World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

Check for updates

Evaluation of the anti-plasmodial effect of *Pleurotus pulmonarius* (Oyster mushroom) incorporated into cookies for potential adjuvant therapy in Nigeria

Caleb Adeyemi Adebiyi [1, *], Bolanle Aishat Akinwande [2], Sunday Samuel Taiwo [1], Rachel Oluwatoyin Adetola [2], Abiodun Adebimpe Adegoke [1] and Yemisi Olukemi Adesiji [1]

[1] *Department of Medical Microbiology and Parasitology, College of Health Sciences, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria.*
[2] *Department of Food Science, Ladoke Akintola University of technology Ogbomoso, Oyo State, Nigeria.*

World Journal of Advanced Research and Reviews, 2025, 27(02), 1662-1675

Publication history: Received on 10 July 2025; revised on 17 August 2025; accepted on 19 August 2025

Article DOI: https://doi.org/10.30574/wjarr.2025.27.2.2977

Abstract

Malaria continues to pose a major global health burden, particularly in sub-Saharan Africa, where drug-resistant strains of *Plasmodium* threaten current therapeutic efficacy. The search for novel, accessible antimalarial agents has highlighted the potential of medicinal mushrooms such as *Pleurotus pulmonarius*.

This study evaluated the anti-plasmodial, hematological and biochemical, effects of *Pleurotus pulmonarius*-fortified cookies as potential adjuvant therapy against *Plasmodium berghei* infection in mice.

Eighty male Swiss albino mice were divided into eight groups (n = 10) and treated with varying ratio of bread flour (BF) and mushroom flour (MF) cookies post-infection. Groups included a normal control, infected control, chloroquine-treated, and five treatment groups containing (20%–60%) MF. Key parameters assessed included parasitemia, hematological indices, liver and kidney function and histopathology.

The 40BF:60MF group (Group 8) showed the highest parasitemia reduction among cookie-treated mice, with post-treatment parasitemia at 13.35% compared to 8.38% in the chloroquine group (Group 3) and 67.43% in the untreated group (Group 2) ($p < 0.001$). Hematological analysis revealed significantly elevated RBC ($13.10 \pm 0.23 \times 10^{12}$/L) and hemoglobin (16.55 ± 0.26 g/dL) in the 80BF:20MF group (Group 4), while Group 8 showed favorable mean corpuscular volume (MCV: 56.08 ± 0.16 fL) and MCHC (53.04 ± 0.25 g/dL), indicating improved erythropoiesis. Platelet recovery was modest in mushroom-treated groups (Group 8: $54.39 \pm 0.20 \times 10^{9}$/L) compared to chloroquine ($1068.37 \pm 0.19 \times 10^{9}$/L).

These findings suggest *Pleurotus pulmonarius* may serve as a safe, nutritionally beneficial, and affordable nutraceutical adjunct in malaria management, meriting further clinical evaluation.

Keywords: *Pleurotus pulmonarius*; Malaria; Antiplasmodial; Nutraceutical; *Plasmodium berghei*; Immunomodulation; Mushroom Cookies; Organ Protection

1. Introduction

Malaria remains a major public health issue, particularly in sub-Saharan Africa, Asia, and South America, where it leads to substantial morbidity and mortality rates(Sarpong *et al.,* 2022). The World Health Organization (WHO) reports millions of malaria cases annually, with young children, pregnant women, and immunocompromised individuals being

* Corresponding author: Caleb Adeyemi Adebiyi

the most vulnerable (Oladipo *et al.,* 2022). Despite extensive efforts in malaria control, drug-resistant strains of *Plasmodium* parasites continue to emerge, undermining the efficacy of current treatments. The economic burden posed by malaria, including direct healthcare costs and loss of productivity, underscores the urgent need for new and effective anti-malarial agents (Li *et al.,* 2024).

Current anti-malarial therapies, such as artemisinin-based combination therapies (ACTs), face challenges in terms of efficacy, accessibility, and affordability, particularly in endemic regions with limited healthcare infrastructure. Moreover, the rise of artemisinin-resistant *Plasmodium falciparum* strains further threatens global malaria control *(*Oshagbemi *et al., 2023*).

Around half of the world's population is at risk of contracting malaria, which is prevalent in the majority of tropical nations (Africa, Asia, and Latin America) (Jin *et al.,* 2022). In 2018, there were 228 million instances of malaria, and an estimated 405,000 people died from the disease, according to the most recent World Malaria Report, which was published in December 2019 (Wang *et al.,* 2022). Plasmodium protozoans, including Plasmodium falciparum, Plasmodium malariae, Plasmodium ovale, and Plasmodium vivax, are the organisms that cause malaria infections; however, P. falciparum is responsible for the majority of severe illnesses. African children under the age of five account for the majority of documented fatalities. For any of the following causes, there is an immediate need for novel anti-malarial treatments:

Pleurotus pulmonarius, commonly referred to as the mini oyster mushroom or lung-shaped oyster mushroom, is a member of the Pleurotaceae family within the Agaricales order of the Basidiomycota phylum. This mushroom is globally recognized and renowned for its culinary appeal. Initially labeled *Pleurotus geesteranus*, commercialized cultivars of the mini oyster mushroom were eventually reclassified as *P. pulmonarius (*Zhang *et al., 2019)*. The basidioma of *P. pulmonarius* has a distinctive crisp-tender texture and an umami flavor profile. Rich in essential nutrients, such as proteins, vitamins, minerals, and essential amino acids (Yu *et al.,* 2024), this mushroom also contains a variety of bioactive compounds, including fungal polysaccharides, ergosterol, and γ-aminobutyric acid (Jin *et al.,* 2022). Its consumption has been associated with a wide range of health benefits, including antioxidative, antidiabetic, antiaging, and immune-enhancing effects (Wang *et al.,* 2022). Due to its impressive adaptability and stress resistance, *P. pulmonarius* can thrive on diverse agricultural and forestry waste materials (Liang *et al.,* 2022).

Mushrooms like *Pleurotus pulmonarius* are rich in bioactive compounds, including polysaccharides, terpenoids, and phenolic compounds, which have exhibited antimicrobial, antioxidant, anti-inflammatory, and anti-cancer properties. *Pleurotus pulmonarius* specifically has been studied for its nutritional and medicinal potential, showing promise in immunomodulatory, anti-diabetic, and anti-tumor applications (Wells, 2011). However, its anti-plasmodial potential remains underexplored. Given that other mushroom species have demonstrated anti-parasitic effects, it is plausible that *Pleurotus pulmonarius* may contain compounds capable of inhibiting *Plasmodium* growth or affecting its life cycle.

Beyond its anti-plasmodial potential, the research could open doors to exploring other therapeutic benefits of edible mushrooms, encouraging their broader integration into medicinal research and public health interventions.

2. Materials and method

2.1. Experimental design

This study employed an in-vivo approach using a Plasmodium-infected mice model to evaluate the anti-plasmodial potential of *Pleurotus pulmonarius*.

2.2. Experimental animals

Eighty male Swiss albino mice with weight of 25-30g were randomly assigned to 8 groups (n=10 per group). The animals were acclimatized for about two weeks under standard animal house conditions (Temperature 24 ± 2^{o}C humidity 50 ± 5%- and 12-hour light -dark cycle). International ethical guidelines regarding animal research handling were approved and followed by our Institutional Animal Ethical committee with the protocol number ERC/FBMS092/2025.

