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Original Research Article

Antimalarial and Antioxidant Effects of *Syzygium cumini* (L.) Skeels (Myrtaceae) Fruit Fraction in *Plasmodium berghei*–Infected Pregnant Mice

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ABSTRACT

Malaria in pregnancy constitutes a principal factor in the enduring prevalence of significant maternal and foetal illness and fatality. This study investigated the potential of *Syzygium cumini* (L.) Skeels (Myrtaceae) fruit fraction in modulating TNF-α, preserving placental spiral artery integrity, and improving foetal outcomes in *Plasmodium berghei*-infected pregnant mice. A true experimental design was conducted using second-trimester pregnant mice (n = 5 per group), which were infected with the *P. berghei* ANKA strain. Each mouse was administered an intraperitoneal dose of 0.2 mL phosphate-buffered saline comprising roughly 1×10^6 parasitized red blood cells obtained from donor mice with 20–30% parasitaemia. The animals were categorized into six groups: normal control (K1), infected untreated (K2), drug control treated with dihydroartemisinin–piperaquine (5 mg/kg body weight, oral), and three treatment groups receiving *Syzygium cumini* fruit (SCF) fraction at doses of 600 mg/kg (P1), 800 mg/kg (P2), and 1200 mg/kg (P3). Placental TNF-α levels were significantly reduced in all treatment groups compared with the infected control (K2), with the greatest reduction observed at 1200 mg/kg (P3, $p \leq 0.05$). Histological analysis revealed that P2 and P3 significantly improved spiral artery diameter and wall thickness, while P1 produced moderate changes. Foetal body length increased significantly in all treatment groups ($p \leq 0.05$), and foetal weight in P3 approached the normal control (K1). The (SCF) fraction demonstrated anti-inflammatory properties and improved placental function and supported foetal growth. These findings demonstrate its potential as a complementary natural therapeutic agent for managing malaria-associated complications during pregnancy.

Keywords: *Syzygium_cumini*, *Plasmodium_berghei*, pregnancy,_malaria, TNF-α, placental histopathology

Introduction

Malaria in pregnancy (MiP) remains a major global health concern,[1] where *Plasmodium falciparum* and *Plasmodium berghei* infections play a major role in maternal and neonatal morbidity and mortality.[2] These conditions are thereby recognized as critical targets for public health intervention. The pathophysiology of MiP is complex. It is driven by parasite sequestration in the placenta,[3] excessive immune-activating cytokine release, notably tumor necrosis factor alpha (TNF-α),[4] and disruption of placental vascular remodelling. These processes culminate in placental insufficiency, intrauterine growth restriction, preterm birth, and increased risk of stillbirth. Despite advances in malaria control strategies, the burden of MiP persists, and safe, effective, and accessible therapeutic options remain limited, particularly in low-resource settings. [5] Conventional antimalarial regimens such as Artemisinin-Based Combination Therapy (ACT) are effective in reducing parasitemia but face growing challenges, including emerging drug resistance, safety concerns during pregnancy, and limited impact on the inflammatory and oxidative pathways that underpin placental pathology. These limitations point to the need for new treatment protocols to improve patient outcomes. Such therapeutic approaches should not only suppress parasitemia but also modulate the host inflammatory response and preserve placental function.[6]

Syzygium cumini (L.) Skeels, widely recognized as Java plum or black plum, has been widely recognized in ethnomedicine for its broad pharmacological activities. It exhibits strong antioxidant, anti-inflammatory, and antimicrobial activities, indicating broad biological efficacy.[7,8] Preliminary studies have suggested its potential in mitigating oxidative stress and modulating cytokine expression in infectious and inflammatory diseases. However, its role in the context of malaria in pregnancy, particularly in preserving placental vascular integrity and improving foetal outcomes remains unexplored.[9]

The present study addresses this critical knowledge gap by investigating the antimalarial and antioxidant effects of *S. cumini* fruit (SCF) fraction in a murine model of *P. berghei*-induced MiP.[10] This is, to our knowledge, the first experimental work to simultaneously evaluate its impact on parasitemia suppression, TNF-α modulation, placental spiral artery architecture, and foetal growth outcomes.[11] By integrating parasitological, histopathological, and inflammatory assessments, this research provides novel insights into the therapeutic potential of a plant-derived fraction as a dual-action antimalarial and placental protective agent, offering a promising adjunct or alternative to conventional therapies in malaria.

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Materials and Methods

Ethical approval
All experimental protocols adhered to established animal care protocols and were fully authorized by the Research Ethics Committee of Universitas Brawijaya, Malang, East Java, Indonesia (Approval No. 001-KEP-UB-2024). In strict adherence to ethical research standards, rigorous efforts were undertaken to refine procedures, thereby minimizing animal suffering and maximizing the statistical efficiency of the study by reducing the total number of subjects required.

