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# *Picrorhiza kurrooa* Royal Ex Benth Exhibits Antimalarial Activity against *Plasmodium berghei* Vincke and Lips, 1948

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## ABSTRACT

Different parts of *Picrorhiza kurrooa* (Kutki) were used to evaluate their antimalarial effect against *Plasmodium berghei in vivo* in a typical 4 day test. Experimental groups of mice were administered with ethanol extracts of roots and leaves of *Picrorhiza kurrooa*. On day 4 parasitaemia in control group of mice was 23.15±1.66 whereas, groups treated with roots and leaves extract of *P. kurrooa* resulted in inhibition of malaria parasite significantly and parasitaemia being 4.14±1.70 and 11.4±1.66, respectively. The effect of root extract was more pronounced compared to leaves.

**Key words:** *Picrorhiza kurrooa*, *Plasmodium berghei*, antimalarial, rodent malaria

## INTRODUCTION

Malaria caused by parasitic protozoan of the genus *Plasmodium* is a major public health problem. An estimated group of 3.4 billion people are at risk of malaria globally, among nations Africa is the most affected one and India has the highest malaria burden followed by Indonesia and Myanmar (WHO, 2013). Development of vaccine against malaria is an area of intensive research, however, there is no effective vaccine introduced into clinical practice, till date. Non availability of malaria vaccine and global scenario of antimalarial drug resistance, research groups are compelled to envisage new antimalarials and plants considered as novel source (Onguene *et al*., 2013). Chemicals produced by plants proved to be useful with antibacterial property, larvicidial, mosquitocidal and repellent properties, explain the potential of specific plant biodiversity. Root extracts of *Picrorhiza kurrooa* inhibited the propagation of *P. berghei* in white Swiss mice (Singh and Banyal, 2011). Different parts of *Picrorhiza kurrooa* has been used to cure cardiac ailments, abdominal pain, hepatitis, jaundice and for promoting bile secretion (Kumar *et al*., 2001; Verma *et al*., 2009). And the rhizome of this plant is used for the treatment of fever, dyspepsia and constipation.

In the present study, extracts of roots and leaves of *Picrorhiza kurrooa* were analysed for their antimalarial activity against *Plasmodium berghei* which was maintained in white swiss mice. *Picrorhiza kurrooa* is a small perennial herb widely used in traditional as well as modern system of medicine. This plant grows in Himalayan region at elevations ranging from 3000-5000 m above the sea level and listed as endangered species due to extensive extraction of picrosides, the medicinally important constituents of *Picrorhiza* (Singh *et al*., 2011).

## MATERIALS AND METHODS

**Parasite:** A rodent malaria parasite, *Plasmodium berghei* (NK-65) was maintained in white Swiss mice, Mus musculus (BALB/c) as per guidelines of Institutional Animal Ethics Committee of Himachal Pradesh University, Shimla vide number IAEC/Bio/2-2012. The erythrocytic stages was maintained by passing the infection to normal mice intraperitioneally (i.p) with $1\times10^5$ *P. berghei* infected erythrocytes in citrate saline (Banyal and Kumar, 1991). The course of parasitaemia was monitored by observing daily blood smears.

**Preparation of extracts:** The parts of the plant i.e., roots and leaves of *Picrorhiza kurrooa* were washed separately with distilled water, air dried and weighed. Homogenisation was done in ethanol and homogenate filtered, centrifuged (Sigma 3k-30) at 2000 rpm for 10 min. Ethanol evaporated and residual concentrated concentrated material was used as plant extract. This extract was stored at 4°C till further use. The screening of extracts of different parts of plant was carried out according to Peter's 4 day test (Peters, 1970). Experimental as well as control groups of animals were inoculated intraperitoneally with $1\times10^5$ *P. berghei* parasitized red blood cells suspended in 0.2 mL of 2% citrate saline, on day 0. The extract suspended in water and given in concentration of 500 mg per kg b.wt. per dose per day per mouse to the experimental animals daily from day 0 to day 3 by oral route. As standard antimalarial, chloroquine was given to another control group of mice at 4 mg $kg^{-1}$ per dose for 4 days. Untreated control receive water only. Blood smears were prepared from the tail vein of all the mice of extract treated groups as well as chloroquine control and untreated control groups, on day 4. Afterward percent infection was monitored.