2.3. Sample collection

Pleurotus pulmonarius mushrooms were sourced from reputable commercial mushroom farms, cultivated in a controlled environment, Samples were collected from ADEPRESH agro allied and food processor Nigeria limited, Eleyele, Ibadan, Oyo State, Nigeria.

2.4. Collection of Mice

Healthy pathogen-free Swiss albino adult mice were obtained from a certified animal research facility. They all meet ethical and health standards for laboratory use. Mice were acclimatized to laboratory conditions for one week before the experiment to minimize stress and variability.

They were allowed to adjust to the environment for two weeks before the commencement of the study. They were kept in a well ventilated room with clean water and fed with standard livestock feed obtained from GLORY Animal feed factory, Ogbomoso, Nigeria.

2.5. Animal Groupings and Treatment

- MF: Mushroom flour
- BF: Bread flour
- Group1- Normal control (uninfected)
- Group 2: Infected and not treated
- Group 3: Infected and treated with chloroquine
- Group4: Infected and treated with 80BF and 20MF cookies
- Group5: Infected and treated with 70BF and 30MF cookies
- Group 6: Infected and treated with 60BF and 40MF cookies
- Group7: Infected and treated with 50BF and 50MF cookies
- Group8: Infected and treated with 40BF and 60MF cookies

2.6. Parasite Collection and Preparation

Plasmodium berghei was obtained from a recognized malaria research center with the necessary ethical approval. Donor mice infected with *P. berghei* were maintained to serve as a source of parasitized red blood cells (RBCs). Blood was collected from the donor mice using tail vein puncture or cardiac puncture under anesthesia to ensure minimal distress. The parasitized blood was then diluted with phosphate-buffered saline (PBS) to prepare a standard inoculum containing approximately 1×10^7 infected RBCs per 0.2 mL, which was used for experimental infection.

2.7. Measurements of Body Weight and Temperature

To monitor physiological responses to treatment, each mouse was weighed daily using a digital weighing scale. Rectal temperature measurements were taken daily using a digital thermometer. These parameters was recorded from Day 0 to Day 7 to observe changes associated with treatment.

2.8. Feeding Rate Assessment

The feeding rate was assessed by monitoring the daily food intake per mouse. The initial amount of food provided were measured at the beginning of each day, and the remaining quantity were recorded at the end of the day. This helped to evaluate the impact of treatment on appetite and overall health status.

2.9. Parasitemia Assessment

The level of parasitemia was determined using thin blood smear technique as described by (CDC, 2022). A small drop of blood was collected from the tail vein of each mouse and spread on a clean, grease-free slide. The thin film was fixed with methanol and air-dried before staining with 10% Giemsa stain for 10 minutes. The slides were washed under slow-running water, dried, and examined under a microscope. Parasitemia were assessed by counting infected red blood cells in randomly selected fields of view.

2.10. Hematology Analysis

Hematological parameters were assessed to determine the impact of *Pleurotus pulmonarius* formulated cookies on blood composition. Blood samples were collected and parameters such as packed cell volume (PCV), hemoglobin concentration (Hb), white blood cell (WBC) count, and red blood cell (RBC) count were measured using an automated hematology analyzer.

2.11. Sample collection and Storage

Animals from each group were anesthetized with ketamine, the mice were sacrificed and examined for tissue abnormalities. Samples of liver and kidney from all groups were immediately fixed in 10% formalin for a week,

embedded in paraffin, cut into 5 µm sections, placed on slides and stained with Hematoxylin-Eosin (H&E). The tissue sections were viewed under a light microscope (Nikon Y-S100, German). The samples were embedded in paraffin, sectioned (5um) and stained with haematoxylin and eosin (H&E) for histopathological examination.

2.12. Histopathology Analysis

For histopathological examinations, liver, kidney, and testicular tissues were collected post-mortem and fixed in 10% formalin (for liver and kidney). The fixed organs were embedded in paraffin, sectioned at 5 µm thickness, and stained with Hematoxylin and Eosin (H&E). Stained sections were examined under a Nikon Y-S100 light microscope for histopathological changes such as cellular degeneration, inflammatory infiltration, and tissue architecture alterations biochemical analysis

Blood sample was collected via cardiac puncture and centrifuged to obtain serum. Biochemical analysis was conducted to evaluate liver and kidney function. The levels of liver enzymes (alanine aminotransferase [ALT] and aspartate aminotransferase [AST]) and kidney function markers (creatinine and urea) were assessed.

2.13. Statistical Analysis

Data were analyzed using statistical software. The Student's *t*-test was used to compare mean parasitemia levels among groups, while one-way analysis of variance (ANOVA) was employed to compare differences in biochemical and hematological parameters across treatment groups. Statistical significance was set at $p < 0.05$.

3. Results

The analysis of temperature and weight changes across the eight groups reveals varying patterns (Figure 1). In terms of temperature, most groups experienced a slight decrease from pre- to post-measurement, except for Group 2, which showed an increase from 35.7°C to 36.9°C. Groups 1, 3, 4, 5, 6, 7, and 8 recorded mild decreases or relatively stable temperatures. Regarding weight, Group 1 showed a notable increase from 21g to 29g, while Group 2 experienced a decrease from 21g to 19g. Similarly, Group 6 showed a slight weight decrease from 23g to 22g. The remaining groups (Groups 3, 4, 5, 7, and 8) exhibited weight gains, with increases ranging between 2g and 5g.

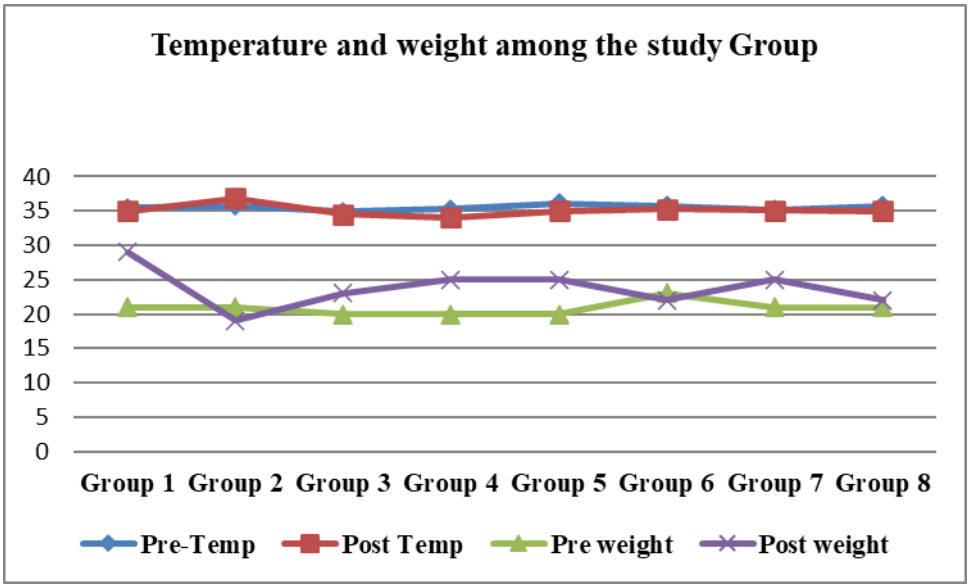

Figure 1 Temperature and weight among the study Group

Table 1 reveals significant differences in parasite burden and treatment response across the groups (p = 0.000). Group 1 (control) showed no change in parasite count (0.40 ± 0.21 pre- and post-treatment). Group 2 exhibited a poor response, with parasite count rising from 50.38 ± 0.20 to 134.46 ± 0.25. Groups 3 and 8 showed the most effective treatment outcomes, with parasite counts reduced from 48.43 ± 0.23 to 16.43 ± 0.23 and 44.47 ± 0.26 to 26.34 ± 0.17, respectively. In contrast, Groups 4 to 7 showed moderate reductions. Table 2 shows significant variations in white blood cell (WBC) counts, lymphocyte percentages, and mid cell counts across the experimental groups (p = 0.000). The highest WBC was recorded in Group 4 (21.89 ± 0.22 × 10^9/L), followed by Group 3 (18.64 ± 0.25), while the lowest was in Group

