Research Article

Antimalarial activity of goat bile against *Plasmodium berghei* ANKA infection in BALB/c mice

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Abstract

Goat bile has been used by some Indonesian people to treat malaria and increase their stamina. This study aimed to prove whether goat bile toxic or not in BALB/c mice and to verify the antimalarial activity of goat bile at various concentrations in mice infected with *Plasmodium berghei* ANKA. Acute toxicity test was performed using twenty male BALB/c mice with an average body weight of 25 grams, which were divided into four groups. Mice were given 25%, 50%, and 100% goat bile, respectively, while negative control was given distilled water. Any change in weight, odor, color, agitation, appearance, color of urine and feces, coma, and death, were recorded. A different set of mice were infected with *P. berghei* ANKA. This study conducted using the posttest only control group design with four treatments and five replications. A four day-treatment of goat bile was given by oral gavage to find out its effect on parasitemia level. Infected mice were divided randomly into 4 groups, where the GBNeg group as negative control was given only distilled water. The GB25, GB50, and GB100 groups were treated with 25%, 50%, and 100% goat bile, respectively. The parasitemia was observed daily on Giemsa-stained tail blood smears of each mice. No death or other sign of toxicity was found in goat bile-treated mice. Goat bile showed anti-malarial activity. The parasitemia in all goat bile treated groups was lower compared with the negative control group. The ED50 of goat bile against the growth of parasite was 48.55%. Goat bile is a potential source of new antimalarial therapies. Further investigations are recommended to yield new anti-malarial drug candidates.
INTRODUCTION

Malaria is one of the deadliest infectious diseases worldwide. World Malaria Report 2018 estimated 219 million cases of malaria occurring worldwide and 435,000 death globally in 2017 (WHO, 2018). Indonesia is one of the countries with the highest malaria cases in Southeast Asia. Indonesia has 10.7 million inhabitants living in middle and highly endemic areas of malaria, especially Papua, West Papua, and NTT (Pusdatin, 2016). Novel approaches and new alternative malarial drugs are important to combat the disease (Zeleke et al., 2017). The development of a new antimalarial drug remains slow, with very little chemical diversity (Muluye et al., 2019).

Artemisinin-based combination therapy (ACT) such as dihydroartemisinin-piperaquine was used to treat malaria in Indonesia as recommended by WHO (Kemenkes, 2016). However, disease treatment in developing countries is often using ethno medicines, which regarded as primary choice as they are most affordable and accessible from available natural sources (Kitua & Malebo, 2004). The antimalarial drug effect is characterized by the inhibition of parasite growth (Penna-Coutinho et al., 2011). Ideal antimalarial candidates should also be able to prevent several conditions in vivo related to the parasite infection, including anemia and body weight loss (Zeleke et al., 2017a). Bodyweight is an indicator of metabolism and gut function. The decrease in body weight in mice infected with malaria may be a consequence of metabolic function disturbance, hypoglycemia or appetite depressant action of the mice (Basir et al., 2012; Fidock et al., 2004).

Traditional Chinese Medicine (TCM) has used various parts of the animal’s body, including goat bile (Wang & Carey, 2014). Some people in Indonesia consumed goat bile to treat malaria and increase stamina. Goat bile has been found to have antimalarial activity against P. berghei ANKA in vivo and P. falciparum in vitro (Hapsari et al., 2014). So far, there is a lack of reports on the the antimalarial activity of goat bile in mice infected with P. berghei ANKA. The aim of this research was to find out antimalarial activity of goat bile in BALB/c mice infected with P. berghei ANKA.

METHODS

Ethical approval

The proposal of this research has been reviewed by the Ethics Committee of Faculty of Medicine, Universitas Airlangga as described on the Ethical Clearance No. 116/EC/KEPK/FKUA/2019

Preparation of goat bile

Goat gallbladders were obtained from the Pegirikan slaughterhouse in Surabaya, East Java. Goat bladders were sprayed with 70% alcohol before removing the bile by syringe and pooled into a sterile tube. Goat bile was then diluted with sterile water to prepare 50% and 25% solutions. The working goat bile was stored in the refrigerator during the course of treatment.

