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The protective effect of *Moringa oleifera* leaf extract on liver damage in mice infected with *Plasmodium berghei* ANKA

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ABSTRACT

Objective: To investigate the protective effect of *Moringa oleifera* leaf extract on liver damage in mice infected with *Plasmodium berghei* ANKA (*P. berghei*)
Methods: For extraction of *Moringa oleifera* (*M. oleifera*) leaves, microwave with hot water method was used and acute toxicity study was then be done. Standard Peters' test was carried out to test the efficacy of *M. oleifera* extract *in vivo*. The ICR mice were inoculated with 1 × 10^7 red blood cells infected with *P. berghei* strain by intraperitoneal injection. They were subsequently given with 100, 500 and 1 000 mg/kg of this extract by intragastric route once a day for 4 consecutive days. Parasitemia was estimated using microscopy and levels of aspartate aminotransferase, alanine aminotransferase and albumin were also measured.
Results: The *M. oleifera* leaf extract showed the protective activity on liver damage in mice infected with *P. berghei* in a dose-dependent fashion. It can be indicated by normal levels of aspartate aminotransferase, alanine aminotransferase and albumin in mice treated with extract. The 1 000 mg/kg of extract was observed to present the highest activity. Interestingly, the dose-dependent antimalarial activity was also found in the mice treated with extract.
Conclusions: The *M. oleifera* leaf extract presented protective effect on liver damage in mice infected with *P. berghei*.

1. Introduction

Malaria is estimated for 300–600 million cases worldwide annually of which 1 million are fatal[1]. Malaria-associated liver injury, one of the causes of death in severe malaria, occurs a mortality up to 50% in the hospitalized patients[2]. The development of liver injury during malaria infection is unclear, but it has been suggested to involve the pro-inflammatory response, adhesion of infected red blood cells (iRBC) and oxidative stress induced hepatotoxicity[3]. The association of inflammatory cytokines in pathogenesis of severe malaria has also been described[4]. During the asexual phase of malaria parasite, free heme is increased from the consumption of hemoglobin by malaria and induce the oxidative stress followed by lipoprotein oxidation and organ damage such as liver[5,6]. Hence, new compounds or extracts have protective effect on liver injury during malaria infection. In this point, the extracts of medicinal plants are suitable targets for studying.

Moringa oleifera (*M. oleifera*) can be found widely throughout Southeast Asia including Thailand[7]. This plant is found to have the highest antioxidant content with a remarkable range of medicinal uses[8]. The *M. oleifera* leaves provide a rich source of vitamins, carotenoids, alkaloids, flavonoids, polyphenols, quercetin, apigenin, and kaempferol. The therapeutic effects of *M. oleifera* including antioxidant, anti-inflammation, anti-cancer, anti-bacterial, anti-diabetic, anti-dyslipidemia, anti-hypertensive and organ-protective activities have been investigated[9]. Polyphenols and flavonoids have been described to have protective effect on organ damage induced by oxidative stress. In addition, it has been described that quercetin and kaempferol have the potent antimalarial effect against *Plasmodium falciparum* and *Plasmodium berghei* ANKA (*P. berghei*)[10]. However, hepatoprotective activity of *M. oleifera* leaf extract in malaria has still not been investigated yet.

Therefore, this study aimed to investigate the protective effect of *M. oleifera* leaf extract on liver injury in mice infected with *P. berghei*.

2. Materials and methods

2.1. Plant material of M. oleifera

The leaves of *M. oleifera* collected in January 2016, at Suphanburi Province, Thailand were used. This plant was identified at the

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All experimental procedures involving animals were conducted in accordance to National Laboratory Animal Center, Mahidol University, Thailand and approved by Ethical Committee for Animal Excperiment, Western University.

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2.2. Preparation of aqueous crude extract

In the present study, hot water method was carried out for extraction[11]. The leaves of *M. oleifera* were washed and dried at 60 ℃ for 3 h in hot-air oven, and then ground using electric blender. Extraction was performed by dispersing 10 g of powdered dried plant material in 100 mL of distilled water and heated in microwave at 360 w for 3–5 min. Overnight incubation at room temperature with continuous shaking was done to ensure complete extraction. Freeze-drying was then performed to collect the crude extract and kept at –20 ℃. Before using, distilled water was used to dissolve the extract at the selected doses.

