**NEPHROPROTECTIVE AND ANTIMALARIAL EFFECTS OF *AZADIRACHTA INDICA* AND *CYMBOPOGON CITRATUS* ETHANOLIC LEAF EXTRACTS IN *PLASMODIUM BERGHEI*-INFECTED MICE**

**Abstract**  
Malaria remains a major public health burden, especially in developing countries, with rising resistance to conventional antimalarial drugs. This study investigated the nephroprotective and antimalarial effects of ethanolic extracts of Azadirachta indica (EAI) and Cymbopogon citratus (ECC) in adult male Wistar mice infected with Plasmodium berghei. Forty mice were randomly assigned into eight groups (n = 5). All groups were induced with malaria except Group A, which served as the normal control. Group B was infected but untreated. Groups C and D received 100 mg/kg and 500 mg/kg of EAI, respectively. Groups E and F received 100 mg/kg and 500 mg/kg of ECC. Group G received 20 mg/kg/day of standard drug (Lonart®), while Group H received a combination of 200 mg/kg each of EAI and ECC. Treatments were administered orally for 14 days. Body weight increased significantly in Group A but showed no significant change in other groups. Relative kidney weights and urea levels varied insignificantly. Uric acid and creatinine levels significantly decreased in all treated groups compared to the untreated group. Parasitemia levels reduced significantly by days 7 and 14 in treated groups. Histological analysis showed preserved kidney structures with mild inflammation. Findings support the nephroprotective and antimalarial potential of EAI and ECC.

Keywords: ***Azadirachta indica***, ***Cymbopogon citratus***, ***Plasmodium berghei***, **Nephroprotection**

1. **INTRODUCTION**

Malaria remains a significant global health challenge, particularly in tropical and developing regions where it continues to cause substantial morbidity and mortality. It is a mosquito-borne infectious disease caused by protozoan parasites of the *Plasmodium* genus, transmitted through the bites of infected female *Anopheles* mosquitoes (WHO, 2018). Among the five *Plasmodium* species known to infect humans (*P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae*, and *P. knowlesi*), *P. falciparum* is the most lethal and prevalent in sub-Saharan Africa (Ajeka *et al*., 2021; Singh, 2011). For experimental modeling in rodents, *Plasmodium berghei*, a rodent parasite with similar characteristics to *P. falciparum*, is widely used (Ogbu *et al*., 2014).

Despite being both preventable and curable, malaria remains endemic in regions with limited healthcare access, poor socioeconomic conditions, and favorable climatic conditions for mosquito breeding. Nigeria alone accounts for more than a quarter of the global malaria burden, with 97% of its population at risk (NPC, 2014). The disease is responsible for approximately 60% of outpatient visits, 30% of hospital admissions, and 11% of maternal deaths (WHO, 2020). If not promptly treated, malaria can lead to complications such as anemia, jaundice, kidney failure, seizures, coma, and death (WHO, 2018).

The World Health Organization currently recommends artemisinin-based combination therapies (ACTs) such as artemether/lumefantrine and artesunate/amodiaquine for malaria treatment (WHO, 2018). However, emerging resistance to these drugs, along with high treatment costs and limited availability, continues to hinder effective malaria control (Zirihi *et al*., 2005; Suresh & Haldar, 2018).

Given these challenges, there has been renewed interest in the use of medicinal plants with antimalarial properties, particularly in rural communities where traditional medicine is accessible and affordable. Several plants used in Nigerian ethnomedicine, including *Artemisia annua*, *Carica papaya*, *Azadirachta indica* (neem), and *Cymbopogon citratus* (lemongrass), have shown promising antiplasmodial activity in both in vitro and in vivo studies (Odugbemi *et al*., 2007; Titanji *et al*., 2008; Nnyaha *et al*., 2021).

While many studies have assessed the individual efficacy of these plants, there remains a gap in understanding their synergistic effects when used in combination. This study seeks to evaluate the separate and combined effects of *Azadirachta indica* and *Cymbopogon citratus* leaf extracts on kidney function and histology in *Plasmodium berghei*-infected mice, providing scientific validation for traditional practices and contributing to the development of novel, plant-based antimalarial therapies.

