ORIGINAL ARTICLE

The Effect of Goat Bile on Parasitemia, IL-12, and IL-6 Levels in Mice Infected With *Plasmodium berghei* ANKA

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ABSTRACT

Introduction: Malaria remains a major global health concern, and increasing resistance to current antimalarial drugs highlights the need to explore alternative therapies. Goat bile (GB) has traditionally been used by some Indonesian communities to improve stamina and treat malaria. This study aimed to investigate the effects of GB on parasitemia and immune responses, particularly interleukin-12 (IL-12) and interleukin-6 (IL-6) levels, in mice infected with *Plasmodium berghei* ANKA. **Methods:** Fifty male BALB/c mice were divided into healthy and P. berghei-infected groups. Both groups received either 50% goat bile (GB50) or no treatment. GB50 was administered orally at 0.5 mL per 20 g body weight for four consecutive days, starting three days post-infection. A positive control group received dihydroartemisinin–piperaquine at 187.2 mg/kg body weight. Parasitemia was monitored daily, while serum IL-6 and IL-12 levels were measured after treatment using ELISA. Data were analyzed using Kruskal–Wallis, Mann–Whitney post hoc, and Spearman's correlation tests. **Results:** GB50 significantly reduced parasitemia in *P. berghei*-infected mice ($p < 0.05$). GB50 treatment increased IL-6 levels in both healthy and infected mice. In contrast, IL-12 levels were significantly lower in the Pb+GB50 group than in the untreated infected group. A similar pattern of increased IL-6 and reduced IL-12 was observed in healthy mice receiving GB50. **Conclusion:** GB50 demonstrated antiparasitic and immunomodulatory effects by reducing parasitemia, increasing IL-6, and decreasing IL-12 levels. These findings suggest that GB50 may regulate immune responses while reducing parasite burden.
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Keywords: Malaria, goat bile, parasitemia, IL-6, IL-12

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INTRODUCTION

Malaria is a life-threatening parasitic disease caused by Plasmodium species and transmitted by Anopheles mosquitoes (1,2). The selection of in vivo research using *Plasmodium berghei* is the most frequently used model in malaria studies including the disease's mechanisms and develop potential treatments because its life cycle and disease symptoms are similar to *Plasmodium* species that infect humans (3). Malaria is an extremely inflammatory disease. Strong host inflammation is caused by the parasite infection, as evidenced by the activation of inflammatory pathways and increased levels of pro-inflammatory cytokines. This inflammation plays a significant role in the pathophysiology of malaria, exacerbating the disease severity and consequences. The initiation of the inflammatory and immunological responses results in noticeable symptoms of malaria. Initially, the inflammatory response helps to alleviate and control the infection (4).

Antimalarial drugs can influence the immune response; in some cases, they may impair it. However, these drugs are generally not regarded as overtly immunosuppressive. While they typically do not suppress the immune system as a whole, they can interfere with the immune response to specific pathogens (5). Despite global efforts to control malaria, the emergence of drug-resistant strains continues to hinder eradication programs (6). The search for alternative therapeutic agents, particularly from natural sources, has gained momentum in recent years (7), mostly from medicinal plants. Bile from various animals has been used for centuries to treat infectious diseases including malaria as well as non infectious diseasei in Traditional Chinese Medicine (TCM), while

GB is utilized less frequently. According to Chinese materia medica, GB was used medicinally in China for the treatment of optic atrophy, acute hemorrhagic conjunctivitis, and other infectious skin diseases (8–10). GB is a traditional remedy used by some Indonesian people for its perceived ability to improve stamina and treat malaria (10). Previous investigations have reported its potential antimalarial activities (11), and suppressive effect in mice infected with *P. berghei* (2). GB does not only cause therapeutic effect but also immunological effect in *P. berghei* infection. Previous studies have reported that the administration of high-dose of 100% goat bile (GB100) in mice infected with *P. berghei* was more therapeutic compared to medium-dose, 50% GB (GB 50%) and low-dose, 25% GB (GB 25). The immunological mechanisms that contribute to the antimalarial effects of GB are still not well understood. The only research addressing the immunological effects of GB focused on the levels of TNF-α and IL-10 in mice infected with *P. berghei* ANKA (12), while the influence on key pro-inflammatory cytokines involved in malaria immunopathogenesis, particularly IL-12 and IL-6 has not been reported. Immunological effect of GB100 was significantly reduced TNF-α levels, indicating an anti-inflammatory effect. This decrease was accompanied by relatively low IL-10 levels, indicating that GB100 is able to reduce inflammation without triggering excessive anti-inflammatory compensation. Conversely, administration of low-dose GB (GB25) caused a fairly high increase in IL-10 levels, but TNF-α remained high, indicating an inflammatory response that was not well controlled. Medium-dose GB (GB 50) showed an intermediate profile between GB25 and GB100(12). Therefore, GB50 was used in this current study to find out the inflammatory effect of GB in mice infected with *P. berghei*.

