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Antiplasmodial activity of certain medicinal plants against chloroquine resistant *Plasmodium berghei* infected white albino BALB/c mice

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Abstract In the present study of antimalarial efficacy, aqueous extracts of leaves and unripe fruits of *Psidium guajava*, leaves of *Ocimum sanctum* and leaves of *Murraya koenigii* are evaluated against *Plasmodium berghei* (chloroquine resistant NK65 strain) infected white albino BALB/c mice. A 7 days oral administration was adopted with different dosage viz., 350 mg, 750 mg and 1,000 mg/kg body weight as treatment schedule along with parasite (Group I) and drug control with Chloroquine, 50 mg/kg body weight (Group II). All the parts were extracted based on the decoction method, which is commonly seen among the villagers/tribes as their usual method of preparation of decoction for most of the ailments. The antimalarial activities were evaluated from the giemsa stained blood smears collected from different treated groups of mice used in this experiment. The antiplasmodial effect that is percent parasitaemia and percent suppression (values in parenthesis) showed by the treated groups of mice at 350 mg/kg b. wt. by the aqueous extracts of *P. guajava* leaves (Group III) was 19.8 ± 1.22 (73.7 %), *P. guajava* unripe fruits (Group IV) was 52.7 ± 2.19 (30.0 %), leaves of *O. sanctum* (Group V) was 64.0 ± 0.73 (15.1 %) and leaves of *M. koenigii* (Group VI) was 28.9 ± 0.81 (61.6 %) whereas at 750 mg/kg b. wt., it all showed 10.3 ± 0.7 (80.2 %), 26.3 ± 0.52 (65.1 %), 42.0 ± 0.47 (44.2 %) and 14.9 ± 0.46 (71.5 %) whereas at 1,000 mg/kg b. wt. dose, it all showed 9.2 ± 0.39 (85.8 %), 25.6 ± 0.40 (62.0 %), 41.8 ± 0.29 (35.5 %) and 14.0 ± 0.42 (76.9 %) respectively.

Keywords Medicinal plants · In vivo antimalarial bioassay · Mice · *P. berghei*

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Introduction

Malaria is an important parasitic protozoan disease, which is widely prevalent in the world. According to the 2011 world malaria report, there were about 216 million cases of malaria with an uncertainty range of 149 million to 274 million and an estimated 6,55,000 deaths in 2010 with an uncertainty range of 5,37,000 to 9,07,000. Malaria mortality rates have fallen by more than 25 % globally since 2000 and by 33 % in the WHO African Region. Most of the deaths occur among children living in Africa where a child dies every minute from malaria (WHO, WMR 2011). Nabarro and Tayler (1998) launched the Roll Back Malaria (RBM) campaign with the stated goal to reduce malaria deaths to 50 % worldwide by 2010 is being confounded by the problem of drug resistance.

Moreover, control and treatment of these *Plasmodium* infections have been complicated as widespread resistance to the available antimalarial drugs such as chloroquine (Kalra et al. 2006). So there is an urgent requirement for

the search of alternative antimalarials to combat the resistance of the existing antimalarial drugs. Natural products are the promising sources for biologically active compounds isolation and have potential for the development of novel antiplasmodial compounds, which are generally safer to man (Klayman 1985).

The parasite causing high mortality, *Plasmodium falciparum* being resistant to currently available antimalarials makes it necessary to search for alternative therapeutic agents with higher efficacy with least toxicity (Bhat and Surolia 2001). The potential of chemotherapeutic compounds against malaria have been proved with some examples such as, quinine from cinchona species, and artemisinin from *Artemisia annua*. Therefore, the present investigation has been carried out with three plants using in vivo animal model infected with *P. berghei* (chloroquine resistant strain). These plants were extracted using water as solvent based on the principles of preparation of decoction.

Materials and methods

Mice strain

White albino mice used in this study were brought from DRDE (Defence R & D organization), Gwalior to animal facility, Defence Research Laboratory, Tezpur. The average weight of mice used for this study is 24.29 ± 0.17 g. The mice were kept at room temperature. Mice were fed with adequate drinking water and feed as per the guidelines given by the CPCSEA. These mice were used only once for the experiment.

