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Resveratrol Reduce the Severity of Anemia and Thrombocytopenia in *Plasmodium berghei* ANKA-Infected Mice

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Abstract

Background*: Malaria is an infectious disease with a high mortality rate. One of the complications of malaria is blood disorders. Hematological disorders such as anemia and thrombocytopenia are common in malaria infection. Resveratrol has been reported to have antimalarial activity.* ***Objective****: This study aimed to evaluate the effect of resveratrol in reducing the severity of anemia and thrombocytopenia in Plasmodum berghei-infected mice.* ***Methods****: The study began with parasite inoculation in mice. After the mice were infected, and randomly grouped into negative control, chloroquine 20 mg/Kg (positive control), and resveratrol with doses 25, 50, and 100 mg/Kg. The mice's blood profile was measured on day 0 and day 4 using a hematology analyzer.* ***Results****: The results showed that after administration of resveratrol at various doses, the number of RBC, hemoglobin, hematocrit and platelets was higher than the control.* ***Conclusion****: It can be concluded that resveratrol can reduce the severity of anemia and thrombocytopenia in mice infected with P. berghei.*

Keywords*: anemia, malaria, Plasmodum berghei, resveratrol, trombocytophenia*

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INTRODUCTION

The prevalence of malaria continues to increase every year. Based on the World Health Organization report in 2021, there were 274 million malaria cases in the world (World Health Organization, 2022). Indonesia is one of the endemic malaria areas, especially in the eastern part of Indonesia. Malaria is an infectious disease caused by *Plasmodium* sp. The *Plasmodium* life cycle occurs in mosquitoes and humans. The human body is divided into two phases: the exoerythrocytic (in the liver) and erythrocytic (in the red blood cells). Parasite invasion and multiplication in red blood cells (RBC) are responsible for the onset of symptoms and pathogenesis of malaria. In infected red blood cells (iRBC), modifications occur on the surface of the erythrocyte membrane so that parasite-infected erythrocytes will be easily attached, especially to the surrounding uninfected RBC (resetting), platelets and endothelial cells (cytoadherence) (Wickramasinghe & Abdalla, 2000). As a result of rosetting and cytoadherence, blood clots occur in the microvascular, which can cause multiorgan disorders such as the liver, spleen, kidney, and brain (Bhutani *et al*., 2020).

Hematological abnormalities such as anemia and thrombocytopenia are common in malaria patients. During malaria, infected red blood cells lyse and release merozoites to complete their life cycle by infecting new red blood cells. This cyclical process repeatedly occurs, causing a reduction in red blood cells and anemia. Infected red blood cells during lysis will release Hb derivative products such as hemin, methemoglobin (MetHb), and hemozoin which are malaria pigments. The release of these products can cause oxidative stress, which triggers oxidative damage to RBC (Balaji *et al*., 2020; Rifkind *et al*., 2015). The pathogenesis of thrombocytopenia is associated with blood clotting disorders, sequestration and cytoadherence in the spleen, destruction of platelets by macrophages, and oxidative stress (Wassmer *et al*., 2008).

Malaria infection needs to be treated quickly to prevent complications of severe malaria. Malaria treatment faces obstacles, including parasites resistant to the available antimalarials (Dondorp *et al*., 2009; Gil & Krishna, 2017). The search for new antimalarial compounds has been developed both from natural and synthetic origins, and medicinal plants are a potential source for investigating and developing new antimalarials.

Resveratrol is a flavonoid compound that is found in the skin and seeds of grapes, nuts, jackfruit, and mulberries. Resveratrol has biological activities such as antioxidant, anti-inflammatory, anticancer, neuroprotective, anticancer, and antimalarial (Galiniak *et al*., 2019; Moon & Sim, 2008). Based on the results of previous studies, resveratrol was reported to have antimalarial activity and can reduce the organ index liver, spleen, and kidney indices in mice infected with *P. berghei*, which is an indicator of inflammation (Hermanto *et al*., 2022). Resveratrol's activity as an antimalarial can inhibit parasite growth, inhibiting parasite invasion of red blood cells, which can cause hemolysis. Oxidative stress caused by malaria infection can trigger tissue damage, including hemolysis (Gomes *et al*., 2022). Resveratrol has antioxidant activity, allowing it to suppress free radicals caused by malaria infection to prevent hemolysis. Research on the effect of resveratrol on the blood profile of malaria-infected mice has not yet been studied. This study aimed to evaluate the effect of resveratrol in reducing malaria complications such as anemia and thrombocytopenia.

