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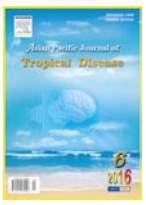

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Ameliorative antimalarial effects of the combination of rutin and swertiamarin on malarial parasites

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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.

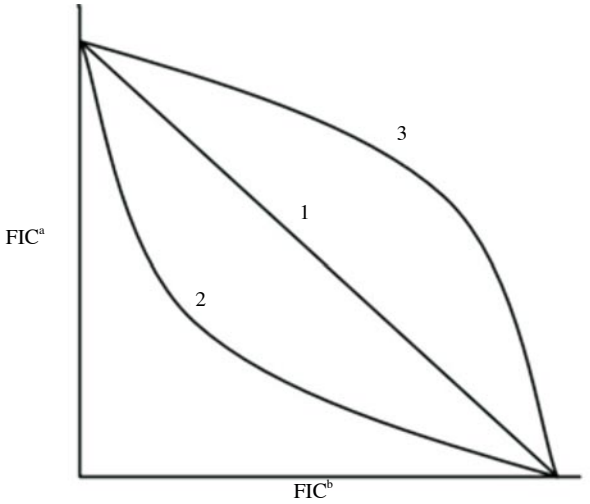

Figure 1. Ideal isobologram showing the possible interaction between two compounds (a and b).
[a]: Rutin; [b]: Swertiamarin; Curve 1: Additivity; Curve 2: Synergism; Curve 3: Antagonisms.

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.

2.3.3. Preparation of drug solutions

Different doses of rutin and swertiamarin were prepared as a suspension in 1% Tween-20 in distilled water and in 0.3% carboxy methyl cellulose in distilled water respectively.

2.3.4. Test on early malaria infection (4-day suppression test)

The method was adopted from Makinde *et al.*[18] and, Peters and Robinson[19]. Swiss albino mice were divided into different groups, each containing six animals and received standard inoculum of 1 × 10^7 *P. berghei* infected erythrocytes through the intraperitoneal route at the commencement of the experiment (Day 1). The malarial control group received 1 mL of 0.3% carboxy methyl cellulose orally. Whereas, test groups received rutin and swertiamarin at three different doses (200, 240 and 280 mg/kg/day, *p.o.*) in their respective group. Chloroquine phosphate (5.0 mg/kg/day, *p.o.*) was given to the standard group. The combination test groups received doses of rutin and swertiamarin in 1:1, 1:2, and 2:1 ratios based on ED_{50} value calculated from DRC. All the treatments were started from the day of infection (Day 1) for four days (*i.e.*, up to Day 4). On the 5th day, blood samples of each animal were collected through tail vein and examined by making thin film stained with Giemsa stain.

2.3.5. Test on established malaria infection (Rane test)

The modified method of Ryley and Peters was used[20]. Animals were divided into different groups similar to test on early malaria infection. Each mouse was inoculated with 1 × 10^7 *P. berghei* infected erythrocytes on Day 1 of the experiment. The mice were not treated until the parasitaemia established. On Day 4, all groups were treated with drugs as before according to their group. The mice were assessed daily for 5 days. On each day of assessment, percentage parasitaemia and percentage chemosuppression relative to the malarial control were determined for each group. After 7th day, the animals were fed *ad libitum* and observed for 28 days. Any death that occurred during this period was noted to determine the mean survival time.

2.3.6. Analysis of results

The blood sample of each animal was fixed with methanol, stained with Giemsa stain and examined under microscope in order to assess the antimalarial activity of the drugs. Percentage parasitaemia in each field and average percentage chemosuppression were calculated as followed:

Level of parasitaemia (%) = (Total No. of packed RBCs / Total No. of RBCs) × 100

where, RBC was red blood cell.

Average chemosuppression (%) = [(Average parasitaemia in control – Average parasitaemia in treated) / Average parasitaemia in control] × 100

2.4. Statistical analysis

Results were expressed as mean ± SEM. The results were analyzed statistically using One-way and Two-way ANOVA methods to identify the differences between multiple groups. The data were considered significant at $P < 0.05$. The ED_{50}/IC_{50} was estimated by nonlinear regression statistical methods.

