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In vivo Antiplasmodial Activity of Byrsocarpus coccineus Leaf Extract in Mice Infected with Plasmodium Berghei

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Abstract

Objective: To investigate the *in vivo* antiplasmodial activity of ethanolic leaf extract of *Byrsocarpus coccineus* in mice infected with *Plasmodium berghei*. **Methods**: Curative effect against established infection and suppressive activity against early infection were screened. **Results:** The extract (100,200 and 400 mg/kg, p.o.) has significant ($p<0.05$) dose dependent activity against the parasites in the curative and suppressive tests. The extract also prolonged the survival time of the infected mice. The oral LD_{50} values were greater than 5000 mg/kg in mice. **Conclusion:** The result shows that the extract possesses considerable antiplasmodial activity which can be exploited in malaria therapy.

Keywords: *Byrsocarpus coccineus,* Medicinal plant, Antiplasmodial activity, *Pasmodium berghei*, Mice.

Introduction

Malaria continues to cause morbidity and mortality on a large scale in tropical countries. It is the most important human parasitic infection (1). Malaria is Africa's leading cause of mortality in patients under five years of age, and constitutes 10% of the continent's overall disease burden (2) and so far little success has been achieved to control it (3,4). Traditionally used antimalarial plants have helped in reducing the problems malaria attack posed (5). Interestingly, some of the plants used have shown real antiparasitic activity (6) and most of them are relatively safe (7,8).

B. coccineus Schum and Thonn. (Connaraceae), a scandent shrub widely dispersed in tropical Africa, is widely used in ethnomedicine for the treatment of diverse ailments. These include mouth and skin sores, swellings, tumors, earache, muscular and rheumatic pains, venereal diseases, jaundice, pile and dysentery (9). The plant extract has also been shown to possess oxytocic (10), antioxidant (11), antidiarrheal (12) activities. The antimicrobial (13,14), analgesic, anti-inflammatory, antipyretic (15, 16) properties of various

extracts of the plant have been reported. However, no work has been reported on the antiplasmodial activity of *B. coccineus*. Hence, the present study was taken up to investigate the antiplasmodial effect of leaf extract of this plant.

Materials and Methods

Plant Collection

The leaves of *B. coccineus* were collected at Chaza, Niger State, Nigeria in July, 2010. The plant was identified and authenticated by Mallam Ibrahim Muazzam and Mrs Jemilat .A. Ibrahim of the Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria where a voucher specimen (No.6416) was deposited at the herbarium unit of the institute for future reference. The International Plant Number Index is Connaraceae *B. coccineus* Schum. and Thonn. Beskr. Guin. Pl. 226. 1827 (Ik).

Preparation of Plant Extract

The leaves of *B. coccineus* were air-dried at room temperature and ground into powder using pestle and mortar. The powdered material (330 g) was macerated with 1.5 l of 70% ethanol in water for 24 h with constant shaking. The resultant mixture was filtered using Whatman® (No. 1) filter paper and the filtrate dried on a water bath to give a yield 35.44 g (10.74% w/w).

Phytochemical Screening

The ethanolic extract of *B. coccineus* was subjected to qualitative phytochemical analysis using standard procedures (17,18).

Acute Toxicity Study

The acute toxicity of the extract was tested to determine the safety of the agent using Lorke's method (19). Dose levels used ranged from 10-5,000 mg/kg. The animals were all kept under the same condition and observed for toxicity signs and mortality for 24 h. LD_{50} values were calculated as geometric mean of the dose that resulted in 100% lethality and that which caused no lethality at all.

Animals Used

Male and female Swiss albino mice (18 – 25 g) obtained from the Animal Facility Centre, NIPRD were used for the study. The animals were kept in cages at room temperature and moisture under naturally illuminated environment of 12:12 h dark/light cycle. They were fed on standard diet and had water *ad libitum* according to the NIH Guide for the Care and use of Laboratory Animals (20).

Rodent parasite (P. berghei berghei and inoculation)

Chloroquine sensitive rodent plasmodia, *Plasmodium berghei berghei* was sourced from National Institute for Medical Research (NIMR), Lagos, Nigeria. Parasites were maintained alive in mice at Animal Facility Centre, Department of Pharmacology and Toxicology, NIPRD by continuous reinfestation (IP) (21), every 4 days. Blood was collected from a donor infested mouse through cardiac puncture and diluted with normal saline. The study mice received 0.2 ml of diluted inoculums IP consisting of $1x10^7$ parasitized red blood cells.

Antiplasmodial Studies

Suppressive Test

Tests were performed in a 4-day suppressive test using the methods of (22). Each mouse was inoculated on the first day (D_o), intraperitoneally with 0.2 ml of infected blood containing $1x10^7$. *P. berghei berghei*. The animals were divided into five groups of six mice per cage. Then, orally administered shortly after inoculation with the extract (100, 200 and 400 mg/kg). Chloroquine (10 mg/kg) and normal saline (10 ml/kg) were given to the positive and negative control groups respectively. Treatment continued daily for four days. On the fifth day (D_5), thin films were made with blood collected from the tail of each mouse. The films were fixed with methanol and stained with Giemsa for 30 min and parasitaemia determined by microscopic examination in 10 different fields.

Curative Test

Evaluation of curative potentials of the extract was done by adopting the methods of (23) with slight modification. Thirty mice were randomized and intraperitoneally injected with standard inoculums of $1x10^7$ *P. berghei berghei* infected erythrocytes and left untreated. Seventy two hours after, the mice were grouped into five groups of six per cage. Group 1 received saline (10 ml/kg) daily, groups 2, 3 and 4 received daily doses of the extract (100, 200 and 400 mg/kg), while group 5 received respective chloroquine (10 mg/kg) daily. All administered orally. Treatment continued until the seventh day (D_7), when thin films were made with blood collected from the tail of each mouse. The films were fixed with methanol, stained with Giemsa and parasitaemia density determined by microscopic examination in 10 different fields.

