IJSCIA
International Journal of Scientific Advances
ISSN: 2708-7972
Volume: 4 | Issue: 6 | Nov – Dec 2023 Available Online: www.ijscia.com
DOI: 10.51542/ijscia.v4i6.4

Immunological Effect of Goat Bile on The Immunity of Mice Infected with *Plasmodium berghei*

Heny Arwati[1,2*], Kholida Nur Aini[3], Kartika Arum Wardani[3,4], and Retno Handajani[2,5]

[1]Department of Medical Parasitology, Faculty of Medicine, Universitas Airlangga, Campus A, Surabaya 60131, Indonesia

[2]Master Program on Immunology, Postgraduate School Universitas Airlangga, Campus B, Surabaya 60286, Indonesia

[3]Graduated student of Master Program on Immunology, Postgraduate School Universitas Airlangga, Campus B, Surabaya 60286, Indonesia

[4]Department of Immunology and Microbiology, Karya Putra Bangsa Institute of Health Science, Tulungagung 66291, Indonesia

[5]Department of Biochemistry, Faculty of Medicine, Universitas Airlangga, Campus A, Surabaya 60131, Indonesia

*Corresponding author details: Heny Arwati; heny-a@fk.unair.ac.id

ABSTRACT

Goat bile (GB) has been used traditionally as antimalarial medicine. Toxicity and antimalarial activity as well as the suppressive effect of GB have been reported previously. This study aimed to find out the immunological effect of GB on the serum level of IgG, TNF-α, and IL-10 in *Plasmodium berghei* ANKA. Fifty male BALB/c mice were infected with *P. berghei* ANKA and were divided into 5 groups. Groups 1-3 were treated with GB at a concentration of 25% (GB25), 50% (GB50), and 100% (GB100), respectively. Group 4 was a negative control and was given only sterile water. Group 5 was a positive control group, which was given 187.2 mg/kg body weight of the dihydroartemisinin-piperaquine antimalarial drug. Simultaneously, 3 groups of uninfected mice were treated with GB as above. Additionally, a group of normal mice without any treatment was used as a comparison control group. Treatments were given for four consecutive days. Parasitemia was observed daily on Giemsa-stained blood smears. Sera were collected on day five before the measurement of IgG anti *P. berghei* ANKA, TNF-α, and IL-10 levels by ELISA kit. Data were analyzed using one-way ANOVA, Mann Whitney, and Pearson correlation tests. Temporary mild diarrhea was seen in GB100-treated mice. All GB concentrations suppressed IgG production. The GB100 was more therapeutic, but GB50 and GB25 increased IL-10 levels as an antiinflammation response against infection. The pro-inflammatory effect of GB in normal mice caused TNF-α levels in GB-treated uninfected mice to be high. Both the toxicity and benefit of GB could be considered potential sources for the development of new anti-malarial candidates. The toxicity of GB should be considered seriously as the contradictive effect.

Keywords: Malaria; goat bile; IgG; TNF-α; IL-10

INTRODUCTION

Malaria is a contagious parasitic infection caused by protozoa of the genus *Plasmodium* which live and reproduce in red blood cells. This disease is transmitted through female *Anopheles* mosquitoes [1]. Malaria as a global disease burden remains a serious concern for WHO because of the large number of deaths that occur, especially in children [2,3]. Globally, malaria cases were estimated at 245 million in 2020 and increased to 247 million cases during 2021 in 84 malaria endemic countries. During the Covid-19 pandemic in 2019-2021, it was estimated that there were an additional 13.4 million cases caused by disruption of services to malaria patients due to the Covid-19 pandemic, such as a decrease of more than 30% in the discovery of malaria cases, delays in the distribution of insecticide-treated bed nets, and stock of antimalarial drugs due to the reductions in drug production and transportation delays [3]. Malaria cases in Indonesia have also continued to increase after the COVID-19 pandemic, since 2020 (235,700 cases), 2021 (305,607 cases), and jumped by 36.29% in 2022 to 415,140 cases. The increase in cases occurred in areas where malaria is still endemic, such as eastern Indonesia, such as Papua, East Kalimantan Province, especially in the District of Penajam Paser where the new capital city of Indonesia is built, and East Nusa Tenggara Province, especially on the island of Sumba.

