**Hepatoprotective and Antiplasmodial Effects of Ethanolic Leaf Extracts of *Azadirachta indica* and *Cymbopogon citratus* in *Plasmodium berghei*-Infected Mice**

**Abstract**

Malaria remains a major public health concern in sub-Saharan Africa, with Nigeria among the most affected countries. This study evaluated the hepatoprotective and antiplasmodial effects of ethanolic leaf extracts of Azadirachta indica (EAI) and Cymbopogon citratus (ECC) in Plasmodium berghei-infected male Swiss albino mice. Forty mice were randomly assigned into eight groups (n=5). Group A served as uninfected control, Group B was infected but untreated, while Groups C–H received varying doses of EAI, ECC, their combination, or a standard drug. Groups C and D received 100 mg/kg and 500 mg/kg EAI; Groups E and F received 100 mg/kg and 500 mg/kg ECC. Group G received Lonart® (standard antimalarial), and Group H received a combination of 500 mg/kg of both extracts. Treatments lasted 14 days. Infected groups showed minimal weight gain, while the uninfected control had significant increases. Relative liver weights were elevated in the untreated group but normalized in most treated groups. Serum AST and ALP levels were elevated in the malaria control group, while ALT levels were paradoxically reduced—possibly reflecting hepatic functional suppression. Extract-treated groups, especially the combination group, showed marked improvement in liver enzyme profiles. Parasitemia significantly declined by Days 7 and 14 in all treated groups, with the combination therapy yielding effects comparable to the standard drug. Histological analysis revealed lymphocytic infiltration and **sinusoidal congestion** in the malaria control group, which were ameliorated in extract-treated groups. These findings suggest dose-dependent hepatoprotective and antiplasmodial activities of EAI and ECC, likely due to their antioxidant and anti-inflammatory constituents.

Keywords: *Plasmodium berghei, Azadirachta indica, Cymbopogon citratus*, Hepatoprotective activity.

**INTRODUCTION**

Malaria remains a major global health concern, especially in sub-Saharan Africa, where it significantly contributes to morbidity and mortality (World Health Organization, 2023). The increasing resistance of Plasmodium species to conventional antimalarial drugs and the potential toxicity of some synthetic medications have spurred renewed interest in natural products as alternative or adjunct therapies (World Health Organization, 2022). Medicinal plants, with their wide range of bioactive phytochemicals, offer promising prospects due to their ethnopharmacological relevance, affordability, and comparatively low toxicity (Felicia et al., 2022; Ekor, 2014; Elioku et al., 2015; Ajayi et al., 2020; Ofoego et al., 2020).

Cymbopogon citratus (DC.) Stapf (Family: Poaceae), commonly known as lemongrass, is a perennial tropical grass widely utilized in traditional medicine to treat fever, headaches, digestive disorders, and malaria (Felicia et al., 2022). The therapeutic efficacy of C. citratus is largely attributed to its rich content of bioactive constituents including essential oils such as citral (neral and geranial), citronellal, myrcene, and limonene, as well as flavonoids like quercetin, kaempferol, and luteolin (Shah et al., 2011; Ajayi et al., 2020). These compounds exhibit potent antioxidant, anti-inflammatory, antimicrobial, and antiplasmodial properties (Tona et al., 1999). The hepatoprotective activity of C. citratus has been demonstrated in experimental models where it significantly reduced elevated liver enzymes and histological damage, likely by enhancing endogenous antioxidant defenses and suppressing lipid peroxidation (Felicia et al., 2022; Rahim et al., 2014).

Azadirachta indica A. Juss (Family: Meliaceae), commonly referred to as neem, is another widely studied medicinal plant with a rich history in Ayurvedic and African traditional medicine. It is a fast-growing evergreen tree native to the Indian subcontinent. Almost every part of the neem tree—leaves, bark, seeds, and flowers—has been used to manage fever, malaria, skin infections, and gastrointestinal disturbances (Brahmachari, 2004). Neem is particularly valued for its antimalarial, anti-inflammatory, antioxidant, and hepatoprotective effects, which are attributed to phytoconstituents such as azadirachtin, nimbin, nimbolide, quercetin, and various limonoids (Biswas et al., 2002; Subapriya & Nagini, 2005). These compounds exert their effects by modulating oxidative stress pathways, enhancing glutathione levels, inhibiting inflammatory mediators, and preserving cellular integrity in liver and kidney tissues affected by infections or toxins (Chattopadhyay, 2003; Bhanwra et al., 2000).

In malaria-infected individuals, oxidative stress plays a significant role in hepatic dysfunction, either directly from parasite metabolism or from the host immune response (Percário et al., 2012; Jaramillo et al., 2005; Guha et al., 2006). Thus, medicinal plants like C. citratus and A. indica, which offer both antimalarial and organ-protective properties, are of high therapeutic interest. Previous studies have shown that the co-administration or combined use of such plants could potentiate their protective effects by targeting multiple pathological pathways, including parasite clearance and the mitigation of oxidative and inflammatory damage (**Chianese** et al., 2010; Nogueira & Lopes, 2011; Anyasodor et al., 2023).

