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Effect of *Foeniculum vulgare* Mill. Leaf Extract on Hemoglobin Levels in Mice with Experimental Malaria

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ABSTRACT

Malaria is a parasitic disease caused by Plasmodium spp. and remains a major public health problem in tropical regions, including Indonesia. One of the clinical manifestations of malaria is anemia resulting from hemolysis, which leads to decreased hemoglobin levels. Although artemisinin-based combination therapy remains the first-line treatment for malaria, the emergence of drug resistance has prompted the search for alternative therapies to prevent malaria-associated anemia. This study aimed to evaluate the effect of Foeniculum vulgare Mill. leaf extract on hemoglobin levels in malaria model mice. A true experimental study with a pretest-posttest with control group design was conducted using 40 mice randomly assigned to eight groups: a negative control (DMSO), an extract control (400 mg/kgBW), a malaria control (Plasmodium berghei), and five treatment groups infected with P. berghei and administered F. vulgare leaf extract at doses of 50, 100, 200, 400, and 800 mg/kgBW. Hemoglobin levels were measured every 24 hours for five days using a hemoglobinometer. Data were analyzed using the Friedman test after the normality test indicated a non-normal distribution ($p<0.05$) and the homogeneity test showed homogeneous data ($p>0.05$). The analysis revealed no significant effect of F. vulgare leaf extract on hemoglobin levels in malaria model mice ($p=0.216$). In conclusion, Foeniculum vulgare Mill. leaf extract did not affect hemoglobin levels in malaria model mice.

1. INTRODUCTION

Malaria remains a major vector-borne infectious disease and an important public health problem in tropical and subtropical regions. The disease is caused by protozoan parasites of the genus *Plasmodium* and transmitted through the bites of infected female *Anopheles* mosquitoes. Among the five *Plasmodium* species that infect humans, *Plasmodium falciparum* is particularly important because of its association with severe disease and malaria-related mortality. Globally, an estimated 263 million malaria cases and 597,000 deaths occurred in 83 endemic countries in 2023. In Indonesia, 418,533 presumed and confirmed malaria cases and 120 indigenous malaria-related deaths were reported in the same year, with a substantial proportion of cases occurring in eastern provinces. Although malaria control and elimination efforts have progressed, these data indicate that malaria remains an important health problem in Indonesia. Beyond parasite infection itself, malaria can cause substantial hematological disturbances, particularly anemia, which contributes to disease morbidity and may influence clinical outcomes (World Health Organization, 2024; Ministry of Health of the Republic of Indonesia, 2024).

Anemia is one of the most important hematological complications of malaria and is characterized by a reduction in hemoglobin concentration and red blood cell mass. Malaria-associated anemia is multifactorial and involves the destruction of parasitized and non-parasitized erythrocytes, splenic clearance of altered erythrocytes, intravascular hemolysis, and impaired erythropoiesis. The clinical importance of malaria-associated anemia is particularly evident in *P. falciparum* infection, in which severe anemia is a recognized manifestation of

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severe malaria. Evidence from clinical studies indicates that very low hemoglobin concentrations are associated with increased mortality, with mortality rising markedly when hemoglobin falls below approximately 3 g/dL in severe falciparum malaria. Accordingly, hemoglobin concentration is not merely a routine hematological parameter but an important indicator of the hematological consequences and severity of malaria infection (Menéndez *et al.*, 2000; White, 2018).

The reduction in hemoglobin during malaria infection results from interconnected processes of erythrocyte destruction and inadequate replacement of circulating red blood cells. During the erythrocytic stage, *Plasmodium* parasites invade and multiply within erythrocytes, resulting in erythrocyte rupture and the release of merozoites and parasite-derived products, including hemozoin. Parasite products and the host inflammatory response can induce the production of pro-inflammatory cytokines, including tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ), which may contribute to suppression of erythropoiesis. In parallel, malaria-associated oxidative stress can promote damage to erythrocyte membranes and increase the susceptibility of infected and uninfected erythrocytes to destruction. These processes collectively contribute to declining hemoglobin concentrations during infection. Therefore, interventions with potential antioxidant, anti-inflammatory, or erythrocyte-protective properties may theoretically help reduce hematological damage and maintain hemoglobin levels, although such effects require direct experimental evaluation (Menéndez *et al.*, 2000; White, 2018).

