ANTIMALARIAL ACTIVITIES OF *DIPLAZIUM ESCULENTUM* (RETZ.) SW. AQUEOUS EXTRACT IN *PLASMODIUM BERGHEI*-INFECTED MICE

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ABSTRACT

Antimalarial drug resistance is a threat to the worldwide effort to control and eliminate malaria and warrants the need to discover new drugs for malarial infection. Thus, this study aims to evaluate the antimalarial activity of *Diplazium esculentum* aqueous extract in *Plasmodium berghei*-infected mice. Forty-two mice were divided into seven groups and the extract was given orally to all treatment groups. The preventive groups (G1 and G2 groups) were given 100 mg/kg of extract 19 days and eight days before infection respectively. Meanwhile, the curative groups were given extract on the same day of infection (G4 group) and four days after infection (G5 group) at different doses, 100 mg/kg and 400 mg/kg respectively. G3 and G5 were the positive control group for each of the treatment that was given primaquine and chloroquine, respectively. All mice were observed for parasitemia level, mortality and body weight changes. For preventive treatment, the plant extract that was given 19 days before infection was found to reduce the parasitemia by 2.67%. Curative treatment showed that the effective dose was found to be 100 mg/kg, which reduced parasitemia by 1.83%. In conclusion, *D. esculentum* extract showed preventive properties but no curative properties. This result indicates that *D. esculentum* could not be compared with commercial antimalarial drugs.

KEYWORDS: Antimalarial, *Diplazium esculentum*, *Plasmodium berghei*.

INTRODUCTION

Malaria is a life-threatening disease caused by *Plasmodium* parasites. People get malaria by being bitten by an infective female *Anopheles* mosquito. Malaysia has seen a reduction in indigenous malaria cases where 5194 cases out of 6141 total malaria cases were recorded in 2010, but in 2017, there were 85 cases

out of 508 total cases. However, 423 cases out of 508 total cases in 2017 were identified as imported cases with 12 malaria deaths (WHO, 2018). WHO has categorized Malaysia as in the pre-elimination phase due to the achievement of a 99% reduction in reported malaria cases in the past twenty years (APMEN, n.d). But, these imported malaria cases cannot be ignored. Malaria is experiencing a sporadic outbreak of disease due to the influx of foreign workers from neighbouring countries, and also due to the failure of some travellers to take antimalarial prophylaxis during travelling (Yong et al., 2018). Thus, the search for new antimalarial drugs from natural sources is imperative.

Diplazium esculentum (Retz.) Sw., is a vegetable fern that is pantropical in distribution and widely grown in countries like Indonesia, Thailand, India, Vietnam and Malaysia (Irudayaraj, 2013). Some societies use *D. esculentum* as a natural insecticide (Halimatussakdiah & Wahyuningsih, 2018). The plant is also used to make a poultice with the bark of *Euphoria hired* to treat jaundice (Sourabh et al., 2009). A study determined that the mature fronds of *D. esculentum* are high in moisture, free fatty acid, lipid content, crude fibre and phenolic content. The plant also has more elevated amounts of flavonoids, glycosides and steroid (Archana et al., 2012) and has significant cytotoxic, antimicrobial and antioxidant properties (Akter et al., 2014). Acute oral toxicity tests revealed that this plant is safe with an LD_{50} estimated to be above 5000 mg/kg body weight (Junejo, 2015). This plant was also claimed to have active larvicidal activity with an LC_{50} value of 149.279 ppm, and it may have potential larvicidal effects against *Culex* (Halimatissakdiah & Wahyuningsih, 2018).

Since there is no documented study on the antimalarial properties of Malaysian *D. esulentum*, this study aims to determine the percentage of parasitized red blood cells of the infected mice after treatment with *D. esculentum* extract and the survival rates of infected mice after treatment.

MATERIAL AND METHODS

Plant material

The plant was collected from the forest and along a riverbank in Kuantan and was identified by a plant taxonomist from University Putra Malaysia. The plant specimen (PIIUM 0310) was deposited in the herbarium of Kulliyyah of Pharmacy, International Islamic University Malaysia (IIUM) as a reference point.

