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Full Length Research Paper

Anti-malarial activity of ethanolic leaf extract of *Piliostigma thonningii* Schum. (Caesalpiniacea) in mice infected with *Plasmodium berghei berghei*

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***Piliostigma thonningii* Schum. (Caesalpiniacea) is used traditionally in the management of fever, cough, wounds and various ulceration. Oral acute toxicity of the ethanolic leaf extract of *Piliostigma thorningii* was evaluated in mice using modified Lorke's method. The ethanolic leaf extract was evaluated for *in vivo* anti plasmodial activity against chloroquine sensitive strain of *Plasmodium berghei berghei NK65* in mice. Four day suppressive, curative effect against established infection and prophylactic models of anti plasmodial studies were carried out. The oral median lethal dose was determined to be 3807.89 mg/kg body weight. The extract (100,200 and 400 mg/kg) exerted dose dependent chemo suppressive effects at the different levels of the infections tested. However the anti-plasmodial effect of chloroquine at 5 mg/kg body weight was higher than the extract in all the test models. This shows that the plant has anti plasmodial property that can be explored for the management of malaria.**

Key words: *Piliostigma thonningii*, anti plasmodial, *Plasmodium berghei berghei* NK65.

INTRODUCTION

Malaria is an endemic infectious disease that is wide spread in tropical and subtropical regions of the world and one of the six most important parasitic disease of man (WHO, 1998). It is a major public health problem in sub-Saharan Africa, where over 85 - 90% of all global burden of malaria exists with up to 50% of all out patient visits in areas with high malaria transmission and 30 - 50% of all hospital admissions are attributed to malaria (WHO, 2005). The disease kills 1.1 million people world wide each year. Approximately 1 million of these deaths are in Africa and an estimated 700,000 of them are children. These malaria deaths account for one out of every four childhood deaths in Africa (UNICEF, 2000).

Although an effective vaccine is the best long term control option for malaria, current research on vaccine development is still at preclinical stage and considering the different phases of vaccine development it is predicted that a reliable malaria vaccine is several years away. The global strategy for malaria mainly focuses on case management through provision of drugs capable of reducing the morbidity and mortality of malaria through provision of drugs capable of reducing or eliminating parasites (WHO, 1993; Schapira, 1994).

However, multiple anti-malarial drug resistant *Plasmodium falciparum* and the emergence of insecticide resistant *Anopheles mosquitoes* is causing not only the spread of malaria to new areas but also its re-emergence in areas

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where it had previously been eradicated (Collins and Jeffery, 1996). This has prompted research towards the discovery and development of new, safe and affordable anti-malarial chemotherapies.

In this respect, plant resources are potential targets for research and development of alternative malarial drugs, with novel modes of action (Muregi et al., 2003). Although up to 80% of the African population uses traditional medicine especially plant remedies for the management of diseases including malaria, plants are not yet fully explored (WHO, 2002b).

Piliostigma thonningii belongs to the family caesalpiniaceous and it is a shrubby tree with alternate compound leaves. The fruits are often pod-like with pods containing one to many seeds. The tree is perennial in nature and its petals are white to pinkish colour produced between November and April. While the fruits, which is a hairy, hard, flattish pod turns rusty brown, woody and twisted which splits at ripening and usually persistent on the tree are produced between June and September (Lock and Simpson, 1999). Locally, the seed is called Abafe in the Yoruba land (Nigeria). Other names include Monkey bread, Camel's foot, Kalgo (Hausa) and Okpoatu (Ibo). *P. thonningii* grows in open woodland and savannah regions that are moist and wooded grassland in low to medium altitudes. It is widely distributed in Africa and Asia.

It is found growing abundantly as a wild uncultivated tree in many parts of Nigeria such as Zaria, Bauchi, Ilorin, Plateau, Lagos and Abeokuta (Schultes and Hofmann, 1973; Djuma, 2003). In Nigeria it is represented by two species that are much alike – *P. thonningii* and *P. reticulatum* (Keay et al., 1964). A warm infusion of the bark and leaves traditionally is used to relieve fever and toothache. The powdered bark or the young inner bark and the scurf scraped from the surface of the pods are applied as dressing for wounds. The bark is also chewed for the relief of cough. The leaves and bark are believed to have expectorant property and are used in infusions or chewed for chest complaints, intestinal troubles, diarrhoea and dysentery (Dalziel, 1937).