Artemisinin-based combination therapy (ACT) remains the recommended standard treatment for uncomplicated malaria because of its rapid parasite clearance and high therapeutic efficacy. However, the emergence and spread of artemisinin resistance remain important concerns for malaria control. Reports of mutations in the *P. falciparum K13* gene associated with artemisinin resistance in Indonesian isolates further emphasize the need to sustain research into strategies that can support malaria management (Fitri *et al.*, 2023). Nevertheless, investigation of medicinal plants in the present context is not intended to replace ACT or other standard antimalarial therapies. Rather, plant-derived preparations may be explored as potential adjunctive or supportive interventions targeting malaria-associated complications, particularly hematological disturbances. Such an approach is conceptually different from developing a plant extract as a substitute for antimalarial treatment because the primary therapeutic objective remains parasite clearance, while the adjunctive approach focuses on mitigating pathological consequences of infection.

Medicinal plants contain diverse secondary metabolites with biological activities that may be relevant to malaria-associated oxidative and inflammatory processes (Hasan et al., 2025). One plant with potential pharmacological relevance is fennel, *Foeniculum vulgare* Mill. However, because the phytochemical composition and biological activity of *F. vulgare* can vary according to the plant part used, evidence obtained from seeds, fruits, essential oils, or whole-plant preparations should not automatically be extrapolated to leaves. Studies specifically investigating *F. vulgare* leaves have demonstrated the presence of phenolic compounds and flavonoids and have reported antioxidant activity of the leaf extract. Sayah *et al.* (2020), using chemical profiling of aqueous extracts, identified caffeic acid, quinic acid, and chlorogenic acid as major phenolic constituents of *F. vulgare* leaves, together with flavonoids including rutin, quercitrin, and kaempferol. The leaf extract also demonstrated substantial radical-scavenging activity, with stronger DPPH activity than the corresponding rootstock extract. These findings indicate that the leaves constitute a biologically active plant material with a phytochemical profile relevant to oxidative-stress-related processes (Sayah *et al.*, 2020; Jayaningtyas et al., 2025).

Additional evidence supports the presence of diverse secondary metabolites in *F. vulgare* leaves. Phytochemical screening of an ethanol extract prepared specifically from the leaves identified flavonoids, tannins, saponins, steroids, terpenoids, alkaloids, and glycosides (Abubakar *et al.*, 2021). The presence of these metabolite classes does not by itself establish a hematoprotective effect; however, the documented antioxidant activity of fennel leaves provides a plausible biological basis for investigating their potential role in conditions involving oxidative

damage. In the context of malaria, this is particularly relevant because oxidative stress is involved in erythrocyte membrane damage and the destruction of both parasitized and non-parasitized erythrocytes. Thus, the antioxidant properties of *F. vulgare* leaves may potentially contribute to preservation of erythrocyte integrity and maintenance of hemoglobin during malaria infection, but this hypothesis requires experimental confirmation rather than being assumed from phytochemical composition alone (Sayah *et al.*, 2020; Abubakar *et al.*, 2021).

Previous studies have provided preliminary evidence that *F. vulgare* may influence hematological parameters, although the available evidence differs substantially from the experimental context of the present study. Abbas *et al.* (2021) evaluated the effect of *F. vulgare* incorporated into the diet of healthy rabbits and reported increased hemoglobin concentrations in animals receiving diets containing crushed fennel seeds. The authors concluded that *F. vulgare* might have a role in maintaining hemoglobin levels. However, the study used seeds rather than leaves and involved healthy rabbits rather than animals with malaria infection. Similarly, Mansouri *et al.* (2015) investigated the effects of a hydro-alcoholic extract of *F. vulgare* on hematological parameters in male rats and reported changes in several hematological indices. Nevertheless, this study did not use a malaria model and therefore could not determine whether *F. vulgare* could influence malaria-associated anemia. These studies provide supportive evidence for the potential hematological relevance of *F. vulgare*, while simultaneously demonstrating the limitations of existing evidence in relation to the specific research question addressed in the present study (Abbas *et al.*, 2021; Mansouri *et al.*, 2015).