Therefore, currently the malaria elimination program in Indonesia is focused on those three malaria-endemic areas. Fortunately, the increase in malaria cases was also accompanied by an increase of 372 out of 514 districts (72.4%) being certified malaria-free [4]

Malaria treatment in Indonesia is based on Artemisinin Combination Therapy (ACT) as recommended by WHO, however, some Indonesian people consume intact goat bladder to treat malaria and increase their stamina [5,6] Ethnomedicine is often considered the main option for treating diseases in developing countries because it is affordable and accessible from available natural sources [7]. For ages, Traditional Chinese Medicine (TCM) has employed bile from diverse animals and certain bile constituents in conjunction with herbal remedies to address both acute and chronic infectious and non-infectious diseases, such as malaria [8].

Recently, the acute and sub-acute toxicity tests of GB in BALB/c mice have been reported, where GB caused mild diarrhea in BALB/c mice indicating a mild intestinal toxicity [5]. GB has also been reported to have antimalarial activity [9] as well as its suppressive effect in BALB/c mice infected with *Plasmodium berghei* ANKA [6]. In Chinese Materia Medica the bile of goat (GB) has been documented that therapeutically used to treat optic atrophy, acute hemorrhagic conjunctivitis, and various skin diseases [8]. However, there is no report on the effect of animal bile treatment on the host immunity especially against malaria infection. *Murine immunoglobulin* G (*IgG*) plays an important role in mediating protective immune responses to *malaria* [10]. Malaria infection is associated with inflammation [11]. Inflammation plays a significant role in the host's defense against pathogens like malaria parasite. An essential pro-inflammatory cytokine, tumor necrosis factor (TNF)-α involved in the immune responses to malaria infection [12]. While, an anti-inflammatory cytokine, IL-10 not only protects against severe disease, but also inhibits protective anti-parasitic immunity. The balance between host pro- and anti-inflammatory immune responses plays a critical role in determining the outcome of malaria infection [13]. Therefore, this study aimed to find out the immunological effect of GB on the level of IgG, TNF-α, and IL-10 in mice infected with *P. berghei.*

MATERIALS AND METHODS

Ethical approval

The research was approved by the Research Ethical Committee of the Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia as stated on certificate number 531/HRECC.FODM/XII/2020.

Parasite and Infection

Parasite used in this experiment was *Plasmodium berghei* strain ANKA obtained from Department of Medical Parasitology Faculty of Medicine, Universitas Airlangga. Donor mice were infected with 200µL of frozen *P. berghei* ANKA- infected mice blood. When parasitemia level reached ±20% mice were sacrificed, blood was collected by cardiac punctured and infected to the test mice. Activity of GB was tested based on a four day test [10]. After one week acclimatization 30 mice were infected with $1x10^6$ *P.berghei*-infected erythrocytes in 0.2 mL of blood from donor mice. Mice were then randomly grouped into five groups. Group 1-3 were treated orally using gavage with 0.5 mL of 25% GB (GB25 Group), 50% GB (GB50 Group) and 100% (GB100 Group), respectively. Group 4 as a positive control (POS group) was given 187.2 mg/kg body weight of antimalarial drug dihydroartemisinine-piperaquin (DHP). Group 5 as a negative control (NEG group) was given 0.5 mL sterile water.

Treatments were commenced at day three post-infection. Physical observation was performed daily including mobility of mice, any changes in fur and skin, eyes and mucous membrane, respiratory and digestive distress, behavior pattern, coma and death.

Determination of parasitemia and curative effect

Parasitemia was observed to find out that the malaria infection had developed in the mice. Parasitemia was observed at day five post-treatment on 10% Giemsa-stained tail thin blood smears under light microscopy with 1000x magnification. The percentage of parasitemia was calculated using the following formula:

$$\% \text{ parasitemia} = \frac{\text{number of infected erythrocyte}}{\text{total number of counted erythrocyts}} x\ 100$$

Curative effect of GB was calculated as reduction of parasitemia in each GB- and DHP-treated groups as follows [11]:

$$\frac{\text{APN-APT x 100}}{\text{APN}}$$

where APN is the average percentage parasitemia in the negative control group and APT is the average percentage parasitemia in test group.

GB treatment in uninfected mice

Prior to measurement of TNF-α and IL-10, simultaneously, 24 normal mice (uninfected) were divided into four groups. Group 6 was normal mice without any treatment (NOR group). Groups 7 to 9 were treated with 25% GB (NOR25 group), 50% GB (NOR50 group) and 100% GB (NOR100 group), respectively to compare with GB-treated infected mice groups. Additionally, 10 male uninfected mice without any treatment were used as comparison control mice.

Serum collection

Sera were collected on day 5 post treatment. After ketamine anesthetized, mouse of each group was sacrificed, blood was collected by cardiac punctured prior to serum collection. Sera were used to measure the IgG, TNF-α and IL-10 levels.