This study was aimed at evaluating the anti - plasmodial activity of ethanolic leaf extract of *P. thonningii on Plasmodium berghei berghei* infection in mice.

MATERIALS AND METHODS

Collection and identification of plant material

Fresh leaves of *P. thonningii* were collected from Suleja, Niger State Nigeria. It was identified by Mal. Ibrahim Muazzam of the Medicinal Plant Research and Traditional Medicine (MPR and TM) Department, NIPRD Idu, Abuja. A sample with voucher number NIPRD/H/6268 has been deposited for future reference at the department's (MPR and TM) herbarium.

Extraction of plant material

The leaves were air dried under shade. The dry leaves were ground to coarse powder in a mortar. Extraction was carried out by dispersing 500 g of the ground plant material in 2.5 L of 70% ethanol and shaking was done with GFL shaker (No 3017 MBH, Germany) for 72 h. This was followed with vacuum filtration and extract concentration using a rotary evaporator at a temperature not exceeding 40°C. The concentrate was heated over a water bath to obtain a solvent free extract, which was stored in a refrigerator at 4°C.

Animals

4 weeks old albino mice weighing 18-22 g obtained from the Animal Facility Centre of NIPRD Abuja were used for the study. They were housed in plastic cages with saw dust as beddings and given food and water *ad libitum.* The mice were used in accordance with NIH Guide for the care and use of laboratory animals; NIH Publication (No. 83 - 23) revised (1985) NIPRD-Standard Operation Procedures (SOPs).

Phytochemical screening

The extract was screened for the presence of secondary metabolites and constituents using conventional protocols for detecting the presence of alkaloids, tannins, saponins and resins (Trease and Evans, 1989).

Acute toxicity test

Acute toxicity of *P. thonningii* ethanolic leaf extract was carried out using using modified Lorke' s method (1983).The study was carried out in two phases . In phase one of the study nine mice were randomized into three groups of three mice each and were given 10,100 and 1,000 mg/kg body weight of the extract orally. The mice were observed for signs of toxicity which include but not limited to paw licking, salivation, stretching of the entire body, weakness, sleep, respiratory distress, coma and death in the first four hours and subsequently daily for 7 days. In the second phase of the study another fresh set of nine mice were randomized into three groups of three mice each and were given 1600,2900 and 5000 mg /kg body weight of the extract orally based on the result of the first phase. These were observed for signs of toxicity and mortality for the first critical four hours and thereafter daily for 7 days. The oral median lethal dose was calculated using the formula:

LD_{50}= √ minimum toxic dose × maximum tolerated dose.

Rodent parasite (*Plasmodium berghei berghei*)

The rodent parasite *Plasmodium berghei berghei* NK 65 was sourced from National Institute for Medical Research (NIMR), Lagos, Nigeria and kept at Animal Facility Centre, NIPRD Idu, Abuja, Nigeria. The parasites were kept alive by continuous intra-

Table 1. Phytochemical composition of ethanolic leaf extracts of *P. thonningii* .

Phytochemicals	Availability
Carbohydrates	Present
Free reducing sugars	Present
Tanins	Present
Flavonoids	Present
Phenols	Present
Saponins	Present
Alkaloids	Present
Balsams	Present
Terpenes	Present
Steroids	Present
Volatile oils	Present
Resins	Absent
Glycosides	Absent

peritoneal passage in mice (Adzu et al., 2007) every four days. The re-infected mice were used for the study. Prior to the beginning of the study, one of the infected mice was kept and observed to reproduce disease symptoms similar to human infection (English et al., 1996).

Suppressive test

The Peter's 4-day suppressive test against chloroquine sensitive *P. berghei berghei* NK 65 infection in mice was employed (Peters, 1965). Adult Swiss albino mice weighing 18 – 22 g were inoculated by intraperitoneal (I.p) injection with standard inoculum of *P. berghei berghei* with 1 x 10^7 infected erythrocytes. The mice were randomly divided into 5 groups of 6 mice per group and treated for 4 consecutive days with 100, 200 and 400 mg extract/kg body weight orally daily respectively. Two control groups were used; the positive control was treated daily with 5 mg chloroquine/kg while the negative control group was given 5 ml/kg normal saline. On day 5 of the experiment, blood was collected from the tail of each mouse and smear on to a microscope slide to make a film (Saidu et al., 2000). The blood films were fixed with methanol, stained with10% Giemsa at pH 7.2 for 10 min and parasitaemia (WHO, 1994) examined microscopically. The percentage suppression of parasitaemia was calculated for each dose level by comparing the parasitaemia in infected controls with those of treated mice.

