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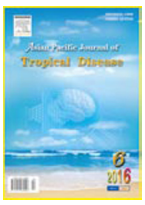

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Ameliorative antimalarial effects of the combination of rutin and swertiamarin on malarial parasites

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ABSTRACT

Objective: To ameliorate the antimalarial activity via the combination of rutin (flavonoid) and swertiamarin (glycoside).

Methods: The antimalarial effects were assessed by *in vitro* and *in vivo* methodology. *In vitro* antiplasmodial activity was assessed by using *Plasmodium falciparum* cultured media and determined the IC_{50} value of individual drugs and their combinations. In *in vivo* methodology, antimalarial effects of rutin, swertiamarin (200–280 mg/kg/day, *p.o.*) and their combination in 1:1, 1:2 and 2:1 ratios were investigated early and established malaria infections using Swiss albino mice infected with *Plasmodium berghei*. Chloroquine phosphate (5 mg/kg/day, *p.o.*) was used as the standard drug.

Results: IC_{50} values of the rutin and swertiamarin via *in vitro* study revealed (9.50 ± 0.29) μg/mL and (8.17 ± 0.17) μg/mL respectively. Whereas, the combination in 1:1 ratio [IC_{50} of (5.51 ± 0.18) μg/mL] showed better antiplasmodial activity against *Plasmodium falciparum*. *In vivo* results showed that rutin and swertiamarin had chemosuppressant effects in a dose-dependent manner, whereas, combination in 1:1 ratio possessed potential antimalarial activity similar to chloroquine phosphate. The drug interaction between rutin and swertiamarin revealed the synergistic effect on 1:1 ratio and additive effect on 1:2 and 2:1 ratios.

Conclusions: The results of the *in vitro* and *in vivo* study clearly indicate that the combination (1:1) of rutin and swertiamarin showed potential antimalarial activity rather than an individual of each and their combinations 1:2 and 2:1.

1. Introduction

Malaria is the most momentous parasitic disease which is a major and constant public health problem throughout the world[1]. It causes more than 1.1 million deaths and affects about 300–500 million people per year globally[2]. *Plasmodium falciparum* (*P. falciparum*) (protozoan parasite) is the most prevalent and virulent causative agents for malaria in human beings[3]. The emergence and rapid spread of *P. falciparum* resistance to commonly used antimalarial drugs including chloroquine pose a serious challenge to the effectiveness of early diagnosis and prompt treatment as a priority strategy within current malaria control efforts[4]. The only hope against drug-resistant severe cerebral malaria in the form of artemisinin combination therapy has also been devastated by the recent reports of clinical artemisinin resistance from South East Asia[5,6]. The potential value of malaria therapy using combinations of drugs was identified as a strategic and feasible option in improving efficacy, delaying development and selection of resistant parasites[7]. Hence, there is a need to search alternative or new antimalarial agents to increase the therapeutic efficacy as well as reduce the resistance.

Natural products contain a great variety of chemical structures and active compounds that have been screened for antiplasmodial activity as potential sources of new antimalarial drugs. There is a renaissance of interest to investigate curative properties of natural products. Green tea and curcumin are a few recently reported active plant products against malaria parasites[8,9]. There are about 360 natural products reported for antimalarial activity and flavonoids are among one of them[10]. The natural compounds like rutin (flavonoid) and swertiamarin (glycoside) both possess antioxidant

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properties and antiplasmodial activity[11-14]. Thus, the literature review suggested that the combination of rutin and swertiamarin might produce ameliorative effects than an individual compound. Therefore, the present research work was carried out to compare the antimalarial activity against *P. falciparum*, *in vitro* and *Plasmodium berghei* (*P. berghei*), *in vivo* of rutin, swertiamarin individually and their different combinations.

2. Materials and methods

2.1. Drugs and chemicals

Rutin and swertiamarin were received as gift sample from Medicinal and Aromatic Plant Research, Boriavi (Gujarat), India. Infected *P. falciparum* blood was obtained from Chhattisgarh Institute of Medical Sciences, Govt. of Chhattisgarh, Bilaspur, India. Roswell Park Memorial Institute media 1640 was purchased from Himedia Chemicals, Mumbai, India. Chloroquine phosphate was obtained from Ipca Laboratories, Mumbai, India. Ampicillin, gentamicin sulphate, Giemsa stain, alcohol, methanol, D-sorbitol, dimethyl sulfoxide (DMSO), heparin and other chemicals used were of analytical grade.

