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Original Article

Evaluation of Experimental Cerebral Malaria of Curcumin and Kaempferol in *Plasmodium berghei* ANKA-Infected Mice

Maulana Yusuf Alkandahri[1,2]*, Afiat Berbudi[3], Anas Subarnas[1]

ABSTRACT

Background: Cerebral malaria (CM) is one of the most severe complications of *Plasmodium falciparum* infection and the leading cause of death from malaria in endemic areas. Natural products with antioxidant and anti-inflammatory activities have become valuable alternative therapeutic options in CM treatment. Therefore, this study aimed to investigate the neuroprotective effects of curcumin and kaempferol in experimental cerebral malaria (ECM) in mice infected with *Plasmodium berghei* ANKA (PbA). **Methods:** After PbA infection, mice were divided into 9 groups, namely Group I (negative control (NC)) with 0.5% HPMC, Group II received chloroquine 20 mg/kg, Group III (normal) with aquadest, Groups IV, V, and VI received curcumin at doses of 20, 40, and 80 mg/kg, respectively, Groups VII, VIII, and IX received kaempferol at doses of 20, 40, and 80 mg/kg, respectively. The antimalarial activity was evaluated using Peter's four-day suppressive test. This was conducted to determine the % parasitemia, survival rate, AST and ALT, blood-brain barrier (BBB) leakage, and neurobehavioral disorders in mice with CM. **Results:** The results showed that all treatments had significant antimalarial activity, with the % suppression depending on the dose. It also indicates that PbA-infected mice had a survival rate of 11-19 days after infection, which was higher than those in the NC group. This suggested that curcumin and kaempferol have a protective effect on the survival of PbA-infected mice. Furthermore, they significantly reduced the AST and ALT concentrations in the sample compared to the NC group. The same was observed in cerebral vessel extravasation, where the Evans Blue stain assay showed significantly less dye extravasation in the brains of PbA-infected mice treated with curcumin and kaempferol. This indicated better-protected integrity of the BBB. Additionally, the results also demonstrated a decrease in neurological disorders arising during ECM in the group treated with curcumin and kaempferol. **Conclusion:** Considering these results, it is concluded that treatments with curcumin and kaempferol could improve animal survival, prevent AST and ALT elevations, as well as protect the BBB and neurobehavioral disorders associated with CM in PbA-infected mice.

Key words: Kurkumin, Kaempferol, Cerebral malaria, *Plasmodium berghei* ANKA, Blood-brain barrier.

Maulana Yusuf Alkandahri[1,2]*, Afiat Berbudi[3], Anas Subarnas[1]

[1]*Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Padjadjaran, Jatinangor, West Java, INDONESIA.*

[2]*Faculty of Pharmacy, Universitas Buana Perjuangan Karawang, Karawang, West Java, INDONESIA.*

[3]*Department of Biomedical Sciences, Parasitology Division, Faculty of Medicine, Universitas Padjadjaran, Bandung, West Java, INDONESIA.*

Correspondence

Maulana Yusuf Alkandahri

Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Padjadjaran, Jatinangor, West Java, INDONESIA.

E-mail: alkandahri@gmail.com

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INTRODUCTION

Malaria remains a health problem worldwide and in Indonesia. There are 241 million cases worldwide, with more than 627,000 people dying yearly.[1] Most people with this disease are at risk for severe anemia or cerebral malaria (CM) which is a life-threatening complication of this infection, often occurring in non-immune individuals and people with failed standard antimalarial medication.[2] The effectiveness of malaria treatment has been hampered by the emergence of parasites resistant to almost all antimalarial drugs available over the years, including the artemisinin drug class.[3] The reduced efficacy of artemisinin-derived drugs on these parasites has recently been reported in parasitic isolates from Southeast Asia. This could compromise efficacy in the CM treatment, which is currently dependent on artesunate or quinine, and is an alarming sign as resistance spreads rapidly to other parts of the world.[4-5] The malaria treatment failure in uncomplicated cases will increase the number of patients susceptible to severe complications. Therefore, there is an urgent need to develop new antimalarial medicines and drug combinations or alternative strategies that prevent the development of drug resistance parasites, with the ultimate goal of reducing this disease burden.[5] Currently, several studies focused on exploring medicinal plants used by rural communities to prevent malaria.[6] They aimed to discover new antimalarial compounds with mild side effects and low toxicity, hence, do not harm the patient.[7] Several studies reported that more than 1200 medicinal plants have been used worldwide for infectious disease treatment, including malaria.[8] Finally, the active compounds from natural ingredients with antimalarial activity are curcumin and kaempferol.[9-11]

Curcumin (1,7-bis (4′hydroxy-3-methoxyphenyl) 1,6-heptadiene, 3,5-dione) is a phytochemical component discovered mainly in *Curcuma longa* and *Curcuma zanthorrhiza*. It is often used as a medicine and traditional herb to treat various diseases in several countries.[12] However, several studies demonstrated the beneficial effects of curcumin as an antimalarial agent. These include the ability to inhibit the histone acetyltransferase of *P. falciparum*, thereby causing damage to the parasite cell.[13] Curcumin can also interact with sarcoplasmic Ca^{2+}ATPase (SERCA) in *P. falciparum*, causing inhibition of a Ca^{2+} ion transporter called *PfATP6*.[14] It also inhibits glycogen synthase kinase-3β (GSK3β), which affects the pro-inflammatory cytokine production by preventing the transcriptional activity of NF-κB.[15] In addition to curcumin, several studies reported the antimalarial effect of kaempferol.[10] This (3,4' 5, 5,7-tetrahydroxyflavone) is a flavonoid-derived compound discovered in various plants and foods such as green tea, grapes, onions, and tomatoes.[16] It was reported to have a high antioxidant effect, inhibiting oxidative stress due to the reactive