Measurement of serum levels of IgG, TNF-α and IL-10

Measurement of IgG, TNF-α and IL-10 level by ELISA kit was done according to the manufacture protocol (Elabscience, Maryland, USA). One hundred microlitters of each mouse serum were used to measure the level of IgG as well as TNF-α- and IL-10. Optical densities (OD) were read by a microplate reader at 450 nm wavelength (Humareader, Faribadad, India).

Statistical analysis

The data with variances of the groups were assumed to be equal were analyzed using one-way analysis of variance (ANOVA), if variances of the groups were not assumed to be equal, Mann–Whitney U-test was used to determine the significance of the group differences. The correlations of GB concentrations and parasitemia with TNF-α and IL-1 were analyzed using Pearson test if data were distributed normally or Spearmen test if data were not distributed normally. The result was considered statistically significant at 95% confidence level and $p<0.05$. All data were analyzed using SPSS version 20 (IBM Corp., NY, USA)

RESULTS

Physical observation of mice

For the first two days of treatment, mice in the GB100 and NOR100 groups experienced mild diarrhea; after that, they appeared normal. At the end of the infection period, only one mouse in the GB100 group perished. It was noted that the mice in the other groups were normal.

Parasitemia and curative effect of GB

Parasitemia in mice treated with GB100 (4.23%) was higher than that of mice treated with DHP (POS) which was reached 0%, but higher than that of GB50 (20.58%) and GB25 (30.85%). Mice in NEG showed the highest parasitemia. Curative effect of GB100 against *P. berghei*-infection was 89.82% which was higher that of GB50 (50.60%) and GB25 (25.77%) (Table 1).

TABLE 1: Parasitemia at day 4 post-GB treatment in P. berghei-infected mice and curative effect of GB.

Groups	Parasitemia (%)	Curative effect (%)
NEG	41.56	
GB25	30.85	25.77
GB50	20.58	50.60
GB100	4.23	89.82
POS	0	100

GB: goat bile; NEG: Negative control, infected mice were given sterile water; GB25: 25% goat bile; 50% goat bile; GB100: 100% goat bile; POS: positive control, mice were given 187.2 mg/Kg body weight once a day.

Effect of GB on serum levels of IgG

All concentration of GB suppressed the IgG level (o.6-0.72 pg/mL) to almost similar to that of normal mice (NOR) (0.42 pg/mL), and compared to that of *P. berghei*-infected mice without any treatment (NEG), which showed high level of IgG (1.43 pg/mL) (Figure 1).

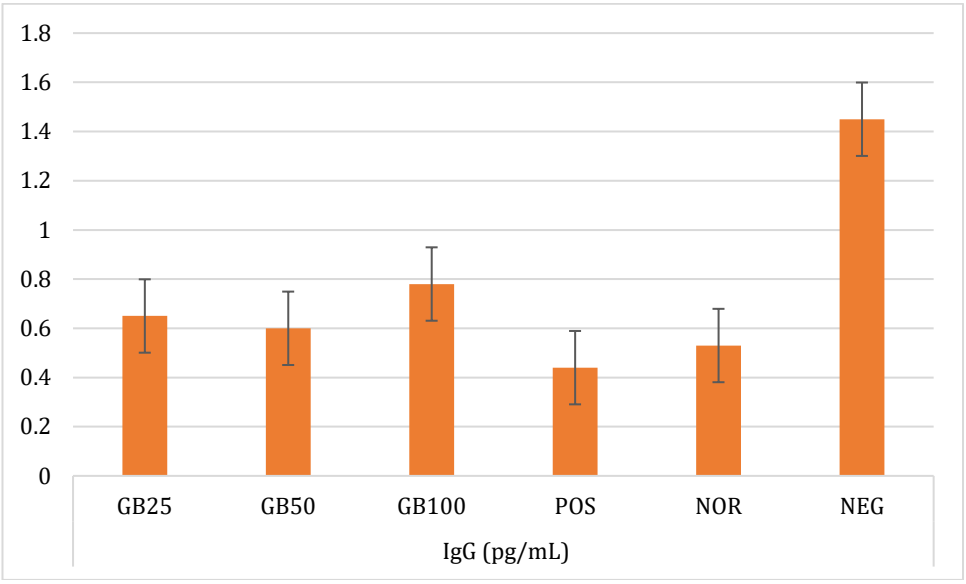

FIGURE 1: The level of IgG in *P. berghei*-infected mice treated with GB25, GB50, GB100 compared with positive control (POS), and negative control (NEG). GB25: 25% goat bile; GB50: 50% goat bile; GB100: 100% goat bile; POS: 187,2 mg/kg body weight dihydroartemisinin-piperaquin once a day; NOR: uninfected mice; NEG: infected mice were given only sterile water.