Given this background, the present study investigates the antimalarial and hepatoprotective potentials of ethanolic extracts of Cymbopogon citratus and Azadirachta indica in Plasmodium berghei-infected mice, with a focus on hepatic function markers and histopathological outcomes. This work seeks to provide scientific evidence supporting their traditional uses and potential application in the development of phytotherapeutics for malaria-associated liver injury.

**METHODOLOGY**

**Collection and Preparation of Plant Materials**

Fresh leaves of Azadirachta indica (neem) and Cymbopogon citratus (lemongrass) were harvested from verified botanical sources and authenticated at the Department of Botany, Nnamdi Azikiwe University. The leaves were thoroughly rinsed, air-dried under shade to preserve their phytochemical content, and pulverized into fine powder using a mechanical grinder. Two hundred and fifty grams (250 g) of each powdered plant material were subjected to cold maceration in 70% ethanol for 72 hours with regular stirring. The extracts were filtered through Whatman No. 1 filter paper, and the filtrates were concentrated using a rotary evaporator under reduced pressure at 40 °C. The extracts were stored in clean, airtight containers and refrigerated at 4 °C until required for experimental use.

**Acute Toxicity Evaluation**

The acute toxicity of the ethanolic extracts of A. indica and C. citratus was evaluated following the protocol described by Lorke (1983). No signs of toxicity or mortality were observed in any of the test subjects, even at the maximum dose of 5000 mg/kg body weight. This indicates that the extracts have an oral LD₅₀ greater than 5000 mg/kg, categorizing them as practically non-toxic according to the Globally Harmonized System of Classification and Labelling of Chemicals (United Nations, 2019).

**Animal Procurement and Ethical Considerations**

Sixty-six Male Swiss albino mice aged 6–8 weeks and weighing 20–22 g were used for the study. They were acquired from the Animal House of the College of Health Sciences and Technology, Nnamdi Azikiwe University. The animals were housed in standard plastic cages with access to clean drinking water and commercial pellet feed. They were maintained under a 12-hour light/dark cycle in a controlled environment and acclimatized for 14 days before the start of the experiment. Twenty-six were used for the acute toxicity tests while forty were used for the experiment proper. Ethical approval for the study was granted by the Department of Anatomy, Faculty of Basic Medical Sciences, Nnamdi Azikiwe University, Nnewi Campus (Ref No: ANA/EA/UG/AS/08/08/2024). Animal care and experimental procedures complied with the guidelines provided by the National Research Council (2011).

**Parasite Inoculation**

Chloroquine-sensitive Plasmodium berghei (ANKA strain) was sourced from the Nigerian Institute for Medical Research (NIMR), Lagos. Blood containing approximately 30% parasitemia was collected from a donor mouse via 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, as described by Fidock *et al*., (2004). Malaria infection was confirmed by preparing thin blood smears stained with Giemsa and examined under a light microscope. Parasitemia was typically detected 72–96 hours post-inoculation of Plasmodium berghei

**Experimental Grouping and Treatment Regimen**

Forty adult male Swiss albino mice were randomly assigned into eight experimental groups (A to H), with five mice in each group. Group A served as the normal (uninfected and untreated) control and was neither infected with Plasmodium berghei nor given any treatment. All other groups (B to H) were intraperitoneally inoculated with P. berghei prior to the initiation of their respective treatment protocols. Group B served as the positive (malaria-infected untreated) control and received no further treatment. Groups C and D were infected and subsequently treated orally with 100 mg/kg/day and 500 mg/kg/day of Azadirachta indica leaf extract, respectively. Groups E and F, also infected, received 100 mg/kg/day and 500 mg/kg/day of Cymbopogon citratus leaf extract, respectively. Group G received Lonart®, a standard artemether-lumefantrine combination antimalarial therapy, while Group H was administered a combination of A. indica and C. citratus leaf extracts at 500 mg/kg each. All treatments were given once daily by oral gavage for 14 consecutive days, beginning 72 hours post-infection.

The dose selections of 100 mg/kg and 500 mg/kg were based on previous studies demonstrating efficacy without observable toxicity in rodents (Sani et al., 2020; Ofoego et al., 2017; Subapriya & Nagini, 2005). The dosage of the standard drug was selected based on its documented efficacy in malaria treatment as recommended by the World Health Organization (WHO, 2022) and the Centers for Disease Control and Prevention (CDC, 2023). Lonart® (artemether:lumefantrine in a 1:6 ratio) was procured from Syleon-C Pharm. Nig. Ltd., Nnewi, and administered at 20 mg/kg/day of artemether and 120 mg/kg/day of lumefantrine, dissolved in distilled water.

#### **Blood Collection and Biochemical Analyses**

Blood samples were collected via cardiac puncture on days 1, 7, and 14 post-infection for parasitemia analysis. At the end of the experiment, 24 hours after the final treatment, mice were anesthetized using chloroform and blood was again collected by cardiac puncture. The collected blood was allowed to clot at room temperature and then centrifuged to separate the serum, which was stored appropriately for biochemical analyses. Serum levels of liver function biomarkers—including alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP)—were measured using diagnostic kits from Randox Laboratories (UK), according to the manufacturer’s instructions.