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oxygen species (ROS) formation during malaria infection, which causes the degradation of hemoglobin.[17-18] Similarly, it also proved to be able to inhibit glycogen synthase kinase-3β (GSK3β) of the malaria parasite,[19-20] thereby preventing the activity of the recombinant protein on *P. falciparum* (PfGSK3β).[21] Despite several studies that described the protective effect of curcumin and kaempferol against malaria infection, there is no systematic study demonstrating the potential protective effect of these compounds in CM. Therefore, this study aimed to investigate the neuroprotective effects of curcumin and kaempferol in experimental cerebral malaria (ECM) in mice infected with *Plasmodium berghei* ANKA (PbA).

MATERIALS AND METHODS

Experimental animals and parasite

Male healthy Swiss albino mice, weighing 20-30 g and aged 6-8 weeks were used for the experiment. The animals were kept in plastic cages at room temperature and 12 h light and 12 h dark cycle, with free access to pellet food and water. The animals were acclimatized to laboratory condition for 1 week prior to the experiment. Chloroquine sensitive strain of *P. berghei* ANKA was used for the antimalarial test was obtained from the Malaria Laboratory, Faculty of Pharmacy, Universitas Jenderal Achmad Yani, Bandung, Indonesia. The donor mice were sacrificed, and blood was collected by cardiac puncture before 0.2 ml of blood suspension containing 1×10^6 *P. berghei* ANKA-infected RBC was inoculated intraperitoneally. The parasite was maintained by passage of blood from infected to non-infected mice on weekly basis.

Animal grouping and dosing

In all models, animals were randomly and equally divided into nine groups, including normal, negative, positive, and six test groups, each consisting of four animals. Group I (negative control) received carrier (10 ml/kg 0.5% HPMC), Group II (positive control) received 20 mg/kg of chloroquine, Group III (normal) was given aquadest, which represents normal conditions, Group IV, V, and VI, received curcumin (catalog no. RC08501, HPLC: ≥99%) at doses of 20, 40, and 80 mg/kg, respectively, Groups VII, VIII, and IX were given kaempferol (catalog no. RC035103, HPLC: ≥99%) at doses of 20, 40, and 80 mg/kg, respectively, purchased from Andalas Sitawa Fitolab Company, Padang, Indonesia.

Determination of antimalarial activity (four-day suppressive test)

The Peter's four-day suppressive test was employed to test the chemo-suppressive activity of the kurkumin and kaempferol against mice infected with chloroquine sensitive *P. berghei* ANKA.[22] A total of 32 mice were used to test the antimalarial activity of curcumin and kaempferol, which were infected with PbA on the first day (day 0). Two hours post-infection, mice were randomly distributed into eight groups and treated as described in the animal grouping and dosing section. The treatment was conducted daily for four consecutive days.

Determination of parasitemia

Blood was collected by cutting the tip of the tail on days 1, 2, 3, and 4, smeared on a slide (thin blood smear), and then fixed with absolute methanol for 10 seconds. After fixation, the slides were dried and stained with 10% Giemsa for 15 min, rinsed with running water, and dried at room temperature. Parasite-infected red blood cells were quantified using a microscope with oil immersion at 100x magnification. The following formula is used to determine the parasitemia percentage:

$$\%\ \text{Parasitemia} = \frac{\text{number of parasitized RBC}}{\text{total number of RBC}} x\ 100\%$$

The percentage of suppression of the parasite was calculated via the following formula:

$$\%\ \text{Suppression} = \frac{\text{mean \% parasitemia of negative group} - \text{mean \% parasitemia of treated group}}{\text{mean \% parasitemia of negative group}} x\ 100\%$$

Measurement of mean survival time (MST)

In all *in vivo* antimalarial experiments, mortality was monitored, and the number of days from the time of infection up to death was then recorded for each mouse for 30 days. The mean survival time (MST) was calculated according to the following formula:

$$\text{MST} = \frac{\text{Sum of survival time (days) of all mice in a group}}{\text{Total number of mice in that group}}$$

Serum biochemical analysis

At 96 hours post-infection, mice in each group were sacrificed with ketamine-xylazine. Blood samples were collected using an EDTA tube and centrifuged at 3000 rpm for 20 minutes. Subsequently, the serum was transferred to an Eppendorf tube for biochemical analysis. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were quantified in mice serum using commercially available kits (Catalog no. GOT (ASAT) IFCC mod. liquiUV and GPT (ALAT) IFCC mod. liquiUV, PT. Wacana Indo Mitra, Jakarta, Indonesia) according to the manufacturer's instructions.

Experimental cerebral malaria (ECM)

PbA-infected red blood cells (iRBCs) were used in all experiments to inoculate mice. A total od 32 Swiss albino mice weighing 20-30 g were infected intra-peritoneally (i.p) 1×10^6 iRBCs (high dose), obtained from those that had been infected intra-peritoneally (i.p) with a stock solution. Afterward, samples were randomly distributed into nine groups and treated as described in the animal group and dose section. Treatment was administered daily for four consecutive days after mice experienced ECM symptoms or parasitemia levels > 20%.