Effect of GB on serum levels of TNF-α and IL-10

GB treatment gave different effect to parasitemia, TNF- α and IL-10 levels (Figure2). The TNF-α level was high (≥40 pg/mL) in almost all mice treated with GB either infected or uninfected mice as well as in DHP-treated mice. Interesting results were seen in the IL-10 level, where GB100 caused low level of IL-10 (21.69 pg/mL), but GB25 (46.28 pg/mL) and GB50 (31.28 pg/mL) caused higher level of IL-10. When GB was not given (NEG), resulted in high parasitemia and caused high level of TNF-α (>40 pg/mL) but moderate IL-10 level (21-45.98 pg/mL). However statistical comparison of NEG with other groups, a significant different was only seen in NOR which completely different in TNF-α (p=0.004). The IL-10 level in NEG was only different significantly with GB100 (p=0.004). In infected mice treated with GB100, where parasitemia was suppressed to low level (4.23%) affected TNF-α to the lowest level among GB-treated mice, while IL-10 was low. TNF-α level in GB100 was different significantly with those in GB25 (p=0.016), GB50 (p=0.018) and POS (p=0.025) groups. While IL-10 level in GB100 was different significantly with those in GB25 (p=0.004), GB50 (p=0.045), NEG (p=0.004), NOR (p=0.004), NOR25 (p=0.010).

Different result was seen in GB25 and GB50 groups, both treatments suppressed parasitemia to be moderate resulted in different level of high level of TNF-α, and high level of IL-10, but GB50 caused moderate levels of IL-10. Serum level of TNF-α and IL-10 in between GB25 and GB50 was different insignificantly (p>0.05). Mice treated with DHP (POS) showed 0% parasitemia, however TNF-α was high and low level of IL-10 (≤11.25 pg/mL). Serum level of TNF-α in POS was different significantly with GB100 (p=0.025), NOR (p=0.004) and NOR50 (p=0.025), however no groups was significantly different in IL-10 level with POS group. Uninfected mice treated with GB treatment resulted in moderate to high level of TNF-α but low level of IL-10. Uninfected mice treated with GB25 resulted in moderate level of TNF-α and low level of IL-10. Different result in NOR100, proved the100% GB caused high level of TNF-α, but IL-10 was also low. TNF-α in NOR100 was different significantly with those in GB100 (p=0.006), NOR (p=0.004) and NOR50 p=0.016), while in IL-10 level there was no group of mice was different significantly with NOR100. This categorization was only applied for the results in this study, other study may result in different categorization.

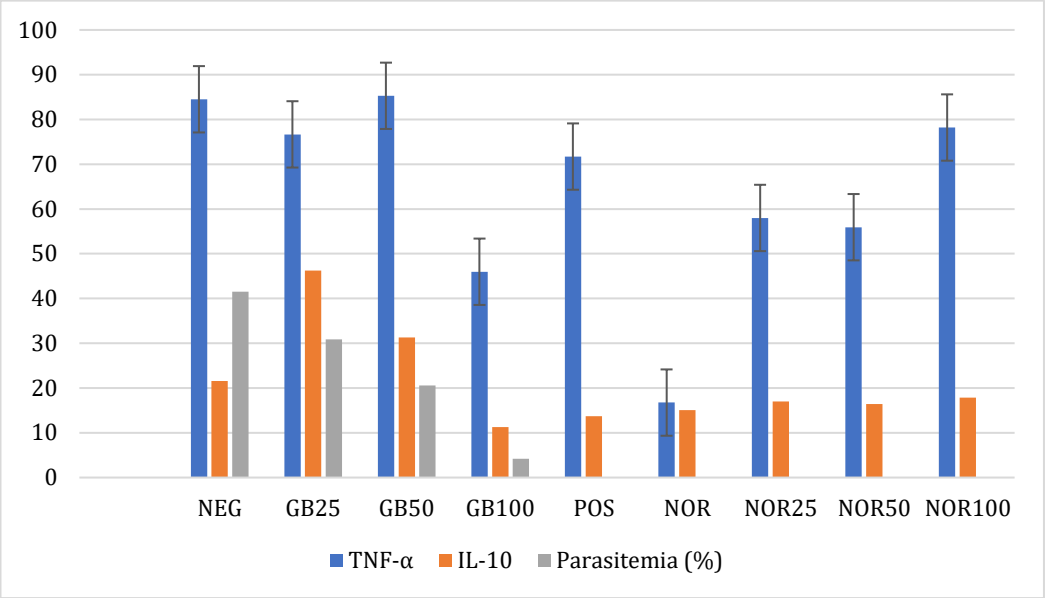

FIGURE 2: TNF-α and IL-10 levels of *P. berghei*-infected mice and normal mice treated with GB25, GB50, GB100 compared with that of positive control (POS), negative control (NEG), and normal mice (NOR). GB25: 25% goat bile; GB50: 50% goat bile; GB100: 100% goat bile; POS: 187,2 mg/kg body weight dihydroartemisinin-piperaquin; NOR: uninfected mice; NEG: infected mice were given only sterile water.