Despite evidence of antioxidant activity in *F. vulgare* leaves and preliminary evidence of hematological effects from other preparations or plant parts, it remains unclear whether *F. vulgare* leaf extract can influence hemoglobin levels during malaria infection. In particular, previous studies have not adequately established whether the antioxidant and potential erythrocyte-protective properties of the leaves translate into preservation of hemoglobin in an experimental malaria model. The specific research gap therefore lies at the intersection of three factors: the use of *F. vulgare* leaves as the plant material, experimental malaria infection as the pathological condition, and hemoglobin concentration as the primary hematological outcome. Addressing this gap is important because a reduction in hemoglobin is a clinically relevant consequence of malaria and may reflect the cumulative effects of erythrocyte destruction, oxidative damage, inflammation, and impaired erythropoiesis.

The present study was therefore conducted to evaluate the effect of *Foeniculum vulgare* Mill. leaf extract on hemoglobin levels in mice infected with *Plasmodium berghei*. The study was designed to assess the potential hematological or hematoprotective effect of the leaf extract rather than to evaluate it as a replacement for standard antimalarial therapy. By examining hemoglobin as the primary outcome in an experimental malaria model, this study is expected to provide evidence regarding whether *F. vulgare* leaf extract has a potential role in maintaining hemoglobin concentrations under malaria-associated hematological stress. The findings may provide a basis for subsequent investigations of the underlying mechanisms and the potential development of *F. vulgare*-derived preparations as adjunctive or supportive approaches to malaria-associated hematological complications.

2. METHOD

This study employed a true experimental design using a pretest–posttest with control group design to evaluate the effect of chloroform extract of *F. vulgare* Mill. leaves on hemoglobin levels in malaria model mice. Dried *F. vulgare* Mill. leaves were obtained from the Center for Research and Development of Medicinal Plants and Traditional Medicine (B2P2TOOT), Tawangmangu, Central Java, Indonesia. Chloroform extract preparation was performed using the maceration method according to the procedure described in our previous study (Ihtiaringtyas *et al.*, 2026). Prior to the biological experiment, phytochemical characterization of the extract was conducted using thin-layer chromatography (TLC). The detailed extraction procedure and phytochemical profile have been published previously and therefore are not presented in this article (Ihtiaringtyas *et al.*, 2026).

Forty healthy male *M. musculus* mice, aged 2–3 months and weighing 18–23 g, were used in this study. The animals were acclimatized for seven days under standard laboratory conditions with free access to food and drinking water before being randomly allocated into eight experimental groups. All animal experiments were conducted at the Animal House, Faculty of Medicine, Jenderal Soedirman University, Purwokerto, Indonesia. Ethical approval for this study was obtained from the Medical Research Ethics Committee, Faculty of Medicine, Jenderal Soedirman University (Ref. No.: 100/KEPK/PE/IX/2025).

The animals were randomly divided into eight groups consisting of three control groups and five treatment groups (n = 5 per group). The negative control group received dimethyl sulfoxide (DMSO), the extract control group received chloroform extract of *F. vulgare* Mill. leaves at a dose of 400 mg/kg body weight without parasite inoculation, whereas the malaria control group was inoculated with *P. berghei* without extract treatment. Mice in the malaria control and treatment groups were inoculated intraperitoneally with 0.2 mL of *P. berghei*-infected blood. The treatment groups subsequently received chloroform extract of *F. vulgare* Mill.leaves at doses of 50, 100, 200, 400, and 800 mg/kg body weight according to the experimental protocol.

Hemoglobin levels were measured beginning 24 hours after *P. berghei* inoculation. Blood samples were collected from the tail vein using the tail-vein sampling technique, and hemoglobin concentrations were determined using a HemoSmart GOLD hemoglobinometer with compatible test strips according to the manufacturer's instructions. Measurements were performed every 24 hours at the same time each day for five consecutive observations. At the end of the experiment, all animals were euthanized by cervical dislocation in accordance with institutional ethical guidelines. Biological waste generated during the experiment was disposed of following institutional biosafety regulations to prevent environmental contamination.

