EFFECTS OF MALARIA PARASITE ON THE SPLEEN USING ALBINO WISTER RATS

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DEPARTMENT OF MEDICAL LABORATORY SCIENCE

SCHOOL OF BASIC MEDICAL SCIENCES

COLLEGE OF MEDICAL SCIENCES

UNIVERSITY OF BENIN, NIGERIA

SEPTEMBER, 2025

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A PROJECT SUBMITTED TO THE DEPARTMENT OF MEDICAL LABORATORY SCIENCE, SCHOOL OF BASIC MEDICAL SCIENCES, COLLEGE OF MEDICAL SCIENCES, UNIVERSITY OF BENIN, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE DEGREE IN MEDICAL LABORATORY SCIENCE

SEPTEMBER, 2025

CERTIFICATION

We the undersigned certify that this research work was carried out by **OMOROTIONMWAN OSADEBAMWEN** in the Department of Medical Laboratory Science, School of Basic Medical Science, University of Benin, Benin City in partial fulfillment of the requirements for the award of Bachelor of Science in Medical Laboratory Science.

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This project is dedicated to Almighty God.

ACKNOWLEDGEMENT

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ABSTRACT

Malaria remains a significant global health challenge, with the spleen playing a crucial role in both parasite clearance and disease pathogenesis. This study investigated the histopathological effects of malaria parasites on the spleen using albino Wistar rats infected with varying concentrations of *Plasmodium berghei*. Sixteen rats were randomly divided into four groups: control (n=4), low infection (10^2 parasitized red blood cells, n=4), medium infection (10^4 parasitized red blood cells, n=4), and high infection (10^6 parasitized red blood cells, n=4). Animals were observed for 14 days before sacrifice and histopathological examination. All infected groups experienced significant weight loss compared to controls, with the high infection group showing the greatest reduction (-25.5±2.5g vs +17.0±0.0g in controls, $p<0.001$). The most striking finding was the paradoxical relationship between infection dose and spleen enlargement. The low infection group demonstrated the most pronounced splenomegaly (1.4±0.1g vs. 0.7±0.1g in controls), representing a 100% increase in spleen weight. The splenic-somatic index confirmed this pattern, with the low infection group showing a two-fold increase (0.91% vs. 0.44% in controls, $p<0.05$). Surprisingly, histopathological examination revealed preserved tissue architecture across all groups, with normal white and red pulp organization despite significant organ enlargement. This suggests that early malaria-induced splenomegaly involves primarily functional rather than structural changes. The inverse dose-response relationship indicates that moderate infections may trigger optimal splenic activation, while severe infections may overwhelm the organ's adaptive capacity. These findings contribute to understanding malaria pathogenesis and emphasize that even low-level infections can have substantial physiological impacts, supporting the need for early intervention regardless of apparent infection severity.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of Study

Malaria remains one of the most pressing public health concerns globally, significantly impacting several regions, particularly in sub-Saharan Africa. Nigeria has one of the highest burdens of malaria worldwide, characterized by substantial morbidity and mortality rates, especially among vulnerable populations, including children under five years old and pregnant women (Kalu *et al*., 2021). The disease is caused by protozoan parasites of the genus *Plasmodium*, with *Plasmodium falciparum* being of critical concern due to its propensity to cause severe clinical manifestations, including cerebral malaria, anemia, and death (Osei-Yeboah *et al*., 2021; Oduola *et al*., 2021).

Transmission of malaria occurs primarily through the bites of infected female Anopheles mosquitoes, which thrive in warm environments, thus perpetuating the cycle of infection in tropical and subtropical regions (NMCP, 2020). As of 2021, global estimates indicated that approximately 240 million cases of malaria were reported, with about 627,000 deaths, demonstrating the urgent need for effective prevention and treatment strategies, especially for high-risk populations (WHO, 2021).

The spleen, a vital organ in the immune response, plays a pivotal role in combating malaria infections. Its functions include filtering damaged or infected blood cells, generating immune responses, and facilitating the removal of malaria-infected red blood cells (RBCs) (Hussein *et al*., 2016). Infection with malaria typically results in splenomegaly, an enlargement of the spleen, which occurs within the first week of infection due to increased lymphocyte influx and heightened erythropoiesis (Alani *et al*., 2021; Oguche *et al*., 2019).

During malaria infections, the spleen actively participates in inducing host immune responses. Macrophages engage in phagocytosis of both infected erythrocytes and portions of the *Plasmodium* parasites, thereby facilitating the presentation of antigens to T cells. This process culminates in a robust immune response that is critical for controlling malaria progression (Moller and Berghöfer, 2019).

Notably, splenomegaly is a hallmark of malaria infection, which underscores its significance in the disease process. It is often associated with enhanced hemolysis, activated red pulp macrophages, and accompanying inflammatory cascades in the microenvironment of the spleen, leading to oxidative stress (Akhtar and Ibrahim, 2019). Importantly, the degree of splenic dysfunction correlates strongly with patient outcomes; individuals who have undergone splenectomy exhibit exacerbated disease severity even at similar levels of parasitemia (Eze, and Ifejika, 2020; Makani and Wambua, 2020).

These observations indicate that a comprehensive understanding of the spleen's functionality in malaria is essential for developing improved therapeutic strategies aimed at enhancing the organ's protective capabilities while mitigating its overactive responses. The recognition of an interdependent relationship between splenic pathology and systemic disease severity emphasizes the need for further research into the spleen's role during malaria infections.

1.2 Statement of the Problem

Despite existing literature on malaria focusing primarily on its systemic and hematological effects, comprehensive investigations into the specific splenic complications associated with the disease are limited. Splenomegaly, while frequently observed, remains poorly understood in terms of the underlying mechanisms of splenic pathology during malaria infection, particularly in related controlled animal models (Obayelu and Adetunji, 2020). Researchers have

acknowledged the necessity for a detailed interrogation of histopathological alterations within the spleen, which may include changes such as red pulp congestion, atrophy or hyperplasia of white pulp, and disruptions in lymphoid structural organization (Ali *et al*., 2021).

The variability in patient outcomes associated with malaria complicates our understanding of splenic involvement in disease progression. Factors such as parasitic load, host genetic predispositions, and concurrent infections contribute to a multifactorial etiology behind splenic dysfunction and related systemic manifestations (Akhtar *et al*., 2017). Thus far, inquiries into the effects of varying doses of *Plasmodium* species on the splenic microarchitecture and cell population dynamics have been sporadic. This underscores the urgent need for systematic, dose-dependent studies that elucidate how acute malaria impacts the cellular composition and functional impairments of splenic tissues (Hwang *et al*., 2021).

