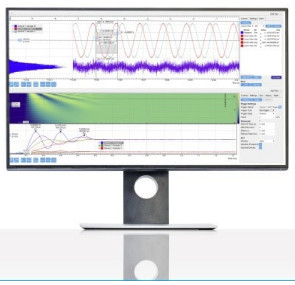
Histopathological effects of ethanolic extract of Gaharu's (*Aquilaria malaccensis* Lamk.) leaves on liver and kidney of mice (*Mus musculus* L.) infected by *Plasmodium berghei*

Cite as: AIP Conference Proceedings **2260**, 040008 (2020); https://doi.org/10.1063/5.0015770
Published Online: 16 September 2020

Ardaning Nuriliani, Deby Lukmawati, Veggy Fazari Ameliya, and Luthfi Nurhidayat



Histopathological Effects of Ethanolic Extract of Gaharu's (*Aquilaria malaccensis* Lamk.) Leaves on Liver and Kidney of Mice (*Mus musculus* L.) Infected by *Plasmodium berghei*

Ardaning Nuriliani[1], Deby Lukmawati[2], Veggy Fazari Ameliya[2] and Luthfi Nurhidayat[1, a)]

[1]*Laboratory of Animal Structure and Development, Faculty of Biology, Universitas Gadjah Mada, st. Teknika Selatan, Sekip Utara, Sleman, Yogyakarta, 55281, Indonesia*
[2]*Undergraduate student, Faculty of Biology, Universitas Gadjah Mada, st. Teknika Selatan, Sekip Utara, Sleman, Yogyakarta, 55281, Indonesia*

[a)]Corresponding author: luthfibio@ugm.ac.id

Abstract. Malaria caused by *Plasmodium* is one of common infectious diseases in tropical country that cause severe problems every year. Several efforts have been developed to eradicate this disease, especially related with new drugs discovery. One of traditional drugs from Gaharu's leaves has been widely known to cure malaria. However, there is no clinical study yet. This study was examining histopathological effects of Gaharu's leaves extract on liver and kidney of mice. Twelve Swiss mice were divided into 4 treatments, those were without DHP (Dihydroarteromisinin-piperaquine as common malaria's drug); with DHP; ethanolic extract of Gaharu's leaves at the concentration of 100 and 200 mg/kg BW. *Plasmodium* infection was done by applying "4 days test" or *Peter's Suppressive Test* method. The mice were sacrificed using overdose ketamine prior to liver and kidney samples collection for histopathological procedure. The samples were fixed with neutral buffered formalin, proceed using paraffin method, and stained with Hematoxylin-Eosin. Histopathological scoring was done using ordinal method and post examination masking. The results reveal that Gaharu's leaves extract treatment exhibit higher histopathological effects in liver and kidney compared to DHP treatment. The histopathological effects that can be detected include cellular damages, hemorrhage, congestion, and inflammation. These results suggested that Gaharu's leaves extract could not overcome histopathological effect of *Plasmodium* in liver and kidney compare to DHP. Thus, we concluded that ethanolic extract of Gaharu's leaves is less effective to be developed as antimalaria compared to DHP.

INTRODUCTION

Malaria is an infectious disease, widely spread in tropical country, and still become serious threat in endemic area. This disease is infected by *Plasmodium* with female mosquito (*Anopheles* spp.) as the vector [1]. Many studies were done using *P. berghei*, a *Plasmodium* which cause malaria in rodent such as mouse, as a model to develop therapy against malaria. The genome size of *P. berghei* is similar with that of *P. falciparum,* which cause malaria in human [2].

Many efforts have been done to minimize the spread of this disease, include its prevention and medication. Vaccine can be the best way to cure malaria. Unfortunately, until now there is no effective vaccine against malaria. Furthermore, many drugs have been developed to eradicate malaria. Since 2008, dihydroartemisinin-piperaquine (DHP) widely use as antimalaria. This drug is an active metabolite from artemisinin with quick response to eliminate parasite from the body, whereas piperaquine has longer half-life around 19-28 days [3].

Development of antimalaria drugs gain serious attention. Recently, many antimalaria has been developed from plants. Some of them are extract of *C. paradisi* [4], aqueous leaf extract of *Mangifera indica* [5], and ethanol bark extract of *B. sapida* [6]. Indonesia also has a lot of potential medicinal plant which can be used as antimalaria such as

The 6th International Conference on Biological Science ICBS 2019
AIP Conf. Proc. 2260, 040008-1–040008-10; https://doi.org/10.1063/5.0015770
Published by AIP Publishing. 978-0-7354-2020-5/$30.00

Gaharu (agarwood), *Aquilaria malaccensis.* Gaharu was known having high antioxidant activity [7], antibacterial [8], and antiinflammation [9]. Alvernita [10] stated that Gaharu also can be used to cure malaria. Gaharu leaves contain secondary metabolite such as alkaloid, tannin, phenol, terpenoid, quinone, flavonoid, sesquiterpenes, and anthraquinone [11, 12]. These phytochemicals can be developed as promising drugs [13] especially as antimalaria.

