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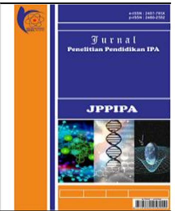

Methanol Extract *Alstonia scholaris* L. R. Br as Hepatoprotective Mice (*Mus musculus*) Infected with Plasmodium berghei ANKA Strains

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Abstract: Alstonia scholaris L. R. Br is one of the traditional plants that contain natural antioxidant compounds which are thought to be able to repair damage to the liver cells of mice (Mus musculus) infected with Plasmodium berghei strain ANKA. This study aimed to determine the effect of methanol extract of Alstonia scholaris L. R. Br stem bark on levels of SGPT enzymes and liver cells of mice (Mus musculus) infected with Plasmodium berghei strain ANKA. Mice with a body weight of 20-30 g were infected with Plasmodium berghei as much as 0.1 ml per head and left until the percentage of parasitemia reached 1-5%. Then mice (Mus musculus) were given methanol extract of Alstonia scholaris L. R. Br stem bark at doses of 1, 10, 100, and 200 mg/kg BW for 4 consecutive days. After that, surgery was performed to take blood to observe SGPT enzyme levels and mice liver cells to be prepared with Hematoxylin Eosin (HE) staining. The results of ANOVA showed that the methanol extract of Alstonia scholaris L. R. Br stems bark doses of 1 mg/kg BW, 10 mg/kg BW, 100 mg/kg BW and 200 mg/kg BW could reduce SGPT enzyme levels and repair damage to the liver cells of mice infected with Plasmodium berghei ANKA strains

Keywords: *Alstonia scholaris L. R. Br*; Methanol extract antioxidants; Liver Cells

Introduction

Malaria is a protozoan infectious disease caused by Plasmodium, which is transmitted through the bite of a female Anopheles mosquito (Mali et al., 2006; Sam et al., 2022). Plasmodium in humans infects erythrocytes and undergoes asexual development in liver tissue and erythrocytes. Infection by malaria gives symptoms such as fever, chills, anemia, and splenomegaly (Dong et al., 2020). Apart from infecting humans, Plasmodium can infect animals such as birds, reptiles and mammals (Baer et al., 2007).

Disturbance to the human liver due to malaria is still a problem in tropical countries. In these countries, there are one million deaths each year (Kaye-Bailey, 2019). More than 45% of the world's population has experienced malaria. In Indonesia, malaria has spread throughout the archipelago, especially in eastern Indonesia (Elyazar et al., 2011). The archipelago comprises seven main islands: Sumatra, Java, Kalimantan, Sulawesi, Maluku, the Lesser Sundas and Papua. Since decentralization of government power in 2000, Indonesia has been considered to consist of 33 provinces, 465 districts/municipalities, 6093 sub-districts and 73,067 villages (Depkes, 2008).

Splenomegaly is one of the signs found in malaria cases which can cause liver damage. Splenomegaly is defined as enlargement of the spleen measured by weight or size (Kang et al., 2019). This is because the sporozoites that enter the blood go to the liver to form schizonts which break down in the liver and form merozoites which then attack other liver cells. Some of the merozoites that remain in the liver will develop continuously causing the liver to secrete large amounts of parasite-infected erythrocytes, causing reduced hemoglobin in the blood and thrombosis in blood vessels, as well as necrosis in the liver. Due to circulation

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disorders, there will be a decrease in blood flow to the liver (Harijanto, 2003).

One of the indicators used to determine impaired liver function is through laboratory tests to increase serum glutamate pyruvate transaminase (SGPT) levels. SGPT is used as an enzyme, because its source is in the liver (Ramadhani et al., 2017). Efforts to eradicate malaria in Indonesia are still experiencing several obstacles, including the emergence of vector resistance to pesticides, the emergence of Plasmodium resistance to antimalarial drugs, and public knowledge of malaria is still low (Elyazar et al., 2011; Habte et al., 2020; Sabbatani et al., 2010). The high incidence of resistance that has been widely reported has further increased the progress of researchers to search for new antimalarial drugs by conducting research using medicinal plants that have the potential to contain natural antioxidants as an alternative to overcome resistance that can safely optimize liver function. These antioxidant compounds are spread in various parts of plants such as roots, stems, bark, twigs, leaves, flowers, fruit, and seeds (Dey et al., 2021).

In addition to antioxidant compounds, there are also secondary metabolites which are useful as antimalarials. These compounds can be grouped into seven groups, namely, alkaloids, quassinoids, sesquiterpenes, triterpenoids, flavonoids, quinones, and miscellaneous compounds (Saxena et al., 2003).

One type of medicinal plant used by the people of Maluku to treat malaria is the pule plant (*Alstonia scholaris* L. R. Br). The part taken by the community from this plant is the bark from the tree. The bark of the pule tree contains saponins, flavonoids, and polyphenols. The bitter substances contain ingredients such as quasinoids, echeretine, and echicherine which can inhibit the growth of the trophozoite and schizont stages of the malaria parasite (Harijanto, 2003; Li et al., 2023).

