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Antimalarial effect of *Tinospora cordifolia* (Willd.) Hook.f. & Thoms and *Cissampelos pareira* L. on *Plasmodium berghei*

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***Cissampelos pareira* L. and *Tinospora cordifolia* (Willd.) Hook.f. & Thoms inhibited the propagation of rodent parasite *Plasmodium berghei in vivo*. In a typical four-day experiment, the BALB/c mice were administered with ethanol extracts of *Cissampelos pareira* L. and *Tinospora cordifolia* (Willd.) Hook.f. & Thoms. The parasitaemian in untreated control group ranged between 17.31% and 30.02% whereas the root extracts of *Cissampelos pareira* L. and stem extracts of *Tinospora cordifolia* (Willd.) Hook.f. & Thoms resulted in inhibition of *Plasmodium berghei* significantly. The inhibitory properties of extracts of two plants require further studies so that the antimalarial activity is elucidated.**

Keywords: Antimalarial activity, *Cissampelos pareira*, malaria, *Plasmodium berghei*, *Tinospora cordifolia*.

MALARIA, a prominent global health hazard is caused by the protozoan parasite of the genus *Plasmodium*. Only four species of *Plasmodium*, namely *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae* infect human beings. Of these *P. falciparum* and *P. vivax* cause a majority of malaria infections. About 3.3 billion people in the world are at risk of malaria. Every year, about 250 million malaria cases are reported and nearly one million deaths occur due to this disease[1]. In addition to its health toll, malaria puts a heavy economic burden on countries where it is endemic. The estimates of expenditure to tackle malaria globally in 2009 and 2010 are US$ 5.335 billion and 6.180 billion respectively which include direct costs for diagnosis, treatment and prevention[2].

Malaria elimination requires robust strategic planning for it to succeed[3]. In the absence of any effective malaria vaccine, chemotherapy plays an important role in containment of the disease but unfortunately, drug-resistant strains of *Plasmodium* have appeared against most of antimalarials introduced till date. *P. falciparum* has developed resistance to practically all the antimalarials used currently[4] and continues to increase in both intensity and geographic distribution[5]. Due to drug resistance and unavailability of malaria vaccine, there is a need of exploring the flora for antimalarial properties.

Artether, artemether and artesunate from *Artemisia annua* have effectively treated drug-resistant *P. falciparum*. Extracts from plants like *Entandro phragma*

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angelense, *Picrolima nitida*, *Schumanniophytan magnificum*, *Thomandersia hensii*[6], *Mammea africana*[7] and *Annona senegalensis*[8] have already shown potential to cure malaria. The leaf extracts of *Craton zambgesicus* showed antiplasmodial activity against chloroquine-sensitive *P. berghei*[9]. Sixteen compounds extracted from roots of *Piper sarmentosum* were tested for antiplasmodial, antimyco-bacterial and antifungal activities[10]. *Alsotonia boonei* De Wild is one of the plants that has been widely used to treat malaria[11] and studies have shown that extracts of this plant possess antimalarial activity[12]. These studies show that ethnopharmacological approach is needed to fight against the disease.

In the present study, extracts of two plants were tested for their antimalarial activity on *P. berghei* maintained in mice. *Cissampelos pareira* L. (Menispermaceae) – popularly called 'Ice Vine' in English, 'Akanadi' and 'Patindu' in Hindi – is a twining, perennial herb. Its leaves are alternate, globular, cordate, kidney-shaped, soft and hairy. Flowers are greenish, dioecious. Male inflorescence is a raceme, drupe avoid, sub globose, red in colour. The plant extracts have pharmacological activities like hypoglycaemic activity, which acts as CNS depressant and show muscular relaxant properties[13,14]. The medicinal effect is mainly confined to root. It is used in malaria, cough, leprosy and its decoction is consumed orally for stomachache caused by indigestion, dysentery, enlarged spleen, skin disease and also as diuretic. *Tinospora cordifolia* (Willd.) Hook.f. & Thoms – commonly known as 'Giloy' in Hindi and 'Heartleaved Mvonseed' in English – is a glabrous climber, succulent shrub with corky grey dotted bar leaves (10–20 cm in diameter), broadly ovate, deeply cordate and shortly acuminate. Flowers are small and greenish yellow on the old wood in 7.5–15 cm long racemes, slender, usually solitary in female and clustered in male. Fruits are of the size of pea and red in colour. Starch from roots is used in chronic diarrhoea and dysentery. Its paste mixed with olive oil is applied to cure pimple. Powder of the root is used to cure breathing problems, piles, ulcer, cough, chronic fever, leprosy, blood pressure, snake bite, headache, hiccups, heart stroke, skin disease, whooping cough, splenomegaly, general debility, dyspepsia, dysentery, fever, urinary diseases, diarrhoea, diabetes, visceral obstruction and as tonic and emetic.

The roots of *C. pareira* L. and stem of *T. cordifolia* (Willd.) Hook.f. & Thoms were washed thoroughly with distilled water, air dried and weighed. After cutting into small pieces these were homogenized in ethanol. The homogenate was filtered and centrifuged at 2000 rpm for 10 min (Sigma 3k-30). The supernatant was air dried and the residual concentrated solid material was used as plant extract. The extract was weighed and stored at 4°C till further use.