Gaharu leaves has been used as traditional drug such as antimalaria for long time. However, there is no empirical study yet especially on its histopathological effects. Thus, it is important to study the safety level of Gaharu as antimalaria, especially in its target organs such as liver and kidney although some studies reported that Gaharu relative save to be use as traditional drugs and beverage [7, 9].

METHODS

Ethanolic Extract of Gaharu's Leaves

Gaharu's leaves (*Aquilaria malaccensis*) randomly picked from its tree at Bogor Botanical Garden. Mature leaves with dark green color, 8-9 cm length, 4-5 cm width were chosen. The leaves were dried, mashed, and extracted with soxhletation method. Two-point one gram of leaves were soxhleted using 96% ethanol to make the filtrate. Then, the filtrate was evaporated using fan in the evaporation chamber.

Four Days Test of *Plasmodium*

Four days test or *Peter's Suppressive Test* were done using 12 male Swiss mice (*Mus musculus* L.), which divided into 4 groups (TABLE 1.). Six to eight weeks mice with weight around 25-30 g were acclimatized for 7 days. The mice were infected intraperitoneally with *P. berghei* using 0.6 mL of blood contained ± 1 x 10^6 *P. berghei*. After 3 hours, ethanolic extract of Gaharu's leaves were orally administrated to the treatment groups for 4 days successively. This research was under ethical clearance no: 00043/04/LPPT/VI/2018.

TABLE 1. The mice grouping of the treatment

Group	Treatment
Negative control	*Plasmodium berghei* + DMSO 1%
Positive control	*Plasmodium berghei* + DMSO 1% + DHP
P1	*Plasmodium berghei* + DMSO 1% + ethanolic extract of Gaharu's leaves 100 mg/kg BW
P2	*Plasmodium berghei* + DMSO 1% + ethanolic extract of Gaharu's leaves 200 mg/kg BW

Necropsy, Organ Collection, and Histological Preparation

The mice were sacrificed intramuscularly using overdose of ketamine. Liver and kidney were collected and fixed using neutral buffered formalin. Histological preparation was done using paraffin method, section thickness ± 6 μm, and stained with Hematoxylin-Eosin as Bancroft and Cook [14] protocols. Histopathological data were gained from 50 fields of view with 10 x 40 magnification in each group.

DATA ANALYSIS

The histopathological data were analyzed using descriptive comparative method. The organs scoring was done using ordinal method and post examination masking (TABLE 2. and 3.).

TABLE 2. Scoring parameter of liver

Histopathological parameter	Score
Celullar damage	
No damage	0
Light necrosis < 15%	1
Mild necrosis 15 - 40%	2
Severe necrosis 41 - 70%	3

TABLE 2. Contd'

Histopathological parameter	Score
Very severe necrosis 71 - 100%	4
Hemorrage	
No hemorrhage	0
< 15%	1
15 - 40%	2
41 - 70%	3
71 - 100%	4
Inflammation	
Absent	0
Focal	1
Diffuse	2
Intense	3

TABLE 3. Scoring parameter of kidney

Histopathological parameter	Score
Tubular	
No damage	0
Reversible injuries	1
Light necrosis (< 25%)	2
Mild necrosis (25 - 50%)	3
Severe necrosis (> 50%)	4
Glomerular	
No damage	0
Thickening of Bowman capsule	1
Retraction of glomerular tuft	2
Glomerular fibrosis	3
Interstitial	
No damage	0
Congestion or hemorrhage	1
Congestion or hemorrhage with necrosis < 25%	2
Congestion or hemorrhage with necrosis 25 - 60%	3
Congestion or hemorrhage with necrosis > 60%	4

RESULTS AND DISCUSSION

Ethanolic Extract of Gaharu's Leaves Have Minor Histopathological Effect in The Liver of Mouse Infected by *Plasmodium berghei*

Some studies reported that malaria could cause serious damage in many organs such as liver and kidney. Liver is one of major target organs of *Plasmodium* infection. The mechanisms of *Plasmodium* infection start after this *Plasmodium* infected the blood. Through blood circulation, the parasite entered liver's parenchyma. Parasite which grown in hepatocytes become a schizont and developed to be a merozoite. Then, hepatocytes which consist of parasite will break down and merozoites freely escape, part of merozoites will remain inside of hepatic phagocyte. Erythrocytic cycle started after merozoite entering erythrocytes. Then, tropozoite will develop become young schizont, then become mature schizont and divide become merozoites. After this phase finish, erythrocytes break down to release merozoites, pigment, and cell debris entering blood plasma. This cycle will repeat when the parasite entering another erythrocyte [15].

Kalia et al. [16] reported that *Plasmodium berghei* infection caused several histopathological effects such as disruption of hepatic architecture because of hepatic necrosis, sinusoidal dilatation and congestion [5, 17], increased number of Kupffer cells, lymphocytes infiltration [5], Kupffer cells hyperplasia, and haemosiderosis [17]. The similar histopathological effects also found in human infection by *Plasmodium falciparum* such as Kupffer cells hyperplasia and portal tract inflammation [18].

