RESEARCH ARTICLE

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In vivo Antimalarial Activity Evaluation of Two *Cryptolepis sanguinolenta* Based Herbal Decoctions

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Abstracts: The antimalarial activity of two *Cryptolepis sanguinolenta* based herbal decoctions namely Cryptoquine and Nibima, were studied using *Plasmodium berghei* (ANKA) in mice. The herbal preparations were evaluated for chemosuppressive activity during an early infection with *P. berghei* in mice and also for possible repository activity to ascertain whether they could be useful as prophylaxis. Each mouse used in the study was infected with a standard inoculum of 1 x 10^6 parasitised RBCs intraperitoneally. *P. berghei* infected mice which were treated with *Cryptolepis sanguinolenta* based herbal preparations showed chemosuppression in both decoctions for the 4-day suppressive test. Also, results of the repository activity of the herbal preparations showed that both decoctions have promising activity judging by their ability to reduce parasitemia in mice. Generally, the results of the mean survival time of mice treated with the decoctions under the 4-day tests compares well with those treated with chloroquine. The results indicate that both *Cryptolepis sanguinolenta* based decoctions used in this study have schizontocidal and repository activity against *Plasmodium berghei* in mice and may be useful in the prevention and treatment of malaria.

Key Words: *Cryptolepis sanguinolenta,* decoction, antimalarial, Plasmodium berghei.

INTRODUCTION

Malaria is a major disease of tropical climates with high mortality rate. According to the WHO, approximately 300 -500 million individuals are infected with the disease annually with total deaths ranging from 1.5 - 3.5 million [(1)].

Due to the increasing resistance of the parasite to conventional antimalarial agents, there is a need to develop more effective new anti-malarial drugs that are inexpensive, routinely available to people especially those in the developing countries[(2)].

Herbal medicines are known to be widely used to treat malaria and are often more available and affordable than Western medicine but only a few of them have been studied and evaluated scientifically for possible medical application[(3)]. The roots of cryptolepis (*Cryptolepis sanguinolenta*, Periplocaceae) are used in traditional African medicine to treat a variety of diseases, including malaria [(4,5)]. In Ghana, dried root decoctions of the herb are prepared by boiling the powdered roots in water, and used in traditional medicine to treat various forms of fever, including malaria, urinary and upper respiratory tract infections, rheumatism, and venereal diseases [(4, 6, 7)].

The *Plasmodium berghei*-infected mouse model has also been used widely in preliminary tests for in *vivo* activity of potential antimalarial agents.

The model provides a preclinical indication of any potential bioactivity as well as possible toxicity of the sample being tested [(8,9,10)]. Preclinical assays are part of the requirements to guarantee the efficacy and safety of natural products and are often requested by regulatory authorities to approve the marketing of these products [(11)].

In the present study, decoctions prepared from the roots of *Cryptolepis sanguinolenta* from two different manufacturers were evaluated against *Plasmodium berghei* in mice to determine their efficacy. The decoctions were Mist Nibima and Cryptoquine 2000 fever mixture. The aim of the study was to screen the decoctions to ascertain their antiplasmodial activity on *Plasmodium berghei* in mice. The study specifically sought to determine the blood schizontocidal activity of the herbal preparations on an early infection, its prophylactic activity as well as its ability to increase the survival time of mice infected with the parasite.

MATERIALS AND METHODS

Herbal Preparations

Mist Nibima and Cryptoquine 2000 fever mixture which were decoctions were supplied by The Centre for Scientific Research into Plant medicine (CSRPM), Mampong-Akuapem and a private herbal drug manufacturer respectively.

Standard Drugs

Chloroquine injection of strength 64.5 mg/ml (Koforidua Infusion Ltd, Koforidua, Ghana) and Amodiaquine hydrochloride (Camoquin) tablets (Parke-Davis, Detroit, USA) were obtained from the Pharmacy Department of the Tetteh Quarshie Government hospital at Mampong-Akuapem, Ghana.

Experimental Animals

Female Swiss albino mice, (8-10 weeks old) and of approximately 25 ± 2 gm weight, bred at the CSRPM animal house were used. The mice for the study were fed on standard diet and water *ad libitum*, and were maintained under standard conditions of humidity and temperature. Prior to the commencement of each experiment, the animals were weighed and assigned to treatment groups. They were allowed to acclimatize for at least a period of one week.