**Evaluation of Antimalarial Efficacy**

Parasitemia levels were assessed daily through thin blood smears prepared 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). Parasitemia was calculated as:

Percentage Parasitemia = 100

To assess treatment efficacy, percentage chemosuppression—a standardized metric indicating the reduction in parasitemia relative to the untreated infected control—was determined using the following formula:

Percentage Chemosuppression =

**Liver Function Tests**

**Aspartate Aminotransferase (AST) Activity**

Serum AST activity was measured using a spectrophotometric method based on the World Health Organization guidelines (WHO, 2017). AST catalyzes the transfer of an amino group from L-aspartate to α-oxoglutarate, forming oxaloacetate. This oxaloacetate subsequently reacts with NADH, resulting in the formation of NAD⁺. Enzyme activity was quantified by monitoring the rate of NADH oxidation.

For the assay, 100 μl of serum was mixed with 0.5 ml of AST Reagent 1 in a test tube and incubated at 37°C for 30 minutes. Then, 0.5 ml of AST Reagent 2 was added, and the mixture was incubated at room temperature for 20 minutes. Sodium hydroxide was subsequently added, and absorbance was measured at 546 nm.

**Alanine Aminotransferase (ALT) Activity**

Serum ALT activity was determined using the spectrophotometric method recommended by the World Health Organization (WHO, 2017). ALT catalyzes the transfer of an amino group from alanine to α-oxoglutarate, yielding pyruvate and L-glutamate. The pyruvate is subsequently reduced to lactate by NADH, and the decrease in NADH absorbance is used to quantify the enzyme activity.

The assay procedure was identical to that used for AST determination.

**Alkaline Phosphatase (ALP) Activity**

Serum ALP activity was assessed using the standard spectrophotometric method recommended by the World Health Organization (WHO, 2017). ALP catalyzes the hydrolysis of *p*-nitrophenyl phosphate to *p*-nitrophenol and inorganic phosphate. The concentration of *p*-nitrophenol, which is directly proportional to enzyme activity, was measured spectrophotometrically.

For the assay, 20 μl of serum was added to test tubes containing ALP reagent. Absorbance was recorded at 1-minute intervals over a 3-minute period. Enzyme activity was calculated using the formula:

**U/L = 2760 × ΔA**

**Histopathological Analysis**

Livers were excised, rinsed in normal saline, and fixed in 10% buffered formalin. The tissues were processed through ascending grades of alcohol for dehydration, cleared in xylene, and embedded in paraffin wax. Sections of 5 μm thickness were obtained using a rotary microtome and stained with hematoxylin and eosin. Microscopic examination of the stained sections was carried out to evaluate pathological features such as hepatic damage, hepatocyte necrosis, and inflammatory infiltration.

**Statistical Analysis**

All results were presented as mean ± standard error of the mean (SEM). Statistical evaluation was performed 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.

**RESULTS**

**Body weight Observation**

Table 1 presents the variations in body weight among the eight experimental groups. No significant differences were observed in body weight changes across most groups, except for the normal control group (Group A), which recorded a statistically significant increase (P = 0.02). In contrast, the malaria-infected and treated groups showed no significant alterations 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 | 0.02a | -6.77 |
| Group B (Malaria control) | 28.67±1.14 | 32.67±0.88 | 4.00 | 0.13b | -2.40 |
| Group C (Malaria + 100mg/kg of EAI) | 27.90±2.30 | 34.00±1.52 | 6.10 | 0.22b | -1.74 |
| Group D (Malaria + 500mg/kg of EAI) | 29.17±1.03 | 34.00±1.73 | 4.83 | 0.22b | -1.79 |
| Group E (Malaria + 100mg/kg of ECC) | 28.40±1.83 | 31.93±1.72 | 3.53 | 0.21b | -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 | 0.52b | 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).

**Relative Organ (Liver) weight Observation**

Relative liver weights showed significant differences when the Malaria-only group (Group B) and Group C (Malaria + 100 mg/kg of EAI) were compared to the Control group (Group A). However, values in the other treatment groups did not differ significantly from the Control, as illustrated in Table 2.

Table 2.0 Effect of ethanolic leaf extracts of *Cymbopogon citratus* and *Azadirachta indica* on relative liver weight following plasmodium berghei induced toxicity

|  |  |
| --- | --- |
|  | Relative liver weight (g) |
|  | MEAN±SEM |
| Group A (Negative control) | 0.04±0.00 |
| Group B (Malaria control) | 0.06±0.00 a |
| Group C (Malaria + 100mg/kg of EAI) | 0.07±0.01a |
| Group D (Malaria + 500mg/kg of EAI) | 0.06±0.01b |
| Group E (Malaria + 100mg/kg of ECC) | 0.06±0.01b |
| Group F (malaria + 500mg/kg of ECC) | 0.06±0.01b |
| Group G (malaria + Standard Drug) | 0.05±0.01b |
| Group H (Malaria + 500mg/kg of EAI +ECC) | 0.06±0.00b |
| **F-ratio** | 7.65 |

Data were analyzed using ANOVA followed by post hoc LSD test. Values were considered significant at p < 0.05. SEM: Standard error of the mean. EAI: Ethanolic leaf extract of Azadirachta indica; ECC: Ethanolic leaf extract of Cymbopogon citratus. Superscripts: a = significant, b = not significant when compared to Group A (Negative control). Relative organ weights were calculated as organ weight (g) divided by body weight (g) and expressed as a decimal ratio (g/g).

