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Pharmacological and Toxicological Evaluation of *Feretia apodanthera* Delile (Rubiaceae) Leaf Extract: Effects on Physiological, Hematological and Biochemical Alterations in *Plasmodium berghei*-Infected Mice, with Acute and Subacute Toxicity Assessments

Saamou Isaac Boni[1,2,3], Nâg-Tero Roland Meda[1], Kouliga Benjamin Koama[1,2], Domonbabele François de Sales Hien[1,2,3], Mariam Youba[2], Zachari Kabre[1,2], Valentin Konsegre[4], Mohamed Haddad[5], Abdoulaye Diabate[2], Rakiswende Serge Yerbanga[2,6]

[1]Laboratoire de Recherche et d'Enseignement en Santé et Biotechnologie Animales, École Doctorale Sciences Naturelles-Agronomie, Université Nazi Boni, Bobo-Dioulasso, Burkina Faso
[2]Institut de Recherche en Sciences de la Sante/Direction Régionale de l'Ouest (IRSS/DRO), Bobo-Dioulasso, Burkina Faso
[3]MIVEGEC, Université de Montpellier, Montpellier, France
[4]Centre Hospitalier Universitaire de Pala, Bobo-Dioulasso, Burkina Faso
[5]Pharma-Dev, Université de Toulouse, Toulouse, France
[6]Institut des Sciences et Techniques (INStech), Bobo-Dioulasso, Burkina Faso
Email: isaacboni303@gmail.com

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Abstract

Malaria remains a major public health burden in sub-Saharan Africa, causing systemic complications beyond parasitemia. While many antimalarial plants have been investigated for their direct antiplasmodial activity, their potential to mitigate malaria-induced host alterations remains poorly explored. Among these, *Feretia apodanthera* Del. is widely used in traditional medicine for malaria and other infectious diseases. Although its antimalarial activity has been demonstrated *in vitro* and *in vivo*, its effects on malaria-associated physiological, hematological, and biochemical disturbances are unclear. Thus, this study aimed to evaluate the capacity of the methanolic leaf extract of *F. apodanthera* to modulate malaria-induced physiological, hematological, and biochemical disorders in *Plasmodium berghei*-infected NMRI mice, while assessing its safety profile. Both restorative and protective models were employed to investigate the extract's impact on body weight, organ weights, and key biological parameters. In parallel, acute and subacute toxicity studies were conducted in ac-

cordance with OECD guidelines to determine its safety. The leaf extract reduced parasitemia by more than 50%. It had limited effects on body weight and did not significantly improve malaria-induced hematological alterations, but attenuated liver and kidney enlargement. Notably, it significantly reduced the elevation of liver enzymes (ALT and AST) and minimized organ alterations, particularly in the restorative model, indicating hepatoprotective and organ-protective effects. In the protective model, moderate biochemical effects were observed, although infection-induced alterations were not fully prevented. Acute toxicity evaluation revealed no mortality or clinical signs of toxicity at 2000 mg/kg, indicating a median lethal dose greater than 2000 mg/kg. Subacute administration over 28 days at all tested doses did not induce significant changes in body weight or organ weights and showed no evidence of dose-dependent toxicity, indicating the absence of systemic toxicity across the administered dose range. Furthermore, at the highest dose (2000 mg/kg), hematological, biochemical, and histopathological analyses revealed no signs of toxicity. Histopathological examination of the heart, lungs, liver, spleen, and kidneys confirmed the absence of tissue damage. Overall, the methanolic leaf extract of *F. apodanthera* is well tolerated and appears to limit malaria-induced physiological disturbances, particularly hepatic and renal alterations, while maintaining a favorable safety profile under both acute and repeated administration.

Keywords

Feretia apodanthera, *Plasmodium berghei*, Malaria, Host Alterations, Acute Toxicity, Subacute Toxicity

1. Introduction

Malaria remains a major global public health concern [1]. It is a parasitic disease caused by protozoa of the genus *Plasmodium* (Apicomplexa) and transmitted to humans through the bite of infected female *Anopheles* mosquitoes [2]. Despite significant advances in prevention and treatment, malaria continues to impose a heavy burden, particularly in sub-Saharan Africa, where nearly the entire population is at risk. Children under five years of age and pregnant women remain the most vulnerable groups [1] [3].

Clinically, malaria is characterized by intermittent fever, chills, sweating, and fatigue. Severe forms, predominantly caused by *Plasmodium* falciparum, may lead to life-threatening complications such as severe hemolytic anemia, cerebral malaria, respiratory distress, and multiorgan failure [4].

Beyond these acute manifestations, malaria also exerts profound systemic effects. It significantly alters organ structure and function, particularly in the liver, spleen, and kidneys, and is associated with a wide range of hematological and biochemical disturbances [5]. These include anemia, thrombocytopenia, leukopenia, elevated liver enzymes, hyperbilirubinemia, hypoglycemia, dyslipidemia, and

renal dysfunction [6]-[8]. In addition, malaria is frequently associated with significant weight loss, which may contribute to or exacerbate malnutrition, especially in endemic areas [8]-[10]. Taken together, these observations highlight that malaria is not only an acute febrile illness, but also a complex systemic disease with interrelated clinical, metabolic, and nutritional consequences [11].

Despite their clinical importance, these systemic manifestations remain insufficiently considered in current malaria management strategies, which primarily focus on parasite elimination and transmission control [4]. Organ dysfunction, metabolic disturbances, and nutritional deterioration are common and clinically relevant, yet they are often underexplored and rarely integrated into therapeutic approaches. This gap highlights the need for complementary strategies targeting host-related complications of malaria [12].

In this context, increasing attention is being given to adjunctive therapies aimed at mitigating malaria-induced physiological alterations [13]. Beyond direct antiplasmodial activity, emerging approaches seek to modulate inflammation, restore metabolic homeostasis, and protect organ function [14] [15]. Various strategies are currently under investigation, including immunomodulatory and anti-inflammatory agents to reduce cytokine-mediated damage, antioxidants to limit oxidative stress, and interventions targeting iron metabolism and erythropoiesis to address anemia [16]. Additional approaches include metabolic support to manage hypoglycemia and acidosis, renal support strategies for malaria-associated kidney injury, and nutritional supplementation to strengthen host resilience [17]. Furthermore, advanced adjuvant systems such as saponins, liposomes, and toll-like receptor agonists have been incorporated into next-generation malaria vaccines, including RTS, S and R21, to enhance immune responses [18].

Among these complementary approaches, medicinal plants represent a promising and accessible source of bioactive compounds [19]. Rich in secondary metabolites such as flavonoids, alkaloids, tannins, saponins, and terpenoids, many plant extracts exhibit antioxidant, anti-inflammatory, immunomodulatory, and antiparasitic properties [19]-[21]. Several studies have demonstrated their ability to mitigate malaria-induced alterations, including anemia, thrombocytopenia, and biochemical dysfunctions [8]. These multi-target effects make medicinal plants particularly attractive as potential adjunctive therapies in malaria management.

Feretia apodanthera Delile (Rubiaceae) is widely used in West African traditional medicine for the treatment of fever and malaria [22]. Previous studies have demonstrated that leaf extracts of this plant possess significant antiplasmodial activity, both *in vitro* and *in vivo* [22]-[24]. Phytochemical investigations have identified a range of bioactive constituents, including flavonoids, alkaloids, tannins, saponins, glycosides, iridoids, and terpenoids [22] [25]-[28], which are associated with diverse pharmacological activities such as antioxidant, anti-inflammatory, antibacterial effects [29]-[32]. In addition to its pharmacological potential, the safety profile of *F. apodanthera* has been partially investigated [28] [33] [34]. Alt-

hough acute toxicity studies on *Feretia apodanthera* have suggested a favorable safety profile at tested doses, investigations remain scarce and geographically limited, with only Boni et al. reporting such data in the western region of Burkina Faso [22]. Moreover, information on subacute toxicity is still lacking. This constitutes a critical knowledge gap, as repeated exposure may induce cumulative or delayed adverse effects that cannot be detected in single-dose toxicity assessments.

In the context of malaria, evaluating *F. apodanthera* extract may help determine whether their therapeutic potential extends beyond antiparasitic activity to include the mitigation of infection-induced weight loss, hematological disturbances, and biochemical alterations. At the same time, assessing its subacute toxicity is essential to better characterize its safety profile under conditions of repeated administration.

In light of these considerations, the present study aimed to investigate the effects of *Feretia apodanthera* leaf extract in a *Plasmodium berghei*-infected NMRI mouse model, with particular emphasis on body weight, organ mass, and hematological and biochemical parameters, while also evaluating the acute and subacute toxicity of the extract. Overall, the findings demonstrate that the leaf extract is capable of limiting malaria-induced physiological disturbances—particularly hepatic, renal, and hematological alterations—while maintaining a favorable safety profile, with no evidence of significant toxicity under repeated administration.

2. Material and Methods

2.1. Plants Materials and Extraction Process

This study focused exclusively on the leaves of *Feretia apodanthera*. Leaves were collected in September 2022 during the rainy season from the classified forest of Dindèrèsso, located between 4˚18'46"W and 4˚26'40"W longitude and 11˚11'05"N and 11˚18'10"N latitude. Dindèrèsso is situated approximately 15 km northwest of Bobo-Dioulasso, Burkina Faso. The plant was identified by Dr. Hermann Ouoba, and a voucher specimen (FAF001) was deposited at LARESBA, Université Nazi Boni.

Collected leaves were cleaned, air-dried under laboratory conditions away from direct sunlight, and ground into a fine powder. For extraction, 25 g of powdered leaves were subjected to soxhlet extraction using 200 mL of analytical-grade methanol for at least 4 hours. The extract was concentrated to dryness using a rotary evaporator at 40˚C under reduced pressure.

2.2. Animals and Parasite

Female and male NMRI mice (8 - 12 weeks old) were obtained from CIRDES (Bobo-Dioulasso, Burkina Faso) and acclimatized under standard laboratory conditions (25˚C, 12 h light/dark cycle, with food and water provided ad libitum). Female mice were used for the restorative, protective, and acute toxicity studies to reduce variability associated with hormonal fluctuations, whereas both sexes

were included in the subacute toxicity study to provide a more comprehensive assessment of systemic safety. Animals were randomly assigned to experimental groups, and outcome assessments were performed under blinded conditions to minimize bias.

