Original Research Article

Herbal Triad Therapy: Antiplasmodial, Immunomodulatory and Antioxidant Effects in Experimental Malaria

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ABSTRACT

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| **Aims**: To evaluate the antiplasmodial, immunomodulatory, and antioxidant effects of combined aqueous extracts of *Carica papaya*, *Ananas comosus*, and *Buxus microphylla* in *Plasmodium berghei*-infected mice.  **Study Design**: A randomized controlled animal experimental study.  **Place and Duration of Study**: Conducted at the Animal House, Gregory University, Uturu, Nigeria, from March 2024 to June 2024.  **Sample**: Sixteen (16) male Wistar mice (20–30 g) randomly assigned to four groups: normal control, negative control (infected untreated), standard drug (lumefantrine-treated), and plant extract combination group.  **Methodology**: Fresh plant materials were collected and authenticated by a botanist. Aqueous extracts of *Carica papaya*, *Ananas comosus*, and *Buxus microphylla* were prepared and administered orally. Mice were inoculated intraperitoneally with Plasmodium berghei on day 0 and treated for four consecutive days. Parasitemia, hematological indices, cytokines (IL-6, TNF-α, IFN-γ, IL-10), antioxidant enzymes (SOD, CAT, GPX, MDA, GSH), and spleen histology were assessed. Data were analyzed using one-way ANOVA and Tukey’s post-hoc test at *P* = .05.  **Results**: The extract combination significantly reduced parasitemia (30.28 ± 1.45%) and parasite density (778,196 ± 78,246 parasites/μL) compared to the negative control (88.48 ± 2.43%; 2,212,000 ± 127,800 parasites/μL). Hematological indices improved, including RBC (2.57 ± 0.25 × 10⁶/μL), hemoglobin (7.27 ± 0.29 g/dL), and platelets (282.33 ± 12.99 × 10³/μL). Pro-inflammatory cytokines IL-6 (17.32 ± 0.28 pg/mL) and TNF-α (86.04 ± 5.70 pg/mL) decreased, with stable IFN-γ (90.00 ± 3.62 pg/mL) and modest IL-10 increase (2.83 ± 0.00 pg/mL). Antioxidant activities improved with increased CAT (38.54 ± 0.48 U/L), reduced MDA (0.89 ± 0.02 µM), and moderate GPX (30.26 ± 3.59 U/L), while SOD and GSH rose slightly.  **Conclusion**: Combined plant extracts demonstrated significant antiplasmodial, antioxidant, and immunomodulatory effects, supporting their potential as complementary malaria therapies. |

*Keywords: Immunomodulation, Cytokines, Spleen histology, Parasitemia, Antioxidant*

1. INTRODUCTION

Malaria, caused by Plasmodium parasites and transmitted by Anopheles mosquitoes, remains a major global health burden (1). It is responsible for significant morbidity and mortality, especially in sub-Saharan Africa (2). Resistance to conventional antimalarial drugs, such as chloroquine and partial resistance to artemisinin-based therapies, poses a growing threat to treatment efficacy (3). Additionally, malaria can lead to complications involving vital organs such as the liver and spleen, intensifying the need for new therapeutic strategies (4,5).

Historically, medicinal plants have played a crucial role in managing infectious diseases (6,7). The World Health Organization recognizes that over 80% of the global population relies on traditional medicine for healthcare needs (8). Various plant extracts have demonstrated antiplasmodial activity in experimental models (9,10). This study investigates the potential antiplasmodial and immunomodulatory effects of combined aqueous extracts of B*uxus microphylla*, *Carica papaya*, and *Ananas comosus* in mice infected with *Plasmodium berghei*. The aim is to assess whether these plant extracts can serve as a viable alternative or adjunct therapy in the treatment of malaria.

2. material and methods

**2.1 Plant Collection and Identification**

Fresh leaves of Buxus microphylla and Carica papaya were collected from within the study area, while Ananas comosus bark was obtained from ripe fruits purchased locally. Plant materials were authenticated by a botanist.

**2.2 Ethical Approval**

Sixteen male Wistar mice (20–30 g) were used. All procedures followed the Animal Research Ethics Committee guidelines (11).