The role of flavonoid compounds in inhibiting the growth of malaria parasites has been proven in several antimalarial medicinal plants. Flavonoids are one of the many secondary metabolite compounds produced by a plant, which can be found in the leaves, roots, wood, bark, and pollen. Flavonoids contained in the bark of the pule tree are thought to have potential as antimalarials. This conjecture is based on the research results of several previous researchers who conducted research using flavonoids from chempedak stem bark as antimalarials (Koram et al., 2005; Li et al., 2023; WHO, 2009). This study aimed to determine the effect of methanol extract of Alstonia schloris L. R. Br stem bark on the histology of liver cells of mice (Mus muculus) infected with Plasmodium berghei ANKA strain.

Method

Types and Research Equipment

This research is a laboratory experiment. The tools used were 3 Erlenmeyer pieces measuring 1000 ml, 1 Erlenmeyer measuring 500 ml, measuring cups, Whatman filter paper No.2, analytical balance, syringe, object glass container, scratch slide, 018 Olympus cover microscope, tubi centrifuge, heparin, sonde tool, volume pipette, spatula, 25 ml test tube, vaporizer cup, spectrophotometer, photometer, analyzer, and digital camera.

While the materials used were *Alstonia scholaris* bark, 2 L absolute methanol, Plasmodium ANKA Strain, and experimental animals, namely mice with body weight intervals of 20-30 grams, and age ± 2 months as many as 30 heads, CMC Na 0.5% (Carboxy Methyl Cellulose sodium), alceiver, Aluminum foil, tissue, cotton, and detergent, 4% Formalin, Aquades, Paraffin, Alcohol 30%, 50%, 70%, 80%, 90%, 100%, Xylol, and Hematoxylin Eosin.

Work Procedures

Extraction. The bark of the pule tree was obtained from the village of Poka with the trunk circumference of the *Alstonia scholaris* tree being 45 cm. The bark was cut into small pieces and dried in the lab for three weeks. After drying, grind it with a blender, and the fine powder is weighed as much as 348 grams. Furthermore, extraction was carried out using the Maceration method. The extraction results were then evaporated using a rotary evaporator for three hours to obtain a concentrated methanol extract.

Negative control setup. In this study, negative controls were used, namely mice that were given Plasmodium berghei infection but were not given the extract (untreated).

Plasmodium berghei infection in donor mice. Donor mice were infected with frozen P. berghei intraperitoneally. As much as 200 µl of Plasmodium enters the body of the donor mice. Furthermore, parasitemia was observed every day until it reached > 20%, then surgery was performed to take blood from the infected mice's heart and put it into a blood tube. The volume of blood that has been taken is dissolved with an alceiver to obtain 5% parasitemia.

Testing the effectiveness of anti-malarial in vivo. The experimental mice were infected with 200 µl of blood from donor mice (Abolins et al., 2017). Observation of the level of parasitemia is carried out in the following way: Mice blood was taken from the tail (cut ± 1 mm), then placed on the slide. However, before a thin blood smear is made, the blood is allowed to spread to the left and right along the edge of the object glass. Furthermore, the glass object is rubbed forward along the surface of

the preparation (preparation) with a thin layer of blood. After being allowed to dry, the blood preparations were fixed with absolute methanol for 3 minutes. Blood preparations were stained with Giemsa solution. The preparation was completely dripped with the solution and left for 45 minutes. The preparation is washed with running water (angle 40°) slowly and dried. The preparations were examined under a microscope after being dripped with immersion oil (magnification 10 x 100). From the examination of the thin blood smear preparations, blood that has been infected with the malaria parasite will be seen. After knowing the percentage of parasitemia, then proceeded with testing the effectiveness of malaria from the extract. The treatment was carried out 6 times. Each treatment I was a negative control, treatment II was a positive control, the dose of methanol extract of pule tree bark was 1 mg/kg BW, group III the dose of methanol extract of pule tree bark was 10 mg/kg BW, group IV dose methanol extract of pule bark bark 100 mg/kg body weight and group V dose of methanol extract pule tree bark 200 mg/kg body weight. Each group consisted of 3 rats (3 replications). Groups II, III, IV, and V were given the extract orally using a sonde at a determined dose. Then the extract is given for 4 days. However, observations of mice parasitemia were still carried out until day 6 to see the drug profile. Finally, on the 7th day, surgery was performed to take blood and liver organs.

Serum collection. Blood collection is done through the heart (intracardial) with a syringe ±3 ml of blood was taken, and then put in a clean and dry test tube. After that, it was centrifuged at 300 rpm for 10 minutes. Separated serum is taken and put in another tube which is clean and closed.

Serum examination. Before measuring SGPT activity serum, a reagent solution was first made by dissolving the reagent tablet in a buffer solution with a ratio of 1:10. After that, SGPT activity can be measured by taking 100µl of blood serum and adding 1000µl of reagent solution then homogenizing and waiting for 1 minute before being measured. After 1 minute, the absorbance (A) was measured with a spectrophoto-meter at a wavelength of 340 nm (Irnawati et al., 2005). Statistical analysis of this concentration was to determine the effect of the methanol extract of the stem bark of *Alstonia scholaris* L. R. Br. on SGPT concentration.