2.2. In vitro assessment of antimalarial activity

2.2.1. Preparation of drug solutions

Stock solutions of rutin and swertiamarin were prepared by dissolving 1 mg of each compound in 100 μL DMSO and 900 μL Roswell Park Memorial Institute 1640 (complete media) to obtain a stock of 1 mg/mL solution. Rutin solution (7–10 μg/mL) and swertiamarin solution (7–10 μg/mL) were prepared by further dilution from stock solutions[13,14]. Chloroquine phosphate (0.06 μg/mL) was taken as standard[13].

2.2.2. Experimental design

In vitro experimental study adopted for the cultivation of the malaria parasite (*P. falciparum*) including method was given by Trager and Jensen[15]. For screening of the drugs, the plates were divided into different groups, each containing 5 plates with 5 mL of *P. falciparum* cultured media. The control group was treated with DMSO (1.2 mL), while test groups of rutin and swertiamarin were treated with three different concentrations (7–10 μg/mL). Chloroquine phosphate (0.06 μg/mL) was used as a standard. Reduction of parasitaemia was determined through microscopic monitoring after 24 h incubation of all the treated plates and their IC_{50} values were calculated via dose-response curve (DRC) or concentration-response curve.

For the evaluation of drugs in combination, the dose ratio of the rutin and swertiamarin were selected by using the method of Berenbaum[16]. The plates were divided into different groups for synergy or additive effects, each plate containing 5 plates with 5 mL of *P. falciparum* cultured media. For the experiment, drug combination was prepared in ratios of rutin and swertiamarin in 1:1, 1:2, and 2:1 of half of their IC_{50} and was tested on a culture plate accordingly. The interaction between rutin and swertiamarin was analyzed by isobologram (Figure 1) and fractional inhibitory concentrations (FIC) techniques[17]. In the FIC techniques, the IC_{50} value of each agent (*n*) was determined. The reference combination made up of 1/*n* of each of these concentrations was titrated to find out a dilution that produced the specified effect. The degree of dilution required was equal to the sum of the FIC (concentration of each agent in combination/concentration of each agent alone) as conventionally determined by checkerboard titrations.

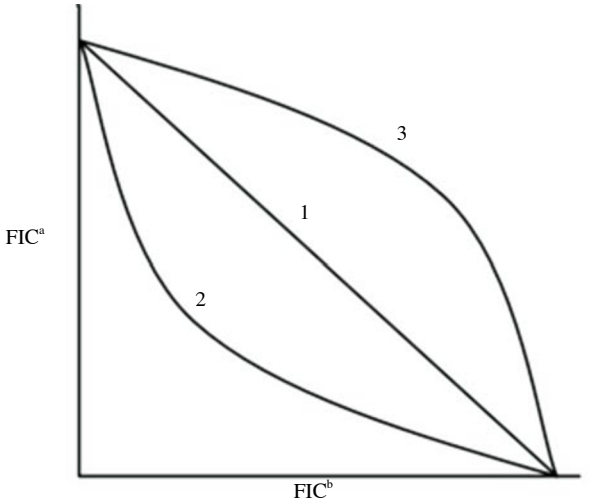

Figure 1. Ideal isobologram showing the possible interaction between two compounds (a and b).
[a]: Rutin; [b]: Swertiamarin; Curve 1: Additivity; Curve 2: Synergism; Curve 3: Antagonisms.

2.3. In vivo assessment of antimalarial activity

2.3.1. Animals

The Swiss albino mice of both sexes weighing 20–25 g (6–8 weeks old) were used as experimental animals and they were obtained from Indian Institute of Chemical Biology, Kolkata, India. The animals were housed under standard environmental condition, according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) [(23 ± 2) ℃, with 55% ± 5% humidity and 12 h light/dark cycle], and pellet diet was provided to them along with free access to water. The animals were acclimatized in a laboratory environment before the experiment. The whole experimental protocol for animal studies was approved by the Institutional Animal Ethics Committee of the Institute of Pharmaceutical Sciences, Guru Ghasidas Vishwavidyalaya, Bilaspur, Chhattisgarh, India (Reg. No. 994/a/GO/06/CPCSEA) and the experiments were conducted according to ethical principles and guidelines provided by CPCSEA, Govt. of India.

2.3.2. Malaria parasite strain

The chloroquine-sensitive *P. berghei* strains were used for *in vivo* study and they were obtained from the National Institute for Malaria Research, Govt. of India, New Delhi. A standard inoculum of 1×10^7 of parasitized erythrocytes from a donor mouse was used to infect the experimental animals.