Parasite strain

A *P. berghei* (chloroquine resistant strain), NK65 strain was used for this study and it is resistant to chloroquine with the concentration of 20 mg/kg body weight. The experimental groups were divided into six each and each group were contained six numbers of mice and placed in a separate animal cages and labeled according to the experimental design viz., Group I, II, III, IV, V, VI and VII.

Medicinal plants

Collections of plants were done in and around Tezpur, Assam, India in the month of March, April and May 2007. The leaves and unripe fruits of *P. guajava*, leaves of *Ocimum sanctum* and leaves of *Murraya koenigii* were collected and dried under shaded conditions at room temperature. After thorough drying, the plant materials were ground into powder form for the preparation of crude extracts.

Preparation of crude extracts based on the principle of decoction

10 g each of powdered plant materials were weighed and placed into sterile beakers containing 100 ml of deionised water (Deionised Millipore Water System, India). It was boiled over 100 °C for 10 min under hot flame and cooled at room temperature. The supernatant were filtered through whatman no.1 filter paper (0.42 μM in diameter) and the filtrates were dried up under hot plate at 40 °C for 5–6 h. Then, the filtered dried extracts were weighed. It was dissolved again in double distilled water (w/v) for their dose preparation. Each mouse was administered orally with 350 mg, 750 mg and 1,000 mg/kg b. wt. of crude filtered extracts for their antimalarial evaluation. A standard 7 days in vivo test was used (Peters 1987). All the mice were administered intraperitoneally with a dose of 3×10^5 *P. berghei* infected erythrocytes per mouse on day 0 according to Eling et al. (1977). Oral administration was started on 0 day with aqueous extracts according to Franssen et al. (1997). Two control groups were included, of which the drug control group (Group II) received chloroquine, 50 mg/kg b. wt. whereas the parasite control group (Group I) was not received chlorquine. Another group of healthy mice were administered only extracts (Group VII) without parasite administration for assessing the toxicity (Ushadevi et al. 2001). At the end of 7 days post treatment, blood smears were collected from all the mice underwent treatments as well as the control groups. This assay was repeated twice. The blood smears were stained using Geimsa's staining method and examined under oil immersion (×1000) for the counting of parasiteamia.

Mean survival time

The mean survival time period was noted arithmetically after post inoculation according to the method by Chandel and Bagai 2010.

Statistical analysis

The mean values of percent parasiteamia among the treatment were compared using one way ANOVA.

Results

The results of the present study showed that the aqueous extracts of leaves of *P. guajava*, unripe fruits of *P. guajava*, leaves of *O. sanctum* and leaves of *M. koenigii* administered at 350 mg/kg b. wt. showed the percent parasitaemia of 19.8 ± 1.22, 52.7 ± 2.19, 64 ± 0.73 and 28.9 ± 0.81 as against the percent parasitaemia of 75.3 ± 0.76 in the

control group with the percentage of suppression of parasiteamia of 73.7, 30.0, 15.1 and 61.6 respectively. When the dose administered at 750 mg of crude extract, leaves of *P. guajava*, unripe fruits of *P. guajava*, leaves of *O. sanctum* and leaves of *M. koenigii*, the percent parasiteamia observed were 10.3 ± 0.7, 26.3 ± 0.52, 42.0 ± 0.47 and 14.9 ± 0.46 with percentage of parasiteamia suppression of 80.2, 65.1, 44.2 and 71.5 respectively. At the dosage rate of 1,000 mg/kg b. wt. of crude extract of leaves of *P. guajava*, unripe fruits of *P. guajava*, leaves of *O. sanctum* and leaves of *M. koenigii* the percent parasiteamia observed were 9.2 ± 0.39, 25.6 ± 0.40, 41.8 ± 0.29, 14.0 ± 0.42 with the percentage of parasiteamia suppression of 85.8, 62.0, 35.5, 76.9 respectively. The mean parasiteamia observed in the parasite control group was 75.3 ± 0.76 %, whereas the drug control group showed no parasiteamia at the end of 7 day post treatment (Table 1). The group of mice (Group VII) for toxicity assay, which received only extracts without parasite, was observed healthy even after 120 days of experiments without any abnormal signs or symptoms. Moreover, the mean survival time period (in days) was also observed among all the treated groups including drug and parasite control. The mean survival period at 350 mg/kg b. wt. administered group of mice showed 15.0 ± 0.40 for *P. guajava* leaves (Group III) followed by 750 mg/kg b. wt. for 18.2 ± 0.24 followed by 18.4 ± 0.51 at 1,000 mg/kg b. wt. The mean survival period for *P. guajava* unripe fruits (Group IV) at 350 mg/kg b. wt. for 12.0 ± 0.48 and 750 mg/kg b. wt. for 14.8 ± 0.37 followed by 15.1 ± 0.32. Whereas for *O. sanctum* leaves (Group V), the mean survival period was 8.0 ± 0.41 at 350 mg, 18.0 ± 0.32 and 18.2 ± 0.37 at 1,000 mg/kg b. wt. The mean survival time period for *M. koenigii* leaves (Group VI) was 13.8 ± 0.37 at 350 mg followed by 15.2 ± 0.37 at 750 mg followed by 15.4 ± 0.24 at 1,000 mg dosage/kg b. wt. of mice. But the group of mice which received only extracts was still surviving except one which died because of other ailment even after 45 days of experiments (Table 2).