6 (7.36 ± 0.15). Lymphocyte percentages peaked in Group 3 (85.45 ± 0.26%) and were lowest in Group 7 (49.30 ± 0.17%). Group 1 had the highest mid cell count (14.37 ± 0.19 × 10^9/L), whereas Group 8 had the lowest (6.38 ± 0.19). These differences suggest variable immune responses among the groups. Table 3 presents statistically significant differences (p = 0.000) in hematological parameters among the experimental groups. Group 4 recorded the highest hemoglobin (16.55 ± 0.26 g/dL) and RBC count (13.10 ± 0.23 × 10^{12}/L), while Group 1 had the lowest hemoglobin (11.10 ± 0.17) and RBC count (7.61 ± 0.18). Group 3 showed an exceptionally high platelet count (1068.37 ± 0.19 × 10^9/L), contrasting with the lowest count in Group 8 (54.39 ± 0.20). The highest neutrophil count was in Group 4 (18.36 ± 0.18), while Group 3 had the lowest (4.34 ± 0.17). Mean corpuscular volume (MCV), MCH, and MCHC were highest in Group 8 (56.08 ± 0.16 fL; 29.36 ± 0.21 pg; 53.04 ± 0.25 g/dL), indicating macrocytic and hyperchromic features. Group 2 had the highest RDW-CV (24.89 ± 0.22), suggesting greater anisocytosis.

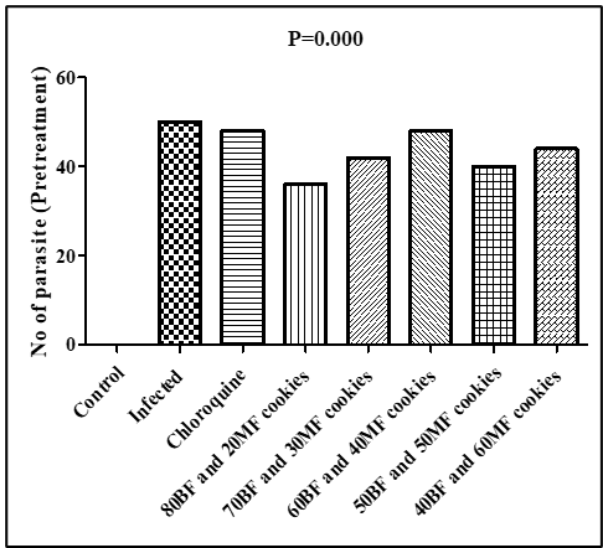

Figure 2 No of parasite (Pretreatment)

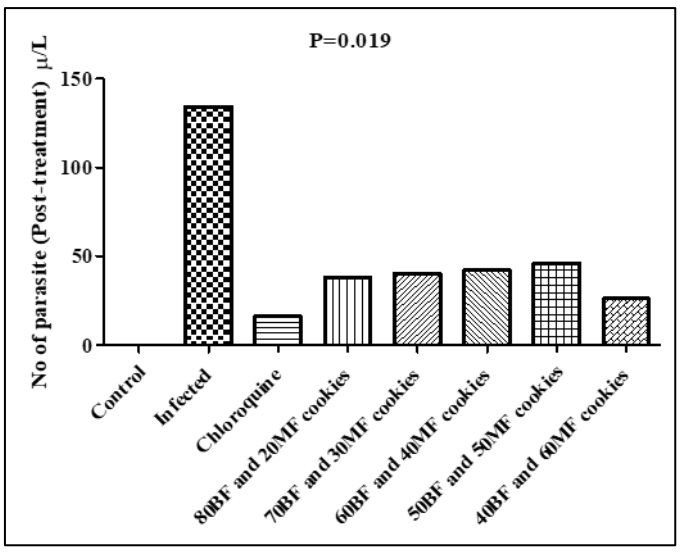

Figure 3 No of parasite (post treatment)

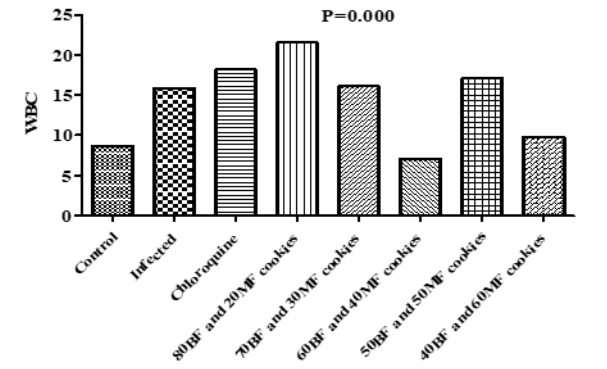

Figure 4 White blood cell (WBC) count

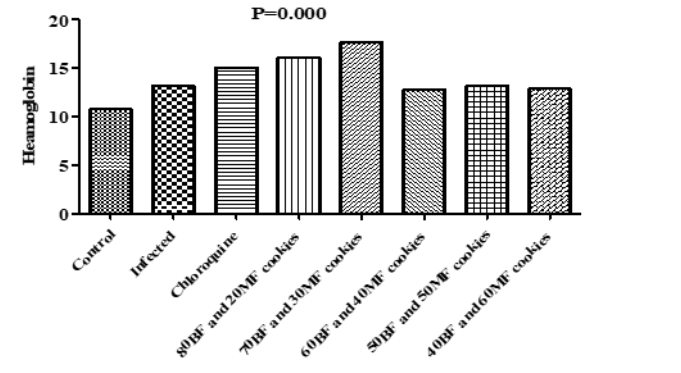

Figure 5 Hemoglobin (Hgb) data g/dL

Figure 6 reveals significant differences in liver enzyme levels AST, ALT, and ALP across the groups (p = 0.000), while total and conjugated bilirubin levels showed no significant variation (p = 0.929 and 0.992, respectively). The highest AST and ALT levels were observed in Group 3 (249.49 ± 0.17 U/L and 99.35 ± 0.16 U/L), indicating potential hepatic stress or damage. ALP was highest in Group 5 (92.34 ± 0.24 U/L) and lowest in Group 6 (63.87 ± 0.14 U/L). Despite enzyme fluctuations, bilirubin values remained relatively stable across all groups, suggesting preserved bilirubin clearance. Table 5 shows statistically significant differences in kidney function and electrolyte parameters across the experimental groups (p < 0.05 for all parameters). Group 4 had the lowest sodium (79.01 ± 0.15 mmol/L), chloride (71.28 ± 0.18 mmol/L), and bicarbonate (10.91 ± 0.16 mmol/L) levels, alongside the highest potassium (6.98 ± 0.18 mmol/L) and urea (52.88 ± 0.12 mg/dL), suggesting impaired renal function. In contrast, Group 6 showed the highest sodium (129.69 ± 0.18 mmol/L) and creatinine (1.72 ± 0.16 mg/dL), indicating possible dehydration or renal stress. Group 1 had normal to near-normal levels, serving as the baseline. Creatinine differences were less pronounced but still significant (p = 0.037).