**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 parasitemia compared to the uninfected control group (Group A). By Day 7, treatment groups administered ethanolic leaf extracts of Azadirachta indica (EAI), Cymbopogon citratus (ECC), or their combination showed substantial reductions in parasite counts relative to the untreated malaria control group (Group B). This downward trend in parasitemia persisted through Day 14, with all treated groups demonstrating further suppression of parasite load, comparable to the effect observed with the standard antimalarial drug (Group G). The uninfected control group (Group A) maintained the lowest parasitemia levels throughout the study, as shown in Table 3.0.

Table 3.0: Effect of Ethanolic extract of *Cymbopogon citratus* and *Azadirachta indica* on plasmodium count at day 0, 7, and 14 following *plasmodium berghei* induced toxicity.

|  |  |  |  |
| --- | --- | --- | --- |
| Group | Plasmodium Count Day 0 | Plasmodium Count Day 7 | Plasmodium Count Day 14 |
| Group A (Negative Control) | 0.00 ± 0.00a | 0.00 ± 0.00ᵃ | 0.00 ± 0.00ᵃ |
| Group B (Malaria Control) | 37.33 ± 1.45 | 60.00 ± 3.00 | 78.00 ± 4.03 |
| Group C (Malaria + 100 mg/kg EAI) | 43.00 ± 1.52b | 8.67 ± 0.88ᵃ | 4.67 ± 0.88ᵃ |
| Group D (Malaria + 500 mg/kg EAI) | 40.33 ± 0.88b | 10.67 ± 0.88ᵃ | 3.33 ± 0.33ᵃ |
| Group E (Malaria + 100 mg/kg ECC) | 44.33 ± 2.64b | 7.00 ± 1.52ᵃ | 4.33 ± 0.33ᵃ |
| Group F (Malaria + 500 mg/kg ECC) | 42.33 ± 1.76b | 5.00 ± 0.57ᵃ | 3.00 ± 0.00ᵃ |
| Group G (Malaria + Standard Drug) | 41.33 ± 1.20b | 3.67 ± 0.88ᵃ | 3.00 ± 0.57ᵃ |
| Group H (Malaria + 500 mg/kg EAI+ECC) | 44.00 ± 2.08b | 5.00 ± 0.00ᵃ | 2.33 ± 0.33ᵃ |

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).

**Percentage Chemosuppression of Parasitemia**

Table 4.0 summarizes the percentage chemosuppression of parasitemia on Days 7 and 14 across all treated groups. On Day 7, the lowest level of chemosuppression was recorded in Group D (500 mg/kg Azadirachta indica extract) at 82.22%, while the highest was observed in Group G (standard drug) at 93.89%. By Day 14, all treatment groups exhibited enhanced chemosuppressive effects, ranging from 94.01% in Group C (100 mg/kg Azadirachta indica extract) to 97.01% in Group H (combined 500 mg/kg Azadirachta indica and Cymbopogon citratus extracts). The standard drug group (Group G) showed a chemosuppression rate of 96.15% on Day 14. No parasitemia was detected in the uninfected control group (Group A) throughout the study.

Table 4.0: Chemosuppression of parasitemia

|  |  |  |
| --- | --- | --- |
| Group | Day 7 Suppression (%) | Day 14 Suppression (%) |
| Group B | - | - |
| Group C | 85.55% | 94.01% |
| Group D | 82.22% | 95.73% |
| Group E | 88.33% | 94.45% |
| Group F | 91.67% | 96.15% |
| Group G | 93.89% | 96.15% |
| Group H | 91.67% | 97.01% |

**Effect of Ethanolic Extracts of Cymbopogon citratus and Azadirachta indica on Liver Enzymes Following Plasmodium berghei-Induced Hepatotoxicity**

Table 5.0 presents the mean ± SEM values of AST, ALT, and ALP across experimental groups. The malaria-infected untreated group (Group B) showed elevated AST (40.67 IU/L) and ALP (45.10 IU/L) compared to the uninfected control (Group A), while ALT was markedly reduced (16.00 IU/L). Treated groups (C–H) displayed variable enzyme responses, with Group C showing the highest ALT (62.67 IU/L) and Group H the lowest ALP (14.63 IU/L). Only ALT levels showed statistically significant differences across groups (F = 7.01, p < 0.05), while AST and ALP did not (F = 2.15 and 0.77, respectively). Superscripts indicate statistical comparisons with Group B.