3. Results

3.1. In vitro antiplasmodial activity

The concentration-response curve of rutin and swertiamarin revealed their IC_{50} values, which were summarized in Table 1. The finding of the results of *in vitro* antiplasmodial activity showed that rutin and swertiamarin both had antiplasmodial activity against chloroquine sensitive strain of *P. falciparum*.

Table 1

IC_{50} values of drugs and combinations screened against *P. falciparum in vitro*.

Drugs	IC_{50} ± SEM (μg/mL)
Rutin	9.50 ± 0.29
Swertiamarin	8.17 ± 0.17
Chloroquine	0.06 ± 0.01
Combination 1:1	5.51 ± 0.18
Combination 1:2	9.13 ± 0.13
Combination 2:1	11.51 ± 0.20

3.2. In vivo antiplasmodial assay

3.2.1. Effects on early malaria infection test

The results of the 4-day suppressive antimalarial screening (Table 2) of rutin and swertiamarin showed significant ($P < 0.001$) reduction in the average percentage of parasitaemia. Rutin and swertiamarin produced the dose-dependent chemosuppressive effect. DRC revealed that rutin and swertiamarin had 221.2 mg/kg and 181.6 mg/kg of ED_{50} value respectively. Different combination of rutin and swertiamarin also showed significant ($P < 0.001$) reduction in the average percentage of parasitaemia. The finding of the results indicated that rutin and swertiamarin in 1:1 ratio possessed potential antimalarial activity against *P. berghei* infection similar to chloroquine phosphate.

Table 2

Effects of rutin and swertiamarin on early malaria infection (%).

Groups	Average parasitaemia	Chemosuppression
Malarial control	12.49 ± 0.21	00.00
Standard	2.22 ± 0.22*	82.24
Rutin-200 (mg/kg/day)	7.02 ± 0.18*#	43.76
Rutin-240 (mg/kg/day)	5.72 ± 0.17*#	54.17
Rutin-280 (mg/kg/day)	4.74 ± 0.19*#	62.00
Swertiamarin-200 (mg/kg/day)	5.62 ± 0.18*#	54.96
Swertiamarin-240 (mg/kg/day)	3.57 ± 0.17*#	71.37
Swertiamarin-280 (mg/kg/day)	3.25 ± 0.42*#	73.99
Combination 1:1	2.50 ± 0.35*	79.95
Combination 1:2	3.40 ± 0.14*#	72.43
Combination 2:1	5.24 ± 0.18*#	58.03

Values were expressed as mean ± SEM ($n = 6$). Data were analyzed by One-way ANOVA with Tukey-Kramer multiple comparisons test. *: $P < 0.001$, significant as compared to control; #: $P < 0.001$, significant as compared to standard.

3.2.2. Effects on established infection test

The results of the established infection test were explored by average percentage of parasitaemia (Table 3), percentage

chemosuppression (Figure 2) and average mean survival time (Figure 3). The data indicated that on the Day 4 (D1 +4), all the treatments showed non-significant chemosupression on parasite except chloroquine phosphate ($P < 0.05$) and swertiamarin-280 (mg/kg/day) ($P < 0.001$). Moreover, on Days 5, 6, 7 and 8, the treatments showed significant ($P < 0.001$) chemosupression effects, except rutin-200 (mg/kg/day) as compared to malarial control. The treatments showed significant ($P < 0.01$) increase in mean survival time as compared to malarial control group except rutin-200 (mg/kg/day). The data showed that only combination in 1:1 ratio potentially increased the mean survival time similar to chloroquine phosphate.