Clinical assessment by rapid murine coma and behavioral scale

For clinical evaluation of neurological symptoms, malaria-infected mice were assessed daily for ECM symptoms. ECM development was scored according to the following symptoms according to the previously described Rapid Murine Coma and Behavioral Scale (RMCBS): 0 = without symptoms, 1 = ruffled fur, 2 = hunching, 3 = wobbly gait, 4 = limb paralysis, 5 = convulsions, 6 = coma.[23] ECM was reported in mice that died or showed symptoms of severe neurological disease, defined as a RMCBS >4.

Evans blue (EB) extravasation

We injected mice intravenously (i.v) with 4 ml/kg BW of 2% Evans Blue (Sigma) as soon as the RMCBS >4 was indicating severe neurological impairment (ECM positive mice). Mice were sacrifced 5 h after injection and brains were weighed and placed in 5 ml of dimethylformamide (Merck) at 37°C for 48 h to extract EB dye from the tissue. Absorbance was measured at λ= 620 nm (Thermo Fisher Scientific, USA) as previously described.[24]

Ethical approval

This study was approved by the research ethics committee, Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia (No.: 267/UN.6.KEP/EC/2021).

Statistical analysis

The results are shown as mean±S.E.M with $p<0.05$ was considered statistically significant. The mean differences of the measured parameters were compared by one-way analysis of variance (ANOVA) using GraphPad Prism version 8, followed by the Tukey HSD post-hoc test.

RESULTS AND DISCUSSION

Evaluation of the antimalarial activity of curcumin and kaempferol (four-day suppressive test)

The curcumin and kaempferol administration resulted in a significant dose-dependent chemosuppressive effect ($p<0.05$) on PbA (Table 1). The suppression percentage of curcumin at 20, 40, and 80 mg/kg were 42.25%, 58.02%, and 69.21%, respectively. Meanwhile, it was 39.25%, 54.26%, and 59.25% for kaempferol at 20, 40, and 80 mg/kg, respectively. The standard drug (chloroquine 20 mg/kg) showed a significant chemosuppressive effect ($p<0.05$) with 99.00% suppression, which was higher than suppression in animals treated with curcumin and kaempferol. Furthermore, the effectiveness of these compounds in mice at all doses correlated with an increase in the mean survival time (MST) compared to animals in the negative control group, as shown in Table 1. Figure 1 indicates that the administration at all doses showed a longer survival rate than animals in the negative group. The MST of the treatment group with the standard drug (chloroquine 20 mg/kg) also increased significantly ($p<0.05$) compared to negative control. Finally, all mice in the negative control group died on day 13.

Table 1: Effect of curcumin and kaempferol on % parasitemia, % suppression, and survival time of *P. berghei* ANKA-infected mice in the four-day suppressive test.

Groups	% Parasitemia	% Suppression	MST (day)
NC	14.65 ± 0.15	0	11.75 ± 1.11
CQ	0.16 ± 0.01**	99.00	27.00 ± 0.91**
Normal	0	0	30(+) ± 0.00**
Cur 20	8.46 ± 0.47**	42.25	12.50 ± 0.96
Cur 40	6.15 ± 0.16**	58.02	13.75 ± 1.03
Cur 80	4.51 ± 0.29**	69.21	16.25 ± 1.49**
Kaemp 20	8.90 ± 0.19**	39.25	12.50 ± 1.04
Kaemp 40	6.70 ± 0.24**	54.26	13.00 ± 1.41
Kaemp 80	5.97 ± 0.06**	59.25	14.00 ± 1.91

The data are presented as mean±SEM; n = 4 per group. ** shows $p<0.05$ compared to the negative control group. NC = negative control; CQ = chloroquine 20 mg/kg BW; Cur 20 = curcumin 20 mg/kg BW; Cur 40 = curcumin 40 mg/kg BW; Cur 80 = curcumin 80 mg/kg BW; Kaemp 20 = kaempferol 20 mg/kg BW; Kaemp 40 = kaempferol 40 mg/kg BW; Kaemp 80 = kaempferol 80 mg/kg BW; + = maximum days of follow up; MST = mean survival time.

Table 2: Effect of curcumin and kaempferol on % parasitemia and survival time of *P. berghei* ANKA-infected mice in experimental cerebral malaria.

Groups	% Parasitemia	MST (day)
NC	28.03 ± 0.38	5.75 ± 0.48
CQ	4.40 ± 0.27**	24.00 ± 1.29**
Normal	0	30(+) ± 0.00**
Cur 20	25.14 ± 0.10	6.25 ± 0.75
Cur 40	23.86 ± 0.27**	6.75 ± 0.48
Cur 80	20.96 ± 0.28**	7.75 ± 0.63
Kaemp 20	26.00 ± 0.11	6.00 ± 0.57
Kaemp 40	24.62 ± 0.13**	6.50 ± 0.86
Kaemp 80	22.12 ± 0.27**	7.25 ± 0.75

The data are presented as mean±SEM; n = 4 per group. ** shows $p<0.05$ compared to the negative control group. NC = negative control; CQ = chloroquine 20 mg/kg BW; Cur 20 = curcumin 20 mg/kg BW; Cur 40 = curcumin 40 mg/kg BW; Cur 80 = curcumin 80 mg/kg BW; Kaemp 20 = kaempferol 20 mg/kg BW; Kaemp 40 = kaempferol 40 mg/kg BW; Kaemp 80 = kaempferol 80 mg/kg BW; + = maximum days of follow up; MST = mean survival time.

