Research Article

Sacha inchi (*Plukenetia volubilis* L.) Seed Mitigates Splenic Histopathological Alterations in *Plasmodium berghei*-Infected Mice

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Abstract | Malaria is a significant global health concern a major global health concern causing significant morbidity and mortality, particularly in tropical regions. It often leads to organ damage, including splenic injury. This study evaluated the effect of sacha inchi seed essential oil extract on splenic histopathology in mice infected with *Plasmodium berghei*. Thirty male BALB/c mice were randomly assigned to six groups: a healthy control group; an infected control group; a positive control group that was treated with dihydroartemisinin–piperaquine; and three treatment groups that received sacha inchi oil at doses of 250, 500, or 1,000 mg/kg of body weight. The mice infected with *Plasmodium berghei*, administered the treatments orally, and sacrificed at the end of the experimental period. Their spleens were collected, processed for histology, and examined for inflammatory cell infiltration, hemorrhage, and hemozoin pigment deposition. The data were analyzed using the Kruskal–Wallis nonparametric test, followed by the Mann–Whitney U test for post hoc pairwise comparisons. The results showed significant differences ($P < 0.001$) were observed among groups in all histopathological parameters. The IC group showed the highest infiltration, hemorrhage, and pigment scores, indicating severe splenic damage. Treatment with sacha inchi oil ameliorated these pathological changes, with the intermediate dose (500 mg/kg) showing the most consistent reduction in inflammation and pigment accumulation. The highest dose (1,000 mg/kg) further reduced hemorrhage and pigment deposition, suggesting enhanced vascular protection. In conclusion, sacha inchi seed oil modulated splenic pathology in *P. berghei*-infected mice in a dose-dependent manner. Although these findings indicate potential organ-protective effects, the absence of parasitemia data and variability across doses warrant cautious interpretation. Further studies incorporating parasite burden and molecular analyses are needed to confirm its therapeutic relevance.

Keywords | Malaria, *Plasmodium berghei*, Sacha inchi seed oil, Spleen, Inflammation, Hemozoin

Received | October 27, 2025; **Accepted** | November 29, 2025; **Published** | March 31, 2026
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Citation | Putri A, Hestianah EP, Suwanti LT, Mufasirin M, Plumeriastuti H, Kuncorojakti S (2025). Sacha inchi (*Plukenetia volubilis* L.) seed mitigates splenic histopathological alterations in *Plasmodium berghei*-infected mice. Adv. Anim. Vet. Sci., 14(4):768-775.
DOI | https://dx.doi.org/10.17582/journal.aavs/2026/14.4.768.775
ISSN (Online) | 2307-8316

INTRODUCTION

Malaria persists as a pervasive global health concern, marked by elevated morbidity and mortality rates, particularly in tropical regions (Hayati *et al*., 2022; Trivendi and Chakravarty, 2022). The number of deaths from malaria worldwide in 2022 exceeded 600,000 (WHO, 2022). In Indonesia, malaria is endemic, with the highest number

of cases occurring in Papua, Maluku, and Nusa Tenggara. Although the national case rate has been declining, malaria continues to pose a serious threat due to its potential to cause severe complications and negatively impact community productivity (Prasetyowati *et al*., 2021; WHO, 2023).

Malaria infection is caused by protozoa of the genus Plasmodium, which is transmitted through the bite of female Anopheles mosquitoes (Plewes *et al*., 2018; Ekasari *et al*., 2020; Ghosh and Stumhofer, 2021). In addition to causing systemic symptoms such as fever, anemia, and weakness, this infection also causes damage to vital organs (Kamarullah *et al*., 2018; Sharma *et al*., 2024). One of the most affected organs is the spleen, which plays an important role in immunity to malaria (Johan *et al*., 2020; Ghosh and Stumhofer, 2021). The spleen functions to filter infected erythrocytes and is the site of immune cell activation. Massive immune system activation during infection does help control the parasite, but it can also trigger tissue damage if it is excessive (Windasari *et al*., 2016; Lacerda-Queiroz *et al*., 2017).

Previous studies have reported that malaria-induced spleen damage has characteristic histopathological features (Siqueira *et al*., 2012). These changes commonly include red and white pulp hypertrophy, leukocyte infiltration, hemozoin pigment deposition, disorganized tissue architecture, necrosis, and fibrosis (Windasari *et al*., 2016; Pujiyanto *et al*., 2020; Maslachah *et al*., 2020). Hemozoin pigment, formed from the degradation of hemoglobin by parasites, accumulates in the spleen, triggering oxidative stress and impaired phagocytic function (Eugenin *et al*., 2019). This histological damage indicates a strong immune response and signifies an inflammatory imbalance that can worsen the disease's progression (Plewes *et al*., 2018).