Data were analyzed using IBM SPSS Statistics software. Descriptive analysis was performed by presenting the median, minimum, and maximum values of hemoglobin levels. Data normality was assessed using the Shapiro–Wilk test, whereas homogeneity of variance was evaluated using Levene's test. Because the data were not normally distributed but met the assumption of homogeneity, differences in hemoglobin levels among observation periods were analyzed using the Friedman test. A p-value of <0.05 was considered statistically significant.

This study has several limitations. First, the evaluation was focused on hemoglobin levels as the primary hematological outcome within a broader research project, while other hematological and related parameters were assessed separately within the main research framework. Therefore, the present study did not include other hematological parameters, such as erythrocyte count, hematocrit, reticulocyte count, or biomarkers of erythropoiesis, which could provide a more comprehensive assessment of anemia and the underlying hematological response. Second, the study was conducted using a murine malaria model; therefore, caution should be exercised when extrapolating the findings to human malaria. Third, the observation period was limited to five days following *P. berghei* inoculation, which may not fully reflect the long-term effects of *F. vulgare* leaf extract on hematological recovery. Further studies involving longer observation periods, additional hematological and molecular parameters, and clinical investigations are warranted to better elucidate the therapeutic potential of *F. vulgare* as an adjunctive treatment for malaria-associated anemia.

3. RESULT

Normality Test

The normality of hemoglobin data was assessed using the Shapiro–Wilk test at baseline (Day 0) and on Days 1–4 after treatment administration. The results are presented in Table 1. Most hemoglobin measurements showed a normal distribution, with Shapiro–Wilk p-values >0.05. However, four observations demonstrated significant deviation from normality ($p<0.05$), namely KN2 on Day 2 ($p=0.037$), P4 on Day 2 ($p=0.001$), P2 on Day 4 ($p=0.028$), and P5 on Day 4 ($p=0.048$). The remaining observations showed p-values >0.05. Overall, the normality assumption was not consistently fulfilled across all experimental groups and observation days.

Therefore, hemoglobin data were subsequently summarized using median and minimum–maximum values, and non-parametric analysis was applied for inferential testing.

Table 1. Shapiro–Wilk normality test of hemoglobin levels in each experimental group

Group	n (mice)	Day 0	Day 1	Day 2	Day 3	Day 4
KN1	5	0.840	0.909	0.685	0.850	0.409
KN2	5	0.453	0.051	0.037	0.802	0.631
KP	5	0.692	0.298	0.961	0.440	0.819
P1	5	0.634	0.861	0.971	0.378	0.394
P2	5	0.259	0.851	0.120	0.103	0.028
P3	5	0.052	0.538	0.729	0.322	0.849
P4	5	0.182	0.510	0.001	0.259	0.969
P5	5	0.660	0.978	0.783	0.612	0.048

Data are presented as Shapiro–Wilk significance (p) values. Values in bold indicate non-normal distribution ($p < 0.05$).

Abbreviations: KN1, healthy control receiving 3% DMSO; KN2, healthy control receiving *Foeniculum vulgare* leaf extract (400 mg/kg body weight); KP, malaria control (*Plasmodium berghei*-infected); P1, *P. berghei* + *F. vulgare* extract 50 mg/kg body weight; P2, *P. berghei* + *F. vulgare* extract 100 mg/kg body weight; P3, *P. berghei* + *F. vulgare* extract 200 mg/kg body weight; P4, *P. berghei* + *F. vulgare* extract 400 mg/kg body weight; P5, *P. berghei* + *F. vulgare* extract 800 mg/kg body weight. Day 0, before extract administration; Days 1–4, 1–4 days after extract administration. n = number of mice in each experimental group.

Hemoglobin Profile

The median hemoglobin concentrations of mice in each experimental group during the five-day observation period are presented in Table 2. Hemoglobin levels showed different patterns across groups and observation days. In KN1, the median hemoglobin concentration decreased from 13.0 g/dL at baseline to 10.9 g/dL on Day 1 and Day 2, followed by an increase to 13.0 g/dL on Day 4. In KN2, median hemoglobin gradually decreased from 12.2 g/dL at baseline to 9.5 g/dL on Day 4. The KP group showed an increase from 10.0 g/dL at baseline to 12.1 g/dL on Day 4. Among the *P. berghei*-infected groups receiving *F. vulgare* leaf extract, P2 showed an increase in median hemoglobin from 10.0 g/dL at baseline to 12.4 g/dL on Day 4. P1 showed relatively fluctuating values, while P3, P4, and P5 also demonstrated fluctuations without a consistent pattern throughout the observation period. Overall, the median hemoglobin concentrations did not demonstrate a consistent dose-related pattern across the *F. vulgare*-treated groups. The highest median hemoglobin concentration on Day 4 was observed in P2 (12.4 g/dL), whereas P4 showed 10.2 g/dL and P5 showed 11.4 g/dL.