The chloroquine-sensitive *Plasmodium berghei* ANKA strain was used and maintained by serial passage in mice. All experimental procedures were conducted in accordance with international guidelines for the care and use of laboratory animals and were approved under protocol number 2021-004.

2.3. Effect of *F. apodanthera* Leaf Extract on Malaria-Induced Alterations

2.3.1. Restorative Effect of Extract on Weight, Biochemical, and Hematological Disorders in *Plasmodium berghei*-Infected NMRI Mice

The restorative potential of the extracts against infection-induced alterations was evaluated in female NMRI mice as previously described [35]. Eighteen mice were randomly allocated into three experimental groups (n = 6 per group): a non-infected negative control group, an infected untreated control group, and an extract-treated group. The dose of 250 mg/kg body weight was selected for treatment. On Day 0, all groups except the negative control were intraperitoneally inoculated with *Plasmodium berghei*-infected erythrocytes (10^7 parasitized red blood cells). Seventy-two hours post-infection (Day 3), treatment was initiated. The extract-treated group received the leaf extract orally once daily for four consecutive days (Day 3 to Day 6), while control groups received the vehicle (10 mL/kg body weight) under the same conditions. Body weight was recorded at baseline (Day 0), prior to treatment initiation (Day 3), and during the follow-up period (Days 5 and 7). On Day 7, all animals were humanely euthanized, and blood samples were collected via cardiac puncture for hematological and biochemical analyses. Major organs, including the liver, kidneys, and spleen, were carefully excised and weighed to assess infection- and treatment-related alterations in organ morphology.

2.3.2. Protective Effect Extract against Weight Loss and Haemato-Biochemical Alterations in Nmri Mice Challenged with *Plasmodium berghei*

The protective potential of the *F. apodanthera* leaf extract was evaluated in female NMRI mice following a protocol adapted from the restorative model [36]. Eighteen mice were randomly divided into three experimental groups (n = 6 per group): a non-infected negative control group, an infected untreated control group, and an extract-treated group. The extract-treated group received the leaf extract orally at a dose of 250 mg/kg body weight once daily for three consecutive days (Day 0 to Day 2). Control groups received the vehicle alone (10 mL/kg body weight) under the same conditions. On Day 3, all groups except the negative control were intraperitoneally inoculated with *Plasmodium berghei*-infected erythrocytes (10^7 parasitized red blood cells). Body weight was recorded at baseline (Day 0), prior

to infection (Day 3), and during infection progression (Days 5 and 7). At the end of the experiment (Day 7), animals were humanely euthanized, and blood samples were collected via cardiac puncture for hematological and biochemical analyses. In addition, major organs including the liver, spleen, and kidneys were carefully excised and weighed to assess the ability of the extract to prevent infection-induced organ alterations.

2.4. Toxicity Assessments

2.4.1. Acute Toxicity

The acute oral toxicity of the methanolic leaf extract of *Feretia apodanthera* was evaluated at a dose of 2000 mg/kg body weight in NMRI mice, in accordance with OECD guidelines [37]. A total of 12 female mice were randomly divided into two groups (n = 6):

- Control group: received 10 mL/kg of vehicle (distilled water containing 5% DMSO);
- Treated group: received a single oral dose of 2000 mg/kg of the leaf extract.

Following administration, animals were closely observed for the first 30 minutes for immediate behavioral changes. Thereafter, observations were conducted twice daily for 14 days to detect signs of toxicity, including changes in behavior, locomotion, feeding, and mortality. Body weight was recorded on Days 7 and 14. At the end of the observation period (Day 15), animals were euthanized, and blood samples were collected for biochemical and hematological analyses. Major organs (liver, kidneys, spleen, heart, and lungs) were excised and examined.

2.4.2. Subacute Toxicity

The subacute toxicity study was conducted over 28 consecutive days in accordance with OECD guideline 407 [38]. Forty-eight NMRI mice (24 males and 24 females) were divided into four groups (n = 12; 6 males and 6 females per group):

- Control group: received vehicle (distilled water + 0.5% DMSO);
- Low dose: 20 mg/kg/bwt of leaf extract;
- Medium dose: 200 mg/kg/bwt of leaf extract;
- High dose: 2000 mg/kg/bwt of leaf extract.

The extract was administered orally once daily. Animals were observed daily for clinical signs of toxicity. Body weight was recorded weekly. On Day 29, animals were sacrificed after fasting. Blood samples were collected for biochemical (ALT, AST, bilirubin, urea, creatinine, triglycerides) and hematological analyses. Organs (liver, kidneys, spleen, heart, lungs) were collected, weighed, and processed for histopathological examination using hematoxylin-eosin staining.

2.5. Statistical Analysis

All statistical analyses were conducted using R software (version 4.4.0). Body weight data, recorded repeatedly over time (D0, D3, D5, and D7), were analyzed using generalized linear mixed models (GLMMs) fitted with the glmmTMB package [39]. Treatment (three levels: uninfected control, infected control, and *Feretia*

apodanthera leaf extract), time, and their interaction were included as fixed effects, while individual mouse identity was included as a random intercept to account for repeated measurements within subjects. Organ weights (liver, spleen, and kidneys), hematological parameters (RBC, WBC, HB, HT, PLT, THT, VMP, LYM, MON, NEU, EOS, BAS), and biochemical markers (AST, ALT, CREA, UREA, BD, BT, TG), each measured once per animal at the experimental endpoint, were analyzed using one-way analysis of variance (ANOVA) with treatment as the fixed factor. When the assumptions of normality and homoscedasticity were not met, the non-parametric Kruskal-Wallis test was applied instead. To control for multiple testing across variables, p-values were adjusted using the Holm-Bonferroni method. When ANOVA assumptions were satisfied, post hoc comparisons were performed using Tukey's honestly significant difference (HSD) test. For non-parametric analyses, pairwise comparisons were conducted using Dunn's test with Holm adjustment. In addition, sex-stratified analyses (male and female animals) comparing two treatment groups (control vs. leaf extract) were performed using the Wilcoxon rank-sum test for non-parametric data.

3. Results

3.1. Restorative Effect of *F. apodanthera* Delile Extracts on Weight, Biochemical, and Hematological Disorders in *Plasmodium berghei*-Infected NMRI Mice

The impact of the leaf extract on parasitemia is presented in Figure 1 and Figure 2 and Table 1. All mice successfully developed *Plasmodium berghei* infection, with a median parasitemia of 17 (range: 8 - 43), confirming the reliability of the experimental model for evaluating treatment effects. At the experimental endpoint (Day 7), parasitemia reached 38.30 ± 2.10 in the untreated infected group (M_Ctrl), compared to 16 ± 5.10 in the leaf extract-treated group. As expected, uninfected control mice (Ctrl) showed no detectable parasitemia (0). These differences correspond to a chemosuppression of 52.89 ± 3.70% in the leaf-treated group.

3.1.1. Effects of *Feretia apodanthera* Leaf Extract on Body Weight-Restorative Model

The evolution of body weight in NMRI mice across the experimental period is presented in Figure 1(A). Overall, a highly significant effect of time was observed ($p < 0.0001$), indicating substantial changes in body weight during the course of the experiment. In contrast, the main effect of treatment was not significant ($p = 0.64$). However, a significant interaction between treatment and time was detected ($p < 0.0001$), suggesting that body weight changes over time differed depending on the treatment group. At baseline (Day 0), no significant differences in body weight were observed between groups ($p > 0.05$), confirming the homogeneity of the experimental groups prior to infection and treatment. In the non-infected control group (Ctrl), body weight increased steadily throughout the study, rising from 32.17 ± 0.30 g at Day 0 to 44.77 ± 0.41 g at Day 7. In contrast, infected

untreated mice (M_Ctrl) showed an initial increase between Day 0 and Day 3 (32.50 ± 0.12 g to 38.26 ± 1.02 g), followed by a progressive decline to 35.10 ± 1.05 g at Day 7. Mice treated with *F. apodanthera* leaf extract exhibited a different pattern. Body weight increased from 31.78 ± 0.22 g at Day 0 to 35.93 ± 0.27 g at Day 3, but subsequently decreased to 33.42 ± 0.78 g at Day 7. Pairwise comparisons revealed no significant differences between groups at Day 0 and Day 3 ($p > 0.05$), except between the control and infected groups at Day 3 ($p = 0.005$), indicating early effects of infection. At Day 5, the control group showed significantly higher body weight compared to both the Leaf-treated group ($p < 0.0001$) and the infected control group ($p = 0.003$). Additionally, a significant difference was observed between the Leaf-treated and infected control groups ($p = 0.004$). By Day 7, body weight in the control group remained significantly higher than in both the Leaf-treated and infected control groups ($p < 0.0001$).

3.1.2. Effects of *Feretia apodanthera* Leaf Extract on Organ Weights-Restorative Model

Liver

A highly significant effect of treatment on liver weight was observed ($p < 0.001$, Figure 1(B)). Compared to the control group (Ctrl), infected untreated mice (M_Ctrl) showed a marked increase in liver weight (1.64 ± 0.10 g vs 2.94 ± 0.17g; $p < 0.001$), indicating hepatomegaly associated with *Plasmodium berghei* infection. Mice treated with *F. apodanthera* leaf extract also exhibited a significant increase in liver weight compared to control (2.27 ± 0.05 g vs 1.64 ± 0.10 g; $p = 0.001$), although this increase was significantly lower than that observed in infected untreated mice (2.27 ± 0.05 g vs 2.94 ± 0.17g; $p = 0.0011$). Importantly, the Leaf-treated group showed a significantly lower liver weight compared to M_Ctrl mice, suggesting a partial attenuation of infection-induced hepatomegaly.

Spleen

A highly significant treatment effect was observed on spleen weight ($p < 0.001$, Figure 1(C)). Infected untreated mice showed a marked increase in spleen weight compared to controls (0.71 ± 0.07 g vs 0.15 ± 0.02 g; $p < 0.001$), reflecting pronounced splenomegaly. Similarly, the Leaf-treated group also exhibited a significant increase in spleen weight compared to control (0.57 ± 0.05 g vs 0.15 ± 0.02 g; $p < 0.001$). However, although spleen weight was lower in the Leaf-treated group than in infected untreated mice, this difference did not reach statistical significance (0.57 ± 0.05 g vs 0.71 ± 0.07 g; $p = 0.06$).

