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Antiplasmodial activity of *Kaempferia galanga* extract against *Plasmodium berghei* infection in mice

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Abstract. Malaria is still one of the deadliest diseases worldwide. Efficient treatment is by artemisinin and its derivatives. Anti-malarial traditional remedies still offer new tracks for identifying promising anti plasmodial molecules. *Kaempferia galanga* was one of ethno medicinal plant The ethanolic extract were assessed for antimalarial activity at 25 and 50 mg/kg. In vivo, anti-plasmodial efficacy was assessed by 7-day suppressive test in malaria murine models of cerebral malaria (*Plasmodium berghei*). Chloroquine (25 mg/kg) was used as positive control. Parasitized red blood cells were enumerated by giemsa staining microscopic methods. Hypothermia, macroscopic organ of liver, spleen and brain were evaluated **.** In vivo, for both oral administration of ethanolic crude extract *K. galanga* (25 and 50 mg/kg) induce minimal inhibition of the parasite growth. The extract caused 1.13%, 2.69% inhibition of parasitaemia at 25, 50 mg/kg body weight respectively while chloroquine cleared the parasitemia 83.29%. The haematological parameters of *K. galanga* prevent decrease ($p<0.05$) in HGB, RBC, PCV value compared to untreated group, and the extract was also prevent the increase of WBC count in treatment groups ($p > 0,05$) The results indicate that the ethanolic extract has minimal potent anti-plasmodia activity against *P. berghei* and necessitates further scientific validation to evaluate its potential antimalarial agents

Keywords: Antimalarial, *Plasmodium berghei*, *Kaemferia galanga*, parasitemia

1 Introduction

Malaria is one of the endemic diseases that causes death in worldwide. There are 5 species of *Plasmodium sp*. and the most dangerous was *Plasmodium falciparum* [1]. Malaria is widespread especially in tropic country such as South-East Asia Region with more than 1.4 million cases and 557 deaths of the population [2]. A study showed that malaria morbidity is higher in Asia and the mortality is high especially in children aged less than 5 years [3]. At this time mosquito vector control to insecticide was not effective because of the increasing reports of resistance to massif insecticide [4,5]. Artemisinin Combination Therapy (ACTs) as the first line drug were also resistant in some country [6,7]. This strongly suggest the need for urgent research into new antimalarials.

Medicinal plants have been complementary used for treatment of any kind of diseases include malaria especially in developing country such as Indonesia. *Kaempferia galanga* (*K. galanga*) in Indonesia are consumed as food supplements. The plant is a monocothyledonous herb from Zingiberaceae and known for its medicinal useful [8]. This plant are rich in antibacterial [9], antiinflamatory [10] and antioxidant component [11,12]. Investigation of these plants in treatment malaria is scarce. The objective of this study is to evaluate the effectiveness of *K. galanga* as anti-plasmodial agent, and haematological parameters in mice infected with *P. berghei*.

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2 Methodology

2.1 Plant collection and extract preparation

Fresh *K. galang*a were obtained from the Wonosari, Gunungkidul, Yogyakarta Farm. The plant materials were air dried at room temperature and then grounden into powder. The 2185 g of extracts was weighed and soaked in 2 Liter ethanol for one week. The mixture was then filtered using Whatman paper. The residues were discarded, and the filtrate was collected and concentrated using rotary evaporator.

2.2 Infection of mice

Twenty-five male Swiss mice (20-30 g) aged 6-8 weeks were purchased from the Department of Physiology, Faculty of medicine Universitas Islam Indonesia. These mice were acclimatized for one week in cages with husk for bedding materials. They were fed with corn and water ad libitum and with 12:12 light day and night. Permission and animal approval were certified by the ethics committee, Faculty of Medicine Universitas Islam Indonesia. Cardiac blood sample from the donor mouse with percentage of parasitemia of 57.9 % was used. The blood sample was dilute with PBS as much 200 µl ($2x10^6$) *P. berghei* infected erythrocytes and inoculated intraperitoneally into each mice groups.

