THE EFFECT OF *Plasmodium spp* ON BLOOD USING ALBINO WISTAR RATS AND BIOCHEMICAL MARKERS

BY

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DEPARTMENT OF MEDICAL LABORATORY SCIENCE
SCHOOL OF BASIC MEDICAL SCIENCES
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THIS PROJECT IS SUBMITTED TO:
THE DEPARTMENT OF MEDICAL LABORATORY SCIENCE,
SCHOOL OF BASIC MEDICAL SCIENCES
UNIVERSITY OF BENIN IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELOR OF MEDICAL LABORATORY SCIENCE DEGREE

SUPERVISOR:

DR. MRS. E. E. ADEYEMI (*fppsn*)

OCTOBER, 2025

CERTIFICATION

This is to certify that this project work was satisfactory carried out by **IGUNBOR PEACE OSARIEMEN (MISS)** with matriculation number: **BMS2001166** in Department of Medical Laboratory Science, University of Benin, Benin City, under my supervision in partial fulfillment for the award of Bachelor of Medical Laboratory Science (BMLS) Degree.

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EXTERNAL EXAMINER **DATE**

DEDICATION

I dedicate this project work to God Almighty, for making this work a great success, to my lovely parent, my dad LATE MR AUGUSTINE OBIAJULU MARTINS, for believing me even unto death, thank you for being best father and continue to rest in the Bosom of the lord.

ACKNOWLEDGEMENTS

I give thanks to God Almighty for His grace upon my life and for seeing me through this project work. My appreciation goes to my supervisor DR. (MRS) B.E OGEYEMHE for her concern, constructive and supportive ideas which has aided this work. A special thanks goes to the Head of Department, Medical Laboratory Science, Dr (Mrs) Zainab Omoruyi for her support in ensuring smooth conclusion this project work and also the entire staff of the department for investing so much in my academic development. My appreciation also goes to my lecturers in histopathology Dr. Mrs. B. E. Ogeyemhe,, Dr. N.T Omorodion, Dr. Mrs Ehizogie Adeyemi, Dr. E. B. Odigie and Prof Akinbo, for their constant advice and encouragement. God bless them abundantly. My deepest gratitude goes to my family and loved ones. my dad LATE MR AUGUSTINE MARTINS and my mom MRS EDITH MARTINS, my siblings(Mrs Orie, Jennifer, Ethel, Clement), my brothers (D' 5 RARE GEM), my friends (MICHAEL and RAPHAEL), my Uniben sister (AKOMEN), my community (JERMART FAMILY), Pastor Cyril, Michalis for their encouragements, prayers, advices and all round support. I am grateful to MR FRANK EZE CHIEDU for his support and unwinding encouragement towards the success of this project and my Academics in General, Lastly, a big shout out goes to my colleague (HISTOPATHOLOGY '25) and coursemates in Medical laboratory science, you made my process through this degree one to cherish and remember for the rest of my life, God bless.

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ABSTRACT

Plasmodium berghei, a rodent malaria parasite, has been widely employed as a model for studying malaria-induced pathophysiology. This study investigated the hematological and biochemical alterations associated with graded parasitemia in albino rats experimentally infected with P. berghei. Sixteen (n = 16) female albino rats weighing 130–174 g were randomly divided into four groups (n = 4 per group): a control group (uninfected) and three treatment groups infected with low (~1×10^2), medium (~1×10^4), and high (~1×10^6) concentrations of parasitized red blood cells (iRBCs). Inoculation was performed intraperitoneally and animals were monitored for 42 days under standard housing conditions. Hematological parameters, including WBC, RBC, Hb, PCV, and differential leukocyte counts, were assessed using an automated hematology analyzer, while serum electrolytes, urea, and creatinine were measured to evaluate renal function. The results revealed significant changes in hematological indices across groups ($p < 0.05$). Rats in the high-infection group showed marked leukocytosis, neutrophilia, and monocytosis, alongside reductions in RBC count, hemoglobin concentration, and packed cell volume compared to controls. Lymphocyte percentages were significantly elevated in medium- and high-infection groups, whereas eosinophil counts were markedly reduced in all infected groups. Biochemical analysis indicated a significant rise in serum urea levels in infected groups ($p = 0.019$), while serum creatinine remained unchanged ($p = 0.184$). These findings suggest that P. berghei infection induces dose-dependent hematological derangements and renal functional alterations, with elevated urea serving as a potential biomarker of malaria-associated renal stress. Further studies are recommended to delineate the mechanisms linking parasitemia severity to hematological and renal pathology.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of Study

Malaria remains one of the most devastating infectious diseases worldwide, with over 240 million cases and nearly 600,000 deaths reported annually, particularly in sub-Saharan Africa (World Health Organization, 2022). Despite significant advancements in diagnostics and vector control, the disease persists due to the complex life cycle of *Plasmodium* species and increasing resistance to antimalarial drugs (Simwela and Waters, 2022). Studying malaria in human populations presents ethical and logistical limitations; thus, animal models have become indispensable for understanding the disease's pathophysiology, especially its hematological impacts (Basir *et al*., 2012). Malaria primarily targets the blood, initiating a cascade of hematological disruptions, including hemolysis, anemia, thrombocytopenia, leukocytosis, and bone marrow suppression (Lamb *et al*., 2006). The erythrocytic stage of the *Plasmodium* life cycle, where parasites invade and rupture red blood cells, is directly responsible for many clinical symptoms observed in infected hosts (Simwela and Waters, 2022). These effects are recapitulated in animal models, such as mice infected with *Plasmodium berghei* or *P. chabaudi*, providing valuable insight into the mechanisms of disease progression (Jiménez-Díaz *et al*., 2014).

Animal models, particularly murine systems, serve as a robust platform to study the hematological consequences of malaria under controlled laboratory conditions (Olatunde *et al*., 2022). The use of inbred strains like BALB/c or C57BL/6 allows for uniform responses to infection, reducing variability and enabling reproducible findings (Basir *et al*., 2012). In these

models, parasitemia levels, anemia severity, and inflammatory responses can be precisely monitored, offering a translational bridge to human pathology (Lamb *et al*., 2006). In experimental models, malaria-induced anemia results from a combination of factors: destruction of parasitized and non-parasitized erythrocytes, suppression of erythropoiesis, and dyserythropoiesis (Adekunle *et al*., 2007). Hemolysis in infected animals is exacerbated by immune-mediated destruction of red cells and oxidative stress induced by the parasite's metabolic activities (Mamudu *et al*., 2025). Platelet counts typically decrease in both human and animal infections, with thrombocytopenia considered a potential biomarker for malaria severity (Ghazanfari *et al*., 2018).

Leukocyte profiles are also altered during infection, with some models showing neutrophilia and lymphopenia, reflective of systemic inflammatory responses (Cornwall *et al*., 2022). Animal studies have demonstrated elevated levels of pro-inflammatory cytokines such as TNF-α and IFN-γ, which contribute to the immunopathology of severe malaria, including cerebral involvement (Hansen, 2012). This immune overactivation further aggravates hematologic injury and may impair host recovery (Murambiwa *et al*., 2020). Beyond erythrocyte destruction, malaria parasites induce biochemical alterations that compromise the host's metabolic and hematological homeostasis (Adekunle *et al*., 2007). Studies in rats and mice reveal significant disruptions in serum proteins, iron metabolism, and liver enzymes during infection, highlighting systemic toxicity beyond the bloodstream (Conrad, 1969). These findings underscore the utility of animal models in tracing the multi-systemic effects of *Plasmodium* infection (Simwela and Waters, 2022).

Emerging research using humanized mouse models provides an even closer approximation to human responses, allowing exploration of drug efficacy, immune dynamics, and blood parameter shifts in response to infection (De Niz and Heussler, 2018). Additionally, animal models are essential for preclinical vaccine testing and screening of blood-stage antimalarial compounds (Langhorne *et al*., 2002). While animal models do not perfectly replicate all aspects of human malaria, they remain critical tools for understanding the hematological consequences of the disease and testing therapeutic interventions (Simwela and Waters, 2022; Hansen, 2012).

1.2 Statement of Problem

Malaria continues to pose a significant global health challenge, particularly in tropical and subtropical regions, accounting for millions of clinical cases and hundreds of thousands of deaths annually, predominantly among vulnerable populations such as children and pregnant women (Cornwall *et al*., 2022; Naser *et al*., 2024). The pathological hallmark of malaria is its impact on the blood, particularly during the erythrocytic stage of the parasite's lifecycle, which directly compromises red blood cell integrity and function (Langhorne *et al*., 2011; Li *et al*., 2001). Despite substantial investment in malaria vaccine and drug development, progress has been hindered by the incomplete understanding of host–parasite interactions and the specific hematological alterations induced by the disease (Leitner *et al*., 2010; Kotepui *et al*., 2015). One of the major limitations in this area of research is the ethical and technical difficulty of studying these effects directly in humans, especially during early or asymptomatic stages of infection (Simwela and Waters, 2022; Tiiba *et al*., 2023).

Animal models, particularly murine models infected with *Plasmodium berghei*, *P. chabaudi*, or *P. yoelii*, are widely used as experimental proxies to study the pathophysiology of malaria (De

Niz and Heussler, 2018; Olatunde *et al*., 2022). However, many of these models exhibit species-specific immune responses and hematological patterns that do not fully replicate human malaria (Buffet *et al*., 2011; Miller *et al*., 2012). Consequently, there is a growing concern that conclusions drawn from these models may not be reliably translated into human contexts (Cornwall *et al*., 2022; Lin *et al*., 2017).

A persistent problem is the variation in hematological outcomes such as anemia, thrombocytopenia, leukocytosis, and dyserythropoiesis observed across different animal species and experimental conditions, which complicates the establishment of standard hematological markers for malaria severity and treatment efficacy (Adekunle *et al*., 2007; Gramaglia *et al*., 2005; Obaldía, 2007; Piguet *et al*., 2002). Moreover, the impact of malaria on biochemical indices like serum iron, bilirubin, and liver enzymes in animals suggests systemic toxicity that extends beyond the blood, yet these systemic interactions are poorly understood and rarely integrated into diagnostic or therapeutic frameworks (Arise *et al*., 2012; Kotepui *et al*., 2015). Given the centrality of hematological changes in the clinical manifestation and diagnosis of malaria, it is imperative to refine animal models that can reliably mimic human blood responses to infection (Leitner *et al*., 2010; Naser *et al*., 2024). The current lack of consensus on which model best represents human hematological changes, coupled with gaps in mechanistic knowledge, undermines our ability to develop precise antimalarial therapies and diagnostic tools (Simwela and Waters, 2022; Hansen, 2012).