Malaria treatment currently still relies on artemisinin-based combination therapy (ACT) (WHO, 2018). While this therapy is effective in reducing parasitemia, it has limitations (Price and Douglas, 2015). For example, resistance to artemisinin has been reported in several Southeast Asian countries, and its ability to protect organs damaged by inflammation is limited (Nugraha, 2011; Zou *et al*., 2022). These limitations underscore the need for alternative therapies that target parasites and modulate the immune response to protect vital organs, such as the spleen (Okell *et al*., 2014).

In this effort, using biological resources with bioactive content is an attractive option (Abdulfatai and Ayotunde, 2022; Firdaus *et al*., 2025). *Plukenetia volubilis* L., also known as sacha inchi , is a plant rich in polyunsaturated fatty acids, vitamin E, flavonoids, and phenolic compounds (Wang *et al*., 2024; Firdaus *et al*., 2025). These compounds are known to exhibit antioxidant and anti-inflammatory properties (Ahmad *et al*., 2024). Therefore, sacha inchi has the potential to suppress excessive inflammatory responses and repair tissue damage caused by a malaria infection (Firdaus *et al*., 2025).

Additionally, essential oil derived from sacha inchi seeds has been reported to exhibit immunomodulatory properties, reducing the secretion of proinflammatory cytokines, such as TNF-α and IL-6, while improving cellular redox status (Rodzi and Lee, 2022; Redjeki *et al*., 2025). These effects are important for preventing excessive immune cell infiltration and reducing hemozoin pigment deposition in the spleen. Therefore, sacha inchi essential oil has the potential to inhibit parasite growth and maintain the histological integrity of the spleen (Firdaus *et al*., 2025).

The antioxidant and anti-inflammatory components of sacha inchi, particularly α-linolenic acid and polyphenols, may mitigate splenic damage by reducing reactive oxygen species and limiting the activation of macrophages overloaded with hemozoin (Aguilar-Olano *et al*., 2025). This could decrease the release of TNF-α and IL-6, which are central to splenic inflammation in malaria. While the oil exerts immunomodulatory effects, its action is not necessarily immunosuppressive but rather regulatory, balancing pro- and anti-inflammatory cytokine production to prevent tissue overactivation while maintaining parasite control (Firdaus *et al*., 2025).

This study was based on the lack of research examining the effects of sacha inchi essential oil on the histopathology of the spleen during malaria infection. Histopathological studies are important because they directly describe the level of tissue damage and serve as an indicator of a therapy's effectiveness in preventing organ complications. Thus, this study aims to evaluate the effect of sacha inchi seed essential oil extract on the histopathology of the spleen in mice (*Mus musculus*) infected with *Plasmodium berghei*. The results are expected to lead to the development of alternative therapies that are both antiparasitic and capable of protecting vital organs from damage caused by excessive inflammation.

MATERIALS AND METHODS

Study design

The study was conducted from March to May of 2024 at the Malaria Laboratory and Animal Facility of the Institute of Tropical Diseases (ITD) at Airlangga University in Surabaya, Indonesia. All procedures were approved by the Animal Ethics Committee of the Faculty of Veterinary Medicine at Airlangga University in Surabaya, Indonesia, and were performed according to national and international animal welfare standards (No: 2.KEH.183.12.2023) (Figure 1).

