IMMUNOLOGICAL EFFECTS OF AQUEOUS CRUDE EXTRACT FROM TEPHROSIA PURPUREA AERIAL PARTS ON PLASMODIUM BERGHEI-INFECTED BALB/C MICE

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ABSTRACT

Malaria is a serious parasitic disease in the developing world, causing high morbidity and mortality. *Plasmodium falciparum*, which causes the deadliest form of the disease, has developed resistance to every drug thrown at it and this has kept researchers up at night fighting it. The stem extract of *Tephrosia purpurea* showed *in vitro* antiplasmodial activity against the chloroquine-sensitive and chloroquine-resistant strains of *Plasmodium falciparum*. A new prenylated flavone, named terpurinflavone, along with the known compounds lanceolatin A, (−)-semiglabrin and lanceolatin B have been isolated from this extract. The new compound, terpurinflavone, showed the highest antiplasmodial activity. The main objective of this study is to determine the immunological effect of aqueous crude extract from *Tephrosia purpurea* aerial parts on the immune system of *Plasmodium berghei* infected BALB/c mice. Highly susceptible BALB/c mice previously infected with *Plasmodium berghei* isolates that cause malaria in rodents will be used for evaluating the ability of the *Tephrosia Purpurea* crude extracts to modulate the immune system in the course of murine malaria. Briefly, the groups of mice will be infected with *P. berghei* and then treated with *T. purpurea* extracts, an already known antimalarial drug candidate, or with Chloroquine, an already known blood stage antimalarial drug or with a saline control. In this study, the parasites will be cultured by parasite passage through 12 BALB/c mice and ultimately infected blood will be harvested from each mouse by cardiac puncture. Subsequently, experimental BALB/c mice will each be injected intraperitoneally with 0.3 ml of the *P. berghei*-infected murine blood. The groups of mice will then be treated orally with 100, 200 and 400 mg/kg/day of aqueous *T. purpurea* extracts using oral gavage needle for mice, or intraperitoneally with 5mg/kg/day of aqueous Chloroquine, or with 0.2ml saline control. Samples will be collected four times in the course of experiment: before infection, after disease development (3 days post infection) but prior to treatment, 4 days post treatment and 12 days post treatment. At every sampling, one drop of blood will be taken from the tail tip of each mouse onto frosted slides for Parasitaemia and leucocytes counts. The slide samples will be air dried and fixed in absolute ethanol then stored in slide boxes till use. Parasitaemia and WBC counts will be done by microscopic enumeration of parasitized RBCs and leukocyte morphology respectively on stained smears. Concurrently, 3 mice will be sacrificed from each cage and blood drawn by cardiac puncture into EDTA-filled tubes for IFN-γ assay. The blood will be centrifuged at 2000 RPM for 5 minutes to extract plasma which will be stored at -20°C till use. IFN-γ in the plasma samples will be measured using mouse IFN-γ ELISA kit (Mabtech, AB, Sweden). In a selective manner, this experiment will characterize the levels of IFN-γ, WBC and parasitaemia in the peripheral blood samples of the infected mice. This study will provide an insight on the possible role of immune system mechanisms of the plant extract in modulating malaria infection. Moreover, it will also attempt to justify its traditional use. Data will be analyzed using ANOVA to compare levels of WBC, parasitaemia and IFN-γ across the groups of mice. P values of less than 0.05 will be considered significant.