Curing mechanism of DHP in malaria treatment through admission of artemisinin to food vacuole of the parasite which further will interact with hemoglobin which release free radicals to destroy vital component of parasite. This will cause the death of the parasite [19]. Whereas, ethanolic extract of Gaharu's leaves will attack the parasite using its secondary metabolites. The secondary metabolites could inhibit the growth of *Plasmodium* by destroying *Plasmodium's* cell membrane at high dose [20].

In this study, we found that *Plasmodium berghei* caused cellular damage (fatty change, cloudy swelling, necrosis, pyknotic, karyolysis, and karyorrhexis), hemorrhage, congestion, and inflammation at different level of treatment in liver (TABLE 4.). The results showed that without drug treatment, *Plasmodium* will cause severe effect to the liver especially cellular injuries and intense inflammation (Fig 1A.). Whereas DHP (Fig 1B.) and Gaharu's leaves extract (Fig 1C. and 1D.) can decrease the effect of cellular injuries or inflammation in liver infected by *Plasmodium berghei.* Singh & Chauhan [21] studied that flavonoid and alkaloid could inhibit the growth of *Plasmodium berghei.*

TABLE 4. Histopathological effects of ethanolic extract of Gaharu's leaves in liver of mouse infected by *Plasmodium berghei*

Type of damage	Treatment			
	No DHP	DHP	Gaharu's leaves extract 100 mg/kg BW	Gaharu's leaves extract 200 mg/kg BW
Cellular damages	2	1	2	2
Hemorrhage	1	1	1	1
Congestion	1	1	1	1
Inflammation	3	1	0	1

Note: score explanation refers to TABLE 2.

We found that Gaharu's leaves extract at the concentration of 200 and 100 mg/kg BW could reduce as well as eliminate inflammation caused by *Plasmodium*, respectively. This result consistent with previous study by Apridamayanti et al. [11] which reported that Gaharu's leaves contain antiinflammation compound. Our result also showed that DHP could decrease inflammation in liver infected by *Plasmodium*. However, the decreasing is not as much as Gaharu's leaves extract treatment.

Previous study reported that DHP could inhibit the growth of *Plasmodium*. Gaharu's leaves extract also could temporarily inhibit the growth of *Plasmodium* if given at lower concentration [20]. Some studies reported that aqueous extract of Gaharu's leaves showed no toxicity in testis of mice up to concentration of 1000 mg/kb BW [22] as well as in liver and kidney of rat at the concentration below 2000 mg/kg BW [23]. Our results showed that ethanolic extract of Gaharu's leaves could ameliorate the histopathological effect of *Plasmodium* in liver especially the inflammation effect at the concentration of 100 mg/kg BW compare to the concentration of 200 mg/kg BW. This result consistent with previous study by Apridamayanti et al. [11] which reported that Gaharu has antiinflammation activity in rats.

In this study, we also found that *Plasmodium* infection caused hemorrhage. Hemorrhage is characterized by blood lose from blood vessel. Blood will escape from blood vessel and fill extracellular areas. Acute hemorrhage may cause hypoxia cellular, decreasing of tissue perfusion, organ damage, and death [24]. In this study, we found that *Plasmodium berghei* only caused very light level hemorrhage in all groups (TABLE 4. and Fig 1.).

The results also showed that there was congestion in liver of all groups (Fig 2.). Congestion or blockage of blood vessel is a condition where there is excessive blood in the blood vessels [25]. Zachary & McGravin [26] reported that in chronic congestion caused fibrosis. Our results showed the congestion level just similar with that of hemorrhage. Only found at very light level. However, Razak et al. [23] reported that aqueous extract of Gaharu's leaves at the concentration of 2000 mg/kg BW caused vascular congestion and lymphocytic infiltration. That is mean the Gaharu's leaves contain secondary metabolite which relative save at the lower concentration and should be use with caution.

DHP is a malaria drug which can kill *Plasmodium berghei*. This drug gives quick response against fever and eliminate asexual phase of the *P. berghei* with fever free and asexual free parasites around 1,6 days and 1 day, respectively followed by increasing of hemoglobin level improvement of patient [15].

Inflammation is a kind of defense mechanisms which cause by tissue's response against parasites. Inflammation is characterized by blood clot and rosy tissue because many erythrocytes escape from blood vessel. Inflammation response proposed to restore tissues condition and suppress the agent that caused necrosis. Inflammation response was done by regeneration of the losing cells, scar formation, and leucocytes infiltration in necrotic area [27]. We found that Gaharu's leaves extract at the concentration of 100 mg/kg BW could eliminates inflammation (Fig 3.).

Inflammation is a protective response to remove necrotic cells. This response will decrease if the concentration of Gaharu's leaves extract which entering the body reduced [28], that is possibly treatment with Gaharu's leaves extract at the concentration of 100 mb/kg BW decrease the level of inflammation.