1.3 Justification of the Study

Understanding the hematological effects of malaria is crucial for enhancing disease management strategies (Simwela and Waters, 2022), particularly because the blood stage of *Plasmodium*

infection causes most clinical symptoms (Buffet *et al*., 2011), including anemia (Basir *et al*., 2012), thrombocytopenia (Basir *et al*., 2012), and leukocytic imbalances (Hansen, 2012). However, human studies are limited by ethical constraints (Tiiba *et al*., 2023), biological variability (Cornwall *et al*., 2022), and the inability to perform invasive or controlled interventions (Simwela and Waters, 2022), which are essential for understanding complex disease dynamics (Buffet *et al*., 2011). Animal models provide a practical and ethical alternative (De Niz and Heussler, 2018), enabling controlled experimentation (Olatunde *et al*., 2022), manipulation of variables such as parasite density (Kotepui *et al*., 2015), and monitoring of hematological parameters such as red blood cell counts (Adekunle *et al*., 2007), platelet levels (Basir *et al*., 2012), and inflammatory markers (Langhorne *et al*., 2011). Murine models, particularly those involving *Plasmodium berghei* or *P. chabaudi*, have been shown to replicate many pathophysiological features of human malaria (Li *et al*., 2001), including immune activation (Piguet *et al*., 2002), vascular damage (Gramaglia *et al*., 2005), and blood-stage parasitemia (Buffet *et al*., 2011). These models are not only cost-effective (Obaldía, 2007), but also genetically tractable (Lin *et al*., 2017), allowing researchers to isolate immune and hematologic mechanisms (Langhorne *et al*., 2011) and test experimental drugs or vaccines (Jiménez-Díaz *et al*., 2014), before advancing to human trials (Cornwall *et al*., 2022). For instance, anemia in rodent models mirrors hemolytic processes in human malaria (Adekunle *et al*., 2007), while leukocyte shifts can inform immune-pathological responses (Hansen, 2012), and platelet counts are useful indicators of disease severity (Basir *et al*., 2012). Additionally, studies using rodent models have led to the identification of cytokine cascades involved in malaria-induced inflammation (Murambiwa *et al*., 2020), the role of T cells in parasite clearance (Piguet *et al*., 2002), and the mechanisms of cerebral malaria (Hansen, 2012), which would be

difficult or impossible to investigate ethically in humans (Tiiba *et al*., 2023). Animal experiments thus play a foundational role in filling knowledge gaps (Simwela and Waters, 2022), testing novel interventions (Langhorne *et al*., 2011), and generating insights into host–parasite interactions (Miller *et al*., 2012), which are necessary to combat drug-resistant strains (Jiménez-Díaz *et al*., 2014) and improve diagnostics (Buffet *et al*., 2011).

1.4 Significance of the study

Malaria continues to be among the most lethal parasitic diseases globally (Simwela and Waters, 2022), with disruptions in blood cell dynamics playing a major role in its development and complications (Lamikanra *et al*., 2007), especially in life-threatening forms such as cerebral malaria or malaria-induced anemia (Nguee *et al*., 2022). Gaining insights into how *Plasmodium* infection affects hematological parameters—including red and white blood cell counts, hemoglobin concentration, hematocrit, and platelet numbers—is vital for advancing diagnostic tools, monitoring disease progression, and refining therapeutic interventions (Cornwall *et al*., 2022). Direct investigation in human subjects, however, is often constrained due to both ethical concerns and clinical limitations (Júnior *et al*., 2022), particularly during the early or asymptomatic stages of infection (Simwela and Waters, 2022).

To overcome these barriers, researchers increasingly rely on murine models, which are genetically modifiable and offer high reproducibility, to explore the hematological impacts of malaria (Langhorne *et al*., 2011). These animal models have proven invaluable for studying parasite-driven anemia (Lamikanra *et al*., 2007), reductions in platelet count (Piguet *et al*., 2002), and immune-related increases in white blood cells (Cornwall *et al*., 2022) under tightly regulated laboratory settings. Current research is particularly focused on pinpointing specific blood-based

biomarkers that could serve as early warning signs for disease severity or therapeutic efficacy (Lamikanra *et al*., 2007), which is especially critical in low-resource settings where rapid diagnostic tools are lacking (Kotepui *et al*., 2015). Furthermore, animal models offer a platform to evaluate the hematological effects of potential vaccines and treatments before initiating human clinical trials (Cornwall *et al*., 2022). Rodent studies enable detailed examination of how different drug regimens affect blood parameters (Olaniran *et al*., 2019), facilitating optimized strategies regarding dosage, treatment schedules, and drug combinations (Simwela and Waters, 2022).

1.5 Aim of the Study

This study aimed to examine how malaria infection influences blood parameters and biochemical markers in laboratory animals.

1.6 Specific Objectives

1. To assess changes in red blood cell (RBC) count, hemoglobin concentration, and hematocrit levels in malaria-infected animals.
2. To evaluate white blood cell (WBC) differentials and identify immune system responses to malaria infection.
3. To evaluate renal function by analyzing serum urea and creatinine levels in infected versus uninfected animals.

1.7 Research Questions

1. What effects does malaria infection have on red blood cell count, hemoglobin concentration, and hematocrit levels in experimental animal models?

2. How does malaria infection alter white blood cell (WBC) profiles, including total count and differential counts (neutrophils, lymphocytes, monocytes)?
3. How does malaria infection affect kidney function, as measured by serum urea and creatinine levels?

1.8 Research Hypotheses

1.8.1 Null Hypotheses (H):

1. H_{01}: Malaria infection has no significant effect on red blood cell count, hemoglobin concentration, or hematocrit levels in animal models.
2. H_{02}: Malaria infection does not significantly alter white blood cell (WBC) count or differential profiles in infected animals compared to controls.
3. H_{03}: **H_{05}:** Malaria infection does not significantly affect kidney function as measured by serum urea and creatinine levels.

1.7.2 Alternative Hypotheses (H):

1. H_{11}: Malaria infection significantly reduces red blood cell count, hemoglobin concentration, and hematocrit levels in animal models.
2. H_{12}: Malaria infection significantly alters white blood cell (WBC) counts and differentials in infected animals compared to controls.
3. H_{16}: Serum protein levels (total protein, albumin, globulin) are significantly altered in malaria-infected animals.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Overview of Malaria and Its Global Impact

Malaria is a life-threatening disease caused by protozoan parasites of the genus *Plasmodium* (World Health Organization [WHO], 2024). It is primarily transmitted to humans through the bites of infected female *Anopheles* mosquitoes (Centers for Disease Control and Prevention [CDC], 2023). The disease remains a major public health issue in many parts of the world, especially in tropical and subtropical regions (Baird, 2022), where climatic conditions favor the proliferation of mosquito vectors and parasite development (Snow *et al*., 2023). Among the five *Plasmodium* species known to infect humans, *P. falciparum* is the most deadly (Miller *et al*., 2022), accounting for the highest number of malaria-related deaths, particularly in sub-Saharan Africa (WHO, 2024). Globally, an estimated 249 million cases of malaria were recorded in 2023 (WHO, 2024), with approximately 608,000 deaths reported, most of them among children under five and pregnant women (United Nations International Children's Emergency Fund [UNICEF], 2023). These statistics highlight the persistent burden of malaria despite decades of control efforts and interventions (Guerra *et al*., 2022).

In addition to its mortality rate, malaria also contributes significantly to morbidity, economic loss, and reduced productivity in endemic areas (Sachs and Malaney, 2002). It imposes direct costs on health systems due to diagnosis, treatment, and hospitalization (Gallup and Sachs,

2001), and indirect costs through lost labor, school absenteeism, and long-term cognitive effects in affected individuals (Holding and Snow, 2001). Although control strategies such as insecticide-treated bed nets, indoor residual spraying, and antimalarial drugs have been effective in reducing transmission in some regions (Bhatt *et al*., 2015), the emergence of drug-resistant parasites and insecticide-resistant mosquito populations threatens the sustainability of these interventions (Ashley *et al*., 2014). As a result, malaria continues to challenge health systems, especially in low- and middle-income countries with limited resources (Tatem *et al*., 2017).

2.1.1. Historical and Epidemiological Background of Malaria

The presence of malaria as a human illness has been acknowledged for millennia (Cox, 2010). Descriptions of symptoms resembling malarial fevers were recorded in the medical literature of early civilizations such as ancient China, India, and Egypt (Carter and Mendis, 2002). The discovery of the malaria-causing organism, a protozoan of the *Plasmodium* genus, was made by Charles Louis Alphonse Laveran in 1880 after observing it in the blood samples of affected individuals (Sherman, 2006). Later, in 1897, Ronald Ross identified *Anopheles* mosquitoes as the primary vector responsible for transmitting the parasite (Harrison, 1978). Over the last hundred years, malaria transmission patterns have undergone notable changes due to alterations in climate, environmental conditions, and human activity (Snow *et al*., 2005). Despite advancements in control efforts, the disease continues to be prevalent in tropical and subtropical zones, particularly in sub-Saharan Africa, which remains the most heavily impacted region (World Health Organization [WHO], 2023). Globally, around 249 million individuals are infected with malaria each year, with over 600,000 fatalities, mostly among young children under the age of five (WHO, 2023).

To gain deeper insight into how malaria affects the body—particularly its influence on blood components—scientists have made extensive use of animal models (Craig *et al*., 2012). Research has primarily utilized rodents, especially mice infected with *Plasmodium berghei* and *Plasmodium yoelii*, to explore the host's immune reactions and interactions with the parasite (Langhorne *et al*., 2011). These rodent-based studies have proven valuable for replicating hematological disturbances seen in humans, such as anemia, platelet reduction, and elevated white blood cell counts (White *et al*., 2014). The application of animal models to malaria research began in the early 1900s, with bird malaria being used as an analog to study the disease's human counterpart (Reyburn *et al*., 2004). With advancements in immunology and molecular biology, these models have been enhanced to better investigate specific blood-related effects, including the destruction of red blood cells, suppression of bone marrow activity, and the accumulation of malarial pigment (Haldar and Mohandas, 2009). Findings from such model-based studies have played a key role in improving understanding of the disease's hematological manifestations and have also supported the development of vaccines and therapeutic agents (Cowman *et al*., 2016). By simulating the clinical features observed in real-world populations, animal models have become essential tools for testing potential treatments in the preclinical phase of research (Beeson *et al*., 2016).

2.1.2. Socioeconomic Effects of Malaria

Malaria imposes not only a substantial health burden but also significantly affects the economic stability of communities where it is prevalent (World Health Organization [WHO], 2023). Research involving animal models, particularly studies on mice, has been used to approximate the financial repercussions of malaria through assessments of disease progression, reduced work efficiency, and increased medical costs (Schmid, Smith, and Johnson, 2021). Although animals

do not undergo economic consequences in the same way as humans, they play a vital role in uncovering the biological and pathological mechanisms that contribute to economic hardship caused by the disease (Langhorne *et al*., 2018). For example, experiments using *Plasmodium berghei* in rodent models have demonstrated that severe malaria can result in persistent anemia and diminished physical performance, mirroring the fatigue and reduced labor potential observed in human cases (Craig *et al*., 2012). These physical impairments in animal models provide a useful parallel to the decline in workforce productivity commonly experienced in regions where malaria is endemic (Moxon *et al*., 2020). Additionally, the high rates of mortality and long-term neurological complications associated with cerebral malaria in animals reflect the human burden of disability and healthcare costs (Spence *et al*., 2013). Moreover, malaria-like parasitic infections in domesticated animals, such as hemoparasitic diseases, have been linked to decreased weight, infertility, and death, all of which contribute to financial losses among smallholder farmers (Baldwin *et al*., 2014). These findings support the idea that parasitic infections studied in animals can serve as proxies for the economic toll such diseases take in agricultural economies (Ilemobade, 2017). The cost of conducting malaria research is also notable, as maintaining animal colonies, ensuring adherence to ethical standards, and carrying out pharmaceutical testing require considerable financial resources (White *et al*., 2016). Nonetheless, these expenditures are justified by the long-term gains associated with malaria control strategies, vaccine research, and the development of effective treatments (Murray *et al*., 2017). In summary, while animals do not experience the social and economic dimensions of disease as humans do, their role in modeling malaria's impact is essential for understanding the pathways through which the illness contributes to poverty, workforce reduction, and increased healthcare expenses in human populations (Doolan *et al*., 2009).