**2.0 Methodology**

**2.1 Plant Materials Collection and Extract Preparation**

Fresh leaves of *Azadirachta indica* (Neem) and *Cymbopogon citratus* (Lemongrass) were collected from authenticated botanical sources and identified at the Department of Botany, Nnamdi Azikiwe University. The leaves were thoroughly washed, shed-dried and then pulverized into fine powder using a mechanical grinder. Each powdered plant material (250g) was subjected to cold maceration in 70% ethanol for 72 hours with intermittent shaking. The resulting extracts were filtered using Whatman No. 1 filter paper and concentrated using a rotary evaporator under reduced pressure at 40°C. The crude extracts were stored in airtight containers at 4°C until further use.

**2.2 Toxicity Tests for Azadirachta indica (Neem) and Cymbopogon citratus (Lemongrass)**

The acute toxicity study of the aqueous leaf extracts of Azadirachta indica and Cymbopogon citratus was conducted using the method described by Lorke (1983). No mortality or observable signs of toxicity were recorded in any of the test animals up to the maximum administered dose of 5000 mg/kg body weight for both extracts. This indicates that the LD₅₀ of each extract is greater than 5000 mg/kg, classifying them as practically non-toxic according to the Globally Harmonized System of Classification and Labelling of Chemicals (United Nations, 2019).

**2.3 Procurement and Housing of Experimental Animals**

Adult male Swiss albino mice weighing between 20 and 22 g were obtained from the Animal House of the College of Health Sciences and Technology, Nnamdi Azikiwe University. The mice were housed in standard laboratory cages under controlled conditions of 12-hour light and dark cycles and were allowed free access to standard pellet diet and water. Animals were acclimatized for two weeks before the commencement of the experiment. Ethical approval for this study was obtained from the Ethical Committee of the Department of Anatomy, Faculty of Basic Medical Sciences, Nnamdi Azikiwe University, Nnewi Campus (Ref No: ANA/EA/UG/AS/07/08/2024). All procedures involving animals were conducted in accordance with the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Sciences and published by the National Institutes of Health (NRC, 2011).

**2.4 Parasite Inoculation**

Chloroquine-sensitive strain of *Plasmodium berghei* (ANKA strain) was obtained from the Nigerian Institute for Medical Research (NIMR), Lagos. Blood containing approximately 30% parasitemia was collected from a donor mouse by cardiac puncture and diluted with normal saline to achieve a final concentration of 1 × 10⁷ parasitized erythrocytes in 0.2 mL. Each experimental mouse was inoculated intraperitoneally with this suspension (Fidock *et al.,* 2004).

**2.5 Experimental Design**

A total of thirty adult Swiss albino male mice weighing between 20-22 grams were randomly assigned into eight groups (A–H), with five mice per group. Group A served as the normal (negative) control and received neither malaria infection nor treatment. All other groups (B–H) were infected with *Plasmodium berghei*. Group B served as the positive (malaria-infected untreated) control. Group C was treated with 100 mg/kg/day of *Azadirachta indica extract*. Group D received 400 mg/kg/day of *Azadirachta indica* extract. Group E was treated with 100 mg/kg/day of *Cymbopogon citratus* extract, while Group F received 400 mg/kg/day of *Cymbopogon citratus* extract. Group G was treated with a standard antimalarial drug (Lonart®), and Group H received a combined treatment of 200 mg/kg each of *Azadirachta indica* and *Cymbopogon citratus* extracts.

All treatments were administered orally once daily for 14 consecutive days, beginning 72 hours’ post-infection. Dose selections were based on prior toxicity and efficacy studies (WHO, 2022; CDC, 2023; Ofoego *et al*., 2017). The standard drug, Lonart®—a fixed-dose combination of artemether and lumefantrine in a 1:6 ratio—was procured from Syleon-C Pharm. Nig. Ltd, Nnewi, and administered orally at 20 mg/kg/day of artemether and 120 mg/kg/day of lumefantrine, dissolved in distilled water.