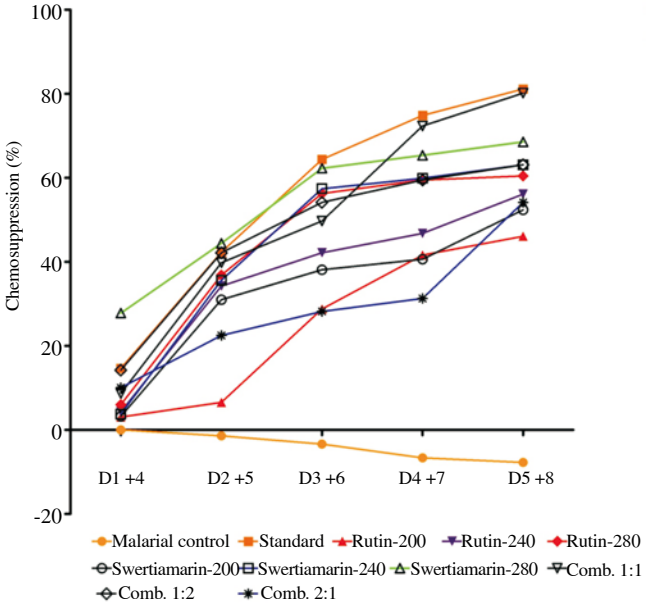

Figure 2. Chemosuppressant effects of rutin and swertiamarin on established infection test.
Comb.: Combination.

RBCs of different treated groups (Figure 4) showed that combination of rutin and swertiamarin in 1:1 ratio potentially reduced the parasitaemia than individuals and other combinations.

3.2.3. In vitro and in vivo interaction of drug in combination

The results of the *in vitro* and *in vivo* interaction of combination of compounds were presented in Table 4. Isobologram (Figure 5) represented the drug interaction between rutin and swertiamarin. The observed interaction between rutin and swertiamarin varied from synergistic to additive one. Synergy was observed in 1:1 combination, whereas, 1:2 and 2:1 showed additive interactions.

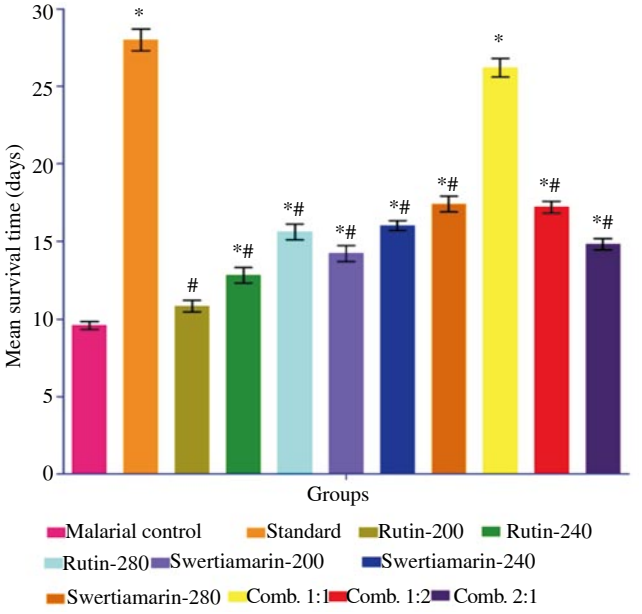

Figure 3. Effects of rutin and swertiamarin on mean survival time.
Data were expressed as mean ± SEM of survival time (days) ($n = 6$). Data were analyzed by One-way ANOVA with Tukey-Kramer multiple comparisons test. *: $P < 0.001$, significant value as compared to malarial control; #: $P < 0.001$, significant value as compared to standard. Doses resulting in survival times greater than that of infected non-treated mice were considered active. Death occurring before Day 5 of infected and treated mice was regarded as toxic death.

Table 4
FIC values of rutin and swertiamarin.

Combinations	*In vitro* drug interactions			*In vivo* drug interactions		
	FIC^R	FIC^S	$\sum FIC^{RS}$	FIC^R	FIC^S	$\sum FIC^{RS}$
1:1	0.31	0.31	0.62[a]	0.31	0.31	0.62[a]
1:2	0.70	0.35	1.05[b]	0.75	0.38	1.13[b]
2:1	0.43	0.86	1.29[b]	0.36	0.87	1.23[b]

[a]: Synergism; [b]: Addition; FIC^R: For rutin; FIC^S: For swertiamarin; FIC^{RS}: For combination. $\sum FIC < 1$: Synergy; $\sum FIC = 1–2$: Additive; $\sum FIC > 2$: Antagonism.