## RESULTS

The antimalarial effect of extracts of roots and leaves of *Picrorhiza kurrooa* was evaluated. These extracts showed varying degree of results. No mortality was observed, within 24 h of drug administration which indicated that the plant products were not toxic to mice. Mice of weight between 20-35 g were used in the present study. On day 4, five mice of control groups exhibited a mean parasitaemia of 23.15±1.66% and the parasitaemia ranged 21-25% (Table 1 and Fig. 1). In the chloroquine control group, no parasite was noticed. In group of mice treated with root extract of *Picrorhiza kurrooa*, mean parasitaemia on day 4 was 4.14±1.70% and the parasitaemia ranged between 2.50-6%. The group of mice treated with leaf extract showed mean parasitaemia of 11.4±1.66% and the percent infection ranged between 9.15-13.5% on day 4. All the treated mice with leaf or root extract finally succumbed to infection and animals died between day 10 and 15 postinoculation (Table 2). The maximum parasitaemia achieved by a mouse was observed in smears prepared on the day given in Table 2 and the animal died later on due to infection.

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

| | | Mouse No. and parasitaemia | | | | | |
|---|---|---|---|---|---|---|---|
| Group of mice | Plant part | 1 | 2 | 3 | 4 | 5 | Mean infection (%) |
| *Picrorhiza kurrooa* (Kutki) | Roots | 3.01 | 2.50 | 3.23 | 5.98 | 6.0 | 4.14±1.70 |
| *Picrorhiza kurrooa* (Kutki) | Leaves | 10.42 | 12.03 | 9.15 | 11.9 | 13.5 | 11.4±1.66 |
| Control | | 21.05 | 24.21 | 25.23 | 22.06 | 23.20 | 23.15±1.66 |
| Chloroquine | | 0 | 0 | 0 | 0 | 0 | 0 |

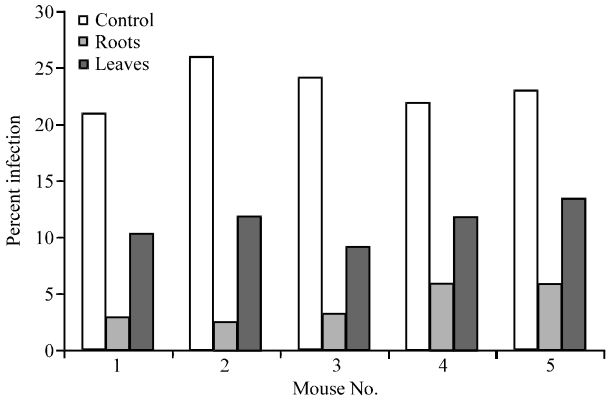


Fig. 1: Parasitaemia on day 4 in control and extracts (roots and leaves) treated mice

Table 2: Maximum parasitaemia attained by mice on postinoculation

| Plant | Part of plant | Extract | Mouse No. | Max. parasitaemia | Max. parasitaemia on day | Death on day |
|---|---|---|---|---|---|---|
| *Picrorhiza kurrooa* | Root | Alcohol | 1 | 49.01 | 9 | 10 |
| | | | 2 | 45.14 | 6 | 15 |
| | | | 3 | 50.26 | 10 | 12 |
| | | | 4 | 44.87 | 9 | 11 |
| | | | 5 | 59.94 | 11 | 12 |
| *Picrorhiza kurrooa* | Leaves | Alcohol | 1 | 49.58 | 8 | 9 |
| | | | 2 | 52.10 | 8 | 10 |
| | | | 3 | 53.90 | 9 | 10 |
| | | | 4 | 48.83 | 8 | 11 |
| | | | 5 | 40.21 | 7 | 9 |

## DISCUSSION

The present study confirms the finding of Singh and Banyal (2011) that roots of *Picrorhiza kurrooa* inhibited *P. berghei* propagation *in vivo,* the leaves used in this study alongwith roots significantly inhibited *P. berghei* infection.

*Picrorhiza kurrooa* is widely used in traditional as well as modern system of medicine. Traditionally, the mature plants of *Picrorhiza kurrooa* are uprooted, rhizomes and roots are traded for preparing medicinal formulations to treat disorders of the liver and upper respiratory tract, dyspepsia, chronic diarrhoea and scorpion sting (Verma *et al.*, 2009). The hepatoprotective action of *Picrorhiza kurrooa* is not fully understood but may be attributed to *Picrorhiza*'s ability to scavenge free radicals and to inhibit the generation of oxygen anions (Russo *et al.*, 2001). The effect of *Picrorhiza*'s antioxidants has been similar to that of superoxide dismutase, xanthine oxidase inhibitors and metal-ion chelators (Chander *et al.*, 1992). During present investigation, extracts of both the parts i.e., roots and leaves of *Picrorhiza kurrooa* showed antimalarial effect, however, extract of roots being more effective than that of leaves. Both the part of the *Picrorhiza kurrooa* have important components showing medicinal properties and antimalarial activity. However, the revelation of the chemical constituent showing the property needs to be elucidated.

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