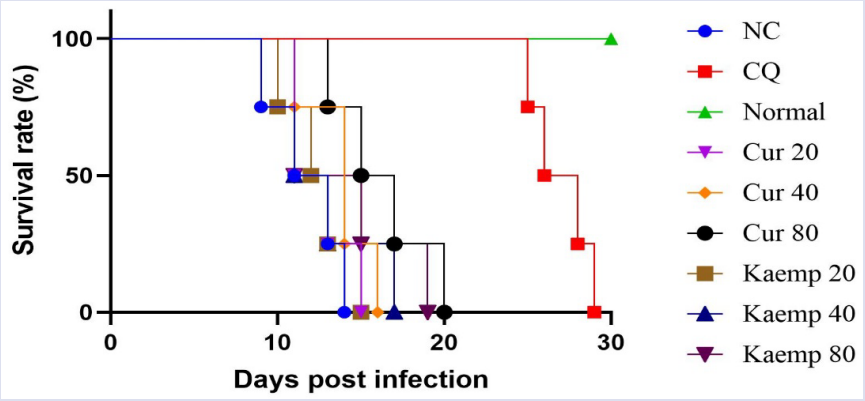

Figure 1: Survival rate after curcumin and kaempferol administration in *P. berghei* ANKA-infected mice (n = 4 per group). NC = negative control; CQ = chloroquine 20 mg/kg BW; Cur 20 = curcumin 20 mg/kg BW; Cur 40 = curcumin 40 mg/kg BW; Cur 80 = curcumin 80 mg/kg BW; Kaemp 20 = kaempferol 20 mg/kg BW; Kaemp 40 = kaempferol 40 mg/kg BW; Kaemp 80 = kaempferol 80 mg/kg BW.

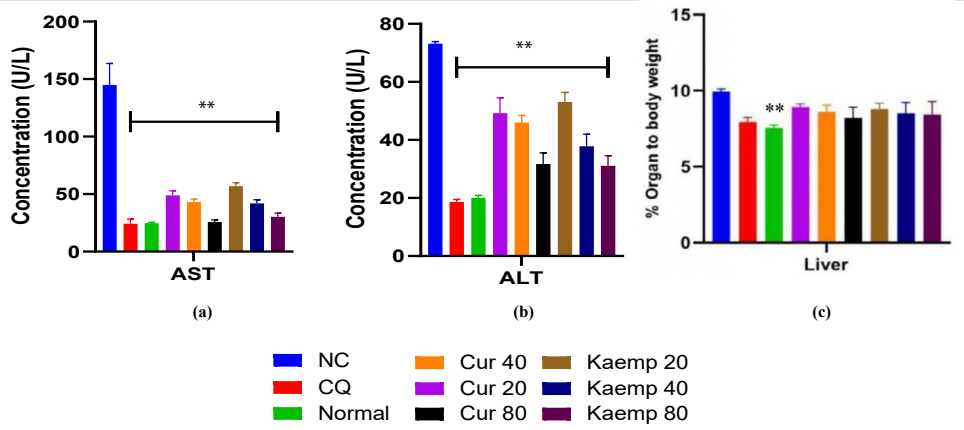

Figure 2: a) Alanine aminotransferase (AST); **b**) Aspartate aminotransferase (ALT) and **c)** Liver-body mass ratio after curcumin and kaempferol administration in *P. berghei* ANKA-infected mice (n = 4 per group). The data are presented as mean±SEM. ** shows $p<0.05$ compared to the negative control group. NC = negative control; CQ = chloroquine 20 mg/kg BW; Cur 20 = curcumin 20 mg/kg BW; Cur 40 = curcumin 40 mg/kg BW; Cur 80 = curcumin 80 mg/kg BW; Kaemp 20 = kaempferol 20 mg/kg BW; Kaemp 40 = kaempferol 40 mg/kg BW; Kaemp 80 = kaempferol 80 mg/kg BW.