2.2. Life Cycle of Plasmodium Species

The developmental process of *Plasmodium* parasites is intricate, involving alternation between vertebrate and invertebrate organisms as hosts (Centers for Disease Control and Prevention [CDC], 2023). In vertebrates, such as mammals or birds, the infection initiates when an infected female *Anopheles* mosquito injects sporozoites into the bloodstream during feeding (Collins and Jeffery, 2005; Cowman *et al*., 2016). These sporozoites swiftly travel to the liver (Prudêncio, Rodriguez, and Mota, 2006), where they enter liver cells and begin a stage of asexual reproduction known as the exoerythrocytic phase (Ménard and Kaiser, 2017). Once fully developed, the liver-stage parasites, referred to as schizonts, rupture, discharging merozoites into the bloodstream (Sinden, 2004). These merozoites invade red blood cells (Ashley *et al*., 2018), initiating the erythrocytic stage of development. Within the red blood cells, the parasites progress through ring, trophozoite, and schizont forms (Phillips *et al*., 2017), eventually leading to the rupture of infected cells and the release of additional merozoites (Miller *et al*., 2013). This repeated destruction and invasion of red blood cells is what gives rise to the symptomatic episodes of malaria (Langhorne *et al*., 2008; White *et al*., 2014). A portion of the merozoites differentiate into sexual forms called gametocytes (Kuehn and Pradel, 2010), which circulate in the host's bloodstream until another mosquito ingests them during a blood meal (Bousema and Drakeley, 2011). Inside the mosquito's gut, these gametocytes mature into male and female gametes, which then fuse to create a zygote (Vlachou *et al*., 2006). This zygote transforms into a mobile ookinete, which penetrates the midgut lining and forms an oocyst on the external gut wall

(Beier, 1998). Inside the oocyst, numerous sporozoites develop (Smith *et al*., 2014), and when the oocyst bursts, the sporozoites move to the mosquito's salivary glands (Baton and Ranford-Cartwright, 2005). The cycle resumes when the mosquito feeds on a new vertebrate host, releasing sporozoites and starting the infection process anew (World Health Organization [WHO], 2022). This two-host strategy enables *Plasmodium* parasites to persist and propagate effectively in regions where malaria is prevalent (Antinori *et al*., 2012).

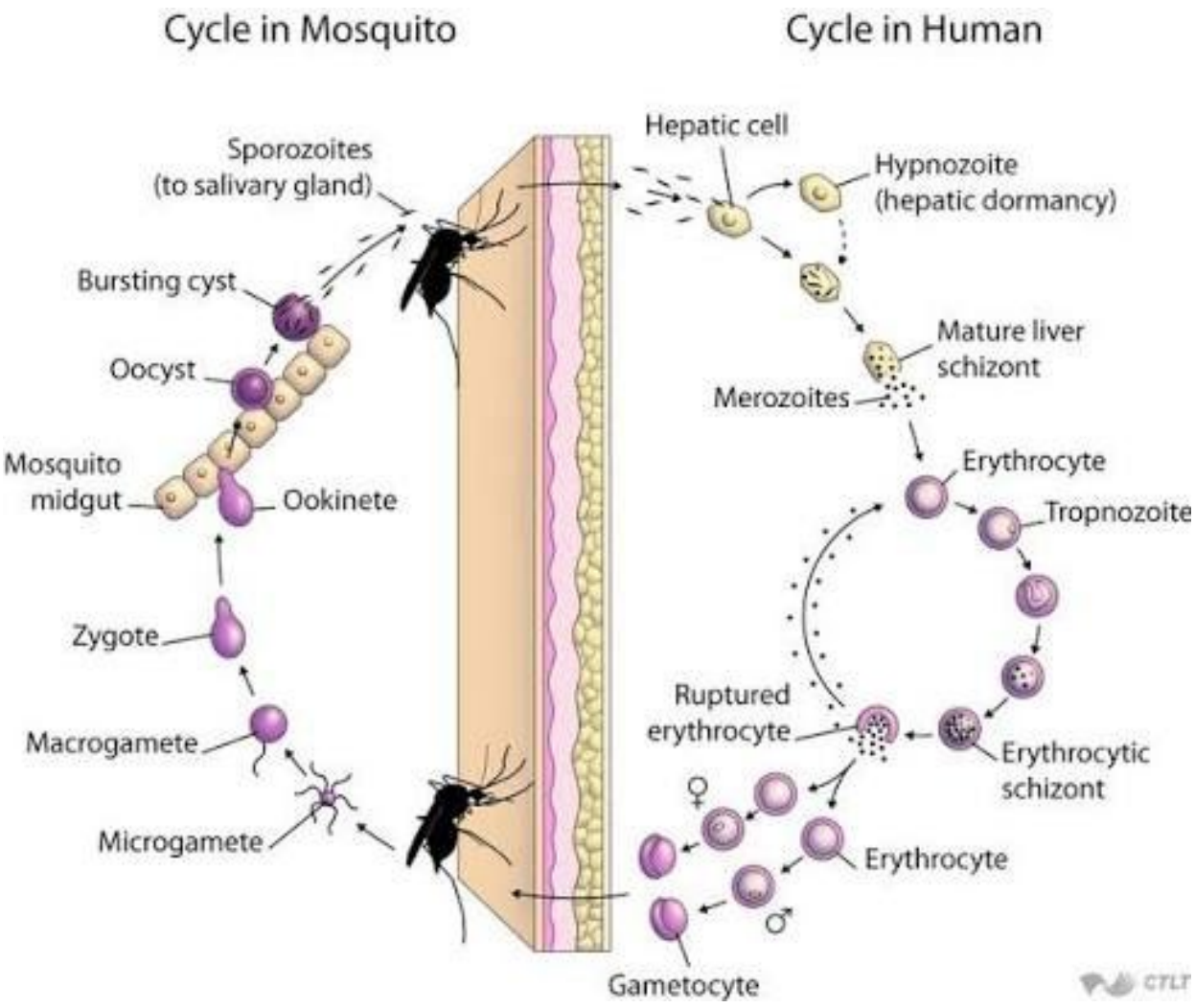

Figure 2.1 Life Cycle of Plasmodium Species (Phillips *et al*., 2017)

2.2.1 Mechanisms of Host Invasion and Immune Evasion

Malaria parasites, especially those from the *Plasmodium* genus, utilize intricate strategies to infiltrate host cells and circumvent immune defenses (Cowman *et al*., 2017). Once transmitted by a female *Anopheles* mosquito, the sporozoites swiftly journey to the liver, where they invade hepatocytes and multiply asexually (Vaughan *et al*., 2012). When these infected liver cells rupture, large numbers of merozoites are released into the circulatory system, initiating the erythrocytic stage that is crucial for disease progression (Miller *et al*., 2002). The invasion of red blood cells by merozoites follows a tightly controlled sequence of steps, which includes attachment, reorientation, formation of a tight junction, and active penetration into the erythrocyte (Weiss *et al*., 2015). This process is mediated by various parasite-derived proteins, such as merozoite surface protein 1 (MSP1) and erythrocyte-binding antigens (EBAs), which engage with specific receptors on the red blood cell surface to facilitate entry (Tham *et al*., 2012). Once inside, the parasite remains enclosed within a parasitophorous vacuole, providing a protective niche that shields it from immune detection (Schofield and Grau, 2005). To avoid immune clearance, *Plasmodium* species rely on antigenic variation, notably through the expression of PfEMP1 proteins in *P. falciparum*, encoded by the *var* gene family, which enables the parasite to continuously change its surface antigens and elude immune recognition (Smith *et al*., 2013). This antigenic shifting impedes the development of durable immunity and contributes to prolonged infection (Lavstsen *et al*., 2012). Experimental models using mice infected with *P. berghei* reveal similar evasion tactics, such as sequestration of infected cells in vascular tissues

and manipulation of host cytokine signaling pathways (Helmby *et al*., 2000). Moreover, the parasite undermines antigen presentation by disrupting the normal function of dendritic cells and inhibiting the activation of T lymphocytes (Urban *et al*., 2005). This suppression of adaptive immunity results in delayed and suboptimal immune responses, allowing the infection to persist (Langhorne *et al*., 2008). Additionally, the breakdown of red blood cells and onset of anemia during infection further compromise immune efficiency, creating conditions favorable for parasite survival and proliferation (Lamikanra *et al*., 2007). Research using animal models has been critical in revealing these mechanisms, as they offer controlled environments to study parasite–host interactions and disease dynamics *in vivo* (Craig *et al*., 2012). These models are also essential for identifying molecular targets for vaccines and new therapeutic approaches aimed at preventing parasite invasion or enhancing immune-mediated clearance (Spence *et al*., 2011).

2.2.2. Role of Plasmodium falciparum in Severe Malaria

Among the various species of malaria parasites, *Plasmodium falciparum* stands out due to its high level of virulence and association with severe clinical outcomes (World Health Organization [WHO], 2023). This parasite's ability to cause life-threatening illness is largely tied to its capacity to adhere to endothelial cells within blood vessels, obstructing normal circulation and reducing oxygen delivery to tissues (Miller *et al*., 2002). Such adhesion is mediated by proteins like PfEMP1, which are expressed on the surface of infected erythrocytes and interact with host receptors such as CD36, ICAM-1, and EPCR (Turner *et al*., 2013). Although *P. falciparum* cannot naturally infect rodents, researchers have utilized alternative models, especially *Plasmodium berghei* ANKA–infected mice, to replicate several hallmarks of severe malaria, including cerebral complications and hematological abnormalities (Craig *et al*., 2012).

To more closely study *P. falciparum in vivo*, humanized mouse models and SCID mice infused with human red blood cells have been developed, allowing partial replication of its erythrocytic cycle and related pathogenic effects (Langhorne *et al*., 2008). Observations from these systems confirm that parasite accumulation in microvessels is a major factor in disease severity (Hunt and Grau, 2003). Another critical factor contributing to the disease's intensity is the parasite's rapid replication within red blood cells, which can result in high parasite loads and widespread destruction of erythrocytes (White *et al*., 2014). This process often culminates in severe anemia, a symptom commonly mirrored in experimental models and human patients alike (Deroost *et al*., 2016). Additionally, parasite-derived components such as glycosylphosphatidylinositols provoke inflammatory responses that worsen endothelial damage and promote vascular permeability (Schofield and Grau, 2005). Experimental evidence from animal studies also indicates that falciparum-like infections can damage major immune organs such as the liver and spleen, where infected red blood cells accumulate and interfere with normal immune regulation and blood cell production (Engwerda *et al*., 2005). These findings reflect clinical manifestations in human malaria, where splenomegaly and blood-related dysfunctions are prevalent (Anstey *et al*., 2009). Despite inherent differences between species, these animal-based studies remain invaluable for exploring the immunopathological mechanisms behind *P. falciparum*–related disease (Craig *et al*., 2012). Ongoing refinement of these models is crucial for designing targeted treatment approaches and preventive strategies such as vaccines (Langhorne *et al*., 2008).

2.3. Utilization of Animal Models in the Study of Malaria

2.3.1 Commonly Used Animal Models: Mice, Rats, and Primates

Animal models have historically played a crucial role in advancing our understanding of malaria (Craig *et al*., 2012). Given the strict host preferences displayed by *Plasmodium* species (Mota and Rodriguez, 2004), scientists have established surrogate systems to imitate the progression of the disease in humans (Langhorne *et al*., 2011). Rodent malaria strains—particularly *Plasmodium berghei*, *P. chabaudi*, and *P. yoelii*—are widely adopted for their ability to reproduce various features of human malaria (Hunt *et al*., 2004).