MATERIALS AND METHODS

Materials

The materials used in this study were resveratrol (Chengdu Biopurify Ltd.), chloroquine (Merck), anticoagulant (Inviclot®), Giemsa dye (Merck), methanol, carboxymethylcellulose (CMC), immersion oil.

Parasites and mice

The parasite culture used in this research was *P. berghei* ANKA obtained from the Malaria Laboratory, Faculty of Pharmacy, Universitas Jenderal Achmad Yani. The test animals used in this study were male Swiss Webster mice aged 7-8 weeks with a body weight of 20-35 g, obtained from PT Biomedical Technology Indonesia, Bogor. This research was conducted after receiving prior approval from the preclinical ethics committee of the Faculty of Pharmacy, Universitas Jenderal Achmad Yani, with number 8015/KEP-UNJANI/VI/2022.

Methods

The effect of resveratrol on the blood profile of mice infected with Plasmodium bergei was tested in February-November 2023. All mice were grouped into six groups: one normal group (uninfected) and five groups *P. berghei* infected-mice. The mouse was inoculated intraperitoneally with *P. berghei* ANKA as much as 200 µL, which contained $1x10^6$ parasites. After the mice were infected with a parasitemia level of

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2%, the mice were randomized and divided into several groups (n = 5). Control (Na CMC 0.5%), chloroquine 20 mg/Kg, resveratrol (25, 50, and 100 mg/Kg). Chloroquine and resveratrol are suspended in 0.5% sodium carboxy cellulose (Na CMC) and given orally. The dose approach used is based on previous research that can inhibit the growth of *Plasmodium* (Hermanto *et al*., 2022). Before being given the treatment (D0), all mice were examined for their blood profile (erythrocytes, hemoglobin, hematocrit, and platelets) by taking 200 µL of blood from the tails, measured using a hematology analyzer (Melet Schloesing®). The treatment was conducted for four days orally, and on the last day (D4), a blood test was carried out again.

RESULTS AND DISCUSSION

Hematological disorders such as anemia and thrombocytopenia are complications that often occur in malaria patients. This study evaluated resveratrol in reducing hematological disorders in *P. berghei* ANKA-infected mice with RBC, hemoglobin, hematocrit, and platelets. The findings on the number of RBC, hemoglobin, hematocrit, and platelets on day 0 in the control, chloroquine, and all resveratrol groups decreased compared to normal. This is because the mice in this group were infected with the parasite. The RBC profile of mice infected with *P. berghei* (Figure 1) showed that the control group had the lowest number of RBCs on day four compared to all the test groups. Meanwhile, the chloroquine and resveratrol groups had a higher RBC count than the control group. In the resveratrol group, the decrease in the number of red blood cells was not dose-dependent, especially at doses of 50 and 100 mg/Kg. However, the number of red blood cells was still higher than the control group, which correlated with the parasite density level. Previous research showed that resveratrol was reported to have antimalarial activity in *P. berghei*-infected mice with an effective dose 50 (ED_{50}) value of 27.25 mg/Kg (Hasugian *et al*., 2014), so resveratrol can suppress parasites, preventing invasion and multiplication of parasites in RBC cause reducing hemolysis.