Kidneys

A significant effect of treatment on kidney weight was observed ($p < 0.001$, Figure 1(D)). Infected untreated mice (M_Ctrl) exhibited a significant increase in kidney weight compared to controls (0.43 ± 0.001 g vs 0.33 ± 0.02 g; $p = 0.0005$), indicating renal involvement during infection. In contrast, mice treated with *F. apodanthera* leaf extract showed no significant difference in kidney weight compared to the control group (0.35 ± 0.01 g vs 0.33 ± 0.02 g; $p = 0.21$). However, kidney weight in the Leaf-treated group was significantly lower than in the in-

fected untreated group (p = 0.0030). These results suggest that the leaf extract effectively prevented or reduced infection-induced kidney enlargement.

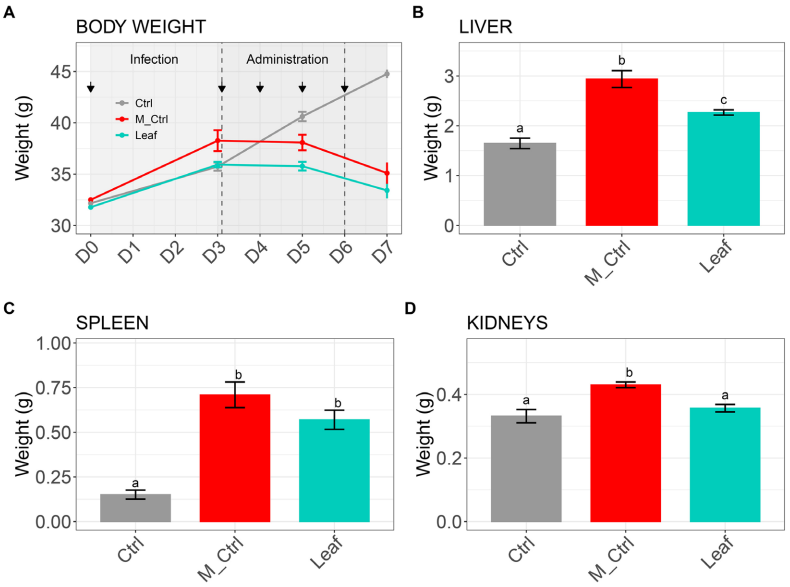

Figure 1. Effect of *Feretia apodanthera* leaf extract on body weight and organ weights during the restorative test assessed at 250 mg/kg/bwt. (A) Weight evolution; (B)-(D) relative organ weights ((B) Liver; (C) Kidneys; (D) Spleen). Note: n = 6 (number of each group); Ctrl: Control uninfected group; M_Ctrl: Malaria control untreated.

3.1.3. Effects of *Feretia apodanthera* Leaf Extract on Hematological Parameters-Restorative Model

The effects of *Feretia apodanthera* leaf extract on hematological parameters in *Plasmodium berghei*-infected mice are presented in Table 1.

Infection induced marked hematological alterations, as evidenced by significant differences between groups for several parameters. A significant decrease in hemoglobin (HB), hematocrit (HT), and red blood cell count (RBC) was observed in infected untreated mice (M_Ctrl) compared to the control group ($p < 0.05$), indicating the development of anemia. Similarly, mice treated with the leaf extract also showed significantly reduced HB, HT, and RBC levels compared to controls ($p < 0.05$).

White blood cell count (WBC) was significantly elevated in both infected untreated (19.90 ± 2.33) and Leaf-treated mice (15.00 ± 1.83 103/mm) compared to controls (1.87 ± 0.09 103/mm) ($p < 0.05$). This increase was accompanied by significant changes in leukocyte subpopulations. Monocyte (MON) levels were significantly higher in both M_Ctrl and Leaf groups compared to controls ($p < 0.05$), while neutrophil (NEU) levels were significantly reduced in infected and treated mice ($p < 0.01$). Basophil (BAS) levels were also significantly increased in both groups ($p < 0.05$). Eosinophil (EOS) counts were significantly reduced in both infected untreated and Leaf-treated mice compared to controls ($p < 0.01$), reaching nearly zero in the M_Ctrl group. Lymphocyte (LYM) levels did not differ significantly between groups ($p > 0.05$), indicating relative stability of this parameter.

Platelet count (PLT) and thrombocrite (THT) showed no significant differences between groups ($p > 0.05$). However, mean platelet volume (VMP) was significantly increased in both infected untreated and Leaf-treated mice compared to controls ($p < 0.05$).

Overall, the hematological profile of the Leaf-treated group closely resembled that of infected untreated mice, with no significant improvement in anemia-related parameters (HB, HT, RBC). However, slight differences were observed in certain leukocyte populations, suggesting a limited modulatory effect of the extract on the immune response.

3.1.4. Effects of *Feretia apodanthera* Leaf Extract on Biochemical Parameters-Restorative Model

Table 1. Effect of extracts in hematological and biochemical parameters of infected mice during restorative model evaluating at 250 mg/kg/bwt.

Parameters	Control	Malaria control	Leaf extract	p-value
Hematological				
RBC (10^6/mm)	9.61 ± 0.27 ab	5.38 ± 0.12 a	5.94 ± 0.25 b	0.018
HB (g/dL)	16.03 ± 0.44 ab	9.65 ± 0.20 a	10.10 ± 0.27 b	0.018
HT (%)	47.27 ± 1.39 ab	27.20 ± 0.40 a	28.45 ± 0.87 b	0.018
PLT (10^3/mm)	573.33 ± 49.35 a	505.50 ± 64.18 b	425.00 ± 5.81 c	0.18
THT (%)	0.30 ± 0.02 a	0.35 ± 0.06 b	0.29 ± 0.00 c	0.80
MPV (μm^3)	5.20 ± 0.06 ab	6.85 ± 0.25 a	6.80 ± 0.04 b	0.018
WBC (10^3/mm)	1.87 ± 0.09 ab	19.90 ± 2.33 a	15.00 ± 1.83 b	0.018
LYM (%)	84.67 ± 0.86 a	81.15 ± 0.74 b	80.70 ± 2.41 c	0.44
MON (%)	1.80 ± 0.22 ab	9.75 ± 0.65 a	10.00 ± 1.16 b	0.01
NEU (%)	12.43 ± 0.92 a	6.10 ± 0.09 b	4.75 ± 0.07 a	0.005
EOS (%)	0.47 ± 0.04 ab	0.00 ± 0.00 a	0.05 ± 0.02 b	0.008
BAS (%)	0.63 ± 0.13 ab	3.00 ± 0.18 a	4.50 ± 1.30 b	0.01
Biochemical				
ALT (U/L)	51.50 ± 5.66 a	203.67 ± 18.25 b	120.33 ± 23.98 a	0.00001
AST (U/L)	132.00 ± 6.67 a	547.67 ± 37.78 b	308.67 ± 54.54 a	0.00001
CREA (mg/L)	62.33 ± 6.98 a	78.50 ± 14.53 a	62.50 ± 6.04 a	0.35
UREA (mg/L)	7.36 ± 0.04 a	6.44 ± 0.34 b	7.18 ± 0.08 a	0.002

Notes: Values are mean ± SEM; n = 6 for each group; RBC: Red blood cells; WBC: White blood cells; HGB: Hemoglobin; PLT: Platelets; THT: thrombocrite; MPV: Mean platelet volume; HT: Hematocrit; LYM: Lymphocytes; MON: Monocytes; NEU: Neutrophil. EOS: Eosinophil; BAS: Basophil; AST: Aspartate Aminotransferase; ALT: Alanine aminotransferase; CREA: Creatinine. Overall differences among groups were assessed using the Kruskal-Wallis test, followed by Holm correction for multiple comparisons. Different letters (a, b, c) indicate significant differences between groups for a given parameter (Dunn post-hoc test, adjusted $p < 0.05$). Groups sharing the same letter are not significantly different.

The effects of *Feretia apodanthera* leaf extract on biochemical parameters are pre-

sented in Table 2. A highly significant effect of treatment was observed on liver enzymes, including alanine aminotransferase (ALT) and aspartate aminotransferase (AST) ($p < 0.001$). Infected untreated mice (M_Ctrl) exhibited a marked elevation of ALT (203.67 ± 18.25 U/L) and AST (547.67 ± 37.78 U/L) compared to the control group (51.50 ± 5.66 U/L and 132.00 ± 6.67 U/L, respectively), indicating severe hepatic injury associated with *Plasmodium berghei* infection.

Treatment with *F. apodanthera* leaf extract significantly reduced ALT (120.33 ± 23.98 U/L) and AST (308.67 ± 54.54 U/L) levels compared to infected untreated mice, although these values remained significantly higher than those of the control group ($p < 0.001$). Serum creatinine (CREA) levels did not differ significantly between groups ($p = 0.35$), suggesting the absence of marked renal impairment or a limited sensitivity of this marker under the experimental conditions. In contrast, a significant difference was observed for urea levels ($p = 0.0022$). Infected untreated mice showed lower urea levels (6.44 ± 0.34 mmol/L) compared to controls (7.36 ± 0.04 mmol/L), while the Leaf-treated group (7.18 ± 0.08 mmol/L) exhibited values closer to those of the control group.

3.1.5. Principal Component Analysis (PCA) of Hematological and Biochemical Parameters-Restorative Model

In the restorative model, PCA revealed that the first two principal components (PC1 and PC2) explained 85.90% of the variance in hematological parameters (PC1: 65.70%; PC2: 20.10%), suggesting that the majority of variability in blood responses to infection and treatment was captured by these components (Figure 2(A)).