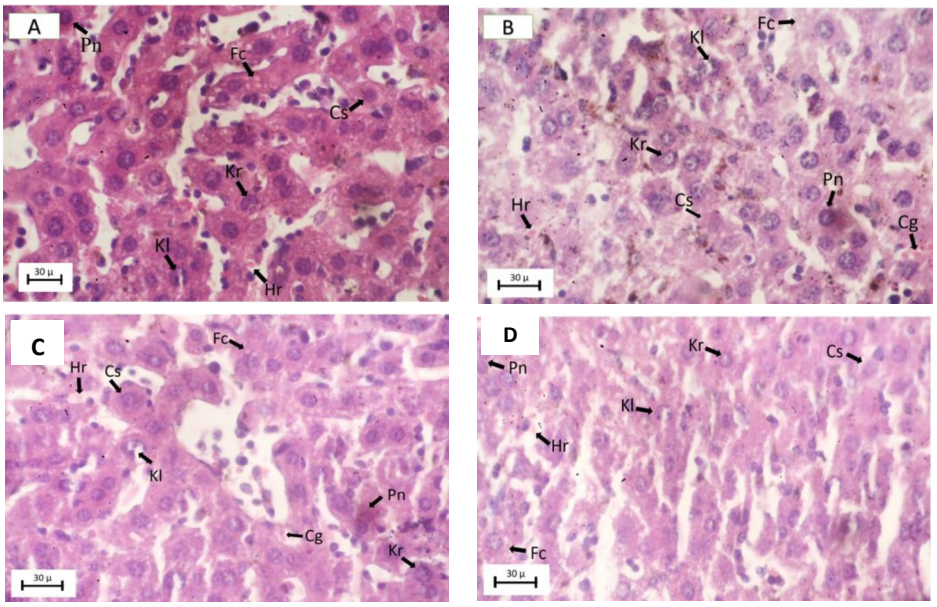


FIGURE 1. Cellular damages in liver of mouse infected by *Plasmodium berghei*. A.without DHP (untreated); B. DHP; C. Gaharu's leaves extract 100 mg/kg BW; D. Gaharu's leaves extract 200 mg/kg BW. Pn: pyknotic, Fc: fatty change, Cs: cloudy swelling, Kr: karyorrhexis, Kl: karyolysis, Hr: hemorrhage, Cg: congestion. Staining: Hematoxylin-Eosin. Bar: 30 µm.

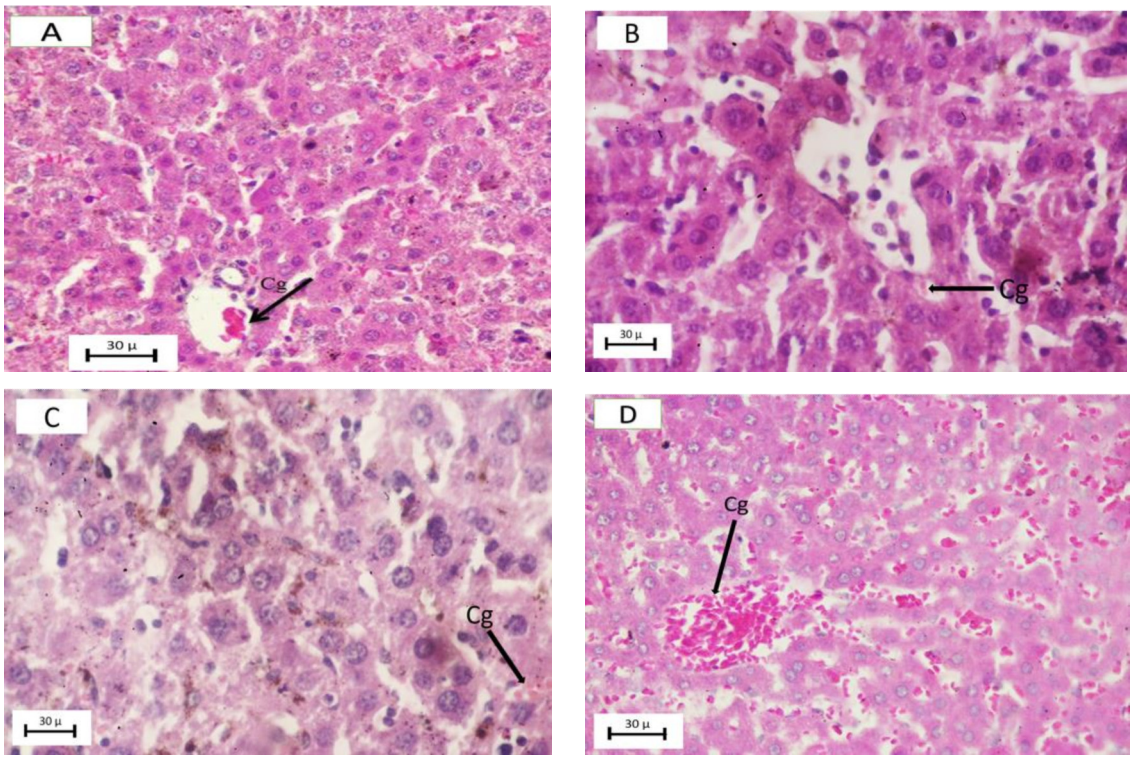


FIGURE 2. Congestion (black arrow) in liver of mouse infected by *Plasmodium berghei*. A. without DHP (untreated); B. DHP; C. Gaharu leaves extract 100 mg/kg BW; D. Gaharu's leaves extract 200 mg/kg BW. Cg: congestion. Staining: Hematoxylin-Eosin. Bar: 30 µm.