Cytokines such as interleukin-6 (IL-6) and interleukin-12 (IL-12) play critical roles in shaping the immune response against Plasmodium infection (13,14). IL-12 is a key cytokine produced by dendritic cells and macrophages represents early innate immune activation following pathogen associated molecular pattern (PAMP) recognition, and crucial for initiating Th1-type immunity (15). In contrast, IL-6 is a pleiotropic cytokine that involved in both innate and adaptive immunity, serving as a bridge between early inflammatory responses and subsequent immune regulation, and involved in both the progression and severity of the disease (13). This study aimed to evaluate the effects of GB50 on parasitemia and serum levels of IL-6 and IL-12 in *P. berghei* ANKA-infected mice. The goal was to investigate whether GB50 influences the immune response in a manner that contributes to its antimalarial activity.

MATERIALS AND METHODS

Animals and Ethics Statement

Fivety five male BALB/c mice aged 6–8 weeks and weighing 20–25 grams were obtained from a licensed laboratory animal supplier. Five mice were used as donor mice, and 50 mice were used as tests mice. The animals were housed under standard laboratory conditions with ad libitum access to food and water. All procedures were conducted following ethical guidelines and approved by the Health Research Ethics Committee of Faculty of Medicine, Universitas Airlangga, as described on the Ethical Clearance No. 79/EC/KEPK/FKUA/2024.

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Preparation of GB Solution

GB used in this study was prepared as described (10). Fresh GB was collected aseptically from five gallbladders of healthy goats (*Capra aegagrus hircus*) and diluted in sterile distilled water to a final concentration of 50% (GB50). The used of GB50 was based on our previous study that GB50 demonstrated an intermediate immunological profile between GB25 and GB100 (12); therefore, GB50 was selected in the present study to further investigate inflammatory responses during *P. berghei* infection. The solution was stored at 4°C before and during experiment.

Experimental Design

This study employed a post-test only control group design in which outcome variables were measured at post treatment and compared across experimental groups. The frozen *P. berghei* ANKA-infected blood were obtained from Institute of Tropical Diseases (ITD) Universitas Airlangga. The parasites of *P. berghei* ANKA was maintained through serial passage in BALB/c mice. Prior to the test mice preparation, 5 mice donors were injected intraperitoneally with 200 µL/mouse of frozen infected blood. When parasitemia reached ± 20%, mice were sacrificed, blood were collected and injected 1 x 10^6 parasitized red blood cells intraperitoneally to each mouse in Pb+DHP, Pb+GB50, and Pb groups. The treatment of GB50 was given 0.5 mL per 20 g body weight of mouse, and was administered orally once daily for 4 consecutive days starting on three days post infection. Mice were randomly divided into five groups (n = 10 per group) as follows, and the timeline schedule is displayed in Fig. 1.

Groups	Treatment
Pb+DHP	*P. berghei* infection + Dihydroartemisinin piperaquin 187.2 mg/kg body weight
Pb+GB50	*P. berghei* infection + GB 50%
Pb	*P. berghei* infection, no treatment
H+GB50	Healthy mice + GB 50%
H	Healthy mice, no treatment

Parasitemia Monitoring

Parasitemia was monitored daily via Giemsa-stained thin tail blood smears during the course of infection. A minimum of 1,000 red blood cells were counted per slide to calculate the percentage of infected erythrocytes (2).