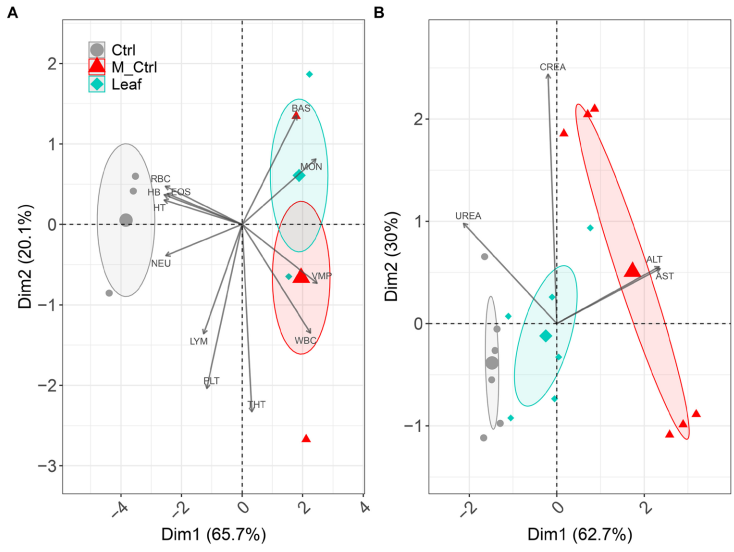

Figure 2. Principal component analysis (PCA) of hematological (A) and biochemical (B) profiles showing clear separation of treatments in the evaluation of the restorative effect of *Feretia apodanthera* leaf methanolic extract. Note: n = 6 for each group; Ctrl: Control uninfected group; M_Ctrl: Infected control untreated; RBC: Red blood cells; WBC: White blood cells; HGB: Hemoglobin; PLT: Platelets; MPV: Mean platelet volume; HT: Hematocrit; THT: Thrombocrite; LYM: Lymphocytes; MON: Monocytes; NEU: Neutrophil. EOS: Eosinophil; BAS: Basophil; AST: Aspartate Aminotransferase; ALT: Alanine aminotransferase; CREA: Creatinine.

For biochemical parameters, PC1 and PC2 accounted for 92.70% of the total variance (PC1: 62.7%; PC2: 30%), highlighting dominant effects on liver and kidney function markers (**Figure 2(B)**). These results indicate that treatment with the methanolic leaf extract of *Feretia apodanthera* exerted stronger restorative effects on biochemical rather than hematological parameters, consistent with observed hepatoprotective and organ-protective actions. The PCA provides a multivariate overview of the extract's capacity to mitigate malaria-induced physiological disturbances in *Plasmodium berghei*-infected mice.

3.2. Protective Effect of *Feretia apodanthera* Leaf Extract against Weight Loss and Haemato-Biochemical Alterations in Nmri Mice Challenged with *Plasmodium berghei*

In the protective model (**Figure 3** and **Figure 4**, **Table 2**), all infected mice successfully developed *Plasmodium berghei* infection. At the experimental endpoint, the infected untreated group (M_Ctrl) showed a median parasitemia of 10 (range: 2 - 37), confirming the robustness of the model. Mean parasitemia levels were 34.6 ± 1.6 in the M_Ctrl group, compared to 12.0 ± 0.6% in the leaf extract-treated group, while uninfected control mice (Ctrl) showed no detectable parasitemia (0). These results correspond to a chemosuppression of 58.23% in the extract-treated group.

3.2.1. Effects of *Feretia apodanthera* Leaf Extract on Body Weight (Protective Assay)

The evolution of body weight in the protective assay is presented in **Figure 3(A)**. A highly significant effect of time was observed ($p < 0.0001$), indicating changes in body weight throughout the experimental period. The main effect of treatment was not significant ($p = 0.4729$). However, a significant interaction between treatment and time was detected ($p = 0.0034$), suggesting that body weight evolution differed among groups over time. At baseline (Day 0), no significant differences in body weight were observed between groups ($p > 0.05$), confirming initial homogeneity. In the control group (Ctrl), body weight increased progressively from 31.44 ± 0.11 g at Day 0 to 38.14 ± 0.42 g at Day 7. In infected untreated mice (M_Ctrl), body weight increased from 31.44 ± 0.23 g at Day 0 to 38.31 ± 0.83 g at Day 3, followed by a slight decrease to 37.17 ± 1.71 g at Day 7. In contrast, mice treated with *F. apodanthera* leaf extract (Leaf group) showed a modest increase from 30.34 ± 0.16 g at Day 0 to 32.49 ± 0.55 g at Day 3, followed by a slight increase to 33.45 ± 0.51 g at Day 5 and stabilization at 33.27 ± 0.84 g at Day 7. Pairwise comparisons revealed no significant differences between groups at Day 0 ($p > 0.05$). At Day 3, body weight in the Leaf-treated group was significantly lower than in both the control ($p = 0.001$) and infected untreated groups ($p < 0.001$). A similar trend was observed at Day 5 and Day 7, where the Leaf-treated group remained significantly lower than the control group ($p < 0.001$) and the infected untreated group ($p < 0.001$). No significant differences were observed between the control and infected untreated groups at Day 5 and Day 7 ($p > 0.05$).

3.2.2. Effects of *Feretia apodanthera* Leaf Extract on Organ Weights (Protective Assay)

Liver

A significant effect of treatment on liver weight was observed ($p < 0.001$, Figure 3(B)). Infected untreated mice (M_Ctrl) showed a significant increase in liver weight compared to the uninfected control group (2.37 ± 0.17 g vs 1.70 ± 0.10 g; $p = 0.006$), confirming the development of infection-induced hepatomegaly. Mice treated with *F. apodanthera* leaf extract also exhibited higher liver weight compared to control; however, this difference was not statistically significant (2.05 ± 0.11 g vs 1.70 ± 0.10 g; $p = 0.09$). Moreover, no significant difference was observed between the Leaf-treated and infected untreated groups (2.05 ± 0.11 g vs 2.37 ± 0.17 g; $p = 0.08$). Although a slight reduction in liver weight was observed in the Leaf-treated group compared to infected untreated mice, this effect did not reach statistical significance.

Spleen

A highly significant effect of treatment was observed on spleen weight ($p < 0.001$, Figure 3(C)). Infected untreated mice showed a marked increase in spleen weight compared to controls (0.32 ± 0.01 g vs 0.13 ± 0.02 g; $p < 0.001$), reflecting pronounced splenomegaly. Similarly, the Leaf-treated group exhibited significantly higher spleen weight compared to controls (0.28 ± 0.02 g vs 0.13 ± 0.02 g; $p < 0.001$). However, no significant difference was observed between the Leaf-treated and infected untreated groups (0.28 ± 0.02 g vs 0.32 ± 0.01 g; $p = 0.13$).

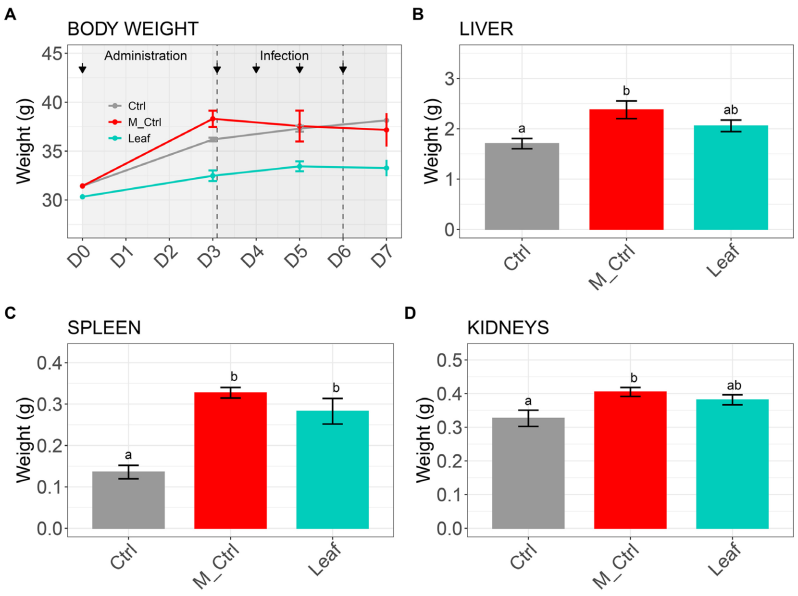

Figure 3. Impact of *Feretia apodanthera* leaf extract on body weight and organ weights during the restorative test assessed at 250 mg/kg/bwt. (A) Weight evolution; B-D: relative organ weights ((B) Liver; (C) Kidneys; (D) Spleen). Note: n = 6 (number of each group); Ctrl: Control uninfected group; M_Ctrl: Malaria control untreated.

Kidneys

A significant treatment effect was observed on kidney weight ($p = 0.002$, Figure 3(D)). Infected untreated mice exhibited a significant increase in kidney weight

compared to controls (0.40 ± 0.01 g vs 0.32 ± 0.02 g; p = 0.02). The Leaf-treated group also showed higher kidney weight compared to control group; however, this difference was borderline non-significant (0.38 ± 0.01 g vs 0.32 ± 0.02 g; p = 0.05). No significant difference was observed between the Leaf-treated and infected untreated groups (0.38 ± 0.01 g vs 0.40 ± 0.01 g; p = 0.34). These results indicate that the leaf extract did not significantly prevent infection-induced kidney enlargement under the conditions of the protective assay.

3.2.3. Effects of *Feretia apodanthera* Leaf Extract on Hematological Parameters (Protective Assay)

The effects of *Feretia apodanthera* leaf extract on hematological parameters in the protective assay are presented in Table 2.

Significant differences were observed between groups for several parameters. Infected untreated mice (M_Ctrl) showed a marked decrease in hemoglobin (HB), hematocrit (HT), and red blood cell count (RBC) compared to the control group ($p < 0.05$), indicating the development of anemia. A similar decrease was observed in the Leaf-treated group, with no significant improvement compared to infected untreated mice.

White blood cell count (WBC) was significantly increased in both infected untreated and Leaf-treated groups compared to controls ($p < 0.05$), reflecting an infection-induced inflammatory response. Neutrophil (NEU) levels were significantly reduced in both groups ($p < 0.05$), while lymphocyte (LYM) and monocyte (MON) levels did not differ significantly between groups ($p > 0.05$). Eosinophil (EOS) levels were significantly decreased in infected and treated mice compared to controls ($p < 0.05$), whereas basophil (BAS) levels showed no significant differences ($p > 0.05$).

Platelet count (PLT) and thrombocrite (THT) were significantly reduced in infected untreated mice compared to controls ($p < 0.05$). Although the Leaf-treated group showed higher values than M_Ctrl, these parameters remained significantly lower than in the control group. Mean platelet volume (VMP) was significantly increased in both infected and treated groups ($p < 0.05$).

Overall, the hematological profile of the Leaf-treated group remained close to that of infected untreated mice, indicating that the extract did not significantly prevent malaria-induced hematological alterations.