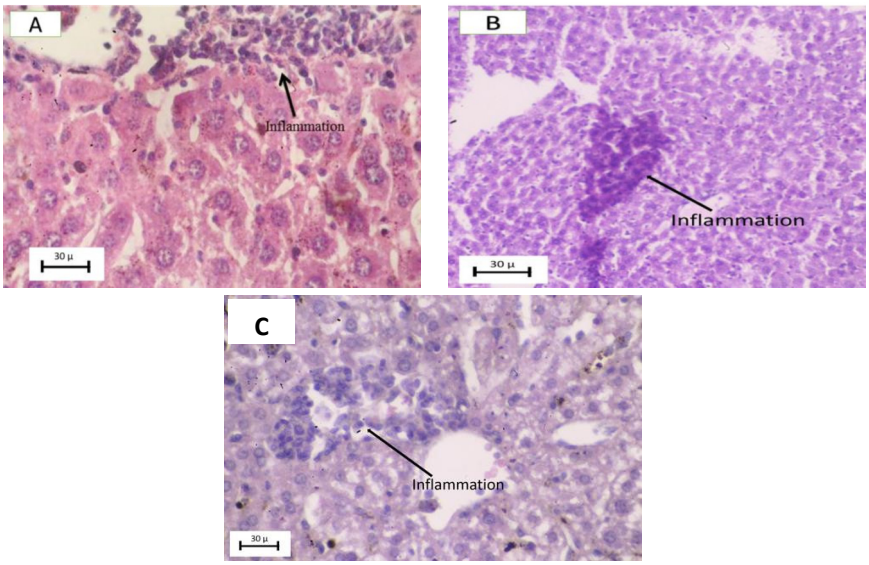


FIGURE 3. Inflammation (black arrow) in liver of mouse infected by *Plasmodium berghei*. A. without DHP (untreated); B. DHP; C. Gaharu leaves extract 200 mg/kg BW. Staining: Hematoxylin-Eosin. Bar: 30 µm.

Gaharu's Leaves Ethanolic Extract Cause Histopathological Effect in Kidney of Mouse Infected by *Plasmodium berghei*

Sibiya et al. [29] reported that malaria can disturb kidney function along with increasing of oxidative stress. In more serious cases, it will be followed by histopathological effect such as necrotic in renal morphology [16] like tubular necrosis [4, 17] and perivascular interstitial mononuclear cell infiltration [17], thickening of glomerular basement membrane and Bowman capsule [29]. In this study, we found that *Plasmodium berghei* infected mouse kidney and caused many histopathological effects in tubular, glomerular, and interstitial areas. The result showed that administration of ethanolic extract of Gaharu leaves at the concentration of 200 mg/kg BW caused higher level of damage among other groups in all kidney areas followed by ethanolic extract of Gaharu leaves at the concentration of 200 mg/kg BW and DHP groups. Whereas untreated group showed lightest damage level. However, despite of many damages in Gaharu's leaves extract and DHP the level is light and reversible (TABLE 5.).

One type of damages in kidney areas was cloudy swelling with light staining of the nuclei and has wider size compare to that of normal cell (Fig 4.). Cloudy swelling is one response to the lesion which cause by trauma, anoxia, immunity disruption, toxic agents, bacteria, virus, or other pathogens [30]. We also found pyknotic cells (Fig 4.) which characterized by shrinkage of nuclei, DNA and basophil mass condensation which consider as irreversible injury [31]. At the higher level this could cause karyolysis and karyorrhexis.

TABLE 5. Histopathological effect of Gaharu's leaves ethanolic extract in the mouse kidney infected by *Plasmodium berghei*

Damage type	Treatment			
	No DHP	(DHP)	Gaharu extract 100 mg/kg BW	Gaharu extract 200 mg/kg BW
Tubular damage	1	2	2	2
Glomerular damage	1	1	2	2
Interstitial damage	1	1	1	2

Note: score explanation refers to TABLE 3.

Gaharu's leaves extract at the concentration of 200 mg/kg BW caused many tubular damages compare to other groups. This might be Gaharu's leaves contain more secondary metabolite which affect the kidney compare to other groups. Some of this secondary metabolite might give benefit effect against *Plasmodium* infection but others compounds may cause adverse effect to kidney although it just categorized as reversible injuries at light level of damage. Medication with some drugs such as dihydroatemisinin might be cause some effect to kidney. However, level of damage from DHP group was lower compare to treatment group with Gaharu's leaves extract.