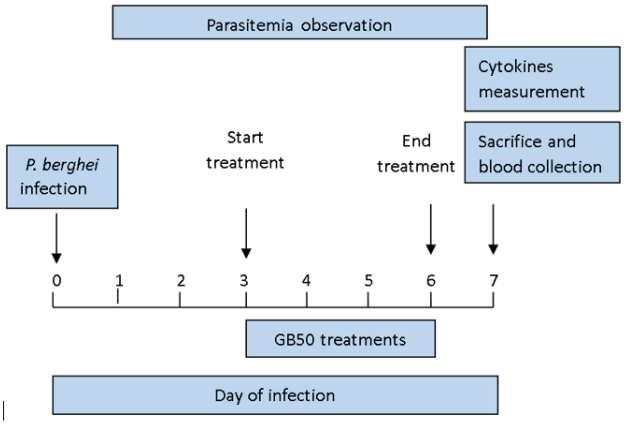

Figure 1: Experimental timeline of *P. berghei* infection and GB50 treatment. Mice were infected with *P. berghei* on day 0. Mice were treated with GB50 for four consecutive days from day 3 to day 6 post infection. Mice were sacrifice on day 7 post infection after completion of the 4-day GB50 treatment for blood collection and cytokines measurement. Parasitemia was observed daily on Giemsa-stained thin tail blood smears during the course of infection.

Cytokine Analysis

Mice were sacrificed after completion of the 4-day treatment period, corresponding to day 7 post-infection, under ketamine anesthesia, for blood collection and cytokine analysis. Blood was collected via cardiac puncture, and serum was isolated. Serum levels of IL-6 and IL-12 were quantified using commercial ELISA kits following the manufacturer's instructions. The measurements of cytokines were done at the end of the treatment period to assess cytokine responses following GB50 administration and to allow comparison between treated and control groups

Statistical Analysis

Data analysis was carried out using SPSS for windows software. Data normality was assessed using the Shapiro–Wilk test. As the data were not normally distributed, the Kruskal–Wallis non-parametric test was applied, followed by Mann–Whitney post-hoc comparisons. Data are presented as mean ± SD, and a p-value < 0.05 was considered statistically significant. Correlation between parasitemia and the IL-12 and IL-6 levels was analysed using Spearman's rank correlation test to assesses the strength and direction of a relationship between those two variables. Differences and correlations between variables are considered significant when the p-value is less than 0.05 ($p<0.05$).

RESULTS

Observational toxicity assessment of GB50 in mice

Physical observation of GB50-treated mice showed that mild diarrhea occurred in non-infected mice (H+GB50 group), consistent with previous reports (10). However, the diarrhea was transient and no mortality related to GB administration was observed. In the present study, GB50-treated *P. berghei*-infected mice showed a 100% survival rate during the observation period. This finding differs from a previous study reporting an 80% survival rate in GB50-treated mice (2). Overall, no overt signs of severe toxicity were observed during the experimental period.

Effect of GB on Parasitemia

Administration of GB50 significantly reduced parasitemia levels in *P. berghei*-infected mice (Pb+GB50) compared to the untreated infected mice (Pb Group) ($p = 0.009$). The Pb+GB50 showed an increase in the percentage of parasitemia on day 1 and 2 post treatment initiation, and decreased on day 3 to reach 1.50% on day 4 post treatment initiation. Although the onset of parasitemia reduction was slower compared to the dihydroartemisinin-piperaquine (DHP) treatment group, the overall decline remained consistent across the treatment period, indicating notable antimalarial activity of GB50 (Figure 2). Statistical analysis of the differences of parasitemia at the end of a 4-day treatments showed a significant difference in parasitemia between the Pb+DHP Group and Pb+GB50 Group ($p = 0.035$), as well as between the Pb+DHP Group and the Pb Group ($p = 0.005$).

