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ANTIPLASMODIAL EFFECTS OF THE AQUEOUS EXTRACT OF *PHYLLANTUS AMARUS* SCHUMACH AND THONN AGAINST *PLASMODIUM BERGHEI* IN SWISS ALBINO MICE

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Summary: Phyllantus amarus Schumach and Thonn is a medicinal plant used commonly for the treatment of malaria-related symptoms by the general public in southeastern Nigeria. The present study determines the possible antiplasmodial effects of the aqueous extract of the leaves and stem of the plant against Plasmodium berghei infection using Swiss albino mice as models. The blood schizonticidal activity of the aqueous extract in early infection and in established Plasmodium berghei infection was assessed and compared to the activities of chloroquine and sulfadoxine/pyrimethamine. The repository activity of the extract was also assessed and compared to the activity of pyrimethamine. The LD50 of the aqueous extract of the leaves and stem of the plant was also determined using albino Wistar rats. The results show that the LD50 of the aqueous extract of Phyllantus amarus Schumach and Thonn was 650 mg/kg. In early infection, the extract at doses of 108.33 mg/kg, 165 mg/kg and 325 mg/kg was found to cause a significant dose-dependent suppression of P berghei parasites ($P<0.05$) sulfadoxine/pyrimethamine caused a similar significant suppression of P berghei parasites ($P<0.05$) while chloroquine at a dose of 5 mg/kg did not cause a significant effect on P berghei parasites. Similarly, the extract was found at all doses to cause a statistically significant ($P<0.05$) suppression of P berghei parasites via a repository action. This effect was comparable to the effects of pyrimethamine a standard repository agent. In established infection, the extract at all doses administered, was found to significantly suppress P berghei parasites at 24 and 72-hour periods ($P<0.05$). Comparatively, sulfadoxine/pyrimethamine caused a similar statistical ($P<0.05$) suppression of the parasites of P berghei. However, the effects of sulfadoxine/pyrimethamine were more sustained over the 72-hour period. The present study therefore validates the local use of the extracts of Phyllantus amarus Schumach and Thonn as an antimalarial agent. Further studies are however recommended to identify and possibly characterize the potential antiplasmodial agents in the aqueous extract of the plant.

Key Words: *Phyllantus amarus; malaria; Antiplasmodial agent; Medicinal plants.*

Introduction

Malaria, a disease caused by *Plasmodium* infection, is still the most important human parasitic disease in the world: 40% of the world population is at risk with an annual fatality of 1.5-2.7 million; highest amongst under-five year olds (Murnigsih *et al* 2005). Research into the identification and production of more effective, cheaper and potentially less toxic remedies for the treatment of malaria would therefore continue to be relevant (Didia *et al*, 2002).

Phyllantus amarus Schumach and Thonn is a plant of the Euphorbiaceae family. It is a wild herb of the Amazonian forest, though widely distributed in the tropics and sub-tropics [Bagchi *et al* 1992; Ross 1999]. Across Nigeria, it is known by several local names and is regarded as a plant of general medicinal application. Traditional local uses in Nigeria include as a treatment of diarrhea and gastro-intestinal disorders (Odetola and Akojenu, 2000) and as a food additive for puerperal and lactating mothers. It is also used in southeastern Nigeria to treat malaria-related symptoms. The parts of the plant used include the dried leaves and the stem.

The medicinal uses and effects of the extracts of *Phyllantus amarus* Schumach and Thonn have been well documented. *Phyllantus amarus* extracts has been found to have

hepatoprotective effects (Lee *et al* 2006), to exhibit hypoglycemic effects and thus useful for the treatment of diabetes mellitus (Raphael *et al* 2002), to have anti-microbial properties (Mazumder *et al* 2006), to interfere with the formation of renal stones and therefore a useful alternative for the treatment and prevention urolithiasis (Barros *et al* 2003). Possible antispasmodic effects of the extract on smooth muscles have been reported to contribute to its effects in urolithiasis (Kassuya *et al* 2003). In addition, analgesic, anti-inflammatory, anti-allodynic and anti-oedematogenic effects have been attributed to extracts of *Phyllantus amarus* (Kassuya *et al* 2005; Kassuya *et al* 2006). A hepatic anti-tumor effect has also been reported in lower animals (Rajeshkumar and Kuttan 2000; Rajeshkumar *et al* 2002). However, reports on the anti-viral properties of extracts of Phyllantus amarus have been extensive but conflicting: A number of reports indicate an effectiveness in the eradication of hepatitis B virus in chronic carriers (Thyagarajan *et al* 1988; Thamlikitkul *et al* 1991; Doshi *et al* 1994), other reports suggests failure to respond (Milne *et al* 1994). Perhaps these conflicts could be resolved with a rigorous clinical trial. An *in vivo* and *in vitro* inhibitory effect on replication of the human immunodeficiency virus has also been documented (Notka *et al* 2004).