Table 3
Effects on average parasitaemia of rutin and swertiamarin on established infection test (%).

Groups	Average parasitaemia				
	D1 +4	D2 +5	D3 +6	D4 +7	D5 +8
Malarial control	7.16 ± 0.08	7.26 ± 0.18	7.40 ± 0.47	7.60 ± 0.30	7.67 ± 0.31
Standard	6.11 ± 0.24[a]	4.13 ± 0.52[c]	2.55 ± 0.35[c]	1.80 ± 0.22[c]	1.35 ± 0.31[c]
Rutin-200	6.94 ± 0.09	6.69 ± 0.19[f]	5.10 ± 0.55[cf]	4.18 ± 0.51[cf]	3.86 ± 0.47[cf]
Rutin-240	6.85 ± 0.10	4.71 ± 0.18[c]	4.14 ± 0.13[cf]	3.81 ± 0.38[cf]	3.14 ± 0.32[cf]
Rutin-280	6.73 ± 0.09	4.52 ± 0.16[c]	3.13 ± 0.21[c]	2.90 ± 0.31[cd]	2.83 ± 0.30[ce]
Swertiamarin-200	6.93 ± 0.08	4.94 ± 0.20[c]	4.43 ± 0.19[cf]	4.25 ± 0.33[cf]	3.41 ± 0.48[cf]
Swertiamarin-240	6.89 ± 0.11	4.61 ± 0.17[c]	3.05 ± 0.16[c]	2.87 ± 0.24[cd]	2.64 ± 0.34[ce]
Swertiamarin-280	5.17 ± 0.07[c]	3.98 ± 0.18[c]	2.70 ± 0.34[c]	2.48 ± 0.41[c]	2.25 ± 0.20[cd]
Combination 1:1	6.54 ± 0.07	4.31 ± 0.19[c]	3.60 ± 0.21[cd]	1.98 ± 0.23[c]	1.42 ± 0.26[c]
Combination 1:2	6.14 ± 0.13	4.14 ± 0.36[c]	3.28 ± 0.17[c]	2.90 ± 0.33[cd]	2.64 ± 0.28[ce]
Combination 2:1	6.44 ± 0.09	5.55 ± 0.39[ce]	5.14 ± 0.35[cf]	4.92 ± 0.19[cf]	3.28 ± 0.24[cf]

Values were expressed as mean ± SEM ($n = 6$). Data were analyzed by Two-way ANOVA followed by Bonferroni *post hoc* tests. [a]: $P < 0.05$; [b]: $P < 0.01$; [c]: $P < 0.001$, significant value as compared to malarial control group and [d]: $P < 0.05$; [e]: $P < 0.01$; [f]: $P < 0.001$, significant value as compared to standard.