Source of experimental animals
Adult female BALB/c mice (aged 8–10 weeks, with a body weight of 25–30 g) were obtained from Biomedical Technology Indonesia, Institute of Technology Bandung, Central Java, Indonesia. Animals were acclimatized for two days in accordance with established laboratory protocols (maintained at 22 ± 2 °C with 50–60% relative humidity, 12:12 h light/dark cycle) with *ad libitum* access to pellet feed and water. Pregnancy was confirmed by the presence of a vaginal plug, and gestational day 10 (GD10) was designated as the onset of the second trimester in mice.[12]

Source and identification of plant materials
Mature SCFs were harvested from Lumajang, East Java, Indonesia (8°08′36.0″S, 113°13′12.0″E), during the peak fruiting season. Only fully ripened fruits exhibiting a dark purple colour were selected for extraction. Collected plant material was taxonomically validated at the Herbal Laboratory of Materia Medica, Batu, East Java, Indonesia, on 27 July 2023, under certificate number 067/1873/102.20/2023.

Preparation of the chloroform–methanol fraction of Syzygium cumini fruits
Fresh ripe *Syzygium cumini* fruits (SCFs) were rinsed extensively with distilled water to effectively remove surface contaminants and surface impurities, deseeded, and dried at ambient temperature (25–28°C) for seven days until the weight remained unchanged. The desiccated fruit pulp was mechanically ground into a fine powder and immediately stored in sealed containers to maintain integrity. A total of 500 g of the powdered fruit was macerated with methanol (100%) at room temperature for 72 hours with intermittent shaking to ensure maximum extraction of polar and semipolar phytoconstituents. Filtration of the mixture was performed employing Whatman No. 1 filter paper, and the collected filtrate was evaporated to concentrate at 40 °C employing a rotary evaporator to recover the crude methanolic extract. Fractionation of the crude extract was carried out using Vacuum Liquid Chromatography (VLC) with chloroform and methanol as mobile-phase solvents. Approximately 5 g of the methanolic extract was mixed thoroughly with 20 g of silica gel 60 GF254 (ratio 1:4 w/w). One-third of the silica mixture was used to prepare a uniform layer in the VLC column, while the remaining portion was blended with the extract to form a homogeneous adsorbent–sample mixture. Elution was carried out using a series of chloroform:methanol gradients (60:40, 50:50, 40:60, 30:70, 20:80, 10:90, and 0:100, v/v). The collected fractions were concentrated under reduced pressure and analyzed using Thin Layer Chromatography (TLC) with a chloroform:methanol (7:3) mobile phase. Fractions exhibiting similar TLC profiles were combined, saturated, and kept at 4°C until needed as the test fraction in experimental treatments.

Parasite strain and inoculation
The *Plasmodium berghei* ANKA strain was maintained through serial passage in donor BALB/c mice, according to Basir *et al*. (2012). Experimental infection was induced on gestational day 9 (GD9) by intraperitoneal injection of 200 µL of phosphate-buffered saline (PBS) containing approximately 1×10^6 parasitized red blood cells (pRBCs).[13]

Plasmodium berghei infection
Gestational dams (day nine of gestation) were challenged via the intraperitoneal route with a total volume of 0.2 mL of source blood, harbouring an estimated 1×10^6 parasitized red blood cells (pRBCs) of *Plasmodium berghei*. Parasitemia was assessed using thin blood smears prepared at 24 hours post-infection and subsequently each day up to day 5 post-infection. Thereafter, the experimental animals were terminated on gestational day 15. Thin smears were prepared on slides from caudal vein blood collected from the tail, fixed in methanol, and stained with 20% Giemsa solution for 30 minutes. The slides were subsequently rinsed with running water and air-dried at room temperature before microscopic examination. The stained blood smears were examined under an Olympus light microscope at 400× magnification using immersion oil. Parasitemia was derived as the percentage of infected erythrocytes relative to the total number of erythrocytes observed.

Preparation of Syzygium cumini fruit chloroform methanol fraction
Ripe fruits of *Syzygium cumini* were harvested from Lumajang, East Java, Indonesia, during the peak fruiting season. The fruit pulp was separated, air-dried, and ground into a fine powder. Maceration of the powdered material in 70% ethanol was performed for 72 hours with periodic agitation. Following filtration, the collected filtrate was evaporated under reduced pressure using a rotary evaporator to obtain the crude extract. Fractionation was then performed using a chloroform–methanol solvent system (60:40 to 0:100 v/v) by Vacuum Liquid Chromatography (VLC) to yield the chloroform–methanol fruit fraction, which was rich in polyphenols and flavonoids.[14] The experimental doses of 600, 800, and 1200 mg/kg body weight (BW) were selected based on preliminary screening and previous studies reporting the pharmacological safety of *S. cumini* fruit extract in rodents.[15] These dose ranges were below the no-observed-adverse-effect level (NOAEL) determined from an acute oral toxicity test conducted according to OECD Guideline 423, in which no mortality or behavioural abnormalities were observed up to 2000 mg/kg body weight. The fraction was freshly suspended in 0.5% carboxymethylcellulose (CMC) before oral administration.