Recent reports on studies of the antibacterial, anti-inflammatory and antimalarial activities of some Nigerian medicinal plants did not include *Phyllantus amarus* Schumach and Thonn (Chukwujekwu *et al* 2005) in spite of its widespread use for fever in the southeastern parts. Adedapo *et al* 2005 in their studies on the effect of *Phyllantus amarus* on the serum biochemistry of rats reported that the plant was toxic (Adedapo *et al* 2005) This study seeks to determine the possible antiplasmodial effects of the aqueous extract of *P amarus* against Plasmodium berghei using Swiss albino mice as models, in order to ascertain the true value of its use in the treatment of malaria.

Materials and Methods

Identification of Plant Material

Fresh leaves and stem of *P amarus* were collected in June 2006 from local gardens at the University of Port Harcourt, Nigeria. Dr. G Obute, an academic staff of the Department of Plant Science and Biotechnology, Faculty of Science, University of Port Harcourt, Nigeria correctly identified the plant. Voucher specimens were deposited in the Malaria Research Unit, Department of Pharmacology, College of Health Sciences, University of Port Harcourt, Nigeria for future reference.

Drugs

Pyrimethamine tablet was obtained from Glaxo-Wellcome. The combination of sulfadoxine and pyrimethamine was obtained from Roche; each tablet contains 500mg sulfadoxine and 25mg pyrimethamine. Each tablet was ground into a fine powder before use. Parentheral chloroquine was obtained from May and Baker.

Preparation of aqueous extract

The leaves and stem were sorted to remove any contaminants, dead matter and sand particles and then air dried for 28 days. The dried leaves and stem were then ground into a fine powder with the aid of an electric dry mill (Moulinex). 100g of the ground powder was then thoroughly soaked in 600ml of distilled water for 48 hours at room temperature. The mixture was filtered into 250ml conical flask with Watman filter paper number one. The filtrate was dried at a temperature of 30°C for 10 hours to produce a gel like extract, which weighed 11.335g. Appropriate concentrations of the extract were then subsequently made by serial dilution with distilled water for further experimentation.

Acute Toxicity Test: Determination of LD_{50}

The acute toxicity of *P amarus* extract was estimated using 42 albino Wistar rats weighing between 125 and 250g. The rats were divided into 7 groups consisting of 6 rats per group. Each group of rat was given a different dosage of the extract. The number of deaths in each group within 24 hours was recorded. The doses were given intraperitoneally using a 1ml disposable syringe. The LD_{50} was calculated using the formula of Kerber (Matselyukh *et al* 2005).

Animals and Inoculation

A total of 40 Swiss albino mice weighing between 15 and 25g were used for the study. The mice and the *P berghei* parasites were obtained from the Nigerian Institute of Medical Research, Lagos, Nigeria. The animals were fed with standard mouse cubes and tap water *ad libitum* and allowed 4-6 weeks to acclimatize to the new environment.

Experimental Design

The experimental design involved three distinct experimental protocols:

Evaluation of Blood Schizonticidal activity in early infection: (The 4-day test)

This was done using a method similar to that described by Knight and Peters (1980). A total of 36 mice were used for studies on the

blood schizonticidal activity. Each mouse was subsequently given standard intra-peritoneal inoculums of 1.02 x 10^5 *P berghei* parasites (chloroquine-sensitive) with the aid of a 1ml disposable syringe. This was done to all the rat groups. The animals were then divided into six groups of 6 mice each: Groups 1 to 3 were given 325, 162.5 and 108.33mg/kg /day of the extract intraperitoneally respectively. Groups 4 to 6 were treated as follows: group 4 was given 5mg/kg /day of chloroquine intraperitoneally; group 5 was given 3mg/kg /day of sulfadoxine/pyrimethamine orally. Group 6 were given 0.2ml of distilled water orally with the aid of a pipette. All the extracts, drugs and distilled water were given for 3 days. Group 6 served as the control group. On the fourth day thick blood smears were made from blood samples obtained from the tails of the animals. The smears were stained with Giemsa stain and examined under the light microscope for the levels of parasitaemia. The average percentage suppression of parasitaemia was calculated in comparison to control.

