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In vivo antimalarial activity of *Stephania japonica* plant extract against *Plasmodium falciparum* in infected mice

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ABSTRACT

Antimalarial activity of the crude aqueous leaf extract of *Stephania japonica* was evaluated using chloroquine-sensitive *Plasmodium falciparum* infection in mice with an objective to antimalarial activity. Different crude extracts of *Stephania japonica* (50, 100 and 200 mg/kg) was administered orally to mice infected with *P. falciparum* in 4 days suppressive test. The extracts produced a dose dependent reduction in parasite density compared to the control group. Percentage parasitemia calculation revealed 62.3, 81.2 and 91.6% inhibition at 50, 100 and 200 mg/kg of the ethanol extract, respectively ($P<0.05$ at the latter two doses). Similarly chloroform extract exhibited dose dependent inhibition, however statistical significant was found to with highest dose (200 mg/kg). Chloroquine exhibits 100% chemosuppression. The result obtained from the present work indicated that *Stephania japonica* has a promising antiplasmodial activity against chloroquine sensitive *Plasmodium falciparum* in a dose dependent manner for which further research is needed to elucidate its active principles.

Keywords: Stephania, Antimalarial, Natural, Herbal products, Chemosuppression.

1. INTRODUCTION

Malaria is the most important parasitic disease worldwide with an incidence of almost 300 millions clinical cases and over one million deaths per year [1]. *Plasmodium falciparum*, the potentially lethal malaria parasite is responsible for one in five childhood deaths in Africa, India and South East Asia and indirectly contributes to illness and deaths from malnutrition, respiratory infections and diarrhoeal disease [2]. In the South East Asia Region of WHO, India is accounted for 58% of malarial cases and it is a problem for centuries which has been reported in the ancient Indian medical literature like Atharva Veda and Charaka Samhita [3, 4].

The effectiveness of chemotherapy constitute the greatest threat to the control of malaria because malaria parasite has shown itself capable of developing resistance to nearly all used antimalarial drugs and resistant strains have rapid extension [5]. Therefore, new knowledge and new products are urgently needed to overcome malaria [6]. The potent anti-malarial compound quinine and artemisinin from plant species, has generated much interest to explore other plant resources for their possible anti-malarial efficacy [7, 8]. In the last few decades medicinal plants has been brought about by the acknowledgement of the value of medicinal plants as potential sources of lead in drug development. In the developing countries, it is estimated that about 80% of the population rely on traditional medicine for primary health care.

The plant *Stephania japonica* is widespread in North Eden to South Coast, also India, throughout Asia to Southern Pacific region. In wet places it is widely distributed. The genus of *Stephania* consists of 18 species have been shown to possess antiplasmodial, anti-inflammatory, antibacterial, anti HIV-1 activities [9-12]. More than 150 alkaloids have been isolated from plants of genus *Stephania* and many of them are bioactive constituents [13]. The roots of *Stephania japonica* are used in the treatment of fever, diarrhea, dyspepsia and urinary diseases [14, 15]. In the present work, the antimalarial activity of *Stephania japonica* (Fam: Menispermaceae) was evaluated against the chloroquine-resistant *Plasmodium falciparum* strain.

2. MATERIALS AND METHODS

2.1. Chemicals

All solvents and chemicals used were of analytical grade.

2.2. Plant material and Extraction

Fresh leaves of *Stephania japonica* was collected from surrounding areas of Komarapalayam and Sankagiri, Namakkal District, Tamilnadu, India. The plant material was taxonomically authentified by a Botanist, Chennai, Tamilnadu. The shade dried leaves were coarsely powdered and stored in airtight container. About 1kg powdered plant material was extracted successively with different solvents of increasing polarity using soxhlet apparatus [16]. The extract was filtered; the filtrates were pooled and concentrated to dryness by removing the solvent under reduced pressure at 50ºC. The percentage yield of each extract was calculated and standardized (Ta**b**le 1). Extracts were stored in airtight glass bottles at 4°C in a refrigerator.

Table - 1: Successive yield of different Solvent Extraction of *Stephania japonica*

Extracts	Colour and Consistency	Percentage yield of extracts of *Stephania japonica* (w/w)
Chloroform	Green with sticky mass	1.05
Ethanol	Green semisolid	9.86
Aqueous	Brown semi solid	13.5

2.3. Phytochemical screening

Freshly prepared crude extracts of *Stephania japonica* were qualitatively tested for the presence of chemical constituents by standard procedures [17].