Animal grouping
Pregnant mice were randomized into six experimental groups, each consisting of five animals. The first group (K1) served as the normal control, comprising pregnant mice that were neither infected with *Plasmodium berghei* nor given any treatment. The second group (K2) represented the infected untreated control, in which pregnant mice were infected with *P. berghei* on gestational GD9 but received no therapeutic intervention. The third group (K3) served as the standard antimalarial control, where infected pregnant mice were administered dihydroartemisinin–piperaquine (DHP) at a dose of 25 mg/kg body weight by oral gavage once daily from GD10 to GD14. For the treatment groups, three different doses of *Syzygium cumini* fruit fraction were evaluated. In group P1, pregnant mice infected on GD9 received 600 mg/kg body weight of the fruit fraction by oral gavage once daily from GD10 to GD14. Group P2 received a medium dose of 800 mg/kg body weight under the same treatment schedule, while group P3 was administered a high dose of 1,200 mg/kg body weight of the fruit fraction once daily from GD10 to GD14.
Treatments were administered orally once daily for five consecutive days, beginning 24 hours post-infection (GD10) and continuing until GD14. The treatment schedule followed the Peter's 4-Day Suppressive Test protocol, which assesses the ability of test substances to inhibit parasitemia progression during the early erythrocytic stages of infection.[16]

Parasitemia monitoring
Parasitemia was monitored daily from GD10, 24 hours after infection, until GD15, covering the entire treatment period. Tail vein blood smears were prepared each day, fixed in methanol, and stained with 10% Giemsa solution. Parasitemia was quantified microscopically under 100× oil immersion, quantifying the number of infected erythrocytes (RBCs) among 1,000 RBCs across five randomly selected microscopic fields per slide. The mean parasitemia value per animal was calculated, and the parasitemia growth rate was expressed as the percentage increase from the baseline count on GD10.

Histopathological analysis of placental spiral artery
On GD15, mice were euthanized under ketamine–xylazine anaesthesia. Placental tissue samples were fixed in 10% buffered formalin,

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embedded in paraffin, and sectioned at a thickness of 5 µm. These sections were then stained with the standard Haematoxylin and Eosin (H&E) staining procedure for microscopic evaluation. Spiral artery morphology was evaluated for lumen diameter, wall thickness, and evidence of fibrinoid necrosis using light microscopy.

Immunohistochemistry for TNF-α
The placental sections were arranged for staining through deparaffinization and rehydration. Following antigen retrieval in a citrate buffer, endogenous peroxidase activity was eliminated (quenched) using 3% H_2O_2. The specimens were incubated overnight with the primary antibody (at the specified dilution), followed by treatment with an HRP-conjugated secondary antibody. Visualization was performed using diaminobenzidine (DAB) substrate, and staining intensity was scored semi-quantitatively.

Measurement of foetal weight and foetal body length
The foetuses were immediately removed from the uterus after dissection while still fresh and carefully cleaned of the embryonic membranes.[12] The mass of the foetuses was quantified employing a precision electronic balance (Ohaus PX124, Ohaus Corporation, Parsippany, NJ, USA) with a precision of 0.01 g. Foetal body length was measured in centimeters using a Fisherbrand stainless-steel ruler (Fisher Scientific, Waltham, MA, USA). The crown–rump length was determined by measuring the distance from the forehead to the base of the tail, projected onto parchment paper and marked with a fine-tip pen for accuracy.

Antioxidant analysis
The DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay was utilized with modifications to assess the antioxidant capacity of the *Syzygium cumini* fruit chloroform–methanol fraction. Test concentrations (25 to 400 µg/mL) were prepared via serial dilution from a methanol stock. For each concentration, a 1 mL aliquot was mixed with 1 mL of 0.1 mM DPPH methanolic solution, mixed by vortexing, and incubated for 30 minutes in the dark. The antioxidant capacity was then measured by recording the absorbance decay at 517 nm against a methanol blank using a UV–Vis spectrophotometer (Shimadzu UV−1800). Ascorbic acid was used as the reference (positive control). The IC_{50} value (concentration providing 50% inhibition) was determined from the linear regression of the scavenging percentage versus concentration curve.[17]

Statistical analysis
Data on TNF-α levels, placental spiral artery diameter and wall thickness, as well as foetal body weight and length, were analyzed using GraphPad Prism version 8.4.2, and the results were presented as graphs with standard deviation (SD). Each experimental group consisted of five pregnant mice (n = 5). For *in vitro* antioxidant testing, each concentration of the SCF was analyzed in triplicate to ensure analytical precision of absorbance readings. Statistical comparisons across the treatment and control groups were performed using one-way ANOVA (analysis of variance) with subsequent Tukey's post hoc analysis. Differences were considered statistically significant at $p < 0.05$. For suppressive and curative tests, a two-way ANOVA followed by Bonferroni's multiple comparison test was applied.