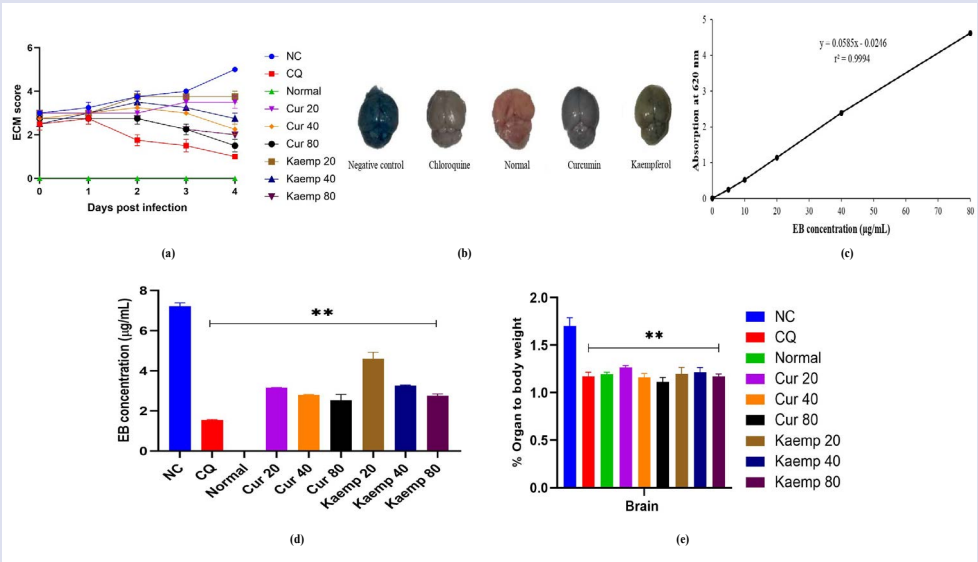

Figure 3: Administration of curcumin and kaempferol protects mice against ECM. **a)** ECM score in each treatment groups; **b)** EB staining of the brains of ECM animals; **c)** Calibration of EB dye concentration using UV-Vis spectrophotometer ($r^2 = 0.9994$); **d)** Quantification of dye extravasation into the brain by measuring the absorbance of brain tissue at 620 nm; **e)** Effect of administration of curcumin and kaempferol on brain-body mass ratio of mice infected with PbA. The data are presented as mean±SEM; n = 4 per group. ** shows $p<0.05$ compared to the negative control group. NC = negative control; CQ = chloroquine 20 mg/kg BW; Cur 20 = curcumin 20 mg/kg BW; Cur 40 = curcumin 40 mg/kg BW; Cur 80 = curcumin 80 mg/kg BW; Kaemp 20 = kaempferol 20 mg/kg BW; Kaemp 40 = kaempferol 40 mg/kg BW; Kaemp 80 = kaempferol 80 mg/kg BW.

Administration of curcumin and kaempferol prevented an increase in serum biochemistry in the liver of mice infected with *P. berghei* ANKA.

The effect of curcumin and kaempferol on liver function was observed by determining the AST and ALT levels in the treated mice group compared to the negative control group. All treatment groups showed lower AST and ALT levels than the negative control ($p<0.05$) (Figure 2a and 2b). Meanwhile, all treatment groups showed an increased liver-body mass ratio (Figure 2c).

Administration of curcumin and kaempferol suppressed the ECM in *P. berghei* ANKA-infected mice

Swiss albino mice were infected with PbA to evaluate the effect of curcumin and kaempferol in preventing ECM. It was observed that curcumin and kaempferol resulted in a dose-dependent reduction of parasitemia compared to the negative control. The parasitemia percentage of curcumin treated groups was 25.14%, 23.86, and 20.96% for the 20, 40, and 80 mg/kg, respectively. Meanwhile, it was 26.00%, 24.62%, and 22.12% for kaempferol at 20, 40, and 80 mg/kg, respectively, while that of the CQ treated group was 4.40%. Concerning the MST, the results showed that all doses of curcumin and kaempferol, and CQ were able to prolong MST as compared to the negative control (Table 2). The signs of ECM were observed between days 1 to 4 post-infection (parasitemia >20%). All mice in the negative control showed neurological signs that increased daily, such as ruffled fur, hunching, wobbly gait, limb paralysis, and convulsions. However, in the standard drug group (chloroquine 20 mg/kg), curcumin and kaempferol decreased these symptoms daily (Figure 3a). Neurological signs in ECM condition are also accompanied by damage to the blood-brain barrier (BBB). To assess the effects of curcumin and kaempferol on BBB integrity, EB dye extravasation was measured in the brains of PbA-

infected mice. The amount detected increased linearly using UV-Vis spectrophotometry as indicated by the calibration curve (r^2 = 0.9994), as shown in Figure 3c, enabling precise quantitation of EB deposition in the ECM mouse brain. At 4 days post-infection, the brains in negative control group showed remarkable extravasation compared to those given standard drug (chloroquine 20 mg/kg), such as curcumin and kaempferol at all doses, as presented in Figures 3b and 3d. This is in line with the brain-body mass ratio of ECM mice. Administration of standard drugs (chloroquine 20 mg/kg) did not show an increase in the ECM brain-body mass ratio (p<0.05) compared to the negative control, as shown in Figure 3e.

Malaria infection is known to disrupt the BBB by two main processes. First, brain vessels are blocked by parasitic erythrocytes, thereby causing local hypoxia and intense oxidative stress.[25] Second, it can also stimulate an inflammatory response in the brain, leading to the overproduction of pro-inflammatory cytokines and reactive oxygen species (ROS).[26-27] These events are followed by endothelial cell degradation leading to a breakdown of the BBB, as demonstrated by bleeding in the cerebrum of CM patients and the extravasation of dyes or antibodies into the brain parenchyma in the ECM.[28] Damage to the BBB is often associated with behavioral dysfunction in the central nervous system (CNS).[29-30] Several studies showed that malaria infection triggers long-term neurological deficits such as neurobehavioral disorders, motor skills, visual acuity, and seizures.[31-32] This study demonstrated that the administration of curcumin and kaempferol could suppress the development of ECM in mice by reducing the breakdown of the BBB and the neurobehavioral disorders associated with the disease. Several studies also reported strong antioxidant and anti-inflammatory activities that can produce a protective effect in the brains of PbA-infected mice.[33-36] Furthermore, the antioxidant effect of curcumin and kaempferol is due to their ability to inhibit the formation of ROS during malaria infection,[37-38] reduce serum malondialdehyde (MDA) levels, and increase the activity of superoxide dismutase (SOD) as well as glutathione peroxidase (GPx).[39-40] The anti-inflammatory effect produced by these compounds is due to their ability to inhibit pro-inflammatory cytokines such as interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), and interleukins (IL-1β, IL-6, IL-8, and IL-10) as a response to inflammation due to malaria infection.[41-42]

Malaria infection also causes hepatomegaly,[43] hence, it triggers elevated levels of AST and ALT as markers of liver damage due to infection.[44] The negative control group observed high levels of AST and ALT and an increased liver mass ratio of mice. Meanwhile, the administration of curcumin and kaempferol can be a remedy for these abnormalities. This may be due to the strong hepatoprotective properties of the compounds.[45-46] This study indicated that curcumin and kaempferol can minimize the harmful effects of ECM in the brains of PbA-infected mice. Also, their administration may be a potentially valuable adjuvant therapy for CM treatment in humans.