Table 2. Median hemoglobin levels in each experimental group during the study period

Group	**n (mice)**	**D0**	**D1**	**D2**	**D3**	**D4**
KN1	5	13.00 (9.90-15.30)	10.90 (8.20-12.40)	10.90 (9.40-12.40)	11.60 (10.20-12.70)	13.00 (9.60-14.20)
KN2	5	12.20 (9.90-13.20)	12.60 (8.70-12.70)	11.50 (8.10-12.50)	10.70 (9.30-12.20)	9.50 (8.80-11.30)
KP	5	10.00 (7.90-12.20)	10.00 (8.80-11.80)	10.70 (8.80-13.00)	11.90 (10.20-12.50)	12.10 (10.70-12.80)
P1	5	11.10 (7.50-12.60)	10.90 (9.70-12.40)	10.60 (8.80-12.50)	12.10 (11.00-12.40)	11.00 (7.80-13.00)
P2	5	10.00 (7.60-14.40)	10.60 (8.80-13.20)	12.40 (10.80-12.60)	11.00 (10.80-13.20)	12.40 (8.60-13.20)
P3	5	10.40 (4.90-12.20)	12.00 (10.70-12.40)	10.30 (8.80-12.10)	11.30 (8.00-12.80)	10.20 (8.60-12.30)
P4	5	11.00 (8.50-11.50)	10.80 (8.70-11.80)	11.80 (8.30-11.90)	11.40 (8.70-12.30)	10.20 (7.70-11.90)

P5	5	10.70 (9.50-13.10)	9.10 (6.30-11.70)	9.60 (7.50-12.30)	11.60 (9.00-12.90)	11.40 (11.10-13.80)

Abbreviations: KN1, healthy control receiving 3% DMSO; KN2, healthy control receiving *Foeniculum vulgare* leaf extract (400 mg/kg body weight); KP, malaria control (*Plasmodium berghei*-infected); P1, *P. berghei* + *F. vulgare* extract (50 mg/kg body weight); P2, *P. berghei* + *F. vulgare* extract (100 mg/kg body weight); P3, *P. berghei* + *F. vulgare* extract (200 mg/kg body weight); P4, *P. berghei* + *F. vulgare* extract (400 mg/kg body weight); P5, *P. berghei* + *F. vulgare* extract (800 mg/kg body weight).

Homogeneity Test

Homogeneity of variance was assessed using Levene's test to determine whether the variances of hemoglobin levels were comparable among the experimental groups at each observation time point. As presented in **Table 3**, the variances were homogeneous at all observation days. The Levene's test yielded p-values of 0.976 on Day 0, 0.571 on Day 1, 0.945 on Day 2, 0.916 on Day 3, and 0.899 on Day 4. All p-values were greater than 0.05, indicating that the assumption of homogeneity of variance was fulfilled throughout the observation period.

Table 3. Homogeneity of variance test of hemoglobin levels among experimental groups

Observation day	Levene's test	p-value	Interpretation
Day 0	—	0.976	Homogeneous
Day 1	—	0.571	Homogeneous
Day 2	—	0.945	Homogeneous
Day 3	—	0.916	Homogeneous
Day 4	—	0.899	Homogeneous

Data are presented as p-values from Levene's test. Homogeneity of variance was considered fulfilled when $p > 0.05$.

Friedman Test

The Friedman test showed no statistically significant difference in hemoglobin levels among the experimental groups ($p = 0.216$). Therefore, administration of *Foeniculum vulgare* leaf extract at doses of 50, 100, 200, 400, and 800 mg/kg body weight did not result in a statistically significant difference in hemoglobin levels during the observation period.

