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The antiplasmodial effect of the extracts and formulated capsules of *Phyllanthus amarus* on *Plasmodium yoelii* infection in mice

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ABSTRACT

Objective: To investigate the antiplasmodial activity of the extracts of *Phyllanthus amarus (P. amarus)* on *Plasmodium yoelii (P. yoelii)* (a resistant malaria parasite strain used in animal studies) infection in mice. **Methods:** The aqueous and ethanol extracts of the whole plant of *Phyllanthus amarus* was administered to Swiss albino mice at doses of 200 mg/kg/day, 400 mg/kg/day, 800 mg/kg/day and 1 600 mg/kg/day and the prophylactic and chemotherapeutic effect of the extracts against *P. yoelii* infection in mice was investigated and compared with those of standard antimalaria drugs used in the treatment of malaria parasite infection. Acute toxicity test was carried out in mice to determine the safety of the plant extract when administered orally. **Results:** The results showed that the extracts demonstrated a dose-dependent prophylactic and chemotherapeutic activity with the aqueous extracts showing slightly higher effect than the ethanol extract. The antiplasmodial effects of the extracts were comparable to the standard prophylactic and chemotherapeutic drugs used in chloroquine resistant *Plasmodium* infection although the activity depended on the dose of the extract administered. The extracts showed prophylactic effect by significantly delaying the onset of infection with the suppression of 79% at a dose of 1 600 mg/kg/day. **Conclusions:** The results obtained indicate that the extracts of the whole plant of *P. amarus* possess repository and chemotherapeutic effects against resistant strains of *P. yoelii* in Swiss albino mice. The findings justify the use of the extract of *P. amarus* in traditional medicine practice, for the treatment of malaria infections.

1. Introduction

Malaria is a public health problem of which about 300-500 million people experience clinical episodes and 1.4-2 million deaths occur annually[1]. Malaria is said to kill a child every 30 seconds, since with the onset of severe malaria, death may occur within 24 h or less[2]. In Nigeria, malaria accounts for 30%-50% morbidity and 25% mortality in infants[3,4]. During the past 30 years, malaria parasites especially *Plasmodium falciparum (P. falciparum)* have rapidly developed resistance to commonly used antimalarial drugs[4]. A dramatic recrudescence of malaria is ongoing due to this increasing resistance to parasites and the progressive resistance of the vectors to insecticides[5]. The greatest impact of the disease is on the poor people of the world and most of these populations are found in the rural settings especially in African communities where the people have poor nutritional status and also lack access to good health facilities. Thus, the rural dwellers depend more on herbs and other forms of traditional medicines for cure[4].

Phyllanthus amarus Schum and Thonn (family Euphorbiaceae) (*P. amarus*) is a perennial herb that has been widely used in Ayurvedic medicines for over 2 000 years. It is a common weed, which grows well in moist, shady and sunny places and is widely distributed in almost all tropical countries and regions including America, India and Nigeria. It is commonly called 'stonebreaker', 'windbreaker', 'gulf leaf flower' or 'gala of wind'. The different plant parts are ethnobotanically used in various diseases and disorders[6]. For example the leaves are used as expectorant and diaphoretic, and the fruits as

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carminative, laxative, astringent, diuretic, diaphoretic and tonic to the liver[7]. Research have shown that the plant has demonstrated anti- viral activity against hepatitis B virus[8,9], hepatoprotective[10,11], anti-carcinogenic[12,13], antimutagenics, anti-nociceptive and anti-inflammatory[14], antidiabetic and antilipidemic[15]activities. In some parts of Nigeria, the whole plant is washed and chewed for menstrual pain, stomach pain and tooth-ache[16]. The phytoconstituents present in *P. amarus* are alkaloids, flavanoids, hydrolysable tannins, polyphenols and lignans[10].