Results and Discussion

Antioxidant activity of Syzygium cumini fraction
The antioxidant activity of the SCF chloroform–methanol fraction was determined using the DPPH free radical scavenging assay. The reaction between DPPH and the sample was indicated by a colour change from deep violet to pale yellow after incubation, and the absorbance was measured at 517 nm using an ultraviolet-visible (UV–Vis) spectrophotometer (UV-1601, Shimadzu, Japan). The *Syzygium cumini* fraction showed an average IC_{50} value of 97.53 µg/mL, indicating strong antioxidant activity, as compounds with IC_{50} values below 100 µg/mL are generally classified as strong antioxidants. In comparison, the reference antioxidant gallic acid showed an IC_{50} value of 9.64 µg/mL. The results of the DPPH radical scavenging activity and IC_{50} values for the *S. cumini* fraction and gallic acid are presented in Table 1.

Table 1: Antioxidant activity of *Syzygium cumini* fruit fractions

Sample	Number IC_{50} (µg/mL)			Average IC_{50}
	1	2	3	
Chloroform-methanol Fraction of *Syzygium cumini* Fruits	91.22	104.60	96.76	97.53± 6.73
Gallic Acid (Positive Control)	1.45	1.45	1.42	1.44± 0.017

1: Replication 1; 2: Replication 2; 3: Replication 3

Effect of Syzygium cumini fruit fraction on placental TNF-α expression
The statistical analysis of placental TNF-α expression showed marked disparities among the control and treatment groups ($p \leq 0.05$), as presented in Figure 1 and Table 2. The infected untreated group (K2) exhibited the highest TNF-α levels, indicating a strong inflammatory response to *Plasmodium berghei* infection. In contrast, the normal control group (K1), which was not infected or treated, showed the lowest TNF-α expression. Treatment with SCF fraction at all doses (P1: 600 mg/kg BW, P2: 800 mg/kg BW, and P3: 1200 mg/kg BW) resulted in a significant reduction in TNF-α expression compared to the infected untreated group (K2) ($p < 0.05$). The most pronounced decrease was observed in the P3 group (1200 mg/kg BW), where TNF-α levels approached those of the normal control (K1). The drug control group (K3), which received dihydroartemisinin–piperaquine (5 mg/kg BW, oral), also showed a marked decrease in TNF-α expression, though not significantly different from P2 and P3. These findings indicate that administration of the *S. cumini* fraction effectively attenuates placental inflammation associated with malaria infection, likely through its anti-inflammatory and antioxidant mechanisms.

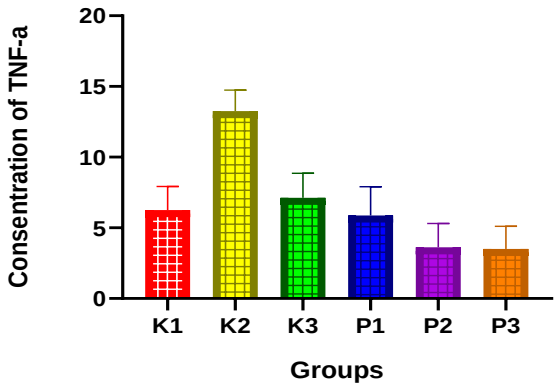

Figure 1: Placental TNF-α concentration (mean ± SEM) in control and treatment groups administered *Syzygium cumini* fruit fraction. K1: normal control; K2: infected untreated control; K3: drug control (DHP); P1, P2, and P3: groups treated with *S. cumini* fruit fraction at 600, 800, and 1200 mg/kg BW, respectively; Bars with different letters (a, b, c) indicate significant differences among groups ($p \leq 0.05$ based on one-way analysis of variance [ANOVA] followed by Tukey's post hoc test).

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Table 2: Placental TNF-α expression in control and treatment groups administered *Syzygium cumini* fruit fraction

Group	Concentration of TNF-α (pg/mL)
K1 (Normal control)	$6.25 \pm 0.59^{b,c}$
K2 (Infected untreated control)	$13.25 \pm 0.53^{a,b,c}$
K3 (Drug control [DHP])	$7.13 \pm 0.61^{a,b}$
P1 (Fraction 600 mg/kg BW)	$6.00 \pm 0.65^{b,c}$
P2 (Fraction 800 mg/kg BW)	4.44 ± 0.56^{b}
P3 (Fraction 1200 mg/kg BW)	4.25 ± 0.57^{b}

Values are presented as mean ± SEM (n = 5); Superscripts with different letters (a, b, c) indicate significant differences among groups ($p \leq 0.05$ based on one-way analysis of variance [ANOVA] followed by Tukey's post hoc test).