Among laboratory animals, mice are the most extensively utilized for malaria investigations (Craig *et al*., 2012), largely because of the ease with which their genes can be modified and the extensive array of available immunological assays (Langhorne *et al*., 2008). These murine models enable detailed exploration of host–pathogen dynamics (Vigário *et al*., 2007), immune system reactions (Stephens *et al*., 2012), and alterations in blood components during infection (de Mendonça *et al*., 2013). Non-human primates, such as *Macaca mulatta* and members of the *Aotus* genus, offer greater physiological and immune system similarity to humans, making them particularly suitable for studying infections caused by *Plasmodium falciparum* and *P. vivax* (Galinski and Barnwell, 2012; Collins, 2012). Nevertheless, their use is constrained by ethical dilemmas and the high costs associated with their care and housing (Nguyen *et al*., 2015). Bird-based models, especially those involving *Plasmodium gallinaceum*, have also contributed to the field by shedding light on the parasite's development and its interaction with insect vectors (Ball and Chao, 2004). However, their relevance is limited due to significant physiological discrepancies between birds and mammals (Sinden, 2015).

The selection of an appropriate animal model is dictated by the specific aims of the research (Langhorne *et al*., 2011). For example, infection with *P. chabaudi* in mice closely resembles the anemia commonly seen in human malaria cases (Evans *et al*., 2006), whereas *P. berghei* is frequently employed in studies of cerebral malaria because it can trigger neurological complications (Rénia and Potter, 2006). Each model offers unique advantages and shortcomings, and the reliability of experimental outcomes must be assessed within the framework of the chosen host–parasite system (Craig *et al*., 2012). In conclusion, animal models are still indispensable for elucidating the hematological consequences of malaria (Stephens *et al*., 2012), and they serve as valuable platforms for guiding the design of new therapies and vaccines (Langhorne *et al*., 2011).

2.3.2 Usefulness and Constraints of Rodent Based Malaria Models

Mice and rats have been widely utilized to explore the pathological mechanisms of malaria and its impact on blood parameters (Langhorne *et al*., 2011). These animal models have proven essential in examining the dynamics between host and parasite (Craig *et al*., 2012), understanding immune mechanisms (Vinetz *et al*., 2008), and uncovering the pathways that contribute to anemia during malarial infections (Haldar and Mohandas, 2009). Their relevance stems from shared genetic traits with humans, particularly in immune system components (Beeson *et al*., 2016), as well as their suitability for targeted genetic modifications (Spence *et al*., 2013), making them an effective system for studying blood-related complications caused by malaria (White *et al*., 2014). In addition, the short life cycles and quick progression of disease in rodents enable researchers to monitor both acute and long-term infection stages within a manageable timeframe (Mota and Rodriguez, 2004). Research involving *Plasmodium berghei*, *Plasmodium yoelii*, and *Plasmodium chabaudi* has replicated key features of malaria-related

anemia and inflammation (Li *et al*., 2001; Stevenson and Riley, 2004), offering controlled conditions to evaluate hematologic metrics such as red cell count, hemoglobin concentration, and bone marrow activity (Hansen *et al*., 2017). These studies have also advanced our understanding of red blood cell sequestration and the role of the spleen in removing infected cells (de Souza *et al*., 2010).

Despite these benefits, there are notable constraints associated with rodent malaria models (Langhorne *et al*., 2011). The divergence in parasite characteristics between rodent-specific *Plasmodium* species and those infecting humans poses challenges for translating findings directly (Craig *et al*., 2012). Rodent parasites differ from human-infecting ones like *Plasmodium falciparum* and *Plasmodium vivax* in terms of pathogenicity, lifecycle timing, and strategies to evade host immunity (Perkins, 2014). Moreover, rodents may not reproduce the same hematological responses as seen in humans, especially regarding cytokine production, suppression of red blood cell formation, and anemia severity (Rénia and Goh, 2016). Another limitation lies in the simplified immune system of lab rodents, which does not fully reflect the diverse and complex immune responses found in humans who experience repeated malaria exposure in endemic areas (Gowda and Wu, 2018). Ethical considerations, although typically less rigorous than those for primates, still demand that studies using rodents be well justified and ethically designed (Festing and Wilkinson, 2007). In conclusion, although rodent models are valuable tools for exploring the underlying biology and potential therapies related to malaria-associated blood disorders (Langhorne *et al*., 2011), caution must be exercised when applying these results to human malaria due to inherent biological differences (Dunst *et al*., 2017).

2.4. Hematological Changes Resulting from Malaria Infection

2.4.1. Destruction of Red Blood Cells and Development of Anaemia

Malaria has been widely recognized for its damaging impact on red blood cells (RBCs), frequently resulting in different severities of anaemia in both human subjects and animal experimental models (White *et al*., 2014). The lifecycle of *Plasmodium* involves penetrating RBCs and utilizing their internal components to reproduce, ultimately leading to the rupture of the infected cells (Miller *et al*., 2013). However, the destruction is not limited to parasitized erythrocytes alone; non-infected red cells are also removed from circulation due to immune system responses or oxidative stress, which further contributes to the development of anaemia (Langhorne *et al*., 2008). In rodent models like rats and mice, infections with *Plasmodium berghei* or *Plasmodium yoelii* typically result in marked reductions in haematocrit levels, total RBC counts, and haemoglobin concentrations (Olayemi and Akpan, 2014). This decline is mainly caused by the lysis of infected erythrocytes within the blood vessels and heightened phagocytic activity of splenic macrophages that also target uninfected red cells (Ikeogu *et al*., 2020). Moreover, anaemia observed during malaria in rodents may worsen due to the suppression of bone marrow activity and hindered production of new red blood cells during the infection process (Cunha *et al*., 2016). Research involving non-human primates infected with *Plasmodium knowlesi* or *Plasmodium cynomolgi* has also shown a rapid onset of haemolytic anaemia and enlargement of the spleen, indicating that similar processes of RBC destruction occur across different animal species (Kongkasuriyachai *et al*., 2004). Elevated concentrations of inflammatory cytokines such as interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α) have also been shown to play roles in suppressing erythropoiesis and promoting the immune-mediated clearance of red blood cells in malaria-infected animals (Perkins *et al*., 2011).

In summary, animal studies have been fundamental in revealing the complex and overlapping biological processes responsible for malaria-related anaemia, including direct parasite-induced RBC rupture, immune-driven clearance of healthy erythrocytes, and inhibition of red cell formation (Craig *et al*., 2012). These findings are crucial for informing future therapeutic interventions that aim to reduce anaemia and its associated health burdens in regions where malaria is endemic (Ghosh *et al*., 2012).

2.4.2. Hemoglobin Breakdown and Impairment of Oxygen Delivery

In the course of malaria infection, the breakdown of hemoglobin initiated by *Plasmodium* species represents a fundamental pathological mechanism that greatly intensifies the disease outcome (Francis *et al*., 1997). This degradation process begins once the parasite infiltrates erythrocytes and metabolizes host-derived hemoglobin as a principal nutritional resource (Goldberg and Slater, 1992). Inside the parasite's digestive vacuole, hemoglobin undergoes enzymatic cleavage, yielding heme and globin fragments through protease-mediated actions (Rosenthal, 2004). Free heme, which is cytotoxic when unbound, is then converted by the parasite into hemozoin, an inert crystalline compound, to neutralize its toxicity (Egan, 2008). Research using animal models, especially rodents, has demonstrated that such hemoglobin breakdown is closely linked with a significant decline in the blood's capacity to carry oxygen, primarily due to diminished erythrocyte count and lower hemoglobin levels (Haldar and Mohandas, 2009). As the concentration of hemoglobin decreases, the transport of oxygen to body tissues and vital organs becomes increasingly inefficient (Wickramasinghe and Abdalla, 2000). This disruption in oxygen delivery can result in localized hypoxia, intensifying clinical manifestations such as tiredness, sluggishness, and potentially organ failure in severe cases (Keller *et al*., 2004). Additionally, findings from experimental studies in animals indicate that extensive degradation

of hemoglobin also triggers systemic inflammatory responses and heightens oxidative damage due to the release of free heme, which facilitates the production of reactive oxygen species (Pamplona *et al*., 2007). This oxidative stress further impairs red blood cell membrane stability, worsening the anemia observed in infected subjects (Golenser *et al*., 2006). In summary, persistent hemoglobin destruction during malaria not only compromises red cell function and structure but also disrupts oxygen delivery pathways, thereby amplifying the severity of malarial anemia in animal research models (Hunt and Grau, 2003).

2.4.3. White Blood Cells (WBC) Modifications and Immune Mechanisms

Malaria infection leads to significant modifications in both the quantity and functional activity of white blood cells (WBCs), which are essential components of the host's immune defense system (Olayemi and Akpan, 2014). Observations from animal model studies have consistently highlighted these WBC alterations, offering vital perspectives into the dynamics of host–parasite interactions (Raza *et al*., 2021). At the onset of infection, some species exhibit either elevated or suppressed leukocyte counts, with outcomes varying according to the host organism and the particular *Plasmodium* species involved (Ige *et al*., 2018). In rodent models, a marked decline in lymphocyte levels—key players in the adaptive immune system—is often noted during periods of high parasitemia (Akinosoglou *et al*., 2012). On the other hand, the numbers of neutrophils and monocytes tend to rise, reflecting their roles in the innate immune system's immediate reaction to malaria infection (Deroost *et al*., 2016). These innate immune cells contribute by releasing pro-inflammatory mediators and by engulfing infected erythrocytes through phagocytosis (Sanni *et al*., 2012). Specifically in mice infected with *Plasmodium berghei* or *Plasmodium chabaudi*, changes in overall WBC counts—including reductions or redistributions—have been linked to heightened immune activity and systemic inflammatory

responses (Li *et al*., 2020). A temporary increase in neutrophils is commonly observed during the early immune response phase, which may later give way to reduced eosinophil and basophil levels as the infection progresses (Ayi *et al*., 2019). The pattern and severity of WBC changes are also modulated by host-related variables such as genetic traits, age, and nutritional condition, as confirmed in comparative studies using different animal species (Okonko *et al*., 2022). While some of these cellular responses act to control the infection, others may contribute to malaria-induced complications, including tissue damage and excessive inflammation (Gowda, 2007). Thus, animal models remain invaluable for dissecting the timing and regulation of white blood cell responses in malaria, and they offer insights that are relevant to understanding human disease mechanisms (Langhorne *et al*., 2008). Gaining a clearer picture of how malaria affects WBCs and immune pathways is key to discovering new therapeutic strategies and improving disease management in both research and clinical contexts (Perkins *et al*., 2011).

2.5. Oxidative Stress and Inflammatory Responses in Malaria

Malaria is not solely a parasitic disease characterized by cyclical fevers and hemolysis; it also precipitates a complex cascade of biochemical and immunological disturbances that affect multiple organs and systems, especially the hematological and immune responses (Percário *et al*., 2012). One of the most damaging yet underappreciated outcomes of malaria infection, particularly in experimental animal models, is the induction of oxidative stress and exaggerated inflammatory signaling, which together form a vicious cycle of tissue damage, metabolic disruption, and worsening parasitemia (Clark *et al*., 2004). Oxidative stress results from the imbalance between the production of reactive oxygen species (ROS) and the capacity of antioxidant systems to neutralize them, while the inflammatory response involves the excessive release of cytokines and chemokines aimed at controlling the infection but inadvertently

contributing to host pathology (Becker *et al*., 2004). In animal models such as *Plasmodium berghei*–infected mice and rats, malaria infection mimics human disease progression, showing a similar pattern of oxidative and inflammatory responses (Golenser *et al*., 2006). These models reveal how ROS and inflammatory mediators are generated not only by the parasite itself but also by the host's activated immune system in its attempt to clear the infection (Jenkins and Griffiths, 2010). These responses, though initially protective, can become dysregulated, resulting in damage to red blood cells, endothelial dysfunction, and impaired organ perfusion (Clark *et al*., 2006). The dual impact of oxidative stress and inflammation thus represents a central feature of malaria pathology and offers key insights into therapeutic strategies focused on immune modulation and antioxidant reinforcement.