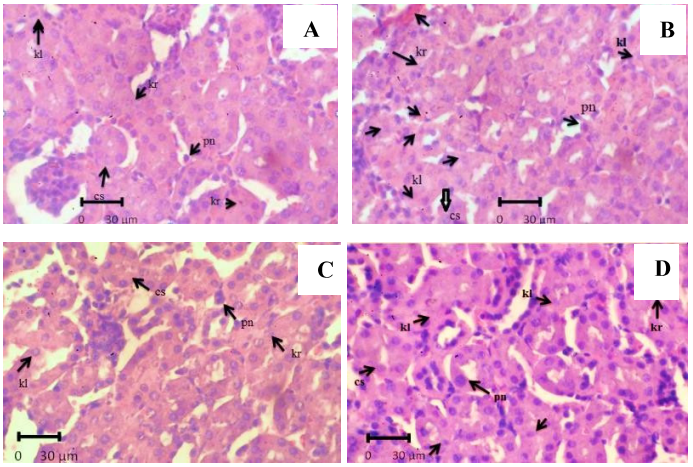


FIGURE 4. Histopatological effects in tubular area of kidney of mouse infected by *Plasmodium berghei*. A. without DHP (untreated); B. DHP; C. Gaharu's leaves extract 100 mg/kg BW; D. Gaharu's leaves extract 200 mg/kg BW. Kl: Karyolysis, Kr: Karyorrhexyis, Pn: Pyknotic, Cs: Cloudy swelling. Staining: Hematoxylin-Eosin. Bar: 30 µm.

We also found glomerular shrinkage and thickening of Bowman capsule (Fig 5.). This was consistent with previous study by Sibiya et al. [29] which found thickening in glomerular membrane and Bowman capsule of mouse's kidney which infected *Plasmodium berghei*. *Plasmodium berghei* infection will stimulate immune system to activate T lymphocyte, macrophage, and cytokine which induced releasing of reactive oxygen intermediate (ROI) and reactive nitrogen intermediate (RNI). ROI and RNI will react to form nitric oxide through activation of inducible nitric oxide synthase which further will eliminate the parasite. However, since the action is not specific sometimes it caused pathological effect [32]. Further, reactive oxygen species will cause cell death [33]. Sibiya et al. [29] reported that in malaria was followed by increasing of oxidative stress.

We also found congestion and hemorrhage (Fig 6.). Congestion happen when blood highly increase in the blood vessel at certain area [34]. Whereas, hemorrhage caused by some factors such as trauma, virus or parasite infection which destruct mucous layer and secreted anticoagulation agent, toxic compound which cause blood clot that induce bleeding, and uremic toxin that can damage the endothelial of blood vessel [35]. In this study, we found hemorrhage in all groups which cause by *Plasmodium berghei* infection.

We found that Gaharu's leaves extract at the concentration of 200 mg/kg BW caused highest histopathological effects such as congestion and hemorrhage in the interstitial area of kidney. Whereas the other three groups suffer similar histopathological effect level. However, the Gaharu leaves extract at the highest concentration was felt at the light level and reversible. Whereas, the other three groups at the level very slight. Although some studies reported that secondary metabolites of Gaharu's leaves quite save to be consumed, Razak et al. [23] reported that at quite high concentration (2000 mg/ kg BW), aqueous extract of Gaharu's leaves caused cytoplasmic vacuolation and pyknotic nuclei of kidney. In our study seem that followed *Plasmodium* infection, Gaharu's leaves extract promotes more tissue injuries in kidney compare to that of in liver through unknown mechanism. Previous study by Apridamayanti et al. [11] reported that Gaharu contain many secondary metabolites such as alkaloid, flavonoid, phenol, antrakuinon, and terpenoid. Among those compounds, they found that flavonoid most potential as antiinflammation in rat at the concentration of 180 mg/kg BW. Other compound of Gaharu's leaves such as flavones, flavonol, isoflavones, glycosides, tannins, and steroid/triterpenoids also potential as antioxidant [7, 36]. At one side, it seems that Gaharu's leaves potential to developed as new antimalaria. However, the potential of ethanolic extract of Gaharu's leaves should be used with caution especially if there is a previous infection by *Plasmodium*.

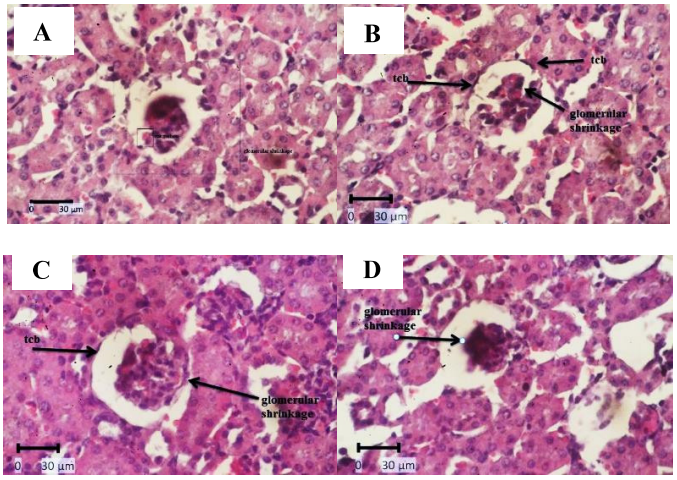


FIGURE 5. Histopatological effects in glomerular area of kidney of mouse infected by *Plasmodium berghei*. A. without DHP(untreated); B. DHP; C. Gaharu leaves extract 100 mg/kg BW; D. Gaharu leaves extract 200 mg/kg BW. tcb: thickening of Capsule of Bowman. Staining: Hematoxylin-Eosin. Bar: 30 µm.