3.2.4. Effects of *Feretia apodanthera* Leaf Extract on Biochemical Parameters (Protective Assay)

The biochemical parameters are presented in Table 2. A highly significant effect of treatment was observed on liver enzymes ($p < 0.001$). Infected untreated mice exhibited markedly elevated ALT (112.00 ± 19.35 U/L) and AST (418.67 ± 32.06 U/L) levels compared to controls (41.50 ± 2.01 and 155.00 ± 5.81 U/L, respectively), indicating hepatic injury. Treatment with *F. apodanthera* leaf extract resulted in lower ALT (54.00 ± 5.48 U/L) and AST (258.00 ± 36.72 U/L) levels compared to infected untreated mice, suggesting a partial attenuation of liver damage, although enzyme levels remained higher than in the control group. Renal param-

eters showed significant differences between groups ($p < 0.001$). Creatinine (CREA) levels were markedly reduced in the Leaf-treated group (18.00 ± 0.45 µmol/L) compared to both control and infected untreated groups. Similarly, urea levels were significantly decreased in both infected untreated (2.91 ± 0.08 mmol/L) and Leaf-treated mice (3.57 ± 0.17 mmol/L) compared to controls (7.36 ± 0.04 mmol/L).

Table 2. Effect of Leaf methanolic extract of *Feretia apodanthera* in hematological and biochemical parameters of mice during protective assay.

Parameters	Control	Malaria control	Leaf extract	p-value
Hematological				
RBC (10^6/mm)	8.99 ± 0.04 ab	6.71 ± 0.39 a	6.57 ± 0.53 b	0.02
HB (g/dL)	15.55 ± 0.16 ab	11.02 ± 0.50 a	9.94 ± 0.90 b	0.02
HT (%)	44.75 ± 0.78 ab	31.85 ± 1.78 a	30.62 ± 2.42 b	0.02
PLT (10^3/mm)	496.50 ± 10.51 a	90.33 ± 2.68 a	259.00 ± 72.57 b	0.019
THT (%)	0.26 ± 0.00 a	0.07 ± 0.00 a	0.17 ± 0.04 b	0.018
MPV (µm^3)	5.25 ± 0.07 ab	7.32 ± 0.14 a	6.97 ± 0.32 b	0.02
WBC (10^3/mm)	1.45 ± 0.20 a	6.95 ± 0.45 a	4.96 ± 0.84 b	0.02
LYM (%)	86.55 ± 1.10 a	89.22 ± 1.34 b	89.77 ± 1.54 c	0.36
MON (%)	2.60 ± 0.31 a	3.58 ± 0.39 b	3.48 ± 0.49 c	0.36
NEU (%)	6.85 ± 0.07 a	3.20 ± 0.26 a	4.78 ± 0.90 b	0.03
EOS (%)	0.70 ± 0.00 ab	0.04 ± 0.02 a	0.16 ± 0.09 b	0.01
BAS (%)	3.30 ± 0.72 a	2.60 ± 0.39 b	1.43 ± 0.27 c	0.17
Biochemical				
ALT (U/L)	41.50 ± 2.01 a	112 ± 19.35 a	54.00 ± 5.48 b	0.003
AST (U/L)	155 ± 5.81 a	418.67 ± 32.06 a	258.00 ± 36.72 b	0.002
CREA (mg/L)	63.50 ± 3.03 a	47 ± 0.45 b	18.00 ± 0.45 a	0.001
UREA (mg/L)	7.36 ± 0.04 a	2.91 ± 0.08 a	3.57 ± 0.17 b	0.001

Notes: Values are mean ± SD; n = 6 for each group; RBC: Red blood cells; WBC: White blood cells; HGB: Hemoglobin; PLT: Platelets; MPV: Mean platelet volume; HT: Hematocrit; THT: Thrombocrite; LYM: Lymphocytes; MON: Monocytes; NEU: Neutrophil. EOS: Eosinophil; BAS: Basophil; AST: Aspartate Aminotransferase; ALT: Alanine aminotransferase; CREA: Creatinine. Overall differences among groups were assessed using the Kruskal-Wallis test, followed by Holm correction for multiple comparisons. Different letters (a, b, c) indicate significant differences between groups for a given parameter (Dunn post-hoc test, adjusted $p < 0.05$). Groups sharing the same letter are not significantly different.

3.2.5. Principal Component Analysis (PCA) of Hematological and Biochemical Parameters (Protective Assay)

To further explore the overall pattern of host responses, a principal component analysis (PCA) was performed on hematological and biochemical parameters in the protective model (**Figure 4(A)**). For hematological variables, the first two principal components (PC1 and PC2) explained 85.40% of the total variance

(PC1: 68.50%; PC2: 16.80%), indicating that the majority of variability in blood parameters among treatment groups could be captured by these components.

For biochemical parameters, PC1 and PC2 accounted for 86.60% of the variance (PC1: 60.30%; PC2: 26.30%), highlighting dominant effects on liver and kidney markers (**Figure 4(B)**). Together, these results suggest that the methanolic leaf extract of *Feretia apodanthera* primarily modulated biochemical parameters associated with organ function, with comparatively minor effects on hematological recovery. The PCA therefore provides a clear multivariate overview of the extract's protective potential, emphasizing its organ-protective and hepatoprotective effects in *Plasmodium berghei*-infected mice.

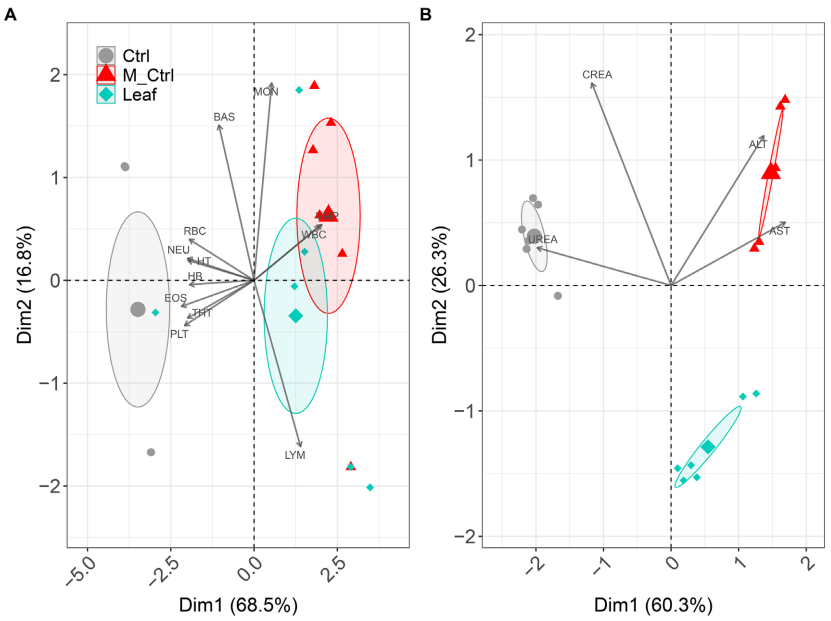

Figure 4. Principal component analysis (PCA) of hematological (A) and biochemical (B) profiles showing clear separation of treatments in the evaluation of the protective effect of *Feretia apodanthera* leaf methanolic extract. Note: n = 6 for each group; Ctrl: Control uninfected group; M_Ctrl: Infected control untreated; RBC: Red blood cells; WBC: White blood cells; HGB: Hemoglobin; PLT: Platelets; MPV: Mean platelet volume; HT: Hematocrit; THT: Thrombocrite; LYM: Lymphocytes; MON: Monocytes; NEU: Neutrophil. EOS: Eosinophil; BAS: Basophil; AST: Aspartate Aminotransferase; ALT: Alanine aminotransferase; CREA: Creatinine.

3.3. Acute toxicity of *Feretia apodanthera* Leaf Methanolic Extract

No mortality or clinical signs of toxicity attributable to the extract were observed in any of the treated mice during the 14-day monitoring period, indicating that the oral median lethal dose (LD_{50}) is greater than 2000 mg/kg body weight. Daily observations revealed no significant changes in general behavior, posture, food or water intake, or locomotor activity.

Oral administration of the extract had no significant overall effect on body weight across treatment groups ($p = 0.32$; **Figure 5(A)**). However, a significant effect of time was observed ($p < 0.001$), along with non-significant treatment × time inter-

action (p = 0.40), indicating similar temporal variation in body weight. The most notable changes occurred between Day 0 and Day 7, particularly in the control group, while minimal variation was observed between Day 7 and Day 14. Pairwise comparisons showed significant differences between groups at Day 0 only, suggesting the absence of treatment-related effects on body weight over time.

Macroscopic examination of major organs on Day 15 revealed no visible lesions or pathological alterations in treated animals. Organ weights were comparable across groups, with no significant differences observed for the heart (p = 0.35; Figure 5(B)), lungs (p = 0.06; Figure 5(C)), liver (p = 0.93; Figure 5(D)), spleen (p = 0.30; Figure 5(E)), or kidneys (p = 0.93; Figure 5(F)).

Overall, these findings indicate that the methanolic leaf extract of *Feretia apodanthera* does not induce acute toxicity at the tested dose and is well tolerated following single oral administration.

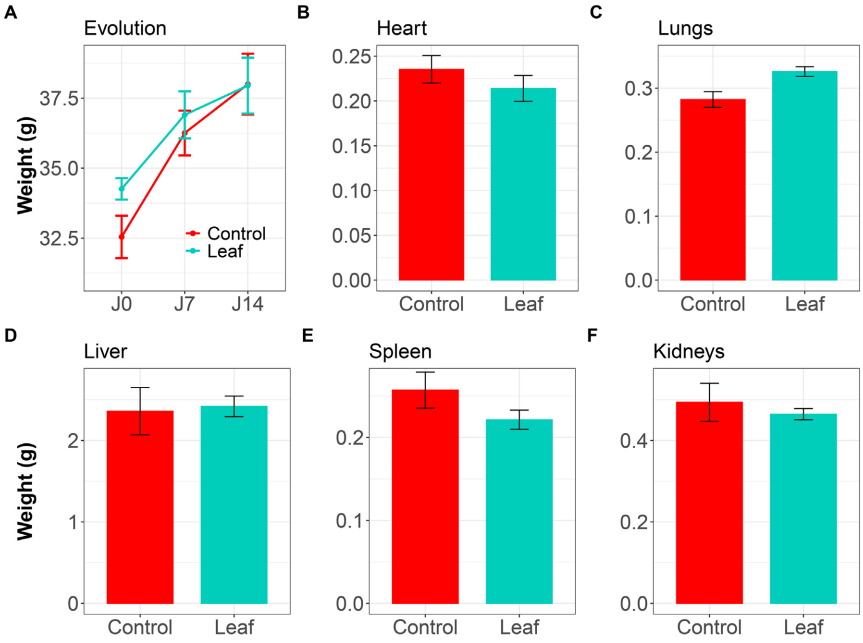

Figure 5. Effects of *Feretia apodanthera* leaf extracts on mice body weight and organ weights in acute toxicity test at a dosage of 2000 mg/kg body weight. (A) Changes in body weight from Day 0 to Day 14. (B)-(F) Organ weights measured during post-mortem examination on Day 15.