Acute toxicity test

Acute toxicity test of goat bile was performed using 25%, 50%, and 100% goat bile. Each mouse in each group was given 0.5mL/25 grams body weight using gavage for four days. (OECD, 2011). The mice in the control group were given 0.5 mL of distilled water. The mice throughout the period of the experiment were under careful watch. Any change in weight, odor, color, agitation, appearance and color of urine and feces, coma, and death were recorded. The body weight of each mice was measured and taken before treatment ($D_0$), at day 4 ($D_4$) and day 30 ($D_{30}$).
Experimental design

Posttest only control group design was applied with a four day-treatment; each treatment consists of five replications. Mice were infected with $1 \times 10^6$ of *P. berghei* ANKA-infected erythrocytes intraperitoneally. Mice were then randomly divided into four groups, where the GBNeg as the negative control group were given only distilled water. Group GB25, GB50 and GB100 were given with 25%, 50% and 100% goat bile, respectively.

**Parasite and Infection** Parasite used in this experiment was *P. bergheri* strain ANKA obtained from the Department of Medical Parasitology Faculty of Medicine, Universitas Airlangga. Donor mice were infected with frozen *P. berghei* ANKA-infected mice blood. When parasitemia level reached 20%, the mice were sacrificed after ketamine anesthetized, blood was collected by cardiac puncture and infected to test mice. Each mouse was infected with $1 \times 10^6$ of *P. bergheri* ANKA-infected erythrocytes intraperitoneally.

**Goat bile treatment**

Goat bile treatment was given daily for four days starting on two days post-infection. Each mouse was given 0.5 mL/25-gram mouse orally of each concentration of goat bile. The mice in the negative control group were given distilled water with the same volume of goat bile.

**Determination of Parasitemia**

Thin smears were prepared on slides from the tail of infected mice. The slides were fixed with methanol and stained with 10% Giemsa, observed under light microscopy with 1000x magnification. The percentage of parasitemia was calculated using the following formula:

$$\text{% parasitemia} = \frac{\text{infected RBC}}{\text{total RBC}} \times 100$$

The percentage of the growth of parasite was calculated for each group using this formula:

$$\text{% growth} = \frac{P(d_1 - d_0) + (d_2 - d_1) + (d_3 - d_2) + (d_4 - d_3)}{(n - 1)}$$

$P(d)$: % parasitemia at day (d)
$n$: total treatment days.

The % inhibition of the growth of parasite was calculated for each group using the following formula.

$$\text{% inhibition} = 100\% - \frac{P_t}{P_c} \times 100\%$$

$P_t$: Parasitemia in treatment group
$P_c$: Parasitemia in control group

**Data analysis**

All treatments and control groups were considered for determination of fifty percent effective dose (ED50) of goat bile against *P. berghei* ANKA in BALB/c mice. The ED50 was determined by Probit analysis on SPSS 16.0 for Windows. P-values <0.05 were considered significant. The two tailed paired t-test was used to compared mean body weight before and after treatment. The level was considered significant at 95% confidence level and p<0.05.

**RESULTS**

**Acute Toxicity**

The physical sign of illness observed during the course of acute toxicity test was mild diarrhea, which occurs only within two days after initial treatment, and then the symptom was disappeared afterward. No death or other sign of toxicity was found in goat bile-treated mice. Figure 1 showed the control and treatment group progressively gain weight until the end of the experiment on day 30. A significant difference (p < 0.05) was observed...
GB50 treatment group in comparison with the negative control. There was no significant difference (p < 0.05) on day 2, 3, and 4 of all treated groups compared with the negative control. A significant difference (p < 0.05) was observed on day 30 of treatment in the GB25 treatment group in comparison with the negative control.

Parasitemia

Figure 2 showed the percentage of parasitemia of mice infected with *P. berghei* ANKA. The parasitemia in all treated group were lower compared with negative control group. Significant difference (p < 0.05) was observed on day 1 of treatment in the GB100 group in comparison with negative control. There was no significant difference (p < 0.05) on day 2, 3, and 4 of all treated groups compared with the negative control. Overall, Figure 1 presents the percentage of parasitemia in *P. berghei* ANKA infected mice with treated group exhibiting lower percentage parasitemia level but a significant difference (p < 0.05) only occur on day 1 of treatment in the GB100 treatment group.

Table 1 showed the percentage of growth and inhibition of parasite in infected mice. The lowest percentage of parasite growth of 1.65% and a maximum percentage of parasite inhibition of 61.11% was shown in 100% goat bile treatment. Statistical analysis showed that the result of ED₅₀ is 48.55%. This result showed that 48.55% of goat bile could inhibit 50% of parasite growth.