The pathogenesis of anemia in malaria infection is multifactorial, including destroying iRBC, impaired RBC production in the bone marrow, and parasite phagocytosis in iRBC (Awoke & Arota, 2019). The spleen is an organ that plays a role in cleaning red blood cells. In malaria infection, changes in blood cleaning are carried out by spleen macrophages. iRBC cells will express marker compounds such as rhoptry-associated protein-2 (RSP-2) and phosphatidylserine (PS) on the surface of the RBC membrane, which will be recognized by lymph macrophages and will then cause phagocytosis. Anemia is caused by the significant number of infected red blood cells cleansed by the spleen, and the burden on the spleen in cleaning parasites is more remarkable, which might interfere with the spleen organ. This is in line with previous research that *P. berghei*-infected mice that were not given treatment showed an increase in the spleen organ index, widening of the white pulp and depletion of the red pulp, which are indicators of spleen damage, while mice treated with resveratrol showed a decrease in the spleen organ index and improvement in histology spleen (Hasugian *et al*., 2014; Intan *et al*., 2017).

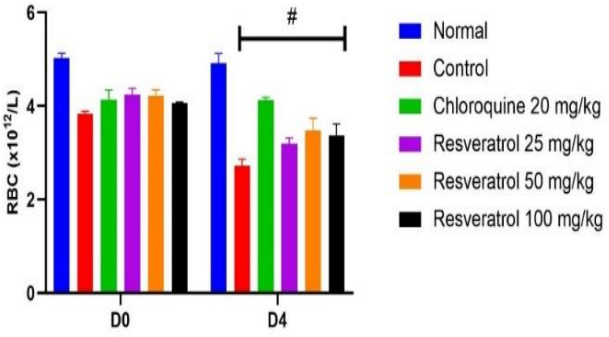

Figure 1. RBC profile of mice treated with resveratrol. The data shown are mean ± SEM. n= 5. *= $p < 0.05$ for the negative control group. #= $P<0.05$ for the standard group

The hemoglobin profile in *P. berghei*-infected mice after being treated with resveratrol can be seen in Figure 2. The hemoglobin level of the control group on day 4 showed the lowest compared to the entire test group. The chloroquine and resveratrol groups had higher hemoglobin than the controls. In the erythrocytic cycle, the parasite will use hemoglobin as a source of nutrition.

Hemoglobin will be degraded into heme and then biomineralized into hemozoin. Compounds resulting from the degradation of hemoglobin, such as heme, methemoglobin, and hemozoin, are free radicals that can cause damage to the RBC membrane and increase the occurrence of hemolysis (Balaji & Trivedi, 2012). Resveratrol's antioxidant action is suggested to prevent hemolysis.

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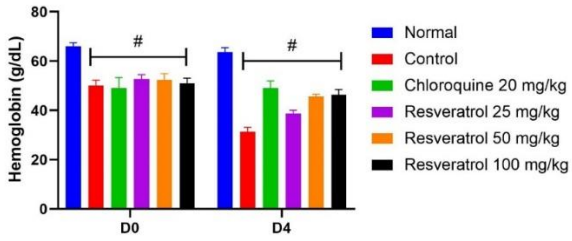

Figure 2. The hemoglobin profile of mice treated with resveratrol. The data shown are mean ± SEM. n= 5. *= p<0.05 for the negative control group. #= P<0.05 for the normal group

Hematocrit is the ratio of the number of red blood cells to whole blood. In malaria infection, the hematocrit will decrease. Changes in the hematocrit profile in *P. berghei*-infected mice are shown in Figure 3. In the control group, the number of hematocrites on day 4 decreased, while in the chloroquine and resveratrol groups, it was higher than the control group and not significantly different from the normal group. The decrease in the number of hematocrit correlated with the level of parasite density. In the erythrocytic phase, the parasite will multiply, resulting in many ruptured RBCs (Akinosoglou *et al*., 2012). Chloroquine and resveratrol have been shown to inhibit parasite growth, reducing the decrease in hematocrit. The findings in this study showed that resveratrol could reduce malaria complications in the form of anemia with higher RBC, hemoglobin, and hematocrit profiles than the control group.