DISCUSSION

The effect of GB treatment to physical condition of mice did not show any damage effect to both infected and uninfected mice. Mild diarrhea was the only symptom occurred which only within two days in mice treated with GB, indicated mild toxicity of GB as reported before, where the increased of the number of mouse suffering from mild diarrhea but the increase of survival rate (5). The death of one mouse in GB100 group at the end of experiment indicated a very low mortality and the safety of GB treatment in mice, at least within 4-day treatment. Furthermore, the increased of GB concentration affected the level of parasitemia and curative effect of GB compared with NEG as shown in Table 1. Both parasitemia and curative effect of GB against *P. berghei* ANKA infection in BALB/c mice were concentration dependent manner. Among the concentration of GB given to the infected mice, GB100 showed the highest therapeutic effect in infected mice. However, the GB25 and GB50 were more effective in increasing the antiinflammation response against infection. The toxicity of GB was seen in uninfected mice treated with GB (GB25, GB50, GB100) by increasing the level of TNF-α. This may relate to the intestinal toxicity that occurred several days post GB treatment.

P. beghei infection in mice has long been used to study the immune response to malaria infection and further understand the development of immunity as occurs in human malaria. However, no report on the effect of animal bile on the host's immunity. Research on the use of bile acids on humoral immunity, especially immunoglobulin production, is still limited to in vitro research, such as immunoglobulin production by peripheral blood mononuclear cells (PBMC) [12] or by mesenteric lymph node (MLN) lymphocytes [13]. Bile acids, both hydrophobic chenodeoxycholic acid (CDCA), and hydrophilic ursodeoxycholic acid (UDCA) have been known to inhibit the production of immunoglobulin M (IgM), IgG, by PBMC in vitro [14,15], and inhibits IgE and IgA by MLN lymphocyte in vitro [13]. This current study proved the GB treatment suppressed IgG production in vivo.

Mice infected with *P. berghei* ANKA and uninfected mice treated with GB100, GB50, and GB25 showed that all concentrations inhibited IgG production by the mice. Although, malaria infection triggers increased levels of IgG, but by GB treatment, the IgG production is suppressed as it is almost the same as in normal mice. Suppression of IgG production is ruled out by UDCA. Although, UDCA as a drug has been reported to increase bile flow, change the hydrophobicity index of bile acid pools, disrupts cholesterol gallstones, and for primary biliary cirrhosis (PBC) which has been approved by the Food and Drug Administration (FDA), UDCA also has the effect of suppressing host immunity [16].

The GB treatment affected the increased of serum levels of TNF-α significantly in both infected and uninfected mice treated with GB compared with that of uninfected mice without GB treatment (NOR). Moderate levels of TNF-α in normal mice treated with GB indicated the toxicity of GB. When compared with NEG it was different insignificantly. This result indicated that the increased of serum levels of TNF-α in infected mice also caused by malaria infection as supported by statistical analysis that TNF-α correlated with parasitemia ($p=0.018$). The highest concentration of GB, GB100, caused moderate serum level of TNF-α in infected mice. These results related to the percentage parasitemia caused by GB100 which reached to the lowest compared with those treated with GB25 and GB50. The ruptured schizont stage in malaria release GPI that leads host cells to release pro-inflammatory cytokine such as TNF-α [17]. This cytokine has both beneficial and detrimental roles during malaria infection [13]. Respond of cytokine type 1 by Th1 cells controls early parasitemia. The optimal TNF-α levels will trigger parasitic clearance to prevent high parasitemia, but excessive levels of TNF-α will lead to various complications such as occurs in severe malaria syndromes, including cerebral malaria and multi-organ failure [14,15, 16]. The high serum level of TNF-α in GB-treated uninfected mice indicated the pro-inflammatory response of mice, that may relate to the intestinal inflammation in mice since GB treatment caused diarrhea.