Parasites

ANKA strain of *Plasmodium berghei* was obtained from the Noguchi Memorial Institute for Medical Research, Legon, Ghana. The parasites were maintained by blood passage in Swiss albino mice.

Preparation of Inoculum

Blood from a donor mouse infected with *P. berghei* was used for inoculum preparation. The red blood cell (RBC) per unit volume was calculated from the inoculum size. Percentage parasitemia was determined from Giemsa stained thin blood film. The required volume of blood was then obtained from the tail vein of the donor mouse and diluted with sterile physiological saline such that the final inoculum (0.2 ml) for each mouse would contain the required number of parasitized RBC (1 x 10^6).

Administration of Parasites

The inoculums, which contained 1 x 10^6 parasitized red blood cells per 0.2 ml in saline preparation, were inoculated intraperitoneally into each mouse using a 25G (0.5 x 16 mm) needle and 1.0 ml syringe.

Drug and Test Sample Preparation

Herbal preparations and standard drugs were all incorporated in the feed of the animals. The drugs and test samples were dissolved in water and added to the feed to make the required dosage. The dosages of the drugs and test material used are shown in table 1 .

The doses of the herbal preparations selected for the study were based on the recommended dose suggested by the manufacturers of the products and also ten times the recommended dose. Acute toxicity tests indicated that the LD_{50} (p.o) of both decoctions was above 300mg/kg bodyweight.

Antimalarial Tests

Blood Schizontocidal Activity of Herbal Preparation on Early Infection, (4-Day Test)

The method used was based on that of Peters [(12)] and Knight and Peters [(13)]. The blood schizontocidal activity of the herbal preparation and that of chloroquine as a standard drug was tested on albino mice receiving a standard inoculum, 1 x 10^6 parasitized RBC intraperitoneally (i.p) on day zero, (Do).

The mice were divided into four groups (n=6) and treated with the normal dose and ten times normal dose of herbal preparation incorporated in the feed. A positive control

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Table 1: The Dosages of The Decoctions and Drugs Used in the Experiment.

Test material	Dosage
Cryptoquine (decoction)	3 ml/kg body weight
	30 ml/kg body weight
Nibima (decoction)	5 ml/kg body weight
	50 ml/g body weight
Chloroquine	5 mg/kg body weight
Camoquine	10 mg/kg body weight

Table2: Blood Schizontocidal activity of 'Nibima' and 'Cryptoquine Decoctions during an Early P Berghei Infection in Mice (4-Day Test).

Treatment	Dose	No. of slides (n)	% Parasitemia ± SEM	Average % chemosuppression
'Nibima'	5 ml/kg	6	1.2 ± 0.1*	61.9
'Nibima'	50 ml/kg	6	1.0 ± 0.1*	67.0
Chloroquine	5 mg/kg	6	0.9 ± 0.1*	70.6
Untreated control	-	6	3.1 ± 0.2	-
'Cryptoquine'	3 ml/kg	6	1.4 + 0.3*	58.2
'Cryptoquine'	30 ml/kg	5‡	1.2 + 0.1*	66.4
Chloroquine	5 mg/kg	5‡	0.9 + 0.1*	75.3
Untreated control	-	6	3.5 + 0.1	-

Key: S.E.M., Standard error of mean; ‡ Some slides not read due to poor preparation; * Statistically significant relative to untreated control, P<0.05

Table 3: Repository (Prophylactic) activity of 'Nibima' and 'Cryptoquine' Decoctions against *P. berghei* Infection in Mice

Treatment	Dose	No. of slides (n)	% Parasitemia ± SEM	Average % chemosuppression
Nibima	5 ml/kg	6	1.3 ± 0.1*	56.5
Nibima	50 ml/kg	5‡	1.0 ± 0.1*	67.5
Camoquine	5 mg/kg	4‡	1.2 ± 0.1*	62.9
Untreated control	-	6	3.1 ± 0.2	-
Cryptoquine	3 ml/kg	6	1.1 ± 0.1*	70.0
Cryptoquine	30 ml/kg	5‡	1.1 ± 0.1*	70.3
Camoquine	10 mg/kg	5‡	1.3 ± 0.2*	66.6
Untreated control	-	6	3.8 ± 0.3	-

S.E.M., Standard error of mean; ‡ Some slides not read due to poor preparation; * Statistically significant relative to untreated control; P<0.05.