**2.3 Extract/ Drug Preparation**

Plant materials were washed, air dried for three weeks, and pulverized. Twenty grams of each were soaked in 200 mL of hot distilled water for 24 hours, filtered, and stored. Lumefantrine (Coartem®, Novartis Pharma AG) was crushed, suspended in water, and dosed based on body weight using standard conversion factors

**2.4 Experimental Design**

Mice were randomized into four groups

**Table 1: Animal Grouping**

|  |  |
| --- | --- |
| Groups | Treatment |
| Normal Control | Mice given normal chow and water *ad libitum*. |
| Negative Control | Infected, untreated mice given normal chow and water *ad libitum*. |
| Standard Drug | Infected, treated with lumefantrine mice given normal chow and water *ad libitum*. |
| Plant Combo | Infected, treated with combined plant extract (same ratio) mice given normal chow and water *ad libitum*. |

**2.5 Determination of Parasitemia and Parasitemia Density** (12,13)

The 4-day suppressive test was used. On day 0, mice were intraperitoneally inoculated with ~10⁷ Plasmodium berghei-infected red blood cells. Treatment commenced 3 hours post-infection and continued for 4 days. On day 5, Giemsa-stained blood smears were examined under a light microscope.

Percentage parasitemia was calculated using the formula:

Parasitemia % =

In this study, a total of 100 RBCs per microscopic field were counted, and parasitemia counts were interpreted accordingly as percentages.

Parasitemia density (parasites/μL of blood) was then estimated using the hematological determined RBC count, applying the formula:

Parasite Density =

**2.6 Determination of Haematological, Immunological, and Antioxidant Parameters**

CBC (RBC, hemoglobin, platelets) was analyzed using an automated haematology analyzer. Cytokines (IL-6, TNF-α, IFN-γ, IL-10) were quantified with ELISA kits (Elabscience®). Antioxidants were measured spectrophotometrically (14-19).

**2.7 Histological Analysis**

Tissue processing was carried out manually according to Burnett and Crocker. (20)

**2.8 Statistical Analysis**

Data were expressed as mean ± SD. One-way ANOVA followed by Tukey HSD post-hoc test was used for group comparisons. Statistical significance was accepted at *P* =.05.

3. results and discussion

3.1: Results

**Table 2: Parasitemia % and Parasitemia Density**

|  |  |  |
| --- | --- | --- |
| **Groups** | **% Parasitemia** | **Parasite Density (parasites/µL)** |
| Normal Control | 10.66 ± 0.60a | 514,878 ±29,005a |
| Negative Control | 88.48 ± 2.43b | 2,212,000 ± 127,800b |
| Standard Drug | 16.04 ± 0.87c | 593,480 ± 65,659c |
| Plant Combo | 30.28 ± 1.45d | 778,196 ± 78,246d |

*Keys: Data reported as mean + standard error determinations, values bearing different superscripts on the same column differ significantly (p<0.05), while ones with the same superscripts indicate no significant difference (p>0.05) using ANOVA.*