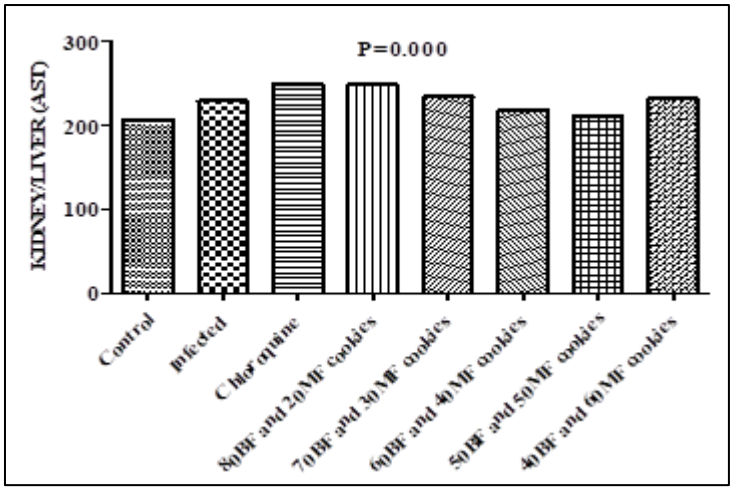

Figure 6 Kidney/ Liver function (AST)

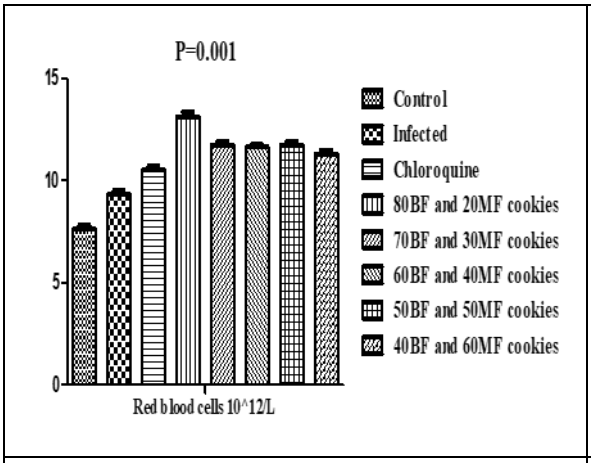

Figure 7 Red blood cell (RBC)

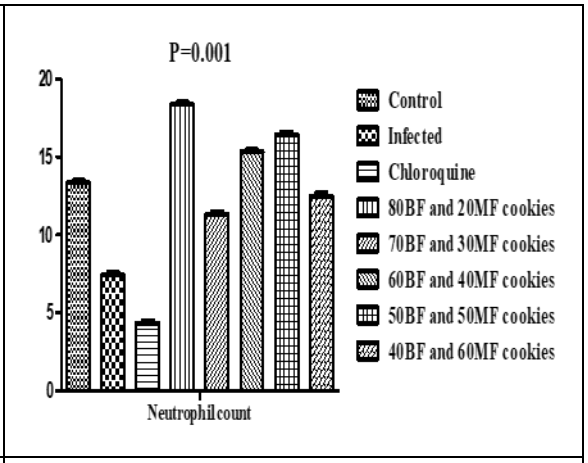

Figure 8 Neutrophil count

Table 1 Comparison of Kidney Function and Electrolyte Levels among Groups

Parameter	Sodium (mmol/L)	Potassium (mmol/L)	Chloride (mmol/L)	Bicarbonate (mmol/L)	Urea (mg/dL)	Creatinine (mg/dL)
Group 1	112.84±0.19	5.69±0.16	86.30±0.17	16.71±0.18	21.96±0.21	1.00±0.17
Group 2	95.99±0.21	5.33±0.23	85.59±0.20	11.10±0.18	43.91±0.26	1.16±0.22
Group 3	105.69±0.18	4.85±0.18	83.31±0.18	15.44±0.19	38.80±0.16	0.80±0.20
Group 4	79.01±0.15	6.98±0.18	71.28±0.18	10.91±0.16	52.88±0.12	0.91±0.21
Group 5	116.70±0.26	4.41±0.16	100.34±0.19	12.50±0.16	43.85±0.16	1.08±0.18
Group 6	129.69±0.18	3.89±0.14	86.30±0.17	15.30±0.17	39.77±0.20	1.72±0.16
Group 7	124.06±0.18	5.19±0.18	96.01±0.17	19.13±0.24	41.40±0.17	1.33±0.17
Group 8	124.34±0.23	4.55±0.18	99.60±0.20	18.68±0.18	45.25±0.14	1.59±0.19
P-value	0.000	0.000	0.000	0.000	0.000	0.037

Figure 4 reveals significant differences in WBC counts across the experimental groups ($p = 0.000$), reflecting varying immune responses due to infection, treatment, and diet. The control group (Group 1) had a normal WBC count of $9.00 \pm 0.17 \times 10^9$/L. Group 2 (infected, untreated) showed a sharp rise to 16.29 ± 0.23, indicating immune activation.

Group 3 (chloroquine-treated) had a further increase to 18.64 ± 0.25, suggesting enhanced immune response during parasite clearance. Among dietary groups, Group 4 (80% BF, 20% MF cookies) had the highest WBC count (21.89 ± 0.22), while Group 6 had the lowest (7.36 ± 0.15), possibly indicating suppressed immunity. Groups 5, 7, and 8 showed intermediate responses, suggesting diet-dependent modulation of immune activity. Figure 8 shows significant differences in neutrophil counts across the groups ($p = 0.000$), indicating varied innate immune responses influenced by infection, treatment, and diet. The control group (Group 1) had a baseline count of 13.34 ± 0.17. Group 2 (infected, untreated) showed a marked decrease to 7.40 ± 0.21, suggesting neutrophil depletion due to infection. Group 3 (chloroquine-treated) had the lowest count (4.34 ± 0.17), possibly reflecting infection resolution or treatment-induced suppression. Dietary groups showed diverse responses: Group 4 had the highest neutrophil count (18.36 ± 0.18), followed by Groups 7 (16.41 ± 0.22) and 6 (15.34 ± 0.17), indicating strong immune stimulation. Groups 5 (11.33 ± 0.17) and 8 (12.47 ± 0.26) showed moderate counts. These findings highlight the immunomodulatory effects of the interventions on neutrophil activity. Figure 7 shows a statistically significant variation ($p = 0.000$) in RBC counts across experimental groups, indicating the influence of infection, treatment, and dietary intervention on hematological status. The control group (Group 1) had a baseline RBC count of 7.61 ± 0.18 × 10^{12}/L. Group 2 (infected, untreated) showed a modest increase to 9.29 ± 0.20, likely due to a stress-induced erythropoietic response. Group 3 (chloroquine-treated) exhibited a further rise to 10.51 ± 0.18, suggesting improved erythropoiesis. Dietary groups (Groups 4–8) showed consistently higher RBC levels, with Group 4 (13.10 ± 0.23) recording the highest, implying that dietary interventions especially those rich in BF may enhance red cell production or stability.