2.3 Determination of percentage of parasitemia

The percentage of parasitemia was count from the tail of infected mice. Thin smears were prepared on slides. The slides were allowed to dry and then fixed with methanol then stained with 10% giemsa for 30 minutes. The percentage of parasitemia were calculated after microscopic examination of giemsa staining. The % parasitemia was calculated using the formula

$$\%\,parasitemia = \frac{\inf.RBC}{1000RBC}(100) \qquad (1)$$

$$\%inhibition = \frac{\%MPUG - \%MPTG}{\%MPUG}(100) \qquad (2)$$

Where MPUG is mean parasitemia in untreated group and MPTG is mean parasitemia in treatment group.

2.4 Experimental design and treatment of mice

Twenty-five infected mice were randomly divided into five groups (two experimental and three control groups (Table 1). The mice in experimental groups were treated with 25 and 50 mg/kg/body weight for the extract for one week. Each mice was inoculated with *P. berghei* intraperitoneally $2x10^6$ parasite/µl. The donor mice was prepared with parasitemia > 50 %. After the percentage parasitemia of treatment groups were raised until 30-35%, mice begin to receive treatment with constant check of the percentage parasitemia every day. Chloroquine (25 mg/kg/weight body was used as positive control and CMC Na 0,5% as negative control. The extract dosage was prepared by dissolving in CMC Na 0,5% in aquadest. Drugs and treatment were given orally started on day 0. After 8 days of treatment, the mice were stopped receiving treatment.

Table 1. Grouping of animal and treatment.

Groups	Treatments
Control	No infection, no treatment
A	Infected with *P. berghei* and treated with *K. galanga* 25 mg/kg weight body
B	Infected with *P. berghei* and treated with *K. galanga* 50 mg/kg weight body
C	Infected with *P. berghei* and treated with chloroquine (positive control)
D	Infected with *P. berghei* and treated with CMC Na 0.5% (negative control).

2.5 Haematological parameter analysis

Twenty-four hour after the last dose on the 8th day of infection, the animals were sacrificed with Ketamin. Blood samples were collected by heart puncture. The blood samples for haematological parameters red blood cell (RBC) count, white blood cell count (WBC), platelet count, packed cell volume (PCV), and haemoglobin (Hb) were collected into EDTA tubes and analyzed using an automated machine.

2.6 Statistical analysis

Percentage parasitemia of the treated and control groups was compared using one-way ANOVA and two-tailed student's t-test, with $p < 0.05$ considered significant.

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3 Results

3.1. Estimation of percentage of parasitemia.

Table 2 showed the mean percentage of parasitemia in control, treatment with *K. galanga* and treated with chloroquine. In addition, Figure 1 showed the mean percentage of parasitemia in both groups in 7 days. However, the results of anti-plasmodial activity in this study showed that the extract were not significantly ($p > 0.05$) reduce the *P. berghei* clearance compared to negative control on seven days of treatment (Tabel 2; Figure 1). Overall, Table 2 presents the percentage inhibition in *P. berghei* infected mice in both groups. However, there is no significant difference ($p > 0.05$) when *K. galanga* treatment groups compared to positive control group. The treatments groups after infection *P. berghei* recorded mortality after 5 days of treatments.

The percentage of parasitemia ethanol extract dose 50 mg/kg weight body were higher than the 25 mg/kg body weight, but this study were showed that those dose of *K. galanga* were not dependent in reduce mean parasitemia. Parasitemia reduction was observed in chloroquine group, it were started from day 2 to day 3 and clearance was 100% in day 4.

Table 2. Suppresive effect to *K. galanga* against *P. berghei*.