Recent studies done on the efficacy of herbal remedies used by herbalist in Oyo state Nigeria for the treatment of malaria infection have shown that majority of the rural dwellers depend on herbal remedies and *P. amarus* was found to be one of the major components in the efficacious remedies[17]. In South eastern Nigeria, the roots are usually soaked in gin (alcoholic spirit distilled from grain or malt) and taken for the treatment of malaria[18]. Chukwujekwu *et al*[19] used the parasite lactate dehydrogenase test to demonstrate the in vitro activity of the petroleum ether extract of *P. amarus* against *P. falciparum*. The aqueous extract of the leaves and stem of *P. amarus* has been reported to possess a dose dependent schizonticidal activity on *P. berghei* when administered intraperitoneally to Swiss albino mice, in early and established infections[20]. However, no work has been done to assess the in vivo antiplasmodial activity of the extracts of the whole plant against *P. yoelii* a resistant malaria parasite used commonly used in animal studies. Thus, the aim of this study is to assess the in vivo antiplasmodial activity of the extracts and formulated capsules of *P. amarus* on Swiss Albino mice infected with *P. yoelii*.

2. Materials and methods

The materials used were chloroquine phosphate obtained from Evans Plc (Lagos, Nigeria), artesunate/amodiaquine combination tablet (G-Sunate® kit) from Greenlife Pharma, (Lagos, Nigeria), pyrimethamine from S-KG Pharma (Lagos, Nigeria), ethanol, methanol, Giemsa stain and 3% Tween 80 all obtained from Sigma Chemical Company (St Louis, USA). Swiss albino mice [weighing (20±2) g] of either sex were obtained from Biovaccines Ltd (former Federal Vaccine Production Laboratory) Lagos, Nigeria. The plant (*Phyllanthus amarus*) was purchased from *Alasalatu* herbal market in Mushin, Lagos-Nigeria and authenticated by Professor Dele Olowokudejo of the department of Botany and Microbiology, Faculty of Science, University of Lagos. A voucher specimen was deposited in the departmental herbarium. The parasite, non lethal strain of *P. yoelii* (17 ×) was obtained from the Nigerian Institute of Medical Research, Lagos, Nigeria and maintained in mice by weekly passage.

2.1. Preparation of plant extract

The whole plant was washed and dried in an oven (Model BS 250, GallenKamp Co., UK) at a temperature of 50 ℃ for 48 h. The dried plant was powdered using a laboratory mill (Kenwood Ltd, Hertfordshire, UK). The aqueous and ethanol extracts were prepared by soaking 350 g of the powdered plant material with 1.3 L of distilled water and 1.5 L of ethanol respectively, using the soxhlet apparatus (Corning, USA). The extract was concentrated at 50 ℃ using a rotary evaporator (Corning, USA) and then evaporated to dryness in a vacuum dessicator. The dried extracts were stored in the refrigerator.

The pH of 5% solution of the extract was determined using pH meter (Model 120, Thermorion) and the microbial limit was determined using established procedure.

2.2. Formulation of capsules

The powder mix with a basic formula of dried aqueous extract (50% w/w) magnesium stearate (1% w/w), maize starch (5% w/w) and lactose (44% w/w) was dry mixed to obtain a homogeneous mixture and the moisture content determined with an Ohaus moisture balance (Ohaus Scale Corporation, USA) and found to be 1.7% w/w. The tapped and bulk densities of the powder-mix were determined using standard methods. Capsule [(400±5) mg] formulations were prepared by manually filling the hard gelatin capsule shells (Therapeutic Laboratories, Lagos, Nigeria). The mean weight and disintegration times of the capsules were determined using established methods.

2.3. Acute toxicity test

Sixty-five Swiss albino mice [(20±2) g] were divided into thirteen groups of five mice per group, housed in standard cages in a well ventilated environment and allowed had free access to food and water. The animals were allowed to acclimatize for seven days before the commencement of the experiment. All animals were fasted for twelve hours before administration of the extracts. Six groups each received either the aqueous or ethanol extract orally using a stainless metallic feeding canula at a dose range of 400-1 600 mg/kg bw. The thirteenth group received 0.2 mL of distilled water to serve as control. The animals were keenly observed for two hours after the administration and then regularly for 48 h.