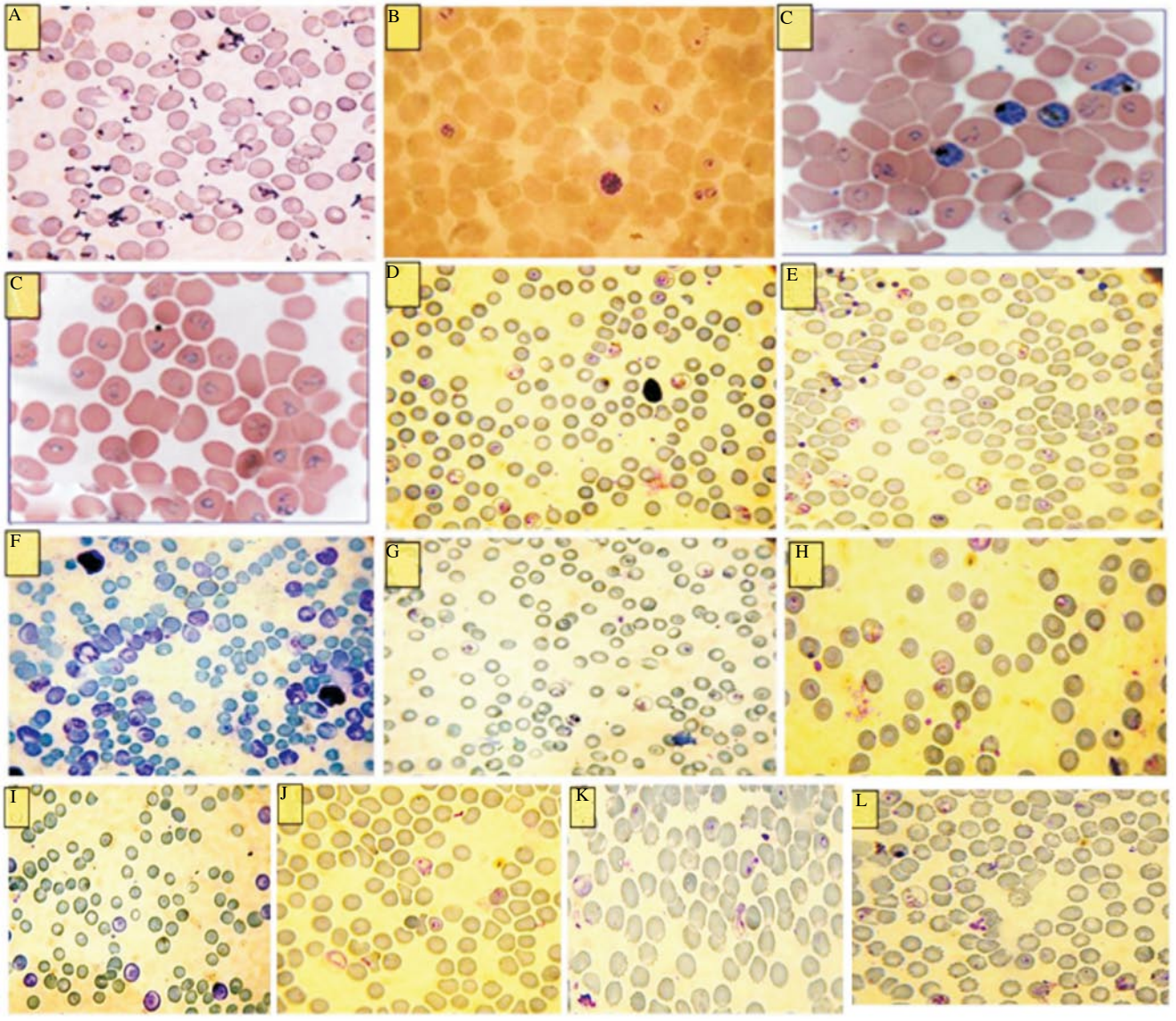

Figure 4. RBCs in different treated conditions.
A: RBCs in culture; B: *P. falciparum* in cultured RBCs; C: Sorbitol treatment of culture for synchronization; D: Blood smear of mice containing *P. berghei*; E: Blood smear of untreated mice (control group); F: Blood smear of mice with established infection; G: Blood smear of mice treated with swertiamarin (200 mg/kg); H: Blood smear of mice treated with rutin (240 mg/kg); I: Blood smear of mice treated with chloroquine (5 mg/kg); J: Blood smear of mice treated with combination (1:1); K: Blood smear of mice treated with combination (1:2); L: Blood smear of mice treated with combination (2:1).

