JTL 11 (1) JUNE 2022
Page: 11-17

Contents list available at Jurnal Teknologi Laboratorium

JURNAL TEKNOLOGI LABORATORIUM

Journal Homepage: www.teknolabjournal.com
ISSN 2580-0191(Online) I ISSN 2338 – 5634(Print)

Original Research

OPEN ACCESS

Prophylactic effects of Kaempferia Galanga against Plasmodium berghei: in vivo study

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Abstract: Recently, medicinal plants have been the main source in treating malaria. *Kaempferia galanga* was investigated as an antioxidant, and anti-inflammation. In vivo study of *K. galanga* had been verified as antimalarial for severe malaria. The objective of this study is to investigate the chemoprophylactic effect, body composition, and blood parameters of *K. galanga* against *Plasmodium berghei*. The prophylactic effects were determined by employing *K. galanga* (50; 100 and 200 mg/kg) in mice infected with *P. berghei*. Mice were subdivided into 4 groups (control negative and 3 treatment groups). The treatment groups received *K. galanga* daily for 3 days before the inoculation of *P. berghei.* Each mice were inoculated with the infected blood containing 10^6 of *P. berghei*. Parasitaemia and body weight were calculated every day until 5 days post-inoculation, and the blood parameters was monitored in day 5. The parasitaemia on the fifth days after inoculation revealed a significant suppresses effect compared to control ($p<0.05$). The suppressed effect by doses 50 mg/kg; 100 mg/kg; 200 mg/kg and negative control were 0.83%; 1.96%; 2.82%, and 17.8% respectively. The body weight on treatment groups was normal, but the control group decreased ($p<0.05$). The blood parameters on treatment groups were normal compared to control group. The *K. galanga* possess the prophylactic effect, normal weight and blood parameters against *Plasmodium berghei*.

Keywords: *Kaempferia galanga*; Malaria; In vivo; Prophylactic.

INTRODUCTION

Malaria is an essential parasitic disease with high morbidity and mortality in the world. The absence of an effective vaccine against malaria and the resistance of the antimalarial agent require a new agent to protect the human from this pathogen. In fact that malaria is a deadly disease, however, these diseases can be prevented.[1] Malaria causes organ dysfunction such as liver, lung, and brain.[2] One of the dangerous sign of malaria is anaemia, hypoglycaemia detected from the blood test.[3,4] These conditions impact cognitive impairment in children and productivity in adults.[5] People who are most at risk are those who live in the malaria-endemic area. This condition creates people in the endemic area to have a habit to consume herbs.[6] One of the wide herbs employed is from Zingiberaceae family.[7]

Kaempferia galanga is a family of *Zingiberaceae* widely distributed in tropical regions.[8] These plants are also identified as kencur, sand ginger, aromatic ginger, and resurrection lily. *Kaempferia galanga* is used not only as medicinal plants but also for cooking.[9,10,11] In Indonesia, these plants are employed as prophylactic from many diseases because these plants are believed has a prophylactic effect.[12] Several studies have revealed the active compounds of *K. galanga* containing alkaloids, saponin, tannin, flavonoids, terpenoids, phytosterols, phenols, and essential oils. [7,10] Based on the previous study, those compounds

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DOI: 10.29238/teknolabjournal.v10i1.283
Received 16 September 2021; Received in revised form 01 January 2022; 30 June 2022

own beneficial effects as an antioxidant.[13] The antioxidant effect of kencur tubers is primarily obtained from flavonoid and phenolic compounds.[14,15] The phenolic compound of *K. galanga* possessed moderate antioxidant activity and less toxicity.[13] *Kaempferia galanga* tubers also own anti-inflammatory effects.[16] The anti-inflammatory effects of kencur tubers are by suppressing the progression of acute and chronic inflammation by inhibiting neutrophil cell infiltration.[17] *Kaempferia galanga* was acknowledged as an antimalarial activity, but there was no report of prophylactic effect. Based on the above background, the objective of this study is to investigate the prophylactic effect of *K. galanga* for malaria prevention.