Groups	Mean % Parasitemia	Mean % inhibition
Control	0	0
A	46.17 ± 13.15^{a2}	1.13
B	45.44 ± 10.7^{a2}	2.69
C	7.48 ± 13.7^{a1}	83.29
D	46.7 ± 13.7^{b1}	-

Parasitemia expressed as mean ± sd, n = 5. Where a=as compared to negative control; b=as compared to positive control; 1=$p<0.05$; 2=$p>0.05$

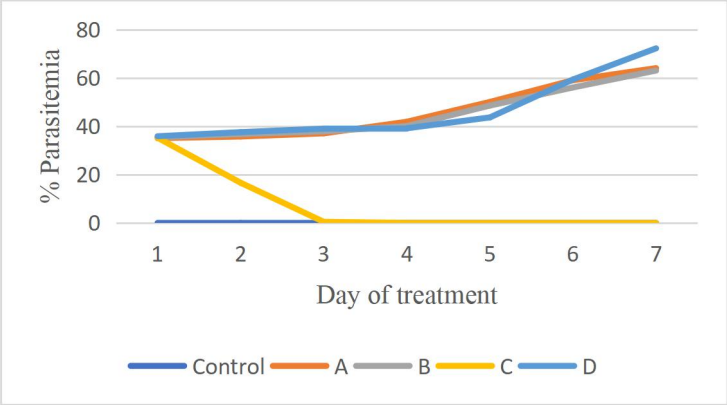

Fig. 1. The percentage of parasitemia of crude extract of *K. galanga* against *P. berghei*.

Control: No infection, no treatment; A: Infected with *P. berghei* and treated with *K. galanga* 25 mg/kg weight body; B: Infected with *P. berghei* and treated with *K. galanga* 50 mg/kg weight body; C: Infected with *P. berghei* and treated with chloroquine (positive control); D: Infected with *P. berghei* and treated with CmCNa 0.5% (negative control).

3.2. Haematological parameter analysis

Haematological parameters of *P. berghei* infected mice groups showed in Table 3. *K. galanga* 25 mg/kg group recorded the lowest WBC count of (26.95±0.46) compared to 50 mg/kg and the untreated group showed the higher WBC count (53.48±16.4). The HGB and RBC count in untreated control group was the lowest while the chloroquine showed highest count in two parameters followed by the *K. galanga* groups. Haematocrit in *K. galanga* 50 mg/kg group highest compared to untreated and *K. galanga* 25 mg/kg group. Neither the extract nor the chloroquine significantly prevent the reduce of platelet, RBC, HMT, and HGB as compared to the untreated control group.

Table 3. Haematological parameters in *P. berghei* treated with *K. galanga*.

Groups	Control	A	B	C	D
WBC ($x10^9$/l)	$12.6 \pm 0.42^*$	$43.21 \pm 12.0^*$	$26.95 \pm 0.46^*$	$12.45 \pm 0.07^*$	53.48 ± 16.4
PCV (%)	$46.5 \pm 1.69^*$	18.8 ± 0.35	$22.0 \pm 0.46^*$	$46.6 \pm 0.14^*$	14.9 ± 0.3
HGB(g/dl)	$14.85 \pm 0.35^*$	4.75 ± 0.21	$6.0 \pm 0.42^*$	$14.7 \pm 0.28^*$	3.2 ± 0.42
RBC ($x10^{12}$/L)	$8.8 \pm 0.02^*$	2.2 ± 0.46	$4.6 \pm 0.06^*$	$8.64 \pm 0.1^*$	1.35 ± 0.4
PLT($x10^3$/µL)	542.5 ± 24.7	602 ± 151.2	514 ± 50.9	550 ± 14.1	82.12 ± 52.01

WBC: White blood cells; PCV: packed cell volume, HGB: haemoglobin; RBC: red blood cells (erythrocytes count); PLT: Platelets count

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4 Discussion

Antimalarial activity of many medicinal plants have been reported [21,22,27,28]. *Kaempferia galanga* are reported to have antimicrobe [13,14], anti-inflamatory effect [10,15], antihelminth activity [16,17], antineoplastic [11], antituberculosis [9], mosquito repellent, larvicidal activity [18,19], also have weak anti-oxidant activity [11,12], that it caused by the extract method [20].

Anti-plasmodial effect of *K. galanga* in this study were tested using *P. berghei*. This research were assessed the percent inhibition of parasitemia, and alteration effect on haematological parameters that assessed after treatment. The percentage parasitemia of the ethanol extract groups were not significantly different compared to negative control in seven-day suppressive test models. The parasitemia clearance and percentage inhibition of *K. galanga* was very low during treatment. Other study showed that parasite clearance can be low in first week but higher in the last week of treatment [21], so it needs further investigation.