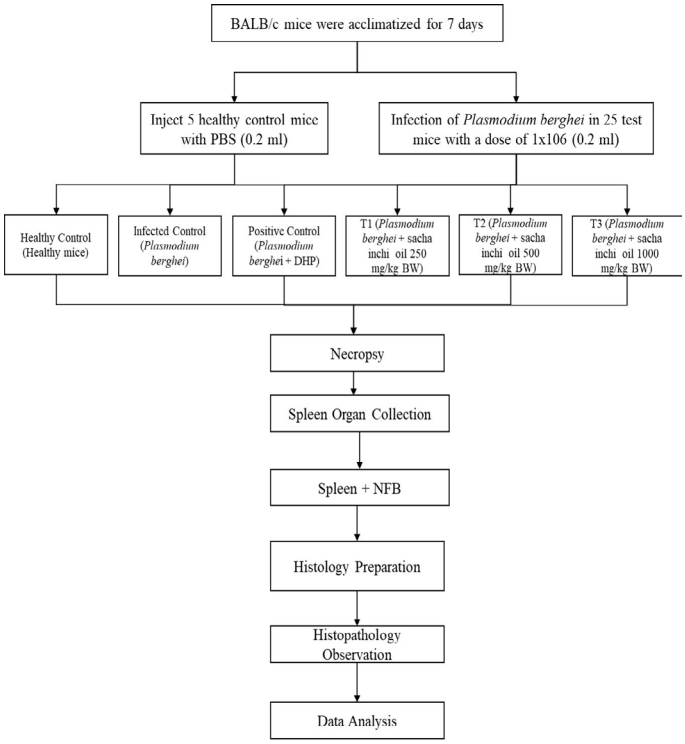

Figure 1: Study design.

The study employed a true experimental design to investigate the impact of *Plukenetia volubilis* (sacha inchi) seed oil extract on the splenic histopathology of *Plasmodium berghei*-infected mice. The post-test only control group design was used, in which assessment was performed at the end of treatment without a baseline measurement. This approach was chosen to enable direct comparison between the treatment and control groups following the intervention.

Animal and sample size

Male BALB/c mice, aged 7–8 weeks and weighing 20–30 grams, were used. The inclusion criteria were normal physiological condition, active movement, clear eyes, and white fur. Exclusion criteria included death before treatment or prior use in other experiments. Mice that died or developed severe clinical symptoms during the study were considered dropouts. The sample size was determined using Federer's formula (1977): $(t - 1)(n - 1) \geq 15$, where $t = 5$ treatment groups and $n \geq 5$ animals per group. To anticipate possible losses, an additional 20% of subjects were added, resulting in six mice per group for a total of 30 animals. Simple random sampling was used to allocate the animals into groups and minimize selection bias.

Sacha inchi oil preparation

P. volubilis seeds were cleaned and dried before being extracted using cold pressing to obtain fixed oil. The oil was filtered through Whatman No. 1 filter paper, stored in a dark container at −20 °C and used without fractional distillation. As this method produces non-volatile oil (triglycerides), the focus of this study is the effects of the fixed oil, rather than the volatile essential oil.

Experimental procedures

Mice were acclimated for seven days prior to the experimental period. They were housed in ventilated cages at a temperature of 22–25°C under a 12-hour light-dark cycle. *Ad libitum* access to standard chow and water was provided to ensure physiological stability before intervention. This acclimatization period is critical for reducing stress and variability in experimental outcomes.

Plasmodium berghei infection

Donor mice were initially infected with a blood suspension containing 200 µL of *Plasmodium berghei* ANKA parasites. The infection was monitored daily until the parasitemia level reached approximately 20%, which indicates a sufficiently high parasite load for experimental transfer. Then, 200 µL of the infected blood was aseptically collected from the donor mice and injected intraperitoneally into the recipient mice to initiate infection. This method of transferring parasitized erythrocytes intraperitoneally is a well-established protocol for ensuring consistent and reproducible infection in rodent malaria models when studying disease progression and therapeutic interventions (Basir *et al.*, 2012).

Treatment protocol

To assess the effects of treatments on *Plasmodium berghei* infection, the experimental mice were divided into six distinct groups (Table 1). The treatment groups were divided based on the administered dose of sacha inchi oil extract. Group T1 received 250 mg/kg body weight, group T2 received 500 mg/kg body weight, and group T3 received 1,000 mg/kg body weight. All treatments were administered orally once daily, beginning three hours post-infection to simulate early intervention. This grouping strategy enabled a comparative evaluation of treatment efficacy at different dosage levels and controls.

Tissue collection and histopathological examination

Mice were anesthetized with ketamine and euthanized at the end of the experimental period to ensure humane treatment. The spleens were excised aseptically, fixed in 10% buffered formalin, and processed using standard histological protocols. The paraffin-embedded tissues were sectioned at 4–5 µm, mounted on glass slides, and stained with hematoxylin and eosin (HE). The histopathological evaluation focused on inflammatory cell infiltrates, hemorrhage, and hemozoin pigment deposition (Table 2) (Moreno *et al.*, 2006; Liu *et al.*, 2009). Image analysis was conducted using ImageJ on five random fields of view. Observations were performed under a Nikon Eclipse Ei light microscope connected to an Optilab device and analyzed with Optilab Viewer 4.0 software.