2.4. Inoculation procedure

Inoculation of mouse was done intra-peritonially (i.p.) with 0.2 mL of infected blood obtained from the eye orbit of the donor mouse which had 30.6% parasitaemia. Thin blood films were made by collecting blood from the tail of the animals and stained with Giemsa stain after fixing with methanol. The percentage parasitaemia was determined by counting the number of parasitized red blood cells out of 1 000 blood cells in ten randomly selected microscopic fields. The mean percentage suppression of parasitaemia was calculated as:

$$\text{Suppression (\%)} = \frac{\text{parasitaemia in control (\%)} - \text{parasitaemia in treated (\%)}}{\text{parasitaemia in control (\%)}} \times 100$$

2.5. Repository (chemoprophylactic) test

The chemoprophylactic test was done using established methods[21,22]. Fifty Swiss albino mice [(20±2) g] were randomly assigned to ten groups of five each. Four groups each received either the aqueous or ethanol extracts, one group was given the standard drug (pyrimethamine 1.2 mg/kg/day) while the tenth group received 0.2 mL distilled water to serve as control. The extracts were administered orally at daily doses ranging from 200-1 600 mg/kg/day for five consecutive days and then on the sixth day, the animals were inoculated intra-peritoneally (i.p.) with 0.2 mL of infected blood containing *P. yoelii* parasitized red blood cells from a donor mouse having 30.6% parasitaemia. Parasitaemia was monitored every two days after administering the infected blood till the sixth day to monitor the parasitaemia.

2.6. Schizontocidal (chemotherapeutic) test

The schizontocidal (chemotherapeutic) effect of the extracts and formulated capsules were done using established methods[21,22]. Seventy Swiss albino mice [(20±2) g] randomly assigned to fourteen groups and infected with parasitized erythrocytes as earlier described and microscopy of the blood smear confirmed the establishment of the infection after five days.

Four groups each received either the aqueous or ethanol extract orally at a dose ranging from 200-1 600 mg/kg/day, three groups received the formulated capsules, one group each received chloroquine 5 mg/kg/day, artesunate/amodiaquine 2 mg/kg/day and 0.2 mL of distilled water for five consecutive days. Parasitaemia was determined on the sixth day after commencing treatment.

2.7. Statistical analysis

Data obtained were expressed as mean ± SEM (standard error of the mean). The student's *t*-test was used to assess if there were any difference in the data obtained. *P* values less than 0.05 were considered statistically significant.

3. Results

The pH of 5% dispersion of the extract was 6.72 and the microbial test showed that there were no microbial contaminations in the extract. The acute toxicity tests revealed that there were no physical signs of toxicity or death recorded at all the doses employed. Thus, the plant extracts can be regarded as safe. The repository effect was determined after daily administration of the extract for five days before the inoculation of the mice with the parasite. The percentage of parasitaemia determined six days post inoculation of *P. yoelii* are presented in Table 1. The result showed that prophylactic administration of both aqueous and ethanol extracts of the plant caused a significant ($P<0.001$) delay in the onset of infection compared with control. The repository effects of *P. amarus* was dose-dependent with the aqueous extract showing higher chemosuppression compared to the ethanol extracts. However, the standard drug, pyrimethamine, given at a dose of 1.2 mg/kg body weight produced a higher (82.51%) chemo-suppression compared to the extracts (78.65% for aqueous extract and 78.53% for the ethanol extract) at the highest dose of 1 600 mg/kg bw. Statistical analysis showed that there were no statistically significant ($P>0.05$) difference in the chemosuppression effect of the extract especially at high doses and the standard drug.