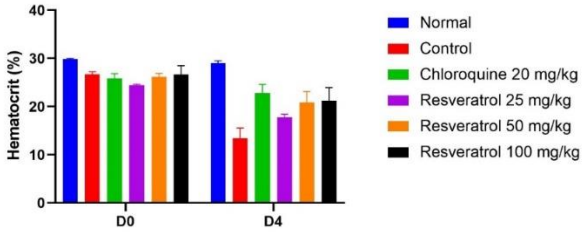

Figure 3. The hematocrit profile of mice treated with resveratrol. The data shown are mean ± SEM. n= 5. *= p<0.05 for the negative control group. #= P<0.05 for the normal group

A hematological disorder in severe malaria infection is thrombocytopenia, which is found to occur in around 50-80% of malaria cases. Figure 4 shows the blood profile of mice infected with *P. berghei* after resveratrol administration. The control group on day 4 showed the lowest platelet count compared to all test groups. In the chloroquine and resveratrol group, the decrease in platelets was not as common as in the control group. Decreased platelets are associated with an increase in the number of parasites. High levels of parasites can trigger an increase in pro-inflammatory cytokines such as TNF-α, INF-γ, and IL12.

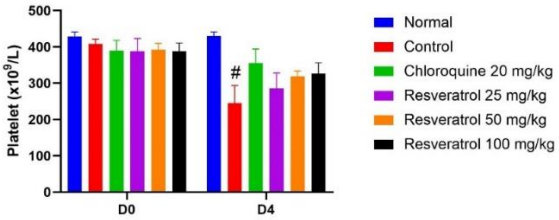

Figure 4. Platelet profile of mice treated with resveratrol. The data shown are mean ± SEM. n= 5. *= p<0.05 for the negative control group. #= P<0.05 for the normal group

This increase in pro-inflammatory cytokines impacts platelets trapped and used for inflammation in blood vessels. Besides that, the immune response increases macrophage activity to destroy platelets which can cause thrombocytopenia (Hasugian *et al*., 2015; Lee *et al*., 1997). Previous studies reported that on day 4, mice infected with *P. berghei* had lower parasitemia values than controls (Hermanto *et al*., 2022), and these results were consistent with their platelet profiles. In addition, resveratrol is also reported to have anti-inflammatory activity by suppressing IL-6, TNF-α, and INF-γ (Galiniak *et al*., 2019). The ability of resveratrol to suppress parasite growth and suppress pro-inflammatory cytokines proves that resveratrol can reduce malaria complications in the form of thrombocytopenia.

All blood parameters in mice infected with *P. berghei* after administration of resveratrol showed improvements in several factors that can cause this. Firstly, resveratrol can inhibit parasite growth, thereby preventing parasite invasion of red blood cells, which will cause hemolysis. Secondly, the antioxidant activity of resveratrol makes it possible to prevent tissue damage due to oxidative stress that occurs during the infection process. Thirdly, anti-inflammatory from resveratrol can prevent an increase in pro-inflammatory cytokines, which is one of the factors that worsens malaria. This study has limitations in not evaluating the effect of resveratrol on antioxidant activity and pro-inflammatory cytokines in mice infected with *Plasmodium*. So that in the future, it can be used as further research regarding the study of the mechanism of action of resveratrol in reducing blood disorders due to complications of malaria.

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CONCLUSION

This study investigated the effect of resveratrol in reducing the severity of anemia and thrombocytopenia. The results show that resveratrol can reduce the severity of anemia and thrombocytopenia by reducing parasitemia levels and antioxidant activity and suppressing pro-inflammatory cytokines. However, further research is still needed.

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AUTHOR CONTRIBUTIONS

Conceptualization, F. H.; Methodology, F. H.; Software, F. H., F. A. H.; Validation, F. H.; Formal Analysis, F. H.; Investigation, F. H., A. R., F. A. H.; Resources, F. H.; Data Curation, A. R., F. A. H.; Writing - Original Draft, F. H., A. R., F. A. H.; Writing - Review & Editing, F. H., F. A. H.; Visualization, F. H., F. A. H.; Supervision, F. H.; Project Administration, F. A. H.; Funding Acquisition, F. H.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

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