Table 4. Friedman test for differences in hemoglobin levels among experimental groups

Variable	Statistical test	p-value
Hemoglobin level	Friedman test	0.216

4. DISCUSSION

The present study showed that administration of *F. vulgare* leaf extract at doses of 50, 100, 200, 400, and 800 mg/kg body weight did not result in a statistically significant difference in hemoglobin levels among *P. berghei*-infected mice during the observation period (p = 0.216). Although the median hemoglobin levels fluctuated among treatment groups, no consistent dose-dependent pattern was observed. The 100 mg/kg body weight group showed the highest median hemoglobin level at Day 4 (12.4 g/dL), whereas the higher-dose groups did not demonstrate a comparable increase. These findings indicate that the administration of F. vulgare leaf extract did not produce a measurable improvement in hemoglobin levels under the conditions of the present study.

The absence of a significant effect should be interpreted in the context of the pathogenesis of malaria-associated anemia. Hemoglobin reduction during malaria is a multifactorial process involving destruction of parasitized erythrocytes, increased clearance of non-parasitized erythrocytes, splenic removal of damaged cells, inflammatory responses, and

impaired or ineffective erythropoiesis (White, 2018; Chang *et al.*, 2016; Looareesuwan *et al.*, 2022). Recent evidence further emphasizes that malaria-associated anemia cannot be explained solely by hemolysis, because disturbances in erythropoiesis may also substantially contribute to the development and persistence of anemia (Looareesuwan *et al.*, 2022).

The hemoglobin pattern observed in this study was therefore biologically plausible. Among the infected groups, hemoglobin levels did not show a uniform decline during the four-day observation period, and the malaria control group also showed an increase in median hemoglobin from 10.0 g/dL at baseline to 12.1 g/dL on Day 4. Similar fluctuations were observed in the extract-treated groups. This pattern suggests that hemoglobin concentration during the relatively short observation period was influenced by several biological processes rather than solely by extract administration. Consequently, the increase observed in some treatment groups cannot be interpreted as evidence of a specific hematoprotective effect of F. vulgare.

Importantly, the interpretation of hemoglobin findings should also be considered together with the parasitemia findings obtained in this study. The extract demonstrated antiplasmodial activity at certain doses, but this response was not accompanied by a statistically significant improvement in hemoglobin. This distinction is important because suppression of parasite growth and restoration of hematological parameters do not necessarily occur simultaneously. A reduction in parasite burden may limit further erythrocyte damage, whereas recovery of circulating erythrocytes and normalization of hemoglobin may require a longer period because they depend on erythrocyte turnover and erythropoietic responses (Looareesuwan *et al.*, 2022).

The present findings differ from previous studies reporting hematological effects of F. vulgare, although direct comparison is limited because of differences in plant material, preparation, experimental models, and study conditions. Abbas *et al.* evaluated healthy rabbits receiving diets containing crushed *F. vulgare* seeds at concentrations of 2% and 4% for one month and reported increased hemoglobin levels compared with controls (Abbas *et al.*, 2021). In another experimental study, hydro-alcoholic extract of F. vulgare seeds was administered orally to male Wistar rats at 250–1000 mg/kg every 48 hours for 30 days, and hematological parameters including hemoglobin, erythrocyte count, and hematocrit were evaluated (Bakhsh *et al.*, 2015). These studies used healthy animals and fennel seeds, whereas the present study used chloroform extract of fennel leaves in *P. berghei*-infected mice. Therefore, their findings cannot be directly extrapolated to malaria-associated anemia.

This difference in plant part and extraction method is particularly relevant because the phytochemical composition of *F. vulgare* varies according to the plant organ, geographical origin, developmental stage, and extraction procedure (Zahi *et al.*, 2025). F. vulgare contains phenolic compounds, flavonoids, terpenoids, coumarins, alkaloids, and other secondary metabolites, but their concentrations may differ substantially between leaves, fruits, and seeds and among different extraction methods (Zahi *et al.*, 2025; Debnath *et al.*, 2023). Thus, the hematological effects reported for seed preparations should not automatically be attributed to the chloroform leaf extract used in the present study.