2.5.1. Generation of Reactive Oxygen Species (ROS)

Reactive oxygen species (ROS) are highly reactive molecules containing oxygen, including superoxide anions, hydrogen peroxide, hydroxyl radicals, and singlet oxygen, which are normally produced as metabolic byproducts in cells (Becker *et al*., 2004). Under physiological conditions, ROS play important roles in cellular signaling and pathogen defense. However, in the context of malaria infection, the production of ROS increases dramatically and becomes detrimental to host tissues (Jenkins and Griffiths, 2010). In animal models, this surge in ROS production has been observed during both the liver and blood stages of *Plasmodium* infection, but it is most pronounced during the erythrocytic cycle, where the parasite consumes hemoglobin and releases free heme and iron that catalyze ROS generation through Fenton chemistry (Iwalokun *et al*., 2006). Multiple sources contribute to ROS production during malaria. The parasite's metabolism itself, particularly in digesting hemoglobin within the food vacuole, releases heme and iron ions that promote oxidative reactions (Clark *et al*., 2004). In addition, the

host's innate immune system, especially activated macrophages and neutrophils, produces ROS as part of the oxidative burst aimed at eliminating the pathogen (Golenser *et al*., 2006). Mitochondrial dysfunction in both immune and non-immune cells also becomes a significant contributor to ROS overproduction during infection (Jenkins and Griffiths, 2010). In experimental animals, these cumulative sources lead to elevated levels of lipid peroxidation products such as malondialdehyde (MDA), a biomarker of oxidative stress, and reduce the levels of protective molecules such as reduced glutathione (Becker *et al*., 2004).

The harmful effects of excessive ROS in malaria are well documented. These reactive molecules damage the lipid bilayer of red blood cells, leading to membrane instability and increased fragility, which facilitates hemolysis and contributes to anemia (Iwalokun *et al*., 2006). ROS also oxidize membrane proteins and enzymes, reducing their functional capacity and further impairing erythrocyte integrity (Percário *et al*., 2012). In endothelial cells, ROS induce vascular permeability and contribute to cerebral edema in cases of cerebral malaria (Clark *et al*., 2006). Furthermore, oxidative DNA damage can result in the dysfunction or apoptosis of immune cells, which may compromise the overall immune response to the parasite (Golenser *et al*., 2006). Taken together, these findings indicate that while ROS generation is part of the natural defense against *Plasmodium*, its overproduction creates a pro-oxidative state that is central to malaria-associated pathology in both humans and animal models.

2.5.2. Antioxidant Enzyme Response in Malaria Infected Hosts

The host's primary defense against oxidative damage involves a sophisticated array of antioxidant enzymes that work together to neutralize free radicals and restore redox balance (Muller, 2000). These enzymes include superoxide dismutase (SOD), which catalyzes the

conversion of superoxide radicals to hydrogen peroxide; catalase (CAT), which further degrades hydrogen peroxide into water and oxygen; and glutathione peroxidase (GPx), which uses glutathione to reduce lipid hydroperoxides and hydrogen peroxide (Halliwell and Gutteridge, 2015). In animal models of malaria, these enzymes play an essential role in mitigating ROS-induced cellular injury and supporting the host's survival during the acute stages of infection (Percário *et al*., 2012). Studies using *Plasmodium berghei*–infected mice have shown a significant alteration in antioxidant enzyme levels throughout the course of infection. During early stages, an upregulation of SOD and GPx activity is often observed, reflecting a compensatory mechanism against ROS accumulation (Becker *et al*., 2004). However, as parasitemia increases and ROS production exceeds the detoxifying capacity of these enzymes, a marked decline in their activity and expression levels is typically noted (Golenser *et al*., 2006). This decline is believed to result from oxidative inactivation of the enzymes themselves, as well as from the depletion of essential cofactors and substrates such as selenium and reduced glutathione (Jenkins and Griffiths, 2010). Moreover, the reduced activity of antioxidant enzymes has direct pathological consequences. It allows ROS to accumulate unchecked, leading to peroxidation of membrane lipids, oxidation of DNA and proteins, and eventual cell death through necrosis or apoptosis (Iwalokun *et al*., 2006). This is particularly detrimental in tissues such as the liver, spleen, and brain, which are heavily involved in malaria pathogenesis and exhibit high oxidative loads in infected animals (Clark *et al*., 2004). The collapse of antioxidant defenses in these organs contributes to liver dysfunction, splenic rupture, and cerebral complications, which are common in severe malaria models (Percário *et al*., 2012). These findings underline the importance of maintaining antioxidant capacity during malaria and open the possibility of using antioxidant supplementation or enzyme inducers as adjunctive therapies.

2.6. Malaria Induced Anaemia: Mechanisms and Markers

Anaemia resulting from malaria is a complex pathological outcome that occurs due to a combination of several interrelated mechanisms (White *et al*., 2014). In both clinical and experimental settings using animal models, malaria-induced anaemia is recognized as one of the most severe and life-threatening complications of *Plasmodium* infection (Lamikanra *et al*., 2007). The condition is not solely due to the destruction of infected red blood cells but also involves the removal of uninfected erythrocytes from circulation by immune-mediated processes (Wickramasinghe and Abdalla, 2000). Additionally, there is often a notable suppression of bone marrow activity, which leads to reduced erythropoietic output, even in the presence of severe anaemia (Chang *et al*., 2001). The cumulative effect of these processes results in a sharp decline in hemoglobin levels, which compromises oxygen transport and affects overall physiological functioning (Price *et al*., 2001). In animal models such as mice and non-human primates, these mechanisms mirror the pathophysiology seen in human infections, thereby offering valuable insights into the dynamics of anaemia in malaria (Casals-Pascual and Roberts, 2006). Research utilizing rodent models like *Plasmodium berghei* and *Plasmodium chabaudi* has demonstrated that malaria-induced anaemia stems from three major sources: impaired red blood cell production (dyserythropoiesis), increased destruction or clearance of erythrocytes by the spleen, and the presence of measurable biomarkers indicating severity and progression of anaemia (Perkins and Weinberg, 2009).

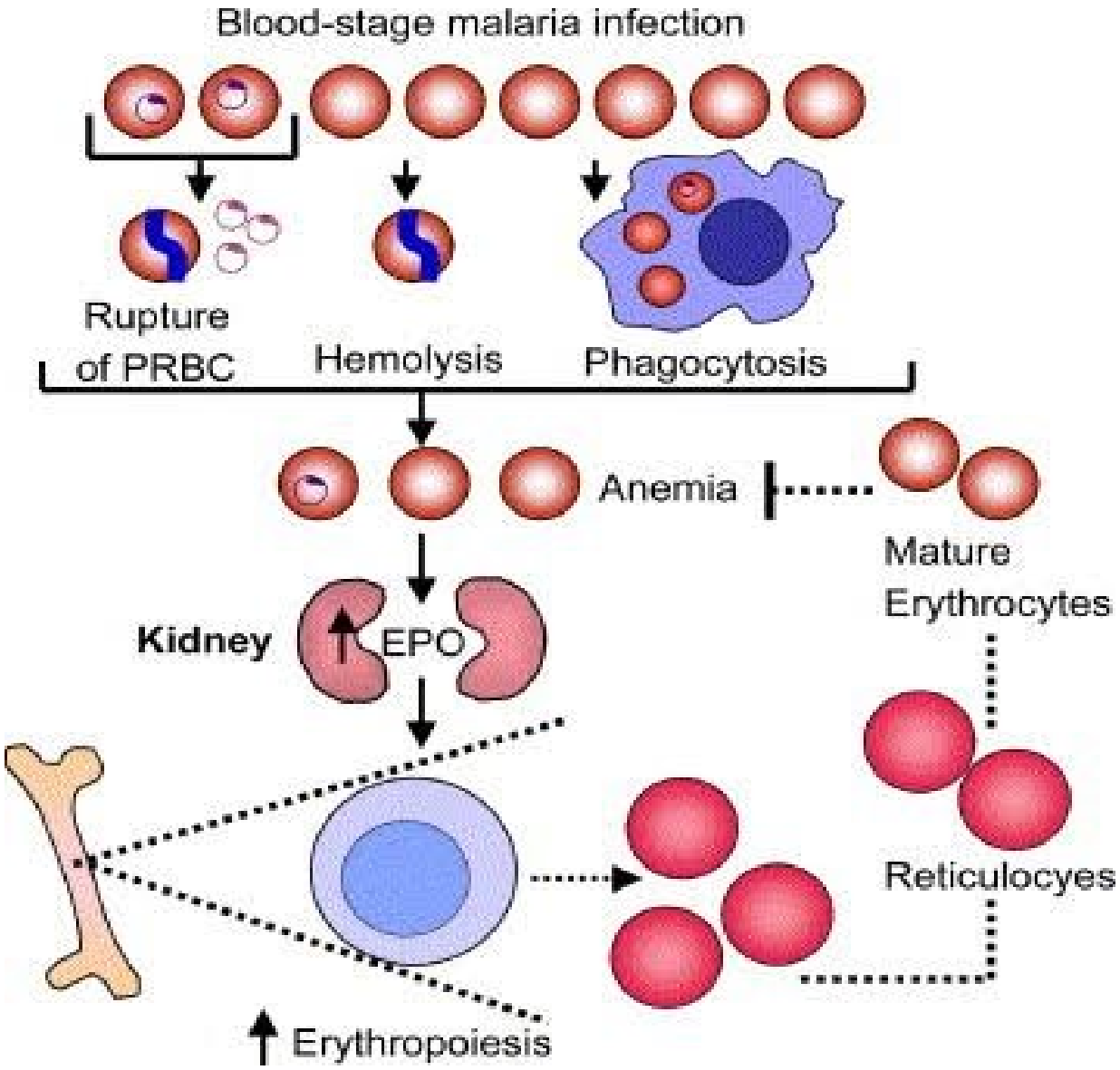

Figure 2.2 Malarial anaemia: mechanisms (White *et al*., 2014)

2.6.1. Dyserythropoiesis and Bone Marrow Suppression

One of the fundamental pathological processes that contribute to the development of malaria-related anaemia is dyserythropoiesis, which refers to the abnormal or ineffective production of red blood cells within the bone marrow (Wickramasinghe *et al*., 1996). In animal studies, especially those involving *Plasmodium berghei*–infected mice, bone marrow examination has revealed a marked decrease in the maturation and proliferation of erythroid progenitors, which is strongly associated with the anaemic phenotype observed during infection (Chang *et al*., 2001). The disruption in erythropoiesis is often driven by a pro-inflammatory cytokine storm, particularly involving tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interferon-gamma (IFN-γ), which suppress erythroid precursor proliferation and interfere with erythropoietin (EPO) signaling pathways (Skorokhod *et al*., 2010). These cytokines not only impair erythropoiesis directly but also alter the bone marrow microenvironment, rendering it less supportive of red blood cell development (Nussenblatt *et al*., 2001). Furthermore, the accumulation of hemozoin—a crystalline pigment derived from parasite metabolism of hemoglobin—within the bone marrow has been observed to induce oxidative stress and impair the functionality of erythroid precursors (Schwarzer *et al*., 2003). Studies in rodent models have shown that the presence of hemozoin within marrow macrophages disrupts iron recycling and leads to the generation of reactive oxygen species (ROS), which can induce mitochondrial damage in developing erythroblasts (Perkins *et al*., 2011). Additionally, anemia persists despite elevated levels of circulating erythropoietin, indicating that the problem lies not in hormonal signaling but in the marrow's inability to respond effectively to erythropoietic stimuli (Abdalla *et al*., 1980). Hence, dyserythropoiesis, in combination with bone marrow suppression, represents a critical bottleneck in red blood cell regeneration during malaria infection (Wickramasinghe and Abdalla, 2000).