Preparation of liver histology preparations. Liver tissue preparation was performed using Hematoxylin Eosin (HE) staining.

Data Analysis

The SGPT data obtained were then analyzed using Analysis Of Variance (ANOVA). If the results of the ANOVA test show a significant difference, then a 95% BNT further test will be carried out. Liver histology will be analyzed descriptively by observing liver histology preparations.

Result and Discussion

SGPT Enzyme Levels in Mice

Plasmodium berghei infection with ANKA strain in mice (*Mus musculus*) can increase SGPT enzyme levels as an indicator of liver cell damage. However, after being given the methanol extract of *Alstonia scholaris* L. R. Br stem bark, it was able to reduce the levels of these enzymes. The increase and decrease in SGPT enzyme levels can be seen in Table 1 below.

Table 1. Average SGPT Enzyme Levels in Mice Infected with Plasmodium berghei Strain ANKA and Given Methanol Extract of *Alstonia scholaris* L. R. Br.

Treatment	Average SGPT Levels (UI/L) ± SD
Control (+)	229.00 ± 1.414[a]
Control (-)	97.00 ± 1.414[b]
1 mg/kg BB	205.00 ± 5.657[c]
10 mg/kg BB	202.00 ± 1.414[c]
100 mg/kg BB	184.50 ± 0.707[d]
200 mg/kg BB	178.00 ± 1.414[e]
(X ± SD)	182.58 ± 43.492

Note: Superscripts with the same letters are not significantly different (P > 0.05). UI/l: International Unit/L

The average SGPT levels in Table 1 show that the levels of the SGPT enzyme in the group of mice that were not infected with Plasmodium berghei strain ANKA (negative control) were 97.00 ± 1.414. After being infected with Plasmodium berghei ANKA strain for two weeks in the positive control group, the SGPT enzyme level was 229.00 ± 1.414. After an increase in SGPT levels, the mice were given methanol extract of *Alstonia scholaris* L. R. Br coolies for 4 days. Administration of methanol extract of stem bark of *Alstonia scholaris* L. R. Br at a dose of 1 mg/kg BW to the group of infected mice, the SGPT enzyme level was 205.00 ± 5.657, the group of infected mice was given methanol extract of the bark of *Alstonia scholaris* L. R. Br at a dose of 10 mg/kg BW. SGPT enzyme was 202.00 ± 1.414, the group of infected mice was given methanol extract of *Alstonia scholaris* L. R. Br bark at a dose of 100 mg/kg BW, the SGPT enzyme content was 184.50 ± 0.707, and the group of infected mice was given methanol extract of pule tree bark at a dose of 200 mg /kg BW SGPT enzyme levels of 178.00 ± 1.414.

Based on the results of the one-way Analysis Of Variance (ANOVA) using the SPSS 16.0 program, it was shown that $F_{count} > F_{table}$, which means that the methanol extract of *Alstonia scholaris* L.R.Br coolie had a significant effect on SGPT enzyme levels (P > 0.05). The results of

further tests using the Least Significant Difference Test (LSD) showed that there were significant differences between each treatment given, but between doses of 1 mg/kg BW and 10 mg/kg BW were not significantly different.

The results of measuring the levels of the SGPT enzyme in each treatment group showed that in the positive control group infected with Plasmodium berghei strain ANKA for 14 days the levels of the SGPT enzyme were higher. Increased levels of the SGPT enzyme in the group of mice infected with Plasmodium berghei strain ANKA indicated liver cell damage. This is due to damage to the hepatocyte cells, especially the cell membrane, this causes the SGPT enzymes present in the hepatocyte cells to come out and flow into the blood. In addition, increased SGPT levels can also be caused by hepatitis virus infection and alcohol.

According to Darmawan et al. (2021), infection with Plasmodium Berghei strain ANKA in mice can increase the production of free radicals in liver cells. Excessive amounts of free radicals in the body are very dangerous because they cause damage to cells, nucleic acids, proteins, and fatty tissues (Basir et al., 2012). Damage to cells by reactive free radicals is preceded by damage to cell membranes, including changing the fluidity, structure, and function of cell membranes. The production of unbalanced free radicals will also cause damage to macromolecules including proteins, lipids, and DNA (Ramadhani et al., 2017). An imbalance between the production of free radicals (reactive oxygen compounds) and the ability to exchange antioxidants results in oxidative stress which can cause damage to cells including liver cells, causing an increase in SGPT (Berawi et al., 2017). One of the aminotransferase enzymes that is often used in the clinical diagnosis of liver cell damage is Serum Glutamate Pyruvate Transaminase (SGPT). Aminotransferases include non-functional plasma enzymes that do not carry out physiological functions in the blood. The presence of aminotransferases in plasma at levels above normal values suggests an increase in the rate of tissue damage. An increase in SGPT concentration will occur if there is release of the enzyme aminotransferase intracellularly into the blood due to liver cell necrosis or acute liver damage (Sodikin, 2011).