Addressing these gaps will not only advance our comprehension of malaria pathogenesis but also facilitate the identification of pertinent therapeutic targets aimed at ameliorating splenic dysfunction. A detailed understanding of immunological responses within the spleen, particularly how varying parasitemia impacts these responses, is critical for potentially informing clinical recommendations for therapeutic interventions (Ali *et al*., 2019; Eze and Udemezue, 2020).

1.3 Significance of the Study

This study seeks to expound upon the mechanisms through which malaria parasites impact spleen functionality and structure, drawing necessary connections between splenic pathology and the severity of malaria manifestations. By addressing these critical gaps in the existing body of research, particularly concerning specific alterations in splenic histopathology and cellular interactions, the findings may enable the development of more effective diagnostic and treatment strategies (Milano and Okeowo, 2019; Eze and Udemezue, 2021).

Employing animal models, especially those using *Plasmodium falciparium*, offers a unique opportunity to delve into the pathological effects of malaria on the spleen. Research has indicated significant variations in splenic microarchitecture due to malaria infection, emphasizing a need for focused investigations that relate these changes to immune competence and overall clinical outcomes (Junaidu *et al*., 2022). Enhancing our understanding of splenic involvement could catalyze improvements across multiple facets of malaria management, particularly concerning anemia and systemic inflammation.

Moreover, elucidating the mechanisms that underpin splenic myelopoiesis—the increased production of myeloid lineage cells within the spleen—holds promise for generating novel therapeutic strategies targeted directly at splenic tissues. Such interventions may enhance beneficial immune cell output or modulate harmful inflammatory pathways, transforming our approach to not only managing malaria but also leveraging the spleen as a therapeutic target (Anukam *et al*., 2021).

In collective terms, a nuanced understanding of the interactions between malaria and splenic pathology can directly contribute to the identification of biomarkers indicative of splenic dysfunction, aiding in the clinical assessment of malaria severity. Furthermore, insights gained from this study could inform broader public health initiatives, particularly in northern Nigeria and other high-burden areas, by providing the groundwork for coherent care protocols and protective measures, ultimately aiming to reduce malaria's toll on vulnerable populations (Mbanefo *et al*., 2022).

1.4 Aim of Study

Aim: The primary aim of this study is to investigate the histopathological effects of malaria parasites on the spleen using albino wister rats.

1.5 Objective Of The Study

The Specific Objectives include:

1. To assess the specific histological changes in spleen tissues of albino rats infected with *Plasmodium* spp
2. To assess the dose-dependent effect of different concentrations of *Plasmodium* spp. on the spleen tissues of albino wister rats.

1.6 Research Questions

1. What specific histological changes occur in the spleen tissues of albino rats infected with *Plasmodium* spp.
2. What is the relationship between the concentration of *Plasmodium* spp. infection and the degree of histopathological changes observed in the spleen of albino wister rats?

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Introduction

Malaria remains one of the most significant global health challenges of the 21st century, affecting millions of people worldwide and causing substantial morbidity and mortality, particularly in tropical and subtropical regions (World Health Organization, 2023). While the clinical manifestations of malaria are well-documented, the complex pathophysiological mechanisms underlying organ-specific damage, particularly splenic involvement, continue to be areas of active research interest (Anstey *et al*., 2009). The spleen plays a crucial role in the malaria parasite life cycle, serving as the initial site of infection and replication following sporozoite inoculation by infected Anopheles mosquitoes (Prudêncio *et al*., 2006).

Understanding malaria's splenic complications is of paramount importance for several reasons. First, the spleen serves as the obligatory site for the pre-erythrocytic stage of parasite development, making it a critical target for both infection establishment and potential therapeutic intervention (Vaughan *et al*., 2008). Second, severe malaria often presents with splenic dysfunction, which can significantly impact patient prognosis and treatment outcomes (Kochar *et al*., 2003). Third, many antimalarial drugs undergo splenic metabolism, making drug-induced hepatotoxicity a significant clinical concern.

Animal models have proven indispensable in malaria research, providing controlled experimental systems that allow for detailed investigation of disease pathogenesis, host-parasite interactions, and therapeutic interventions. The use of animal models in malaria research addresses several limitations inherent in human studies, including ethical constraints, the need

for tissue sampling at various time points, and the requirement for standardized experimental conditions (Craig *et al*., 2012). Rodent malaria models, particularly those utilizing *Plasmodium berghei*, *P. yoelii*, and *P. chabaudi*, have been extensively employed to study splenic pathology and have provided valuable insights into the mechanisms of spleen damage during malaria infection.

This literature review aims to provide a comprehensive examination of current knowledge regarding the effects of malaria parasites on spleen function and morphology, with particular emphasis on findings from animal model studies. The review is organized to progress from fundamental concepts of malaria biology and epidemiology to specific aspects of splenic pathology, animal model applications, and therapeutic implications. The scope encompasses both historical perspectives and recent advances in the field, providing a foundation for understanding the complex relationship between malaria parasites and splenic dysfunction.

2.2 Malaria: An Overview

2.2.1 Global Burden and Epidemiology

Malaria continues to impose a tremendous burden on global public health, with the World Health Organization (2023) reporting an estimated 247 million cases and 619,000 deaths in 2021. The disease exhibits a distinctly unequal geographical distribution, with sub-Saharan Africa bearing approximately 95% of the global malaria burden (Bhatt *et al*., 2015). This disproportionate impact reflects complex interactions between environmental factors, socioeconomic conditions, vector ecology, and healthcare infrastructure availability (Gething *et al*., 2011).

The epidemiological landscape of malaria has evolved significantly over the past two decades, with substantial progress in disease control and prevention efforts (Alonso and Noor, 2017). Despite these advances, certain regions continue to experience high transmission rates, particularly in areas with limited access to preventive measures such as insecticide-treated bed nets and indoor residual spraying (Lengeler, 2004). Climate change and environmental modifications have further complicated malaria epidemiology, potentially altering vector distribution patterns and transmission dynamics (Caminade *et al*., 2014).

Regional distribution patterns reveal significant heterogeneity in malaria burden across different geographical areas. Sub-Saharan Africa accounts for the vast majority of malaria cases and deaths, with countries such as Nigeria, Democratic Republic of Congo, Uganda, and Mozambique contributing disproportionately to the global burden (Snow *et al*., 2005). The West African region, including Nigeria, faces particular challenges due to high transmission intensity, widespread drug resistance, and limited healthcare infrastructure (Okiro *et al*., 2009).

Nigeria, as the most populous country in Africa, carries the heaviest malaria burden globally, accounting for approximately 27% of all malaria cases and 32% of deaths worldwide (National Malaria Elimination Programme, 2020). The country's tropical climate, extensive breeding sites for Anopheles vectors, and complex socioeconomic challenges contribute to sustained high transmission rates across all ecological zones (Oyewole *et al*., 2007). Urban malaria has emerged as a growing concern in Nigerian cities, challenging traditional assumptions about malaria transmission patterns (Klinkenberg *et al*., 2006).