Plasmodium berghei (NK-65), a rodent malaria parasite, was maintained in white Swiss mice, *Mus musculus* (BALB/c). The parasite was maintained by passing the infection to normal mice intraperitoneally with 1×10^5 *P. berghei*-infected erythrocytes. The course of parasitaemia was monitored by preparing daily blood smears.

The animals were treated according to the guidelines of the Institutional Animal Ethics Committee (IAEC) of H.P. University, Shimla.

Plant extracts for their antimalarial activity were screened following Peter's 4-day test[15]. This test was followed to evaluate the blood schizontocidal action against *P. berghei*. In brief, on day 0, experimental and control groups of animals were inoculated intraperitoneally with 1×10^5 *P. berghei* parasitized red blood cells suspended in 0.2 ml of 2% citrate saline. The inoculated animals were categorized into untreated control, chloroquine-treated control and extract-treated experimental groups. The test extract was prepared in water and given daily from day 0 to day 3 by oral route. The extract was given in concentration of 500 mg/kg body weight per dose per day to the experimental animals. The plant extracts were administered orally with the help of syringe and rubber tube. The tube was inserted deep into the oral cavity of the animal taking care that not a single drop of extract is spilled over during the administration. A control group of mice received chloroquine at 4 mg/kg daily from 0 to day 3 as standard antimalarial. Untreated control group was given only plain water. On day 4, thin blood smears were prepared from the tail vein of all the extract-treated animals, chloroquine treated and untreated control groups and the percentage of infection was monitored. If any mouse died within 24 h of extract administration, this was considered as toxicity of the extract.

Plasmodium berghei, a rodent malaria parasite, is lethal to white Swiss mice, *M. musculus* (BALB/C) as the mice of untreated control group died between day 6 and day 7 because of high infection. Parasite appeared in the blood smear on day 2 post-inoculation. Monitoring the course of parasitaemia revealed asexual stages of ring, tropozoite and schizont were present asynchronously in smear every day.

No mortality of mice was observed within 24 h of drug administration. On day 4, five mice of untreated control group exhibited a mean parasitaemia of $25.48 \pm 4.88\%$ and parasitaemia ranged between 17.71% and 30.02% (Table 1). In the chloroquine control group, no parasitaemia was noticed. In the group of mice treated with the extract of *C. pareira* L. mean parasitaemia was $11.64 \pm 4.20\%$ and infection ranged between 6.28% and 16.06% on day 4 (Table 1). In this group one mouse, i.e. mouse number 2 showed maximum parasitaemia on day 12 and died on day 13 post-infection. Other mouse, i.e. mouse number 5 showed maximum percentage of infection on day 12. Mouse number 5 also died on day 13. The other mice of this group, i.e. 1, 3 and 4 showed maximum parasitaemia on day 7, 9 and 5 respectively. Mouse number 1 died on day 8 and mouse number 3 on day 10. Mouse

Table 1. Per cent of infection (parasitaemia) on day 4 in mice treated with various plant extracts along with controls

Group of mice	Plant part used	Mouse number and parasitaemia					Mean % of infection
		1	2	3	4	5	
Cissampelos pareira L.	Root	15.01	6.28	12.43	16.06	8.42	11.64 ± 4.20
Tinospora cordifolia	Stem	10.42	14.63	13.27	16.44	14.73	13.90 ± 2.25
Untreated control		30.02	17.71	28.71	26.84	24.13	25.48 ± 4.88
Chloroquine treated control		0	0	0	0	0	

number 4 lived for six days post-infection and died on day 7.

The group of mice which were treated with extracts of *T. cordifolia* (Willd.) Hook.f. & Thoms showed mean parasitaemia of 13.90 ± 2.25%, with parasitaemia ranging between 10.42% and 16.44% (Table 1). Three mice of this group died on day 8 post-infection and showed maximum percentage of infection a day before death, i.e. on day 7. The remaining two mice died on day 11 post-inoculation showing maximum percentage of infection on day 9 and 10.

In the present study, *P. berghei* was found lethal to white Swiss mice and the animals died due to infection in a week's time. The parasite induced reticulocytosis in some cases[16]. In the absence of effective vaccine and increasing drug resistance against the available drugs, extracts of these plants have shown promising antimalarial results. Alcohol soluble extracts of two plants have significantly inhibited the propagation of *Plasmodium*. This dose of the given extracts was not toxic to the mice as none of them died during four-day test. The antimalarial effect of the two plant extracts is evident from the variations in parasitaemia of the experimental and untreated control. Mean parasitaemia of 25.48 ± 4.88 in untreated group is almost double the mean parasitaemia of 11.64 ± 4.20 for *C. pareira* L. and 13.90 ± 2.25 for *T. cordifolia* (Willd.) Hook.f. & Thoms of treated group. However, the inhibitory effect of *C. pareira* L. is more than *T. cordifolia* (Willd.) Hook.f. & Thoms.

Aqueous extracts of *T. cordifolia* (Willd.) Hook.f. & Thoms along with choroquine were studied in the treatment of three cases of hyper reactive malarious splenomegaly in District Hospital, Datenganj town, Jharkhand, India[17]. The main constituents are tinosporin and a furanoid diterpene identical with columbine have been isolated from the plants. A new hypoglycaemic agent and a new phenolic lignan have also been isolated[18].

Both these plants have important constituents exhibiting medicinal properties and the present study clearly establishes their antimalarial effects. The particular ingredients of the plant, responsible for antimalarial properties, may be extracted, chemically analysed and biochemically studied to know its nature and hence its effective role in checking the propagation of malaria parasite.

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