Alstonia scholaris L. R. Br stems bark extract can reduce SGPT enzyme levels in mice infected with Plasmodium berghei. This can be seen by the low levels of the SGPT enzyme in the group of mice infected with Plasmodium Berghei and given *Alstonia scholaris* L. R. Br extract at a dose of 100 mg/kg BW and 200 mg/kg BW when compared to the positive control group of mice. The decrease in GPT enzyme levels was due to the presence of secondary metabolites such as flavonoids, saponins, and polyphenols contained in the stem bark of *Alstonia scholaris* L. R. Br.

As antioxidants, flavonoids function to reduce the impact of free radicals on lipid peroxidation. Flavonoids also capture free radicals by releasing hydrogen atoms in their hydroxyl groups. The decrease in SGPT enzyme levels in this study indicates that the content of flavonoid compounds present in the stem bark of *Alstonia scholaris* L. R. Br has hepatoprotective activity which can inhibit oxidation reactions through free radical scavenging mechanisms by donating an electron to an unpaired electron in free radicals so that the number of free radicals decreases. Flavonoids are thought to affect inhibiting liver damage by binding to free radicals so that their impact on the liver is reduced.

Histology of Mice Liver Cells

The results of observing the livers of mice using Hematoxylin Eosin (HE) staining showed that in the group of mice that were given distilled water (Figure 1A), there was no damage to the liver. The group of mice infected with Plasmodium berghei but not given the methanol extract of *Alstonia scholaris* L. R. Br stem bark for 14 days (Figure 1B) showed liver cell damage. This can be seen by the presence of dilated sinusoids, swelling of the hepatocytes, and liver cell necrosis. Administration of methanol extract of stem bark of *Alstonia scholaris* L. R. Br at a dose of 1 mg/kg BW (Figure 1C), methanol extract of stem bark of *Alstonia scholaris* L. R. Br at a dose of 10 mg/kg BW (Figure 1D) for 4 days showed no improvement in liver cells mice. Administration of methanol extract of stem bark of *Alstonia scholaris* L. R. Br at a dose of 100 mg/kg BW (Figure 1E) and methanol extract of stem bark of *Alstonia scholaris* L. R. Br at a dose of 200 mg/kg BW (Figure 1F) for 4 days showed an improvement in the cells mouse liver cells. A histological picture of the liver cells of mice infected with Plasmodium berghei can be seen in Figure 1.

Histological observations of the liver cells of mice (*Mus musculus*) infected with Plasmodium berghei strain ANKA in vivo (Figure 1) showed that in the group of mice that were given distilled water (Figure 1A), there was no necrosis (cell death) in the liver cells. The group of mice infected with Plasmodium berghei ANKA (Figure 1B) showed dilated sinusoids, hepatocytes experienced swelling (hydropic degeneration) of endothelial cells in small and irregular numbers, and necrosis of liver cells. The group of mice infected with Plasmodium Berghei and given methanol extract of *Alstonia scholaris* L. R. Br stem bark at a dose of 1 mg/kg BW (Figure 1C) and a dose of 10 mg/kg BW (Figure 1D) showed that the sinusoids were still dilated, the hepatocytes were still experiencing swelling (hydropic

degeneration) of endothelial cells are still small and irregular in number and necrosis occurs in liver cells. The group of mice infected with Plasmodium berghei and given the methanol extract of *Alstonia scholaris* L.R. Br doses of 100 mg/kg BW (Figure 1E) and doses of 200 mg/kg BW (Figure 1F) showed that the sinusoids looked tight and regular and the endothelial cells began to be numerous and orderly but necrosis still occurred.