Vulnerable populations bear disproportionate malaria burden, with children under five years and pregnant women experiencing the highest rates of severe disease and mortality (Dellicour *et al*.,

2010). Children in endemic areas are particularly susceptible due to limited acquired immunity, with cerebral malaria and severe anemia representing leading causes of childhood mortality. Pregnant women face increased risks of maternal anemia, low birth weight, and adverse pregnancy outcomes, particularly during first pregnancies when immunity levels are typically lower (Desai *et al*., 2007). Immunocompromised individuals, including those with HIV/AIDS, also experience increased malaria susceptibility and severity (Flateau *et al*., 2011).

2.2.2 Etiology and Classification of Malaria Parasites

Malaria is caused by obligate intracellular protozoan parasites belonging to the genus *Plasmodium*, which comprises over 200 species, with five species known to infect humans (Cox, 2010). These parasites exhibit complex life cycles involving both vertebrate hosts and arthropod vectors, requiring sophisticated molecular machinery for successful transmission and pathogenesis (Cowman *et al*., 2016).

Plasmodium falciparum represents the most virulent and clinically significant malaria parasite affecting humans, responsible for the majority of severe malaria cases and deaths worldwide (Miller *et al*., 2002). This species exhibits several unique characteristics that contribute to its pathogenicity, including its ability to cause cytoadherence of infected erythrocytes to vascular endothelium, leading to microvascular obstruction and organ dysfunction (Rowe *et al*., 2009). *P. falciparum* demonstrates remarkable genetic diversity and adaptability, facilitating the development of drug resistance and immune evasion mechanisms (Volkman *et al*., 2007).

Plasmodium vivax, while generally causing less severe disease than *P. falciparum*, remains a significant global health concern due to its wide geographical distribution and unique biological

characteristics (Gething *et al*., 2012). This species exhibits the ability to form dormant spleen stages (hypnozoites), which can cause relapsing infections weeks to months after initial treatment (White, 2011). *P. vivax* preferentially invades reticulocytes and exhibits a predilection for the Duffy blood group antigen, limiting its distribution in populations lacking this receptor.

Plasmodium ovale and *P. malariae* represent less common but nonetheless important human malaria parasites. *P. ovale*, similar to *P. vivax*, can form hypnozoites and cause relapsing infections, though it exhibits a more restricted geographical distribution primarily in West Africa (Mueller *et al*., 2007). *P. malariae* is characterized by its longer erythrocytic cycle (72 hours versus 48 hours for other species) and its association with nephrotic syndrome in chronic infections. *Plasmodium* knowlesi, primarily a simian parasite, has emerged as the fifth human malaria species, causing severe disease in Southeast Asia through zoonotic transmission (Singh *et al*., 2004).

Parasite morphology and identification remain fundamental aspects of malaria diagnosis and research. Each *Plasmodium* species exhibits distinctive morphological features in blood smears, including characteristic shapes, sizes, and staining patterns of different developmental stages. *P. falciparum* typically shows small, compact ring forms with occasional double infections per erythrocyte, while *P. vivax* displays larger, more amoeboid forms with enlarged infected cells. Modern molecular diagnostic techniques have complemented traditional microscopy, providing increased sensitivity and species-specific identification capabilities.

2.2.3 Life Cycle of Malaria Parasites

The malaria parasite life cycle represents one of nature's most complex and fascinating examples of multi-host pathogen biology, involving intricate developmental stages in both mosquito vectors and vertebrate hosts (Sinden, 2015). Understanding this life cycle is crucial for identifying potential intervention targets and developing effective control strategies (Targett, 2005).

The sporogonic cycle occurs within the Anopheles mosquito vector and begins when a female mosquito ingests gametocytes during a blood meal from an infected human host (Sinden *et al*., 2007). Within the mosquito midgut, male and female gametocytes undergo gametogenesis, forming motile male gametes and receptive female gametes through a process regulated by environmental cues such as temperature and pH changes (Billker *et al*., 2004). Fertilization results in the formation of diploid zygotes, which differentiate into motile ookinetes capable of traversing the mosquito midgut epithelium (Han *et al*., 2000).

Following successful midgut wall penetration, ookinetes develop into oocysts on the outer surface of the midgut, where they undergo extensive DNA replication and cellular division (Vlachou *et al*., 2004). The oocyst maturation process typically requires 10-14 days under optimal environmental conditions, during which thousands of sporozoites are produced through sporogony. Mature sporozoites are released into the mosquito hemocoel, where they migrate to the salivary glands through mechanisms involving circumsporozoite protein interactions with salivary gland receptors (Kariu *et al*., 2006).

The human infection cycle begins with sporozoite inoculation during mosquito blood feeding, initiating the schizogonic cycle in the vertebrate host (Amino *et al*., 2006). Sporozoites demonstrate remarkable motility and tissue-invasive capabilities, rapidly migrating through skin tissues to reach the bloodstream and subsequently homing to the spleen. The splenic phase represents a critical bottleneck in the parasite life cycle, as sporozoites must successfully invade hepatocytes to establish infection (Mota *et al*., 2001).