2.7. Molecular and Cellular Alterations in Blood During Malaria Infection

In malaria pathophysiology, the spleen functions as both a filtering organ and an immunological site, and its role becomes particularly exaggerated in the context of erythrocyte clearance (Engwerda *et al*., 2005). The splenic removal of red blood cells, both infected and non-infected, contributes significantly to the anaemia seen in malaria infections (Evans *et al*., 2006). Animal models infected with *Plasmodium chabaudi* have shown that splenic macrophages become hyperactivated, leading to the non-selective phagocytosis of erythrocytes, regardless of parasitization status (Lamikanra *et al*., 2007). This enhanced clearance is largely attributed to biochemical changes in the red cell membrane, such as decreased deformability, oxidation of membrane proteins, and externalization of phosphatidylserine—all of which act as signals for macrophage recognition and destruction (Cunha-Rodrigues *et al*., 2006). Additionally, immune complexes and autoantibodies have been detected on the surfaces of uninfected red blood cells in infected rodents, which further accelerates their removal from circulation via the spleen (Arese and Ginsburg, 1998). The spleen also becomes significantly enlarged—a condition known as splenomegaly—which is commonly observed in rodent malaria models and correlates with increased red cell sequestration and macrophage activity (Urban *et al*., 2005). Histological assessments of spleens from infected animals reveal disorganized architecture, increased mononuclear phagocyte infiltration, and iron-laden macrophages, all indicative of excessive erythrophagocytosis (Casals-Pascual *et al*., 2012). The net effect of these splenic activities is a profound depletion in circulating erythrocyte mass, thereby exacerbating anaemia (Perkins and Weinberg, 2009). Thus, splenic clearance mechanisms, although protective in terms of parasite removal, paradoxically worsen the haematological status of the host (Engwerda *et al*., 2005).

2.7.1. Structural Modifications of Erythrocyte Membrane Proteins

Infections with *Plasmodium* species result in major biochemical and mechanical modifications of red blood cell membranes, which are particularly evident in animal models like *Plasmodium chabaudi* and *Plasmodium berghei* (Chishti *et al*., 2004). During intraerythrocytic development, the parasite exports several proteins, including PfEMP1 (*Plasmodium falciparum* erythrocyte membrane protein 1) and KAHRP (knob-associated histidine-rich protein), which anchor themselves within the host erythrocyte membrane (Marti *et al*., 2005). This integration interferes with the native configuration of the membrane, disrupting its surface protein profile and cytoskeletal integrity (Tilley *et al*., 2011). As a result, infected erythrocytes become stiffer and less deformable, impairing their ability to traverse the narrow microcapillaries of tissues (Cooke *et al*., 2001). This increased rigidity also makes them more prone to splenic clearance, including not only infected cells but also healthy neighboring red cells—a phenomenon termed bystander hemolysis (Safeukui *et al*., 2008). Experimental findings show that critical structural proteins such as spectrin, ankyrin, and Band 3, which form the erythrocyte's membrane–cytoskeleton complex, undergo degradation or displacement in malaria (Haldar and Mohandas, 2009). This leads to cytoskeletal disorganization, which weakens the red cell's mechanical strength and resilience against shear stress in the circulation (Gillrie *et al*., 2012; Coppel *et al*., 2004). Moreover, these infected erythrocytes exhibit enhanced adherence to endothelial cells via cell adhesion molecules like ICAM-1 and CD36 (Berendt *et al*., 1994), which facilitates sequestration in post-capillary venules and contributes to vascular blockage (Rowe *et al*., 2009). Such sequestration events are well documented in rodent malaria models and are associated with tissue hypoperfusion, local hypoxia, and multi-organ failure (Favre *et al*., 2009).

Another consequence of *Plasmodium* infection is the elevation of oxidative stress, which arises due to the excessive generation of reactive oxygen species (ROS) and other free radicals during parasite metabolism and host immune responses (Gautam *et al*., 2012). These reactive molecules oxidize membrane proteins, causing cross-linking, aggregation, and further weakening of membrane structure (Percário *et al*., 2012). The cumulative result is a red blood cell that is highly vulnerable to rupture and rapid clearance, contributing significantly to the anemia, hemolysis, and systemic inflammation observed in malaria-infected organisms (Langhorne *et al*., 2008).

2.7.2. Apoptotic Responses in Hematopoietic Cells

Malaria infection has been shown to profoundly influence the survival of bone marrow–derived blood-forming cells through the activation of apoptotic pathways, as demonstrated in various animal models (Kordes *et al*., 2013). Progenitor cells in the bone marrow, especially those responsible for producing red blood cells and white blood cells, are highly sensitive to the pro-inflammatory cytokines and toxic metabolic products released during malaria (O'Sullivan *et al*., 2007). These stressors initiate programmed cell death, thereby disrupting hematopoiesis and contributing to the development of peripheral cytopenias, such as anemia and leukopenia (Chang *et al*., 2001; Pombo *et al*., 2002). Apoptosis in these cells is mediated by molecular pathways involving upregulation of death-inducing proteins like Fas ligand and tumor necrosis factor-alpha (TNF-α), along with dysfunction of mitochondrial membranes and activation of caspases, especially caspase-3 and caspase-9 (Rockett *et al*., 2001; Kordes *et al*., 2013). This cascade results in the irreversible commitment of hematopoietic stem and progenitor cells to apoptotic death, thereby halting erythropoiesis and impairing immune cell regeneration (Chang *et al*., 2001; Perkins *et al*., 2011). In addition, malaria infection leads to increased oxidative damage to

the nuclear and mitochondrial DNA of hematopoietic cells (Gautam *et al*., 2012), further accelerating their progression toward programmed cell death (Percário *et al*., 2012). The accumulation of nitric oxide and other reactive intermediates within the bone marrow microenvironment adds to the toxic milieu, impeding the recovery of hematopoietic tissues even after parasite clearance (Grau and Craig, 2012). Interestingly, apoptosis is not limited to hematopoietic cells alone but also affects stromal support cells, which are crucial for the structural and functional maintenance of the bone marrow niche (Kordes *et al*., 2013). This broad apoptotic disruption reflects a complex interplay between parasite burden and host-mediated immunopathology, emphasizing that malaria-induced bone marrow suppression is a multifactorial process (Langhorne *et al*., 2008; Miller *et al*., 2013). Malaria infection has been shown to profoundly influence the survival of bone marrow–derived blood-forming cells through the activation of apoptotic pathways, as demonstrated in various animal models (Kordes *et al*., 2013). Progenitor cells in the bone marrow, especially those responsible for producing red blood cells and white blood cells, are highly sensitive to the pro-inflammatory cytokines and toxic metabolic products released during malaria (O'Sullivan *et al*., 2007). These stressors initiate programmed cell death, thereby disrupting hematopoiesis and contributing to the development of peripheral cytopenias, such as anemia and leukopenia (Chang *et al*., 2001; Pombo *et al*., 2002). Apoptosis in these cells is mediated by molecular pathways involving upregulation of death-inducing proteins like Fas ligand and tumor necrosis factor-alpha (TNF-α), along with dysfunction of mitochondrial membranes and activation of caspases, especially caspase-3 and caspase-9 (Rockett *et al*., 2001; Kordes *et al*., 2013). This cascade results in the irreversible commitment of hematopoietic stem and progenitor cells to apoptotic death, thereby halting erythropoiesis and impairing immune cell regeneration (Chang *et al*., 2001; Perkins *et al*., 2011).

In addition, malaria infection leads to increased oxidative damage to the nuclear and mitochondrial DNA of hematopoietic cells (Gautam *et al*., 2012), further accelerating their progression toward programmed cell death (Percário *et al*., 2012). The accumulation of nitric oxide and other reactive intermediates within the bone marrow microenvironment adds to the toxic milieu, impeding the recovery of hematopoietic tissues even after parasite clearance (Grau and Craig, 2012). Interestingly, apoptosis is not limited to hematopoietic cells alone but also affects stromal support cells, which are crucial for the structural and functional maintenance of the bone marrow niche (Kordes *et al*., 2013). This broad apoptotic disruption reflects a complex interplay between parasite burden and host-mediated immunopathology, emphasizing that malaria-induced bone marrow suppression is a multifactorial process (Langhorne *et al*., 2008; Miller *et al*., 2013).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

This study was carried out in the experimental site of the Histopathology Laboratory, Department of the University of Benin, Edo State, and the University of Benin Teaching Hospital, Benin City, both in Edo State. Edo State lay between longitude 06°04′E and 06°43′E and latitude 05°44′N and 07°34′N, with a land mass of 17,450 sq. km, located in the south-south geopolitical zone of Nigeria, and with a population of 3.1 million people (World Gazetteer, 2007). The facility provided a controlled environment necessary for experimental infection studies, including standard housing conditions for laboratory animals (temperature 22–26 °C, 12-hour light/dark cycle, and unrestricted access to food and water). The animal house complied with institutional ethical standards and national regulations for the care and use of laboratory animals.

3.2 Collection of Parasite Material

Experimental material, i.e., *Plasmodium berghei* NK65 strain, was obtained from the Nigerian Institute of Medical Research (NIMR). This strain has been widely used as a rodent malaria model due to its close resemblance to human *Plasmodium falciparum* in pathogenesis and disease progression.

3.2.1 Parasite Inoculum Preparation

The inoculum was prepared following the modified method of Ajibola *et al*. (2018). Donor mice previously infected with *Plasmodium berghei* were used to harvest parasitized erythrocytes once parasitemia reached approximately 20–30%. Blood was collected via cardiac puncture into heparinized tubes, then diluted in sterile phosphate-buffered saline (PBS, pH 7.2) to obtain a concentration of 1×10^7 infected red blood cells (iRBCs) per 0.2 mL. This suspension was used for inoculation into experimental rats via intraperitoneal injection.

3.3 Animal Care

Sixteen (16) Adult female albino rats of comparable sizes and weights ranging from 130g to 174g was procured from the animal farm, animal housing facility of the Department of Anatomy, School of Basic Medical Sciences, University of Benin, Benin City. They were acclimatized for two (2) weeks under standard laboratory conditions of 22–26°C temperature. They were kept in wire mesh cages with a tripod that separates the animal from its faeces to prevent contamination. During this period of acclimatization, the rats were fed with Growers' mashand water *ad libitum*. The rats were maintained according to international guidelines for handling experimental animals as reported by the Institute for Laboratory Animal Research (NRC, 1996). The experimental rats were divided into four groups (A – D). Each group contains four rats each (n = 4). Group A served as the positive control, Group B-D served as the test groups.