The mean survival time for each group was determined by finding the average survival time (days) of the mice in each

Table 1. Suppressive activity of *B. coccineus* in *P. berghei* infected mice.

Treatment	Dose	Mean Parasitaemia Density	Percentage inhibition
Nornal saline	10 ml/kg	28.17±1.55	-
B. coccineus	100 mg/kg	5.30±1.53	81.5*
B. coccineus	200 mg/kg	3.13±0.29	88.9*
B. coccineus	400 mg/kg	2.17±0.49	92.3*
Chloroquine	10 mg/kg	1.28±0.1.7	95.5*

D3=Day three; D7=Day seven; *Significant as compare to control with p<0.05

Table 2. Curative effect of *B. coccineus* in *P. berghei* infected mice

Treatment	Dose	Mean Parasitaemia Density		Survival time
		Pre-(D_3) treatment	Post-(D_7) treatment	Days
Nornal saline	10 ml/kg	32.60±1.00	41.70±0.55	9.20±1.20
B. coccineus	100 mg/kg	28.90±2.5	13.16±1.03*	20.17±0.01
B. coccineus	200 mg/kg	29.63±1.20	9.12±1.62*	22.33±0.01
B. coccineus	400 mg/kg	31.45±3.10	7.48±0.25*	26.0 ±0.01
Chloroquine	10 mg/kg	30.20 ± 2.18	2.10±0.31*	30.0±00

D3=Day three; D7=Day seven; *Significant as compare to control with p<0.05

group over a period of 30 days.

Data Analysis

Results obtained were expressed as mean ± SEM and were analyzed using one-way ANOVA followed by the Turkey Kramer multiple comparison test. P<0.05 was considered significant in all cases.

Results

Phytochemical Tests

The ethanolic extract of *B. coccineus* gave positive test for saponins, tannins, terpenes, steroids, flavonoids and alkaloids.

Acute toxicity test

There was no mortality recorded in mice upon oral administration even at doses as high as 5000 mg/kg. This indicates that the experimental doses used are relatively safe.

Suppressive test

As shown in table 1, the ethanolic extract of *B. coccineus* demonstrated dose-dependent antiplasmodial activity at doses employed in the study. The extract at 400 mg/kg/day gave 92.3% suppression as against 88.9% and 81.5% suppression induced by 200 mg/kg/day and 100 mg/kg/day. Chloroquine (10 mg/kg/day) however, gave 95.5% suppression (table1).

Curative test

In established *P. berghei* infection in mice, there was a dose-dependent reduction in the level of parasitaemia in the treated groups unlike in the saline control group in which there was a consistent increase in the blood

parasite density. The standard drug, chloroquine (10 mg/kg) gave a mean survival time of 30.0±0.00 days compared to 20.17±0.01, 22.33±0.01, 26.0±0.01 days respectively, observed for the groups treated with 100, 200.and 400 mg/kg of ethanolic leaf extract of *B. coccineus*. However, the mice in the control group survived for 9 days only (table 2).

Discussion

In the study, the ethanolic leaf extract of *B. coccineus* exhibited significant (P<0.05) antiplasmodial activity *in vivo* following oral administration to infected mice. The extract demonstrated good antilpasmodial activity against early infection at various doses employed in the study. This method of suppressive testing for antiplasmodial activity has become popular during scientific evaluation of potential phytomedicines for treatment of experimental malaria (24). Our investigation of the scientific reasons behind the folkloric use of *B. coccineus* in treatment of malaria attack in traditional African settings can be partially satisfied with this result (25). In addition, the result of chemosuppressive study can be interpreted to be that the leaf extract of *B. coccineus* can suppress parasite growth to non-detectable levels in erythrocytes. It is important that scientific evaluation of traditional medicine preparations for claimed antimalarial efficacy be carried out even up to the level of finding out the degree of suppression of parasite growth in erythrocytes (26).

The leaf extract of *B. coccineus* also exerted significant curative effect in established infection induced by an intraperitoneal inoculation of $1x10^7$ red blood cells parasitized with *P. berghei berghei*. The leaf extract has a noteworthy antimalarial activity as the mean survival time values at some employed doses were twice or more than that of the control group (27). The observed antimalarial activity of the plant extract is consistent with the traditional use of the plant as herbal medication against the disease and indicative of its potential as a chemotherapeutic antimalarial agent. *P. berghei* has been used in studying the activity of potential antimalarials *in vivo* in rodents (28,29) and it produces diseases similar to those of human plasmodial infection (3o, 31). Agents with suppressive activity against *P. berghei* were known for antimalarial activity (32). However, the mechanism of antiplasmodial action of the extract at this stage is not clear, there is that possibility that the agent might have acted through metabolic activation of the immune system (33), which can be attributed to the useful phytochemicals in the plant. Plant substances like terpenes, alkaloids and flavonoids (detected in the extract), as well as xanthones were reported to have antiplasmodial effect (34, 35). Earlier studies on extracts of *B. coccineus* showed that it possesses analgesic, anti-inflammatory and antipyretic properties (15, 16). Agents with such properties are known to produce additional remedy to malaria patients (36).

However, it is hoped that the screening of the locally used medicinal plants for antimalarial properties can fully be investigated with a view to establishing their efficacy and to determine their potentials as sources of new antimalarial agent.

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