Discussion

In the present study, three medicinal plant extracted materials were used for its antimalarial potential against *P. berghei* (chloroquine resistant strain), a mice infecting malarial parasite. The plants are *P. guajava* (both leaves and fruits), *O. sanctum* (only leaves) and *M. koenigii* (only leaves). The extraction method followed were based on the principles of the preparation of decoctions and the route of administration was conducted per oral.

The *P. guajava* is ubiquitous, growing in both tropical and subtropical climatic conditions. The fruits are edible and are rich source of vitamin C and minerals. The leaf extract has a broad spectrum of activities against cough, bacterial infections, heamostasis (Jairaj et al. 1999, 2000), diarrheas and narcotic properties (Lozoya et al. 1990), antidiabetic (Obatomi et al. 1994), antioxidant (Qian and Nihorimbere 2004), antimutagenic (Grover and Bala 1993; Matsuo et al. 1994), abdominal pain, convulsions, epilepsy, cholera, insomnia and hypnotic effects (Lutterodt and Maleque, 1988; Meckes et al. 1996). Our effort was to evaluate its leaf and unripe fruits for antimalarial activity. According to Nundkumar and Ojewole (2002), the *P. guajava* stem bark aqueous extract showed higher activity than the leaves and fruits. This showed IC_{50} values of 10–20 µg/ml against chloroquine sensitive strain of *P. falciparum* D10 under in vitro study and the phytochemical analysis of stem bark of *P. guajava* revealed the presence of anthraquinones, flavanoids, seccoridoids and terpenoids (Nundkumar and Ojewole 2002). However, the mean length of survival period observed for the present study for the leaf extracts were 18.33 ± 2.23 whereas for the unripe fruit, 14.67 ± 2.64 days which were not observed by other workers.

O. sanctum has a wide variety of biological activity and ayurvedic practice recommends tulsi in several formulations to enhance immunity and metabolic functions as well as in the management of respiratory problems (Shwaskasa).

Table 1 Percent parasitaemia and percent suppression of parasitaemia after seven days of treatment with aqueous extracts of plants

Treatment	Group	Dosage 350 mg Percent parasitaemia* (% suppression)	Dosage 750 mg Percent parasitaemia* (% suppression)	Dosage 1,000 mg Percent parasitaemia* (% suppression)	F	*p*
Psidium guajava leaves	GP III	19.8 ± 1.22^{b} (73.7)	10.3 ± 0.7^{a} (80.2)	9.2 ± 0.39^{a} (85.8)	47.9	0.000
Psidium guajava unripe fruits	GP IV	52.7 ± 2.19^{b} (30)	26.3 ± 0.52^{a} (65.1)	25.6 ± 0.40^{a} (62)	136.9	0.000
Ocimum sanctum leaves	GP V	64 ± 0.73^{b} (15.1)	42 ± 0.47^{a} (44.2)	41.8 ± 0.29^{a} (35.5)	581.5	0.000
Murraya koenigii leaves	GP VI	28.9 ± 0.81^{b} (61.6)	14.9 ± 0.46^{a} (71.5)	14 ± 0.42^{a} (76.9)	200.9	0.000
Parasite control	GP I	75.3 ± 0.76				
Drug control	GP II	0				