2.3. Experimental mice

Female ICR mice, 4 week olds, weighting 25–30 g used in the present study were obtained from the National Laboratory Animal Center, Mahidol University, Thailand. These mice were housed at animal room with temperature control between 25–28 ℃ and fed with standard mouse pellet food and drinking water *ad libitum*. The experiments involving the mice were approved by the Ethical Committee for Animal Excperiment, Western University.

2.4. Acute toxicity assay in vivo

M. oleifera leaf extract was tested for acute toxicity in mice using Lorke's method[12]. Five groups of naive ICR mice (5 mice/group) were given 100, 500, 1 000, 2 000 and 4 000 mg/kg of the extract by intragastric route. The signs of toxicity were then observed in these mice including reduced activity, salivation, sleep, weakness, coma, and death for 7 days. The calculation of median lethal dose was shown below:

$$LD_{50} = \sqrt{\text{minimum mortality dose} \times \text{maximum survival dose}}$$

2.5. P. berghei rodent malaria

Chloroquine-sensitive *P. berghei* strain was used. This parasite was propagated in ICR mice with 1×10^7 iRBC by intraperitoneal injection. Parasite growth was subsequently monitored by microscopy of thin smear with Wright-Giemsa staining. Parasitemia was subsequently calculated using formula below:

$$\text{Parasitemia (\%)} = \frac{\text{No. of iRBC} \times 100}{\text{No. of RBC}}$$

2.6. Measurement of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and albumin

The tail blood collected into heparinized hematocrit tube was done and centrifuged at maximum speed for 5 min. Plasma was then collected for the measurement of liver injury markers including AST, ALT and albumin levels with the available commercial kits, according to manufacturer's direction.

2.7. Efficacy test of M. oleifera leaf extract in vivo

For the efficacy of *M. oleifera* leaf extract, standard Peters' test was performed[13]. Six groups of naive ICR mice, 5 mice of each, were inoculated intraperitoneally with 1×10^7 iRBC of *P. berghei*. The extract (100, 500, and 1 000 mg/kg) was subsequently administered orally by gavage once a day for 4 consecutive days. Three control groups were used in this experiment including normal mice treated