CONCLUSION

This study showed that the administration of curcumin and kaempferol provided good antimalarial activity in PbA-infected mice. These results also demonstrate these compounds' significant and potent neuroprotective effect on the ECM model in protecting the BBB and neurobehavioral disorders associated with CM. Furthermore, it showed that the application can prevent the increase in AST and ALT due to malaria infection. This study confirmed the potential use of herbal medicines as an effective approach to cerebral malaria treatment.

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CONFLICTS OF INTEREST

Authors declare no conflict of interest.

REFERENCES

1. World Health Organization. World malaria report 2021. World Health Organization, Geneva, Switzerland, 2021.
2. Schmidt KE, Kuepper JM, Schumak B, Alferink J, Hofmann A, Howland SW, et al. Doxycycline inhibits experimental cerebral malaria by reducing inflammatory immune reactions and tissue-degrading mediators. PLoS ONE. 2018;13(2):1-20.
3. Owoloye A, Olufemi M, Idowu ET, Oyebola KM. Prevalence of potential mediators of artemisinin resistance in African isolates of *Plasmodium falciparum*. Malar J. 2021;20(1):1-12.
4. Dondorp AM, Fanello CI, Hendriksen ICE, Gomes E, Seni A, Chhaganlal KD, et al. Artesunate versus quinine in the treatment of severe falciparum malaria in African children (AQUAMAT): an open-label, randomised trial. Lancet. 2010;376:1647-57.
5. Mimche PN, Taramelli D, Vivas L. The plant-based immunomodulator curcumin as a potential candidate for the development of an adjunctive therapy for cerebral malaria. Malaria J. 2011;10(Suppl 1):1-9.
6. Prakash BN, Unnikrishnan PM. Ethnomedical survey of herbs for the management of malaria in Karnataka, India. Ethnobot Res App. 2013;11:289-98.
7. Alkandahri MY, Berbudi A, Utami NV, Subarnas A. Antimalarial activity of extract and fractions of *Castanopsis costata* (Blume) A.DC. Avicenna J Phytomed. 2019;9(5):474-81.
8. Willcox ML, Bodeker G. Traditional herbal medicines for malaria. British Med J. 2004;329(7475):1156-9.
9. Alkandahri MY, Patala R, Berbudi A, Subarnas A. Antimalarial activity of curcumin and kaempferol using structure-based drug design method. J Adv Pharm Educ Res. 2021;11(4):86-90.
10. Alkandahri MY, Berbudi A, Subarnas A. Active compounds and antimalaria properties of some medicinal plants in Indonesia - A Review. Sys Rev Pharm. 2018;9(1):64-69.
11. Somsak V, Damkaew A, Onrak P. Antimalarial activity of kaempferol and its combination with chloroquine in *Plasmodium berghei* infection in mice. J Pathog. 2018;2018:1-7.
12. Alkandahri MY, Yuniarsih N, Berbudi A, Subarnas A. Antimalaria activities of several active compounds from medicinal plants. Pharmacogn J. 2022;14(1):245-52.
13. Cui L, Miao J. Cytotoxic effect of curcumin on malaria parasite *Plasmodium falciparum*: inhibition of histone acetylation and generation of reactive oxygen species. Antimicrob Agents Chemother. 2007;51:488-94.
14. Ji HF, Shen L. Interactions of curcumin with the PfATP6 model and the implications for its antimalarial mechanism. Bioorg Med Chem Lett. 2009;19:2453-55.
15. Ali AH, Sudi S, Basir R, Embi N, Sidek HM. The antimalarial effect of curcumin is mediated by the inhibition of glycogen synthase kinase-3β. J Med Food. 2017;20(2):152-61.
16. Calderón-Montaño JM, Burgos-Morón E, Pérez-Guerrero C, LópezLázaro MA. Review on the dietary flavonoid kaempferol. Mini Rev Med Chem. 2011;11(4):298-344.
17. Wallqvist A, Fang X, Tewari SG, Ye P, Reifman J. Metabolic host responses to malarial infection during the intraerythrocytic developmental cycle. BMC Syst Biol. 2016;10(1):1-18.
18. Tripathy S, Roy S. Redox sensing and signaling by malaria parasite in vertebrate host. J Basic Microbiol. 2015;55(9):1053-63.
19. Wang H, Kumar A, Lamont RJ, Scott D. GSK3-beta and the control of infectious bacterial diseases. Trends Microbiol. 2014;22(4):208-17.