2.4. Acute toxicity test: Animals

Twenty-five Swiss albino mice of both sexes were obtained from the animal house of JKK Nattraja College of Pharmacy. The mice were acclimatized for a period of 10 days. The animals were fed standard mouse cubes and clean drinking water. The animals were caged in five (5) groups of separate cages of five animals in each. The acute toxicity of plant extracts were tested on mice as per OECD guidelines No. 420 and recorded using 3 doses (500, 1000 and 3000 mg/kg body weight) administered orally. Control rats were kept under the same conditions without any treatments. The animals were routinely inspected for appearances or signs of toxicity such as tremors, weakness and refusal of feeds, falling off of hair, coma or even death for 48 hours. Hence all the mice were subjected for *in vivo* antimalarial screening.

2.5. Parasite strain and *in vitro* culture

Strain of *P. falciparum* sensitive to chloroquine (MRC-pf-20) was obtained from the Malaria Parasite bank, maintained by the National Institute of Malaria Research, New Delhi, India. Parasites were maintained through weekly passage in mice by inoculation of known amount of parasite into healthy mice weighing 20-24 g.

2.6. *In vivo* antimalarial test in early infection (4-day suppressive test)

The method described by Trager and Jensen [18] with slight modification was used. Briefly, three hours after inoculation of the parasite, the mice in the three treatment groups were administered with the extracts in doses of SJC 50, SJE 100 and SJA 200 mg/kg for four consecutive days by dissolving the extract in distilled water and tween 80 for each. Two control groups were used in the experiment, the negative control treated with distilled water for four consecutive days while the positive control administered chloroquine phosphate, a standard anti-malarial drug at 10 mg/Kg/day. The dug, the vehicle and the extracts used in this study were administered by oral route with the aid of an oral gavage. Treatment was continued for 4 days: parameters body weight and PCV were taken at day 0 and day 5 using Wintrobe's method [19]. Then parasitemia was measured on the 5 day.

2.7. Parasitemia Measurement

Blood smears from tail were applied on Menzel-Glaser microscope slides (Germany), fixed with absolute methanol and stained with 10% Geimsa stain at pH 7.2 for 15 minutes as described by David *et al.* [20]. The slides were taken out, washed with gentle passage of tap water and dried with the room temperature. The number of parasitized red blood cells (PRBC) were counted using Olympus microscope (CHK2-F-GS, Taiwan) with an oil immersion nose piece of 100x magnification power. Two fields were counted for each slide, average was taken and percentage parasitemia was determined by using the formula described by David *et al.* and Sanni *et al*. [20, 21].

2.8. Statistical analysis

Data obtained was carried out in triplicate and were analyzed using student's t-test and ANOVA. Values for $^{**}p \leq 0.01$ were taken to be significant.

3. RESULTS

The phytochemical screening of different extracts of plant material was shown in table 2.

Table - 2: Phytochemical screening of *Stephania japonica* extracts

Phytoconstituents	Chloroform extract	Ethanol extract	Aqueous extract
Alkaloids	(+)	(+)	(-)
Carbohydrates	(-)	(+)	(+)
Glycosides	(+)	(+)	(-)
Flavonoids	(+)	(+)	(+)
Phytosterols	(+)	(+)	(+)
Fixed oils and Fats	(-)	(-)	(-)
Saponins	(+)	(+)	(+)
Phenolic Compounds and Tannins	(-)	(+)	(+)
Lignins	(-)	(-)	(-)
Proteins and free Amino acids	(-)	(+)	(+)
Gums and Mucilage	(-)	(+)	(+)

Table - 3: Response of mice infected with *Plasmodium falciparum* to various doses of *S. japonica* extracts

Treatment	Dose (mg/kg)	Parasitema (%)	Inhibition (%)	Survival time (days)
Vehicle control	0.2mL	32± 1.2	0	10.1±0.78
S. japonica chloroform extract	50	46±1.1	37.40±1.2	15.4±1.42
	100	23.34±0.5	58.20±1.8	17.6±1.08
	200	10.15±0.5	79.60±1.5**	19.7±0.42
S. japonica ethanol extract	50	31.2±1.5	62.3±1.5	23.3±1.02
	100	13.16±1.5	81.2±0.6 **	25.±0.43
	200	5±0.5	91.6±1.9 **	28.3±1.22
S. japonica aqueous extract	50	61±2.1	27.3±0.7	17.4±0.12
	100	46.8±1.7	37.01±0.3	18.3±0.52
	200	42.3±1.2	39.1±1.5	18.7±0.87
Chloroquine	10	1.1	100	27.3±0.22

Values are mean ± SEM; n=5 animals in each group; ** P < 0.05.