**2.6 Assessment of Antimalarial Activity**

Parasitemia levels were assessed daily using thin blood smears made from tail vein blood. The smears were fixed with methanol, stained with Giemsa, and examined under a light microscope at 100× magnification (Chessbrough, 2006; Schmidt, 2013). Percentage parasitemia was determined using the formula:

Percentage Parasitemia = 100

**2.7 Termination of Experiment and Evaluation of Nephroprotective Effects**

On days 1, 7, and 14 post-inoculation, blood samples were collected via cardiac puncture, allowed to clot at room temperature, and subsequently centrifuged to obtain serum. These serum samples were used to determine percentage parasitemia levels. Twenty-four hours after the final treatment administration, animals were anesthetized using chloroform, and blood was collected immediately via cardiac puncture. The collected blood was allowed to clot, and serum was separated by centrifugation and stored under appropriate conditions for subsequent biochemical analysis. Serum levels of urea and creatinine were determined using commercially available diagnostic kits (Randox Laboratories, UK) following the manufacturer’s instructions. Kidneys were excised, rinsed in normal saline, and fixed in 10% buffered formalin for histopathological evaluation.

**2.8 Histopathological Examination**

Formalin-fixed kidney tissues were dehydrated in graded alcohol, cleared in xylene, and embedded in paraffin wax. Sections of 5 µm thickness were cut using a rotary microtome and stained with hematoxylin and eosin. Histological slides were examined under a light microscope for pathological changes such as glomerular distortion, tubular necrosis, and inflammatory cell infiltration.

**2.9 Statistical Analysis**

All data were expressed as mean ± standard error of the mean (SEM). Statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by Tukey’s post hoc test for multiple comparisons. Differences were considered statistically significant at p < 0.05.

**3.0 RESULTS**

**3.1 Body weight Observation**

Table 1.0 summarizes body weight changes across eight experimental groups. Body weight changes across groups were generally not statistically significant, except in the positive control group (Group A), which showed a significant increase (P = 0.02). All other groups, including treated and malaria control groups, exhibited non-significant differences in body weight before and after treatment.

Table 1.0 Effect of ethanolic extract of *Cymbopogon citratus* and *Azadirachta indica* on body weight following *plasmodium berghei* induced toxicity.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **Initial weight (g)** | **Final weight (g)** | **BWC** | **P-value** | **T-value** |
|  | **MEAN±SEM** | **MEAN±SEM** |  |  |  |
| Group A (Negative control) | 22.46±0.96 | 28.56±0.31 | 6.10 | 0.02**a** | -6.77 |
| Group B (Malaria control) | 28.67±1.14 | 32.67±0.88 | 4.00 | 0.13**b** | -2.40 |
| Group C (Malaria + 100mg/kg of EAI) | 27.90±2.30 | 34.00±1.52 | 6.10 | 0.22**b** | -1.74 |
| Group D (Malaria + 500mg/kg of EAI) | 29.17±1.03 | 34.00±1.73 | 4.83 | 0.22**b** | -1.79 |
| Group E (Malaria + 100mg/kg of ECC) | 28.40±1.83 | 31.93±1.72 | 3.53 | 0.21**b** | -1.85 |
| Group F (malaria + 500mg/kg of ECC) | 28.70±1.47 | 28.87±2.00 | 0.17 | 0.91 **b** | -0.13 |
| Group G (malaria + Standard Drug) | 30.97±1.46 | 30.88±0.76 | -0.09 | 0.92 **b** | 0.11 |
| Group H (Malaria + 500mg/kg of EAI +ECC) | 29.83±1.02 | 28.31±2.04 | -1.53 | 0.52**b** | 0.78 |

Data was analyzed using T-test, and values considered significant at *p<0.05*. SEM: Standard error of mean. BWC: Bodyweight change, EAI: ethanolic leaf extract of *Azadirachta indica*, ECC: ethanolic leaf extract of *Cymbopogon citratus* (**a**= significant, **b**= not significant when initial weight was compared with final weight).

**3.2 Relative Organ (Kidney) weight Observation**

The relative kidney weights across all groups showed no statistically significant differences, as indicated by the F-ratio of 0.32. Values remained comparable among treated and control groups as shown in table 2.0.

Table 2.0 Effect of ethanolic extract of *Cymbopogon citratus* and *Azadirachta indica* on relative kidney weight following *plasmodium berghei* induced toxicity.

|  |  |
| --- | --- |
|  | **Relative kidney weight (g)** |
|  | **MEAN±SEM** |
| Group A (Negative control) | 0.72±0.14 **b** |
| Group B (Malaria control) | 0.77±0.01 |
| Group C (Malaria + 100mg/kg of EAI) | 0.79±0.07 **b** |
| Group D (Malaria + 500mg/kg of EAI) | 0.71±0.04 **b** |
| Group E (Malaria + 100mg/kg of ECC) | 0.82±0.01 **b** |
| Group F (malaria + 500mg/kg of ECC) | 0.72±0.02 **b** |
| Group G (malaria + Standard Drug) | 0.74±0.02 **b** |
| Group H (Malaria + 500mg/kg of EAI +ECC) | 0.75±0.05 **b** |
| **F-ratio** | 0.32 |