20. Wong SK, Jann MLS, Sudi S, Hassan WRM, Chin LP, Embi N, et al. Antimalarial and antiinflammatory effects of Gynura procumbens are mediated by kaempferol via inhibition of glycogen synthase kinase-3β (GSK3β). Sains Malaysiana. 2015;44(10):1489-500.
21. Droucheau E, Primot A, Thomas V, Mattei D, Knockaert M, Richardson C, et al. *Plasmodium falciparum* glycogen synthase kinase-3: molecular model, expression, intracellular localisation and selective inhibitors. Biochim Biophys Acta. 2004;1697(1-2):181-96.
22. Peters W, Portus JH, Robinson BL. The chemotherapy of rodent malaria. XXII. The value of drug-resistant strains of *Plasmodium berghei* in screening for blood schizonticidal activity. Ann Trop Med Parasitol. 1975;69:155-71.
23. Amante FH, Stanley AC, Randall LM, Zhou Y, Haque A, McSweeney K, et al. A role for natural regulatory T cells in the pathogenesis of experimental cerebral malaria. Am J Pathol. 2007;171(2):548-59.
24. van der Heyde HC, Bauer P, Sun G, Chang WL, Yin L, Fuseler J, et al. Assessing vascular permeability during experimental cerebral malaria by a radiolabeled monoclonal antibody technique. Infect Immun. 2001;69(5):3460-65.
25. Gun SY, Claser C, Teo TH, Howland SW, Poh CM, Chye RRY, et al. Interferon regulatory factor 1 is essential for pathogenic CD8+ T cell migration and retention in the brain during experimental cerebral malaria. Cell Microbiol. 2018;20(5):1-15.
26. Dunst J, Kamena F, Matuschewski K. Cytokines and chemokines in cerebral malaria pathogenesis. Front Cell Infect Microbiol. 2017;7:1-16.
27. Kariuki SN, Marin-Menendez A, Introini V, Ravenhill BJ, Lin YC, Macharia A, et al. Red blood cell tension protects against severe malaria in the Dantu blood group. Nature. 2020;585(7826):579-83.
28. Jensen AR, Adams Y, Hviid L. Cerebral *Plasmodium falciparum* malaria: The role of PfEMP1 in its pathogenesis and immunity, and PfEMP1-based vaccines to prevent it. Immunol Rev. 2020;293(1):230-52.
29. Guha SK, Sarkar I, Patgaonkar M, Banerjee S, Mukhopadhyay S, Sharma S, et al. A history of juvenile mild malaria exacerbates chronic stress-evoked anxiety-like behavior, neuroinflammation, and decline of adult hippocampal neurogenesis in mice. J Neuroimmunol. 2020;348:1-14.
30. Christensen SS, Eslick GD. Cerebral malaria as a risk fator for the development of epilepsy and other long-term neurological conditions: a meta-analysis. Trans R Soc Trop Med Hyg. 2015;109(4):233-38.
31. Oluwayemi IO, Brown BJ, Oyedeji OA, Oluwayemi MA. Neurological sequelae in survivors of cerebral malaria. Pan Afr Med J. 2013;15:1-9.
32. Song X, Wei W, Cheng W, Zhu H, Wang W, Dong H, Li J. Cerebral malaria induced by Plasmodium falciparum: Clinical features, pathogenesis, diagnosis, and treatment. Front Cell Infect Microbiol. 2022;12:1-14.
33. Alabdali A, Kzar M, Chinnappan S, Mogana R, Khalivulla SI, Rahman H, et al. Antioxidant activity of curcumin. Res J Pharm Technol. 2021;14(12):6741-46.
34. Wang J, Fang X, Ge L, Cao F, Zhao L, Wang Z, et al. Antitumor, antioxidant and anti-inflammatory activities of kaempferol and its corresponding glycosides and the enzymatic preparation of kaempferol. PLoS One. 2018;13(5):1-12.
35. Boroumand N, Samarghandian S, Hashemy SI. Immunomodulatory, anti-inflammatory, and antioxidant effects of curcumin. J Herbmed Pharmacol. 2018;7(4):211-19.
36. Ren J, Lu Y, Qian Y, Chen B, Wu T, Ji G. Recent progress regarding kaempferol for the treatment of various diseases. Exp Ther Med. 2019;18(4):2759-76.
37. Wu X, Huang L, Zhou X, Liu J. Curcumin protects cardiomyopathy damage through inhibiting the production of reactive oxygen species in type 2 diabetic mice. Biochem Biophys Res Commun. 2020;530(1):15-21.
38. Somsak V, Damkaew A, Onrak P. Antimalarial activity of kaempferol and its combination with chloroquine in *Plasmodium berghei* infection in mice. J Pathog. 2018;2018:1-7.
39. Jakubczyk K, Drużga A, Katarzyna J, Skonieczna-Żydecka K. Antioxidant potential of curcumin - A meta-analysis of randomized clinical trials. Antioxidants (Basel). 2020;9(11):1-13.
40. Kouhestani S, Jafari A, Babaei P. Kaempferol attenuates cognitive deficit *via* regulating oxidative stress and neuroinflammation in an ovariectomized rat model of sporadic dementia. Neural Regen Res. 2018;13(10):1827-32.
41. Lee SY, Cho SS, Li Y, Bae CS, Park KM, Park DH. Anti-inflammatory effect of *Curcuma longa* and *Allium hookeri* co-treatment via NF-κB and COX-2 pathways. Sci Rep. 2020;10(1):1-11.
42. Alam W, Khan H, Shah MA, Cauli O, Saso L. Kaempferol as a dietary anti-inflammatory agent: Current therapeutic standing. Molecules. 2020;25(18):1-12.
43. Scaccabarozzi D, Deroost K, Corbett Y, Lays N, Corsetto P, Salè FO, et al. Differential induction of malaria liver pathology in mice infected with *Plasmodium chabaudi* AS or *Plasmodium berghei* NK65. Malar J. 2018;17(1):1-9.
44. Khairani S, Fauziah N, Wiraswati HL, Panigoro R, Salleh A, Setyowati EY, et al. Piperine enhances the antimalarial activity of curcumin in *Plasmodium berghei* ANKA-infected mice: A novel approach for malaria prophylaxis. Evid Based Complement Alternat Med. 2022;2022:1-11.
45. Farzaei MH, Zobeiri M, Parvizi F, El-Senduny FF, Marmouzi I, Coy-Barrera E, et al. Curcumin in liver diseases: A systematic review of the cellular mechanisms of oxidative stress and clinical perspective. Nutrients. 2018;10(7):1-28.
46. Zang Y, Zhang D, Yu C, Jin C, Igarashi K. Antioxidant and hepatoprotective activity of kaempferol 3-*O*-β-d- (2,6-di-*O*-α-l-rhamnopyranosyl)galactopyronoside against carbon tetrachloride-induced liver injury in mice. Food Sci Biotechnol. 2017;26(4):1071-76.