These result showed that the plant had less antimalarial activity and also less in suppress multiplication of severe infection of *P. berghei* in mice. This less activity may be caused by the dose, so its needs loading dose to optimize or higher dose as report [22]. Current study showed that an extract has an anti-plasmodial effect if the percent suppression of parasitemia was 30 % or more [23]. Anti-plasmodial activity of plant extract can be divided into 3 groups (very good, good and moderate) if the extract have 50% parasitemia clearance at 100, 250 and 500 mg/kg weight body [24].

Anti-plasmodial activities of plants were depend on the presence of bioactive secondary compounds in material plants. Some research showed the phytochemical screening of ethanolic extract of *K. galanga* were contain of alkaloids, saponins, glycocides, phenols, terphenoids, quinone and sterol, flavonoids [11], and tannins [25,26]. Active compounds such as alkaloids, have been reported as anti-plasmodial [27]. Anti-plasmodial screening of phytochemical compounds have been shown to be caused of alkaloid, tannin, flavonoid, saponins [28]. Those compounds have been suggested act by reduce the oxidative damage that caused by *Plasmodium sp*. [29]. The minimum anti-plasmodial activity from this plant could resulted from single or combined of secondary metabolites [24].

In this models of study, chloroquine was used as positive control and it showed high parasitemia clearance (Figure 1) in day 3. It protect from severe malaria by interfering the metabolite of parasite by reducing intake nutrient of the parasite related to iron [30]. The effect of chloroquine in this study was similar with other studies [21,23].

This study was also evaluate of haematological parameters such as haemoglobin (HGB), red blood cell (RBC), white blood cell (WBC) count, pack cell volume (PCV), platelet (PLT), SGOT, SGPT, ureum, and creatinine. The result of this study showed that the extract could prevent significant decrease of PCV, HGB, RBC compared to untreated control. This study show that HGB concentration in 50 mg/kg group was higher than 25 mg/kg and also untreated group. Very low concentration of HGB is a clinical manifestation of severe malaria [31,32]. Consequently that *K. galanga* has anti-anemic extract, it mechanism could because antihaemolytic effect to eritrocyte by *K. galanga* [14]. PCV was also measured in this study, It result showed that at 50 mg/kg group was significantly different with untreated control. Prevention of PCV reduction in treatment group could probably be related to the antihaemolytic effects in the extract [14]. Increase of WBC was associated with severe malaria [33]. Result of this study showed that WBC in treatment groups were higher than untreated group.

Conclusion

This study indicates that ethanol extract of *K. galanga* have minimum anti-plasmodial. The extract appeared to be superior in prevent the severe condition on haematological parameters than suppressive activity to parasite. The findings suggested that further studies on the plant regarding antimalarial activity should be conducted to isolate compounds responsible

Competing Interests

The author declare that they have no competing of interest

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References

[1] P. M. Nmadu, E. Pete, P. Alexander A. Z. Koggie, J. I. Maikenti, The prevalence of malaria in children between the ages 2-15 visiting gwarinpa general hospital Live-Camp, Abuja Nigeria, JHSCI, 5:47-51 (2015)
[2] WHO (World Health Organization). World Malaria Report 2017. 195, WHO, Press, Genewa, Switzerland.
[3] S. Roy, T. Khatun, Analysis of trend of malaria prevalence in the ten Asia countries from 2006

* Corresponding author: 107110411@uii.ac.id

to 2011: A longitudinal study, Malaria Research and Treatment, 2015: 620598 (2015)

[4] L. A. Messenger, J. Shililu, S. R. Irish, G. Y. Anshebo, A. G. Tesfaye, Y. Ye-Ebiyo , et al., Insecticide resistance in *Anopheles arabiensis* from Ethiopia (2012-2016): a national wide study for insecticide resistance monitoring, Malaria Journal, 16:469 (2017). https://doi.org/10.1186/s12936-017-2115-2

[5] R. Kusriastuti, A. Surya, New treatment policy of malaria as a part of malaria control program in Indonesia. *Acta Med Indones*, 44(im):265-269 (2012). http://www.ncbi.nlm.nih.gov/pubmed/22983085.