![Graph showing body weight vs day post treatment](image)

**Figure 1.** The Bodyweight of BALB/c mice treated with goat bile. GB25: goat bile 25%. GB50: goat bile 50%. GB100: goat bile 100%. NEG: Negative control.
ABSTRACT

Diabetes insipidus in patients with traumatic severe brain injury of Indonesia are more than 50,000 deaths and 500,000 incidents of polyuria of 300cc/hour urine production and 149mmol/l of hypernatremia. Although the immediate administration of desmopressin, the patient’s clinical and hemodynamic was sufficient, the signs of diabetes insipidus were presented by severe brain injury in the Intensive Care Unit (ICU). The definitive data on the incidence of diabetes insipidus in cases of brain injury requires complicated treatment. Therefore, diabetes insipidus in cases of traumatic severe brain injury of Indonesia requires complicated treatment. Hence, the mortality rate of up to 50%. About 1.5 million people experience severe brain injury in the United States. There are more than 50,000 deaths and 500,000 incidents of diabetes insipidus.

DISCUSSION

The acute toxicity test resulted in mild intestinal toxicity, as shown by mild diarrhea in two mice treated with 100% goat bile. However, the increased of the mice body weights were not affected by goat bile treatment, indicated no significant toxicity of goat bile to intestinal effect. Body weight was measured to monitor the effects of treatment on factors such as metabolism and intestinal function (Basir et al., 2012). An intestinal disturbance may occur due to goat bile treatment that may affect intestinal metabolism. Metabolic function disturbance such as hypoglycemia and appetite depressant action of the mice (Basir et al., 2012; Fidock et al., 2004).

The in vivo evaluation proved the antimalarial activity of goat bile against P. berghei ANKA infection in mice. An ideal antimalarial candidate should prevent several conditions such as anemia, hemolysis, body temperature reduction, and body weight loss (Zeleke, Kebebe, Mulisa, & Gashe, 2017). The effect of the antimalarial drugs is characterized by the inhibition of parasite growth in the treatment group compared with a drug-free control group (Penna-Coutinho et al., 2011). The effects of goat bile on malaria infection in mice is shown by the percentages of inhibition and parasite growth in Table 1. The data shows the antimalaria activity of goat bile against P. berghei infection in BALB/c mice. Goat bile
that the higher concentration affected the level of parasitemia compared with the negative control shown in Figure 2. Parasitemia level is influenced by various factors, including the host immune system (Kotepui et al., 2015). The density of parasites is one of the factors that influence the severity of clinical manifestations (Avrina et al., 2011).

Goat bile was one of the various animals’ bile used in TCM. Even there was no report on the use of goat bile to treat infectious disease; goat bile has been believe to treat eye diseases, various infectious skin diseases, and constipation (Wang & Carey, 2014). Animal bile contains various compounds, including amino acids, steroids, enzymes, cholesterol, bile salts, bilirubin, phospholipids, vitamins, heavy metal, and environmental toxins. Goat bile contains taurocholate (TC), taurochenodeoxycholate (TCDC), taurodeoxycholate (TDC), glycocholate (GC), and monoglucuronide bilirubin pigment. About 5% of bile salts consist of organic and inorganic solutes. Dry matter of goat bile contains 15.7% protein (Boyer, 2013; Wang & Carey, 2014);

Although the active compound in goat bile for the antimalarial effect is not known or yet to be identified, bile acid and bile salt have known to have roles in therapeutical actions. Bear bile was used in TCM to reduce inflammation, liver diseases, and fever. Bear bile has taurine conjugate form of bile acid, such as taurochenodeoxycholic acids (TCDCa), taurodeoxycholic acids (TDCa), taurursodeoxycholic acids (TUDCA), and taurocholic acids (TCA) (Coleman et al., 2006; Li et al., 2016). A role of bile acid in neuroprotection of age-related neurodegenerative disorder has been found (Ackerman & Gerhard, 2016). The beneficial effect of bile salt is as a natural detergent to prevent sexually transmitted disease and protection against bacterial growth (de Buy Wenniger & Beuers, 2010; Herold et al., 1999).

CONCLUSION

Although goat bile possessed mild intestinal toxicity in BALB/c mice, however, this result suggested that goat bile is one of the potential sources for new anti-malarial therapies. Further investigations are recommended, as there are no published studies on anti-malarial activity for specific compounds of goat bile that can yield new anti-malarial drug candidates.

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ABSTRAK

CASE REPORT