Histopathological changes of the spiral artery of the placenta

The statistical analysis of histopathological changes in the placental spiral artery revealed significant differences in arterial diameter among the experimental groups. Notably, disparities were observed between the control group K2 and K1 (p = 0.024), K2 and K3 (p = 0.0123), K2 and P2 (p = 0.0109), and K2 and P3 (p = 0.0013). Meanwhile, the comparison between the K2 and P1 groups showed no significant difference ($p \geq 0.05$). These findings indicate that malaria infection may affect changes in vascular structure in pregnancy, with responses varying by treatment group. The results of statistical analysis of histopathological changes in the thickness of the spiral artery in pregnant mice infected with malaria contrasted sharply with the K2 control group and all treatment groups, namely K1 (P = 0.0106), K3 (P = 0.0101), P1 (P = 0.0232), P2 (P = 0.0317), and P3 (P = 0.0319). These findings indicate that malaria infection contributes to changes in the thickness of the spiral arteries during pregnancy, which can impact uteroplacental perfusion and foetal development. Histopathological changes of the placenta spiral artery are presented in Figures 2 and 3, and Table 3.

Influence of Syzygium cumini treatment on foetal weight and body length

Statistical analysis showed a significant difference in foetal body length between the negative control group (K2) and all other groups: K1 (p=0.0129), K3 (p=0.0121), P1 (p=0.012), P2 (p=0.025), and P3 (p=0.034). Analysis of foetal weight revealed significant differences

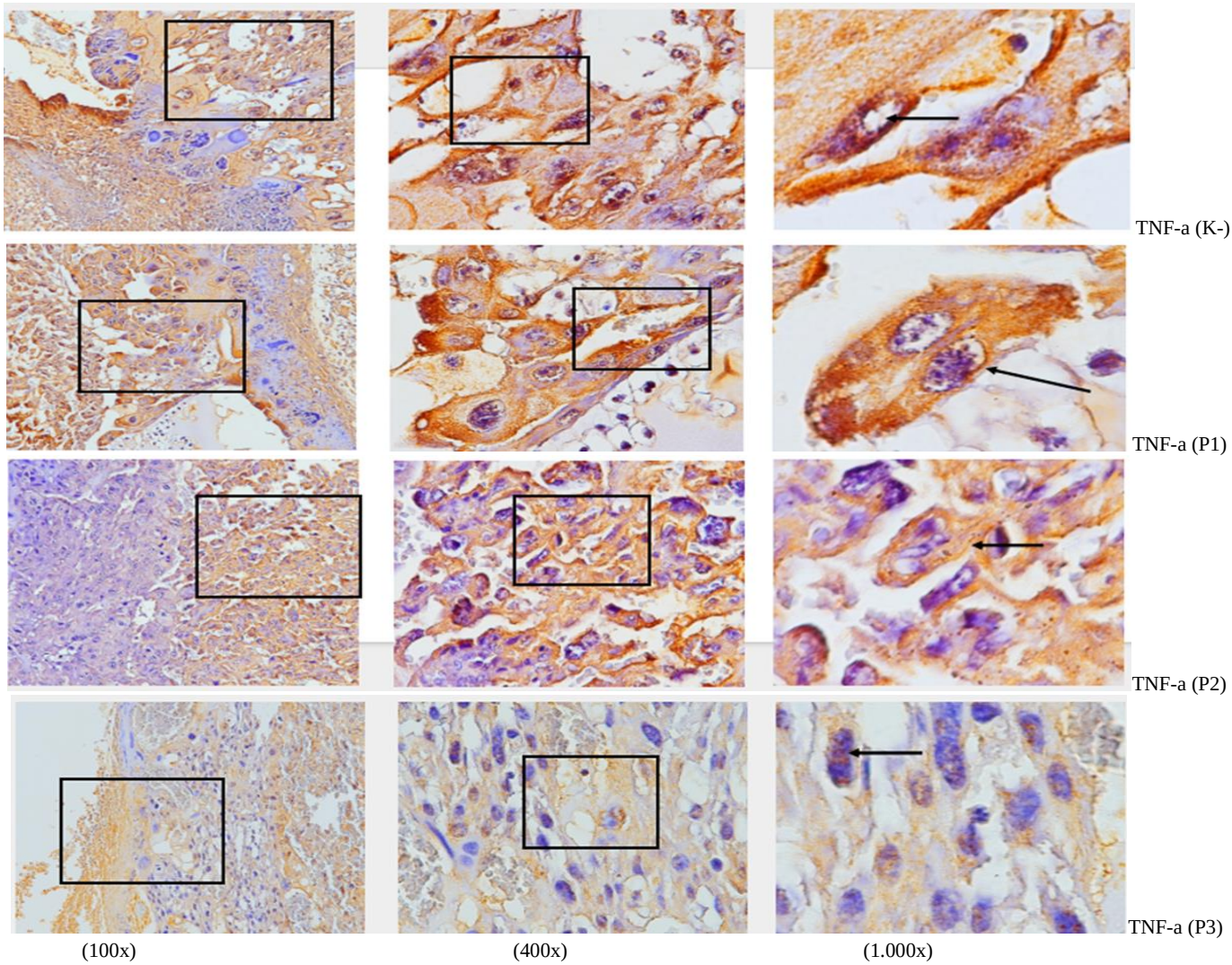

Figure 2: Immunohistochemical expression of TNF-α in placental tissues from control and treatment groups administered *Syzygium cumini* fruit fractions. K: infected untreated control; P1, P2, and P3: treatment groups receiving *S. cumini* fruit fraction at 600, 800, and 1200 mg/kg BW, respectively; Brown cytoplasmic staining indicates positive TNF-α expression; Images were captured at 100×, 400×, and 1000× magnification using an Olympus CX43 microscope with a digital imaging system