The results of the chemotherauptic activity of *P. amarus* extract on established *P. yoelii* infection are presented in Table 2. The results show that the extracts of *P. amarus* showed a dose-dependent chemotherapeutic effect with the aqueous extract showing better chemotherapeutic activity than the ethanol extract at corresponding doses although there were no statistically significant ($P>0.05$) difference in their effects. The chemotherapeutic effect of the extracts were comparable to the standard chemotherapeutic drug used in chloroquine resistant *Plasmodium* infection, artesunate/amodiaquine, especially at higher doses (1 600 mg/kg/day). The plot of the percentage of parasitaemia vs. time is presented in Figure 1. The graph shows that there was a reduction in the percent parasitaemia with time. The percentage of parasitaemia obtained for the extract at a dose of 200 mg/kg/day bw was similar to those of chloroquine at a dose of 5 mg/kg/day bw, while the percentage of parasitaemia obtained at high doses of 1 600 mg/kg/day bw was similar to the effects obtained for the artesunate/amodiaquine combination tablet at a dose of 2 mg/kg bw. This indicates that the extract would be useful in the treatment of different degree of malaria infection depending on the dose administered.

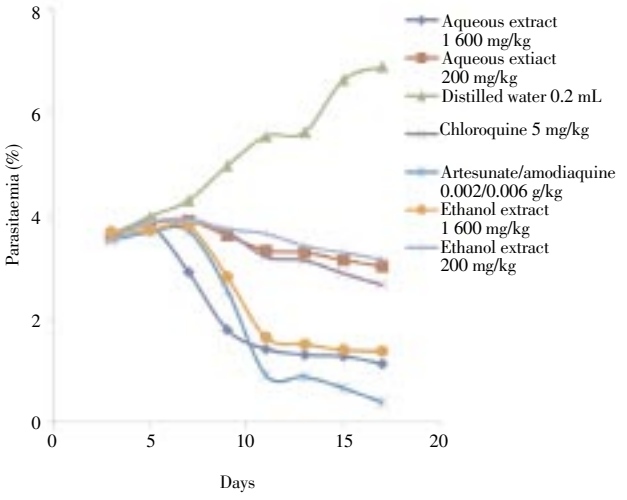

Figure 1. The effect of different doses of *Phyllanthus amarus* extracts on *Plasmodium yoelii* infection in mice.

Table 1
Chemoprophylactic (repository) effects of *Phyllanthus amarus* extracts (mean±SEM, *n*=5).

Drug	Dose (mg/kg/day)	Parasitaemia (%)	Suppression (%)
Aqueous extract	200.0	4.86±0.05	41.37
	400.0	3.85±0.02	53.56
	800.0	1.92±0.02	76.56
	1 600.0	1.77±0.18	78.65
Ethanol extract	200.0	5.12±0.23	38.24
	400.0	4.16±0.16	49.82
	800.0	2.14±0.29	74.19
	1 600.0	1.78±0.43	78.53
Pyrimethamine (Standard)	1.2	1.45±0.03	82.51
Distilled water (Control)	0.2 mL	8.29±0.02	–

Table 2
Chemotherapeutic (Schizontocidal) activity of the different doses of the extracts of *Phyllanthus amarus* during established infection (mean±SEM, *n*=5).

Drug description	Dose (mg/kg)	Parasitaemia (%)	Suppression (%)
Aqueous extract	200	3.33±0.12	56.07
	400	2.36±0.20	68.02
	800	1.67±0.18	77.97
	1 600	1.42±0.42	81.27
Ethanol extract	200	3.66±0.04	51.72
	400	2.43±0.12	67.94
	800	1.95±0.10	74.27
	1 600	1.65±0.01	78.23
Chloroquine	5	2.69±0.05	52.37
Artesunate/amodiaquine	2/6	0.92±0.04	87.86
Distilled water (control)	0.2 mL	5.54±0.01	0.00

Table 3
Chemotherapeutic activity of the formulated capsules using aqueous extract of *Phyllanthus amarus* during established infection (mean±SEM, *n*=5).