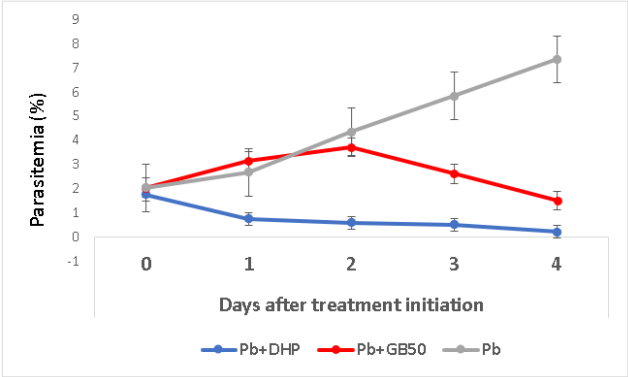

Figure 2: Mean percentage of parasitemia during the four-day treatment period. Data are presented as mean ± SD. Pb+DHP Group: Pb infection + dihydroartemisinine-Piperaquine; Pb+GB50 Group: P. berghei infection + GB 50%; Pb Group: P. berghei infection, no treatment; H+GB50 Group: Healthy mice + GB 50%; H Group: Healthy mice, no treatment.

Effect of GB on IL-12 and IL-6 Levels

The serum level of cytokines in all groups of mice is shown in Figure 3 and Figure 4. Serum IL-12 levels differed among the experimental groups (Figure 3). The untreated infected group (Pb) showed the highest IL-12 level compared with the other groups. Mann–Whitney analysis demonstrated that IL-12 levels in the Pb group were significantly higher than those in the Pb+GB50 group ($p = 0.047$), Pb+DHP group ($p = 0.009$), and H+GB50 group ($p = 0.016$). IL-12 levels in the Pb group were also significantly higher than those in the healthy control group (H) ($p = 0.009$). No significant differences were observed between the Pb+GB50 and Pb+DHP groups or between the treated groups and healthy controls. These results indicate that malaria infection was associated with increased IL-12 production, while treatment with GB50 or DHP was associated with lower

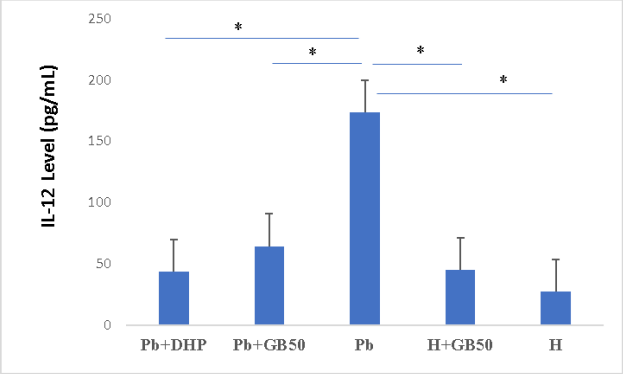

Figure 3: The mean IL-12 levels after completion of the 4-day treatment period. Pb+DHP Group: Pb infection + dihydroartemisinine-Piperaquine; Pb+GB50 Group: *P. berghei* infection + GB 50%; Pb Group: *P. berghei* infection, no treatment; H+GB50 Group: Healthy mice + GB 50%; H Group: Healthy mice, no treatment. Data are presented as mean ± SD. Statistical differences between groups were analyzed using the Mann–Whitney test. $p < 0.05$ was considered statistically significant. Asterisks indicate statistically significant differences between groups (*$p < 0.05$).

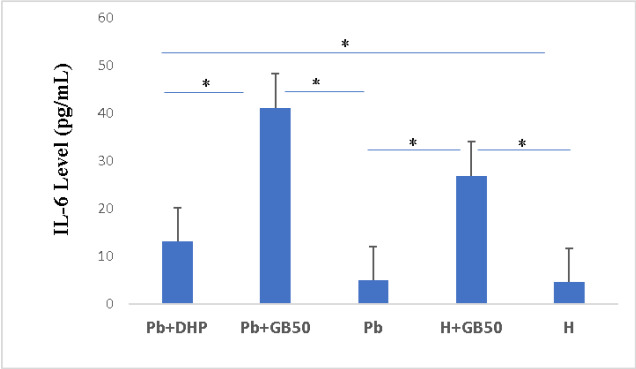

Figure 4: The mean IL-6 levels after completion of the 4-day treatment period. Pb+DHP Group: Pb infection + dihydroartemisinine-Piperaquine; Pb+GB50 Group: *P. berghei* infection + GB 50%; Pb Group: *P. berghei* infection, no treatment; H+GB50 Group: Healthy mice + GB 50%; H Group: Healthy mice, no treatment. Data are presented as mean ± SD. Statistical differences between groups were analyzed using the Mann–Whitney test. $p < 0.05$ was considered statistically significant. Asterisks indicate statistically significant differences between groups (*$p < 0.05$).