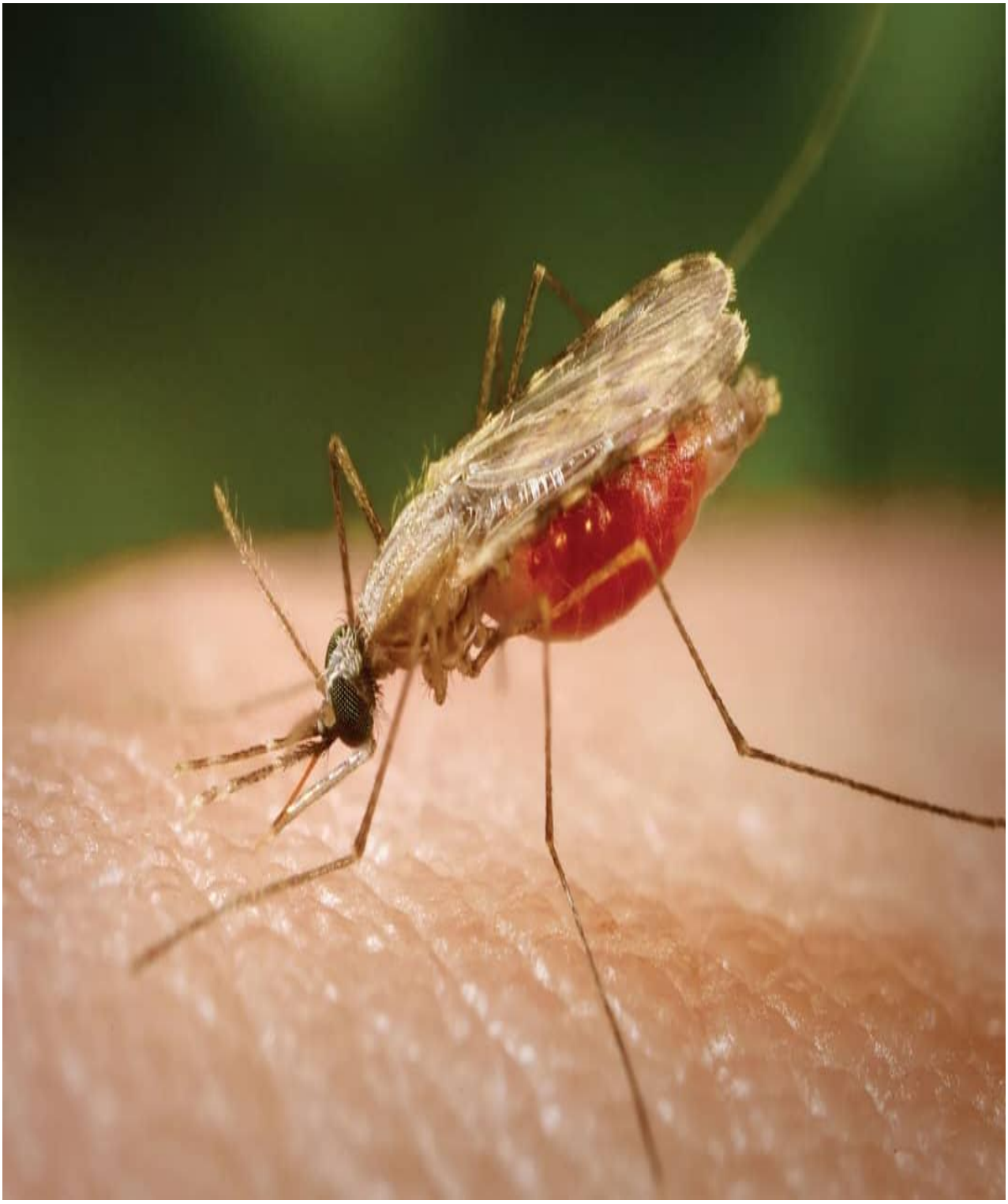

Figure 1: Anopheles mosquito: malaria vector (Encyclopedia Britannica, 2025).

The splenic or pre-erythrocytic phase occurs entirely within spleen parenchymal cells and represents the first major site of parasite replication in the human host (Prudêncio *et al*., 2006). Following hepatocyte invasion, sporozoites differentiate into spleen-stage parasites enclosed within a parasitophorous vacuole, where they undergo extensive growth and nuclear division (Bano *et al*., 2007). This splenic schizogony process typically requires 5.5-7 days for *P. falciparum*, during which a single sporozoite can produce 10,000-30,000 merozoites.

The erythrocytic phase begins with the release of merozoites from hepatocytes into the bloodstream, where they rapidly invade red blood cells through complex receptor-ligand interactions (Cowman and Crabb, 2006). Within erythrocytes, merozoites develop through ring, trophozoite, and schizont stages, ultimately producing 6-32 daughter merozoites depending on the parasite species (Bannister and Mitchell, 2003). This erythrocytic cycle repeats every 24-72 hours, leading to exponential parasite multiplication and clinical symptom development (White, 2004).

Gametogony represents the sexual phase of parasite development, occurring within erythrocytes when some merozoites differentiate into male and female gametocytes rather than continuing asexual reproduction (Baker, 2010). This process is regulated by complex molecular mechanisms involving environmental stress factors and parasite density-dependent signals (Kafsack *et al*., 2014). Mature gametocytes circulate in peripheral blood for 7-22 days, remaining available for mosquito uptake and continuation of the transmission cycle (Bousema and Drakeley, 2011).