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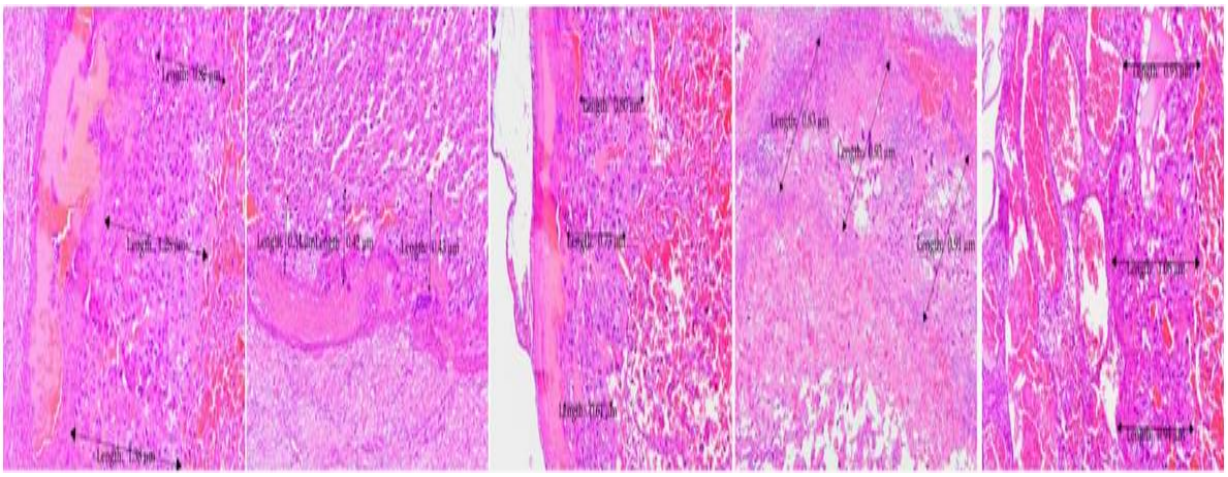

Figure 3: Histopathological features of placental spiral arteries in control and treatment groups administered *Syzygium cumini* fruit fraction. Placental sections were stained with hematoxylin and eosin (H&E) and observed under an Olympus CX43 light microscope at 400× magnification; K1: normal control; K2: infected untreated control; K3: drug control (DHP); P1, P2, and P3: treatment groups receiving *S. cumini* fruit fraction at 600, 800, and 1200 mg/kg BW, respectively; Improved spiral artery remodeling was observed in treated groups, characterized by larger luminal diameter and thinner vascular walls compared with the infected control group

Table 3: Histopathological changes in the diameter and wall thickness of placental spiral arteries in control and treatment groups administered *Syzygium cumini*fruit fractions

Groups	Spiral Artery Diameter (μm)	Spiral Artery Thickness (μm²)
K1 (Normal control)	25.06 ± 4.94^{b}	$42.95 \pm 20.16^{b,c}$
K2 (Infected untreated control)	$16.64 \pm 3.68^{a,c}$	$96.50 \pm 16.66^{a,c}$
K3 (Drug control [DHP])	20.08 ± 5.53^{b}	$62.79 \pm 8.04^{a,b}$
P1 (Fraction 600 mg/kg BW)	$19.21 \pm 6.03^{a,c}$	$75.16 \pm 8.04^{a,b}$
P2 (Fraction 800 mg/kg BW)	$29.87 \pm 6.03^{b,c}$	69.11 ± 15.04^{b}
P3 (Fraction 1200 mg/kg BW)	22.06 ± 7.31^{b}	54.79 ± 27.22^{b}

Values are presented as mean ± SEM (n = 5); Superscripts with different letters (a, b, c) indicate significant differences among groups ($p \leq 0.05$ based on one-way analysis of variance [ANOVA] followed by Tukey's post hoc test).

between the K2 group and the normal control group (K1) (p=0.0125) and P3 (p=0.054), indicating that the SCF fraction at a dose of 1200 mg/kg BB had a beneficial effect. No significant differences were observed between the K3, P1, and P2 groups. Overall, administration of the SCF fraction significantly improved foetal body length and foetal weight in *Plasmodium berghei*-infected pregnant mice, bringing fetal parameters closer to normal values ($p < 0.001$). Detailed measurements of foetal weight and foetal body length are presented in Figure 4 and Table 4.