IL-12 levels compared with the untreated infected group.

Mann–Whitney analysis showed that IL-6 levels in the Pb+GB50 group were significantly higher than those in the Pb group ($p = 0.009$) and Pb+DHP group ($p = 0.047$). IL-6 levels in the Pb+GB50 group were also significantly different from the healthy control group ($p = 0.016$). In healthy mice, administration of GB50 (H+GB50) resulted in significantly higher IL-6 levels compared with both the Pb group ($p = 0.009$) and the untreated healthy control group ($p = 0.016$). No significant differences were observed between the Pb+DHP group and the other groups.

Correlation between cytokine levels and parasitemia

Correlation analysis using Spearman test between high level of IL-6 and parasitemia resulted in an insignificant correlation ($p = 0.270$, $r = -0.304$). However, a strong significant and positive direction correlation was seen between IL-12 and parasitemia ($p = 0.001$, $r = 0.821$).

DISCUSSION

This study demonstrates that GB50 possesses not only antimalarial activity but also potential immunomodulatory properties in *P. berghei*-infected mice. The significant difference in parasitemia between the Pb+GB50 group and the untreated infected group indicates that GB50 has an antiparasitic effect against *P. berghei*. Although the reduction in parasitemia was not as rapid as that observed in the DHP-treated group, the consistent decline in parasite levels suggests that components within GB may interfere with parasite growth or survival. These findings support the hypothesis that GB contains bioactive compounds with antimalarial potential (2).

One possible explanation for the inhibitory effect of GB on parasite multiplication involves the complex composition of bile acids. Hydrophobic bile acids such as deoxycholic acid (DCA) are known to disrupt cellular membranes by increasing lipid polarity and membrane fluidity (16,17), which may contribute to parasite damage. In contrast, hydrophilic bile acids such as tauroursodeoxycholic acid (TUDCA) and ursodeoxycholic acid (UDCA) may counteract the cytotoxic effects of hydrophobic bile acids by exerting protective and stabilizing actions (8,18). Additionally, bile acids may influence the parasite's acidic food vacuole by increasing the intracellular pH. Since hemoglobin digestion by malaria parasites depends on an acidic environment, alkalinization of the food vacuole may interfere with parasite metabolism. This mechanism is conceptually similar to the action of chloroquine, which disrupts hemoglobin digestion by altering the pH of the parasite food vacuole (19,20).

The serum level of cytokines in all groups of mice is shown in Fig.3 and Fig.4 The effect of GB50 as an immunomodulator can be observed in healthy mice treated with GB (H+GB50 group) compared with those of untreated healthy mice (H group), where IL-12 levels were low while IL-6 levels were elevated. A similar pattern was also observed in the Pb+GB50 group. This finding suggests that bile components in GB50 may stimulate mild inflammatory signaling even in the absence of infection. In contrast, the low IL-12 level may indicate reduced activation of macrophages and dendritic cells following GB50 administration. However, the presence of parasites resulted in a different immunomodulatory profile after GB50 treatment. In the Pb+GB50 group, serum IL-12 levels were significantly lower than those in untreated infected mice (Pb group), indicating that GB50 modulates the immune response during malaria infection.

IL-12 is a key cytokine involved in the early immune response to malaria infection, particularly through the induction of Th1 responses and IFN-γ production (14). In the present study, IL-12 levels were highest in the untreated infected group (Pb group), indicating activation of pro-inflammatory immune responses during *P. berghei* infection. Elevated IL-12 levels during malaria infection have been widely reported and are associated with activation of macrophages and dendritic cells following parasite recognition (21). Interestingly, IL-12 levels in the Pb+GB50 group were significantly lower than those in untreated infected mice (Pb group), and a similar reduction was observed in the Pb+DHP group. This finding suggests that treatment, either with conventional antimalarial drugs or with GB50, may reduce inflammatory stimulation during infection. Therefore, the lower IL-12 levels observed in treated groups likely reflect decreased immune activation following partial parasite control rather than direct suppression of protective immune responses.

