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Antimalarial effect of *Ocimum sanctum* Linn. and *Bauhinia variegata* Linn. on *Plasmodium berghei*

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Abstract: *Extracts of leaves and roots of Ocimum sanctum Linn. and leaves of Bauhinia variegata Linn. were used to evaluate their antimalarial effect against Plasmodium berghei in vivo in a typical 4 day test. Water and ether soluble extracts were administered orally to experimental mice along with placebo controls. On day 4 parasitaemia in control group of mice was 25.20% ± 9.44% while in mice treated with water soluble extracts of leaves and roots of Ocimum sanctum showed 2.80% ± 2.17% and 7.60% ± 5.32% infection respectively while in mice treated with water soluble extract of leaves of Bauhinia variegata showed 23.60% ± 13.35% infection.*
Keywords: *Ocimum sanctum, Bauhinia variegata, Plasmodium berghei, sporozoa, malaria*

I. Introduction

Malaria remains one of the major health hazards with high morbidity and mortality rate in the developing countries. This disease transmitted by female *Anopheles* mosquito and caused by *Plasmodium*, is the most deadly and devastating parasitic disease of mankind. In the absence of any effective malaria vaccine, chemotherapy plays an important role to combat malaria. Plants have proved good source of new antimalarial drugs in view of the success of chemotherapeutic agents, quinine and artemisinin (ACTs). ACTs are recommended as first line of treatment for malaria (WHO, 2013). Therefore, there is a great need of exploring the flora for antimalarial properties. *Ocimum sanctum* Linn. popularly known as Tulsi (Family-Lamiaceae), is a sacred herb worshipped by Hindus. *Ocimum sanctum* is renowned Indian medicinal plant for its immune-modulating activity. *Ocimum sanctum* possesses anti-diabetic, cardioprotective, anti-cancer, neuro-protectant, anti ulcer , anti-malarial, anti-inflammatory, analgesic and anti-oxidant activity (Singh *et al*., 2013; Bhasker *et al*., 2014 ; Mishra *et al*., 2014). Leaf extract of *O. sanctum* has been found to be toxic to mosquito larvae and exhibit highly deleterious effects on adult mosquitoes (Priyanka *et al*., 2014). The leaves of this plant contain eugenol, carvacrol, limatrol, methylchavicol, and caryophylline (Jain and Argal, 2013). Another plant used in this study was *Bauhinia variegata* Linn. commonly known as kachnar (Family-Caesalpiniaceae). *Bauhinia* leaves are used for their anti-inflammatory and decongestant properties, whereas the bark is traditionally used in bronchitis, leprosy, tumors and ulcers (Kassem *et al*., 2013). The extract of *Bauhinia variegata* leaves exhibited antioxidant, antibacterial and anticancer activities. Since these two plants, *Ocimum sanctum* and *Bauhinia variegata* have been demonstrated to possess antimalarial properties in the ancient Indian medicine system. The extracts of these two plants were employed for a systematic study to evaluate their antimalarial effect in *Plasmodium berghei* parasitized mice.

II. Materials And Methods

Plasmodium berghei (NK-65), a rodent malaria parasite, was maintained in white Swiss mice, *Mus musculus* (BALB/c). The present work was carried out with prior permission of Institutional Animal Ethics Committee (IAEC) of H.P. University, Shimla vide number IAEC/Bio/4-2011. 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 plants, *Ocimum sanctum* and *Bauhinia variegata* were collected from the districts of Kullu and Bilaspur in Himachal Pradesh, India respectively, brought to laboratory and identified. After proper identification roots and leaves were washed with distilled water, air dried, weighed and homogenized with alcohol, ether or water separately. The homogenate was filtered and centrifuged at 2,000 rpm for 10 minutes (Sigma, 3k-30). Supernatant was boiled and concentrated. The concentrated residual material used as plant extract. The extract was stored at 4^0C till further use.

The antimalarial activity of plant extracts was screened by 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% (w/v) citrate saline. The test extract was given daily through oral route from day 0 to day 3. The concentration of the extract given was 500 mg per kg body weight per dose per day to the experimental animals. Placebo controls administered similar volume of solvent in which extract prepared. Another control group of mice received chloroquine 4 mg/ kg per dose for 4 days as standard antimalarial. On

day 4 smears were prepared from the tail vein of all the animals, stained and examined.