Data was analyzed using ANOVA followed by post Hoc LSD, and values considered significant at *p<0.05*. SEM: Standard error of mean. EAI: ethanolic leaf extract of *Azadirachta indica*, ECC: ethanolic leaf extract of *Cymbopogon citratus* (**a**= significant, **b**= not significant when compared with the positive control – Group B).

**3.3 Effect of Ethanolic Extracts of Cymbopogon citratus and Azadirachta indica on Urea, Creatinine, and Uric Acid Levels in Plasmodium berghei-Infected Mice**

Table 3.0 shows the levels of urea, uric acid, and creatinine varied across treatment groups following Plasmodium berghei infection. The F-ratios for urea (4.34), uric acid (5.90), and creatinine (4.96) indicate intergroup differences, with statistical significance observed in certain treatment comparisons as denoted by superscripts.

Table 3.0 effect of ethanolic extract of *Cymbopogon citratus* and *Azadirachta indica* on urea, creatinine, and uric acid level following *plasmodium berghei* induced toxicity.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Urea level (mg/dl)** | **Uric acid level (mg/dl)** | **Creatinine level (mg/dl)** |
|  | **MEAN±SEM** | **MEAN±SEM** | **MEAN±SEM** |
| Group A (Negative control) | 45.55±16.35 **b** | 2.74±1.26 **a** | 2.94±0.01**b** |
| Group B (Malaria control) | 61.86±4.32 | 8.33±0.47 | 2.83±1.26 |
| Group C (Malaria + 100mg/kg of EAI) | 47.42±4.62 **b** | 3.40±1.61 **a** | 0.83±0.49 **a** |
| Group D (Malaria + 500mg/kg of EAI) | 46.30±4.27 **b** | 1.52±0.47 **a** | 0.34±0.04 **a** |
| Group E (Malaria + 100mg/kg of ECC) | 48.63±6.95 **b** | 1.36±0.10 **a** | 0.77±0.04 **a** |
| Group F (malaria + 500mg/kg of ECC) | 45.76±14.67 **b** | 1.15±0.53 **a** | 0.67±0.08 **a** |
| Group G (malaria + Standard Drug) | 32.65±5.68 **a** | 2.33±0.91 **a** | 0.69±0.07 **a** |
| Group H (Malaria + 500mg/kg of EAI +ECC) | 99.07±10.92 **a** | 2.68±1.22 **a** | 0.33±0.04 **a** |
| **F-ratio** | 4.34 | 5.90 | 4.96 |

Data was analyzed using ANOVA followed by post Hoc LSD, and values considered significant at *p<0.05*. SEM: Standard error of mean. EAI: ethanolic leaf extract of *Azadirachta indica*, ECC: ethanolic leaf extract of *Cymbopogon citratus* (**a**= significant, **b**= not significant when compared with the positive control – Group B).

**3.4 Percentage parasitemia (%) at Day 0**, **Day 7**, and **Day 14 Following Treatment with Ethanolic Extracts of Cymbopogon citratus and Azadirachta indica**

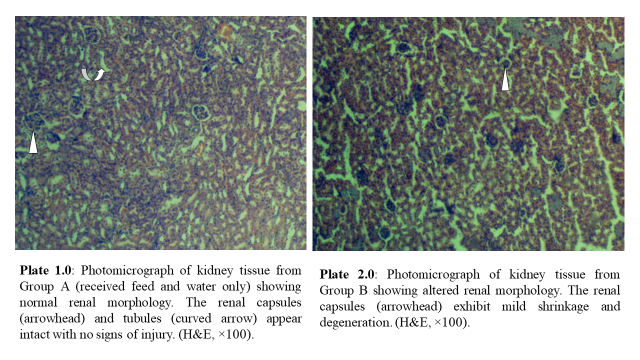
At Day 0, all malaria-infected groups (Groups B to H) exhibited significantly elevated Plasmodium counts compared to the uninfected positive control group (Group A). By Day 7, all treatment groups, including those receiving ethanolic extracts of Azadirachta indica (EAI), Cymbopogon citratus (ECC), and their combination, showed marked reductions in parasite counts relative to the malaria control group (Group B). This downward trend continued by Day 14, with all treatment groups demonstrating further suppression of parasitemia, comparable to the standard drug treatment (Group G). The positive control group maintained the lowest parasite load throughout the study as shown in table 4.0.