**Table 3. Effect of plant extract combination on haematological parameters of *Plasmodium berghei* infected mice**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Parameters | Normal Control | Negative Control | Standard Drug | Plant Combo |
| WBC (103/uL) | 5.12 ± 0.47a | 7.79 ± 1.66a | 5.82 ± 0.89a | 5.59 ± 1.26a |
| Neu (%) | 44.03 ± 1.56a | 76.70 ± 1.51b | 50.20 ± 1.85ac | 55.53 ± 1.74c |
| Lym (%) | 41.03 ± 0.98a | 14.83 ± 0.90b | 34.13 ± 2.83ac | 30.17 ± 0.44c |
| Mon (%) | 5.27 ± 1.22a | 7.03 ± 0.20a | 5.57 ± 0.18a | 7.77 ± 0.46a |
| Eos (%) | 5.40 ± 1.92ab | 0.97 ± 0.38b | 8.13 ± 0.30a | 1.0 ± 0.12b |
| Bas (%) | 0.71 ± 0.06a | 0.20 ± 0.00b | 0.56 ± 0.09a | 1.67 ± 0.04c |
| NLR | 0.95 ± 0.12a | 5.20 ± 0.45b | 1.41 ± 0.19a | 1.77 ± 0.15a |
| PLR | 99.20 ± 15.13a | 155.92 ± 30.75a | 154.05 ± 31.84a | 204.55 ± 18.57a |
| RBC (106/uL) | 4.83 ± 0.22a | 2.50 ± 0.23b | 3.70 ± 0.40ab | 2.57 ± 0.25b |
| HGB (g/dL) | 13.73 ± 0.65a | 6.87 ± 0.18b | 8.43 ± 1.26b | 7.27 ± 0.29b |
| HCT (%) | 44.57 ± 3.33a | 18.80 ± 1.56a | 28.93 ± 12.00a | 21.13 ± 0.41a |
| MCV (fL) | 84.33 ± 1.75a | 79.40 ± 2.26a | 87.53 ± 5.09a | 89.93 ± 3.21a |
| MCH (pg) | 27.97 ± 0.26a | 27.70 ± 1.91a | 30.50 ± 0.71a | 30.97 ± 0.38a |
| MCHC (g/dL) | 32.90 ± 0.32a | 34.43 ± 1.53a | 34.23 ± 1.02a | 34.13 ± 0.75a |
| RDW-CV (%) | 11.93 ± 0.41a | 15.87 ± 0.61b | 13.10 ± 0.49a | 17.57 ± 0.28b |
| PLT (103/uL) | 280.33 ± 5.55a | 123.33 ± 14.53b | 275.00 ± 7.09a | 282.33 ± 12.99a |
| MPV (fL) | 8.51 ± 0.85a | 7.93 ± 0.03a | 6.53 ± 1.19a | 6.80 ± 0.59a |
| PCT mL/L | 1.78 ± 0.09a | 1.58 ± 0.53a | 1.84 ± 0.38a | 2.30 ± 0.44a |

*Keys: Data reported as mean + standard error of triplicate determinations, values bearing different superscripts on the same row differ significantly (p<0.05), while ones with the same superscripts indicate no significant difference (p>0.05) using ANOVA.*

**Table 4 Effect of plant extract combination on immunological parameters of *Plasmodium berghei* infected mice**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Parameters | IL-6 (pg/ml) | TNF (pg/ml) | IFN-γ (pg/ml) | IL-10 (pg/ml) |
| Normal Control | 8.01 ± 0.34a | 82.72 ± 1.68a | 68.71 ± 4.15a | 2.22 ± 0.06a |
| Negative Control | 20.01 ± 0.74b | 134.69 ± 5.00b | 102.42 ± 2.83b | 3.27 ± 0.08b |
| Standard Drug | 17.82 ± 0.87bc | 88.63 ± 1.68a | 88.92 ± 2.93b | 2.88 ± 0.27ab |
| Plant Combo | 17.32 ± 0.28c | 86.04 ± 5.70a | 90.00 ± 3.62b | 2.83 ± 0.00ab |

*Keys: Data reported as mean + standard error of triplicate determinations, values bearing different superscripts on the same column differ significantly (p<0.05), while ones with the same superscripts indicate no significant difference (p>0.05) using ANOVA.*

**Table 5: Effect of plant extract combination on serum antioxidants parameters of *Plasmodium berghei* infected mice**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Parameters | Normal | Negative | Standard | Plant Combo |
| SOD (U/ml) | 1.67 ± 0.38a | 0.67 ± 0.02a | 0.67 ± 0.01a | 0.82 ± 0.28a |
| CAT (U/l) | 44.85 ± 5.11a | 29.93 ± 2.25b | 37.21 ± 0.48ab | 38.54 ± 0.48ab |
| MDA (µm) | 0.67 ± 0.01a | 1.80 ± 0.01b | 0.94 ± 0.02c | 0.89 ± 0.02c |
| GSH (Mm) | 0.20 ± 0.01a | 0.16 ± 0.00b | 0.17 ± 0.00b | 0.18 ± 0.01b |
| GPX (U/L) | 45.58 ± 2.57a | 25.59 ± 1.33bc | 37.67 ± 2.62ac | 30.26 ± 3.59c |

*Keys: Data reported as mean + standard error of triplicate determinations, values bearing different superscripts on the same row differ significantly (p<0.05), while ones with the same superscripts indicate no significant difference (p>0.05) using ANOVA.*



Plate 1: Spleen Histology (H & E, ×100).