Plant extraction

360 g of the bud section furls were ground, soaked in water and heated in a water bath at 60 °C for 3 hours. Subsequently, the aqueous mixture was filtered to obtain the concentrated solution of the plant and subjected to freeze-drying to obtain the crude extract. The crude extract was stored at 4 °C until use (Baba et al., 2015).

Animals

Female ICR mice (20-25 g) were used and housed under standard conditions (22 °C with 12 hours dark and 12 hours light) and were maintained on a standard pelleted feed and water *ad libitum*. Permission and approval for animal studies were obtained from the Institutional Animal Care and Use Committee (I-ACUC), International Islamic University Malaysia (I.D. number: IIUM/IACUC-2019(3)).

Parasite inoculation

The mice were given the same amount of inoculum, which is 1×10^7 *P. berghei* parasitized red blood cells. The inoculum was prepared by calculating the percentage of parasitemia and the red blood cell count of the donor mouse before diluting the blood with isotonic saline to get 1×10^7 *P. berghei* parasitized red blood cells in 0.1 ml solution. On day 0, each mouse was inoculated with 0.1 mL of infected blood intraperitoneally (i.p) using the prepared inoculum (Chung et al., 2009).

In vivo erythrocytic antimalarial assay

Aqueous extract of *D. esculentum* was freshly prepared daily with the desired dosage. 0.9% normal saline was prepared as the negative control. The positive control included two standard drugs, namely primaquine 12.6 mg/kg and chloroquine 20 mg/kg.

Experimental mice were inoculated with malaria parasite that was established in donor female ICR mice by the intraperitoneal administration of blood containing 1 x 10^7 *P. berghei* parasitized red blood cells (Chung et al., 2009). Two different treatment groups of malaria infections were set up as a preventive and curative group (Figure 1).

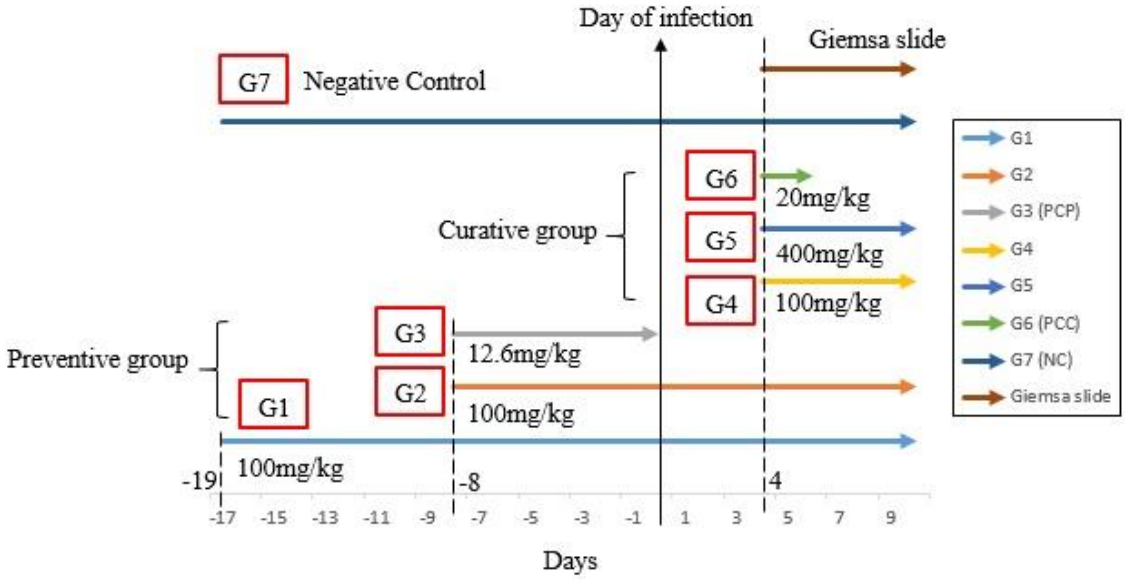

Figure 1 Study scheme of the study

In preventive treatment, two groups of mice (G1 and G2) were treated 19 days and eight days respectively before infection (D0) until the end of the experiment (D10). Blood smears were prepared from each mice (from the tail blood of each mouse) daily starting day 4 (D4) after infection and stained with Giemsa's stain. The mean survival day (in days) for each group was determined over 30 days.

