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Bioefficacy of *Centella asiatica* (Linnaeus) Urban on *Plasmodium berghei* Vincke and Lips, 1948

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ABSTRACT

Extracts of roots and leaves of *Centella asiatica* (Linnaeus) Urban were used to evaluate their antimalarial effect against *Plasmodium berghei* *in vivo* in a typical 4 day test. Alcohol soluble extracts were administered orally in experimental mice along with placebo controls. On day 4 parasitaemia in control group of mice was $21.54 \pm 1.75\%$ while in mice treated with roots and leaves extracts showed $5.73 \pm 1.14\%$ and $11.89 \pm 1.25\%$ infection, respectively.

Key words: *Centella asiatica*, *Plasmodium berghei*, sporozoa, malaria

INTRODUCTION

Malaria caused by parasitic protozoan of the genus *Plasmodium* remains the most important tropical infection and it poses major public health challenge. Plants being valuable source used in practices as repellants throughout developing countries (Azokou *et al.*, 2013). Plants have always been considered to be a possible alternative and rich source of new drugs and most of the antimalarial drugs in use today such as quinine and artemisinin derivatives either obtained directly from plants or developed using chemical structures of plant derived compounds as templates (Basco *et al.*, 1994). The *Centella asiatica* cultivated in India showed good antioxidant activity as assessed by DPPH (2,2-diphenyl-1-picrylhydrazyl), reducing power and ferrous ion chelating capacity methods (Gupta and Prakash, 2009). The leaves of *Centella asiatica* are used for the treatment of diarrhoea due to the presence of flavonoids (Schuier *et al.*, 2005).

In the present study, ethanol soluble extracts of roots and leaves of *Centella asiatica* were analysed for their antimalarial activity against *Plasmodium berghei*. *Centella asiatica* is a herbaceous creeper of family Apiaceae and grows in moist areas of tropical and sub-tropical countries (Rakotondralambo *et al.*, 2013). The extracts and some fractions of *Centella asiatica* possess several medical uses against rheumatism, syphilis, leprosy, ulcer and eczema (Visweswari *et al.*, 2010). *Centella asiatica* possesses triterpenes and saponins and these may be responsible for its therapeutic effect (Orhan *et al.*, 2013).

MATERIALS AND METHODS

Plasmodium berghei (NK-65) was maintained in white Swiss mice, *Mus musculus* (BALB/c). The present study was carried out with prior permission of Institutional Animal Ethics Committee (IAEC) of H.P. University, Shimla vide number IAEC/Bio/4-2013. The asexual form of parasite was maintained by passing the infection to normal mice intraperitoneally with 1×10^5 *P. berghei* infected erythrocytes in citrate saline (Banyal *et al.*, 1991). The fresh whole plant, *Centella asiatica*

(Linnaeus) Urban used in present study was collected from Hamirpur, Himachal Pradesh (India), brought to laboratory and identified. After proper identification, roots and leaves were collected, washed with distilled water, air dried, weighed and homogenized in ethanol. The homogenate was filtered and centrifuged at 2,000 rpm (Sigma 3k-30) for 10 min. Supernatant was boiled and concentrated. The residual concentrated material used as plant extract was stored at 4°C till further use.

The screening of plant extracts for their antimalarial activity was carried out following Peter's 4 day test (Peters, 1970). This test was followed to evaluate the blood schizontocidal action against *P. berghei*. Experimental as well as 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 test extract was given through oral route daily from day 0-3. The extract was given in concentration of 500 mg per kg body weight per dose per day to the experimental animals. Placebo controls received similar volume of phosphate buffered saline, pH 7.4. Another control group of mice received chloroquine 4 mg kg⁻¹ per dose for 4 days as standard antimalarial. On day 4 smears were prepared all the animals, stained and examined.

RESULTS

Centella asiatica exhibited antimalarial properties, roots being more active as compared to leaves. After 24 h of extracts administration no mortality was observed indicating that the plant products showed no toxicity to mice. On day 4, five mice of control group exhibited mean parasitaemia of 21.54±1.75% and the parasitaemia ranged between 21-24% (Table 1). In chloroquine control group mice, no parasite infection was noticed. Mice treated with root extract of *Centella asiatica* showed mean parasitaemia on day 4 of 5.73±1.14% and ranged between 4.20 and 6.90%. Leaf extract treated mice showed mean parasitaemia of 11.89±1.25% and the percent

Table 1: Percentage infection on day 4 in mice treated with roots and leaves extracts

Material	Plant part	Mouse number and parasitaemia					Mean infection (%)
		1	2	3	4	5	
<i>Centella asiatica</i>	Roots	5.30	6.82	4.20	6.90	5.44	5.73±1.14
<i>Centella asiatica</i>	Leaves	12.00	10.20	11.42	12.34	13.60	11.89±1.25
Placebo (Control)	-	21.16	19.90	22.24	20.18	24.20	21.54±1.75
<i>Chloroquine</i> (Control)	-	0	0	0	0	0	0

Table 2: Maximum parasitaemia attained by mice treated with extracts of *Centella asiatica* on postinoculation

Part of plant and mouse No.	Maximum parasitaemia (%)	Maximum parasitaemia on day	Death on day
Root			
1	53.00	9	12
2	55.70	7	11
3	28.20	8	11
4	43.30	9	14
5	51.40	10	13
Leaves			
1	55.20	8	12
2	46.10	8	11
3	52.25	7	10
4	53.00	9	10
5	40.21	9	11

infection ranged between 10.20-13.60%. All the treated mice with root or leaf extract finally succumbed to infection and mice died between day 10 and 14 postinoculation (Table 2). The maximum parasitaemia achieved by each mouse was observed in smears prepared on the day given in Table 2.

DISCUSSION

Centella asiatica is one of the chief herbs for treating skin problems, to heal wounds, for revitalizing the nerves and the brain cells (Singh *et al.*, 2010). There is a growing interest in co-relating phytochemical constituents of plants with its pharmacological activities. Scientists have started co-relating the botanical properties of plants with their pharmacological activities (Cheng and Koo, 2000). The extract of *Centella asiatica* exhibited antiallergic, antipruritic and antiinflammatory activities (George and Joseph, 2009).

During present investigation, extracts of both the parts of plant i.e., roots and leaves of *Centella asiatica* showed antimalarial effect, however, extract of roots being more effective than that of leaves. Both the parts of *Centella asiatica* have important components showing medicinal properties. The particular ingredients of the plant which are responsible for their antimalarial effect are unknown, however, may be extracted, chemically analyzed and biochemically studied to know its nature and role in preventing the propagation of malaria parasite.

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