3.4. Subacute Toxicity of *Feretia apodanthera* Leaf Extract

3.4.1. Body Weight

The effects of repeated oral administration of the methanolic leaf extract of *Feretia apodanthera* over 28 consecutive days on body weight were evaluated in NMRI mice (Figure 6). The analysis revealed significant effects of treatment (p < 0.001), day (p < 0.0001), sex (p = 0.005), and dose (p = 0.03) on body weight. In addition, a significant treatment × day interaction was observed (p = 0.01), indicating that the impact of the extract on body weight varied over the course of the study. Post-hoc comparisons demonstrated that body weight changes over

time were largely driven by the control group, while mice receiving the leaf extract exhibited minimal variations, suggesting no adverse effects attributable to repeated oral administration at the tested doses. These results indicate that the methanolic leaf extract of *F. apodanthera* is well tolerated in NMRI mice under subacute exposure conditions.

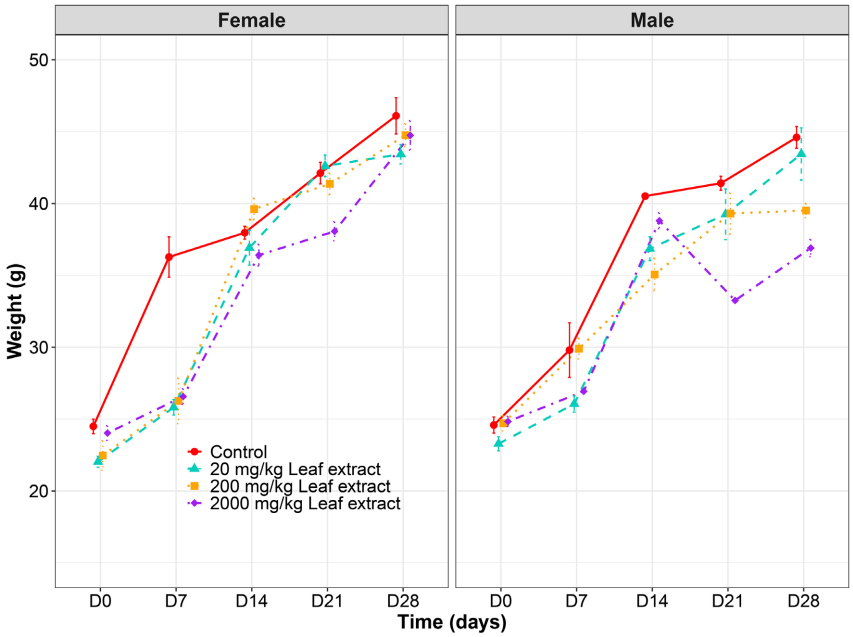

Figure 6. Effect of *Feretia apodanthera* methanolic leaf extract on body weight changes in mice during a 28-day subacute toxicity study (20, 200, and 2000 mg/kg).

3.4.2. Organ Weights

The effects of repeated oral administration of the methanolic leaf extract on organ weights were evaluated in NMRI mice after 28 days of treatment at doses of 20, 200, and 2000 mg/kg. Statistical analysis assessed the effects of treatment, dose, sex, and their interactions on the weights of the heart, lungs, liver, spleen, and kidneys (Figure 7). Macroscopic examination of these organs at the end of the study revealed no visible lesions or pathological alterations. In agreement with these observations, relative organ weights remained comparable across all treatment groups, further supporting the absence of subacute toxicity (Figure 7).

Heart

No significant effect of treatment ($p = 0.67$) or dose ($p = 0.65$) was observed. Sex had a small but significant effect ($p = 0.040$), whereas all interaction terms were not significant. Mean heart weights ranged from 0.16 to 0.19 g across groups, indicating no treatment-related hypertrophy or atrophy.

Lungs

Treatment had a marginally significant effect on lung weight ($p = 0.04$), while dose and sex were not significant. Mean lung weights varied between 0.23 g and 0.27 g, with slightly higher values observed in some extract-treated groups, though all values remained within normal physiological ranges.

Liver

Neither treatment ($p = 0.26$) nor dose ($p = 0.08$) significantly affected liver weights. No sex or interaction effects were observed. Liver weights ranged from 1.78 g to 2.11 g across groups.

Spleen

No significant effects of treatment, dose, sex, or their interactions were detected on spleen weight (all $p > 0.19$). Spleen weights ranged from 0.173 g to 0.21 g across all groups.

Kidneys

Kidney weights were not significantly affected by treatment ($p = 0.57$), dose ($p = 0.62$), sex, or interactions. Mean kidney weights ranged from 0.393 g to 0.427 g, consistent with normal values.

Overall, repeated oral administration of the leaf extract did not induce significant changes in major organ weights, supporting the absence of organ-specific toxicity under the experimental conditions.

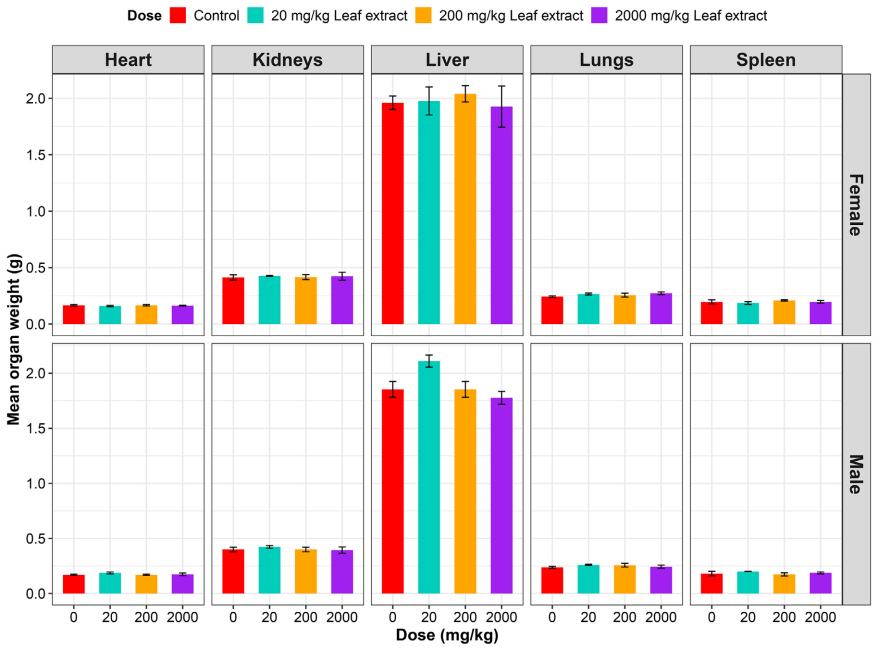

Figure 7. Effect of *Feretia apodanthera* leaf methanolic extract on mice organ weight in subacute toxicity test.

3.4.3. Hematological Parameters Following Subacute Oral Administration

The effects of repeated oral administration of the methanolic leaf extract of *Feretia apodanthera* on hematological parameters in NMRI mice are presented in **Table 3**. Overall, no statistically significant differences were observed between control and treated groups for most hematological parameters in both sexes ($p > 0.05$). In female mice, hemoglobin (HB), hematocrit (HT), red blood cells (RBC), white blood cells (WBC), platelets (PLT), and differential leukocyte counts (BAS, EOS, LYM, MON, NEU) remained comparable between treated and control groups.

Similarly, in male mice, no significant alterations were detected in erythrocytic parameters (HB, HT, RBC), leukocyte counts (WBC, LYM, MON, NEU), or platelet indices (PLT, THT, VMP) following extract administration ($p > 0.05$). Although slight variations were observed in some parameters, such as increased RBC and WBC levels in treated animals, these changes were not statistically significant and remained within physiological ranges. Overall, these findings indicate that repeated oral administration of *F. apodanthera* leaf extract does not induce hematological toxicity in NMRI mice, regardless of sex.

Table 3. Effect of *Feretia apodanthera* leaf methanolic extract on hematological parameters of mice in subacute toxicity tested at 2000 mg/kg/bwt.

Parameters	Sex	Control	Leaf extract	p-value
RBC (10^6/mm)	Male	7.84 ± 0.54 a	9.61 ± 0.62 a	0.37
	Female	9.54 ± 0.07 a	10.28 ± 0.32 a	0.20
HB (g/dL)	Male	15.12 ± 0.23 a	15.85 ± 1.02 a	0.57
	Female	15.37 ± 0.61 a	14.85 ± 0.67 a	0.64
HT (%)	Male	43.93 ± 0.73 a	47.87 ± 2.87 a	0.56
	Female	47.00 ± 0.87 a	49.13 ± 0.68 a	0.47
PLT (10^3/mm)	Male	503.67±15.67 a	587.67±59.74 a	0.56
	Female	652.67±73.57 a	657.67±48.25 a	0.83
THT (%)	Male	0.27 ± 0.01 a	0.47 ± 0.03 a	0.20
	Female	0.66 ± 0.05 a	0.29 ± 0.07 a	0.20
MPV (µm^3)	Male	6.30 ± 0.43 a	8.18 ± 0.86 a	0.47
	Female	7.90 ± 0.31 a	5.85 ± 0.91 a	0.38
WBC (10^3/mm)	Male	1.78 ± 0.32 a	4.95 ± 1.55 a	0.36
	Female	2.57 ± 0.32 a	5.28 ± 0.62 a	0.19
LYM (%)	Male	84.23 ± 2.11 a	87.50 ± 1.16 a	0.36
	Female	90.10 ± 0.72 a	89.87 ± 3.16 a	0.68
MON (%)	Male	4.07 ± 1.02 a	4.90 ± 0.53 a	0.56
	Female	4.63 ± 0.75 a	5.63 ± 0.30 a	0.47
NEU (%)	Male	9.43 ± 0.17 a	4.50 ± 1.15 a	0.18
	Female	7.63 ± 0.58 a	7.17 ± 0.42 a	0.71
EOS (%)	Male	0.30 ± 0.06 a	0.33 ± 0.12 a	0.66
	Female	0.70 ± 0.06 a	0.67 ± 0.12 a	0.72
BAS (%)	Male	1.17 ± 0.17 a	2.37 ± 0.57 a	0.20
	Female	4.17 ± 1.27 a	1.93 ± 0.23 a	0.47

Notes: Values are mean ± SE; n = 6 for each group; extreme values for each parameter are highlighted in bold. RBC: Red blood cells. WBC: White blood cells. HB: Hemoglobin; PLT: Platelets; MPV: Mean platelet volume; HT: Hematocrit; LYM: Lymphocytes; MON: Monocytes; NEU: Neutrophil; EOS: Eosinophil; BAS: Basophil. Differences between groups within each sex were assessed using the Wilcoxon rank-sum test. Distinct letters (a, b) indicate significant differences between treatments for a given parameter within the same sex ($p < 0.05$), whereas shared letters indicate no significant difference.