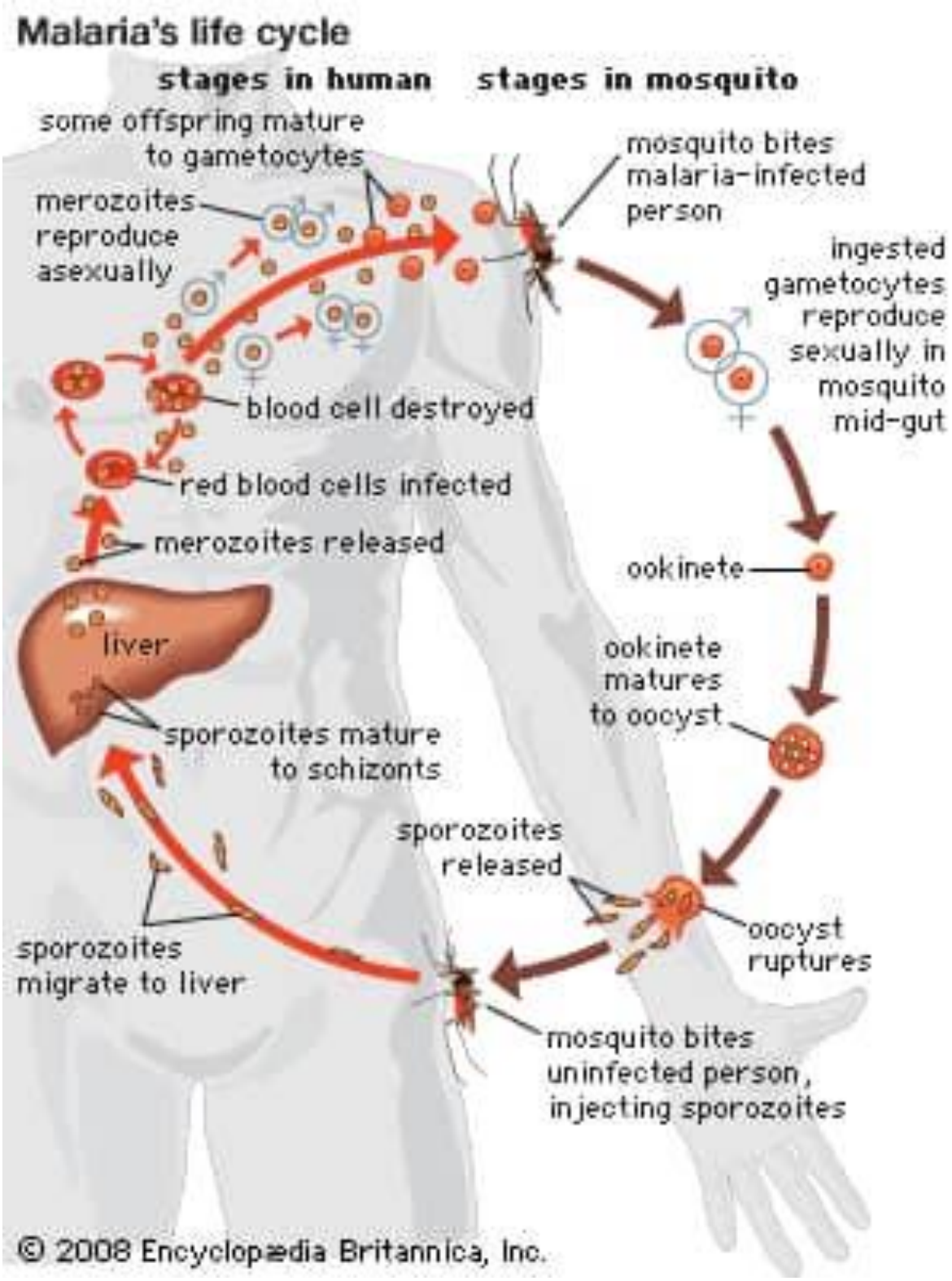

Figure 2: Malaria Life Cycle (Encyclopedia Britannica, 2025)

2.2.4 Pathophysiology and Mechanisms of Malaria Infection

The successful invasion of host cells by malaria parasites represents a highly sophisticated process involving multiple molecular interactions and cellular mechanisms that have evolved over millions of years (Cowman *et al*., 2017). Understanding these invasion mechanisms is crucial for developing targeted therapeutic interventions and provides insights into host-parasite coevolutionary relationships (Miller *et al*., 2002).

Sporozoite invasion of hepatocytes represents the initial critical step in establishing malaria infection following mosquito bite transmission (Mota and Rodriguez, 2004). Sporozoites exhibit remarkable motility mediated by a specialized form of locomotion called gliding motility, powered by an actomyosin motor complex located beneath the parasite plasma membrane. This motility enables sporozoites to traverse various cell types and tissue barriers during migration to the spleen, including skin cells, blood vessel endothelium, and Kupffer cells (Amino *et al*., 2008).

Hepatocyte invasion by sporozoites involves complex molecular recognition events mediated by specific surface proteins and host cell receptors (Sinnis and Coppi, 2007). The circumsporozoite protein (CSP), the most abundant sporozoite surface antigen, plays crucial roles in both salivary gland invasion in mosquitoes and hepatocyte recognition in mammals. Additional sporozoite surface proteins, including thrombospondin-related adhesive protein (TRAP) and TRAP-related protein (TRP), facilitate attachment to hepatocyte surface proteoglycans and subsequent invasion.

The invasion process involves formation of a moving junction between the sporozoite and hepatocyte, mediated by secretion of proteins from specialized organelles called micronemes. This junction serves as a molecular sieving mechanism, excluding certain host membrane proteins while allowing others to be incorporated into the nascent parasitophorous vacuole membrane. Following successful invasion, sporozoites undergo dramatic morphological and metabolic changes as they differentiate into spleen-stage trophozoites (Vaughan *et al*., 2008).

Merozoite invasion of red blood cells represents the most clinically relevant invasion process, as it directly correlates with symptom development and disease pathogenesis (Cowman and Crabb, 2006). This process occurs through a series of well-characterized steps involving initial attachment, reorientation, tight junction formation, and membrane invagination. Merozoite surface proteins, particularly MSP-1, MSP-2, and MSP-3, mediate initial erythrocyte recognition and binding through interactions with glycophorin A and other red blood cell surface receptors.

The formation of tight junctions between merozoites and erythrocytes involves secretion of proteins from rhoptry organelles, including rhoptry-associated protein complexes that restructure the host cell membrane and establish the parasitophorous vacuole (Alexander *et al*., 2006). This invasion process is remarkably rapid, typically completed within 1-2 minutes, and results in complete internalization of the merozoite within a membrane-bound vacuole derived from the host erythrocyte membrane (Gilson and Crabb, 2009).

Host-parasite interactions at the cellular level extend beyond simple invasion mechanisms to include complex metabolic exchanges, immune evasion strategies, and host cell remodeling processes (Kirk, 2001). Infected erythrocytes undergo extensive modifications, including insertion of parasite-derived proteins into the host cell membrane, establishment of new

permeation pathways for nutrient uptake, and development of cytoadherence properties that contribute to disease pathogenesis (Marti *et al*., 2004). These cellular modifications represent potential targets for therapeutic intervention and provide insights into parasite adaptation mechanisms.

2.3 The Spleen in Malaria Infection

2.3.1 Spleen Anatomy and Physiology

The spleen represents a complex secondary lymphoid organ that occupies a central position in the body's defense against blood-borne pathogens, particularly malaria parasites. Understanding its intricate anatomy and physiology is fundamental to comprehending its role in malaria pathogenesis and host defense mechanisms. The spleen's unique architectural organization comprises two functionally distinct compartments: the red pulp and the white pulp, each serving specialized roles in immune surveillance and blood filtration (Buffet *et al*., 2011; del Portillo *et al*., 2012).

The red pulp, constituting approximately 75% of the splenic volume, functions as the primary blood filtration system. This compartment contains a network of splenic cords and venous sinuses lined with specialized macrophages that possess remarkable capability to recognize and eliminate abnormal red blood cells (RBCs). The splenic cords house various immune cells including macrophages, dendritic cells, and plasma cells, creating an environment optimized for phagocytic clearance of parasitized erythrocytes. The venous sinuses, with their unique fenestrated endothelium, allow for selective passage of healthy RBCs while trapping those that have been compromised by parasitic infection (Buffet *et al*., 2011).

The white pulp serves as the immune-reactive center of the spleen, organized around central arterioles and comprising distinct zones including the periarteriolar lymphoid sheaths (PALS), marginal zones, and germinal centers. The PALS contains predominantly T lymphocytes, while the marginal zone harbors specialized B cells, marginal zone macrophages, and dendritic cells. This architectural organization facilitates efficient antigen presentation and immune cell activation following exposure to malaria parasites (Stevenson and Riley, 2004). The marginal zone macrophages are particularly important in malaria immunity, as they serve as the first line of defense against blood-borne parasites and play crucial roles in both innate and adaptive immune responses.

