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Research Article

ANTIMALARIAL ACTIVITY OF *CRYPTOLEPIS SANGUINOLENTA* BASED HERBAL CAPSULES IN *PLASMODIUM BERGHEI* INFECTED MICE

Sammy C. K. Tay[1], Kwabena Dankwa[2], Stephen Y. Gbedema[3]*, Archibald Sittie[4]
[1]Department of Clinical Microbiology, School of Medical Sciences, College of Health Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana
[2]Department of Microbiology, School of Medical Sciences, University of Cape Coast, Ghana
[3]Department of Pharmaceutics, Faculty of Pharmacy and Pharmaceutical Sciences, College of Health Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.
[4]Centre for Scientific Research into Plant Medicine, Mampong – Akwapim, Ghana

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*Email: sgbedema.pharm@knust.edu.gh

ABSTRACT
Two *Cryptolepis sanguinolenta* based herbal capsules prepared at the Centre for Scientific Research into Plant Medicine, Mampong-Akwapim, Ghana, were evaluated for antiplasmodial activity using *Plasmodium berghei* in mice. The herbal capsules were evaluated for chemosuppressive activity on an early infection as well as for possible repository activity in which each mouse was infected with a standard inoculum of 10^6 parasitized RBCs. Chloroquine and Amodiaquine were used as standard drugs. The results indicate that the herbal capsules possessed significant blood schizontocidal activity following chemosuppression of parasitemia in treated mice compared to the untreated control groups. The herbal based capsules of *C. sanguinolenta* have both schizontocidal as well as repository activity and may be useful in the treatment and prevention of malaria.
KEY WORDS: *Cryptolepis sanguinolenta* capsules, antiplasmodial, malaria.

INTRODUCTION

The WHO estimates that each year, between 300-500 million people living in the tropical and subtropical regions of the world become infected with malaria and almost 3 million of them die, especially young children[1].

In Ghana, malaria is a major disease that causes poverty and low productivity, highly endemic and accounts for over 44% of reported out-patient visits in hospitals[2]. Of malaria cases reported at out-patient units in public health facilities, 36-38% is typically children under 5 years of age[1].

Effective control of the *Plasmodim* parasite that causes malaria has been hampered by the complexity of the life cycle, absence of vaccines, drug resistance and non-availability of good quality prophylactic drugs[3]. The high cost of treatment for malaria coupled with the threat posed by resistant strains of malaria parasites to existing therapies have made the need for cheaper alternative treatment more relevant now than ever.

For thousands of years, plants have formed the basis of sophisticated traditional medicine systems and more recently, natural products have been a good source of lead compounds especially against infectious diseases[4]. Herbal medicines provide an option for malaria treatment which may eventually reduce the need for importation of conventional anti-malarial drugs which are usually expensive and unavailable to the people who need it most.

Lack of clinical data on safety and efficacy of herbal antimalarials, and the variation in the concentration of active compounds in plants has been a major hindrance to the use of herbal antimalarials[5]. However, traditional practices are now being coupled with up-to-date scientific methodology and processed dosage forms are now being produced[6].

The need for newer therapies has led to intensive research on *Cryptolepis sanguinolenta*, a plant that has been used traditonally to treat a variety of diseases including malaria[7,8]. This plant has also been used in clinical therapy of malaria at the Centre for Scientific Research into Plant Medicine (CSRPM) at Mampong-Akwapim in Ghana for nearly 30 years. Unfortunately the present dosage form (decoction) in which the plant is formulated at the CSRPM requires the administration of large volumes of the decoction, (100 ml x tid x 6 days) which often causes nausea, vomiting and poor

compliance. CSRPM has therefore developed two solid dosage forms of *C. sanguinolenta* which needs to undergo preclinical evaluation for safety and efficacy.

In this study, we report on the efficacy studies of two solid dosage forms (capsules) of the plant namely NBO1 and NBO2 which were prepared from the decoctions and tinctures of the plant respectively.

The aim of this study was to screen the two *C. Sanguinolenta* based herbal solid dosage forms to ascertain their antiplasmodial effect on *Plasmodium berghei* in mice. The study specifically sought to determine;

i. the blood schizontocidal activity of the herbal preparations when used in mice
   - during an early infection (4-Day test)
   - as a prophylactic

ii. the survival time of mice treated with the herbal preparations.

MATERIALS AND METHODS

Herbal Preparations

Herbal preparations employed in the study were 'NBO1' and 'NBO2' which were in the form of capsules supplied by CSRPM, Mampong-Akwapim.