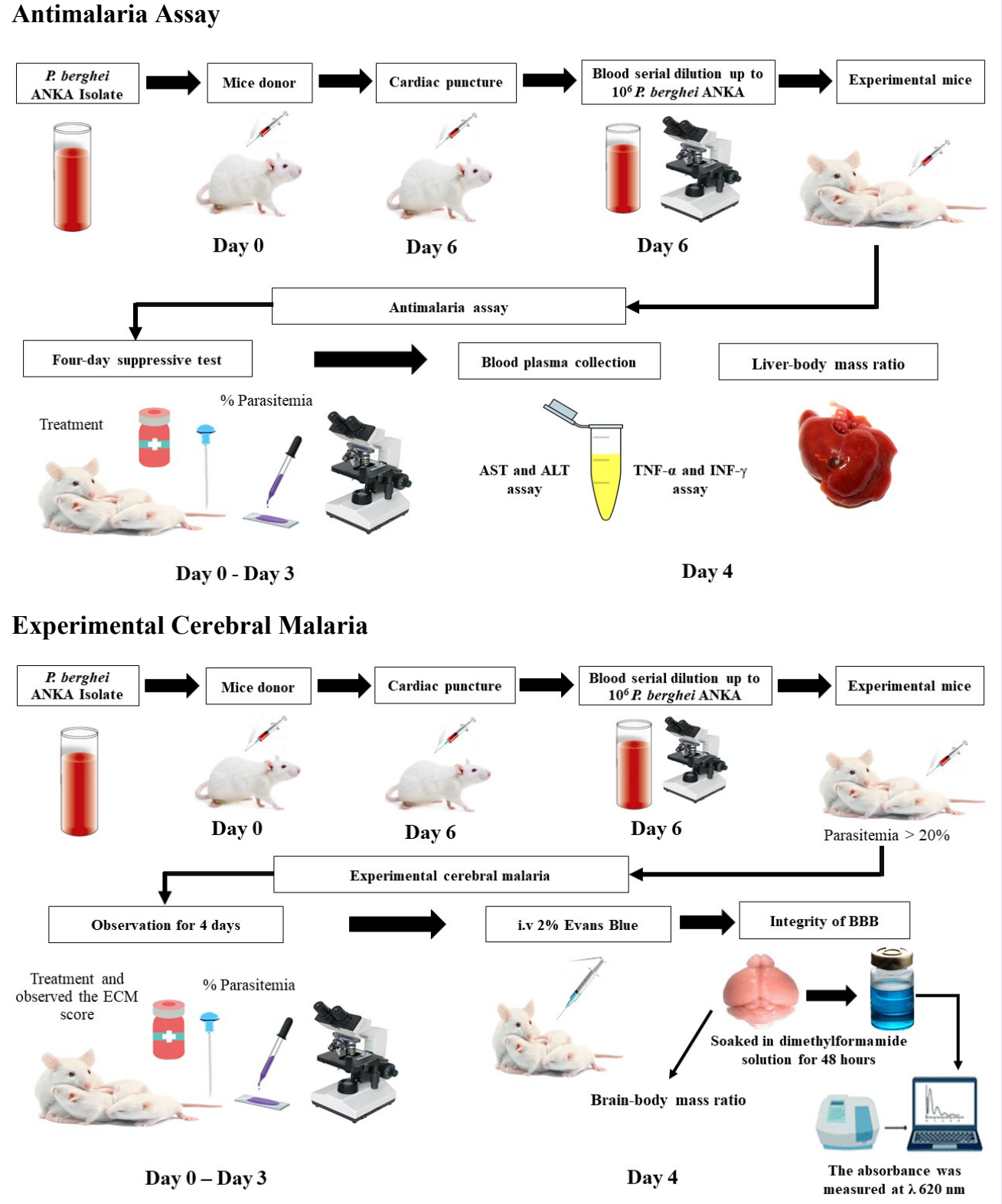
ABOUT AUTHORS

Maulana Yusuf Alkandahri: A Doctoral student at the Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Padjadjaran, Indonesia. He is also a lecturer at the Faculty of Pharmacy, Universitas Buana Perjuangan Karawang, Indonesia.

Afiat Berbudi: A lecturer at the Department of Biomedical Sciences, Parasitology Division, Faculty of Medicine, Universitas Padjadjaran, Indonesia.

Anas Subarnas: A Professor at the Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Padjadjaran, Indonesia.

GRAPHICAL ABSTRACT



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