[6] A. Ogouyemi-Hounto, G. Damien, A. B. Deme, N. T. Ndam, C. Assohou, D. Tchonlin, et al., Lack of artemisisnin resistance in *Plasmodium falciparum* in Northwest Benin after 10 years of use artemisinin-based combination therapy. Parasite. 2016; 23:28 (2016). Doi:10.1051/parasite/2016028.

[7] E. A. Ashley, M. Dhorda, R. M. Fairhurst, C. Amaratunga, P. Lim, S. Suon, et al., Spread of artemisinin resistance in *Plasmodium falciparum* malaria, NEJM, 371:411-423 (2014). Doi:10.1056/NEJMoa1314981.

[8] K. R. Kirtikar, B. D. Basu, Indian Medicinal Plants, IV:2426-2427

[9] P. N. Fauziyah, E. Y. Sukandar, D. K. Ayuningtyas, Antituberculosis drugs and ethanolic extract of selected medicinal plants against Multi-Drug Resistant Mycobacterium tuberculosis isolates, Sci. Pharm, 85:14 (2017).

[10] I. Kusumaswati and H. Yusuf, Phospholipid complex as a carrier of *Kaempferia galanga* rhizome extract to improve its analgesic activity, Int. J. Pharm. Sci., 3:44-46 (2011).

[11] H. Ali, R. Yesmin, A. A. Satter, R. Habib, T. Yeasmin, Antioxidant and antineoplastic activities of methanolic extract of *Kaempferia galanga* Linn. Rhizome against Ehrlich ascites carcinoma cells, Journal of King Saud University-Science, 30:386-392(2018).

[12] C. Mekseepralard, N. Kamkaen, J. M. Wilkinson, Antimicrobial and antioxidant activities of traditional Thai herbal remedies for aphthous ulcers, Phytother. Res., 24:1514-1519 (2010).

[13] K. P. Kochuthressia, S. J. Britto, M. O. Jaseentha, R. Raphael, In vitro antimicrobial evaluation of *Kaempferia galanga* L rhizome extract, AJBMS, 21-5 (2012).

[14] B. Kaushita, L. Priya, K.V.B. Rao, HPLC analysis and antioksidan activities of hydroethanolic leaf extract of *Kaempferia galanga* Linn, Int. J. Pharm. Tech. Res., 2014-2015. 7(2):422-432.

[15] M. R. Sulaiman, Z. A. Zakaria, I. A. Daud, F. N. Ng, M. T. Hidayat. Antinociceptive and anti-inflamatory activities of the aqueous extract of *Kaempferia galanga* leves in animal model, J. Nat. Med, 62:221-227 (2008)

[16] H. Tae-Kyun, L. E.E. Jae-Kook, H. E. O. Jae-Won, K. I. M. Soon-Il, C. Dong-Ro, A. H. N. Young-Joon. Toxicity of *Kaempferia galanga* rhizome derived extract and steam distillate to *Meloidogyne incognita* juveniles and eggs, and their effects on *Lycopersicon esculentum* germination and growth, Nematology, 12: 775-782 (2010).

[17] C. In-Ho, P. Ju-Yong, S. Sang-Chul, P. Il-Kwon, Nematicidal activity of medicinal plant extracts and two cinnamates isolated from *Kaempferia galanga* L. (Proh Hom) against the pine wood nematode, *Bursaphelenchus xylophilus*, Nematology, 8:359-365 (2006).

[18] N. J. Kim, S. G. Byun, J. E. Cho, K. Chung, Y. J. Ahn, Larvicidal activity of *Kaempferia galanga* rhizome phenylpropanoids towards three mosquito species, Pest Manage. Sci., 64: 857-862 (2008).

[19] W. Choochote, U. Chaithong,, K. Kamsuk, A. Jitpakdi, P. Tippawangkosol, B. Tuetun, D. Champakaew, B. Pitasawat, Repellent activity of selected essential oils against *Aedes aegypti*, Fitoterapia, 78: 359-364 (2007).