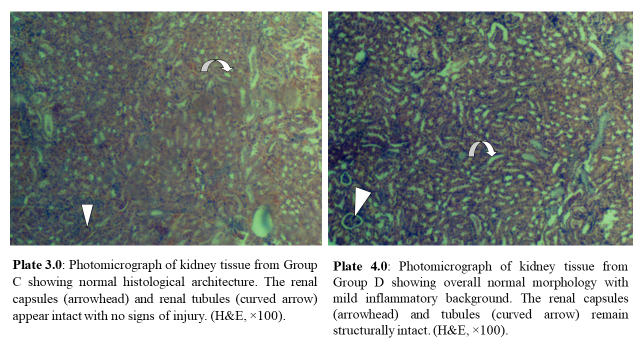
Table 4.0 effect of ethanolic extract of *Cymbopogon citratus* and *Azadirachta indica* on plasmodium count at day 0, 7, and 14 following *plasmodium berghei* induced toxicity.

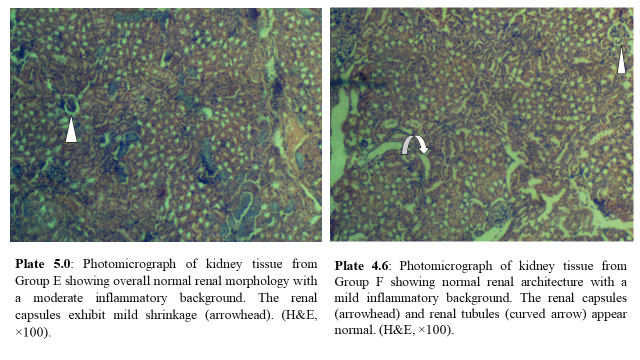
|  |  |  |  |
| --- | --- | --- | --- |
|  | **Plasmodium count Day 0** | **Plasmodium count Day 7** | **Plasmodium count Day 14** |
|  | **MEAN±SEM** | **MEAN±SEM** | **MEAN±SEM** |
| Group A (Negative control) | 4.33±0.67 | 2.67±0.67a | 1.33±0.33 **a** |
| Group B (Malaria control) | 37.33±1.45a | 31.67±3.38 | 20.00±2.89 |
| Group C (Malaria + 100mg/kg of EAI) | 43.00±1.52**a** | 8.67±0.88 **a** | 4.67±0.88**a** |
| Group D (Malaria + 500mg/kg of EAI) | 40.33±0.88 **a** | 10.67±0.88 **a** | 3.33±0.33**a** |
| Group E (Malaria + 100mg/kg of ECC) | 44.33 ±2.64 **a** | 7.00±1.52 **a** | 4.33±0.33 **a** |
| Group F (malaria + 500mg/kg of ECC) | 42.33±1.76 **a** | 5.00±0.57 **a** | 3.00±0.00 **a** |
| Group G (malaria + Standard Drug) | 41.33±1.20 **a** | 3.67±0.88 **a** | 3.00±0.57**a** |
| Group H (Malaria + 500mg/kg of EAI +ECC) | 44.00±2.08**a** | 5.00±0.00 **a** | 2.33±0.33**a** |
| **F-ratio** | 66.78 | 41.99 | 29.63 |

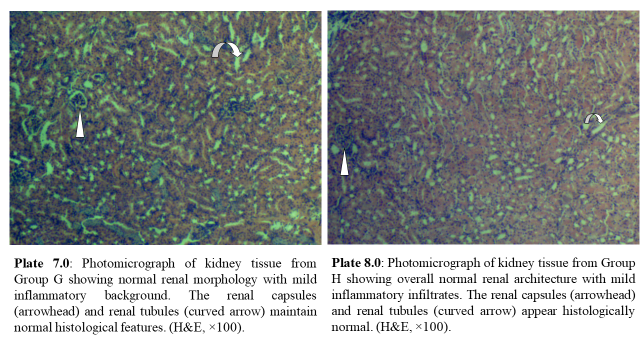
Data was analyzed using ANOVA followed by post Hoc LSD, and values considered significant at *p<0.05*. SEM: Standard error of mean. EAI: ethanolic leaf extract of *Azadirachta indica*, ECC: ethanolic leaf extract of *Cymbopogon citratus* (**a**= significant, **b**= not significant when compared to the Positive Control – Group B).