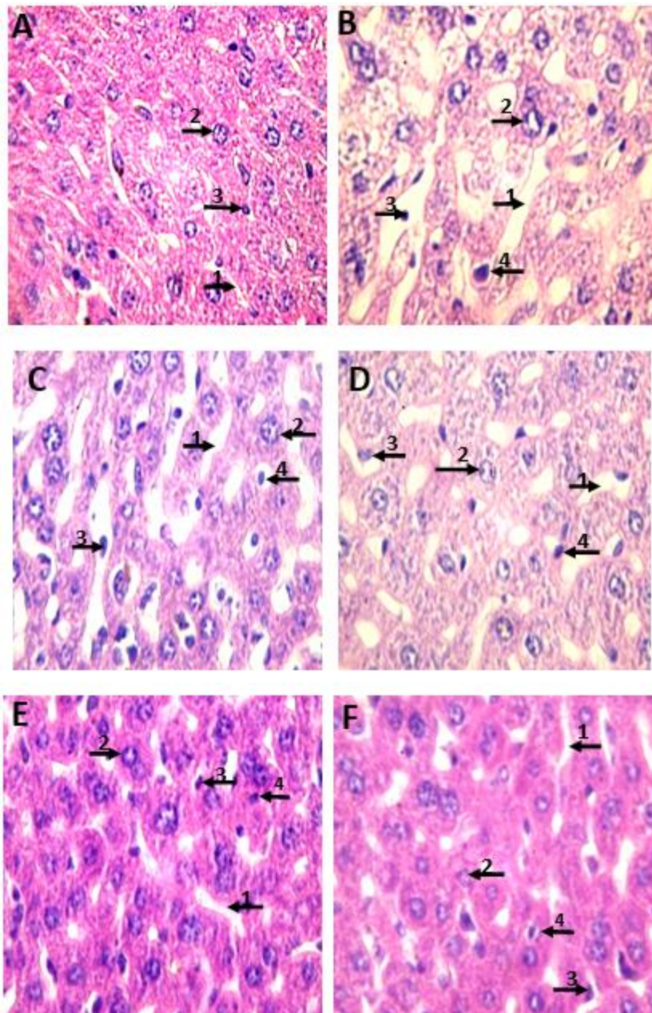

Figure 1. Histological picture of mice liver cells (1000x magnification). (A) Control (-), B (Control (+), (C) methanol extract of stem bark of *Alstonia scholaris* L. R. Br dose of 1 mg/kg BW, (D) methanol extract of stem bark of *Alstonia scholaris* L. R. Br dose of 10 mg/kg BW, (E) methanol extract of stem bark of *Alstonia scholaris* L. R. Br dose of 100 mg/kg BW and (F) methanol extract of stem bark of *Alstonia scholaris* L. R. Br dose of 200 mg/kg BW Description: (1) Sinusoids, (2) Hepatocytes, (3) Endothelial cells and (4) Necrosis.

The results of histological analysis of the liver of mice (*Mus musculus*) infected with Plasmodium berghei strain ANKA using Hematoxylin Eosin (HE) staining in this study showed widening of the sinusoids, swollen hepatocytes (hydropic degeneration), endothelial cells in small and irregular numbers, and the presence of necrosis or apoptosis in liver cells (Figure 1B). Hydropic degeneration is the initial response to toxic substances. One of the causes of hydropic degeneration is hypoxia. Hypoxia causes a decrease in the process of exidative phosphorylation in mitochondria which results in a decrease in ATP. Pathological conditions will cause the fluid around the cells to seep into the cells and cause cell swelling. Microscopically, there is water in the cytoplasm of the cell, so that it looks like the cytoplasm is swollen and contains clear spaces surrounding the nucleus. In general, hepatocytes that experience necrosis show changes in the nucleus and cytoplasm. The nucleus will shrink and turn blue due to aggregation of nuclear chromatin, this process is called pyknotic (Cotran et al., 1989; Jubb et al., 1993).

In contrast to necrosis, apoptosis is cell death that occurs in an orderly and controlled manner. Apoptosis can occur in normal (physiological) or abnormal (pathological) processes. The morphological characteristics of apoptotic cells are cell shrinkage or cells become shriveled or small; chromatin condensation, namely chromatin condenses at the edges, under the nuclear membrane and the nucleus can also break into several fragments; formation of protrusions in the cytoplasm and apoptotic bodies as well as phagocytosis of apoptotic cells or apoptotic bodies (Spector, 1993).

In malaria infection, antigens from parasites are processed by antigen presenting cells via MHC molecules, resulting in activation of T lymphocytes which will produce IFN-γ cytokines. These cytokines activate phagocytic cells, especially monocytes and macrophages which then release materials as a host response to parasites, namely oxygen derived free radicals (ODFR) and cytokines (TNF-α and IL-6) (Hunt et al., 1992).

In liver cells, TNF-α will cause protein synthesis in the acute phase. In muscle and fat cells, TNF-α will cause catabolism and in some cells will cause apoptosis. The biological action of TNF-α in principle is to stimulate the uptake of neutrophils and monocytes at the site of infection, and then activate them to kill bacteria. The biological actions of TNF-α include: (1) TNF-α in vascular endothelial cells will cause the expression of adhesion molecules on the endothelial surface, this expression makes the endothelial surface able to bind to leukocytes, neutrophils, monocytes, and lymphocytes. (2) TNF-α stimulates endothelial cells and macrophages to secrete chemokines, this chemokines secretion causes chemotaxis and more leukocyte recruitment. TNF-α also stimulates mononuclear phagocytic cells to express IL-1, where IL-1 functions similarly to TNF-α. (3) TNF-α induces apoptosis of several cell types, but the mechanism of this apoptosis is unclear. Besides TNF and

cytokines, the damage that underlies the pathogenesis of malaria is also caused by those produced by activated macrophages (Basir et al., 2012; Natsume et al., 2014).