Drug	Dose (mg/kg)	Parasitaemia (%)	Suppression (%)
Capsule	400	3.89±0.03	48.68
	800	2.36±0.20	62.27
	1 600	2.32±0.03	69.13

The result of the material properties of the powder mix (extract and excipients) showed that the bulk density of the powder was 0.588 g/cm^3. The capsules formulated with the aqueous extract of *P. amarus* had a mean weight of (399.6 ±0.1) mg and an average disintegration time of 4.34 min. The results of the chemotherapeutic activity of the capsule formulation are presented in Table 3. The capsules showed a dose dependent chemotherapeutic activity similar to those of the aqueous extract. The plot of suppression vs. the dose of the aqueous extract present in the capsule formulation is presented in Figure 2. From the graph, the dose of extract in the capsule equivalent to those of the extract was extrapolated. The results showed that the percentage of suppression for the capsule formulation equivalent to 200 mg/kg bw and 400 mg/kg bw of the extract was 55% and 64% respectively. This indicates that the capsule formulations showed better activity than when the extract was administered alone. Statistical analysis showed that there were no significant difference (P>0.05) in the antiplasmodial activity of both extract and capsule formulation. This indicates that the excipients included in the formulation did not affect the activity of the extract against the parasite.

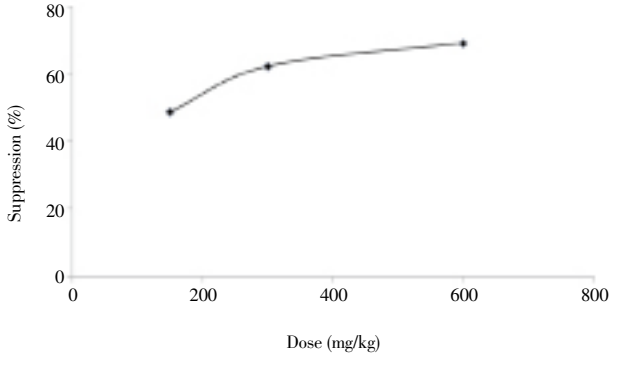

Figure 2. The plot of the percentage of suppression of *Plasmodium yoelii* infection in mice *vs*. doses of *Phyllanthus amarus* extracts present in the capsule formulation.

4. Discussion

Plants have always been a good source of chemotherapeutic

agents. The first antimalarial drug was quinine, isolated from the bark of *Cinchona* species (Rubiaceae). Chloroquine is a first line drug in the treatment of malaria until recently, when it has become resistant to the malaria parasite, *P. falciparum*[1]. The aqueous extract demonstrated a slightly higher antiplasmodial (chemotherapeutic and prophylactic) activity than the ethanol extract at all doses administered. Statistical analysis showed that there were no significant difference in the percent reduction of parasitaemia of the two extracts. The antiplasmodial activity of the extracts against the resistant strains of the parasite was dose-dependent. This is similar to the results obtained for the aqueous extract of the stem and leaves of *P. amarus* against the chloroquine sensitive strains of *P. berghei*[20].

The repository effect of the extract was dose dependent delay in the onset of *Plasmodium* infection in the animals. There were significant ($P<0.05$) differences in the repository activity of the extracts and the standard drug, pyrimethamine except at high doses where the repository effects were comparable. This indicates that high doses of aqueous and ethanol extract of *P. amarus* could be used as a substitute for the standard drug in the prevention of malaria infection. The schizonticidal activity of the extract in established infection was also dose dependent. Chloroquine which is the standard drug for the treatment of malaria infection showed 52.3% suppression which is similar to the activity of the extracts at low doses (200 mg/kg/day). On the other hand, the schizonticidal activity of the extract at high doses (1 600 mg/kg/day) was similar to those of artesunate/amodiaquine. The results also showed that there was significant ($P<0.05$) difference in the chemotherapeutic effects of chloroquine and artesunate/amodiaquine tablets, indicating that the artesunate/amodiaquine combination are more effective in the treatment of resistant strains of *Plasmodium*.

The results showed that capsule formulation equivalent to the dose administered for the extract showed percent suppression equivalent to those obtained when the extract was administered alone. There were no significant ($P>0.05$) difference in the antiplasmodial activity of both extract and capsule formulation. This indicates that the excipients included in the formulation did not affect the activity of the extract against the parasite.

The results obtained indicate that the extracts of the whole plant of *P. amarus* possess repository and chemotherapeutic effects against resistant strains of *P. yoelii* in Swiss albino mice.

Conflict of interest statement

We declare that we have no conflict of interest.

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