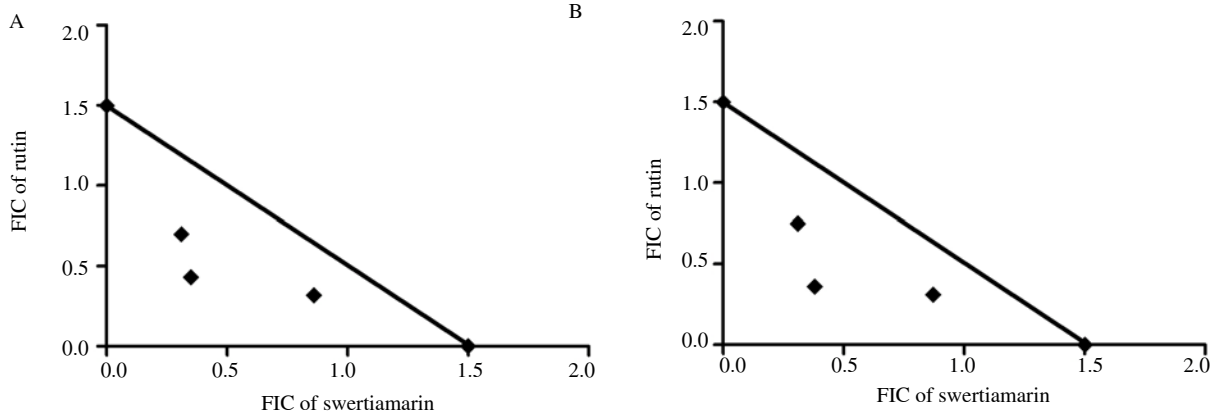

Figure 5. Isobologram of drug interaction between rutin and swertiamarin. A: *In vitro* study; B: *In vivo* study. The point on Y axis showed FIC of rutin in the presence of swertiamarin and on X axis showed FIC of swertiamarin in the presence of rutin. The straight line showed additive interactions, as points of interaction, fell on the line joining FIC of both drugs, while points below the line showed synergism.

4. Discussion

Malaria is a well-known protozoan parasitic killer disease and the spread of drug resistance has played an important role in the occurrence and sternness of epidemic diseases in the world, which is a major cause of malaria morbidity and mortality. It has been proved that the *Plasmodium*-infected erythrocyte is under invariable oxidative stress through exogenous reactive oxidant species and reactive nitrogen species produced by the immune system of the host and by endogenous production of reactive oxidant species generated during the digestion of host cell haemoglobin and concomitant biochemical reactions[21,22]. The compounds exhibiting both antiplasmodial and antioxidant activities could be very interesting as leads in the search for new antimalarial agents. Therefore, the present study included the test of individual flavonoid (rutin), and glycoside (swertiamarin) for antimalarial activity and evaluation of the potentiating antimalarial effects of each other through different combination ratios.

The *in vitro* results of present study showed that rutin [IC_{50} of (9.50 ± 0.29) μg/mL] and swertiamarin [IC_{50} of (8.17 ± 0.17) μg/mL] both have good antiplasmodial activity against chloroquine sensitive strain of *P. falciparum*. While, combination in ratio of 1:1 [IC_{50} of (5.51 ± 0.18) μg/mL] was found to be more effective than individuals and other combinations in ratio of 1:2 [IC_{50} of (9.07 ± 0.13) μg/mL] and 2:1 [IC_{50} of (11.70 ± 0.20) μg/mL]. The *in vivo* study was designed to test the effects of antiplasmodial activity on early malaria infection (4-day test) and on established malaria infection (Rane test). Results indicated that rutin and swertiamarin possessed blood schizontocidal activity as evident from dose-dependent chemosuppression effects obtained during the 4-day early infection test. The results for test on established infection were also encouraging. After observing the results, it was clear that rutin and swertiamarin both showed a consistent increase in chemosuppressive activity as the dose increased from 200 to 280 mg/kg/day similar to chloroquine treated group. While the control group showed a daily increase in parasitaemia. On established infection, both rutin and swertiamarin showed significant blood schizontocidal activity. The result of the mean survival time of rutin and swertiamarin in established infection showed that the mean survival period was dose-dependent. It is noteworthy that the antiplasmodial activity of the individual rutin and swertiamarin at all the doses during early and established infection was not comparable to standard drugs.