Standard Drugs

Chloroquine injection (64.5 mg/ml) and Amodiaquine hydrochloride (Camoquine) tablets (200 mg /tablet) manufactured by Koforidua Infusions Ltd, Ghana and Parke Davis & Co, UK respectively were employed as the standard drugs..

Experimental Animals

Male Swiss albino mice of the ICR strain, (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, 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, University of Ghana, Legon, Accra. 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 number of parasitized RBC in a volume of blood was then calculated. The required volume of blood was then obtained from the tail vein of the donor mouse and was diluted with sterile physiological saline so that the final inoculum (0.2 ml) for each mouse would contain 1 x 10^6 parasitized RBC.

Administration of Parasites

The inocula (1 x 10^6 parasitized red blood cells per 0.2 ml in saline preparation) were injected 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 doses of the drugs and test material used are as 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 ten times the recommended dose. Acute toxicity tests indicated that the LD_{50} (p.o) of both capsules were above 3000mg/kg bodyweight.

Blood schizontocidal activity of herbal preparation on early infection, (4 Day test)

The method used was based on that of Knight and Peters[9,10]. 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 (Dose 1) and ten times normal dose (Dose 2) of herbal preparation incorporated in the feed.

A positive control group was given 5 mg/kg body weight/day of chloroquine while the negative control/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 level of parasitemia. The slides were examined by light microscope using x 100 oil immersion. The mean percentage parasitemia was calculated as follows :

$$\% \text{ Parasitemia} = \frac{\text{Total number of Parasitized RBCs}}{\text{Total number of RBCs}} \times 100$$

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

A method similar to that described by Peters[9] was used in assessing the prophylactic activity of the herbal capsules. The mice were divided into four groups (n=6) and treated with the normal dose (Dose 1) and ten times normal dose (Dose 2) of herbal capsule incorporated in the feed. The positive control group received Amodiaquine hydrochloride (Camoquine) 10 mg/kg body weight/day incorporated in the feed while the negative/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 untreated control group. The survival time for each treatment group was also monitored.

Phytochemical screening

Phytochemical screening methods adapted from previous work on plant analysis by Harbone[11] and Sofowora[12] were used to screen the extracts for the presence of 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 test for multiple group comparisons. Data were considered significant at $P < 0.05$.

RESULTS

Determination of anti-malarial activity

The NBO1 and NBO2 capsules were tested for anti-malarial activity against *Plasmodium berghei* in mice. Results in table 2 show that at the recommended dose and ten times this dose, there was significant reduction in parasitemia levels with respect to the control groups of both NBO1 and NBO2 capsules ($p<0.05$). NBO1 capsule appears to show dose-dependent chemosuppression effect with the normal and recommended doses giving chemosuppression of 25.4% and 47.0% respectively. NBO2 at the two dose levels also produced a dose dependent chemosuppression of 66.2 and 74.65% respectively compared to the 77.5% by chloroquine.

Repository Test

Table 3 shows the repository activity of NBO1 and NBO2 capsules. Both capsules produced significant reduction of parasitemia with respect to the untreated control groups ($p< 0.05$). NBO1 in doses of 30 and 300 mg/kg body weight produced dose dependent chemosuppression of 59.5% and 63.0% respectively compared to the untreated control group. NBO2 at the recommended dose and ten times the recommended dose gave a dose dependent chemosuppression of 49.6% and 76.9% respectively.

Mean survival time (4 –Day test)

Table 4 shows the mean survival times of NBO1 and NBO2 capsules. NBO1 at the 2 dose levels appears to increase the survival time of mice infected with *P. berghei* above the untreated control group though the differences are not significant ($p>0.05$). NBO2 at the recommended dose on the other hand showed a significant increase in mean survival time from the untreated control group. Surprisingly NBO2 at 500 mg/kg bodyweight did not appear to increase the mean survival time of mice.

Mean Survival time (Repository test)

Table 5 shows the mean survival times of mice treated with NBO1 and NBO2 capsules. NBO1 capsules at the two dose levels did not increase the survival time of mice beyond that of the untreated control group. Result for the 30 mg/kg NBO1 group was unexpected as it showed mean survival time lower than the untreated control group. However NBO2 at 500 mg/kg body weight and Camoquine groups had mean survival times, which were significantly different from the untreated control groups ($p<0.05$).

Phytochemical screening

The phytochemical screening of the herbal capsules revealed the presence alkaloids and reducing sugars in both herbal preparations and polyamides in NBO1 capsules only (Table 6).

DISCUSSION

This study focused primarily on the preliminary evaluation of antiplasmodial activity of two solid dosage forms of *Cryptolepis sanguinolenta* based herbal preparations on *Plasmodium berghei* in mice.