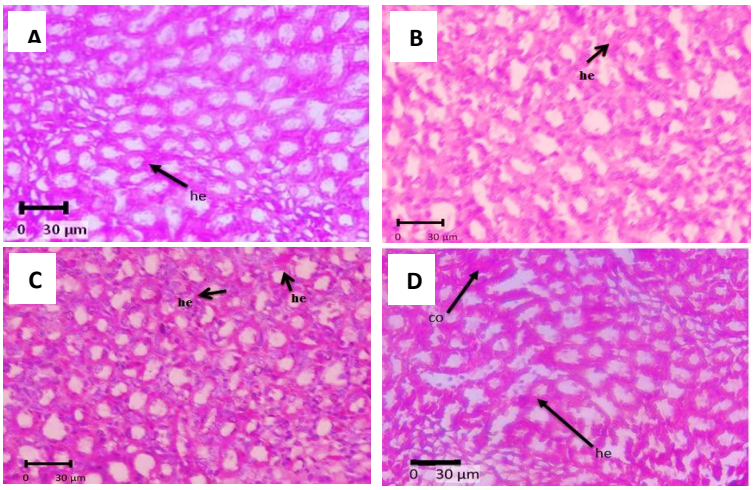


FIGURE 6. Histopathological effects in interstitial area of kidney of mouse infected by *Plasmodium berghei*. A. without DHP (untreated); B. DHP; C. Gaharu leaves extract 100mg/kg BW; D. Gaharu leaves extract 200mg/kg BW. Staining: Hematoxylin-Eosin. Bar: 30 µm.

CONCLUSION

The results suggested that Gaharu's leaves extract could not overcome histopathological effect of *Plasmodium* in liver and kidney compare to DHP. Thus, we concluded that ethanolic extract of Gaharu's leaves is less effective to be developed as antimalaria compared to DHP.

ACKNOWLEDGEMENT

This research is funded by Research Collaboration Grant of Lecturer and Undergraduate Student of Faculty of Biology, Universitas Gadjah Mada 2019 (Hibah Penelitian Kolaborasi Dosen dan Mahasiswa, Dana Masyarakat mengikat tahun 2019, No: UGM/BI/1715/M/02/05). We also would like to say thank you to Soenarwan Heri Poerwanto, S.Si., M.Kes. from Laboratory of Parasitology, Faculty of Biology, Universitas Gadjah Mada, Yogyakarta who kindly gave us the *Plasmodium* and important discussion of the method.