3.4.4. Biochemical Parameters Following Subacute Oral Administration

The biochemical profile of mice treated with the methanolic leaf extract of *Feretia apodanthera* is summarized in Table 4. Most biochemical parameters—including alanine aminotransferase (ALT), aspartate aminotransferase (AST), total and direct bilirubin, creatinine (CREA), and triglycerides (TG)—did not show significant differences between control and treated groups in both sexes ($p > 0.05$), indicating stable hepatic and lipid profiles. AST levels, however, were significantly reduced in both males (155.67 ± 11.39 → 74.67 ± 6.36 U/L, $p = 0.02$) and females (165.67 ± 11.39 → 66.00 ± 6.43 U/L, $p = 0.01$), suggesting a potential protective effect rather than toxicity. Urea levels also showed significant, but moderate, changes ($p = 0.0302$): a slight increase in females (6.49 ± 0.45 vs 5.92 ± 0.18 mmol/L) and a decrease in males (7.44 ± 0.66 vs 8.92 ± 0.18 mmol/L), all remaining within physiological ranges. Bilirubin (total and direct), ALT, and creatinine levels remained unchanged, confirming the absence of hepatotoxicity and nephrotoxicity after subacute administration of the extract. Overall, these results support the good biochemical tolerance of the methanolic leaf extract in mice.

Table 4. Effect of *Feretia apodanthera* leaf methanolic extract on biochemical parameters of mice in subacute toxicity tested at 2000 mg/kg/bwt.

Parameters	Sex	Control	Leaf extract	p-value
ALT (U/L)	Male	95.00 ± 3.46 a	82.00 ± 5.29 a	0.28
	Female	108.00 ± 4.73 a	74.67 ± 10.73 a	0.28
AST (U/L)	Male	155.67 ± 11.39 a	74.67 ± 6.36 b	0.02
	Female	165.67 ± 11.39 a	66.00 ± 6.43 b	0.01
CREA (mg/L)	Male	155.00 ± 25.54 a	78.00 ± 6.81 a	0.17
	Female	128.33 ± 21.53 a	61.33 ± 9.53 a	0.28
UREA (mg/L)	Male	8.92 ± 0.18 a	7.44 ± 0.66 a	0.29
	Female	5.92 ± 0.18 a	6.49 ± 0.45 a	0.83
TG	Male	2.60 ± 0.19 a	2.12 ± 0.13 a	0.28
	Female	3.49 ± 0.38 a	2.91 ± 0.35 a	0.83
BD	Male	6.59 ± 0.58 a	3.22 ± 0.14 b	0.02
	Female	5.62 ± 0.52 a	4.37 ± 0.59 a	0.76
BT	Male	6.46 ± 0.60 a	2.46 ± 0.04 b	0.01
	Female	5.60 ± 0.64 a	4.70 ± 0.32 a	0.83

Notes: Values are mean ± SE; n = 6 for each group; AST: Aspartate Aminotransferase; ALT: Alanine aminotransferase; CREA: Creatinine; TG: Triglycerides; BT: Bilirubin total and BD: Bilirubin direct. Differences between groups within each sex were assessed using the Wilcoxon rank-sum test. Distinct letters (a, b) indicate significant differences between treatments for a given parameter within the same sex ($p < 0.05$), whereas shared letters indicate no significant difference.

3.4.5. Histopathological Analysis

Histological examination of major organs (heart, lungs, liver, spleen, and kidneys)

was performed to complement macroscopic and organ weight assessments and to detect potential microscopic alterations. Analysis of hematoxylin-eosin stained sections revealed no significant histopathological changes in mice treated with the methanolic leaf extract of *Feretia apodanthera* compared to the control group (**Figure 8**). The normal architecture of all examined organs was preserved. Specifically, myocardial fibers in the heart (**Figure 8(A)**) were intact, pulmonary alveoli showed normal structure (**Figure 8(B)**), hepatic lobular organization was maintained (**Figure 8(C)**), splenic architecture (red and white pulp) remained well defined (**Figure 8(E)**), and renal glomeruli and tubules exhibited normal morphology (**Figure 8(D)**). No evidence of cellular degeneration, necrosis, inflammatory infiltration, or vascular congestion was observed.

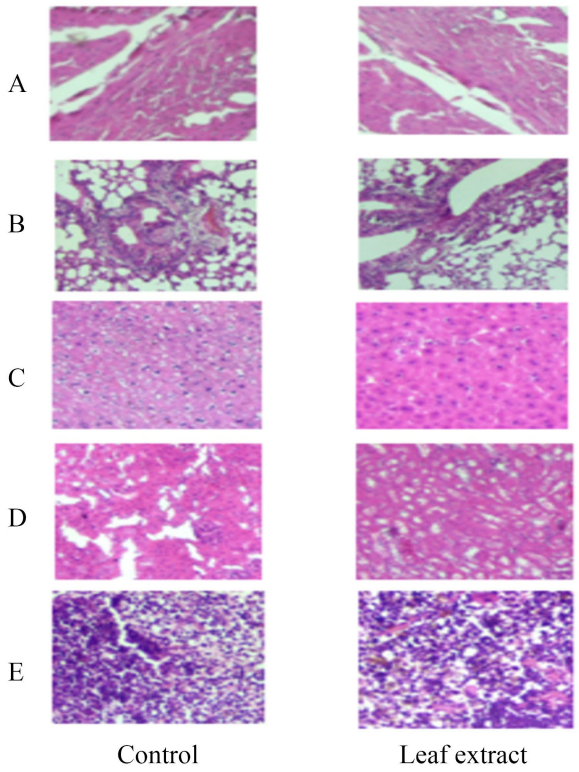

Figure 8. Histopathological evaluation of major organs ((A) Heart, (B) Lungs, (C) Liver, (D) Spleen, and (E) Kidneys) in control and *Feretia apodanthera* leaf extract-treated mice (2000 mg/kg), showing preserved tissue architecture.

4. Discussion

Malaria infection is associated with a progressive deterioration of the host's general condition, resulting from the complex interplay between parasite burden, immune and inflammatory responses, and visceral damage, particularly affecting the liver and spleen, as well as hematological and biochemical parameters [5] [40]. In this context, the combined evaluation of both restorative and protective effects of the methanolic leaf extract of *Feretia apodanthera* provides valuable insight into

their capacity to modulate malaria-induced pathophysiological alterations beyond direct antiplasmodial activity.

The leaf extract of *Feretia apodanthera* significantly reduced parasitemia, decreasing from 38.3 ± 2.1% in untreated infected mice to 16.0% ± 5.1% in the restorative model (chemosuppression ≈ 52.9%), and from 27.6% ± 1.6% to 12.0% ± 0.6% in the protective model (chemosuppression ≈ 58.2%). According to the classification proposed by Rosanaivo's and Deharo, this level of suppression reflects good antimalarial activity [41] [42]. These findings are consistent with previous reports on *F. apodanthera*, which have demonstrated comparable reductions in parasitemia under both therapeutic and prophylactic conditions [22].

These antiparasitic effects are particularly relevant given that parasitemia is a key driver of malaria-associated physiological alterations. Among these, body weight loss is a common clinical feature observed during malaria infection in both experimental models and humans. In murine models, it is closely associated with increasing parasitemia and disease severity [43]. Therefore, an effective antimalarial agent should not only reduce parasite burden but also mitigate associated physiological disturbances, including weight loss [44]. In the present study, the restorative model clearly illustrates the impact of *Plasmodium berghei* infection on host metabolism. While uninfected mice exhibited a steady and expected weight gain, infected untreated animals showed a biphasic pattern, with an initial increase followed by a significant decline, consistent with previous reports [11] [45]. This transient increase may reflect early compensatory mechanisms or fluid retention, whereas the subsequent decrease likely results from anorexia, increased catabolism, and systemic inflammatory responses [45] [46]. Notably, mice treated with the leaf extract of *Feretia apodanthera* followed a similar trajectory, indicating that the extract did not effectively prevent infection-induced weight loss. A comparable pattern was observed in the protective model. Although pre-treatment slightly delayed the onset of weight decline, it did not ultimately prevent it, and treated animals maintained lower body weights compared to controls throughout the experiment. This suggests that the extract does not exert a significant prophylactic effect on the metabolic disturbances associated with early infection. The persistence of weight loss despite leaf treatment highlights the difficulty of modulating systemic energy imbalance during malaria, which is driven not only by parasite burden but also by host immune and inflammatory responses. When both models are considered together, a consistent picture emerges: malaria infection induces a progressive metabolic disruption leading to weight loss, and the methanolic leaf extract of *F. apodanthera* does not significantly alter this trajectory. This may be explained by the nature of the bioactive compounds present in the extract [22]. Although rich in polyphenols, flavonoids, and tannins—compounds known for their antioxidant and anti-inflammatory properties [29] [30] [32]—these metabolites are not typically associated with appetite stimulation or metabolic recovery [47]. Overall, these findings indicate that the leaf extract of *F. apodanthera* has a limited capacity to counteract malaria-induced weight loss,

whether administered before or after infection, suggesting that its effects do not extend to the regulation of systemic metabolic alterations induced by the disease.