Figure 4 Shows the results of the post-treatment % parasite count reveal varied responses across the different treatment groups. In the Control group, one subject showed a low parasite count post-treatment, suggesting some natural reduction of parasitemia without intervention. In the Infected group, one subject had a high parasite count, indicating that the infection persisted without treatment. The Chloroquine group demonstrated a positive effect, with one subject showing a low parasite count, indicating the drug's effectiveness. For the 80BF and 20MF cookies and 70BF and 30MF cookies groups, each had one subject with a moderate parasite count, indicating a partial reduction in parasitemia but not complete eradication. In contrast, the 60BF and 40MF cookies and 50BF and 50MF cookies groups had subjects with high parasite counts, suggesting these interventions did not effectively reduce the parasite load. Lastly, the 40BF and 60MF cookies group had one subject with a low parasite count, demonstrating that this specific composition showed some efficacy in reducing parasitemia.

The results of the post-treatment % parasite count reveal varied responses across the different treatment groups (Figure 7). In the Control group, one subject showed a low parasite count post-treatment, suggesting some natural reduction of parasitemia without intervention. In the Infected group, one subject had a high parasite count, indicating that the infection persisted without treatment. The Chloroquine group demonstrated a positive effect, with one subject showing a low parasite count, indicating the drug's effectiveness. For the 80BF and 20MF cookies and 70BF and 30MF cookies groups, each had one subject with a moderate parasite count, indicating a partial reduction in parasitemia but not complete eradication. In contrast, the 60BF and 40MF cookies and 50BF and 50MF cookies groups had subjects with high parasite counts, suggesting these interventions did not effectively reduce the parasite load. Lastly, the 40BF and 60MF cookies group had one subject with a low parasite count, demonstrating that this specific composition showed some efficacy in reducing parasitemia.

Table 2 % parasite Count (Post treatment)

% parasite Count (Post treatment)	Low	Moderate	High
Control	1	0	0
Infected	0	0	1
Chloroquine	1	0	0
80BF and 20MF cookies	0	1	0
70BF and 30MF cookies	0	1	0
60BF and 40MF cookies	0	0	1
50BF and 50MF cookies	0	0	1
40BF and 60MF cookies	1	0	0
Total	3	2	3

This study shows the distribution of post-treatment parasite count percentages reveals varying levels of response to the treatment among participants. Of the 8 individuals assessed, 37.5% (3) demonstrated a low parasite count, indicating a strong response to treatment with a substantial reduction in parasitemia. Half of the participants (50.0%, n=4) showed a moderate parasite count, reflecting partial treatment effectiveness. Only 12.5% (1) had a high parasite count after treatment, suggesting limited or poor response to the intervention. Figure 6 shows comparison of pre- and post-treatment parasite count percentages across the eight groups reveals diverse responses to the administered treatment. Group 1 had no detectable parasitemia before or after treatment, indicating no infection. Groups 3, 4, 5, 6, 7, and 8 exhibited reductions in parasite count post-treatment, suggesting a favorable response to the intervention. Notably, Group 3 showed a marked decrease from 24% to 8%, while Group 8 reduced from 22% to 13%, indicating significant parasitemia clearance. However, Group 2 showed an increase in parasite count from 25% to 67%, suggesting a poor or adverse response to treatment.

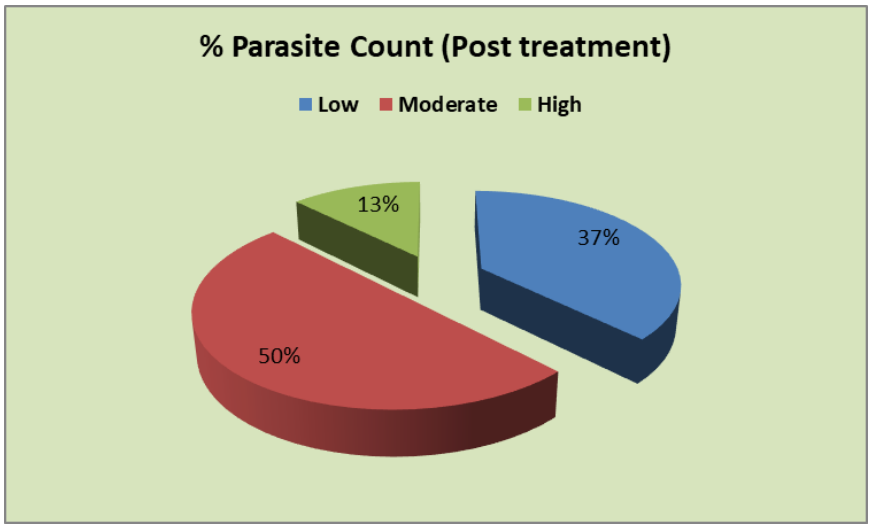

Figure 9 Percentage parasite count (Post-treatment)

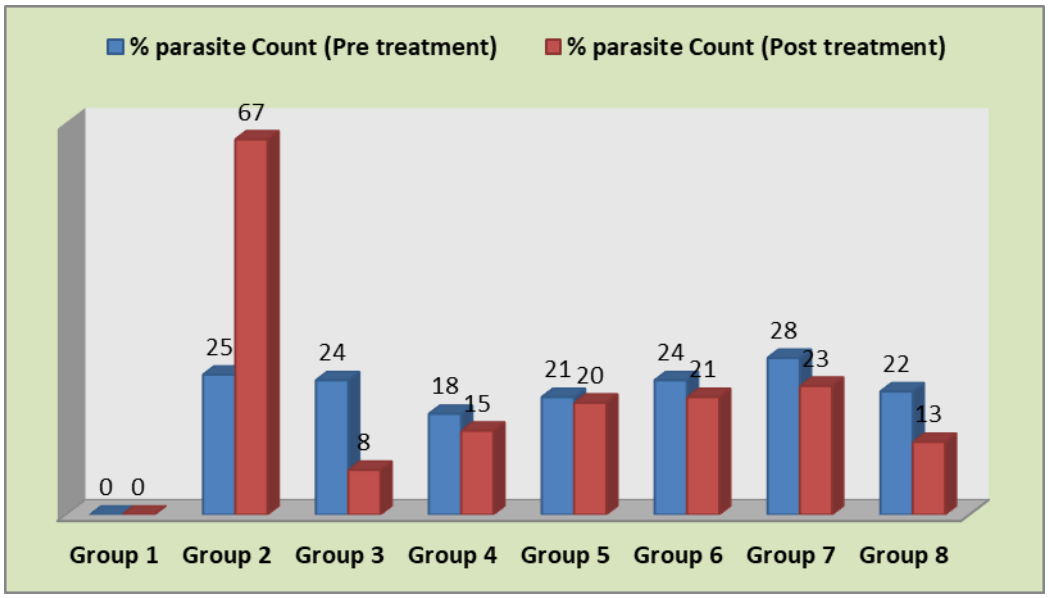

Figure 10 Comparison of pre- and post-treatment parasite count

The correlation analysis (Table 3) reveals several significant relationships among hematological parameters. Notably, a strong negative correlation exists between neutrophil percentage and WBC count ($r = -0.960$, $p = 0.000$), indicating that higher neutrophil levels are associated with reduced total WBC. Similarly, the mid cell value correlates negatively with WBC ($r = -0.722$, $p = 0.043$), suggesting an inverse relationship between mid-range cells and total leukocyte count. RBC count is moderately positively correlated with hemoglobin ($r = 0.642$, $p = 0.086$), though not statistically significant at