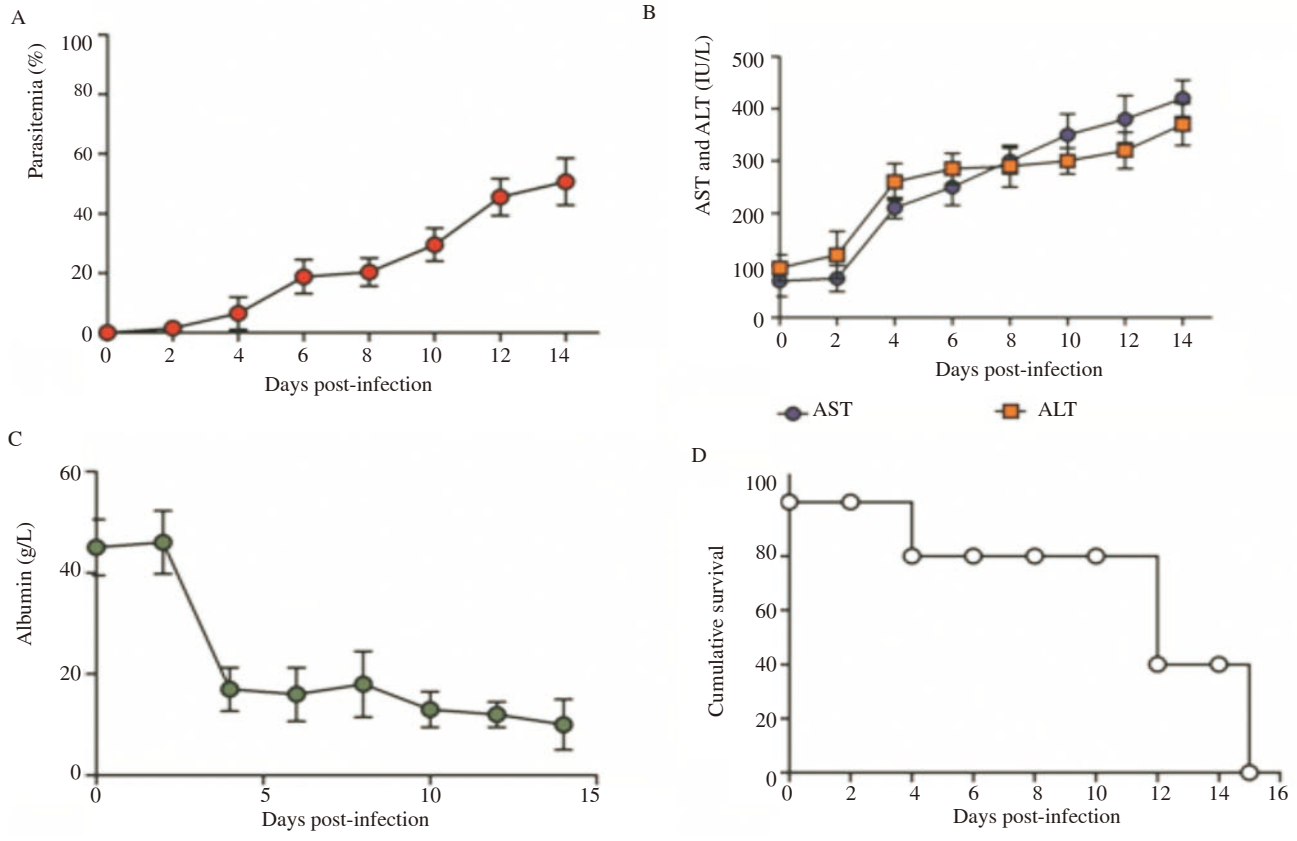

Figure 1. Malaria-associated liver injury.
ICR mice were infected intraperitoneally with 1×10^7 parasitized erythrocytes of *P. berghei*. Parasitemia, AST and ALT, albumin levels were daily monitored. Cumulative survival time was also observed. Results were expressed as mean ± SEM.

with distilled water, normal mice treated with extract of 1 000 mg/kg, and *P. berghei* infected mice treated with distilled water as healthy, toxicity, and disease controls. On Day 5, tail blood was collected to measure parasitemia, AST, ALT and albumin levels as described above.

2.8. Statistics

All data was analyzed by GraphPad Prism Software and the results were shown as mean ± SEM. ANOVA with Tukey *post hoc* test was done to compare the mean between the groups and significant difference was considered at 95% confidence, $P < 0.05$.

3. Results

3.1. Acute toxicity of *M. oleifera* leaf extract

The *M. oleifera* leaf extract administered orally at dose up to 4 000 mg/kg, presented no mortality effect throughout the 7 days of observation. None of these mice showed any signs of toxicity. They were physical active. In addition, the LD_{50} was estimated to be above 4 000 mg/kg.

3.2. Malaria-associated liver injury

Parasitemia was observed firstly on Day 1 after infection with < 1% parasitemia and increased to 60% on Day 14 (Figure 1A). During *P. berghei* infection, liver injury was developed as indicated by AST, ALT, and albumin levels. The levels of AST and ALT were markedly increased in mice infected with *P. berghei* (Figure 1B). Additionally, the decrease of albumin in these infected mice was also observed (Figure 1C). The marked alternation in levels of AST ALT, and albumin was observed firstly on Day 4 after infection. Moreover, these mice infected with *P. berghei* died within 2 weeks (Figure 1D).

3.3. Protective effect of *M. oleifera* leaf extract on liver injury induced by *P. berghei* infection

During *P. berghei* infection, significant ($P < 0.001$) increase levels of AST and ALT were found (Figure 2A and 2B in UN group). Moreover, significant ($P < 0.01$) decrease of albumin level was also found in untreated mice (Figure 2C in UN group). Interestingly, aqueous leaf extract of *M. oleifera* exerted protective activity on liver injury induced by *P. berghei* infection which indicated significant ($P < 0.01$) decrease levels of AST and ALT and significant ($P <$