Evaluation of the Repository Activity

The repository activity was determined using the method described by Peters (1965). The mice were divided into 5 groups (A to E) consisting of 6 mice each. Groups A, B and C were given 325, 162.5 and 108.333mg/kg /day of the extract respectively. Group D were administered 1.2 mg/kg of pyrimethamine; while group E were administered 0.2ml of distilled water. The drugs, extracts and distilled water were given for three consecutive days. Group E served as the control group. On the fourth day all the animals were inoculated intra-peritoneally with 1.02 x 10^5 *P berghei* parasites with the aid of a 1ml disposable syringe. 72 hours after the inoculation thick blood smears were made and the level of parasitaemia determined as described above.

Evaluation of the Blood Schizonticidal activity during established infection: (Rane Test)

This was determined using a method similar to that described by Ryley and Peters (1970). In this instance the animals were inoculated intra-peritoneally with 1.02 x 10^5 *P berghei* parasites with the aid of a 1ml disposable syringe. On the third day, the animals were subsequently divided into 6 groups similar to and as described for the evaluation of blood schizonticidal activity above. Groups 1 to 3 were given 325, 162.5 and 108.33mg/kg /day of the extract respectively. Group 4 was given 5mg/kg /day of chloroquine; group 5 was given 3mg/kg /day of sulfadoxine/pyrimethamine. Group 6 was given 0.2ml of distilled water. In this experiment, group 6 mice served as the control group. The drugs were administered for five days. After 24 and 72 hours of drug administration, blood smears were obtained as described earlier and the level of parasitaemia determined. The mean survival period was for each group within a 28day period was determined and noted.

Statistical Analysis

Results obtained are presented as mean±standard error of mean. Statistical analysis was done using the student's t-test; a 'p' value less than 0.05 were considered significant.

Results

The results obtained for each experimental protocol are as presented in Tables 1 to 4.

LD_{50} values

The LD_{50} value of *P amarus* extracts in albino wistar rats was found to be 650-mg/kg.

Blood schizonticidal activity of aqueous extract of P amarus in early infection: (The 4-day test)

Table 1 shows the blood schizonticidal activity of various doses of *P amarus* extract, chloroquine, sulfadoxine/pyrimethamine and distilled water. The average percentage parasitaemia and percentage suppression of *P amarus* at the highest dose administered was found to be 13.40±0.58 and 64.70 respectively; these values were found to be higher and lower respectively, than values obtained for sulfadoxine/pyrimethamine at a dose of 3mg/kg which were found to be 9.60±2.00 and 74.74 for average percentage parasitaemia and percentage suppression respectively. At all doses administered, *P amarus* extract and sulfadoxine/pyrimethamine at a dose 3mg/kg bw both caused a statistically significant suppression of *P berghei* activity in early infection ($p<0.05$). Chloroquine at a dose of 5mg/kg caused an average percentage parasitaemia and average percentage suppression of 31.70±3.17 and 16.40 respectively; these differences were however not significant ($p>0.05$).

Table 1: Blood schizonticidal activity of aqueous extract of P amarus in early infection [4 day Test]

Drug/Extract	Dose (mg/kg/day)	Average percentage parasitaemia	Average percentage suppression	Significant differences (t-test)
P amarus extract	325.00	13.40±0.58	64.70	Yes p<0.05
P amarus extract	165.50	18.40±0.58	51.58	Yes p<0.05
P amarus extract	108.33	27.53±1.45	27.55	Yes p<0.05
Chloroquine	5.0	31.70±3.17	16.40	No p>0.05
Sulfadoxine/ pyrimethamine	3.0	9.60±2.00	74.74	Yes p<0.05
Distilled water (control)	0.2ml	38.00±5.77	0	

Table 2: Repository activity of aqueous extract of P amarus

Drug/Extract	Dose (mg/kg/day)	Average percentage parasitaemia	Average percentage suppression	Significant differences (t-test)
P amarus extract	325.00	6.93±0.88	80.62	Yes p<0.05
P amarus extract	165.50	8.70±0.87	72.21	Yes p<0.05
P amarus extract	108.33	10.27±1.20	70.74	Yes p<0.05
Pyrimethamine (standard)	1.20	4.00± 0.58	88.60	Yes p<0.05
Distilled water (control)	0.2ml	35.40±2.60		

Repository activity of aqueous extract of P amarus

Table 2 shows the repository activity of *P amarus* compared to pyrimethamine. The highest dose of *P amarus* extract caused an average percentage parasitaemia and average percentage suppression of 6.93±0.88 and 80.62 respectively. Pyrimethamine at a dose of 1.20mg/kg caused an average percentage parasitaemia and average percentage suppression of 4.0±0.58 and 88.60 respectively. The effects of pyrimethamine and *P amarus* at all doses administered were found to be statistically significant (p<0.05).