the 0.05 level. A strong positive correlation is observed between hematocrit and hemoglobin (r = 0.940, p = 0.001), confirming their close physiological link. NCH shows a very strong positive correlation with MCHC (r = 0.993, p = 0.000), while platelet count is negatively correlated with both NCH (r = -0.878, p = 0.004) and MCHC (r = -0.892, p = 0.003), suggesting that as platelet levels rise, red cell indices decrease. Additionally, RBC and RDW-CV are negatively correlated (r = -0.745, p = 0.034), implying that elevated RBC counts are associated with less variation in red cell size. Platelets show no significant correlation with RBC or HCT but have a weak, non-significant positive correlation with RDW-CV (r = 0.298, p = 0.473).

Total bilirubin shows a strong positive correlation with sodium (r = 0.849, p = 0.008) and a moderate negative correlation with potassium (r = -0.734, p = 0.038), suggesting that higher sodium levels are linked with increased bilirubin, while higher potassium levels correspond to reduced bilirubin levels. It also shows a positive, though marginally non-significant, correlation with bicarbonate (r = 0.683, p = 0.062), indicating a possible trend. In contrast, conjugated bilirubin does not significantly correlate with any measured electrolytes or metabolites, as all p-values exceed 0.05. Sodium (Na^+) correlates positively with chloride (r = 0.771, p = 0.025) and bicarbonate (r = 0.815, p = 0.014), suggesting coordinated electrolyte balance. It is negatively correlated with potassium (r = -0.825, p = 0.012), implying that an increase in sodium tends to coincide with a decrease in potassium. Potassium also shows a moderate, non-significant negative correlation with bicarbonate (r = -0.609, p = 0.109), hinting at an inverse relationship. Bicarbonate itself is positively associated with sodium and creatinine (r = 0.815, p = 0.014; r = 0.807, p = 0.015, respectively), linking electrolyte regulation with renal function and acid-base homeostasis. These findings highlight the intricate interplay between liver function, electrolyte balance, and renal markers in the experimental context.

Table 3 Correlation among hematological analysis

		Lymph %	**RBC**	**Hgb**	**HCT**	**MCV**	**NCH**	**MCHC**	**RDW-CV**	**Platelet**
WBC	r	-0.120	0.424	0.689	0.661	0.541	-0.078	-0.147	0.092	0.256
	p	0.777	0.295	0.059	0.074	0.166	0.854	0.728	0.828	0.541
Lymph %	r	1	-0.158	0.274	0.303	0.159	-0.089	-0.108	0.205	0.232
	p		0.709	0.511	0.465	0.708	0.834	0.799	0.626	0.580
Mid	r	-0.722*	-0.440	-0.432	-0.271	-0.282	-0.428	-0.429	0.403	0.256
	p	0.043	0.275	0.285	0.516	0.498	0.290	0.289	0.323	0.541
Neut	r	-0.960**	0.382	-0.143	-0.240	-0.077	0.285	0.308	-0.421	-0.399
	p	0.000	0.350	0.735	0.567	0.857	0.494	0.458	0.299	0.328
RBC	r	-0.158	1	0.642	0.390	0.372	0.360	0.350	-0.745*	-0.185
	p	0.709		0.086	0.339	0.364	0.381	0.395	0.034	0.661
Hgb	r	0.274	0.642	1	0.940**	0.456	0.050	0.005	-0.334	0.094
	p	0.511	0.086		0.001	0.256	0.906	0.991	0.419	0.826
HCT	r	0.303	0.390	0.940**	1	0.270	-0.170	-0.207	-0.172	0.201
	p	0.465	0.339	0.001		0.519	0.688	0.622	0.684	0.633
MCV	r	0.159	0.372	0.456	0.270	1	0.463	0.356	0.248	-0.192
	p	0.708	0.364	0.256	0.519		0.248	0.386	0.554	0.648
MCH	r	-0.089	0.360	0.050	-0.170	0.463	1	0.993**	-0.314	-0.878**
	p	0.834	0.381	0.906	0.688	0.248		0.000	0.449	0.004
MCHC	r	-0.108	0.350	0.005	-0.207	0.356	0.993**	1	-0.381	-0.892**
	p	0.799	0.395	0.991	0.622	0.386	0.000		0.351	0.003
	r	0.205	-0.745*	-0.334	-0.172	0.248	-0.314	-0.381	1	0.298

RDW-CV	p	0.626	0.034	0.419	0.684	0.554	0.449	0.351		0.473
Platelet	r	0.232	-0.185	0.094	0.201	-0.192	-.878**	-0.892**	0.298	1
	p	0.580	0.661	0.826	0.633	0.648	0.004	0.003	0.473	

*. Correlation is significant at the 0.05 level (2-tailed); **. Correlation is significant at the 0.01 level (2-tailed).

Table 4 Correlation analysis among biochemical parameters

		SODIUM NA+ (MMOL/L)	**POTASSIUM K+ (MMOL/L)**	**CHLORIDE CL- (MMOL/L)**	**BICARBONATE HC03- (MMOL/L)**	**UREA (MG/DL)**	**CREATININE(MG/DL)**
TOTAL BILURIBIN (MG/DL)	r	0.849**	-0.734*	0.569	0.683	-0.541	0.507
	p	0.008	0.038	0.141	0.062	0.166	0.200
CONJUGATED BILURIBIN	r	0.408	-0.336	0.285	0.201	-0.526	-0.123
	p	0.315	0.416	0.494	0.633	0.180	0.772
SODIUM NA+ (MMOL/L)	r	1	-0.825*	0.771*	0.815*	-0.374	0.768*
	p		0.012	0.025	0.014	0.362	0.026
POTASSIUM K+ (MMOL/L)	r	0.825*	1	-0.687	-0.609	0.157	-0.628
	p	0.012		0.060	0.109	0.710	0.095
CHLORIDE CL- (MMOL/L)	r	0.771*	-0.687	1	0.282	-0.122	0.496
	p	0.025	0.060		0.498	0.773	0.211
BICARBONATE HC03- (MMOL/L)	r	0.815*	-0.609	0.282	1	-0.315	0.807*
	p	0.014	0.109	0.498		0.447	0.015
UREA (MG/DL)	r	-0.374	0.157	-0.122	-0.315	1	0.021
	p	0.362	0.710	0.773	0.447		0.960
CREATININE(MG/DL)	r	0.768*	-0.628	0.496	0.807*	0.021	1
	p	0.026	0.095	0.211	0.015	0.960	

Table 5 presents the histopathological effects of Pleurotus pulmonarius (mushroom flour) at different doses in comparison to chloroquine therapy and a control group with no treatment in malaria-infected mice. Tissues from the liver, kidney, and spleen were assessed for indications of inflammation, necrosis, and degenerative changes. Mild inflammation, necrosis, and degeneration were found in the liver and kidneys of the untreated infected group. Mice treated with chloroquine had few inflammatory alterations, indicating excellent protection. Mice administered with escalating quantities (20%–60%) of mushroom flour demonstrated dose-dependent enhancements. Moderate pathology persisted at lower concentrations (20%–30%), but elevated dosages (50%–60%) exhibited less tissue damage, particularly in the spleen and kidneys.