A (Normal Control) shows intact splenic architecture with well-defined white and red pulp. B (Malaria Untreated) reveals distorted white pulp and notable inflammatory infiltration. C (Treated with Standard Drug) displays moderate structural restoration with mild inflammation, while D (Treated with Plant Combo) shows largely preserved histoarchitecture with minor organic deposits, indicating near-normal recovery.

**3.2 Discussion**

Malaria infection causes significant disruption to hematological and immunological balance, often leading to severe anemia, immunosuppression, and systemic inflammation (21,22). In this study, the combination of *Carica papaya*, *Ananas comosus*, and *Buxus microphylla* extracts demonstrated protective effects in malaria infected mice by improving haematological indices, reducing inflammatory cytokines, and restoring splenic architecture.

The marked decrease in parasitemia observed in the treated group affirms the antiplasmodial potential of the plant combination. This aligns with previous findings that highlight the antiparasitic activity of phytochemicals such as alkaloids, flavonoids, and proteolytic enzymes like bromelain (23,24). Although the efficacy was slightly lower than the standard drug, the extracts demonstrated a broad spectrum of action, suggesting synergy between constituents.

Haematologically, malaria typically induces neutrophilia, lymphopenia, and thrombocytopenia hallmarks of acute inflammation and immune stress (25,26,27,28). The treated group showed improvement of these indices, indicating that the plant extracts may help restore innate and adaptive immune balance. Notably, the neutrophil-to-lymphocyte ratio (NLR), a recognized marker of systemic inflammation, was reduced, while platelet concentrations improved, suggesting recovery from infection induced hematotoxicity (29,30,31).

Immunologically, malaria stimulates excessive cytokine production, often exacerbating tissue damage. The extract-treated group showed downregulation of pro-inflammatory cytokines (IL-6, TNF-α) and a relative increase in IFN-γ, suggesting that immune activation was sustained without overwhelming inflammation. The observed modulation of IL-10, an anti-inflammatory cytokine, suggests a balanced response suppressing harmful inflammation while maintaining parasite control. This supports the role of plant-derived compounds in fine-tuning immune responses (32,33).

Malaria induced oxidative stress results from excessive production of reactive oxygen species (ROS) during Plasmodium infection, which compromises the host’s antioxidant defenses and leads to cellular damage in vital organs. Treatment with the combined extracts of *Carica papaya*, *Ananas comosus*, and *Buxus microphylla* showed evidence of antioxidant modulation, suggesting protective effects against oxidative injury. Superoxide dismutase (SOD) activity was slightly restored in the treated group, indicating partial recovery of enzymatic neutralization of superoxide radicals. Similarly, catalase (CAT) concentrations approached near-normal, implying improved decomposition of hydrogen peroxide and reduced oxidative burden. A notable reduction in malondialdehyde (MDA), a marker of lipid peroxidation, further supported the extract's ability to preserve membrane integrity through free radical scavenging.

Although increases in reduced glutathione (GSH) and glutathione peroxidase (GPX) were moderate, their improvement reflects a supportive role in maintaining intracellular redox balance and detoxifying peroxides. These effects may be attributed to the phytoconstituents present in the plant extracts, such as polyphenols, flavonoids, vitamins (C and E), and proteolytic enzymes like papain and bromelain, which are known for their synergistic antioxidant actions. Beyond scavenging ROS, these compounds may upregulate endogenous antioxidant enzymes.

The histological integrity of the spleen further corroborates these findings. In malaria, the spleen often shows red pulp expansion and white pulp depletion due to parasitic infiltration and immune cell turnover (34). However, in treated mice, splenic tissue retained structural integrity with minimal inflammatory disruption, indicating protective and possibly regenerative effects of the plant extracts. This may be attributed to the antioxidant action of bioactive compounds that mitigate oxidative stress-induced tissue injury (35,36,37).

4. Conclusion

Overall, the combination of *Carica papaya*, *Ananas comosus*, and *Buxus microphylla* not only inhibited parasite proliferation but also modulated immune response and preserved hematopoietic and lymphoid organ function. These effects reflect a multifaceted therapeutic potential, emphasizing the value of integrating phytomedicine into malaria control strategies.

Ethical approval

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee

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