The effect of GB treatment to the serum level of IL-10 among *P. berghei* ANKA-infected mice was decreased along with the increased of concentration of GB (Figure 1), and supported by statistical analysis which was different significantly among GB-treated infected mice. Anti-inflammatory cytokine, IL-10, plays a role in protection and pathology against malaria infection [17]. This cytokine is necessary for suppressing severe pathology during *Plasmodium* infection [15]. High concentrations of IL-10 inhibits the development of malaria by controlling the effects of excessive serum TNF-α [12]. In this study, the highest serum level of IL-10 with the high serum level of TNF-α was seen in GB25-treated mice. In this case, the GB25 was able to control the multiplication of parasite and triggered the cytokine productions tend to balance. The high level of both TNF-α and IL-10 protect the host against asexual stages of malaria parasites and the development of severe clinical symptoms [17]. Serum level of IL-10 in GB25- and GB50-treated infected mice showed a host defense response not only against infection but also against GB treatment. The high serum level of TNF-α with low serum level of IL-10 associated with severe disease [18] as seen in NEG. In contrast, the low level of IL-10 in GB100-treated mice and POS associated with the low level of parasitemia. The GB100 treatment were able to kill parasites and suppressed the serum level of TNF-α to moderate, however, unable to increase the serum level of IL-10 similarly it occurred in POS. Low level of IL-10 lead to the increased production of nitric oxide, which influenced erythropoiesis in vivo and has been implicated in pathogenesis of anemia in malaria [19]. Bile acids has also been reported to induce synthesis and secretion of inflammatory cytokines such as TNF-α and IL-1β by Kupffer cells and repress CYP7A1 expression in mouse hepatocytes and macrophage cell line [20]. The balance of pro- and anti-inflamatory cytokine responses is important than absolute level of any single cytokine. Ratio of pro-inflamatory cytokines to IL-10 is consistently correlated with increasing hemoglobin concentration [21]. The nature and outcome of malaria infection are governed by the balance of pro-inflammatory and anti-inflammatory immune response [22].

Bile contains various compounds, including amino acids, steroids, enzymes, cholesterol, bile salts, bilirubin, phospholipids, vitamins, heavy metal, and environmental toxins. Bear bile was one of animals' bile which has been used to reduce inflammation, fever, and liver diseases. Bear bile was also used for infectious diseases such as ascariasis and oxyuriasis [23]. Bile acids of bear bile are in the form of taurine conjugate, such as taurochenodeoxycholic acids (TCDCA), taurodeoxycholic acids (TDCA), tauroursodeoxycholic acids (TUDCA), and taurocholic acids (TCA) [24]. Bear bile's composition, however, is not constant; rather, it varies depending on a number of circumstances, including the season, species, physical condition, and current style [25]. Therefore, in this study the male goat of Java strain was chosen to minimize the difference in component of GB.

Bile acid has known to have a major role in therapeutic actions. Bile acids have a unique amphipathic property due to their hydrophobic and hydrophilic side on their component that may play dual role. Hydrophobic acid such as deoxycholic acid (DCA) can cause damage of the cell membrane [26], while hydrophilic acids such as ursodeoxycholic acid (UDCA) and tauroursodeoxycholic acid (TUDCA) are able to reverse the effect [27] and protect against toxicity of hydrophobic bile acids [28]. In this study both bile acids act synergistically, where in one side hydrophobic bile acids killed the parasite and in other side hydrophilic bile acids protect the damage of erythrocytes and other cells that led the increased of curative effect of GB. This property may explain why some people of Indonesia consume whole gall bladder to increase their stamina and prevent and treat malaria infection. However further investigation is needed to explain this phenomenon.

Limitation of the study

The small volume of GB has limited this study. The GB from several goat gallbladders must be pooled to obtain sufficient volume to perform one set of in vivo tests. Due to different content of different type of goat, therefore, the same gender and strain of goat was chosen to minimize the difference of GB content.

CONCLUSION

Indonesian traditional antimalarial medicine, GB, was able to cure malaria in concentration dependent manner in curative test. All concentration of GB suppressed IgG production in mice infected with *P. berghei* ANKA. The GB100 was more therapeutic compared with that of GB50 and GB25 which were more likely to increase the anti-inflammatory response against infection by increasing level of IL-10. Since component of GB possess both toxic and beneficial properties, and learnt from the results of this study suggested that GB could be one of the potential sources for new anti-malarial therapy, however, the toxicity of GB should be considered seriously as the contradictive effect against therapeutic effect of GB. Further investigation is recommended to develop new anti-malarial drug candidates based on the unique property of GB.

ACKNOWLEDGMENT

We thank Mr. Hery and Mr. Choirul who have helped in taking care of and sacrificing the mice as well as Mr. Iswahyudi for the ELISA.