MATERIAL AND METHOD

Plant collection and extract preparation

The *K. galanga* tubers were obtained from Gunung Kidul Yogyakarta. The tubers were air-dried at room temperature and powdered. One hundred grams of *K. galanga* was macerated in 1000 ml of 80% ethanol for 72 hours and then filtered. The filtrate was concentrated with a rotary evaporator to dryness. The residue was stored in a desiccator until it was used.

Ethical consideration

The protocol and experimental procedures employed in this study were confirmed with the Ethical Commission, Faculty of Medicine, Universitas Islam Indonesia with number 35/Ka.Kom.Et/70/KE/V/2018.

The parasite and treatment

Plasmodium berghei in this study was procured from the Faculty of Medicine, Universitas Islam Indonesia. Male Swiss mice weighing 20-30 g administered in this study were acclimatize for a week before treatment. The animal was kept in a standard laboratory with temperature 4±7^{o}C; humidity 70±5% with 12 hour light/dark cycles. Foods for mice were provided daily and water supplied ad libitum. Treatment mice were infected with 10^6 parasitized erythrocytes intra peritoneal. The day of infection was defined as days D1, D2, D3, D4, and D5.

Experimental design

Experimental groups were divided in accordance with wether they were control or treatment groups**.** Mice were divided into five groups of 4 mice for each group. The groups were as follows:

Group 1 (negative control): received solution solvent only
Groups 2 (treatment): ethanol extract of *K. galanga* 50 mg kg^{-1}
Groups 3(treatment): ethanol extract of *K. galanga* 100 mg kg^{-1}
Groups 4 (treatment): ethanol extract of *K. galanga* 200 mg kg^{-1}

Four animals per group were housed together in a cage with food, and water ad libitum. Food, water, and weight gain were monitored every day at 09.00 am. Treatment mice were treated orally by oral cannular to intra gastric (i.g). A ball-tipped, 18-gauge gavage needle was attached to a 1 cc syringe. Prophylactic treatment was performed once a day for 3 days. Four mice in each group were provided with an oral dose of 50, 100, and 200 mg kg^{-1} of *K. galanga* and observed for the mortality for 5 days. After the prophylactic treatment, mice were injected by 10^6 of *P. berghei* intra peritoneal.

Each mice was inoculated with 0.2 µl intra peritoneal which contain 10^6 *P. berghei* from donor mice which possess 30% parasitemia. Parasitemia was calculated by Giemsa stained thin blood smears from the mice's tail until day 5 (D5). The percentage of parasitemia was determined by calculating the number of parasitized red blood cells out of 1000 erythrocyte in random microscopic fields.

Full blood count was applied to identify the influence of treatment on malaria and its co-morbidities. The blood count was obtained from a cardiac puncture on day 6 after the re-passage of *P. berghei*. The blood count analysis found hemoglobin, hematocrit, and white cell count (WBC).

Statistical analysis

Data were presented as mean plus standard deviation of the mean. The analysis was performed by statistical analysis administering Kruskal's Wallis, followed by Mann Whitney. The $p < 0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION

The prophylactic effect of *Kaempferia galanga*

Most of the people believe that traditional herbs could prevent infectious diseases. The result of this study revealed that local traditional herbs can indeed prevent people from the *Plasmodium* sp. infection. This study supports other research which local herbs own a potential effect as a prophylactic against *Plasmodium* infection.[18] The lack of local herbs consumption evidence in the community is the absence of standard dose. The herbal preparation is based on experience and drunk ad libitum. This practice induces adverse effect due to overdose or accumulation of herbal ingredient in several organs such as kidney.[18]

This study revealed that pretreatment of mice with *K. galanga* for 3 days, delayed the establishment of parasitemia compared to control (Figure 1). On day 3 after *P. berghei* injection, average parasitemia of mice pretreated with *K. galanga* doses 50 mg kg^{-1} was 0.03%, while the group 100 mg kg^{-1}, and 200 mg kg^{-1} were 0.06% and 0.65%, and the control group was 10.15%. The lowest decrease of parasitemia was discovered in 50 mg kg^{-1} group among groups and control groups. The similar result has been discovered until the fifth day after induction