**3.5 Histological Findings**









**4.0 DISCUSSION**

Malaria remains one of the most serious infectious diseases globally, caused by Plasmodium species and transmitted through the bite of an infected Anopheles mosquito. Among the species, *P. falciparum, P. malariae, P. vivax, P. ovale, and P. knowlesi* are most implicated in human infections, with *P. falciparum* being the most lethal (Babatunde *et al*., [2018](https://isevjournals.onlinelibrary.wiley.com/doi/full/10.1002/jev2.12305#jev212305-bib-0010); Ajeka *et al*., 2021).

The current study investigated the effect of ethanolic extracts of *Cymbopogon citratus* and *Azadirachta indica* on kidney function in mice infected with *Plasmodium berghei*. Both plants are well-documented for their phytochemical richness and medicinal properties. *Cymbopogon citratus* (lemongrass) contains compounds such as alkaloids, saponins, tannins, anthraquinones, steroids, phenols, and flavonoids (Asaolu *et al*., 2009), while *Azadirachta indica* (neem) possesses antibacterial, antifungal, and antimalarial properties due to its secondary metabolites like azadirachtin and nimbolide (Islas *et al*., 2020; Roshan & Venar, 2015).

Weight changes observed during the study revealed a significant gain in Group A (positive control), possibly due to normal physiological growth with unrestricted feeding and absence of stress or infection. In contrast, Group B (malaria control) showed an insignificant increase in weight, suggesting that *P. berghei* infection may have altered normal metabolic processes without a statistically significant impact. This partially contrasts with findings by Atkinson *et al*., (2000), who reported reduced food intake and weight loss in infected birds.

Interestingly, Groups C to F, which received plant extracts, showed varied weight changes. Some exhibited increases, which might be attributed to the nutritional and metabolic benefits of the phytochemicals present in the extracts, as supported by Sarker *et al*., (2014), who noted improved growth performance in poultry supplemented with neem extract. However, this contradicts Agbafor and Akubugwo (2007), who observed weight loss in rats administered *C. citratus*.

Relative kidney weight changes were mostly insignificant across treatment groups, suggesting minimal structural organ impact. Group D (500 mg/kg *A. indica*) showed a slight decrease, possibly due to the diuretic and detoxifying effects of alkaloids. This aligns with findings from Ofoego *et al*., (2019) and Kpela *et al*., (2013), who reported increased relative kidney weights in nephroprotective studiesusing neem extract, and Ogbuewu *et al*., (2015), who observed lower kidney weights in rabbits fed neem-based diets.

In terms of biochemical parameters, urea levels decreased insignificantly in most treatment groups except for Group H, which showed a significant increase, possibly due to extract interaction effects at combined doses. The significant reductions in uric acid and creatinine levels across treatment groups reflect nephroprotective potential, likely mediated by the antioxidant activities of flavonoids and alkaloids. These findings are supported by Serina *et al*., (2015), who demonstrated improved renal markers in neem-treated rats.

Parasitemia assessment showed high parasite loads on day 0 for all infected groups. By day 7 and 14, a significant reduction in parasitemia was observed in extract-treated groups (C to H), indicating the antimalarial potential of both *C. citratus* and *A. indica*. These results are consistent with previous reports by Okokon *et al*., (2012) and Onwusonye *et al*., (2017), which documented the suppression of parasitemia in mice treated with these plants.

Histological examination of the kidneys showed preserved architecture in most groups, with occasional signs of mild to moderate inflammation, especially in Group H, which received a combined dose. The absence of structural damage supports the nephroprotective effect of the plant extracts, though the presence of inflammatory changes suggests possible dose-related tissue response.

**5.0 Conclusion**

This study demonstrates that ethanolic extracts of *Cymbopogon citratus* and *Azadirachta indica* possess antimalarial and nephroprotective properties in *Plasmodium berghei*-infected mice. The extracts significantly reduced parasitemia and improved renal biomarkers, especially at individual doses. However, combined high doses may induce mild kidney inflammation, indicating a need for dose optimization.

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