In curative treatment, the mice (G4, G5 and G6) were treated on day 4 (D4) after infection, and the treatments were continued daily until day 10 (D10). On day 0 (D0), all mice were infected with 1 x 10^7 *P. berghei*. Four days after infection (D4), blood smears were prepared. The smears were then stained with Giemsa's stain to determine parasitized erythrocytes. The percentage of parasitemia and inhibition rate was calculated by the following formula:

% Parasitaemia = [No. of parasitized RBC/Total no. of RBC counted] × 100

% Inhibition rate = [Parasitemia of negative control – Parasitemia of treatment group/Parasitemia of negative control] × 100

The mean survival time (in days) for each group was determined over a period of 30 days. From that, the survival rate (%) was determined. The mice were weighed daily, and the status was observed daily until Day 10 survival post-infection.

2.6 Statistical analysis

Values are expressed as mean ± Standard deviation (S.D.) and was analyzed using SPSS version 25.0. Results were statistically analyzed using the Friedman Test and One-Way ANOVA. The significant difference between controls and treated groups were considered significant at value $p<0.05$.

RESULTS

Antimalarial assay

Daily body weight

Body mass of the mice for each treated group was weighed daily beginning day of infection until day 10. Fluctuations were seen in the weight but showed decreasing weight a few days before the end of the study. All mice had shown a loss of body weight beginning day four after the day of infection except for G11 (preventive group administered with 100 mg/kg extract 19 days before infection) that showed a stable pattern before decreasing starting day 6. However, mice for G6 (chloroquine group) had shown a steady pattern of body weight throughout the study.

Parasitemia and Inhibition rate

The preventive treatments showed preventive properties in *D. esculentum* aqueous extract. Table 1 shows the percentage of parasitemia from G1 (a group administered with 100 mg/kg dose extract) and G2 (a group administered with 100 mg/kg extract given on day -8) with the percentage of 2.67% and 4.78% on day ten respectively. Both groups, G1 and G2, showed the inhibition rate of 85.65% and 78.41% respectively. However, they were not comparable with G3 (primaquine group).

For curative treatments, Table 1 showed the percentage of parasitemia from G4 (a group administered with 100 mg/kg extract given on day 4) and G5 (a group administered with 400 mg/kg extract given on day 4) with the percentage of 1.83% and 3.23% respectively. The inhibition rate of both groups is 86.12% and 84.69% respectively. However, the plant showed no significant curative properties in infected mice.

Table 1 Table of result summarization for parasitemia rate and inhibition rate for day 10

Test	Group	% Parasitemia (mean ± S.D.)	% Inhibition rate (mean ± S.D.)	% Survival rate (mean ± S.D.)
Preventive	G1	2.67 ± 2.33*[1]	85.65 ± 13.59	100.00 ± 0.00
	G2	4.78 ± 3.11	78.41 ± 18.38	97.78 ± 5.44
	G3 (PCP)	1.70 ± 0.35	87.72 ± 5.29	98.89 ± 2.72
Curative	G4	1.83 ± 1.37*[2]	86.12 ± 13.90	97.78 ± 5.44
	G5	3.23 ± 1.01*[3]	84.69 ± 7.82	100.00 ± 0.00
	G6 (PCC)	0	100.00 ± 0.00	100.00 ± 0.00
Control	G7 (NC)	17.61 ± 8.81	0	99.45 ± 1.36

PCP = Positive Control Primaquine, PCC = Positive Control Chloroquine, NC = Negative Control. *[1]= significant when compared with G7, *[2] = significant when compared with G6, *[3] = significant when compared with G6.