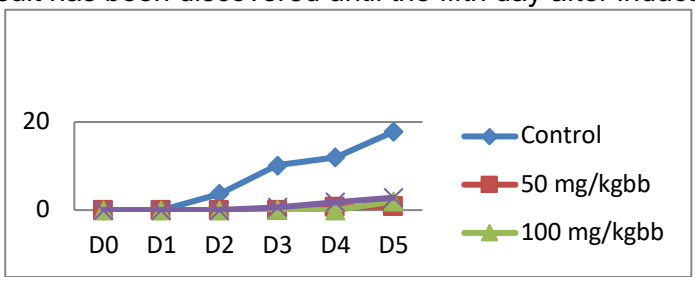

Figure 1. The prophylactic effect of *K. galanga* among groups and control

Based on Table 1 and Figure 1, five days after the *Plasmodium* injection, the average of parasitemia in the treatment groups 50 mg kg^{-1}, 100 mg kg^{-1}, and 200 mg kg^{-1} was 0.83%, 1.96%, and 2.82% respectively. The prophylactic effect was good in the treatment group with a dose of 50 mg kg^{-1} compared to other treatment doses. The parasitemia rate of treatments groups was lower and significantly different compared to the control groups (p=0.47). The prophylactic effect evidence is able to reduce parasitemia about 15% of the negative control group.

This study presents that *K. galanga* may delay the parasitemia growth until 5 days after the injection of *P. berghei* compared to control, indicating the chemoprophylactic effects. Based on the resulting study displayed, it is implied that ethanol extract of *K. galanga* had a good potential prophylactic effect. The result of this study is also supported by other studies which discovered that traditional plants have an effect as prophylactic against *Plasmodium*.[19,18] Several in vitro and in vivo studies had also investigated the antiplasmodial activity of the *K. galanga's* family.[20]

The best prophylactic effect was discovered in low concentration, whereas at the high dose, the parasitemia was not lower than low and middle concentration. Another study exhibited that the best prophylactic effect was identified at the highest concentration.[18] However, other studies had presented that the best prophylactic effect was noticed at low doses.[19] This study consisted of other studies that *K. galanga* had a chemo-prophylactic effect on human cells.[10]

Kaempferia galanga effect to hematological profile

The hematological parameter of hemoglobin in all treatment groups was normal compared to the control group. Table 1 presents that at all dosage of *K. galanga* extract, the hemoglobin, white blood cells were normal compared to the control group. All treatment groups show a normal value of those parameters than control groups. The 100 mg kg^{-1} group displays the highest value on the parameters of hemoglobin (13.1 g/dl), hematocrit (45.23 %), and the lowest white blood cell count (5.33 mmk), implying that this dose has better result than other doses (50 mg kg^{-1} and 200 mg kg^{-1}). The lowest dosage and highest dosage possess an almost similar parameter in blood count hemoglobin, hematocrit, and white blood cell. This result demonstrates that both in the low and high dosage groups, there was no different effect in a blood test.

Table 1. Parasitemia and hematological profile of *K. galanga* on infected mice

Information	Groups (mg kg^{-1})			
	Control	50	100	200
Parasitemia (%)	17.8±4.38	0.83±0.61	1.96±0.61	2.82±2.94
Hemoglobin (g/dl)	9.6±3.25	11.97±1.00	13.1±0.78	11.97±1.55
Hematocrit (%)	32.05±7.0	40.1±4.54	45.23±2.46	40.1±6.03
WBC (mmk)	18.205±3.57	13.09±7.04	5.33±2.19*	13.092±5.39

Kaempferia galanga contains ethyl-methoxycinnamate which is a derivative of cinnamic acid. Cinnamic acid is considered to have many benefits, such as an antimalarial. The antimalarial mechanism of cinnamic acid inhibits the ATP production in the parasite.[21] That information might explain the normal hemoglobin in the treatment group, compared to the control group. When the production of ATP Plasmodium decreased, it also reduced the Plasmodium ability to degrade the hemoglobin. Thus, the result of our study discovered that the ethanol extract of *K. galanga* revealed the ability to prevent anemia. In treatment groups, the Hb concentrations were normal than the control groups. In the control group, it was shown the Hb levels < 10 mg/dl indicating that anemia occurred. *Kaempferia galanga* contains phenolic compounds and flavonoids, understood as their antioxidant properties.[22,14,23] The phenolic compound of *K. galanga* possesses moderate and high antioxidant activity.[24,14] Typically, as antioxidants, flavonoids may reduce oxidative stress or increase the antioxidant capacity,[23], and radical scavenging.[24] Although the total phenolic level and radical scavenging activity were not as high as other Zingiberaceae family.[23] Phenolic compounds have also been widely acknowledged as an antimalarial.[25] Antimalarial activity of phenol compounds is accurately inhibiting heme polymerization.[26]