When these two drugs were tested in combination, it produced more prominent effects. The combination in 1:1 ratio of these drugs ameliorated the anti-malarial activity (Table 3 and Figure 2) as compared to the others. They were associated with a rapid reduction of parasites in the blood of *P. berghei*-infected mice. The speed of effect of this combination is important in a clinical way because a rapid elimination of parasite from the bloodstream is required for the treatment of severe malaria[23]. The mean survival times (days) of mice treated with combinations of rutin and swertiamarin in 1:1 (26.2 ± 0.6) μg/mL was increased as compared to individual drug and was somewhat similar to that of chloroquine phosphate (28.0 ± 0.7) μg/mL and showed a good level of efficacy of the combination. The mean survival time for other two combinations (1:2) and (2:1) was similar to an individual drug. The test combination (1:1) was able to suppress parasites significantly thereby alleviating deaths associated with parasite infection. The overall results clearly indicated the better effectiveness of these two natural compounds in combination therapy especially in 1:1 ratio rather than individual compound.

Ideally, combination chemotherapy for malaria should take advantage of synergistic interactions as these would enhance therapeutic efficacy and lower the risk of resistance emergence. The findings from studies with animal models may be more predictive of the drug action in humans[7]. Results of *in vitro* and *in vivo* studies of drug interactions suggested that interaction was synergistic in the case of 1:1 combination and additive in the case of 1:2 and 2:1. However, the mechanism of synergistic and additive interactions of the rutin and swertiamarin could not be addressed in this study. The additive effect may be due to the two separate entities binding to the same receptor in the parasite while in synergy, different sites on the parasite may be the target points[24].

Although the mechanism of action of two compounds (rutin and swertiamarin) has not been elucidated, they were selected based on literature survey as rutin possesses iron chelating property and antioxidant property that can help in diminishing the oxidative stress on erythrocytes induced during *Plasmodium* infection through free radical scavenging and also reported for *in vitro* antiplasmodial activity[12]. Swertiamarin also possesses the antioxidant property and is proved for *in vitro* antiplasmodial activity too. So their combination might help in producing better activity compared to the individual drug. The intra-erythrocytic developmental stages of parasites thrive in highly aerobic situations thereby generating oxidants which disrupt the oxidative defense of the RBC. It has been reported that plasmodial infection results in increased oxidative stress damage and decreased antioxidant defense of the host. Metal chelators and antioxidants are well-known scavengers of reactive oxygen species and some of them have been used in medicinal products. Iron chelator can be profitable through another way also as for nucleic acids synthesis and the *Plasmodium* enzyme ribonucleotide reductase needs Mg^{2+} and Fe^{3+} ions as cofactors. That is why many metal chelators are well-known antimalarial drugs[25,26]. Rutin also protects against hemoglobin oxidation[27]. Swertiamarin may be acting due to its antioxidant property. The combination of two antioxidants, a metal chelator and an antioxidant has been tested for other activity and in malaria for suppressing oxidative stress and proved active[28,29]. The antimalarial activity of rutin may be through its antioxidant or as an iron chelator property, whereas, for swertiamarin, it may be due to its antioxidant property.

These findings demonstrate that it is useful to combine drugs as it is usually done in traditional preparations and encourage the test of other plant product combinations[30]. This investigation had two aims, *i.e.* enhancement in the survival time and protection of the erythrocytic oxidant defence mechanism of the malaria-infected host by a combination of metal chelators and antioxidants. The results suggest that a combination of rutin and swertiamarin could form the basis of a new antimalarial combination for the treatment of human malaria.

The observations from the study concluded that combination of the rutin and swertiamarin in 1:1 ratio had a potential antimalarial activity, and is very useful as in the normal practice of traditional health practitioners. Therefore, the combinations need further

evaluation to identify the possible mechanism of action of drug, mechanism of interaction and to establish the therapeutic value in the treatment of malaria.

Conflict of interest statement

We declare that we have no conflict of interest.

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