Table 5.0: Effect of ethanolic extract of *Cymbopogon citratus* and *Azadirachta indica* on liver enzymes following *plasmodium berghei* induced toxicity

|  |  |  |  |
| --- | --- | --- | --- |
|  | Aspartate Transaminase (IU/L) | Alanine Transaminase (IU/L) | Alkaline Phosphatase (IU/L) |
|  | MEAN±SEM | MEAN±SEM | MEAN±SEM |
| Group A (Positive control) | 24.33±5.89b | 49.67±7.79a | 23.33±6.01 b |
| Group B (Malaria control) | 40.67±3.71 | 16.00±2.00 | 45.10±15.45 |
| Group C (Malaria + 100mg/kg of EAI) | 32.67±1.33 b | 62.67±8.41 a | 46.56±27.87 b |
| Group D (Malaria + 500mg/kg of EAI) | 68.00±26.63 b | 51.67±6.96 a | 40.33±4.13 b |
| Group E (Malaria + 100mg/kg of ECC) | 86.00±26.63 a | 52.67±2.33 a | 38.87±13.68 b |
| Group F (malaria + 500mg/kg of ECC) | 39.33±1.67 b | 51.00±6.00 a | 28.23±0.36 b |
| Group G (malaria + Standard Drug) | 49.33±0.88 b | 51.67±0.88 a | 29.56±7.10 b |
| Group H (Malaria + 500mg/kg of EAI +ECC) | 44.33±2.02 b | 58.00±0.57 a | 14.63±0.97 b |
| F-ratio | 2.15 | 7.01 | 0.77 |

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 Group B – Positive control).

**Histological Findings**

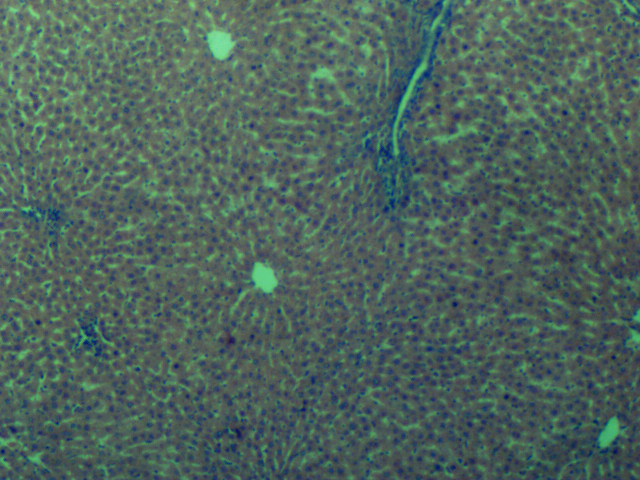
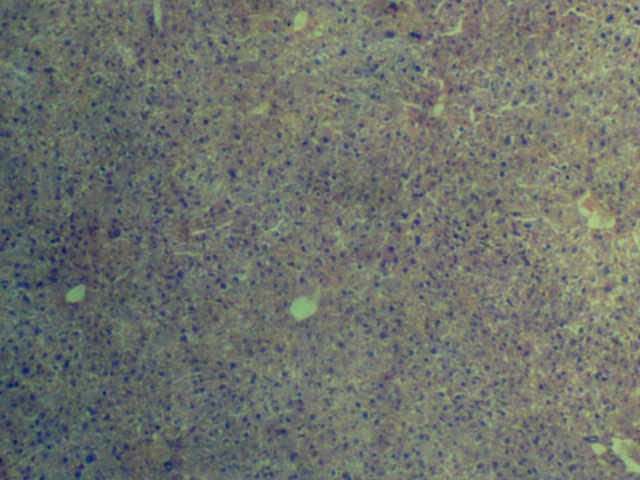


Plate 1.0: **Photomicrograph of liver tissue of Group A (received standard feed and distilled water *ad libitum*) reveals normal histological architecture, with hepatocytes (arrow) and central vein (arrowhead) appearing intact and unaltered (H&E, ×100).**



SC

Plate 2.0: Photomicrograph of liver tissue of Group B shows the central vein (arrowhead), sinusoids (arrow), and hepatocytes (curved arrow). However, mild to moderate lymphocytic infiltration and **sinusoidal congestion** (SC) are visible, indicative of inflammatory activity and structural injury at this stage of infection (H&E, ×100).

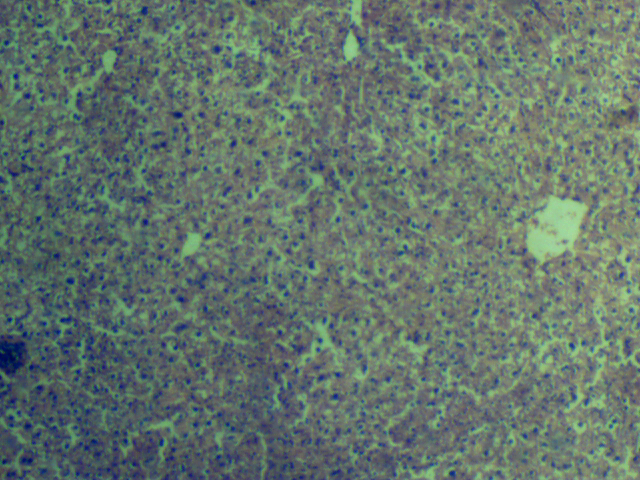


Plate 3.0: Photomicrograph of liver tissue of Group C showing morphology with normal liver histology but with moderate lymphocytic infiltration. The central vein is (arrowhead) in intact (H&E, ×100).