Table 4: Mean Survival time of Mice Treated with 'Nibima' and 'Cryptoquine' Decoctions during an early *P. berghei* Infection in Mice (4-Day test)

Treatment	No. of mice (n)	Dose	Survival time ± S.E.M/days
Nibima	6	5 ml/kg	13.5 ± 2.5
Nibima	6	50 ml/kg	16.2 ± 2.3*
Chloroquine	6	5 mg/kg	17.8 ± 3.0*
Untreated control	6	-	10.2 ± 1.2
Cryptoquine	6	3 ml/kg	15.2 ± 2.7 *
Cryptoquine	6	30 ml/kg	14.0 ± 2.9*
Chloroquine	6	5 mg/kg	16.5 ± 3.1*
Untreated control	6	-	9.5 ± 1.5

Key: S.E.M., Standard error of mean, * Statistically significant relative to untreated control, P<0.05.

Table 5: Mean Survival time of Mice Treated with 'Nibima' and 'Cryptoquine' Decoctions during the Repository activity test using *P. Berghei* in Mice

Treatment	No. of mice (n)	Dose	Survival time ± S.E.M/days
Nibima	5†	5 ml/kg	13.8 ± 2.9
Nibima	6	50 ml/kg	12.5 ± 2.5
Camoquine	6	10 mg/kg	17.5 ± 2.5*
Untreated control	6	-	12.5 ± 2.0
Cryptoquine	5†	3 ml/kg	10.8 ± 2.6
Cryptoquine	6	30 ml/kg	7.8 ± 0.4
Camoquine	6	10 mg/kg	8.0 ± 0.3
Untreated control	6	-	7.5 ± 0.2

Key: S.E.M., Standard error of mean, † Some mice died due to poor handling during inoculation, * Statistically significant relative to untreated control, P<0.05

group was given 5 mg/kg body weight/day of chloroquine while the negative/untreated group received an equivalent amount of untreated feed.

The 'drugs' were administered for four consecutive days (Day 0 to Day 3). On Day 4, tail blood was taken from each animal for a Giemsa stained thin blood film to determine the level of parasitemia. The slides were examined by light microscope using x100 oil immersion. The mean percentage parasitemia was calculated as follows:

$$\%\text{Parasitemia} = \frac{\text{Total No. of Parasitized RBCs}}{\text{Total Number of RBCs}} \times 100$$

The average percentage suppression of parasitemia by the "drugs" was assessed by comparison with the control. The survival time for each treatment group was also monitored.

Repository (Prophylactic) Activity of Herbal Extract

Table 6: Phytochemical Screening of *C. Sanguinolenta* based Decoctions.

Phytoconstituent	Nibima	Cryptoquine
Alkaloids	+	+
Reducing sugars	+	+
Saponins	-	+
Polyamides	-	+
Phenolic compounds	-	-
Cyanogenic glycosides	-	-
Flavonosides	-	-

A method similar to that described by Peters [12] was used in assessing the prophylactic activity of the herbal preparations. The mice were divided into four groups (n=6) and treated with the normal dose and ten times normal dose of herbal preparation incorporated in the feed. The positive control group received Camoquine (10 mg/kg body weight/day) incorporated in the feed while the untreated group received an equivalent amount of untreated feed on Day 0.

On Day 4, each mouse was inoculated with 1 x10^6 parasitized RBC. Giemsa stained thin films were made 72 hours after the inoculation to determine level of parasitemia. The average percentage suppression of parasitemia by the drugs was assessed by comparison to the control group. The survival time for each treatment group was also monitored.

Phytochemical Screening

Methods used for phytochemical screening were adapted from previous work on plant analysis [14]. The herbal decoctions were screened for alkaloids, saponins, polyamides, phenolic compounds, cyanogenic glycosides, flavonosides, and reducing sugars.

Statistical Analysis

Graph Pad Prism 4.0 statistical package software was used for statistical analysis. Averaged data are presented as mean ± SEM. Statistical significance was accessed by one-way ANOVA followed by the Dunnett's test. Data were considered significant at $P < 0.05$.