Blood schizonticidal activity of aqueous extract of P amarus in established infection: [Rane Test]

Tables 3 and 4 show the blood schizonticidal activity of the extract *P amarus* at 24 and 72-hour periods respectively following established infection of *P berghei*: At 24 hours, the highest dose of *P amarus* extract administered caused an average percentage parasitaemia and average percentage suppression of 5.10±0.88 and 93.64 respectively. At the dose of 3.0mg/kg the values for sulfadoxine/pyrimethamine were 3.60±2.0 and 95.51 respectively; and values for chloroquine at the dose of 5.0mg/kg were found to be 40.50±1.44 and 49.50 respectively. These values were found to be statistically significant (p<0.05). However, at 72 hours, the highest dose of *P amarus* administered caused an average percentage parasitaemia and average percentage suppression of 18.87±0.88 and 79.93 respectively suggesting a reduction in its effectiveness. Comparatively, the effects of sulfadoxine/pyrimethamine were sustained as the average percentage parasitaemia and average percentage suppression were found to be 2.70±0.50 and 97.13 respectively. These values were however also found to be statistically significant (p<0.05). At 72hours chloroquine at the dose of 5mg/kg bw actually induced a negative percentage average suppression.

Table 3: Blood schizonticidal activity of aqueous extract of P amarus during established infection [24 hour Rane Test]

Drug/Extract	Dose (mg/kg/day)	Average percentage parasitaemia	Average percentage suppression	Significant differences (t-test)
P amarus Extract	325.00	5.10±0.88	93.64	Yes p<0.05
P amarus Extract	165.50	11.20±0.57	86.03	Yes p<0.05
P amarus Extract	108.33	13.87±0.66	82.72	Yes p<0.05
Chloroquine	5.0	40.50±1.44	49.50	Yes p<0.05
Sulfadoxine/ pyrimethamine	3.0	3.60±2.0	95.51	Yes p<0.05
Distilled water (control)	0.2ml	80.20±0.57		

Table 4: Blood schizonticidal activity of aqueous extract of P amarus during established infection [72 hour Rane Test]

Drug/Extract	Dose (mg/kg/day)	Average percentage parasitaemia	Average percentage suppression	Significant differences (t-test)
P amarus Extract	325.00	18.87±0.88	79.93	Yes p<0.05
P amarus Extract	165.50	24.40±0.88	73.69	Yes p<0.05
P amarus Extract	108.33	28.40±1.52	69.79	Yes p<0.05
Chloroquine	5.0	110.00±5.77	-17.02	No (p>0.05)
Sulfadoxine/ pyrimethamine	3.0	2.70±0.50	97.13	Yes p<0.05
Distilled water (control)	0.2ml	94.00±5.77	0	

Discussions

This study suggests that the aqueous extract of *P amarus* possesses antiplasmodial actions against *P berghei* parasites in early infection and in established infections. The extract also exhibits repository actions against *P berghei* parasites and its effects are dose dependent. The highest dose of Phyllantus amarus Schumach and Thonn administered in the present study was half of the LD_{50}.

The results of the present study would suggests that the effects of the aqueous extract of *P amarus* are not sustained compared to the effects of the combination of pyrimethamine and sulfadoxine in established *P berghei* infection. The average percentage parasitaemia at 24-hours following the administration of the extract was found to be 5.10±0.88; this however increased to 18.87±0.88 at 72-hours. Comparatively the corresponding values for the combination of pyrimethamine and sulfadoxine at 24-hours was 3.60±2.0, the value however reduced to 2.70±0.5 at 72-hours. The data obtained therefore suggests that the antiplasmodial effects of the aqueous extracts of *P amarus* unlike that of sulfadoxine/pyrimethamine are not sustained. The actual agent(s) in the extract of *P. amarus* causing these antiplasmodial effects and the possible mechanisms of action need to be determined further.

The results of the present study suggest that the aqueous extracts of Phyllantus amarus Schumach and Thonn has potential antiplasmodial and thus antimalarial properties. These results therefore validate its local use as an antimalarial agent in southeastern Nigeria.

In conclusion, the present study reports that the aqueous extracts of *Phyllantus amarus* Schumach and Thonn possess antiplasmodial activity against *P berghei* infection in Swiss albino mice. The study therefore fairly validates the local use of the extracts of *Phyllantus amarus* as an antimalarial agent in southeastern Nigeria.

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