
Antimalarial Activity of a New Herbal Formulation

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Accepted 27 January 2005

Revised 29 June 2004

Received 28 February 2004

Malaria is recognized as a highly widespread infectious disease of world caused by sporozoa of genus *Plasmodium*. Most antimalarial drugs were developed on the basis of their action against asexual erythrocytic forms of malaria parasites, which are responsible for the clinical illness. More recently, chloroquine-resistant strains of *P. vivax* also have been reported. Practical, effective and safe drugs, insecticides and vaccines still are needed to combat malaria. The object of the present study was to develop a more effective, scientific-based herbal formulation by using traditional medicines. The screening was done by Peter's 4 day test by means of parasite counts. There was no parasitaemia from 15th day post infection in both drug treated animals. All animals were found infection free and healthy. The preliminary pharmacological studies revealed that the prepared formulation possesses promising antimalarial activity justifying its use for the management of malarial infections.

Malaria is a major health problem in many countries and according to an estimate of the WHO, more than 500 million infections occur per year¹. It is recognized as a highly widespread infectious disease of world caused by sporozoa of genus *Plasmodium* and is characterized clinically by periodic fever, anemia and enlargement of liver and spleen. It is the cause of higher sickness and death rate than any other disease, particularly in tropical and subtropical countries of Asia, Africa and South America. In India, about 70% of infections are reported to be due to *P. vivax*, 25-30% due to *P. falciparum* and about 4-8% due to mixed infections². Since 1960, transmission of malaria has risen in most regions where the infection is endemic, chloroquine-resistant and multidrug resistant strains of *P. falciparum* have spread, and the degree of drug resistance has increased. More recently, chloroquine-resistant strains of *P. vivax* also have been reported³.

Most antimalarial drugs were developed on the basis of their action against asexual erythrocytic forms of malaria parasites, which are responsible for the clinical illness. Highly effective chemotherapeutic agents in this category are chloroquine, quinine, mefloquine and halofantrine. Pyrimethamine, sulfonamides, sulfones and tetracyclines share

this property but are slower acting, less effective and nearly always used in combination with other antimalarial compounds⁴. With increasing drug resistance and the high cost of pharmaceutical drugs, the use of herbal antimalarials is popular. Artemisinin, a sesquiterpene lactone endoperoxide isolated from *Artemisia annua* L., and a number of its semisynthetic derivatives have shown to possess antimalarial properties⁵. They are all effective against *Plasmodium* parasites that are resistance to the newest and commonly used antimalarial drugs⁶. *Bidens pilosa*⁷, *Ampelozizyphus amazonicus*⁷, *Strychnous myrtoides*⁸, *Dichroa febrifuga* Lour⁹, *Spirostachys African*¹⁰, *Bridelia cathartica*¹⁰ and *Scoparia dulcis*¹¹, were studied extensively for antimalarial property. Practical, effective and safe drugs, insecticides and vaccines still are needed to combat malaria. The objective of the present study was to develop a more effective, scientific-based herbal formulation by using traditional medicines.

The herbal formulation prepared for the present study is an amalgam of natural plant ingredients comprising of; *Tinospora cordifolia* Sat, *Caesalpinia crista* seed powder, *Encostemma littorale* leaves extract, *Godanti bhasma* and Purified alum, which are in traditional use for several diseases and disorders¹². After authentication and collection, *Tinospora cordifolia* sat was prepared with cut and crushed pieces of stems with water by sedimentation process. *Caesalpinia crista* seed kernel powder was prepared by re-

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moving hard coat of these nuts and their subsequent pulverization. *Enicostemma littorale* sat was prepared with dried and powdered leaves with 60% alcohol by percolation method. Alum was purified by losing its water for crystallization and powdered. All the ingredients were pulverized and passed through sieve No. 44 to get uniform fine powder separately and they were stored in airtight containers. For preparation of antimalarial formulation, each ingredient was taken in an equal proportion and further pulverized thoroughly and stored in airtight container. Ten grams of the prepared formulation was extracted in a Soxhlet apparatus by successive solvent extraction method. The solvents used were petroleum ether (60-80°), benzene, ethyl acetate, chloroform and methanol in succession in increasing order of polarity. Extraction with each of the solvent was continued till the material exhausted completely. Each extract portion was collected, dried, weighed and percent yield of each extract was 1.9, 2.85, 1.1, 0.63 and 17.5, respectively. The percent yield of methanol extract of formulation and its constituents was highest and hence the methanol extract was considered for the present study.