Evaluation of schizontocidal activity of *P. thonningii* on established infection (Curative or Rane test)

Evaluation of the curative potential of *P. thonningii* leaf extract was carried out according to the method described by Ryley and Peters (1970). The mice were injected intraperitoneally with standard inoculum of 1 x 10^7 *P. berghei berghei* NK 65 infected erythrocytes on the first day (day 0). Seventy two hours later, the mice were divided into five groups of five mice each. The groups were orally treated with *P. thonningii* extract (100, 200 and 400 mg/kg/day), chloroquine (5 mg/kg/day) was given to the positive control and an equal volume of distilled water was given to the negative control group. The treatment was carried out once daily for 5 days and blood smears were collected and examined micro-scopically to monitor the parasitaemia level. 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 over a period of 28 days (0 - 27).

Evaluation of prophylactic activity of *P. thonningii* (Repository test)

Evaluation of the prophylactic potential *P. thonningii l*eaf extract was carried out according to the method of Peters (1965). Treatments were initiated on D0 and continued till D4 when the mice were all infected with the parasite. Blood smears were then made from each mouse 72 h after treatment (Abatan and Makinde, 1986) and increase or decrease in parasitaemia determined.

Statistical analysis

The one way ANOVA test was used to analyse and compare the results at a 95% confidence level. Values of $p < 0.05$ were considered significant. Results were expressed as mean ± standard error of mean.

RESULTS

Phytochemical screening

Results obtained from the phytochemical screening of the ethanolic leaf extract of *P. thonningii* showed the presence of carbohydrates, free reducing sugars, tanins, flavonoids, phenols, saponins, alkaloids, balsams, terpenes, steroids and volatile oils while resins and glycosides were absent as shown in Table 1.

Acute toxicity test

Behavioural signs of toxicity observed in mice given 1,000 mg extract/kg body weight and above include; paw licking, salivation, stretching and reduced activity. There was however no mortality at all the dose levels used. The median lethal dose LD_{50} was estimated to be 3807.89 mg/kg body weight.

Suppressive effect

The ethanolic extract of *P. thonningii* exerted dose dependent chemosuppressive effect against *P. berghei berghei* malaria parasite. The extract caused a significant ($p < 0.05$) chemo suppression when compared to the control. The standard drug, chloroquine caused chemo

Table 2. Suppressive effect of *P. thonningii* ethanolic leaf extracts and chloroquine against *P. berghei berghei* infection in mice.

Treatment	Parasite count	% Suppression
Normal saline 5 ml/kg (control)	6.2 ± 1.16	-
Extract 100 mg/kg	2.36 ± 1.20*	61.94
Extract 200 mg/kg	1.34 ± 0.18*	78.39
Extract 400 mg/kg	0.5 ± 0.28**	91.94
CQ 5 mg/kg	0.074 ± 0.018**	98.81

*Significantly different ($p < 0.05$) from the control.
**Highly significant different ($p < 0.01$) from the control.

Table 3. Curative effect of *P. thonningii* ethanolic leaf extracts and chloroquine against *P. berghei berghei* established infection in mice.

Treatment	Parasite count	% suppression
Normal saline 5 ml/kg (control)	18 ± 0.63	-
Extract 100 mg/kg	9.5 ± 0.29*	47.22%
Extract 200 mg/kg	8.5 ± 0.65*	52.78%
Extract 400 mg/kg	7.0 ± 0.41*	61.11%
CQ 5 mg/kg	4.25 ± 0.48**	76.39%

*Significantly different ($p < 0.05$) from the control.
**Highly significant different ($p < 0.01$) from the control.

suppression of 98.81%, which was higher than those of the extract treated groups (Table 2).

Curative effect

It was observed that the ethanol ic leaf extract of *P. thonningii* produced daily, dose dependent reduction in parasitaemia levels in the extract treated groups, with a similar reduction in the chloroquine treated group (positive control). However, there was a daily increase in parasitaemia in the negative control group. The average percentage suppression of parasitaemia of the extract treated groups on day 7 were 47.22, 52.78 and 61.11% for the 100, 200 and 400 mg/kg/day of the extract respectively. While that of the chloroquine treated group was 76.39% (Table 3).