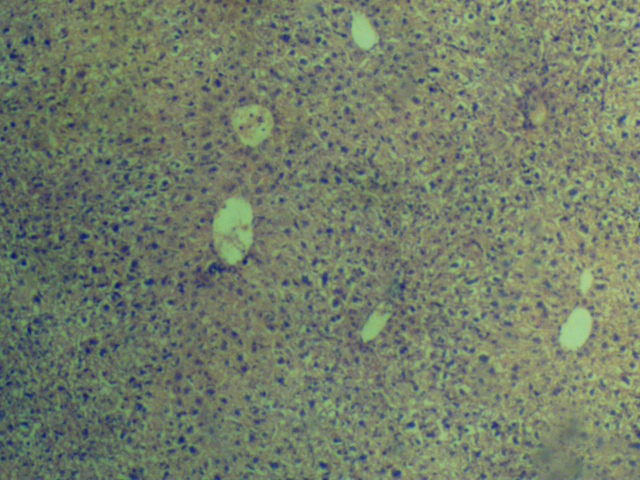


Plate 4.0: Photomicrograph of liver tissue of Group D showing morphology with normal liver histology but with mild inflammatory background. The central vein, (arrowhead) sinusoids (arrow) and hepatocytes (curved arrow) are normal with no obvious sign of injury (H&E, X100).

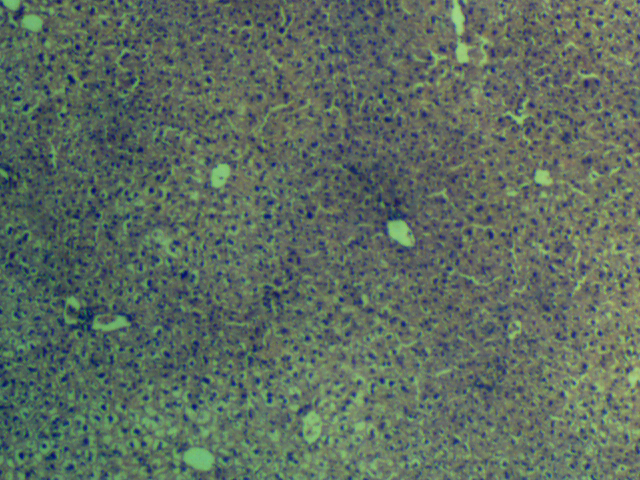


Plate 5.0: Photomicrograph of liver tissue of Group E showing morphology with normal liver histology but with mild inflammatory background. The central vein, (arrowhead) sinusoids (arrow) and hepatocytes (curved arrow) are normal with no obvious sign of injury (H&E, X100).

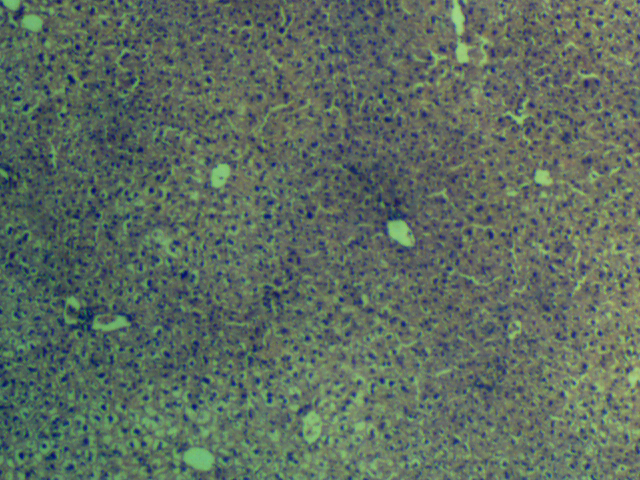


Plate 6.0: Photomicrograph of liver tissue of Group F showing normal Central vein (arrow) and hepatocytes (arrowhead) (H&E, X100).

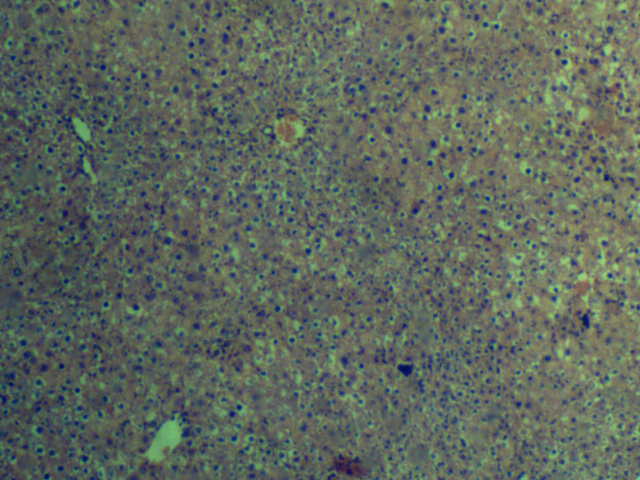


Plate 7.0: Photomicrograph of liver tissue of Group G showing morphology with normal liver histology but with mild inflammatory background. The central vein (arrowhead), sinusoids (arrow) and hepatocytes (curved arrow) are normal with no obvious sign of injury (H&E, X100).

