate readings \pm SEM and + denote present.

Our present results indicate that the aqueous leaf extract of *M. pruriens* extract significantly reduced the parasitaemia in chemosuppressive, prophylactic and curative models in dose dependent fashion. The reduction in parasitaemia might be attributed to the presence of phytochemical compounds (Ishih *et al.*, 2001; Mendonca-Filho, 2006). Plants are known to exert antiplasmodial activity either by direct or indirect action on the parasite depending on their phytochemical constituents (Misra *et al.*, 1991). Previous study on *Mucuna pruriens* plant has implicated alkaloids to be the bioactive compound responsible for its various activities (Reddy *et al.*, 2008). This correlate with the result obtained from quantitative phytochemical analysis where

alkaloids gave the highest value (see Table 4). Alkaloids have also been implicated in antiplasmodial activities of many plants. For example, the alkaloid constituents of *Hydrangea macrophylla* var. Otaka was found to affect the host immune mechanism contributing to acquired immunity to blood-stage malaria instead of a direct action on parasites (Ishih *et al.*, 2001). This effect was further attributed to macrophage activation which includes production of oxygen radicals that ultimately lead to the clearance of infected Red blood cells (Hisaeda *et al.*, 2005). Thus, it is possible that the active alkaloids of *Mucuna pruriens* leaf extract might be the same principles as those isolated from other plants. Further experiments are at present being undertaken to examine in detail the characteristics of antimalarial activity of *Mucuna pruriens* leaf extract. The results of this study demonstrated that *M. pruriens* possess considerable intrinsic antiplasmodial activity, which can be attributed to its phytochemical compound. This confirms its use in treatment of fever and other malaria-related diseases in the southeastern region of Nigeria.

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Conflict of interest statement

We declare that we have no conflict of interest.

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