Protective antioxidant effects of Syzygium cumini fruit fraction on placental cells

The SCF fraction exhibited strong antioxidant activity. Antioxidant activity is inversely related to the IC_{50} value: the lower the IC_{50}, the stronger the activity. Specifically, IC_{50} values < 50 μg/mL indicate very strong antioxidant activity, 50–100 μg/mL indicate strong activity, 100–150 μg/mL indicate moderate activity, and 150–200 μg/mL indicate weak activity in the DPPH reduction percentage test.[18] In this study, the administration of SCF fraction in 2nd trimester pregnant mice infected *with Plasmodium berghei* protected placental cells from oxidative stress caused during malaria infection. Oxidative stress can cause cellular damage and impair placental function. Antioxidant compounds can neutralize free radicals and help maintain the integrity of placental cells.[19] The protection against oxidative stress can contribute to the restoration of placental function and support foetal growth.[20] The results of this study show that the administration of SCF fraction not only reduces placental damage caused by malaria infection but also affects the improvement of maternal and foetal health for optimal foetal development.[21]

Protective mechanisms of Syzygium cumini fraction against placental damage in malaria-infected pregnancy

The results of this study indicated that the SCF may help reduce malaria-induced inflammation, as evidenced by decreased placental TNF-α expression in the second trimester of pregnant mice infected with *Plasmodium berghei*. TNF-alpha is a key immune mediator of inflammation against pathogens.[4] However, excessive increases in TNF-α levels can lead to tissue damage and worsen the clinical condition of malaria patients. Malaria infection during pregnancy can lead to a variety of complications, including a reduced number of foetuses and impaired foetal growth.

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Table 4: Foetal weight and foetal body length in control and treatment groups administered *Syzygium cumini* fruit fractions

Group	Foetal Weight (g)	Foetal Body Length (cm)
K1 (Normal control)	1.21 ± 0.08^{b}	$2.030 \pm 0.082^{b,c}$
K2 (Infected untreated control)	$0.71 \pm 0.09^{a,c}$	1.762 ± 0.064^{a}
K3 (Drug control [DHP)]	1.05 ± 0.08^{b}	1.799 ± 0.045^{a}
P1 (Fraction 600 mg/kg BW)	1.14 ± 0.15^{b}	1.831 ± 0.071
P2 (Fraction 800 mg/kg BW)	1.19 ± 0.08^{b}	1.884 ± 0.077
P3 (Fraction 1200 mg/kg BW)	1.26 ± 0.08^{b}	$2.145 \pm 0.105^{b,c}$

Values are presented as mean ± SEM (n = 5); Superscripts with different letters (a, b, c) indicate significant differences among groups ($p \leq 0.05$ based on one-way analysis of variance [ANOVA] followed by Tukey's post hoc test).

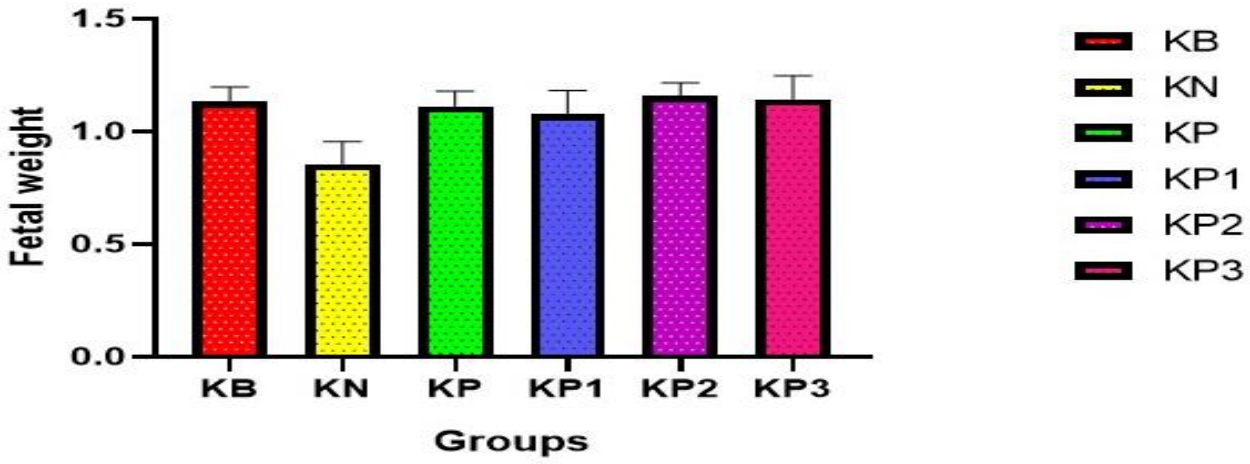

Figure 4: Foetal weight and crown–rump length in control and treatment groups administered *Syzygium cumini* fruit fraction. K1: normal control; K2: infected untreated control; K3: drug control (DHP); P1, P2, and P3: treatment groups receiving *S. cumini* fruit fraction at 600, 800, and 1200 mg/kg BW, respectively; Values are expressed as mean ± SEM (n = 5); Bars with different letters (a, b, c) indicate significant differences among groups ($p \leq 0.05$ based on one-way analysis of variance [ANOVA] followed by Tukey's post hoc test).