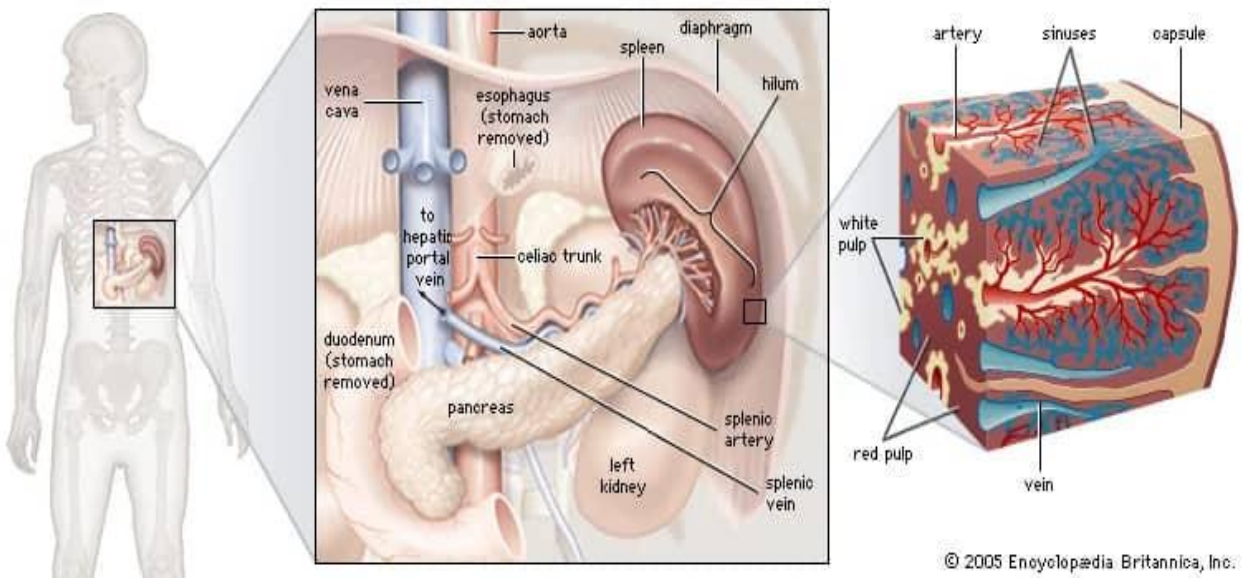

Figure 3: The Spleen (Editors of Encyclopaedia Britannica, 2025).

The spleen's blood flow dynamics are uniquely adapted to facilitate pathogen detection and clearance. Blood entering through the splenic artery flows through progressively smaller vessels before reaching the red pulp, where it undergoes either rapid transit through the closed circulation or slower passage through the open circulation system. This dual circulation pattern

ensures that all blood cells are subjected to mechanical and immunological screening, with parasitized RBCs being selectively retained for destruction (del Portillo *et al*., 2012).

2.3.2 Splenic Role in Malaria Pathogenesis and Host Defense

The spleen functions as the epicenter of malaria infection and immunity, serving dual and sometimes conflicting roles as both a site of parasite clearance and potential parasite sequestration. During malaria infection, the spleen undergoes dramatic structural and functional modifications that reflect its central role in host-parasite interactions. These changes represent adaptive responses aimed at controlling parasitemia while simultaneously contributing to the pathophysiology observed in malaria patients (Wykes *et al*., 2014; Lamikanra *et al*., 2007).

In the host defense, the spleen employs multiple mechanisms to recognize and eliminate parasitized RBCs. The mechanical filtering function relies on the spleen's ability to detect subtle changes in RBC deformability caused by parasite-induced modifications to the erythrocyte membrane and cytoskeleton. Parasitized RBCs become progressively less deformable as the parasite matures, making them susceptible to splenic retention and subsequent phagocytic clearance (Safeukui *et al*., 2008). This mechanical clearance mechanism operates independently of specific immune recognition and represents a fundamental innate defense mechanism against malaria parasites.

The immunological clearance mechanisms involve complex interactions between various splenic cell populations and parasitized RBCs. Marginal zone macrophages serve as sentinel cells that rapidly recognize and phagocytose parasitized erythrocytes through pattern recognition receptors and complement-mediated mechanisms. These cells also play crucial roles in antigen presentation, initiating adaptive immune responses that contribute to long-term protection against

malaria (Wykes *et al*., 2014). The activation of splenic T and B lymphocytes leads to the production of specific antibodies and the development of cellular immune responses that enhance parasite clearance in subsequent infections.

However, the spleen's role in malaria extends beyond simple parasite clearance. Recent evidence suggests that the spleen may also serve as a reservoir for malaria parasites, particularly during chronic infections. Studies have demonstrated the presence of intact parasites within splenic tissue, indicating that the organ may provide a protected environment for parasite survival and potential transmission (Barber *et al*., 2015). This dual role of the spleen as both a site of parasite clearance and potential sequestration adds complexity to our understanding of malaria pathogenesis and has important implications for treatment strategies.

The spleen also plays a critical role in extramedullary hematopoiesis during malaria infection, compensating for the massive destruction of RBCs that occurs during acute infection. This process involves the activation of resident hematopoietic stem cells and the recruitment of progenitor cells from the bone marrow, leading to increased production of new RBCs within the splenic microenvironment (Buffet *et al*., 2011). While this response helps maintain adequate RBC levels, it also contributes to splenomegaly and may provide additional targets for parasite invasion.

2.4 Malaria-Induced Splenic Pathology

2.4.1 Histopathological Changes in Malaria-Affected Spleen

Malaria infection induces profound histopathological alterations in splenic architecture that reflect both the organ's defensive responses and the pathological consequences of parasitic infection. These changes vary depending on the species of malaria parasite, the stage of

infection, host factors, and the chronicity of the disease process. Understanding these histopathological modifications is essential for interpreting the clinical significance of splenic involvement in malaria and for developing appropriate therapeutic interventions.

In acute malaria infections, the most prominent histopathological feature is marked splenomegaly accompanied by congestion of the red pulp with parasitized and uninfected RBCs. The red pulp sinusoids become engorged with blood, and there is significant expansion of the macrophage population, reflecting increased phagocytic activity. Hemozoin deposits, the insoluble pigment formed from hemoglobin degradation by malaria parasites, become prominently visible within macrophages throughout the red pulp, serving as a pathognomonic marker of malaria infection (Lamikanra *et al*., 2007). These pigment-laden macrophages, often referred to as malaria pigment macrophages, represent sites of active parasite destruction and hemoglobin processing.

The white pulp undergoes significant structural disorganization during malaria infection, with disruption of normal follicular architecture and loss of distinct boundaries between different lymphoid compartments. The periarteriolar lymphoid sheaths show increased cellularity with activation of T lymphocytes, while the marginal zones become expanded and populated with activated macrophages and dendritic cells. Germinal center formation may be impaired in severe infections, potentially compromising the development of effective adaptive immune responses (Wykes *et al*., 2014).

Inflammatory infiltrates comprising neutrophils, eosinophils, and mononuclear cells are commonly observed throughout the splenic parenchyma, particularly in areas of intense parasitized RBC sequestration. These inflammatory cells contribute to tissue damage through the

release of cytotoxic mediators and may exacerbate the pathological changes associated with malaria infection. The presence of microthrombi and areas of ischemic necrosis may be observed in severe cases, reflecting the systemic coagulation abnormalities that characterize complicated malaria (Lamikanra *et al*., 2007).

