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Original Research Article

Anti-*plasmodial* Effects of Aqueous Extract of *Phyllanthus amarus* schum et Thonn on SWISS Abino Mice

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Abstract

Plasmodium berghei infections which usually kill mice in 7-14 days can be terminated in most cases by administration of leaf and stem extracts of *Phyllanthus amarus*, in doses of 325-108.3mg/kg after inoculation of the animals with $6.2x10^3$ parasitized red blood corpuscles. The methods employed in the course of the study were evaluation of the blood schizontocidal activity on early evaluation of the blood schizontocidal activity during established infection using mice as a case study. The 3-test showed a merozoiticidal action on *Plasmodium berghei* after taking 4-5µ1 of blood and preparing a thick smear and viewing with x 100 magnification. From the result obtained, blood schizontocidal activity during established infection showed a more promising result from 82.72-93.64%. Thus from all indications of my result it shows that the aqueous extract of *P. amarus* is therapeutically efficacious in the treatment of *P. berghei* in all the three test carried out in this study, and this tally closely to the claims by Nigeria Traditional healers of its effectiveness in human *P. falciparum* infection.

Keywords: *Plasmodium berghei*, swiss Abino mice, anti-*plasmodium* agents, extracts of *phyllanthus amarus.*

INTRODUCTION

Malaria is a parasitic disease that is transmitted from person to person by the bite of an Anopheles mosquito. Historically, ancient Chinese mythology describes three demons who brought a debilitating disease. One demon carried a hammer to cause a pounding Headache. The second carries a pail of ice water to chill its victims and the third carried a stove to produce fever. Thus the demons afflicted humans with malaria, a disease that plagued the ancient civilizations of China, India and Mesopolamia and also infected people in Europe until World War II (Lear Month, 1988).

The name of the disease comes from the Italian words mala "bad" and aria "air". For centuries, people avoided building homes near marshes because they believed that marash air caused the illness.

Anopheles mosquitoes are present in almost all countries in the tropics and subtropics. The incidence of fatal *plasmodium falciparum* infections has been increasing in Africa since the early 1990s, coincidental with the rapid expansion of resistance to first line therapies. Chloroquine was the basis of malaria treatment in Africa but since the 1980s its clinical efficacy has precipitously declined and sulfadoxine pyrimethamine has had a short useful therapeutic life. Artemisinin based combination therapy has proved highly efficacious in Africa and is now recognized as the therapy of choice form countries with failing monotherapies.

By 2002 in Zambia overwhelming audience showed that choloroquine was failing to cure over 50% of patients by day 14. Furthermore, by 2003, sulfadoxine – pyrimethamine was failing in 8-33% of patients. In December 2002 after a series of meetings, the Zambia ministry of health announced that chloroquine would be replaced as the first line therapy for uncomplicated malaria by artemetter – lumefantrine for patient weighing 10kg or more and by sulfadoxine – pyrimethamine in children weighing less than 10kg until more information on the efficacy and safety of artemetter – lumefantrine became available in this patient group.

Changing and implementing new drugs policies is complex when the transition includes familiar drugs that are commonly prescribed in a country are easy to administer, are inexpensive and are readity available. Zambia was the first African country to change its policy towards using more expensive artemether lumefantrine, characterized by a complex dosing regimen complicated procurement and distribution and little national prescribing experience.

Malaria can be cured with the use of anti malaria drugs and certain local medicinal plants. These plants has long been used traditionally in Nigeria for the treatment of human *plasmodia* infections. Out of many useful available drug plants *phyllanthus amarus*, *phyllanthus Nirun* is employed either alone or in combination with other plants as concoction, and it is assumed to be very effective in malaria. *Phyllanthus* amanus has long been used traditionally in Hindu Ayurvedic system of medicine and it is said to be a medicinal agent in cultures around the world *Phyllanthus amarus* has been found in central and Southern India, where it grows from 30-40cm in height and it blooms with green buds. *Phyllanthus amarus* can also be found in many other countries including the Philippines, cuba, Nigeria, Ghana etc.

The extracts of phyllanthus either aqueous extract or methanol extracts of this plants are useful in the treatment of many diseases that includes, diabetic neuropathy Gall stone, cancerous cells etc.

Phyllanthus amarus (locally called quinine weed), chanca piedra "meaning stone breaker" by the Spanish people. Is a tropical green plant. And this plant can be classified as follows.

MATERIALS AND METHODS

Some of the materials used include: Animal cage, Rotra evaporator, Conical flask, Slides, Microscope.

Preparation of Plant Extract (*Phyllantus Amarus*)

The leaves were collected from a farm around the botanical garden in the university park, University of Port Harcourt and they were collected whole i.e. leaves, stems and roots; around 10-2pm in the month of April. The plants were then washed and the roots were cut off. The leaves and stems were weighed and the wright recorded and it was found to be 1.35kg then air dried for one month.