Table 5 Histopathological Effects of Treatments on Organs in Malaria-Infected Mice

Group	**Table**	**Inflammation**	**Necrosis**	**Degenerative changes**
Infected and not treated(Group 2)	Liver	None	None	None
	Kidney	None	None	None
	Spleen	None	None	None

	Tissue	Inflammation	Necrosis	Degenerative changes
Infected and not treated(Group 2)	Liver	Mild with mild oedema and mild inflammatory cells	+	+
	Kidney	None	+	None
	Spleen	None	None	None
Infected and treated with chloroquine (Group 3)	Liver	Mild with scanty inflammatory cells, no oedema	None	None
	Kidney	None	None	None
	Spleen	None	None	None
	Tissues	Inflammation	Necrosis	Degenerative
Infected and treated with 20% mushroom flour (Group 4)	Liver	Mild oedema and inflammatory cells	Mild to none	Mild
	Kidney	Mild inflammation of the glomeruli and interstitium	Moderate tubular necrosis	Moderate
	Spleen	Mild oedema	None	None
Infected and treated with 30% mushroom flour (Group 5)	Liver	Mild oedema	Mild to none	None
	Kidney	Mild	Mild necrosis	mild
	Spleen	Mild to Moderate inflammation	None	None
Infected and treated with 40% mushroom flour (Group 6)	Liver	Mild to moderate inflammation	Mild necrosis	Mild
	Kidney	Mild to moderate inflammation	Mild tubular necrosis	Mild
	Spleen	Mild inflammation	None	None
Infected and treated with 50% mushroom flour (Group 7)	Liver	Moderate inflammation and oedema	Mild	Mild
	Kidney	Moderate inflammation and oedema	Mild necrosis	Mild
	Spleen	Moderate oedema	None	None
Infected and treated with 60% mushroom flour (Group 8)	Liver	Moderate inflammation and oedema	Mild to moderate	Mild
	Kidney	None to mild	Mild	None to Mild
	Spleen	Mild	None	None

4. Discussion

This study demonstrates the dose-dependent anti-plasmodial and immunomodulatory effects of *Pleurotus pulmonarius* cookie formulations in *Plasmodium berghei*-infected mice. Among the various treatment groups, the 40BF:60MF formulation (Group 8) was the most effective cookie-based intervention, achieving a post-treatment parasitemia of 13%, outperforming other formulations (19–23%) but remaining slightly less effective than chloroquine-treated mice (8%). This finding is consistent with the report by Oluranti *et al.* (2018), where *P. pulmonarius* extract at 0.4 mg/mL significantly reduced parasitemia in infected mice, with comparable efficacy to chloroquine. The present results suggest that embedding the extract in a food matrix may enhance its bioavailability and therapeutic effect.

Comparison with other studies highlights the interspecies variability in anti-plasmodial efficacy. For instance, *Pleurotus ostreatus* demonstrated relatively lower activity with an IC_{50} of 25.18 µg/mL, while *Myrianthus libericus* stem bark extract suppressed parasitemia by 77.11% at 200 mg/kg, albeit at a much higher dose. These observations suggest that while *P. pulmonarius* is moderately effective, its therapeutic potential may be optimized through improved formulation, standardization of active compounds, or combination therapies. The safety and nutritional profile of the mushroom, however, positions it as a valuable candidate for adjunctive malaria therapy.

Hematological parameters revealed the immunomodulatory potential of *P. pulmonarius*, with Group 8 displaying normalized leukocyte values (WBC: 9.8 $\times 10^3/\mu L$, Lymphocyte%: 82%) similar to uninfected controls. This contrasts with Group 2 (infected, untreated), which showed leukocytosis (WBC: 15.9 $\times 10^3/\mu L$) and elevated lymphocyte levels (83.2%), indicative of a strong but dysregulated immune response. Chloroquine-treated mice (Group 3) demonstrated even greater leukocytosis (18.2 $\times 10^3/\mu L$), aligning with reports that chloroquine can provoke excessive immune stimulation in *Plasmodium yoelii*-infected mice (Zhou *et al.,* 2015). The immunoregulatory properties of *P. pulmonarius* observed in this study are supported by previous findings (Jonathan *et al.,* 2012), which demonstrated enhanced leukopoiesis and phagocytic activity following mushroom extract administration.

Notably, in this study neutrophil counts varied significantly among groups. Group 4 , group 6 and group 7 showed a significant increase in neutrophil count relatively to the control group, this increased could be as a result of significant increase in the mean parasite counts observed in each of the group, this findings corroborate with the submission of stimulation of cytokines may increase immune response and activate neutrophil complement receptors as described by (Torres-Martínez *et al.,* 2022) hematological indices such as RBC count, hemoglobin (Hgb), hematocrit (HCT), mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC) provided additional insights. Group 4 (80BF:20MF) recorded the highest RBC and Hgb levels, suggesting protective effects against malaria-induced anemia. Elevated MCV in infected and some treated groups suggested macrocytosis, a typical response to hemolytic stress or recovery from anemia. Meanwhile, chloroquine-treated mice exhibited normalized erythropoietic parameters, including lower MCHC and nucleated RBCs (NCH), indicating treatment-induced stabilization of red cell production. These observations align with reports that *P. pulmonarius* supports hematopoietic recovery following parasitic infections (Rijia *et al.,* 2025).

Platelet counts further supported the therapeutic potential of *P. pulmonarius*. Group 3 (chloroquine-treated) showed significantly elevated platelet levels (1068 $\times 10^9/L$), suggesting a rebound thrombopoietic effect. Group 2 (infected) had markedly reduced platelets (108 $\times 10^9/L$), consistent with malaria-induced thrombocytopenia (WHO, 2005). Among the mushroom-treated groups, platelet recovery was observed but remained lower than in chloroquine-treated animals, suggesting moderate but beneficial effects. Group 5 (70BF:30MF) showed relatively improved values for platelet count and other hematological indices, indicating that moderate MF inclusion might offer a more balanced therapeutic effect.

Liver function analysis revealed differential impacts across treatment groups. Elevated liver enzymes (AST and ALT) in the chloroquine group indicated possible hepatotoxic effects, as previously reported by (Song *et al.,* 2018). In contrast, groups treated with *P. pulmonarius* formulations generally exhibited lower liver enzyme values, implying reduced hepatic stress and safer profiles. This suggests that mushroom-based interventions may avoid the hepatic side effects associated with conventional antimalarial.

Electrolyte and renal function analysis provided additional evidence for the organ-protective effects of *P. pulmonarius*. Group 2 showed significant renal impairment, characterized by low sodium (Na^+), bicarbonate (HCO_3^-), and elevated urea and creatinine, indicating malaria-induced kidney dysfunction. Chloroquine partially normalized these values but did not fully restore electrolyte balance. Interestingly, Group 6 (60BF:40MF) had the highest sodium and bicarbonate levels, with stable urea and creatinine levels, suggesting potential nephroprotective effects. However, other formulations showed variable efficacy, underscoring the importance of formulation optimization.