REFERENCES

1. U. Frevert, E. Sabine, Z. Sergine, S. Jorg, Ng. Bruce, M. Kai , L. Leonard, and H. Yee, PLoS Biology. **3(6)**, 1034-1046 (2005).
2. M. J. Gardner, N. Hall, E. Fung, O. White, M. Berriman, R.W. Hyman, J.M. Carlton, A. Pain, K.E. Nelson, S. Bowman, I.T. Paulsen, K. James, J.A. Eisen, K. Rutherford, S.L. Salzberg, A. Craig, S. Kyes, M.S. Chan, V. Nene, S.J. Shallom, B. Suh, J. Peterson, S. Angiuoli, M. Pertea, J. Allen, J. Selengut, D. Haft, M.W. Mather, A.B. Vaidya, D.M. Martin, A.H. Fairlamb, M.J. Fraunholz, D.S. Roos, S.A. Ralph, G.I. McFadden, L.M. Cummings, G.M. Subramanian, C. Mungall, J.C. Venter, D.J. Carucci, S.L. Hoffman, C. Newbold, R.W. Davis, C.M. Fraser, and B. Barrell, Ann Soc belge Med trop. **45(3)**, 259-266 (2002).
3. H. Siswantoro, A. R. Hasugian, R. Avrina, Y. Risniati, and E. Tjitra, Media Litbang Kesehatan. **21(3)**, 135-144 (2011).
4. A. Adetutu, O. S. Olorunnisola, and K. Iyanda, Functional Foods in Health and Disease. **5(10)**, 339-349 (2015).
5. A. A. Olayode, D. A. Ofusori, T. A. B. Ogunniyi, and O. S. Saka, Anatomy. **9(3)**,168–176 (2015).
6. O. O. Otegbade, J. A. Ojo, D. I. Adefokun, O. O. Abiodun, B. N. Thomas, and O. Ojurongbe, Drug Target Insights. **11**, 1–8 (2017).
7. N. N. A. N. Wil, N. A. M. Omar, N. A. Ibrahim, and S. N. Tajuddin, Journal of Chemical and Pharmaceutical Research. **6(12)**, 688-693 (2014).
8. Y. Begum, Pharma Tutor. **4(2)**, 47-50 (2016).
9. M. Eissa, Z. Yumi, H-Y. Hashim and N. A. A. Zainurin, International Journal on Advanced Science Engineering Information Technology. **8(4-2)**, 1625-1632 (2018).
10. G. Alvernita, "Gambaran Histopatologi Hati Mencit Setelah Pemberian Suspensi Kepel Secara Intragastrik Selama 14 Hari [Histopathological Overview of Mice Liver After Intragastric Kepel Suspension For 14 days]", Skripsi, Insitut Pertanian Bogor, 2011.
11. P. Apridamayanti, F. Sanera, and R. Robiyanto, Pharmaceutical Sciences and Research. **5(3)**, 152-158 (2018).
12. S. Wang, Z. Yu, C. Wang, C. Wu, P. Guo, and J. Wei, Molecules. **23(342)**, 1-21 (2018).
13. A. S. Khalil, A. A. Rahim, K. K. Taha, and K. B. Abdallah, Journal of Applied and Industrial Sciences. **1(3)**, 78-88 (2013).
14. J.D. Bancroft and H.C. Cook. *Manual of Histology Techniques and Their Diagnostic Application* (Churchill Livingstone, London, 1994).
15. J. P. Vanderberg and U. Frevert, Int J Parasitol. **34(9)**, 991–996 (2004).
16. S. Kalia, U. Bagai, and V. Gorki, International Journal of Pharma Sciences and Research (IJPSR). **6(3)**, 575-581 (2015).
17. O. T. Soniran, O. A. Idowu, O. L. Ajayi, and I. C. Olubi, Malaria Research and Treatment. **2012**, Article ID 960758, 7 pages (2012).
18. P. Viriyavejakul, V. Khachonsaksumet, and C. Punsawad, Malaria Journal. **13(106)**, 1-9 (2014).
19. Muti'ah, R, Jurnal Fakultas Sains dan Teknologi UIN Malang. **2(1)**, 80-91 (2012).
20. B. Havsteen, Biochem Pharmacol. **32**, 1141-1148 (1983).
21. V. Singh and D. Chauhan. Indo American Journal of Pharmaceutical Research. **14(12)**, 5943-5948 (2014).
22. N. H. C. Musa, H. H. M. Zain, H. Ibrahim, and N. N. M. Jamil, Natural Product Sciences. **25(2)**, 157-164 (2019).
23. R. N. H. A. Razak, S. A. Rahman, A. H. Hamdan, R. Ramli, M. L. M. Isa, H. Muhammad, and N. F. N. Hassan, AsPac J. Mol. Biol. Biotechnol. **27(1)**, 20-32 (2018).
24. G. Gutierrez, H. D. Reines, and M. E. Wulf-Gutierrez, Critical Care. **8**, 373-381 (2004).
25. M. Danciu, M.S. Mihailovici, A. Dima, and C. Cucu, Atlas of Pathology, 3rd edition, pp 356-357 (2009)
26. J. F. Zachary and M. D. McGavin, *Pathologic Basic of Veterinary Disease* (Elsevier Mosby, Missouri, 2012), pp 1112-1115.
27. F.H.S. Jerry, "Pengaruh Pemberian Minyak *Pandanus conoideus* Terhadap Gambaran Histologis Hepar pada Mencit Swiss yang Diinfeksi *Plasmodium berghei* ANKA [Effects of Pandanus conoideus Oil on Liver Histological Representations in Swiss Mice Infected by Plasmodium berghei ANKA]", (Skripsi, Universitas Diponegoro, 2006).
28. S. Q. Wang, E. Christophel, S. G. Lin, F. Meng, X. M. Hu and G. Z. Wang, *Chinese journal of parasitology & parasitic diseases*. **26(1)**, 50-2 (2008).
29. H. P. Sibiya, C. T. Musabayane, and M. V. Mabandla, Journal of Endocrinology and Thyroid Research. **1(3)**, 1-9 (2017).

30. S. L. Mera, *Pathology and Understanding Disease prevention* (Stanley Thornes Publisher, United Kingdom, 2003), pp 27-29.
31. W.C. Langley, *Pancreatitis Research Advances* (Nova Science Publisher, New York, 2007), p:294.
32. F.Y. Liew, Y. Li, D.M. Yang, A. Severn, and F.E. Cox, Immunology. ***74*(2)**, 304 (1991).
33. E. Fitri, Jurnal Kedokteran Brawijaya. **26(4)**, 222-260 (2011).
34. A. A. Suttie, J. R. Leininger, and A. E. Bradley. *Boorman's Pathology of The Rat* (Academic Press, London, 2018), pp 125.
35. M. D. McGavin, Zachrafy. *Pathology Basis of Veterinary Disease.* (Mosby, USA, 2007).
36. R. Nugraha, R. Batubara, and H. Ginting, Peronema Forestry Science. **4(1)**, 1-9 (2015).