Our study revealed that the WBC parameter was normal in treatment groups compared to the control group. This result confirmed the previous study that *K. galanga* did not change the blood parameters.[27] The anti-inflammatory effects of *K. galanga* tubers were performed by suppressing the progression of acute and chronic inflammation which is by inhibiting neutrophil cell infiltration.[28] The anti-inflammatory effect of *K. galanga* caused by ethyl-methoxycinnamate was conducted by inhibiting the pro-inflammatory cytokines TNFα, IL-1, and also by inhibiting cyclooxygenase.[22,29,30] Research by [31] discovered that *K. galanga* tubers have anti-inflammatory benefits.

Kaempferia galanga effect to body weight

Bodyweight in all treatment groups increased every day (Figure 2). Control mice group owned significant decreased water and food intake as well as body weight until day 5 post-*Plasmodium* inoculation compared to treatment groups ($p<0.05$). *Plasmodium sp*. infection may cause weight loss both in human or animal models.[19,4] This study presented that weight loss appeared at 2 days after *Plasmodium* injection, but in the following day, the weight of treatment groups increased within the normal range. Bodyweight in the control group proved a

continuous decline until the end of the observation. The infected mice which are not treated indicated low food and water intake as well as negative weight gain.

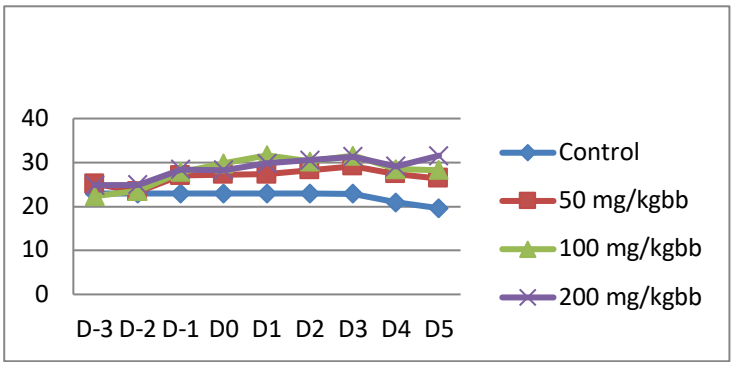

Figure 2. Bodyweight among groups

The result of this study is similar to the other study[19] and weight gain is better than other studies.[32] *Plasmodium* infection is contributing to the clinical manifestation of weight loss by producing IL 1 from the neutrophil.[30] This result occurred in the following control group but did not occur in the treatment group. *Kaempferia galanga* containing ethyl-methoxycinnamate suppresses the production of IL 1. This result also indicates that *K. galanga* is good received orally and possesses high tolerated dose.[33]

CONCLUSION

Kaempferia galanga demonstrates a positive effect on *Plasmodium* infection by suppressing the parasitaemia progression, maintaining body composition and normal blood parameters. It is indicated that *K. galanga* are save and good as a prophylactic candidate agent for malaria.

ACKNOWLEDGEMENT

The acknowledgement was given to Parasitology laboratory, Fakultas Kedokteran Universitas Islam Indonesia.

FUNDING INFORMATION

This study was funded by Unit Penelitian dan Pengabdian Masyarakat Fakultas Kedokteran Universitas Islam Indonesia.

DATA AVAILABILITY STATEMENT

The utilized data to contribute to this investigation are available from the corresponding author on reasonable request.

DISCLOSURE STATEMENT

The views and opinions expressed in this article are those of the authors and do not necessarily reflect the official policy or position of any affiliated agency of the authors. The data is the result of the author's research and has never been published in other journals.

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