REFERENCES

[1] White N. Severe malaria. Malar J. 2022;21(284):1–8.

[2] Laishram DD, Sutton PL, Nanda N, Sharma VL, Sobti RC, Carlton JM, et al. The complexities of malaria disease manifestations with a focus on asymptomatic malaria. Malar J [Internet]. 2012;11(1):29. Available from: http://malariajournal.biomedcentral.com/articles/10.1186/1475-2875-11-29

[3] WHO. World Malaria Report 2022 [Internet]. Geneva; 2022. Available from: https://www.mmv.org/sites/default/files/uploads/docs/publications/World Malaria Report 2022.pdf

[4] P2PM Ke kes RI. Informasi Malaria Tahun 2022 [Internet]. 2023 [cited 2023 Sep 15]. Available from: https://p2pm.kemkes.go.id/publikasi/infografis/informasi-malaria-tahun-2022

[5] Arwati H, Hapsari WT, Wardhani KA, Aini KN, Bahalwan RR, Wardhani P, et al. Acute and subacute toxicity tests of goat bile in BALB/c mice. Vet World. 2020

[6] Arwati H, Bahalwan R, Hapsari W, Wardhani K, Aini K, Apsari P, et al. Suppressive effect of goat bile in Plasmodium berghei ANKA infection in mice. Vet World. 2021;14(8):2016–22.

[7] Taek M, Banilodu L, Neonbasu G, Watu Y, Prayogo B, Agil M. Ethnomedicine of Tetun ethnic people in West Timor Indonesia: philosophy and practice in the treatment of malaria. Integr Med Res. 2019;8(3):139–44.

[8] Wang DQ-H, Carey MC. Therapeutic uses of animal biles in traditional Chinese medicine: An ethnopharmacological, biophysical chemical and medicinal review. World J Gastroenterol. 2014;20(29):9952–9975.

[9] Aini KN, Hapsari WT, Wardhani KA, Arwati H, Sandhika W. Antimalaria activity of goat bile against Plasmodium berghei ANKA infection in BALB/c mice. J Kedokt Qanun Med. 2020;04(02).

[10] Adame-Gallegos J, Shi J, McIntosh R, Pleass R. The generation and evaluation of two panels of epitope-matched mouse IgG1, IgG2a, IgG2b and IgG3 antibodies specific for Plasmodium falciparum and Plasmodium yoelii merozoite surface protein 1-19 (MSP1(19)). Exp Parasitol. 2012;130(4):384–393.

[11] Kwiatkowski D, Hill A, Sambou I, Twumasi P, Castracane J, Monague K, et al. TNF concentration in fatal cerebral, non-fatal cerebral, and uncomplicated plasmodium falciparum malaria. Lancet. 1990;336(8725):1201–1204.

[12] Penha-Gonçalves C. Genetics of malaria inflammatory responses: a pathogenesis perspective. Front Immunol. 2019;10(1771):1–151.

[13] do Rosario A, Langhorne J. T cell-derived IL-10 and its impact on the regulation of host responses during malaria. Int J Parasitol 42549–55. 2012;42:549–55.

[14] Ryley J, Peters W. The antimalarial activity of some quinoline esters. Ann Trop Med Parasitol. 1970;84:209–22.

[15] Adebajo AC, Odediran SA, Abosede F, Nwafor AP., Nwoko N and, Umana US. In Vivo Antiplasmodial Potentials of the Combinations of Four Nigerian Antimalarial Plants. Molecules. 2014;19(9):13136–13146.

[16] Correia L, Podevin P, Borderie D, Verthier N, Montet J, Feldmann G, et al. Effects of bile acids on the humoral immune response. A mechanistic approach. Life Sci. 2001;69:2337–2348.

[17] Lim B, Yamada K, Sugano M. Effects of bile acids and lectins on immunoglobulin production in rat mesenteric lymph node lymphocytes. Vitr Cell Dev Biol. 1994;30A:407–13.

[18] Yamada K, Lim B, Sugano M. Suppression of immunoglobulin production of rat lymphocytes by bile acids. Vitr Cell Dev Biol. 1993;29A:840–841.

[19] Yoshikawa M, Tsujii T, Matsumura K, Yamao J, Matsumura Y, Kubo R, et al. Immunomodulatory effects of ursodeoxycholic acid on immune response. Hepatology. 1992;16:358–364.

[20] Kotb M. Molecular Mechanisms of Ursodeoxycholic Acid Toxicity & Side Effects: Ursodeoxycholic Acid Freezes Regeneration & Induces Hibernation Mode. Int J Mol Sci. 2012;13(7):8882–8914.