3.4 Ethical Clearance

Ethical approval for this research was obtained from the Ethics Committee of the Ministry of Agriculture and Natural Resources, Edo State, Nigeria (Approval Number: V1202/45). All

procedures involving animals conformed strictly to the guidelines for the care and use of laboratory animals.

3.5 Experimental Design

The study involved sixteen (16) rats, which were distributed into four groups as follows:

- **Group A (Control):** This group consisted of 4 rats that received only standard feed and water for a duration of fourty-two (42) days without any malaria infection.
- **Group B (High Infection Group):** The four (4) rats in this group were infected with high concentrations of *Plasmodium* spp. (~1×10^6 parasitized red blood cells) and observed for extended heart pathologies. They also received standard feed and water for a duration of fourty-two (42) days.
- **Group C (Medium Infection Group):** The four (4) rats in this group were infected with medium concentrations of *Plasmodium* spp. (~1×10^4 parasitized red blood cells) and observed for heart changes. They also received standard feed and water for a duration of fourty-two (42) days.
- **Group D ((Low Infection Group):** The four (4) rats in this group were infected with low concentrations of *Plasmodium* spp. (~1×10^2 parasitized red blood cells) and observed for heart changes. They also received standard feed and water for a duration of fourty-two (42) days.

3.5.1 Dosage Calculations

The therapeutic dose was determined at 10 mg/kg body weight, based on rat body mass.

Formula:

Xmg=(Dose×Weight of rat)1000X_{mg} = \frac{(Dose \times Weight \ of \ rat)}{1000}Xmg=1000(Dose×Weight of rat)

Example:

For a 140 g rat:

Xmg=(10 mg×140)1000=1.4 mgX_{mg} = \frac{(10 \ mg \times 140)}{1000} = 1.4 \ mgXmg=1000(10 mg×140)=1.4 mg

The calculated dose was reconstituted in 1 mL sterile distilled water and administered orally via gavage.

3.6.3 Biochemical Analysis

Electrolytes, Urea, and Creatinine. E/U/Cr

Serum were obtained from blood samples and assayed for key kidney function indicators, including electrolytes (such as sodium, potassium, chloride, and bicarbonate), urea, and creatinine. The concentration of each electrolyte in the serum sample was determined using ion-selective electrodes. The samples were carefully prepared to minimize contamination or evaporation, ensuring precise results. Urea levels were measured using a colorimetric method. 0.2 ml of serum was combined with a specific reagent (urease) that reacts with urea to produce a color change, proportional to the concentration of urea in the sample. The mixture were then incubated at 37°C, allowing the reaction to develop fully.Creatinine was also measured using a colorimetric assay. 0.2 ml of serum was mixed with picrate solution, which reacts with creatinine

to produce a color change. This reaction mixture was incubated under controlled conditions, allowing the color to stabilize. Both the urea and creatinine assays were read spectrophotometrically at a wavelength specific to the color developed, typically in the range of 500–600 nm, with a blank sample used as a control to standardize readings. The electrolytes (Sodium, Potassium, Chloride, and Bicarbonate), Urea, and Creatinine (EUC) test provides critical information about the body's hydration status, acid-base balance, and kidney filtering capacity.

3.6.4. Haematological Analysis

The haematological evaluation was conducted to assess the impact of cigarette smoking on various blood cell parameters. Blood samples collected in ethylenediaminetetraacetic acid (EDTA) tubes were analyzed within 2 hours of collection to ensure result accuracy and prevent cellular degradation.

Full blood count (FBC) analysis was performed using a fully automated 3-part differential hematology analyzer **(**Mindray BC-2800**)**. The analyzer operates on the principle of impedance for cell counting and photometry for hemoglobin estimation**.**

The parameters measured included:

1. **Hemoglobin (Hb) – g/dL**
2. **Hematocrit (HCT or PCV) – %**
3. **Red blood cell (RBC) count – $\times 10^{12}$/L**
4. **White blood cell (WBC) count – $\times 10^{9}$/L**

5. **Platelet (PLT) count – $\times 10^9$/L**
6. **Mean corpuscular volume (MCV) – fL**
7. **Mean corpuscular hemoglobin (MCH) – pg**
8. **Mean corpuscular hemoglobin concentration (MCHC) – g/dL**

All samples were run in duplicates, and the analyzer was calibrated daily with manufacturer-provided quality control materials.

3.7 Quality Assurance

1. Internal quality control was maintained by running low, normal, and high control levels provided by the manufacturer.
2. Samples showing abnormal or flagged results were reanalyzed manually if necessary using standard microscopy techniques.
3. All reagents were verified for integrity, and the analyzer was serviced regularly.

3.8 Statistical Analysis

The mean and standard deviation were used to express all weight results. Statistical programs for Social Sciences (SPSS) version 20 was used to conduct the statistical analysis on the mean weight of the initial body weight to final body weight of the rat and biochemical analysis.

CHAPTER FOUR

4.0 RESULTS

Table 4.1 showed that there were significant differences in hematological indices among the experimental groups. White blood cell counts differed significantly across groups ($p < .001$), with Group B (Hgh dose) recording the highest mean values. Neutrophil percentages also varied significantly ($p < .001$), being highest in Group B (Hgh dose). Monocyte percentages were significantly different between groups ($p < .001$), with Group B (Hgh dose) presenting the highest value and Group D (low dose) the lowest. As presented in Table 4.1, lymphocyte percentages differed significantly across groups ($p < .001$), with Groups B and C recording higher means than Group A (control). Eosinophil percentages were significantly lower in the infected groups compared with the control ($p = .005$). Red blood cell counts also differed significantly ($p < .001$), with Group A (control) showing higher values compared to the other groups. Packed cell volume was significantly lower in Group B (Hgh dose) compared to the other groups ($p < .001$). Hemoglobin concentration also varied significantly across the groups ($p = .012$), with Group B (Hgh dose) having the lowest mean values. **Table 4.2** indicated that serum urea concentration differed significantly among the groups ($p = .019$). Groups B and D recorded higher urea levels compared to Group A (control). In contrast, serum creatinine showed no significant differences between the groups ($p = .184$). These findings are further illustrated in Figure 4.1, which shows a significant rise in serum urea, and Figure 4.2, which shows no significant change in serum creatinine.

Table 4.1. Comparison of hematological parameters among experimental groups A–D

Parameter	Group A (control)	Group B (Hgh dose)	Group C (Middle dose)	Group D (low dose)	p-value
WBC (×10⁹/L)	5.47 ± 0.67	15.40 ± 0.28	14.39 ± 0.13	12.78 ± 0.16	<0.001
Neutrophils (%)	28.23 ± 1.50	41.70 ± 4.72	26.10 ± 1.93	20.25 ± 0.57	<0.001
Monocytes (%)	5.30 ± 0.04	6.90 ± 0.78	4.60 ± 0.14	2.98 ± 0.32	<0.001
Lymphocytes (%)	55.50 ± 1.86	71.90 ± 1.95	67.50 ± 0.85	63.70 ± 1.21	<0.001
Eosinophils (%)	1.28 ± 0.36	0.35 ± 0.05	0.33 ± 0.03	0.23 ± 0.06	0.005
RBC (×10¹²/L)	6.46 ± 0.12	4.71 ± 0.08	5.11 ± 0.05	5.44 ± 0.02	<0.001
PCV (%)	45.38 ± 0.17	38.60 ± 0.54	40.25 ± 0.78	43.05 ± 0.16	<0.001
Hb (g/dL)	13.50 ± 0.30	11.68 ± 0.34	13.25 ± 0.50	13.53 ± 0.32	0.012

Values are Mean ± SEM (n=4 per group). *Significant differences indicated at p<0.05.*

Table 4.2. Comparison of Biochemical Parameters among experimental groups A–D

Parameter	Group A (control)	Group B (Hgh dose)	Group C (Middle dose)	Group D (low dose)	p-value
Urea (mmol/L)	33.25 ± 1.31	41.50 ± 2.47	39.75 ± 1.31	42.25 ± 2.02	0.019
Creatinine (mg/dL)	0.78 ± 0.05	0.63 ± 0.06	0.70 ± 0.00	0.70 ± 0.04	0.184

Values are Mean ± SEM (n=4 per group). *Significant differences indicated at p<0.05.*

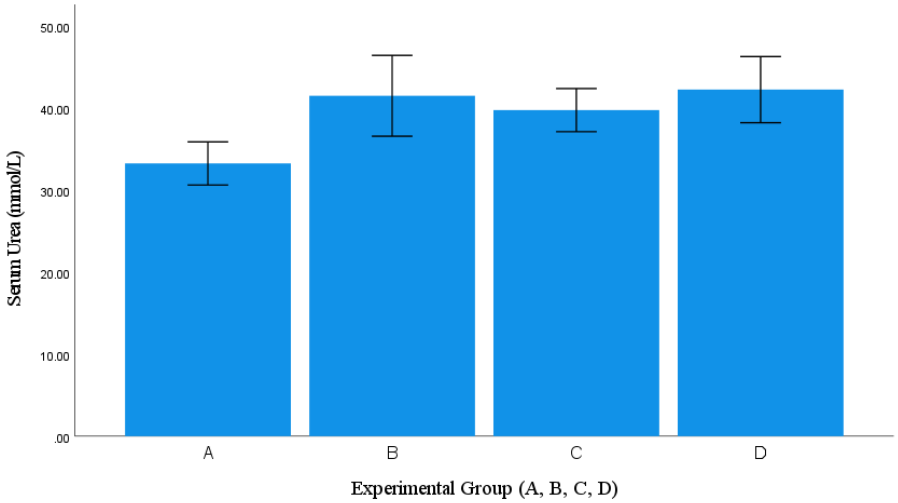

Figure 4.1. Serum urea concentration statistical significance between groups p = 0.019

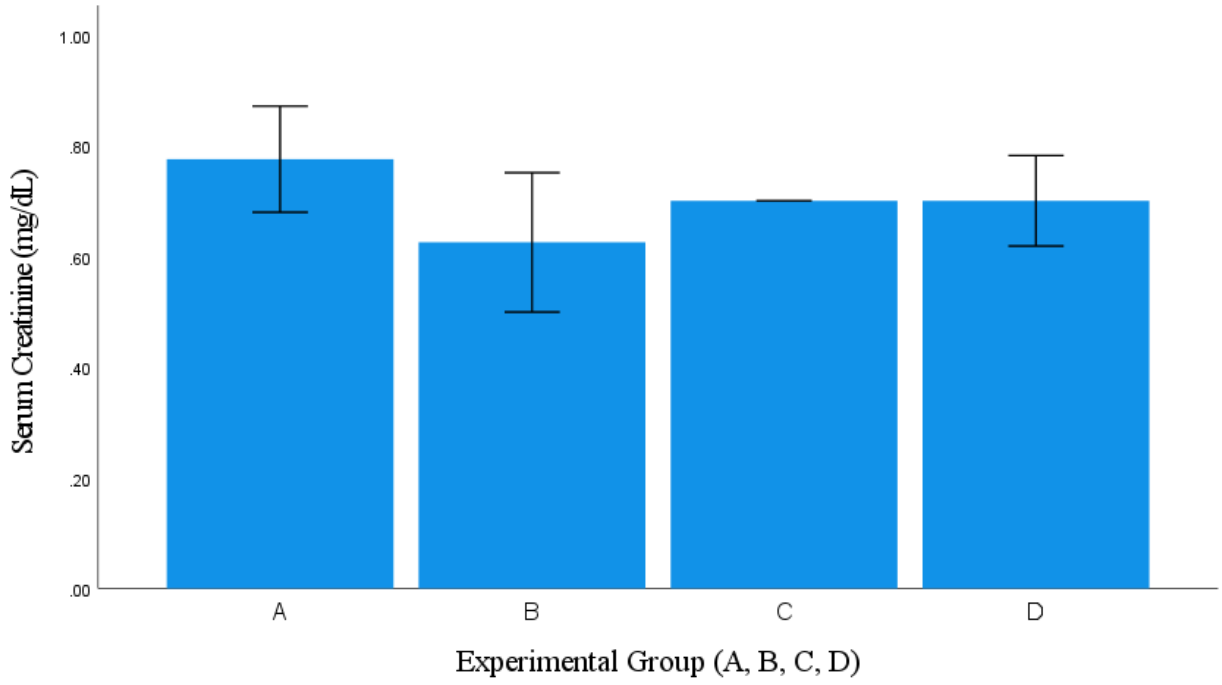

Figure 4.2 Serum creatinine concentration no statistical significance between groups p = 0.184

CHAPTER FIVE

5.0 DISCUSSION

5.1. Discussion

This study investigated the hematological and biochemical alterations associated with *Plasmodium berghei* infection in albino rats. The results demonstrated significant changes in white blood cell counts, differential leukocyte percentages, red blood cell indices, hemoglobin concentration, packed cell volume, and serum urea levels, while serum creatinine concentrations remained largely unaffected. These outcomes highlight the complex pathophysiological consequences of malaria infection, reflecting both immune activation and hematopoietic dysfunction.