The screening was done by Peter's 4 day test by means of parasite counts¹³. Each male mouse (4 w old Kasuali strain) received an intraperitoneal inoculum of 1×10^7 parasitized red blood cells on day of infection (D+O) and was treated once daily from day D+O to D+3 (for 4 d). A suspension of the test material prepared in distilled water and 1% Tween 80 and administered orally after 4 h of injection. Each group consists of five mice. Blood films were prepared from tail blood and stained with Geimsa. The blood smears were

prepared on D+4 and then on days 6, 8, 10, 13, 16, 25, 30, 40 and 60 post infection. Percent reduction in the treated mice was compared with untreated controls. The drug cycloguanil-HCl (25 mg/kg) was taken as the standard drug for comparison. Death occurring within 48 h of treatment is classified as death due to toxicity. Mean survival time of drugs was observed in increasing dosage of 20, 40, 80 and 160 mg/kg after treatment with the compounds, respectively as shown in Table 1.

In this study we found that out of five mice, three mice and one mouse were found surviving in the prepared formulation and purified alum, respectively on the d 60 of post infection. There was no parasitaemia from d 15 post infection in both drug treated animals. All animals were found infection free and healthy. The data reveal that the prepared formulation comprising of *Caesalpinia crista* powder, *Tinospora cordifolia* sat, *Godanti bhasma*, Purified alum and *Enicostemma littorale* sat proving antimalarial activity.

The formulation is in traditional use for the treatment of malaria¹⁴. The purpose of its evaluation was proving antimalarial activity on scientific base. The preliminary pharmacological studies revealed that the prepared formulation possesses promising antimalarial activity justifying its use for the management of malarial infections. By the use of formulation, the side effects of cinchona use like anorexia, ringing of ear, rashes and other allergic reactions, mental disturbance and abnormalities in GI system can be avoided. After all, it is suggested that the formulation should be taken up for rigorous pharmacological studies including synergistic

TABLE 1: ANTIMALARIAL ACTIVITY OF PREPARED FORMULATION AND ITS INGREDIENTS

Name of Compound	Mean Survival Time (days)				No. of mice survived at 160 mg/kg dose levels on 60 th post infection day
	20 mg/kg	40 mg/kg	80 mg/kg	160 mg/kg	
<i>Caesalpinia crista</i> powder	8.4	9.2	15.2	17.4	-
<i>Tinospora cordifolia</i> sat	9.8	13.6	14.8	16.4	-
<i>Godanti bhasma</i>	9.4	12.8	14.2	15	-
Purified alum	10.4	13.2	15.8	32.6	1/5
<i>Enicostemma littorale</i> sat	10.0	13.8	15.8	18.3	-
Formulation	10.6	14.8	16.6	39.0	3/5

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effects, screening for different malarial parasites including the chloroquine and multiresistant strains of *Plasmodium* on different developmental stages, toxic manifestations, minimum therapeutic concentration and dose regimen and comparative studies on prepared formulation and in use antimalarial formulation with regard to dose and duration of treatment.

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Spectrophotometric Methods for The Estimation of Nicorandil in Tablet Dosage Forms

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Accepted 5 February 2005

Revised 5 August 2004

Received 29 December 2003

Two simple, sensitive, accurate and rapid spectrophotometric methods have been developed for the estimation of nicorandil in tablets. Method A is based on the reaction of nicorandil with sulphanilic acid reagent in presence of cyanogen bromide solution giving yellow chromogen, which show maximum absorbance at 460 nm against reagent blank while method B is based on the estimation of nicorandil in 0.1 N HCl at 262 nm. Beer's law was obeyed in the concentration range of 10-80 µg/ml in method A and 5-40 µg/ml in method B. Results of the analysis were validated statistically and by recovery studies.

Nicorandil, a nitro vasodilator and potassium channel activator used as an antianginal drug. Chemically, nicorandil is N-[2-(Nitrooxy)ethyl]-3-pyridine carboxamide¹. Nicorandil is not official in any pharmacopoeia, hence there is no official method for the estimation of nicorandil in pharmaceuti-

cal formulations. Only spectrofluorimetric², HPLC^{3,5}, LC and LC-MS^{6,7} methods are reported for the estimation of nicorandil especially in the biological fluids. The present work describes two new, simple spectrophotometric methods involving nicorandil with reagents such as sulphanilic acid and cyanogen bromide (method A) and UV measurement of nicorandil in 0.1N HCl (method B)

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