Table 1: Treatment group.

Group	Description
HC (healthy control)	Healthy mice without infection
IC (infected control)	Mice infected with *Plasmodium berghei* without treatment
PC (positive control)	Mice infected with *Plasmodium berghei* and treated with dihydroartemisinin–piperaquine (DHP)
T1	Mice infected with *Plasmodium berghei* and treated with sacha inchi oil 250 mg/kg BW
T2	Mice infected with *Plasmodium berghei* and treated with sacha inchi oil 500 mg/kg BW
T3	Mice infected with *Plasmodium berghei* and treated with sacha inchi oil 1000 mg/kg BW

Table 2: Histological scoring.

	Score	Indication
Inflammatory cell Infiltrates	0	None
	1	Rare or around ductal margins
	2	In the parenchyma (<50% of the lobules)
	3	In the parenchyma (>50% of the lobules)
Hemorrhage	0	No evidence of erythrocyte extravasation
	1	1–2 small foci of erythrocyte extravasation each focus defined as localized accumulation of ≥10 extravascular erythrocytes)
	2	3–5 hemorrhagic foci per section
	3	>5 foci or diffuse hemorrhage involving large tissue areas
Hemozoin pigment deposition	0	None
	1	<10%
	2	10-40%
	3	>40%

Data analysis

Data were analyzed using SPSS version 25.0. The Kolmogorov–Smirnov test was first used to assess normality, considering the data to be normally distributed when $p>0.05$. Homogeneity of variance was examined next using the same significance threshold. One-way ANOVA was then performed to determine differences among groups when both assumptions were met. Significant pairwise differences were examined further using the Tukey's HSD post hoc test. All analyses were performed at a 95% confidence level ($\alpha = 0.05$).

Parasitemia assessment (Limitation)

Although parasite counts were not measured in the present study, future experiments should include parasitemia quantification to differentiate between antiparasitic and organ-protective effects.

RESULTS AND DISCUSSION

This study conducted a histopathological evaluation of the spleen in mice infected with *Plasmodium berghei* to determine the protective and restorative effects of sacha inchi seed oil on tissue damage caused by malaria. The assessment parameters included inflammatory cell infiltrates, hemorrhage, and hemozoin pigment deposition. These indicators were used to assess the level of spleen tissue damage caused by *Plasmodium berghei* infection and the effect of sacha inchi seed oil administered at specific doses.

The spleen was selected as an observation parameter because it plays a central role in the immune response to *Plasmodium berghei* infection. As a secondary lymphoid organ with complex molecular mechanisms, the spleen filters and eliminates infected or parasitized red blood cells (pRBCs). Additionally, the spleen plays a role in activating innate and adaptive immune cells, including CD4+ and CD8+. Previous studies have reported that the absence of the spleen's role in malaria can weaken the host's ability to defend against parasitemia, thereby increasing the risk of death (Ghosh and Stumhofer, 2021).

A histopathological examination of the spleen revealed significant differences ($P<0.05$) in the level of inflammatory cell infiltration ($P < 0.001$) between the treatment groups (Table 3). The infected control group (IC) had the highest inflammatory cell infiltration score (3.32 ± 0.17^{f}), indicating massive inflammation and immune cell activation in the spleen tissue due to Plasmodium berghei infection. In contrast, the healthy control group (HC) had the lowest infiltration score (0.12 ± 0.11^{a}), indicating normal conditions.

Table 3: Splenic pathology score in *Plasmodium berghei*-infected mice. Results are represented as mean ± SE. Different superscripts (a–f) within the same column indicate significant differences (P<0.05).

Group	Inflammatory cell Infiltrates	Hemorrhage	Hemozoin pigment deposition
HC (Healthy Control)	0.12 ± 0.11^{a}	1.40 ± 0.20^{a}	0.12 ± 0.11^{a}
IC (Infected Control)	3.32 ± 0.17^{f}	2.68 ± 0.22^{e}	3.44 ± 0.26^{e}
PC (Positive Control)	2.12 ± 0.26^{d}	2.08 ± 0.11^{cd}	1.88 ± 0.22^{c}
T1 (250 mg/kg BW)	2.80 ± 0.20^{e}	2.28 ± 0.11^{d}	2.72 ± 0.26^{d}
T2 (500 mg/kg BW)	1.56 ± 0.32^{c}	1.84 ± 0.09^{bc}	1.16 ± 0.16^{b}
T3 (1000 mg/kg BW)	0.88 ± 0.17^{b}	1.65 ± 0.07^{ab}	0.52 ± 0.17^{a}

The treatment groups showed varying degrees of reduction in infiltration levels. The PC group had a value of 2.12 ± 0.26^{d}, which indicates moderate inflammation in response to infection, though this was lower than for the IC group. Administering treatments T1, T2 and T3 showed a consistent downward trend as the doses or therapeutic efficacy increased. The highest infiltration value was found in T1 (2.80 ± 0.20^{e}), while the lowest was found in T3 (0.88 ± 0.17^{b}).