When the leaves and stem were crunchy we then blended with corona blender to fine powder. 10g of the plant was soaked in 600g of distilled water. At the end of 72hrs at a degree of 4^0C, about 70mg of the aqueous extract was obtained and this was filtered and put into a stainless container and then oven dried at 30^0C to avoid some of the active substances from been destroyed by heat. After 10hrs the extract was 11.37g (grams) and further diluted with 600g of distilled H_20 to get a concentration of 20mg/ml. (stock solution).

Malaria (*Plasmodium Berghei*)

Both *plasmodium berghei* and *plasmodium Yoeli* were collected from;

The department of parasitology, Nigerian institute of medical research, Yaba, Lagos Nigeria.

And *Plasmodium Yoeli* was known to be chlonquine sensitive. And *Plasmodium berghei* chloroquine resistant and were maintained in the laboratory by serial passages into albino mice every forthright.

Laboratory Animal

Both male and female albino mice were used exdusively throughout the experiment and they weighed 19±5.0 grams. The mice were feed with vital, pelletized finisher feed that contained crude protein 195, fat 8.60% crude fibre 5.4%, calcium 1.2% and available phosphorus, gotten from grand cereals and oil mill limited, Port Harcourt, Rivers State, Nigeria and they were free of any parasitic infection. Ordinary pipe-borne water was made available for their drinking and they were kept at room temperature of 37^0C. Before using the mice for experiment, I allowed them to acclimatize for fourteen (14) days.

Each mice was intraperitoneally or subcutaneously given the plant extract and drug.

Determination of LD_{50} (Lethal Close 50)

The concentration of the stock used for LD_{50} determination was 50mg/ml. This test LD_{50} was created by J.W. Trevan in 1927, in animal testing studies and those of other organism. LD_{50} or semi lethal close of a particular substance is a measure of how much constitutes a lethal close i.e. the dosage administered that kills $\left(1/2\right)$ half the test population (50%).

6,00mg of the extract was diluted with 120mls of distilled water to give 50mg/ml. in determining the LD_{50} of *phyllantus amarus* 28 rats were used and they were within the ranges of 150-275g. Different doses of the extract were administered into the rats based on the wright. The rats were divided into 7 groups, 4 in each group, each group was administered 50, 550, 600, 650, 700, 750 and 80mg/kg. and the following table is a detailed account of the experiment carried out in the laboratory.

RESULTS

Table 1: Determination of Lethal Dose (LD_{50})

Dose mg/kg	No of death	Mean death	Dose diff	Mean death x dose different
500	0	0	0	0
650	1	0.5	50	25
600	2	1.5	50	75
650	2	2.0	50	100
700	2	2.0	50	100
750	3	2.5	50	125
800	4	3.5	50	175
				$600/4 = 150mg/kg$

Using Kerber's formular

$$LD_{50} = LD_{100} - \frac{Sum\ of\ \ dose\ diff\ \times mean\ death}{No\ of\ \ animals\ in\ \ the\ \ group}$$

$LD_{50} = LD_{100} - 150$

$LD_{50} = 650mg/kg$

Table 2: Blood Schizontocidal Activity of the Aqueous extract of P. Amarus in the mice early infection (4-day east)

Drug/extract	Dose mg/kg/day	Average % parasitaemia	Average % Suppression
P. amarus	325.0	13.4 ± 0.58	64.7%
P. amarus	165.5	18.4 ± 0.58	51.58%
P. amarus	108.3	27.5 ± 0.45	51.55%
Chloroquine	5.0	31.7 ± 3.18	16.58%
Fansidar	3.0	9.6 ± 2.0	74.74%
Dil-H_20	0.2ml	38.0 ± 5.77	-

The suppressive activity of aqueous extract of *P. amarus* against *plasmodium berghei* in mice is shown in table 4.2 above. The extract produced a dose dependent-chemo suppressive effect between the different doses form high dose to low dose (325, 165.5, 108.3mg/kg/day) chloroquine was also administered into a group of mice and it served as a test group. The result showed that it had no chemosupprerssive effect on the strain of plasmodium *berghei* used.

The standard reference fanisidar produced a suppression of 74.74%. The extract suppressed parasitaemia significantly on 325-108mg/kg/day.

Table 3: Repository Activity of Aqueous extract of *P. amarus*

Drug/extract	Dose mg/kg/day	Average % parasitaemia	Average % Suppression
P. amarus	325.0	6.93 ± 0.88	80.62%
P. amarus	165.5	8.7 ± 0.87	72.21%
P. amarus	108.3	10.27 ± 1.20	70.74%
Pyrimetamine	1.2	4.0 ± 0.58	88.60%
Dil-H_2O	0.24ml	35.1 ± 2.6	-

Table 3 above shows the average % suppressive activity of *P. amarus* extract against *plasmodium berghei* in mice. The aqueous extract of *P. amarus* not only reduced parasitaemia level but also produced a dose

dependent chemosuppressive effect between 325.0mg/kg/day and 108.3mg/kg/day – the standard drug (pyrimethamine) produced a profound suppression of 88.60% at $P < 0.05$ all the doses of the extract showed significant different between treated and control groups.

Comparison of mean survival time of plasmodium berghei infected mice, treated with aqueous *P. amarus*, chloroquine, fansidar and distilled H_2O.