These effects are primarily caused by placental damage and disrupted blood flow resulting from inflammation and oxidative stress induced by the infection. In pregnant mice, similar pathophysiological mechanisms are observed: *Plasmodium berghei* infection can reduce foetal survival and impair foetal development by disrupting nutrient and oxygen delivery to the foetuses. Therefore, the health of the pregnant mouse directly influences both foetal count (number of viable foetuses) and foetal growth parameters such as body weight and body length.

Administration of SCF at doses of 600 mg/kg BB, 800 mg/kg BB, and 1200 mg/kg BB can reduce the expression of the pro-inflammatory cytokine TNF-α. This is in line with previous research showing that malaria infection increases levels of TNF-α, which plays a role in the immune response to infection but can also cause tissue damage if it is excessive. In addition, other research suggests that flavonoids, such as apigenin, can lower levels of mRNA induced by TNF-α, thereby reducing the expression of adhesion molecules and inflammatory responses.[10] This is in line with studies on the administration of *Syzygium cumini* at certain doses shown to downregulate the production of inflammatory mediators, such as TNF-α and IFN-γ, as well as increase anti-inflammatory cytokines, which play a role in reducing inflammation and placental damage due to malaria infection. This anti-inflammatory effect is thought to be related to the flavonoid and phenolic content in *Syzygium cumini*, which has immunomodulatory and antioxidant activity. By lowering the inflammatory response excessively, *Syzygium cumini* can be a natural therapeutic agent,[22] to protect the health of pregnant women and foetuses during malaria infection. The flavonoid compounds in *Syzygium cumini* can inhibit inflammatory signalling pathways, including those that stimulate TNF-α production, thereby reducing tissue damage and improving placental health. Decreased expression of TNF-alpha in the group given the SCF at a dose of 1200 mg/KgBB (P3) may reduce inflammation, minimize tissue damage and promote the restoration of physiological function.

The results of this study show that malaria infection affects changes in the thickness of spiral arteries during pregnancy, which can have an impact on uteroplacental perfusion and foetal development. The placenta serves as a link between the mother and the foetus through the spiral artery.[23] These arteries transport nutrients and oxygen to tissues while removing metabolic waste. However, malaria infection can affect the spiral arteries of the placenta and can interfere with this function. Increased levels of these cytokines can cause damage to placental tissue, disrupt blood flow, and reduce the efficiency of nutrient transport to the foetus. Malaria infection can lead to the infiltration of immune cells into placental tissue, which contributes to structural and functional damage to the placenta. In addition, malaria infection can also lead to the formation of abnormal placental tissue, such as increased necrosis and decreased number of chorionic villi. This can result in a decrease in the surface area of the placenta available for gas and nutrient exchange, impacting the growth and development of the foetus.[24] The damage to the chorionic villi can result in foetal hypoxia, potentially leading to premature birth or low birth weight. The malaria infection in pregnant

women elevates the likelihood of adverse pregnancy outcomes, including preeclampsia and postpartum bleeding.

Role of Syzygium cumini in enhancing foetal development during malaria infection

The results of the study on the administration of SCF fraction on the body weight and length of the second trimester of pregnancy in mice infected with *Plasmodium berghei* showed an effect on the number of foetuses, foetal length, and foetal weight. Malaria infection in pregnancy can cause a variety of complications, including decreased foetal count and stunted foetal growth. This is caused by placental damage and blood flow disorders resulting from inflammation and oxidative stress produced during infection.[13,25] This study demonstrated that mice treated with SCF fraction exhibited a significant increase in the number of foetuses compared to the control group that did not receive the treatment. This increase is related to the ability of SCF fraction to reduce inflammation and improve placental health,[23] and hence improve fetal growth. In addition, the fruit fraction *of Syzygium cumini* also contains antioxidant compounds that can protect cells from damage due to oxidative stress, thereby supporting optimal foetal development. The increase in foetal weight and length in second trimester of pregnancy in mice infected with *Plasmodium berghei* following treatment suggests that SCF can contribute to foetal growth.

Conclusion

Administration of the SCF fraction significantly reduced parasitemia and placental TNF-α expression, improved spiral artery diameter and wall thickness, and enhanced foetal weight and crown-rump length particularly at the dose of 1200 mg/kg body weight. These findings indicate that the SCF fraction possesses strong antimalarial, anti-inflammatory, and vasculoprotective activities, supporting its potential as a safe and promising natural therapeutic candidate for malaria in pregnancy. The future direction of this research could focus on conducting clinical trials to validate the efficacy of *Syzygium cumini* in pregnant women with malaria. Further investigations on its mechanism at the molecular level, its potential synergistic effects with current antimalarial drugs, and its safety profile in humans would also be crucial.

Conflict of interest

The authors declare no conflicts of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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