A possible explanation for the absence of a significant hemoglobin response is related to the antioxidant properties of *F. vulgare*. Previous studies have demonstrated that fennel contains phenolic and flavonoid compounds with antioxidant activity, while recent evidence indicates that extracts of *F. vulgare* can exhibit substantial free-radical-scavenging activity (Akbari *et al.*, 2023; Zahi *et al.*, 2025). In malaria, oxidative stress is closely associated with hemoglobin degradation, heme metabolism, inflammatory responses, and erythrocyte injury. A recent systematic review and meta-analysis found reduced total antioxidant status in malaria patients, with greater oxidative imbalance associated with more severe disease and higher parasite density (Kotepui *et al.*, 2024). Therefore, the antioxidant constituents of *F. vulgare* may potentially contribute to erythrocyte protection; however, this mechanism cannot be confirmed in the present study because oxidative stress biomarkers such as malondialdehyde, superoxide dismutase, catalase, or glutathione were not measured.

The absence of a dose-dependent response also warrants consideration. Increasing the dose from 50 to 800 mg/kg body weight did not produce a progressively greater hemoglobin response. Interestingly, the 100 mg/kg body weight group showed the highest median hemoglobin at Day 4, whereas the 400 and 800 mg/kg body weight groups did not show a similar pattern. A non-linear response may occur with complex plant extracts because different constituents can have different pharmacological activities, bioavailability may vary with dose, and the biological response may not increase proportionally with the administered amount. However, because each group contained only five mice, random biological variation may also have contributed substantially to the observed differences. Therefore, the present data do not provide sufficient evidence to establish an optimal dose for hematological protection.

The comparison with the healthy controls also provides an important perspective. The healthy control receiving *F. vulgare* extract alone (KN2) showed a progressive decline in median hemoglobin from 12.2 g/dL at baseline to 9.5 g/dL on Day 4, whereas the DMSO control returned to a median hemoglobin of 13.0 g/dL by Day 4. However, because the overall inferential analysis did not demonstrate a significant difference among groups, the decline in KN2 should not be interpreted as evidence of a direct adverse hematological effect of the extract. Previous toxicity research on F. vulgare preparations has generally reported no major changes in several hematological parameters under specific experimental conditions, although the findings vary according to extract type and dose (Dai *et al.*, 2020).

Several limitations should be considered when interpreting these findings. First, the sample size was relatively small, with five mice per experimental group, which may have limited the statistical power to detect modest differences. Second, hemoglobin was evaluated as the principal hematological outcome; therefore, changes in erythrocyte count, hematocrit, erythrocyte indices, and reticulocyte response could not be assessed. Third, although the study included parasitemia assessment, the present analysis did not directly establish a statistical relationship between parasite density and hemoglobin concentration. Fourth, oxidative stress and inflammatory biomarkers were not measured, preventing direct confirmation of the proposed antioxidant or anti-inflammatory mechanisms. Finally, the use of a crude chloroform leaf extract means that the specific compounds responsible for the observed biological activity could not be identified or quantified.

Further studies should therefore integrate parasitemia with a broader hematological profile, including erythrocyte count, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and reticulocyte count. Measurement of oxidative stress markers such as MDA, SOD, catalase, and glutathione, together with inflammatory mediators, would help determine whether the potential antioxidant and anti-inflammatory properties of *F. vulgare* contribute to erythrocyte protection during malaria infection. Characterization and standardization of the active constituents of the leaf extract are also necessary to determine whether specific fractions or compounds have greater hematoprotective potential. These approaches would provide a stronger basis for determining the optimal dose and clarifying whether *F. vulgare* leaf extract has a potential role as an adjunctive therapy alongside established antimalarial treatment rather than as a replacement for standard antimalarial therapy.

5. CONCLUSION

This study demonstrated that administration of fennel leaf extract (*Foeniculum vulgare* Mill.) did not significantly affect hemoglobin levels in malaria model mice (*Mus musculus*) infected with *Plasmodium berghei*. The administration of different doses of fennel leaf extract showed no significant improvement or reduction in hemoglobin levels compared with the control groups. These findings indicate that, under the conditions of this experimental model, fennel leaf extract did not exhibit a measurable effect on hemoglobin maintenance during malaria infection. Further studies are required to investigate the potential effects of *Foeniculum vulgare* extract on other hematological parameters and to elucidate the underlying mechanisms associated with its bioactive compounds in malaria infection.

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