RESULTS

Antimalarial Tests

Blood Schizontocidal Activity of 'Nibima' and 'Cryptoquine' Decoctions during an Early *P Berghei* Infection in Mice (4-Day Test):

Table 2 shows results of the blood schizontocidal activity of Nibima decoction and Cryptoquine in mice. There was significant suppression of parasitemia in all the treatment groups compared to the untreated control ($P < 0.05$). Nibima at 5 ml/kg body weight of mice gave 61.9% suppression of parasitemia and at a dose of 50 ml/kg it produced a chemosuppression of parasitemia of 67.0% compared to 70.6% by chloroquine. Cryptoquine decoction at 3 ml/kg body weight produced a chemosuppression of 58.2% but at 30 ml/kg body weight it gave chemosuppression of 66. 4% while chloroquine had a chemosuppression of 75.3%.

Repository (Prophylactic) Activity of 'Nibima' and 'Cryptoquine' Decoctions against *P. Berghei* Infection in Mice

Table 3 shows the repository activity of Nibima and Cryptoquine. Prophylactic activity of the herbal decoctions and Camoquine were significant compared to the untreated control group ($P < 0.05$). Nibima at doses of 5 and 50 ml/kg body weight produced chemo-suppression of 56.5 and 67.5% respectively compared to 62.9% by Camoquine. Cryptoquine at the 2 dose levels produced almost the same chemosuppression of 70% while Camoquine, the positive control drug produced a chemosuppression of 66.6% which was lower than that of Cryptoquine.

Mean Survival Time of Mice Treated with the Decoctions during Early *P. Berghei* Infection (4-Day Test)

Table 4 shows increased mean survival times for both Nibima and Cryptoquine treatment groups above that of the untreated controls. Mean survival time for Nibima at 50ml/kg and that of chloroquine were comparable and significantly different from the control groups ($P<0.05$). However the mean survival time of Nibima at 5 ml/kg body weight though high was not significantly different from the untreated control group. The survival times of the two treatment groups of Cryptoquine and chloroquine were all significantly different from that of the untreated control group, $p<0.05$.

Mean Survival Time of *P. berghei* Infected Mice Treated with the Decoctions during the Repository Activity Test

Data in table 5 does not appear to show significant differences in mean survival times between Nibima and Cryptoquine treatment groups and their untreated control groups. The increased mean survival time for the Camoquine group was however significantly different from the untreated control group ($p < 0.05$). This confirms the use of Camoquine as a positive control drug.

Phytochemical Screening

Phytochemical screening revealed the presence of alkaloids and reducing sugars in both decoctions. Polyamides and saponins were also present in Cryptoquine cryptoquine decoction (Table 6).

DISCUSSION

The antimalarial activity of the two *Cryptolepis sanguinolenta* based decoctions was evaluated in this study using *Plasmodium berghei* in mice.

Both decoctions namely Nibima and Cryptoquine 2000 fever mixture showed marked schizontocidal activity against *P. berghei*. This resulted in the reduction of parasitemia which was significant in all treatment groups compared to the untreated controls ($p<0.05$).

The decoctions also showed repository activity by producing significant reduction in parasitemia in the treatment groups compared to the untreated controls. It must be pointed out that chemosuppression by chloroquine; the positive control drug in the 4-day test was higher than the herbal decoctions which show that the parasite is more sensitive to chloroquine than the herbal decoctions. Chemosuppression of the herbal preparations also appear to be dose dependent.

Results of the mean survival times showed increased survival times for mice treated with the herbal preparations compared to the untreated control groups. The herbal preparations demonstrated ability to extend the survival time of mice beyond those which were untreated which is indicative of antiplasmodial activity.

The antimalarial activity, which is indicated by chemosuppression of parasitemia in both tests used in this study might be attributed to the presence of alkaloids that were identified during phytochemical screening of the two herbal preparations. Alkaloids, particularly cryptolepine are known to have biological activity against the parasite [15]. Grellier [16] has also reported the activity of cryptolepine against *P. berghei* in mice. Other alkaloids apart from cryptolepine could also be responsible for the antiplasmodial activity of the herbal preparations [17].

CONCLUSION

The results of the present study has established that both decoctions of *Cryptolepis sanguinolenta* used in this work have blood schizontocidal and repository activity and this may justify and confirm their use as remedies for malaria. These decoctions which have been used over the years without any serious side effects may therefore be useful in the treatment and prevention of malaria. It would therefore be interesting if clinical trial is carried out to evaluate the antimalarial activity of the herbal preparations in humans.

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