The results indicate that the two capsules possess blood schizontocidal activity as evident from the chemosuppression obtained in the 4 - day early infection test. The herbal preparations also exhibited repository activity which in some cases was better than Camoquine, the standard prophylactic drug used in this study.

It must be noted however that the antiplasmodial activity of the herbal capsules on an early *P. berghei* infection

were below that of chloroquine, the standard drug. *P. berghei* infected mice treated with the *C. sanguinolenta* based herbal capsules during an early infection also showed dose dependent chemosuppression.

Generally the mean survival times of mice treated with the herbal capsules did not show dose dependence as expected. It appears that the use of mean survival time in evaluating anti-malarial activity is a less sensitive parameter. This may be attributed to the influence of host genetic factors on the virulence and pathology of *P. berghei* as well as the varied immune responses of mice[13]. These factors enabled some mice that are moribund to linger on for longer time before death, thereby extending their survival times. Despite these limitations, the ability of the herbal capsules to prolong the life of the infected mice beyond that of the untreated control groups demonstrated their antiplasmodial activity.

Phytochemical screening of the herbal capsules revealed the presence of alkaloids and other phytocompounds. Alkaloids have been implicated in the antiplasmodial activities of many plant extracts[14]. It is speculated that Cryptolepine and other minor alkaloids in *Cryptolepis sanguinolenta* might be the main group of secondary metabolites responsible for the observed antiplasmodial activity. Cryptolepine is known to target the DNA and inhibits the enzyme topisomerase II[15] in DNA synthesis which may affect cell division. Some plants are also known to exert their antiplasmodial action by elevation of red blood cell oxidation[16]. These may be the basis for its effect on *P. berghei*.

CONCLUSION

The results of the present study show that the *Cryptolepis sanguinolenta* based herbal capsules NBO1 and NBO2 have antiplasmodial activity as seen in its ability to suppress parasitemia in mice in all the two evaluation tests. This underscores the possibility of it being used in the treatment of malaria especially when the capsules are relatively convenient to patients than liquid forms of the herbal preparation.

REFERENCES

1. WHO. Severe *Falciparum* malaria (severe complicated malaria - 3rd edn.). *Trans R Soc Trop Med Hyg.*, 2000;94: S1/1-S1/11 and S1/34-S1/35.
2. WHO. "Roll back malaria monitoring and evaluation: Country Profile"2005; [www.rbm.who.int/wmr2005/profiles/ghana], (accessed 2006 April 5)
3. Krettli AU, Andrade-Neto V, Brandao LM, Ferrari WM. The search for new antimalarial drugs from plants used to treat fever and malaria or plants randomly selected: A review. *Mem. Inst. Oswaldo. Cruz*. 2001;**96** (8): 1033-1042
4. Sianne S, Fanie R. Antimalarial activity of plant metabolites. *Nat Prod. Rep*., 2002;**19**, 675-692.
5. Bodeker G, Willcox ML. Traditional herbal medicines for malaria clinical review. *BMJ*, 2004;**329**,1156-1159
6. Orwa JA. Herbal medicine in Kenya: Evidence of safety and efficacy. *East African Journal*. 2002;341-42
7. Boye GL, Ampofo O. Medicinal Plants in Ghana. In: Wagner and Farnsworth NR, editors. *Economic and Medicinal Plants Research*. Vol. 4. *Plants and Traditional Medicine*. London: Academic Press; 1990;32-3.
8. Oliver-Bever BEP. Medicinal Plants in Tropical West Africa. Cambridge University Press; 1986; 18, 41, 131, 205.
9. Peters W. Drug resistance in *Plasmodium berghei*. Chloroquine resistance. *Exptl. Parasitol.,* 1965;**17**, 80-89.
10. Knight DJ, Peters W. The antimalarial action of N-benzyl-oxydihydrotriazines. The action of Clociguanil (BRL50216) against rodent malaria and studies on its mode of action. *Annals Trop. Med. Parasitol*. 1980;**74,** 393-404.
11. Harborne JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis, Chapman A & Hall. London, 1973: 279.
12. Sofowora A. Medicinal plants and traditional medicine in Africa. Chichester John Wiley & Sons New York, 1993; 97 - 145.
13. Janse CJ, Waters A. The *Plasmodium berghei* research model of malaria 2006; [http://www.lumc.nl/1040/research/malaria/model.html]. (accessed 2006 23 October)
14. Milliken W. Malaria and Antimalarial Plants in Roraima, Brazil. *Trop Doct*., 1997;**27**,20-4.
15. Bonjean K, DePauw-Gillet MC, Defranse MP. The DNA intercalating alkaloid cryptolepine interacts with topoisomerase II and inhibits Primary DNA synthesis in B16 melanoma cells. *Biochem.* 1988;**37**,5136 - 46
16. Etkin NL. Antimalarial plants used by Hansa in northern Nigeria. *Trop Doct*, 1997;27:12-6.