The involvement of NO and superoxide free radicals in the pathogenesis of complications in malaria is now becoming a concern. This is supported by facts and theories that induction of NO by cytokines is thought to mediate secondary pathological abnormalities in cerebral malaria by severing important aspects of synaptic signal transmission (Maneerat et al., 2013). In addition, reactive oxygen compounds can cause oxidative stress on endothelial cells, causing damage and even cell death. NO via endothelial cells plays an important role in regulating blood vessels and blood flow. NO also has a significant contribution to homeostasis in blood vessels, through the regulation of platelet aggregation and adhesion to the endothelium, in regulation of clotting in blood vessels, (Radomski et al., 1991), regulates the adhesion of neutrophil cells to the endothelium so that it can inhibit the increased process of inflammation (Oza et al., 2022). Organ failure in patients with falciparum malaria is associated with neutrophil activation and endothelial damage.

Administration of methanol extract of stem bark of *Alstonia scholaris* L.R to mice (*Mus musculus*) infected with Plasmodium bergehi strain ANKA showed repair of damage to the liver cells of mice. Administration of methanol extract of stem bark of *Alstonia scholaris* L.R.Br at a dose of 1 mg/kg BW (Figure 1C) and 10 mg/kg BW (Figure 1D) could not repair damage to the liver cells of mice. This can be seen with the sinusoids still dilating, hepatocytes still experiencing swelling (hydropic degeneration), endothelial cells in small and irregular numbers, and necrosis still occurring. This is due to the small dose given so that the antioxidant content contained in the stem bark of *Alstonia scholaris* L.R.Br at doses of 1 mg/kg BW and 10 mg/kg BW cannot neutralize free radicals.

Administration of methanol extract of stem bark of *Alstonia scholaris* L.R at a dose of 100 mg/kg BW (Figure 1E) and 200 mg/kg BW (Figure 1F) can improve the level of damage to the liver cells of mice (Wang et al., 2014). This can be seen by the sinusoids starting to arrange tightly, the hepatocytes starting to return to normal and the endothelial cells starting to multiply. The methanol extract of *Alstonia scholaris* L.R.Br stem bark at a dose of 200 mg/kg BW (Figure 1F) showed that the sinusoids began to be tightly arranged, intact and normal, intact hepatocytes and endothelial cells in large numbers and arranged tightly and regularly but necrosis still occurred. Liver cell necrosis in the group of mice infected with Plasmodium berghei strain ANKA and given methanol extract of stem bark of *Alstonia scholaris* L.R.Br at doses of 100 mg/kg BW and 200 mg/kg BW was very little when compared to the group of mice infected with Plasmodium berghei strain ANKA, and the group of infected mice Plasmodium berghei strain ANKA and given the methanol extract of *Alstonia scholaris* L.R.Br stem bark at a dose of 1 mg/kg BW and 10 mg/kg BW.

This is because T cells as regulators activate cytotoxic T cells, macrophages, and other phagocytes through the Th-1 subset. The Th-1 subset through the secretion of IFN and TNF (tumor necrosis factor) will activate cellular immunity carried out by macrophages, monocytes, and leukocytes. Th-1 also activates NK (nature killer) cells through the mechanism of Antibody antibody-dependent cellular Cytotoxicity (ADCC), which is the mechanism of action of antibodies against parasites mediated by eosinophil cells (Tizard, 2000). These antibodies generally coat the parasite making it easier for it to be phagocytosed. The Th-1 subset will activate both specific and non-specific cellular immunity mechanisms to kill intra-erythrocyte Plasmodium.

All mechanisms of immunity against malaria, especially at the erythrocyte stage, are regulated and coordinated by CD4 T lymphocytes, while effectors are the production of antibodies by B lymphocytes, activation of CD8 cytotoxic lymphocytes, or the production of cytokines, and is related to the activation of non-specific immune responses, especially phagocytes. In general, it is said that antibodies work against extracellular parasites in blood and other body fluids, whereas cellular immunity primarily fights against intracellular parasites. In malaria, antibodies work by inhibiting the parasite's ability to enter new cells, especially in sporozoites and merozoites, while cellular immunity inhibits parasite development in hepatocytes and erythrocytes (Keswani et al., 2014).

Improvement occurred in the liver cells of mice infected with Plasmodium berghei strain ANKA which were given methanol extract of pule bark at doses of 100 mg/kg BW and 200 mg/kg BW due to the high content of natural antioxidants found in the methanol extract of pule bark. Natural antioxidants found in the bark of the pule tree include flavonoids, saponins, and polyphenols. Flavonoids include natural phenolic compounds that have potential as antioxidants and have bioactivity as drugs (Panche et al., 2016). These compounds can be found in stems, leaves, fruit, twigs, roots and flowers. Flavonoids in the human body function as antioxidants and protect cell structures, increase the effectiveness of vitamin C, are anti-inflammatory, prevent bone loss, and as antibiotics (Li et al., 2023; Ullah et al., 2020).

Conclusion

Alstonia scholaris L. R. Br stem bark extract can act as a hepatoprotective as evidenced by a decrease in SGPT enzyme levels in mice infected with Plasmodium berghei. Administration of methanol extract of stem bark of *Alstonia scholaris* L.R.Br at a dose of 100 mg/kg BW and 200 mg/kg BW to mice infected with Plasmodium berghei ANKA can prevent damage to the liver cells of mice such as the sinusoids begin to form tightly packed, intact and normal, intact hepatocytes and endothelial cells in large numbers and arranged tightly and regularly but necrosis still occurs..