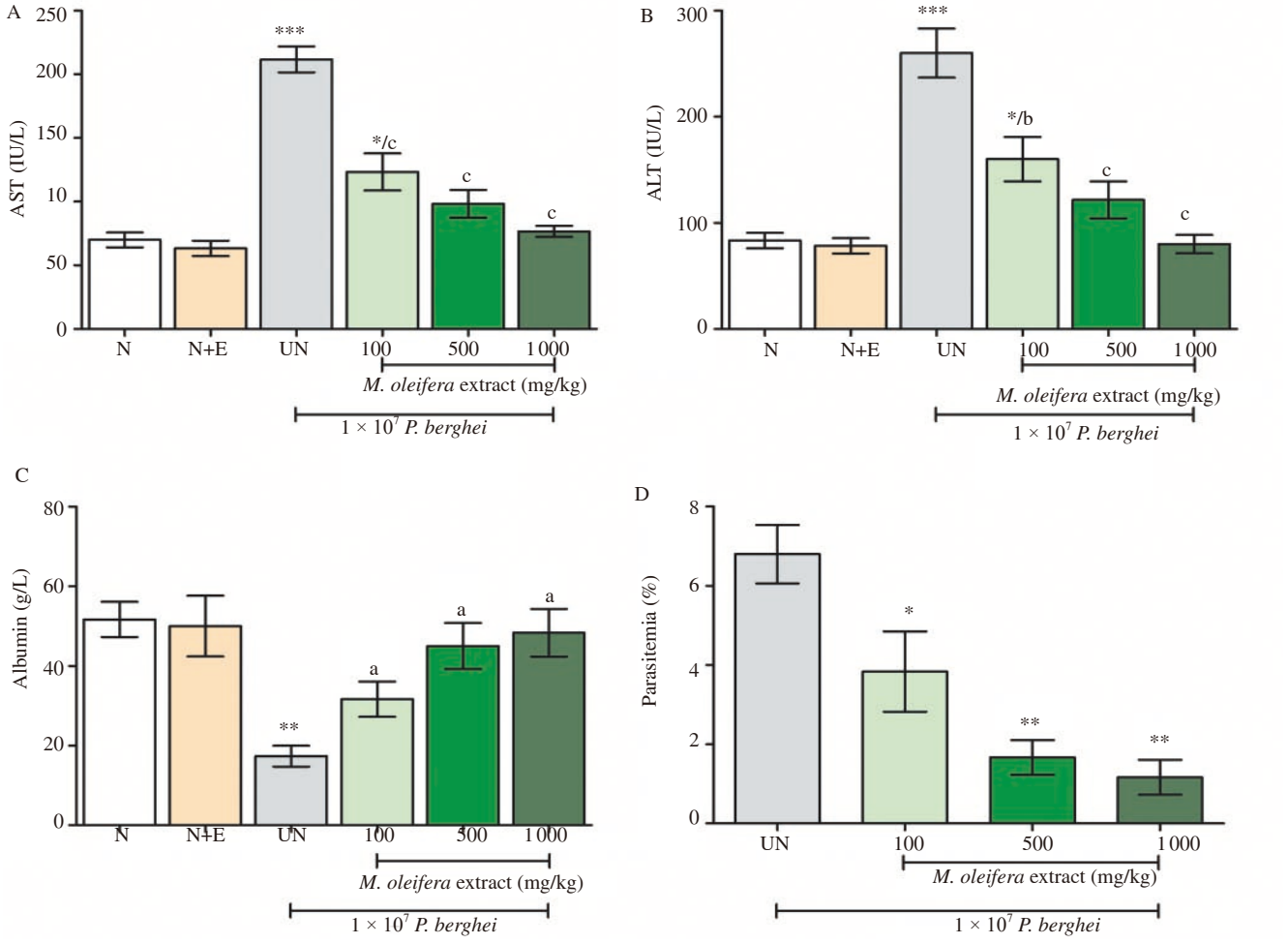

Figure 2. Protective effect of *M. oleifera* leaf extract on liver injury induced by *P. berghei* infection in mice.
Groups of ICR mice (5 mice of each) were infected intraperitoneally with 1×10^7 parasitized erythrocytes of *P. berghei* and given the extract (100, 500, and 1 000 mg/kg) orally for 4 consecutive days. AST, ALT, albumin and parasitemia levels were measured. Results were expressed as mean + SEM. *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$, compared to normal group. ^{a}: $p < 0.05$; ^{b}: $p < 0.01$; ^{c}: $p < 0.001$, compared to untreated group. N: Normal mice; N+E: Normal mice treated with 1 000 mg/kg of extract; UN: Untreated mice.

0.05) increase level of albumin levels with a dose-dependent manner (Figure 2A, 2B, and 2C in extract treated groups). The 1 000 mg/kg of this extract presented the highest activity. Additionally, no effects on AST, ALT, and albumin levels in the extract treated normal mice (Figure 2A, 2B, and 2C in N+E groups).

3.4. Antimalarial activity of *M. oleifera* leaf extract

The *M. oleifera* leaves extract showed significant ($P < 0.05$) inhibition of parasitemia in mice infected with *P. berghei* in a dose-dependent fashion (Figure 2D). The percent inhibitions of 42.86%, 71.43% and 85.71% were found at doses of 100, 500, and 1 000 mg/kg of the extract, respectively.