It has been reported that reduced IL-12 levels in human malaria are associated with severe disease and impaired immune responses (14). However, in the present study, the decrease in IL-12 levels was observed together with reduced parasitemia and improved survival in GB50-treated mice. This suggests that the reduction in IL-12 is more likely due to decreased parasite-driven inflammatory stimulation rather than impaired immune function. Therefore, the observed cytokine profile does not indicate severe malaria, but rather reflects a modulation of immune responses following parasite reduction.

IL-6 is a pleiotropic cytokine involved in both inflammatory signaling and immune regulation (22). In this study, IL-6 levels were elevated in mice treated with GB50, both in infected and non-infected groups. The increased IL-6 levels observed in the Pb+GB50 group compared with Pb group suggest that GB50 administration may influence inflammatory signaling during *P. berghei* infection. IL-6 participates in early host defense by promoting immune cell activation and coordinating inflammatory pathways, but it also contributes to the regulation of immune homeostasis (23). Therefore, the increase in IL-6 observed following GB50 administration may represent modulation of host immune responses rather than a direct antiparasitic mechanism.

Interestingly, IL-6 levels in the Pb+DHP group were not significantly different from those in Pb group, suggesting that conventional antimalarial therapy primarily acts through parasite clearance rather than through direct modulation of inflammatory cytokine production (24). In contrast, the elevation of IL-6 in the GB50-treated groups (Pb+GB50 and H+GB50) may indicate that GB influences immune responses through mechanisms distinct from those of standard antimalarial drugs (DHP).

Correlation analysis further supports the relationship between parasite burden and cytokine responses. A strong positive correlation was observed between IL-12 levels and parasitemia ($r = 0.821$, $p = 0.001$), whereas IL-6 showed no significant correlation with parasitemia ($r = -0.304$, $p = 0.270$). These findings indicate that IL-12 production is closely associated with parasite burden during infection, reflecting immune activation in response to parasite replication. This is consistent with the established role of IL-12 in driving Th1-mediated immune responses during malaria infection. IL-12 stimulates the production of interferon-γ (IFN-γ) by T cells and natural killer cells, which in turn activates macrophages to enhance parasite clearance (21). During malaria infection, parasite-derived molecules such as glycosylphosphatidylinositol (GPI) anchors, hemozoin, and parasite DNA act as pathogen-associated molecular patterns (PAMPs) that stimulate macrophages and dendritic cells to produce IL-12 through pattern recognition receptors (25). Therefore, the reduction in parasitemia observed following GB50 treatment may lead to decreased PAMP-mediated immune stimulation, resulting in lower IL-12 production. The reduction of IL-12 levels together with decreased parasitemia, may reflect a more balanced immune response in which inflammatory activation is reduced as parasite burden declines. Such modulation of inflammatory pathways could be beneficial in limiting excessive immune responses that contribute to malaria pathology while maintaining adequate host defense against the parasite (21). In contrast, the lack of correlation between IL-6 and parasitemia suggests that IL-6 production may be regulated by additional host factors rather than directly reflecting parasite density. Taken together, these findings suggest that GB50 treatment may contribute to both parasite reduction and modulation of inflammatory responses during malaria infection.

An interesting observation in this study is the differential pattern between IL-12 and IL-6 responses following GB50 treatment. While parasitemia and IL-12 levels decreased, IL-6 levels were elevated in GB50-treated mice. This pattern may suggest a shift from strong pro-inflammatory activation toward a more regulated immune response during infection. Such cytokine modulation could contribute to maintaining immune activity while preventing excessive inflammatory responses that are often associated with malaria pathology.

CONCLUSION

GB50 demonstrated moderate antiparasitic activity and altered cytokine profiles in infected mice, characterized by reduced IL-12 and elevated IL-6 levels. These findings suggest that GB50 may modulate host immune responses during malaria infection; however, the precise mechanisms underlying these effects require further investigation.

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