The results of this study demonstrate that the administration of sacha inchi seed essential oil extract modulates splenic histopathological alterations in *Plasmodium berghei* -infected mice. The intermediate dose (T2) was the most effective among the treatment groups, as indicated by reduced inflammatory cell infiltration and hemozoin pigment deposition, as well as lower hemorrhage scores. These results imply that sacha inchi oil has organ-protective properties in addition to its potential antiparasitic activity.

Similar variations were observed in haemorrhage scores ($P < 0.001$), with the IC group exhibiting the highest bleeding score (2.68 ± 0.22^{e}). This indicates vascular damage and increased permeability due to parasitic infection. In contrast, the HC group had the lowest bleeding score (1.40 ± 0.20^{a}), indicating normal conditions without vascular disorders. Compared to the IC group, all treatment groups showed a reduction in hemorrhage levels.

The apparent non-linear response, with the intermediate dose (500 mg/kg) being most effective, may indicate a hormetic effect. At low doses, the concentration of active compounds might be insufficient to counteract inflammation, whereas at high doses, excessive fatty acid intake could promote lipid peroxidation and pro-inflammatory signaling. This U-shaped pattern has been observed in other studies of polyunsaturated fatty acid-rich oils.

A congruent configuration was also identified in the observation of pigment metabolite scoring parameters ($P < 0.001$). The IC group showed the highest value (3.44 ± 0.26^{e}). By contrast, the HC group had the lowest value (0.12 ± 0.11a) as there was no parasite infection and therefore no hemozoin pigment was formed. In the treatment groups, a gradual decrease in pigment accumulation was observed. The PC group had a value of 1.88 ± 0.22^{c}, while the T1 and T2 groups had values of 2.72 ± 0.26^{d} and 1.16 ± 0.16^{b}, respectively. The most significant decrease was found in the T3 group (0.52 ± 0.17^{a}), indicating high effectiveness in suppressing parasite metabolic activity and accelerating the phagocytosis and elimination of hemozoin pigment.

The presence of different superscripts across groups confirmed that these variations were statistically significant ($P<0.05$). These results suggest that sacha inchi seed oil administration influences histopathological changes in the spleen of *Plasmodium berghei*-infected mice by modulating inflammatory responses, hemorrhage, and hemozoin deposition (Figure 2).

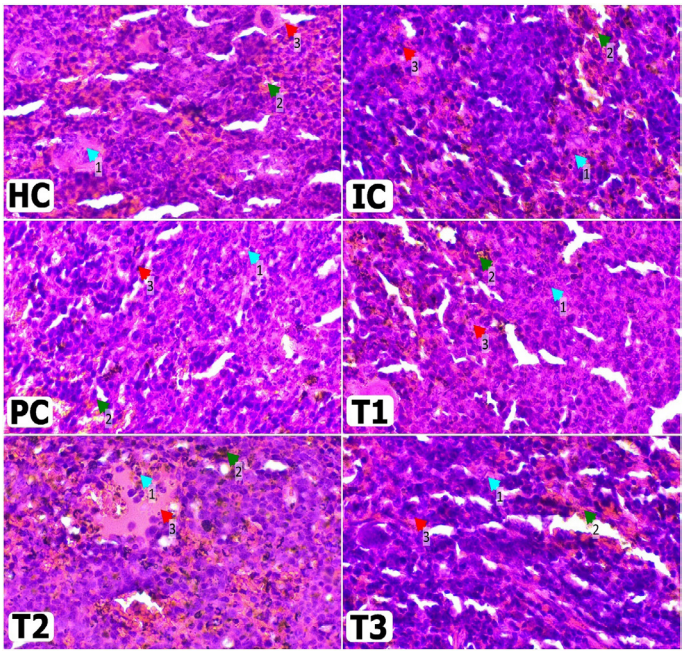

Figure 2: Histopathological features of the spleen showing (1) inflammatory cell infiltrates, (2) hemorrhage, and (3) hemozoin pigment deposition. (HC): healthy control; (IC): infected control; (PC): positive control (DHP); (T1): Sacha inchi oil 250 mg/kg BW; (T2): Sacha inchi oil 500 mg/kg BW; (T3): Sacha inchi oil 1000 mg/kg BW. Sections stained with H&E, magnification ×400.