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Author Contributions
Authors listed in this article contributed to the research and development of the article. The authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest
The authors declare no conflict of interest.

References

Abolins, S., King, E. C., Lazarou, L., Weldon, L., Hughes, L., Drescher, P., Raynes, J. G., Hafalla, J. C. R., Viney, M. E., & Riley, E. M. (2017). The comparative immunology of wild and laboratory mice, Mus musculus domesticus. *Nature Communications*, *8*(1), 14811. https://doi.org/10.1038/ncomms14811

Baer, K., Klotz, C., Kappe, S. H. I., Schnieder, T., & Frevert, U. (2007). Release of Hepatic Plasmodium yoelii Merozoites into the Pulmonary Microvasculature. *PLoS Pathogens*, *3*(11), e171. https://doi.org/10.1371/journal.ppat.0030171

Basir, R., Rahiman, S. S. F., Hasballah, K., Chong, W. C., Talib, H., Yam, M. F., Jabbarzare, M., Tie, T. H., Othman, F., Moklas, M. A. M., Abdullah, W. O., & Ahmad, Z. (2012). Plasmodium berghei ANKA infection in ICR mice as a model of cerebral malaria. *Iranian Journal of Parasitology*, *7*(4), 62–74. http://doi.org/11.1144/SP470.3

Berawi, K. N., & Agverianti, T. (2017). Efek Aktivitas Fisik pada Proses Pembentukan Radikal Bebas sebagai Faktor Risiko Aterosklerosis. *Jurnal Majority*, *6*(2), 85–90. Retrieved from https://juke.kedokteran.unila.ac.id/index.php/majority/article/view/1019

Darmawan, M. R., Rahardjo, D., Tyasningsih, W., Kurnijasanti, R., Legowo, D., & Setiawan, B. (2021). Acute Toxicity Test Of The Green Viper Snake (Trimeresurus albolabris), Macroscopic Description Of The Kidney And Liver Of Mice (Mus musculus). *Journal of Basic Medical Veterinary*, *10*(2), 59. https://doi.org/10.20473/jbmv.v10i2.31135

Depkes. (2008). *Indonesia Health Profile 2007* (p. 308). Center for Data and Information, Indonesian Ministry of Health. Retrieved from https://www.kemkes.go.id

Dey, D., Quispe, C., Hossain, R., Jain, D., Ahmed Khan, R., Janmeda, P., Islam, M. T., Ansar Rasul Suleria, H., Martorell, M., Daştan, S. D., Kumar, M., Taheri, Y., Petkoska, A. T., & Sharifi-Rad, J. (2021). Ethnomedicinal Use, Phytochemistry, and Pharmacology of Xylocarpus granatum J. Koenig. *Evidence-Based Complementary and Alternative Medicine*, *2021*, 1–16. https://doi.org/10.1155/2021/8922196

Dong, Y., Deng, Y., Xu, Y., Chen, M., Wei, C., Zhang, C., Mao, X., & Xue, J. (2020). Analysis of initial laboratory diagnosis of malaria and its accuracy compared with re-testing from 2013 to 2018 in Yunnan Province, China. *Malaria Journal*, *19*(1), 409. https://doi.org/10.1186/s12936-020-03477-1

Elyazar, I. R. F., Hay, S. I., & Baird, J. K. (2011). Malaria Distribution, Prevalence, Drug Resistance and Control in Indonesia. In *PMC PubMed Central* (Vol. 74, pp. 41–175). https://doi.org/10.1016/B978-0-12-385897-9.00002-1

Habte, G., Nedi, T., & Assefa, S. (2020). Antimalarial Activity of Aqueous and 80% Methanol Crude Seed Extracts and Solvent Fractions of Schinus molle Linnaeus (Anacardiaceae) in Plasmodium berghei -Infected Mice. *Journal of Tropical Medicine*, *2020*, 1–9. https://doi.org/10.1155/2020/9473250

Harijanto, P. N. (2003). *Malaria: Epidemiology, Pathogenesis, Clinical Manifestations, & Management*. University Library. Retrieved from http://elib.unisa-bandung.ac.id:80/

Irnawati, R., A, W., & Studiawan, H. (2005). Effect of Ethanol Extract and Aqueous Extract of Artocarpus champeden Stem Bark on SGPT and SGOT Enzyme Levels in Mice. *Airlangga Journal of Pharmacy*, *5*(3). Retrieved from https://journal.unair.ac.id/

Kang, D. W., & Kim, S. H. (2019). Clinical aspects of splenomegaly as a possible predictive factor of coronary artery changes in Kawasaki disease.