Table 3.1: Mean survival times of the extract (*P. amarus*) chloroquine, tansider and distilled H_2O.

Drug/extract	Dose (mg/kg/day)	Mean survival time (day)
P. amarus	325.0	16.3 ± 1.45
P. amarus	162.5	13 ± 0.577
P. amarus	108.3	10 ± 1.73
Pyrimetamine	0.5	6 ± 0.577
Dil-H_2O	0.24ml	4 ± 0.577

As show in table 3.1 the mean survival time for the test drug/extract 9 doses 325, 165.5, 108.mg/kg (day). The range of (16.3 ± 1.45, days, 13 ± 0.577 days 10 ± 1.73 (days). For chloroquine at 5mg/kg, the mean survival period was 6 ± 577, days. The mean survival time value for chloroquine and the (increase in parasitaemia) confirms the ineffectiveness or non sensitivity of *P. berghei* to chloroquine.

Table 3.2: Full Blown 24 hours after drug administration

Drug/extract	Dose mg/kg/day	Average % parasitaemia	Average % Suppression
P. amarus	325.0	5.1 ± 0.882	93.64%
P. amarus	162.5	11.2 ± 0.577	86.03%
P. amarus	108.3	13.87 ± 0.667	82.72%
Chloroquine	5.0	40.5 ± 1.44	49.50%
Fansidar	3.0	3.6 ± 2.0	95.5%
Dil-H_2O	0.24ml	80.2 ± 0.577	-

The suppressive activity of aqueous extract of *P. amarus* against *plasmodium berghei* in mice is shown in table (3) above. The extract produced not only a chemosuppressive effect but also reduced the parasitaemia level. The doses were from high dose to low dose (325, 1652.5, 108.3, mg/kg/day). The standard reference fansidar produced a suppression of 95.51%.

Table 3.3: 72 hours after drug administration

Drug/extract	Dose mg/kg/day	Average % parasitaemia	Average % Suppression
P. amarus	325.0	18.87 ± 0.882	79.93%%
P. amarus	162.5	24.40 ± 0.882	73.69%
P. amarus	108.3	28.40 ± 1.528	69.79%
Chloroquine	5.0	110.0 ±5.774	-17.02%
Fansidar	3.0	2.70 ± 0.50	97.13%
Dil-H_2O	0.24ml	94.05.774	-

The suppressive activity of aqueous extract of *P. amarus* against *plasmodium berghei* in mice is shown in table 3.3 above. The extract produced not only a chemosuppressive effect but also reduced the parasitaemia level. The doses were from high dose to low dose (325, 162.5, 108.3mg/kg/day). Here the result showed that the level of suppression reduces after 72 hours compared to 24 hours, apart from fansidar that the suppressive activity increased from 95.51% - 97.13% this is because the effect of the drug fansidar (sulfodoxine pyrimethamine) carries out its effect after three days.

DISCUSSION

Results from this study on the effect of medicinal plant *P. amarus* in *P. berghei*, infected mice provided strong evidence for its significance in the treatment of *P. berghei* infections in man.

The fact that chloroguine 5mg/kg/day gave suppression effect is a clear indication that the parasite used for this study is not sensitive to that drug.

During early infection the various doses of extract showed a chemosuppressive effect for doses 325.0mg/kg, the suppression was 64.7 – 27.05% indicating that the parasite was sensitive to the extract fansidar has a promising effect of 74.74%.

During repository test, the % suppression was more impressive compared to that in early infection for

doses within the ranges of (325, 162.5, 108.3mg/kg/day) the % suppression was (80.62, 72.21, 70.74%) compared to distilled H_2O that showed no suppressive effect.

During established infection, the result was most promising because the plant extract showed schizontocidal effect as there was a significant difference between the daily increase in parasitaemia level in the untreated (control) group than in the treated) group/hose groups administered the various doses of the extract. This was further confirmed by the accompanying high (m.s.t.) mean survival time. Values in the groups administered the doses of the extract and fansidar (standard) while the daily increase in parasitaemia level obtained to the distilled H_2O (control) was accompanied by low m.s.t. value of 4 ± 0.26 daysout of 28 days of observation.

The concentration and potency of the extract increases with increase in the number of days, the leaves and stems are soaked. These decrease after a longer period of days. This is as a result of the biodegradation of the extract.

CONCLUSION

Based on the findings of my results, it is concluded that aqueous extract of *P. amarus* is effective in the treatment of malaria. When taken prophylactically or after established infection. The drug is more potent when taken as recommended or prescribed discontinuity in the course of drug administration reduces its potency. So! The drug should be taken as prescribed or as recommended.

RECOMMENDATIONS

i) It is recommended that prophylactic intake of extract of *P. amarus* is more effective.
ii) The drugs should be taken as recommended or prescribed to avoid systemic toxicity.
iii) The dietary supplement should standardized to 3% bitter principle to avoid additive effects.
iv) You should always communicate to your health care provider the dietary supplement you are taking to avoid adverse side effects when combined.

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