The accumulation of hemozoin and the associated inflammatory response are key features of malaria pathogenesis, leading to splenic dysfunction and tissue injury (Olivier *et al*., 2014; Maslachah *et al*., 2019). The attenuation of these changes in the T2 group is consistent with the known immunomodulatory and antioxidant properties of bioactive compounds in sacha inchi, particularly omega-3 fatty acids and polyphenols, which have been reported to suppress pro-inflammatory cytokines and oxidative stress (Ambulay *et al*., 2020, 2021; Wang *et al*., 2024; Ilmayani *et al*., 2024).

The positive control (DHP) showed higher pathology scores despite its known antimalarial efficacy. This could result from drug-induced oxidative stress or residual inflammation during parasite clearance, suggesting that histopathological changes may persist even when parasitemia is reduced.

Compared to standard therapy, which primarily targets parasite clearance but often fails to prevent host tissue damage, sacha inchi oil appears to confer additional benefits by preserving splenic architecture (Firdaus *et al*., 2025). These findings align with previous reports on the protective effects of plant-derived oils rich in polyunsaturated fatty acids against infection-induced inflammation (Dkhil *et al*., 2015). Taken together, the present results highlight the potential of sacha inchi seed oil as a natural adjunctive therapy in malaria, capable of modulating pathological changes in the spleen and reducing the burden of excessive inflammation.

However, without parasitemia data, it is not possible to definitively separate the oil's direct organ-protective effects from potential antiparasitic actions. Further investigation is required to validate these findings at the molecular level and to evaluate its combined use with conventional antimalarial drugs.

CONCLUSION AND RECOMMENDATIONS

In conclusion, sacha inchi seed oil modulated splenic histopathology in a dose-dependent manner of splenic histopathology in *Plasmodium berghei*-infected mice, with the intermediate dose showing the most consistent improvement. Although these results indicate potential organ-protective effects, the absence of parasitemia data and variability across doses warrant cautious interpretation. Further studies incorporating parasite burden measurement and molecular analyses are essential to clarify its mechanism and therapeutic relevance. Future studies are recommended to incorporate parasitaemia measurements, immune cell profiling and molecular analyses of inflammatory and oxidative stress pathways, in order to elucidate the underlying mechanisms of action. Furthermore, evaluating longer treatment durations and combining histopathological findings with functional immunological outcomes would offer a more thorough insight into the therapeutic relevance of sacha inchi seed oil as a supportive intervention in the management of malaria.

ACKNOWLEDGMENT

The authors would like to express their sincere gratitude to the Faculty of Veterinary Medicine, Airlangga University, for the facilities and support provided during the course of this study. Appreciation is also extended to the Division of Veterinary Anatomy and the Division of Veterinary Parasitology, as well as the Research Group of Toxoplasma, Institute of Tropical Disease, Universitas Airlangga, for their valuable guidance, technical assistance, and constructive input throughout the research process.

NOVELTY STATEMENT

This study provides the first evidence that sacha inchi (*Plukenetia volubilis*) seed oil has a dose-dependent protective effect on the spleen of mice infected with *Plasmodium berghei*, with an intermediate dose yielding the most consistent improvement. This highlights the oil's potential as an immunomodulatory adjunct in the treatment of malaria.

AUTHORS'S CONTRIBUTION

Aliya Putri and Lucia Tri Suwanti conceptualized and designed the study. Aliya Putri, Eka Pramyrtha Hestiana-ah, and Mufasirin Mufasirin conducted the experimental work and data collection. Aliya Putri and Lucia Tri Suwanti performed data analysis and interpretation. Aliya Putri prepared the original draft of the manuscript. Lucia Tri Suwanti, Hani Plumeriastuti, and Suryo Kuncorojakti critically reviewed and edited the manuscript. Lucia Tri Suwanti supervised the study. All authors read and approved the final manuscript.

Generative AI and AI-assisted technology statement

The authors declare that no generative AI or AI-assisted technologies were used in the writing, data analysis, or preparation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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