Prophylactic effect

The ethanolic leaf extracts of *P. thorningii* exerted significant ($P < 0.05$) dose dependent reduction in level of parasiteamia of 34.21, 44.74, 60.53% and 78. 90% at 100 mg/kg, 200 mgkg, 400 mg/kg and the chloroquine treated groups, respectively (Table 4).

DISCUSSION

The rodent model of malaria has been employed in this study for prediction of efficacy of ant malaria effect of *P. thoningii* leaf extract. Several conventional anti malaria agents such as chloroquine, halofantrine, mefloquine and more recently artemisinin derivatives have been identified using rodent malaria model (David et al., 2004). *P. berghei berghei* are used in the prediction of treatment outcomes, hence it was an appropriate parasite for the study. Since this parasite is sensitive to chloroquine, this drug was used as the standard drug in this study.

The choice of 4 weeks old mice for the study was done to avoid the effect of anaemia in the old mice and the effect of possible physiological changes associated with ageing may induce on the treatment outcome (Pierrot et al., 2003). The *in vivo* model was employed for this study because it takes into account possible prodrug effect and possible involvement of immune system in eradication of infection (Waako et al., 2005). The oral median lethal dose of 3807.89 mg/kg body weight obtained for the ethanolic leaf extract of *P. thoningii* is 19 times greater than the minimum effective dose of 200 mg/kg. Earlier reports have shown that if the median lethal dose of a test substance is three times more than the minimum effective dose, the substance is considered a good candidate for

Table 4. Prophylactic effect of *P. thonningii* ethanolic leaf extracts and chloroquine against *P. berghei berghei* (Repository test).

Treatment	Parasite count	% Suppression
Normal saline 5 ml/kg(control)	7.6 ± 0.68	-
Extract 100 mg/kg	5.0 ± 0.48	34.21%
Extract 200 mg/kg	4.2 ± 0.37*	44.74%
Extract 400 mg/kg	3.0 ± 0.32*	60.53%
CQ 5 mg/kg	1.6 ± 0.19**	78.90%

*Significantly different ($p < 0.05$) from the control.
**Highly significant different ($p < 0.01$) from the control.

further studies. It was also reported that oral administration is about 100 times less toxic than the intraperitonial (Jutamaad et al., 1998). The extract is therefore safe and this could explain the safe use of the plant by the local people who have been using it in traditional management of malaria in Nigeria. The ethanolic extract is therefore a good candidate for further studies.

The 4-day suppressive test is a standard test commonly used for antimalarial screening and the determination of percent inhibition of parasitaemia is the most reliable parameter. A mean group para sitaemia level of less than or equal to 90% of the mock-treated control animals usually indicate that the test material is active in standard screening studies (Peter and Anatoli., 1998). The results obtained from our study showed significant decrease in parasitaemia of *P. berghei berghei* infected mice treated with the ethanolic leaf extract of *p. thorningii*. This significant suppression of parasitaemia observed was dose dependent. The crude extract caused 91.94% suppression in parasitaemia of *P berghei* infected mice while chloroquine a standard antimalaria drug exerted 98.81% suppression. When a standard antimalarial drug is used in mice infected with *P. berghei*, it suppresses parasitaemia to non-detectable levels (Kiseko et al., 2000), just like the effect of chloroquine in this study. The observed antimalaria activity is consistent with the traditional use of the plant as a herbal medication against the disease in Nigeria. The extract exerted significantly repository effect in mice treated with 200 and 400 mg/kg body weight (Table 2). This effect was however lower in group that received low dose. This effect may be due to short duration of action of the extract occasioned by rapid metabolism and so parasite clearance could not be total. It may also be explained by the fact that not all antimalarials are completely active against the *P. berghei* model (Dow et al., 1998). The extract demonstrated a significant pro-phylactic effect against *P. berghei* infected mice as demonstrated by the reduction in the level of parasitaemia dose dependently (Table 3). It is evident based on these findings that *P. thorningii* possess potent anti-plasmodial effect justifying its folkloric usage in the management of malaria. This effects may be attributed to the presence of alkaloids, terpenes and flavonoids that have been implicated in antiplasmodial activity (Phlipson and Wright, 1990; Christensen and Kharazmi, 2001). However, the active principle(s) are yet to be identified and there is need for the identification. In view of this fact, attempts are being made to carry out anti-plasmodial guided fractionation of the ethanolic extract to isolate the active compounds and also to test for the cytotoxicity of the extract.

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