Survival Rate

The mean of survival day was calculated throughout 30 days of observation from day -19 until day 10. Table 2 showed that mice in groups G1, G5 and G6 survived until the end of the study with a survival rate of 100% each. Meanwhile, mice in G2, G3, G4 and G7 survived until day 29 from the start of the

experiment (D-19) with a survival rate of 97.78%, 98.89%, 97.78% and 99.45%, respectively. However, there was no significant difference between each of the treated groups.

Table 2 Mean of survival day and survival rate for each of treated group [Day -19 until day 10]

Treated groups	Survival day (mean ± S.D.)	% Survival rate (mean ± S.D.)
G1	30 ± 0.00	100.00 ± 0.00
G2	29 ± 1.63	97.78 ± 5.44
G3	29 ± 0.82	98.89 ± 2.72
G4	29 ± 1.63	97.78 ± 5.44
G5	30 ± 0.00	100.00 ± 0.00
G6	30 ± 0.00	100.00 ± 0.00
G7	29 ± 0.41	99.45 ± 1.36

DISCUSSION

This study found that the bodyweight of mice decreased over time after the infection. It is proved that when the mice have an infection, they will experience loss of body mass during that period because of other symptoms (Birru et al., 2017; Davis, 2012; Ramli et al., 2014). This also might be due to the pathophysiologic changes of the mice towards the infection and the imbalance of the extract's protective effect (Birru et al., 2017).

From parasitemia level results on Day 10, G2 from the preventive treatment and G5 from the curative treatment showed higher parasitemia level than the other treated groups. These two groups showed the inability to control the rising of parasitemia level. Plasmodia species metabolize the haemoglobin and other RBC proteins for them to create hemozoin, a toxic pigment that would infect other RBCs. This leads to anaemia and splenomegaly, which then cause them to die (Herchline, 2019). In addition, G5 showing higher parasitemia level than G4 might be due to the higher dosage that may have exacerbated the infection as the extract may have immunosuppressive compounds. A study reported that the oral administration of phytochemicals like saponins, tannins and phenols can suppress cellular immunity (Sankari et al., 2010).

G1 and G4 had lower parasitemia level on Day 10 (D10) compared to G2 and G5 probably due to the accumulation of phytochemical constituents that may exert antimalarial action such as flavonoids, alkaloids and terpenoids as previously reported (Zannah et al., 2017). The antimalarial activity of this plant may be attributed to the phytochemical properties that have also been found to contribute to the antimalarial properties in many plants. Antimalarial activity of some plants is exhibited by either causing elevated red blood cell oxidation or by protein synthesis inhibition (Christian et al., 2012).

The inhibition rate is inversely correlated with parasitemia level. There was no parasite inhibition at all in the negative control group as the group did not receive any treatment to inhibit the parasite activity inside the mice. Both positive control groups, as in Table 1, G3 and G6 showed the highest inhibition rate towards the parasite at 87.72% and 100% rate respectively. It is because they received the commercial drugs that were scientifically proven to be able to inhibit the parasite in the mice. Several studies show the resistance of these drugs towards malaria parasite. A review on the ineffectiveness of primaquine stated that there are several reasons for the failure, including the use of many different daily dosages, inadequate dosage and the duration of therapy using primaquine (Fernando et al., 2011).

Survival rates showed that G1 survived better than G2 as G1 survived till Day 30 of the experiment, but no significant difference in survival rate was determined between G1 and G2. As supported by the parasitemia level result, it is suggested that the extract showed preventive properties, but it is not comparable with the positive and negative control. This is because there is no significant difference between both preventive groups with the controls. For the curative test, the result showed

that there is no significant difference in the survival rate between G4 and G5 even though G5 survived till Day 30 of the experiment compared to G4. This suggested that the plant extract has no potential to be used as a curative agent against *P. berghei* as both G4 and G5 are not statistically significant with both positive and negative control.

CONCLUSION

The study proved that the plant extract does have preventive properties but no curative properties. However, it could not be compared with the commercial antimalarial drugs. Hopefully, these findings may become a stepping stone in fundamental knowledge, and extensive research can be done to explore this plant for malaria treatment since there was no documented study on the antimalarial properties of Malaysian *D. esculentum*.

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