Chronic malaria infections are associated with progressive fibrosis of the splenic parenchyma, particularly affecting the red pulp architecture. This fibrotic response may represent an attempt to contain ongoing inflammation but ultimately leads to impaired splenic function and reduced efficiency of parasite clearance. The deposition of collagen and other extracellular matrix proteins results in the formation of fibrous septa that compartmentalize the splenic tissue and may contribute to the development of hypersplenism in patients with repeated malaria infections (Buffet *et al*., 2011).

Vascular changes are also prominent features of malaria-induced splenic pathology, including endothelial activation, increased vascular permeability, and the formation of microvasculopathy. These changes may contribute to splenic infarction in severe cases and are associated with the systemic endothelial dysfunction observed in complicated malaria. The splenic vasculature may also serve as a site for parasite sequestration, particularly for mature stages of *Plasmodium* falciparum that exhibit cytoadherence properties (del Portillo *et al*., 2012).

2.4.2 Clinical Presentations of Splenic Malaria

The clinical manifestations of splenic involvement in malaria encompass a broad spectrum of presentations ranging from mild splenomegaly to life-threatening complications such as splenic rupture. The clinical presentation is influenced by multiple factors including the infecting parasite species, parasitemia levels, host immune status, previous exposure to malaria, and the

presence of comorbid conditions. Understanding these clinical presentations is crucial for early recognition and appropriate management of patients with malaria-associated splenic pathology.

Splenomegaly represents the most common clinical manifestation of splenic involvement in malaria, occurring in 50-90% of patients depending on the population studied and the endemicity of the region. The degree of splenomegaly correlates with the severity and duration of infection, with acute infections typically causing moderate enlargement while chronic or repeated infections may result in massive splenomegaly. The enlarged spleen is typically soft, tender, and may extend several centimeters below the left costal margin. Patients often complain of left upper quadrant pain, early satiety, and abdominal discomfort related to the mechanical effects of the enlarged organ (Lamikanra *et al*., 2007).

The clinical syndrome of hypersplenism may develop in patients with chronic malaria, particularly those living in hyperendemic areas with repeated infections. This condition is characterized by pancytopenia resulting from increased sequestration and destruction of blood cells within the enlarged spleen. Patients present with symptoms related to anemia, thrombocytopenia, and leukopenia, including fatigue, bleeding tendency, and increased susceptibility to infections. The development of hypersplenism significantly complicates the clinical management of malaria and may require specific therapeutic interventions (Buffet *et al*., 2011).

Splenic pain is a frequent complaint in patients with malaria-associated splenomegaly, typically described as a dull, aching sensation in the left upper quadrant that may radiate to the left shoulder. The pain is often exacerbated by movement, deep inspiration, or palpation and may be associated with a sensation of abdominal fullness. In some cases, the pain may be severe enough

to limit physical activity and significantly impact the patient's quality of life (Wykes *et al*., 2014).

The clinical presentation may also include symptoms related to splenic dysfunction, such as increased susceptibility to bacterial infections, particularly those caused by encapsulated organisms. This increased infection risk reflects the impaired immune function associated with splenic pathology and may persist even after successful treatment of the malaria infection. Patients may present with recurrent respiratory tract infections, sepsis, or other invasive bacterial diseases that require specific antibiotic prophylaxis and vaccination strategies (del Portillo *et al*., 2012).

Laboratory findings in patients with splenic malaria typically include evidence of hemolysis, such as elevated lactate dehydrogenase, indirect bilirubin, and reticulocyte count. Thrombocytopenia is common and may be severe, contributing to bleeding complications. The presence of malaria pigment in peripheral blood monocytes may provide additional evidence of splenic involvement and active parasite clearance. Imaging studies, particularly ultrasonography, can provide valuable information about splenic size, echogenicity, and the presence of complications such as infarction or hematoma formation.

2.4.3 Complications and Prognosis

Splenic complications in malaria represent some of the most serious and potentially life-threatening manifestations of the disease, with significant implications for patient morbidity and mortality. These complications range from relatively common conditions such as splenic infarction to rare but catastrophic events like spontaneous splenic rupture. The recognition and

management of these complications require a thorough understanding of their pathophysiology, risk factors, and clinical presentations.

Spontaneous splenic rupture represents the most feared complication of malaria-associated splenomegaly, with a reported incidence ranging from 0.1% to 2% of patients with malaria. This complication can occur at any stage of the infection but is most commonly observed during the acute phase when the spleen is maximally enlarged and congested. The pathophysiology involves acute distension of the splenic capsule combined with increased fragility of the splenic parenchyma due to congestion and inflammatory changes. Risk factors include rapid parasite clearance following antimalarial treatment, physical activity, abdominal trauma, and pre-existing splenomegaly (Renzulli *et al*., 2009).

The clinical presentation of splenic rupture is typically dramatic, with sudden onset of severe left upper quadrant pain, signs of hypovolemic shock, and peritoneal irritation. However, the presentation may be subtle in some cases, particularly when rupture is contained by surrounding structures. The diagnosis requires a high index of suspicion and may be confirmed by imaging studies such as computed tomography or ultrasonography. Management typically requires emergency surgical intervention, including splenectomy or splenorrhaphy, depending on the extent of injury and the patient's hemodynamic status (Buffet *et al*., 2011).

Splenic infarction is another significant complication that may occur in patients with severe malaria, particularly those infected with *Plasmodium falciparum*. The pathophysiology involves occlusion of splenic arteries by sequestered parasitized RBCs, microthrombi, or inflammatory processes leading to ischemic necrosis of splenic tissue. Patients typically present with severe left upper quadrant pain, fever, and leukocytosis. The diagnosis can be confirmed by imaging

studies showing characteristic wedge-shaped areas of decreased perfusion. Management is primarily supportive, focusing on pain control and treatment of the underlying malaria infection (Lamikanra *et al*., 2007).

The development of splenic abscess is a rare but serious complication that may occur in patients with severe or complicated malaria. This condition typically results from secondary bacterial infection of necrotic splenic tissue or hematogenous seeding from other infected sites. Patients present with persistent fever, left upper quadrant pain, and systemic signs of sepsis despite appropriate antimalarial treatment. The diagnosis requires imaging studies and may necessitate percutaneous drainage or surgical intervention in addition to appropriate antibiotic therapy (Wykes *et al*., 2014).