[20] E. w. C. Chan, Y. Y. Lim, S. K. Wong, K. K. Lim, S. P. Tan, F. S. Lianto, M. Y. Yong, Effects of different drying methods on the antioxidant properties of leaves and tea of ginger species, Food Chem., 113:166-172 (2009).

[21] A. Adetutu, O. S. Olorunnisola, A. O. Owoade, P. Adegbola, Inhibition of in vivo growth of *Plasmodium berghei* by *Launaea taraxacifolia* and *Amaranthus viridis* in mice, Malaria Research and Treatment, vol. 2016(2016)

[22] L. Bantie, s. Assefa, T. Teklehaimanot, E. Engidawork, In vivo antimalarial activity of the crude leafe extract and solvent fractions of *Croton macrostachyus* Hocsht. (Euphorbiaceae) against *Plasmodium berghei* in mice, BMC Complementary and Alternative Medicine, 14:79 (2014).

[23] A. B. Muluye, E. Melese, M. Adinew, Antimalarial activity of 80% methanolic extract of *Brassica nigra* (L.) Koch. (Brassicaceae) seeds against *Plasmodium berghei* infection in mice, BMC Complementary and Alterative Medicine,15, 367 (2015).

[24] E. Deharo, G. Bourdy, C. Quenevo, V. Munoz, G. Ruiz, M. A. Sauvain, Search for Natural Bioactive Compounds in Bolivia through a Multidisciplinary Approach. Part V. Evaluation of the Antimalarial Activity of Plants Used By the Tacana Indians, J Ethnopharmacol, 77:91–8 (2001).

* Corresponding author: 107110411@uii.ac.id

[25] N. Rao. V, Biochemical and phytochemical analysis of the medicinal plant, *Kaempferia galangal* rhizome extracts, IJSR, 3:18-20.
[26] N. Aziman, N. Abdullah, Z. M. Noor, K. S. Zulkifli, W. S. S. W. Kamarudin, Phytochemical constituents and in vitro bioactivity of ethanolic aromatic herb extracts, Sains Malaysiana, 41:1437-1444 (2012).
[27] T. P. C. Chierrito, A. C.C. Anguiar, I. M. de Andrade, I. P. Ceravolo, R. A.C. Goncalves, A. J. B. de Oliveira, et al, Anti-malarial activity of indole alkaloids isolated from *Aspidosperma olivaceum*, Malaria Journal, 13:142 (2014).
[28] J. Momoh, A. O. Longe, C. A. Campbell, In vivo anti-plasmodial and in vitro antioxidant activity of ethanolic leaf extract of *Alstonia boonie* (Eweahun) and its effect on some biochemical parameters in Swiss albino mice infected with *Plasmodium berghei* NK 65, ESJ, 10:68-82 (2014).
[29] S. A. Adesegun, C. I. Orabueze, H. A. B. Coker, Antimalarial and antioxidant potentials of extract and Fractions of aerial part of *Borreria ocymoides* DC (Rubiaceae), Pharmacogn J, 9:534-540 (2017).
[30] A. Ferreira, J. Ball, V. Jeney, B. Balla, M. P. Soares, A central role for free heme in the pathogenesis of severe malaria: the missing lnk?, J. Mol. Med., 86:1097-1111 (2008).
[31] Q. O. Junaid, L. T. Khaw, R. Mahmud, K. C. Ong, Y. L. Lau, P. U. Borade, Pathogenesis of *Plasmodium berghei* ANKA infection in the gerbil (*Meriones unguiculatus*) as an experimental model for severe malaria, Parasite, 24:38 (2017).
[32] A. A. Lamikanra, D. Brown, A. Potocnik, C. Casals-Pascual, J. Langhore, D. J. Roberts, Malaria anemia: of mice and men, Blood, 110:18-28 (2007).
[33] D. Modiano, B. S. Sirima, A. Konate, I. Sanou, A. Sawadogo, Leucocytosis in severe malaria, Transactios of the royal society of tropical medicine and Hygiene, 95:175-176 (2001).

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