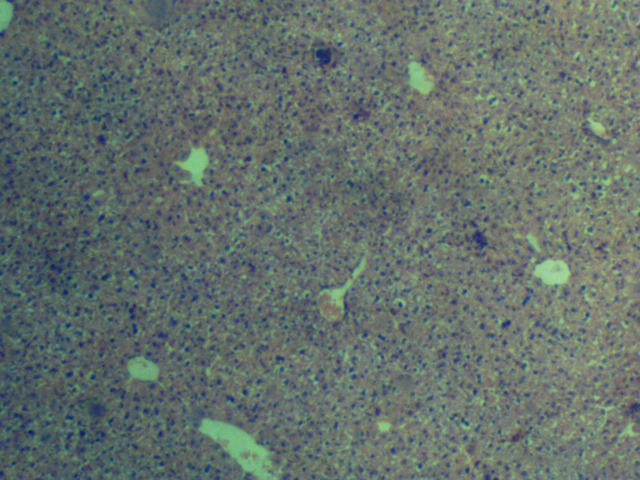


Plate 8.0: Photomicrograph of liver tissue of Group H showing morphology with normal liver histology but with mild inflammatory background. The central vein, (arrowhead) sinusoids (arrow) and hepatocytes (curved arrow) are normal with no obvious sign of injury (H&E, X100).

**DISCUSSION**

Malaria remains a significant global health burden, particularly in sub-Saharan Africa, due to its high morbidity and mortality. The disease, caused by *Plasmodium* species and transmitted by *Anopheles* mosquitoes (Babatunde et al*.,* 2018; Weiss et al*.,* 2024), continues to challenge public health systems, especially with the growing resistance to conventional antimalarial drugs. Consequently, attention has shifted towards plant-derived alternatives, such as *Azadirachta indica* (neem) and *Cymbopogon citratus* (lemongrass), which are widely recognized for their therapeutic potential and ethnomedicinal use (Shah et al., 2011; Acharaike et al., 2022: Ofoego et al., 2025).

In this study, the hepatoprotective effect of ethanolic leaf extracts of *A. indica* and *C. citratus* were evaluated in *Plasmodium berghei*-infected mice. These plants are rich in phytochemicals, including flavonoids, alkaloids, tannins, saponins, and phenolic compounds, which contribute to their broad pharmacological activities (Asaolu et al., 2009; Islas et al., 2020; Subapriya & Nagini, 2005).

Post-infection weight changes reflected systemic stress and treatment responses. The uninfected control group exhibited significant weight gain, consistent with normal physiological development, whereas infected groups showed negligible weight changes. This pattern is similar to our earlier findings on kidney function, where *P. berghei* infection modestly impaired weight gain (Ofoego et al., 2025), potentially due to metabolic disruption (Ubua et al., 2019). Interestingly, weight stabilization in treated groups could be attributed to the metabolic benefits of bioactive compounds, including alkaloids and flavonoids (Agbafor & Akubugwo, 2007), which may support nutrient absorption and counteract infection-related catabolism.

Relative liver weights differed significantly among groups. The malaria-only group (Group B) and the group treated with 100 mg/kg of Azadirachta indica (Group C) showed notable increases compared to the control, which may indicate hepatomegaly due to parasitic infection and/or phytochemical effects. This observation aligns with earlier studies suggesting that Plasmodium infection and certain plant-derived compounds can induce hepatic enlargement as part of oxidative or inflammatory stress responses (Arinola, 2005; Sani et al., 2020). In contrast, groups treated with higher doses of extracts, the combination therapy, or standard drug showed relatively stable or slightly reduced liver weights, possibly reflecting protective, anti-inflammatory, or hepatoregulatory effects (Ofoego et al., 2025; Abdel Moneim et al., 2014).

The antiplasmodial efficacy of the extracts was clear: all infected groups had high parasitemia at baseline, but significant **parasite clearance occurred by day 7**, and further reduction by day 14. The combination of Azadirachta indica and Cymbopogon citratus leaf extracts achieved chemosuppressive activity **comparable to standard antimalarial drugs** (Slater & Cerami, 1992; Mgbemena et al., 2010; Ukpai & Amaechi 2012). These findings align with earlier reports attributing antimalarial effects to multiple mechanisms, including disruption of parasite mitochondria, inhibition of hemozoin formation, and interference with parasite DNA and protein synthesis. For example, Martiney et al., (1996) demonstrated that hemozoin formation can be effectively blocked, offering a therapeutic strategy against Plasmodium falciparum. Additionally, natural products from neem and lemongrass have been shown to impair parasite metabolic pathways, including interference with mitochondrial function and nucleic acid processes (**Chianese** et al., 2010; Nogueira & Lopes, 2011).