The table indicates the presence of alkaloids, saponins, tannins, flavonoids, carbohydrates, steroids and anthraquinones. Selectivity of the antimalarial activities of *S.japonica* extracts are shown in table 3. The comparison analysis indicated that only two dose levels (100 mg/kg and 200 mg/kg) of the extract showed statistically significant (P<0.05) difference on the fifth day parasitemia compared to the negative control. The 50 mg/kg dose level, however, showed a statistically non-significant reduction. Percentage inhibition analysis indicated that inhibition was dose dependent with 37.4%, 58.2 and 79.6% inhibition by SJC 50, 100 and 200 mg of the extracts, respectively, for the 4 day suppressive test (P<0.05 in the last dose) when compared the negative control. Similarly 50, 100 and 200 mg of *S.japonica* ethanol extract showed a significant (P<0.05) inhibition with 62.3, 81.2 and 91.6 %. However, no significant difference was observed with all doses of *S.japonica* aqueous extract. Chloroquine had shown a statistically significant (P <0.05) inhibition compared to vehicle controls as well as *S.japonica* chloroform extract 100 and *S.japonica* ethanol extract 100 and 200.

A body weight changes was observed between days 0 and 4 in all groups of mice treated with different crude extract of *S.japonica*. Treatment with ethanolic extract of *S.japonica* prevented loss of weight associated with the increase in parasitemia level at all the 3 dose levels compared to the negative controls. However, the increase in body weight was not

found to be dose dependent, the highest increment being caused by *S.japonica* ethanol extract 200 followed by 100 and the least was by 50. There were no measurable differences in preventing weight reduction associated with increasing parasitemia between different doses of the chloroform extract as well as aqueous extract (Table 3).

4. DISCUSSION

In this preliminary study, we used crude plant extracts with different solvents, since extracts contain complex mixtures of different active compounds.

The present study assessed the anti-malarial properties of *Stephania japonica* and compared the activity with chloroquine. The use of ethanol and water as solvents for the extraction of the active metabolites is in consonance with folkloric procedure of the use of decoctions and alcohol extracts. The percent (%) yield of the extracts varied, probably due to the solvent medium.

Highest level of inhibition was observed with 200 mg/kg of *S.japonica* ethanol extract (91.6%). The overall result showed a higher reduction in mean percentage parasitaemia with increased concentration of ethanol extract of *Stephania japonica*. The difference in the efficacy of the chloroform and aqueous extracts may be due to the concentration of the active metabolites in the two extracts. Although the parasitemia was slightly inhibited in the group treated with the chloroform as compared with aqueous extract with no significant reduction in body weight of animals (Table 3). Numerous plant extracts and its active phytoconstituents have been evaluated and reported to inhibit *Plasmodium falciparum* [22, 23]. Similarly present study also showed intrinsic antimalarial activity in mice and the antimalarial effects of different species of *Stephania* have been documented [24-26].

In the study, the ethanol extract demonstrated higher antimalarial activity than the crude aqueous extract. Ethanol is less dense than water and might possess greater diffusibility in the same medium than water. This might account for the greater efficacy exhibited by ethanol extract over the chloroform and aqueous. It might also be possible that the most active metabolites were more soluble in ethanol than water conferring this advantage on the ethanol extract.

The phytochemical composition of the plant is notable, containing alkaloids, tannins, terpenoids, flavonoids and steroids. The high preponderance of these metabolites and phytochemicals may be responsible for the antimalarial activity exhibited by the plant [27, 28].

5. CONCLUSION

The antimalarial activity showed by *Stephania japonica* could be attributable to the presence of alkaloids which was one of its constituents [29]. However, the active compound(s) known to give this activity need to be identified. This study adds important information to the area of malaria research, which always is in need of alternative anti-malarial drugs and this finding provides a foundation for further exploration of a new effective herbal drug or identification of active phytoconstituents.

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