Cardiology in the Young, *29*(3), 297–302. https://doi.org/10.1017/S1047951118002238

Kaye-Bailey, J. (2019). *World Malaria Day. This entry was posted in Health*. Health, Malaria, News, Uncategorized; Uncategorized. Retrieved from https://www.thebutterflytree.org.uk/pages/2019/world-malaria-day-4/

Keswani, T., & Bhattacharyya, A. (2014). Differential role of T regulatory and Th17 in Swiss mice infected with Plasmodium berghei ANKA and Plasmodium yoelii. *Experimental Parasitology*, *141*, 82–92. https://doi.org/10.1016/j.exppara.2014.03.003

Koram, K. A., Abuaku, B., Duah, N., & Quashie, N. (2005). Comparative efficacy of antimalarial drugs including ACTs in the treatment of uncomplicated malaria among children under 5 years in Ghana. *Acta Tropica*, *95*(3), 194–203. https://doi.org/10.1016/j.actatropica.2005.06.018

Li, M., Qian, M., Jiang, Q., Tan, B., Yin, Y., & Han, X. (2023). Evidence of Flavonoids on Disease Prevention. *Antioxidants*, *12*(2), 527. https://doi.org/10.3390/antiox12020527

Mali, S., Steele, S., L, S., & Arguin, P. M. (2006). Centers for Disease Control and Prevention (CDC). *Malaria Surveillance--United States, MMWR Surveill Summ*, *57*(5), 24–39. Retrieved from https://pubmed.ncbi.nlm.nih.gov/18566568/

Maneerat, W., Phakhodee, W., Ritthiwigrom, T., Cheenpracha, S., Deachathai, S., & Laphookhieo, S. (2013). Phenylpropanoid derivatives from Clausena harmandiana fruits. *Phytochemistry Letters*, *6*(1), 18–20. https://doi.org/10.1016/j.phytol.2012.10.006

Natsume, M., & Baba, S. (2014). Suppressive Effects of Cacao Polyphenols on the Development of Atherosclerosis in Apolipoprotein E-Deficient Mice. *Sub-Cellular Biochemistry*, *77*(4), 189–198. https://doi.org/10.1007/978-94-007-7920-4_16

Oza, P. P., & Kashfi, K. (2022). Utility of NO and H2S donating platforms in managing COVID-19: Rationale and promise. *Nitric Oxide*, *128*, 72–102. https://doi.org/10.1016/j.niox.2022.08.003

Panche, A. N., Diwan, A. D., & Chandra, S. R. (2016). Flavonoids: an overview. *Journal of Nutritional Science*, *5*, e47. https://doi.org/10.1017/jns.2016.41

Ramadhani, M. R., Bachri, M. S., & Widyaningsih, W. (2017). Effects of Ethanolic Extract of Arrowroot Tubers (Maranta Arundinacea L.) on the Level of MDA, SGPT and SGOT in Ethanol Induced Rats. *Jurnal Kedokteran Dan Kesehatan Indonesia*, *8*(1), 10–18. https://doi.org/10.20885/JKKI.Vol8.Iss1.art3

Sabbatani, S., Fiorino, S., & Manfredi, R. (2010). The emerging of the fifth malaria parasite (Plasmodium knowlesi). A public health concern? *The Brazilian Journal of Infectious Diseases*, *14*(3), 299–309. https://doi.org/10.1016/S1413-8670(10)70062-3

Sam, J., Shamsusah, N. A., Ali, A. H., Hod, R., Hassan, M. R., & Agustar, H. K. (2022). Prevalence of simian malaria among macaques in Malaysia (2000–2021): A systematic review. *PLOS Neglected Tropical Diseases*, *16*(7), e0010527. https://doi.org/10.1371/journal.pntd.0010527

Saxena, D., Kannan, K. B., & Brandriss, M. C. (2003). Rapamycin Treatment Results in GATA Factor-Independent Hyperphosphorylation of the Proline Utilization Pathway Activator in Saccharomyces cerevisiae. *Eukaryotic Cell*, *2*(3), 552–559. https://doi.org/10.1128/EC.2.3.552-559.2003

Sodikin. (2011). *Asuhan keperawatan anak: gangguan sistem gastrointestinal dan hepatobilier* (Vol. 3). Jakarta Salemba Medika. Retrieved from https://onesearch.id/Record/IOS3107.49144

Tizard, I. R. (2000). *Immunology: An Introduction* (6th ed.). Saunders College Publishing. Retrieved from https://www.amazon.com/Immunology-Introduction-Ian-Tizard.

Ullah, A., Munir, S., Badshah, S. L., Khan, N., Ghani, L., Poulson, B. G., Emwas, A.-H., & Jaremko, M. (2020). Important Flavonoids and Their Role as a Therapeutic Agent. *Molecules*, *25*(22), 5243. https://doi.org/10.3390/molecules25225243

Wang, C. M., Chen, H. T., Li, T. C., Weng, J. H., Jhan, Y. L., SX, L., & Chou, C. H. (2014). The role of pentacyclic triterpenoids in the allelopathic effects of Alstonia scholaris. *Journal of Chemistry Ecology*, *40*(1), 90–8. https://doi.org/10.1007/s10886-013-0376-y.

WHO. (2009). *Methods For Surveillance of Antimalarial Drug Efficacy*. Retrieved from https://www.who.int/docs/