Table 1. Doses of the herbal capsules and standard drugs incorporated in feed

Test material	Dosage
NBO1 (capsules)	30 mg/kg body weight
	300 mg/kg body weight
NBO2 (capsules)	50 mg/kg body weight
	500 mg/kg body weight
Chloroquine	5 mg/kg body weight
Camoquine	10 mg/kg body weight

Table 2: Blood Schizontocidal activity of 'NBO1' and 'NBO2' capsule during an early *P. berghei* infection in mice (4-Day test)

Treatment	Dose	No. of slides (n)	% Parasitemia ± SEM	Average % chemosuppression
'NBO1' capsule	30 mg/kg	6	2.9 ± 0.5*	25.4
'NBO1' capsule	300 mg/kg	6	2.1 ± 0.3*	47.0
Chloroquine	5 mg/kg	4†	1.1 ± 0.1*	70.9
Untreated control	-	6	3.9 ± 0.2	-
'NBO2' capsule	50 mg/kg	6	1.9 ± 0.1*	66.2
'NBO2' capsule	500 mg/kg	6	1.4 ± 0.1*	74.6
Chloroquine	5 mg/kg	5‡	1.3 ± 0.2*	77.5
Untreated control	-	4‡	5.6 ± 0.4	-

S.E.M., Standard error of mean
† Some mice died due to poor handling during inoculation
‡ Some slides not read due to poor preparation
* Statistically significant relative to untreated control, P<0.05

Table 3. Repository (prophylactic) activity of 'NBO1' and 'NBO2' capsule against *P. berghei* infection in mice

Treatment	Dose	No. of slides (n)	% Parasitemia ± SEM	Average % chemosuppression
'NBO1' capsule	30 mg/kg	4‡	1.6 ± 0.1*	59.5
'NBO1' capsule	300 mg/kg	6	1.4 ± 0.1*	63.0
Camoquine	10 mg/kg	6	1.8 ± 0.1*	52.4
Untreated control	-	6	3.9 ± 0.9	-
'NBO2' capsule	50 mg/kg	6	2.6 ± 0.3*	49.6
'NBO2' capsule	500 mg/kg	6	1.2 ± 0.1*	76.9
Camoquine	10 mg/kg	5‡	1.8 ± 0.2*	65.3
Untreated control	-	5‡	5.1 ± 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 NBO1 and NBO2 capsules during an early *P. berghei* infection in mice (4-Day test)

Treatment	No. of mice (n)	Dose	Mean Survival time ± S.E.M /days
NBO1	4†	30 mg/kg	11.3 ± 2.5
NBO1	5†	300 mg/kg	13.2 ± 2.0
Chloroquine	4†	5.0 mg/kg	12.8 ± 2.4
Untreated control	4†	-	8.0 ± 0
NBO2	6	50 mg/kg	17.5 ± 2.2*
NBO2	4†	500 mg/kg	8.5 ± 0.3
Chloroquine	6	5.0 mg/kg	10.6 ± 0.2*
Untreated control	6	-	8.5 ± 0.2

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

Table 5: Mean Survival time of mice treated with NBO1 and NBO2 capsules during the repository activity test using *P. berghei* in mice

Treatment	No. of mice (n)	Dose	Mean Survival time ± S.E.M / Days
NBO1	6	30 mg/kg	7.5 ± 0.2
NBO1	6	300 mg/kg	10.0 ± 0.7
Camoquine	5†	10 mg/kg	13.6 ± 2.9
Untreated control	4†	-	8.25 ± 0.3
NBO2	6	50 mg/kg	12.3 ± 2.3
NBO2	6	500 mg/kg	13.7 ± 2.3*
Camoquine	6	10 mg/kg	11.2 ± 1.5*
Untreated control	6	-	9.2 ± 1.3

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

Table 6. Phytochemical screening of *C. sanguinolenta* herbal based capsules.

Phytoconstituent	NBO1 capsule	NBO2 Capsule
Alkaloids	+	+
Reducing sugars	+	+
Saponins	-	-
Polyamides	+	-
Phenolic compounds	-	-
Cyanogenic glycosides	-	-
Flavonosides	-	-

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