Following the evaluation of body weight changes, organ weights were analyzed to further assess the impact of infection and the potential protective effects of the leaf extract of *Feretia apodanthera*, particularly at the hepatic level, a key target during malaria infection. Overall, untreated infected mice exhibited marked enlargement of the liver and spleen in both models, reflecting the systemic impact of *Plasmodium* infection on organs involved in metabolism, detoxification, and immune response [48]. In the restorative model, the significant increase in liver weight observed in infected untreated mice confirms the development of hepatomegaly, a common feature of malaria associated with hepatic congestion, inflammation, and parasite sequestration [49]. Treatment with the leaf extract led to a moderate but significant reduction in liver weight, suggesting a partial attenuation of infection-induced hepatic alterations. In the protective model, similar but less pronounced changes were observed, with pre-treatment resulting in intermediate liver weights. This indicates that the extract can limit the extent of hepatic enlargement, although it does not fully prevent it. Taken together, these results point to a more pronounced effect under curative conditions than in a preventive context. A comparable pattern was noted for kidney weights. Infection induced a significant increase in kidney mass in both models, consistent with renal involvement during malaria [50] [51]. In the restorative model, treatment with the extract restored kidney weight to values comparable to those of the control group, suggesting a near-complete normalization. In the protective model, the effect was more moderate, with only partial attenuation of kidney enlargement. These findings again support a stronger therapeutic rather than prophylactic effect of the leaf extract. In contrast, spleen weight was markedly increased in infected mice in both models, reflecting splenomegaly associated with immune activation and erythrocyte clearance [48] [52]. Although treatment with the extract resulted in a slight reduction in spleen weight, this change remained limited and did not significantly differ from untreated infected animals. This indicates that the extract has a weaker influence on splenic alterations compared to its effects on the liver and kidneys. The differential effects observed across organs suggest an organ-specific activity of the methanolic leaf extract of *Feretia apodanthera*. This pattern can be related to its phytochemical composition, particularly its richness in polyphenolic compounds such as flavonoids and tannins [22] [32]. These metabolites are known for their antioxidant and anti-inflammatory properties, which may contribute to limiting tissue alterations induced by malaria, especially in organs with high metabolic activity such as the liver and kidneys [30] [32]. Their ability to reduce oxidative stress and modulate inflammatory processes may explain the partial normalization of organ weights observed in treated animals. In contrast, splenomegaly is primarily driven by intense immune activation and increased clearance of parasitized erythrocytes, processes that may be less responsive to the mechanisms of action of these compounds. As a result, the extract appears less effective in

modulating spleen enlargement [53]. Overall, these findings indicate that the methanolic leaf extract of *Feretia apodanthera* exerts selective protective effects against malaria-induced organ alterations, with greater efficacy on metabolically active organs than on immune-related responses. This supports the hypothesis that its biological activity is mainly mediated through antioxidant and cytoprotective mechanisms.

The hematological and biochemical alterations observed in both restorative and protective models further confirm the systemic impact of *Plasmodium berghei* infection and provide insight into the pharmacological profile of the *Feretia apodanthera* leaf extract [54]. In the restorative model, infection induced pronounced hematological disturbances, mainly characterized by a marked decrease in erythrocyte parameters (RBC, hemoglobin, and hematocrit), indicative of severe anemia. These alterations are consistent with malaria pathophysiology, involving hemolysis of infected erythrocytes, impaired erythropoiesis, and increased splenic clearance [7] [8]. Treatment with the leaf extract resulted in only a slight improvement in these parameters, which remained significantly below normal values. Similarly, infection triggered a strong leukocytosis associated with inflammatory and immune responses, partially reduced by treatment, suggesting a moderate modulation of inflammation [6] [55]. However, platelet parameters were not significantly improved [6]. In the protective model, pre-treatment did not prevent the onset of anemia, as erythrocyte parameters remained altered. Nevertheless, a partial preservation of platelet levels and attenuation of leukocyte elevation were observed, indicating a limited protective effect on certain hematological components. Overall, these findings suggest that the extract exerts only modest effects on malaria-induced hematological disorders, both in curative and preventive conditions.

In contrast, biochemical parameters revealed more pronounced effects of the extract, particularly regarding liver function. In the restorative model, infection led to a marked elevation of transaminases (ALT and AST), reflecting hepatocellular damage [56]. Leaf extract treatment significantly reduced these enzyme levels, although not to baseline values, indicating a partial attenuation of liver injury and hepatoprotective potential [57]. In the protective model, pre-treatment also limited the increase in transaminases, suggesting a protective effect against infection-induced hepatic alterations. Renal parameters showed less pronounced changes overall. While creatinine levels remained relatively stable in the restorative model, variations observed in the protective model did not indicate severe renal impairment. Urea levels also fluctuated but remained within a relatively narrow range, supporting the absence of major kidney dysfunction [58]. These results reveal a differential effect of the methanolic leaf extract of *Feretia apodanthera* on hematological and biochemical parameters. While its impact on hematological alterations—particularly anemia—remains limited, its effects on biochemical markers, especially liver enzymes, are more substantial. This suggests that the extract is more effective in mitigating infection-induced functional disturbances at the bio-

chemical level than in restoring hematological balance. Such a profile supports the hypothesis that its pharmacological activity is primarily related to the modulation of metabolic and cellular stress rather than direct correction of hematological deficits.

While the methanolic leaf extract of *Feretia apodanthera* demonstrated moderate restorative and protective effects against malaria-associated physiological disturbances, evaluating its safety profile remains essential to support its therapeutic potential. To this end, both acute and subacute toxicity studies were conducted in NMRI mice. In the acute toxicity assay, a single oral dose of 2000 mg/kg body weight resulted in no mortality or observable signs of toxicity during the 14-day observation period. Behavioral patterns, locomotor activity, food and water intake, and general appearance remained normal. These findings indicate that the median lethal dose (LD_{50}) exceeds 2000 mg/kg, classifying the extract as practically non-toxic according to OSHA and OECD guidelines [37] [59]. Subacute toxicity was assessed following daily oral administration over 28 days at doses of 20, 200, and 2000 mg/kg. Across all tested doses, no significant changes were observed in body weight evolution or relative organ weights (liver, kidneys, spleen, heart, and lungs) compared to control animals, indicating the absence of dose-dependent systemic toxicity. Based on these results, hematological and biochemical evaluations were subsequently performed at the highest dose (2000 mg/kg), in line with standard toxicological approaches focusing on the worst-case exposure scenario. Hematological analysis at 2000 mg/kg revealed no evidence of adverse effects associated with the administration of the leaf extract. Erythrocyte-related parameters, including red blood cell count, hemoglobin, and hematocrit, remained comparable between treated and control groups in both sexes, with no statistically significant differences observed. Although slight increases in erythrocyte indices and hematocrit were noted in treated animals, these variations were not significant and do not indicate altered erythropoiesis or anemia [60]. Platelet indices were also unaffected, suggesting preserved thrombopoietic function. Similarly, total and differential leukocyte counts remained stable. A non-significant increase in white blood cell counts was observed in treated animals, but values remained within physiological ranges and were not associated with signs of inflammation or immune dysregulation [61] [62]. Overall, these results indicate the absence of hematological toxicity. Biochemical analyses further supported a favorable safety profile. Liver function markers showed no evidence of hepatotoxicity, as alanine aminotransferase levels were not significantly altered in either sex. In contrast, aspartate aminotransferase levels were significantly reduced in treated animals, suggesting a potential hepatoprotective effect [63]. This interpretation is reinforced by the observed decrease in total and direct bilirubin in males, indicating improved hepatic function rather than impairment [64]. Renal function remained unaffected, as creatinine and urea levels showed no significant differences between treated and control groups, despite a non-significant downward trend in creatinine values. Likewise, metabolic parameters such as triglycerides were not

significantly modified. These results indicate that the extract does not induce biochemical alterations associated with hepatic or renal toxicity [61]-[64]. Histopathological examination of major organs further confirmed these observations, revealing no evidence of cellular degeneration, necrosis, inflammation, or structural alterations [65].

This safety profile is consistent with previously reported data on *F. apodanthera*. Boni et al. previously demonstrated that the acute toxicity of a methanolic leaf extract exceeded 5000 mg/kg [22]. Similarly, Taiwe et al. reported that aqueous extracts and alkaloid fractions from the stem bark produced no mortality or clinical signs of toxicity in mice, even at very high doses [66]. In the same line, Njimoh et al. confirmed the acute safety of these extracts in albino rats, with no mortality, behavioral alterations, or body weight changes observed at high oral doses [33]. More recently, Silué et al. reported that a lyophilized aqueous leaf infusion administered at 2000 mg/kg induced no toxic effects in female rats, with no alterations in body weight, food and water intake, hematological or biochemical parameters, or relative organ weights. In a subsequent subacute study, repeated daily administration over 28 days at increasing doses also produced no mortality or systemic toxicity, with only transient and mild somnolence reported, without physiological consequences [34].

Taken together, these findings demonstrate that the methanolic leaf extract of *Feretia apodanthera* is well tolerated under both acute and subacute conditions, with a No Observed Adverse Effect Level exceeding the highest tested dose (>2000 mg/kg/day). In addition, the observed reductions in liver injury markers suggest that, beyond its safety, the extract may exert protective effects on hepatic function. Overall, these results consistently support the low toxicity and wide safety margin of *F. apodanthera* leaf extract. This favorable toxicological profile reinforces the relevance of the present findings and supports its potential use as a safe therapeutic or prophylactic adjunct in malaria management, while also justifying further pharmacological investigations.

5. Conclusion

The methanolic leaf extract of *Feretia apodanthera* is well tolerated and demonstrates a high safety profile, with an acute oral LD_{50} and subacute NOAEL both exceeding 2000 mg/kg. While the extract showed limited effects on body weight and malaria-induced hematological disturbances, it significantly mitigated liver enzyme elevations and attenuated hepato- and renomegaly, particularly in the restorative model. These results suggest that *F. apodanthera* possesses hepatoprotective and organ-protective properties, supporting its potential use as a safe adjunct in malaria management. Further studies are warranted to elucidate its mechanisms of action and optimize its therapeutic application.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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