Values are given as mean ± SEM

*ANOVA followed by Tukey HSD. Values in rows followed by same letter are not significantly different ($p > 0.05$)

Table 2 Showing the mean survival period (in days) of mice after administration of crude plant extracts at 350, 750, 1,000 mg/kg body weight dosage

S. no.	Treatment groups	Materials used	Mean survival time in days		
			350 mg[a]	750 mg[a]	1,000 mg[a]
1	GP III	*Psidium guajava* leaves	15.0 ± 0.40	18.2 ± 0.24	18.4 ± 0.51
2	GP IV	*Psidium guajava* unripe fruits	12.0 ± 0.48	14.8 ± 0.37	15.10 ± 0.32
3	GP V	*Ocimum sanctum* leaves	8.0 ± 0.41	18.0 ± 0.32	18.2 ± 0.37
4	GP VI	*Murraya koenigii* leaves	13.8 ± 0.37	15.2 ± 0.37	15.4 ± 0.24

[a] Values are indicated with standard error mean

Leaves and roots of *O. sanctum* were believed to have anti-malarial effects. The ethanol extracts of aerial parts showed ED_{50} value of greater than 2,000 µg/ml against chloroquine sensitive *P. falciparum* strains in vitro (Badam et al. 1988). The 50 % ethanolic extract of roots showed 24.11 % inhibition in vivo and 11.62 % in vitro (Misra et al. 1991) but the same extract of whole plant, excluding root showed no inhibition and 29.65 % inhibition respectively. Similar observation against *P. berghei* was made using aqueous extracts of *O. sanctum* with different dosage of crude extract injected intraperitoneally at 100, 300, 500 and 1,000 mg showed the results of 5.27 ± 0.38, 3.96 ± 0.35, 3.6 ± 0.28 and 3.8 ± 0.57 respectively (Usha et al. 2001) with the percentage reduction in parasitaemia of 5.61, 34.65, 40.59 and 37.29 % respectively. Ethanol extracts of aerial parts of *O. sanctum* showed ED_{50} value of >2,000 µg/ml against chloroquine sensitive *P. falciparum* strains in vitro. 50 % ethanolic extract of its root showed 24.11 % of inhibition in vivo and 11.62 % in vitro in *P. berghei* while the same extract of whole plant excluding root showed nil and 29.65 % inhibition respectively.

Leaves of *M. koenigii* is taken up for its antimalarial properties. The aqueous extracts of leaves of this plant showed 61.6, 71.5 and 76.9 percentage of parasiteamia suppression at different dosage scheduled. The present study showed the percentage of parasiteamia with leaf extracts of *M. koenigii* were 28.9 ± 0.81 (350 mg) followed by 14.9 ± 0.46 (750 mg) and 14.0 ± 0.42 (1,000 mg) respectively. The mean survival period of those mice treated with *M. koenigii* showed 15.17 ± 1.14 days. As far our knowledge is concerned, this could be the first report of antimalarial evaluation of *M. koenigii* against *P. berghei*. The LC_{50} values estimated for *M. koenigii*, *C. paradisi* and two of these combinations were 1.96, 1.85 and 1.67 % respectively. Natural herbalists revealed that the method of treatment of onchocerciasis and plants were mentioned as anti-onchocerciasis herbs and two seeds were mentioned as possessing antilarvicidal properties (Ebigwai 2012).

The leaves of aqueous extracts of *O. sanctum* and *M. koenigii* treated mice were survived for length of days than the *P. guajava* (11.83 ± 0.30 days with leaves and 12.35 ± 0.31 with unripe fruits). The present study showed encouraging results against the *P. berghei* malarial parasite. Different combination of the herbal extracts of the plants were not tried for the evaluation for the antimalarial study, which might have produced any additive effects or added efficacy against *P. berghei* in white albino BALB/c mice. Further research is on progress to identify its phyto components which might be giving better efficacy against this plasmodium spp.

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