The markedly reduced ALT level observed in the malaria-infected, untreated group (Group B) compared to the uninfected control group (Group A) is counterintuitive, as hepatocellular injury typically elevates ALT. However, emerging evidence suggests that Plasmodium berghei infection can impair hepatocyte function without causing widespread necrosis, leading to suppressed enzyme synthesis or release rather than leakage. Deroost et al., (2014) reported that P. berghei induces hepatic inflammation and immune activation with limited structural damage, while Khan et al., (2024) noted that oxidative stress and parasite-derived products such as hemozoin can downregulate hepatic transaminase production. This suggests that the low ALT may reflect functional hepatic injuries, where hepatocytes, though stressed, are no longer actively releasing certain enzymes satisfactorily due to metabolic shutdown. Our histological findings of lymphocytic infiltration with congested sinusoids support this explanation, indicating early or sub-lethal hepatocellular dysfunction rather than extensive necrosis.

The elevated ALT observed in the uninfected control group may reflect normal physiological variability in laboratory mice, potentially influenced by factors such as diet, circadian rhythm, or individual metabolic differences. This reinforces the importance of interpreting biochemical results alongside histological findings and broader physiological context. Future studies using additional biomarkers—such as LDH, GGT, and pro-inflammatory cytokines—and employing serial time-point measurements could better clarify the dynamics of liver enzyme changes during malaria infection and enhance understanding of how functional impairment relates to structural liver injury.

Biochemical analyses revealed that ethanolic leaf extracts of *A. indica* and *C. citratus* significantly reduced serum AST and ALT levels compared to the malaria control group. This suggests a hepatoprotective effect, likely mediated by antioxidant flavonoids that stabilize hepatocyte membranes and inhibit oxidative stress-induced enzyme leakage. These findings align with other studies reporting protective enzyme profiles following phytotherapeutic interventions (Yanpallewar et al., 2003; Ozims et al., 2017; Ogundipe et al., 2025).

Histopathological evaluation of liver tissues from the malaria-infected, untreated group (Group B) revealed **lymphocytic infiltration and sinusoidal congestion**. These findings suggest that although Plasmodium berghei infection had initiated hepatic immune activation and structural injury (sinusoidal congestion), the degree of structural injury remained limited—likely reflecting **moderate inflammation and localized oxidative stress** rather than extensive hepatocellular necrosis. Similar histological patterns have been reported in earlier studies of P. berghei infection (Adetutu et al., 2016; Adachi et al., 2004). However, livers from extract-treated groups, particularly those receiving the combination treatment, showed relatively preserved architecture with minimal inflammatory changes. This aligns with previous renal findings, where extract-treated groups exhibited preserved glomerular and tubular structures despite infection (Ofoego et al., 2025; Ullah et al., 2014; Naqvi, 2015**)**. The improved liver histology further confirms the antioxidant, anti-inflammatory, and membrane-stabilizing properties of the phytochemicals.

In summary, the present findings reaffirm the hepatoprotective and antiplasmodial potentials of *Azadirachta indica* and *Cymbopogon citratus*, complementing our earlier observations on their nephroprotective roles. These extracts may offer a promising adjunct or alternative to conventional antimalarial therapies, especially in regions burdened by drug resistance and limited healthcare access. Previous studies have shown that the leaves of Azadirachta indica and Cymbopogon citratus contain higher concentrations of bioactive compounds, particularly flavonoids and phenolics, compared to other parts of the plants. This phytochemical richness contributes to their superior biological activities (Subapriya & Nagini, 2005; Shah et al., 2011).

**Conclusion**  
Ethanolic extracts of *Cymbopogon citratus* and *Azadirachta indica* exhibited notable antimalarial and hepatoprotective effects in *Plasmodium berghei*-infected mice. The combined administration of both extracts at 500 mg/kg each produced therapeutic outcomes comparable to standard antimalarial treatment, as evidenced by significant reductions in serum ALT and AST levels, decreased parasitemia, and improved liver histoarchitecture.

These findings indicate that both the individual and combined ethanolic leaf extracts of Azadirachta indica and Cymbopogon citratus may offer effective plant-based therapeutic options for mitigating malaria-induced hepatic injury. While the extracts were generally well-tolerated, the mild hepatic inflammation observed at higher doses underscores the need for dose optimization and careful administration. Notably, the combination treatment demonstrated enhanced efficacy in reducing parasitemia and improving hepatic histoarchitecture, suggesting potential additive or synergistic effects that warrant further investigation.

Future studies should aim to optimize dosing regimens, assess long-term safety, and elucidate the molecular mechanisms responsible for their antiplasmodial and hepatoprotective actions, paving the way for potential therapeutic development.

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Ethical approval

Ethical approval for the study was granted by the Department of Anatomy, Faculty of Basic Medical Sciences, Nnamdi Azikiwe University, Nnewi Campus (Ref No: ANA/EA/UG/AS/08/08/2024). Animal care and experimental procedures complied with the guidelines provided by the National Research Council (2011).

**Disclaimer (Artificial intelligence)**

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, manuscript.

**Competing Interests:**

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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