[21] Arinola O, Onubogu D, Salimonu L. Spleen Weight, Liver Weight And Levels Of Circulating Immune Complexes In Vitamin Deficient Mice Infected With Plasmodium berghei. African J Clin Exp Microbiol. 2005;6(2):95–9.

[22] Depinay N, Franetich JF, Grüner AC, Mauduit M, Chavatte JM, Luty AJF, et al. Inhibitory effect of TNF-α on malaria pre-erythrocytic stage development: Influence of host hepatocyte/parasite combinations. PLoS One. 2011;6(3):e17464.

[23] May J, Lell B, Luty AJF, Meyer CG, Kremsner PG. Plasma Interleukin-10:Tumor Necrosis Factor (TNF)–α Ratio Is Associated with TNF Promoter Variants and Predicts Malarial Complications. J Infect Dis. 2000;182(5):1570–3.

[24] Kumar R, Ng S, Engwerda C. The role of IL-10 in malaria: A double edged sword. Front Immunol. 2019;10:229.

[25] Ihuarulam OC, Obiora AC, Chukwuebuka IF, Francis IU, Kenneth OK, Ikechukwu NR, et al. The Relationship Between the Ratio of Interleukin-10(IL-10) and Tumor Necrosis Factor (TNF) with Plasmodium falciparum Density in Nigerian Children. Eur J Prev Med. 2018;6(14):58–63.

[26] Angulo I, Fresno M. Cytokines in the Pathogenesis of and Protection Against Malaria. Clin Diagn Lab Immunol. 2002;9(6):1145–52.

[27] Perera MK, Herath NP, Pathirana SL, Phone-Kyaw M, Alles HK, Mendis KN, et al. Association of High Plasma TNF-alpha Levels and TNF-alpha/IL-10 Ratios With TNF2 Allele in Severe P. Falciparum Malaria Patients in Sri Lanka. Pathog Glob Heal. 2013;107(1):21–9.

[28] Kurtzhals JA, Adabayeri V, Goka BQ, Akanmori BD, Oliver-Commey JO, Nkrumah FK, et al. Low Plasma Concentrations of Interleukin 10 in Severe Malarial Anaemia Compared With Cerebral and Uncomplicated Malaria. Lancet. 1998;35 1768-72(9118):1768–72.

[29] Li T, And AJ, Chiang JYL. Bile acids and cytokines inhibit the human cholesterol 7α-hydroxylase gene via the JNK/c-Jun pathway. Hepatol c. 2006;43(6):1202–1210.

[30] Dodoo D, Omer F., Todd J, Akanmori B., Koram K., Riley E. Absolute Levels and Ratios of Proinflammatory and Anti-Inflammatory Cytokine Production In Vitro Predict Clinical Immunity to Plasmodium falciparum Malaria. J Infect Dis. 2002;185:971–9.

[31] Medeiros MM, Da Silva HB, Reis AS, Barboza R, Thompson J, Lima MRDI, et al. Liver accumulation of Plasmodium chabaudi-infected red blood cells and modulation of regulatory T cell and dendritic cell responses. PLoS One. 2013.

[32] Wang DQH, Carey MC. Therapeutic uses of animal biles in traditional Chinese medicine: An ethnopharmacological, biophysical chemical and medicinal review. World Journal of Gastroenterology. 2014.

[33] Li S, Tan HY, Wang N, Hong M, Li L, Cheung F, et al. Substitutes for bear bile for the treatment of liver diseases: Research progress and future perspective. Evidence-based Complementary and Alternative Medicine. 2016.

[34] Li S, Tan HY, Wang N, Hong M, Li L, Cheung F, et al. Substitutes for Bear Bile for the Treatment of Liver Diseases: Research Progress and Future Perspective. Evid Based Complement Altern Med. 2016;2016.

[35] Zhou Y, Doyen R, M.Lichtenberger L. The role of membrane cholesterol in determining bile acid cytotoxicity and cytoprotection of ursodeoxycholic acid. Biochim Biophys Acta. 2009;1788(2):507–13.

[36] Solá S, Brito MA, Brites D, Moura JJG, Rodrigues CMP. Membrane Structural Changes Support the Involvement of Mitochondria in the Bile Salt-Induced Apoptosis of Rat Hepatocytes. Clin Sci 2475-85. 2002;103(5):2475–85.

[37] Rodrigues C, Steer C. Mitochondrial membrane perturbations in cholestasis. J Med Chem. 2012;55(33):10662-10673.