4. Discussion

In the present study, protective effect of *M. oleifera* leaf extract on liver injury during *P. berghei* infection in mice was investigated. Moreover, antimalarial activity of this extract was also evaluated using standard animal model. In the present study, *M. oleifera* leaf extract did not show toxicity indicated by the dose at 4 000 mg/kg which caused no behavioral change or mortality of these normal mice. For acute toxicity test, it has been described that the dose of the extract up to 5 000 mg/kg caused no toxicity and mortality which can be suggested that it is safety[14]. Moreover, if the LD_{50} of tested compounds or extracts is more than the minimum effective dose about 3 times, they are considered good candidates for further studies[15]. Therefore, aqueous crude extract of *M. oleifera* leaves was safe and good candidate for further studies.

Liver injury in malaria patients has been reported by clinical cases[16]. From our results, liver injury in mice infected with *P. berghei* was shown on Day 4 after infection as indicated by increasing of AST and ALT, as well as decreasing of albumin levels. For acute liver injury, AST and ALT levels were increased as the leaking of these enzymes from hepatocytes, while liver function was decreased in order to synthesize the albumin. Malaria-associated liver injury is suggested to be a result of malarial parasite adhesion as well as inhibited immune response against oxidative stress[17]. Additionally, accumulation of toxic free heme from erythrocyte destruction during malaria infection could induce oxidative stress from the hydroxyl radical production via Fenton/Harber-Weiss reaction followed by liver injury[18]. Moreover, during malaria infection, overproduction of oxidized low density lipoprotein can trigger adverse effect in vascular lesion, reactive oxygen species, and liver cell damage[19]. The sequestration of malarial parasite at the microvessel is a critical feature of severe malaria associated with liver damage[20]. Therefore, proinflammatory molecules and oxidative stress products have a critical role for the development of malaria-associated liver injury. In addition, the extent of free radical-induced oxidative stress and liver injury can be exacerbated by decreasing the efficiency of antioxidant system and cytoprotective mechanisms[21].

The *M. oleifera* leaf extract showed the significant protective effect on liver injury induced by *P. berghei* infection in mice with a dose-dependent manner. It can be indicated by significant decreasing of AST and ALT, and increasing of albumin levels in the extracted treated mice, compared to untreated control. This extract was reported to have different active compounds such as flavonoid, polyphenol, alkaloids, saponin, terpenoids, quercetin and kaempferol[22]. In particular, polyphenols and flavonoids of this extract have potent antioxidant activity which might contribute to protect liver injury during malaria infection[7]. It has been reported that the antioxidant enzymes including catalase and superoxide dismutase, as well as glutathione system were decreased in mice treated with this extract[23]. Moreover, the *M. oleifera* leas extract also has free radical scavenging activity. Hence, these active compounds of this extract might play the protective activity to liver injury induced by malaria infection.

Antimalarial activity was seen in the *M. oleifera* leaf extract against *P. berghei* infected mice. This extract showed significant reduction of parasitemia at a dose-dependent manner. Terpenoids, flavonoids, saponin, alkaloids, quercetin and kaempferol were known to have antimalarial activity and antioxidant activity which might also play antimalarial activity[24,25]. It has been reported and suggested that quercetin and kaempferol presented antimalarial activity against *in vitro* culture of *Plasmodium falciparum* and *P. berghei* infected mice[26,27]. In addition, antimalarial activity of these compounds was found evidently on the ring stage of the malaria parasite[28]. The point of action corresponds with the protein and nucleic acid synthesis. The inhibition of heme polymerization followed by toxic free heme accumulation by antioxidant activity from these active compounds in the extract has been also reported[29]. However, the identification of these active compounds that present the antimalarial activity should be performed. The test compound is considered active when the inhibition of parasitemia is higher than 30%[30]. In the present study, all the extract in treated groups induced more than 30% inhibition. Therefore, the present finding indicated that the *M. oleifera* leaf extract has a good antimalarial activity.

From all the study, it could be concluded that the *M. oleifera* leaf extract showed protective effect on liver injury induced by *P. berghei* infection in mice. Additionally, this extract also presented good antimalarial activity and could serve as the target of new antimalarial drug for the treatment of malaria.

Conflict of interest statement

We declare that we have no conflict of interest.

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