5. Conclusion

This study highlights the dose-dependent anti-plasmodial and immunomodulatory effects of *Pleurotus pulmonarius* cookie formulations in *Plasmodium berghei*-infected mice. Among the treatment groups, the 40BF:60MF formulation demonstrated the most effective parasitemia suppression and normalization of hematological and biochemical parameters, though it remained less potent than chloroquine. The mushroom formulations exhibited beneficial effects on leukocyte profiles, red blood cell counts, platelet recovery, and liver and kidney function, with lower toxicity risks compared to standard antimalarial. These findings underscore the potential of *P. pulmonarius* as a nutraceutical adjunct in malaria therapy, especially in resource-limited settings. However, the variability in therapeutic outcomes across different formulations emphasizes the need for further research to optimize dosage, enhance bioavailability and identify

active compounds. *pulmonarius* presents a promising, safe, and affordable alternative or complementary intervention in malaria management, warranting further investigation through clinical studies and mechanistic exploration.

Compliance with ethical standards

Acknowledgments

This study was funded by the Tertiary Education Trust Fund (TET Fund), Nigeria, under the 2024 Institution-Based Research (IBR) intervention (Grant No. TETF/DR&D/CE/UNI/[015]/2024)

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

Ethical approval (ERC Approval Number: ERCFBMSLAUTECH:105/04/2025) was obtained from Ladoke Akintola University of Technology Ethical Research Committee, Ogbomoso, Oyo State with an Issuance of an ethical clearance certificate from the ethics committee. All participants were informed of the details of the study before samples were collected.

References

[1] Akyüz, M., İnci, Ş., & Kırbağ, S. (2023). Evaluation of Antimicrobial, Antioxidant, Cytotoxic and DNA Protective Effects of Oyster Mushroom: Pleurotus pulmonarius (Fr.) Quel. *Arabian Journal for Science and Engineering*, *48*(6), 7273–7283. https://doi.org/10.1007/s13369-022-07418-9

[2] dos Reis, E. E., Schenkel, P. C., & Camassola, M. (2022). Effects of bioactive compounds from Pleurotus mushrooms on COVID-19 risk factors associated with the cardiovascular system. *Journal of Integrative Medicine*, *20*(5), 385–395. https://doi.org/10.1016/j.joim.2022.07.002

[3] Jin, M., Xie, Y., Xie, P., Zheng, Q., Wei, T., Guo, L., Lin, J., Ye, Z., & Zou, Y. (2022). Physicochemical and functional properties of Pleurotus geesteranus proteins. *Food Research International (Ottawa, Ont.)*, *162*(Pt A), 111978. https://doi.org/10.1016/j.foodres.2022.111978

[4] Li, J., Docile, H. J., Fisher, D., Pronyuk, K., & Zhao, L. (2024). Current Status of Malaria Control and Elimination in Africa: Epidemiology, Diagnosis, Treatment, Progress and Challenges. *Journal of Epidemiology and Global Health*, *14*(3), 561–579. https://doi.org/10.1007/s44197-024-00228-2

[5] Liang, Z., Yin, Z., Liu, X., Ma, C., Wang, J., Zhang, Y., & Kang, W. (2022). A glucomannogalactan from Pleurotus geesteranus: Structural characterization, chain conformation and immunological effect. *Carbohydrate Polymers*, *287*, 119346. https://doi.org/10.1016/j.carbpol.2022.119346

[6] Oladipo, H. J., Tajudeen, Y. A., Oladunjoye, I. O., Yusuff, S. I., Yusuf, R. O., Oluwaseyi, E. M., AbdulBasit, M. O., Adebisi, Y. A., & El-Sherbini, M. S. (2022). Increasing challenges of malaria control in sub-Saharan Africa: Priorities for public health research and policymakers. *Annals of Medicine and Surgery (2012)*, *81*, 104366. https://doi.org/10.1016/j.amsu.2022.104366

[7] Oshagbemi, O. A., Lopez-Romero, P., Winnips, C., Csermak, K. R., Su, G., & Aubrun, E. (2023). Estimated distribution of malaria cases among children in sub-Saharan Africa by specified age categories using data from the Global Burden of Diseases 2019. *Malaria Journal*, *22*, 371. https://doi.org/10.1186/s12936-023-04811-z

[8] Rijia, A., Krishnamoorthi, R., Kaviyadharshini, M., & Mahalingam, P. U. (2025). Unlocking fungal fortunes: Cutting-edge extraction techniques and therapeutic possibilities of mushroom bioactive compounds. *Food Chemistry Advances*, *6*, 100895. https://doi.org/10.1016/j.focha.2025.100895

[9] Sarpong, E., Acheampong, D. O., Fordjour, G. N. R., Anyanful, A., Aninagyei, E., Tuoyire, D. A., Blackhurst, D., Kyei, G. B., Ekor, M., & Thomford, N. E. (2022). Zero malaria: A mirage or reality for populations of sub-Saharan Africa in health transition. *Malaria Journal*, *21*(1), 314. https://doi.org/10.1186/s12936-022-04340-1

[10] Song, X., Liu, Z., Zhang, J., Yang, Q., Ren, Z., Zhang, C., Liu, M., Gao, Z., Zhao, H., & Jia, L. (2018). Anti-inflammatory and hepatoprotective effects of exopolysaccharides isolated from Pleurotus geesteranus on alcohol-induced liver injury. *Scientific Reports*, *8*(1), 10493. https://doi.org/10.1038/s41598-018-28785-0

[11] Torres-Martínez, B. del M., Vargas-Sánchez, R. D., Torrescano-Urrutia, G. R., Esqueda, M., Rodríguez-Carpena, J. G., Fernández-López, J., Perez-Alvarez, J. A., & Sánchez-Escalante, A. (2022). Pleurotus Genus as a Potential Ingredient for Meat Products. *Foods*, *11*(6), 779. https://doi.org/10.3390/foods11060779

[12] Wang, Q., Meng, L., Wang, X., Zhao, W., Shi, X., Wang, W., Li, Z., & Wang, L. (2022). The yield, nutritional value, umami components and mineral contents of the first-flush and second-flush Pleurotus pulmonarius mushrooms grown on three forestry wastes. *Food Chemistry*, *397*, 133714. https://doi.org/10.1016/j.foodchem.2022.133714

[13] Wells, T. N. (2011). Natural products as starting points for future anti-malarial therapies: Going back to our roots? *Malaria Journal*, *10*(1), S3. https://doi.org/10.1186/1475-2875-10-S1-S3

[14] Yu, Y., Liu, T., Wang, Y., Liu, L., He, X., Li, J., Martin, F. M., Peng, W., & Tan, H. (2024). Comparative analyses of Pleurotus pulmonarius mitochondrial genomes reveal two major lineages of mini oyster mushroom cultivars. *Computational and Structural Biotechnology Journal*, *23*, 905–917. https://doi.org/10.1016/j.csbj.2024.01.021

[15] Zhang, Z., Zhang, X., Xin, G., Gong, X., Wang, Y., Wang, L., & Sun, B. (2019). Umami taste and its association with energy status in harvested Pleurotus geesteranus stored at different temperatures. *Food Chemistry*, *279*, 179–186. https://doi.org/10.1016/j.foodchem.2018.12.010