III. Results

Ocimum sanctum and *Bauhinia variegata* plant extracts of different parts exhibited antimalarial properties. No mortality was observed after 24 hours of extracts administration indicating that the plant products showed no toxicity to mice. On day 4, five mice of control group exhibited mean parasitaemia of 25.2% ± 9.44 % and the parasitaemia ranged between15% to 37% (Table 1). In chloroquine control group mice which received 4mg/kg chloroquine orally mean parasitaemia ranged between 0% to 2%. The extracts of leaves and roots of *O. sanctum* were prepared in water and ether. In mice treated with water soluble extracts of leaves and roots the mean parasitaemia was 2.80% ± 2.17% and 7.60% ± 5.32% respectively. The ether soluble extracts treated mice showed slightly high parasitaemia as compared to water soluble extract and the mean parasitaemia was 6.40%± 3.04% and 7.0% ± 4.83% while the parasitaemia ranged between 2% to 11%. Similarly the extracts of leaves of *B. variegata* was prepared in water and alcohol separately. On day 4, the water soluble extract treated mice exhibited mean parasitaemia 23.60% ± 13.35% and parasitaemia ranged between 15% to 47% while alcohol soluble leaves extracts treated mice exhibited high mean parasitaemia 46.2% ±12.97% and parasitaemia ranged between 35% to 66%. Maximum parasitaemia attained by mice treated with extracts showed in Table 2.

Table 1: Percent infection (parasitaemia) on day 4 in mice treated with roots and leaves extracts.

Plant	Plant part	Extract	Mouse number and parasitaemia					Mean infection (%)
			1	2	3	4	5	
Ocimum sanctum	Leaves	Water	6	2	1	4	1	2.80 ± 2.17
		Ether	7	5	8	2	10	6.40 ± 3.04
Ocimum sanctum	Roots	Water	14	1	5	6	12	7.60 ± 5.32
		Ether	0	8	11	9	Died	7.0 ± 4.83
Bauhinia variegata	Leaves	Water	22	15	16	18	47	23.60 ± 13.35
		Alcohol	66	35	42	36	52	46.2 ± 12.97
Placebo Control			37	25	15	32	17	25.2 ± 9.44
Chloroquine control			0	0	0.63	2	0	0.53 ± 0.87

Table 2: Maximum parasitaemia attained by mice treated with extracts.

Plant	Part of plant	Extract Of	Mouse no.	Maximum parasitaemia (%)	Maximum parasitaemia on day	Death on day
Ocimum sanctum	Leaves	Water	1	56	5	6
			2	52	6	7
			3	42	6	7
			4	48	5	6
			5	36	6	7
		Ether	1	7	4	5
			2	31	11	Recovered
			3	38	9	Recovered
			4	53	7	8
			5	42	5	6
Ocimum sanctum	Roots	Water	1	31	5	7
			2	17	8	Sacrificed on day 11
			3	35	5	Sacrificed on day 11
			4	35	5	7
			5	23	5	7
		Ether	1	65	8	9
			2	53	7	8
			3	55	7	8
			4	51	5	6
			5	Died		
Bauhinia variegata	Leaves	Water	1	46	7	8
			2	15	4	5
			3	69	7	Sacrificed
			4	78	6	7
			5	47	4	5
		Alcohol	1	66	4	5
			2	54	6	7
			3	42	4	5
			4	62	6	7
			5	52	4	5

IV. Discussion

Ocimum sanctum has excellent antimalarial properties, eugenol the main constituent of this plant is responsible for its repellant property (Singh *et al*., 2010). Different parts of plant *Ocimum sanctum* possess anticancer, antifertility, antidiabetic, antifungal, hepatoprotective, cardioprotective and analgesic properties (Vishwabhan *et al*., 2011). Ethanolic and alcoholic extracts of stem bark and root of *Bauhinia variegata* have shown antioxidant property (Rajani *et al*., 2009). Roots of *Bauhinia variegata* used as antidote to snake poison and also useful for inflammatory conditions (Mishra *et al.*, 2013). Leaf extracts of this plant showed antimicrobial and anticancer properties

During present investigation, extracts of both the parts of plant i.e. roots and leaves of *Ocimum sanctum* has eliminated the propagation of parasite to a greater extent as compared to *Bauhinia variegata* plant used. Leaves extract of the former plant exhibited better antiparasite inhibitory properties compared to the roots. However, water soluble extract of the leaves of *B. variegata* when administered though inhibited the infection in animals to some extent. The present study thus elucidates that of the two plants used *Ocimum sanctum* definitely exhibits antimalarial activity in vivo. The extract of this plant may further be purified to determine the constituents of the plant responsible for parasite inhibitory activity.

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