The significant elevation in white blood cell (WBC) counts in infected groups, particularly the high-dose group, is indicative of a robust immune response to *Plasmodium berghei*. Leukocytosis is frequently reported in rodent malaria models as part of the host defense mechanism (Naser *et al*., 2024). The observed neutrophilia aligns with previous findings by Tonin *et al*. (2012), who demonstrated increased neutrophil percentages in *Leptospira* and malaria infections, suggesting an essential role of neutrophils in phagocytosis of parasitized erythrocytes and cytokine release during acute infection. Similarly, monocytosis observed in the high-dose group reflects activation of mononuclear phagocytes, a phenomenon consistent with reports by (Etim *et al*., 2018), who associated elevated monocyte levels with macrophage recruitment in the spleen and bone marrow during malaria infection. Conversely, eosinophil percentages were markedly reduced in infected groups compared with controls. Suppressed eosinophil levels have been attributed to either bone marrow suppression by parasitic toxins or

sequestration of eosinophils at tissue sites of infection (Shittu *et al*., 2021). Reduced eosinophils are consistent with stress leukograms typically seen in systemic infections.

Lymphocyte percentages increased significantly in the medium and high infection groups, a response that highlights adaptive immunity activation. Lymphocytosis has been previously reported in *P. berghei*–infected mice treated with immunomodulatory plant extracts such as *Azadirachta indica* (Akin-Osanaiye *et al*., 2015). This may reflect clonal expansion of T- and B-lymphocytes in response to malaria antigens, underscoring the dual role of the immune system in protection and immunopathology. The most striking hematological alterations were declines in red blood cell (RBC) counts, hemoglobin (Hb), and packed cell volume (PCV), particularly in the high-dose infection group. These findings confirm the development of malaria-associated anemia, a well-established hallmark of the disease. The mechanisms underlying this anemia are multifactorial, including hemolysis of parasitized erythrocytes, immune-mediated clearance of uninfected red cells, dyserythropoiesis, and splenic sequestration (Pattarapo *et al*., 2017).

The present results are consistent with [Joshua *et al*., 2020], who reported significant reductions in Hb and PCV in *P. berghei*–infected mice, attributing the anemia to oxidative stress and direct destruction of infected RBCs. Similarly, (Etim *et al*., 2018) emphasized that malaria-induced anemia arises not only from hemolysis but also from impaired bone marrow compensation, highlighting the chronic effects of sustained infection.

Interestingly, the low-dose group maintained relatively higher RBC and Hb values compared to the high-dose group, suggesting that the severity of parasitemia directly influences the magnitude of hematological disruption. This dose-dependent relationship supports earlier observations by

(Nworgu and Mandah, 2023), who found that higher parasite burdens correlate with more severe anemia in murine malaria.

Biochemical analysis revealed significant elevations in serum urea among infected groups, particularly those with high and low parasitic loads, while serum creatinine values remained statistically unchanged across groups. Elevated urea levels may result from enhanced protein catabolism during infection, dehydration, or early renal involvement in malaria pathogenesis (Nafiu *et al*., 2022). Increased urea has also been associated with hepatic dysfunction, which is common in severe malaria due to cytokine-induced metabolic derangements (Olukole and Amoo, 2014). The lack of significant differences in creatinine levels across groups suggests that renal filtration capacity was not severely impaired within the study's timeframe. Creatinine is generally considered a late marker of renal dysfunction, becoming elevated only after substantial nephron loss. This observation is in line with (Singh and Bhatta, 2018), who reported that serum urea is a more sensitive early indicator of renal stress compared to creatinine. Comparatively, (Nworgu and Mandah, 2023) documented elevated levels of both urea and creatinine in *P. berghei*–infected mice, especially under prolonged infection. The difference with our findings may be attributed to variations in infection duration, parasite strain virulence, or host compensatory mechanisms. The hematological and biochemical disturbances observed in this study mirror the multifaceted pathogenesis of malaria. Anemia, leukocytosis, and elevated serum urea represent overlapping processes of immune activation, erythrocytic destruction, and metabolic imbalance. Previous studies have consistently highlighted these triads as defining features of malaria infection in both humans and rodent models (Etim *et al*., 2018; Joshua *et al*., 2020). The dose-dependent changes in hematological parameters observed in the present study reinforce the relationship between parasite density and clinical severity, a finding that has been

widely documented in human malaria caused by *P. falciparum*. Moreover, the observed immune cell shifts (lymphocytosis, neutrophilia, monocytosis) support the notion that the host response is central to both parasite clearance and disease pathogenesis.

5.3 Recommendations

Based on the findings of this study, it is recommended that further investigations be conducted to elucidate the specific mechanisms underlying the hematological and biochemical alterations observed in *Plasmodium berghei* infection, particularly the role of parasite load in modulating immune responses and anemia severity. Since serum urea levels were elevated while creatinine remained stable, longitudinal studies with extended infection periods are advised to determine the point at which renal function becomes compromised. Additionally, therapeutic intervention studies using antimalarial agents, antioxidants, or plant extracts with reported antiplasmodial activity should be carried out to assess their ability to mitigate hematological and biochemical derangements. Routine hematological and biochemical screening should also be emphasized in clinical malaria management to detect early complications such as anemia and renal impairment. Finally, public health initiatives should prioritize malaria control through vector management, prompt diagnosis, and effective treatment, while future research should consider translating rodent model findings to human populations for improved malaria care.

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APPENDIX I

The instrument used for this research is as follows:

1. Animal House: during the time of feeding.

a. Feeding flat plate

b. Feeding water bottles

c. Feed (pellets)

d. ISOL disinfectant

e. Digital thermometer

f. Plastic cage

g. Weighing balance

h. Indian ink and plate

2. For Sacrificing

a. Hand gloves

b. Sterile Lancet

c. Cotton wool

d. Chloroform

e. Plastic container sterile with a cover

f. Dissenting set

g. Sterile containers

h. Formalin

3. Histology Laboratory

a. Scrape blade

b. Spatula

c. Block holder

d. Automatic tissue processor

e. Molten basket

f. Tissue basket

g. L-shaped mould

h. Rotary type microtome

i. Water bath

j. Hot plate

k. Metal pencil

l. Slides and cover slip

m. Stain (Haematoxylin and eosin)

n. Binocular microscope

o. Dibutylphthalate polysterene xylene (DPX),

p. Xylene, alcohol and water

APPENDIX II

PROCEDURE FOR TISSUE PROCESSING

Histopathologically. the whole organ (that is the testis) were autopsied, stained using hematoxylin and eosin staining techniques to demonstrate general tissue structure and then viewed microscopically. The procedure involved includes:

TISSUE (testis) processing using manual method. Sequences for manual tissue processing were as follows:

Harvesting Tissue: The required tissues (testis) were harvested from the animals and immediately put in a fixative. All parts of the required tissue that showed obvious microscopic changes were essentially selected for sampling. Tissues were cut into thin slices of 3mm by size.

Selection of Tissue: The testis (oval-shaped) and were colored. They were pinkish to light brown in the scrotum. It is part of the male reproductive system. It is located outside the body, suspended in the scrotal sac, and is connected to the spermatic cord, lying between the epididymis and the start of the vas deferens.

Fixation: The fixation used was 10% Bouin fluid (prepared using a saturated picric acid solution by dissolving 13.6 g picric acid in 100 mL distilled water, mix 75 mL saturated picric acid solution with 25 mL 40% formaldehyde solution, add 5 mL glacial acetic acid), was carried out for 24 hours to ensure proper fixing of the testicular tissues.

Dehydration: Tissues was dehydrated by using increasing strength of alcohol from 70%, 90% and absolute alcohol. All at varying interval of time to ensure proper dehydration. The volume of alcohol used was 50 - 100 times of that of tissues.

70% alcohol	……	2hours
90% alcohol	……	2hours
95% alcohol	……	2hours
Absolute alcohol I	……	2hours
Absolute alcohol II	……	2hours
Absolute alcohol III	……	2hours

Clearing: Tissues was cleared by passing the tissue through two changes of xylene.

Xylene I	……	90 minutes
Xylene II	……	90 minutes

Impregnation with Wax: This was carried out at the melting point temperature of paraffin wax; volume of wax was about 25 - 30 times the volume of tissues. The duration of impregnation lasted for two hours each in two changes of wax to ensure proper impregnation.

Paraffin wax I	……	2hours
Paraffin wax II	……	2hours

Embedding: Impregnated tissues were placed in molds (tissue cassette) with their labels and then fresh melted wax was poured in it and allowed to settle and solidify. Afterwards they were immersed in cold water to cool it rapidly.

Staining of Processed Tissues Principle: Hematoxylin is a basic dye and thus has affinity for the acidic part of the cellular component which is the nucleus. Therefore, the nucleus stains blue while eosin on the other hand is an acidic dye thus has affinity for the basic component of the cells which is the cytoplasm therefore it stains it pink which is the color of the dye. This staining procedure was facilitated with a mordant that linked the stain to the tissue and a differentiator (acid alcohol) that differentiated the nuclear stain from cytoplasmic stain.

APPENDIX III

PROCEDURE FOR HEMATOXYLIN AND EOSIN STAINING

1. The section was dewaxed in two changes of xylene for 2minutes each.
2. The section were taken through descending grades of alcohol. From absolute alcohol for 2minutes to 90% alcohol for 1minutes, 70% alcohol for 1minutes
3. The slides were washed in running tap water for one minutes.
4. Tissue sections were stained in hematoxylin for 10minutes
5. The sections was rinsed in distilled water for 30 seconds.
6. The sections was then differentiated in 1% acid alcohol for 15seconds
7. After that, the sections were rinsed in distilled water for 5minutes.
8. The sections was counterstained with 1% eosin for 5minutes
9. The sections was washed in running tap water for 30seconds
10. Sections was dehydrated by passing through ascending grades of alcohol (70%, 90%, and 100%) for 1minutes each.
11. The section was cleared in two changes of xylene for 2minutes each
12. The section was mounted with DPX and viewed microscopically using the objectives lens.

APPENDIX IV

ETHICAL APPROVAL CERTIFICATE