Chronic complications of splenic malaria include the development of tropical splenomegaly syndrome (TSS), also known as hyperreactive malarial splenomegaly. This condition occurs in patients with repeated malaria infections and is characterized by massive splenomegaly, hypergammaglobulinemia, and pancytopenia. The pathophysiology involves an exaggerated immune response to repeated antigenic stimulation, leading to progressive splenic enlargement and hypersplenism. Patients may present with symptoms related to mechanical compression by the enlarged spleen and complications of pancytopenia. Treatment typically involves long-term antimalarial prophylaxis and may require splenectomy in severe cases (del Portillo *et al*., 2012).

The prognosis for patients with splenic complications of malaria varies depending on the specific complication, the timeliness of diagnosis and treatment, and the presence of other organ involvement. Patients with uncomplicated splenomegaly generally have a good prognosis with appropriate antimalarial treatment and supportive care. However, those who develop splenic

rupture or other serious complications face significant morbidity and mortality risks that may exceed 50% without prompt recognition and appropriate intervention. Long-term follow-up is essential for patients with splenic complications, as some may develop chronic problems requiring ongoing management.

2.5 Animal Models in Malaria Research

2.5.1 Rationale for Animal Models

The utilization of animal models in malaria research has been instrumental in advancing our understanding of disease pathogenesis, host-parasite interactions, and therapeutic interventions over the past several decades. The complexity of malaria biology and the ethical constraints associated with human experimentation necessitate the use of well-characterized animal model systems that can recapitulate key aspects of human disease while allowing controlled experimental manipulation (Craig *et al*., 2012).

Limitations of human studies present significant obstacles to comprehensive malaria research, particularly regarding mechanistic investigations and interventional studies. Ethical considerations preclude many types of experimental manipulations in human subjects, including tissue sampling at multiple time points, investigation of lethal disease progression, and testing of potentially toxic therapeutic agents. Additionally, the variability inherent in human populations regarding genetic background, immune status, concurrent diseases, and environmental exposures makes it difficult to isolate specific variables of interest (Craig *et al*., 2012).

The heterogeneity of human malaria infections further complicates research efforts, as factors such as previous exposure history, acquired immunity levels, nutritional status, and concurrent

medications can significantly influence disease presentation and progression. Natural infections often involve mixed parasite species, variable parasite loads, and unpredictable timing of presentation, making it challenging to study specific aspects of disease pathogenesis or evaluate therapeutic interventions under controlled conditions.

Ethical considerations in human malaria research extend beyond simple subject safety to include complex issues regarding vulnerable populations, informed consent in resource-limited settings, and equitable benefit distribution (Craig *et al*., 2012). Controlled human malaria infection studies, while valuable, are limited to healthy volunteers and cannot investigate severe disease manifestations or test interventions with significant toxicity risks (Sauerwein *et al*., 2011). Observational studies of natural infections, while informative, cannot control for confounding variables or establish definitive causal relationships.

Controlled experimental conditions represent a fundamental advantage of animal model systems, allowing researchers to standardize variables such as parasite strain, inoculum size, infection route, environmental conditions, and genetic background. These standardized conditions enable precise investigation of dose-response relationships, temporal progression of pathological changes, and mechanisms of therapeutic action that would be impossible to study in human populations (Craig *et al*., 2012). The ability to control experimental variables also facilitates statistical analysis and interpretation of results, improving the reliability and reproducibility of research findings.

Animal models provide unique opportunities to investigate tissue-level pathology through serial sampling and detailed histopathological analysis that would be unethical or impractical in human subjects. The availability of genetically modified animal strains allows investigation of specific

gene functions and pathogenic mechanisms, while the ability to manipulate immune responses through pharmacological or genetic means provides insights into host-parasite interactions (Craig *et al*., 2012). Additionally, animal models enable investigation of pre-clinical interventions and serve as essential stepping stones for translating laboratory discoveries to human applications.

Reproducibility and standardization represent critical advantages of animal model systems that enhance the reliability and comparability of research findings across different laboratories and studies. Inbred animal strains provide genetic uniformity that reduces variability and improves statistical power, while standardized housing conditions, diets, and experimental protocols ensure consistent results (Craig *et al*., 2012). The availability of well-characterized parasite strains with known biological properties facilitates comparison of results between studies and enables meta-analysis of findings across multiple investigations.

2.5.2 Rodent Models of Malaria

Rodent malaria models have served as the cornerstone of experimental malaria research for over seven decades, providing invaluable insights into parasite biology, disease pathogenesis, immune responses, and therapeutic interventions. These models utilize naturally occurring *Plasmodium* species that infect rodents, offering experimental systems that closely parallel many aspects of human malaria while allowing detailed investigation under controlled laboratory conditions.

Plasmodium berghei represents one of the most widely used rodent malaria models, infecting both mice and rats and providing a versatile system for studying various aspects of malaria biology. This parasite species exhibits several characteristics that make it particularly valuable for research, including rapid growth rates, high parasitemia levels, and the ability to cause severe

disease manifestations resembling human malaria complications. *P. berghei* infections typically progress rapidly, with parasitemia reaching 20-80% within 7-10 days of infection, making it suitable for both acute pathogenesis studies and drug efficacy testing (Craig *et al*., 2012).

The *P. berghei* model has been particularly valuable for investigating cerebral malaria pathogenesis, as certain strains (particularly *P. berghei* ANKA) reliably induce experimental cerebral malaria in susceptible mouse strains (Hunt and Grau, 2003). This model has provided crucial insights into the role of inflammatory responses, vascular dysfunction, and blood-brain barrier disruption in cerebral malaria development (Rénia and Potter, 2006). Additionally, *P. berghei* infections cause significant splenic pathology, making this model suitable for investigating spleen involvement in malaria.

Plasmodium yoelii models encompass several subspecies with distinct biological characteristics, providing researchers with systems for studying different aspects of malaria pathogenesis and immunity. *P. yoelii* yoelii 17XNL represents a self-limiting infection in most mouse strains, making it valuable for studying naturally acquired immunity and memory responses. In contrast, *P. yoelii* yoelii 17XL causes lethal infections in most mouse strains, providing a model for studying severe malaria pathogenesis (Craig *et al*., 2012).

The *P. yoelii* model system has been particularly important for investigating spleen-stage biology and immunity, as this parasite produces large, easily visualized spleen schizonts that facilitate detailed study of pre-erythrocytic development (Vaughan *et al*., 2008). *P. yoelii* infections also provide excellent models for studying erythrocytic stage immunity, as infected animals can develop sterile immunity following drug cure, enabling investigation of protective immune

mechanisms. The genetic tractability of *P. yoelii* has made it valuable for molecular studies of parasite gene function and host-parasite interactions.

Plasmodium chabaudi represents another important rodent malaria model that has contributed significantly to our understanding of malaria immunology and pathogenesis. This parasite typically causes chronic infections with periodic parasitemia peaks, closely resembling the natural history of human *P. malariae* infections. *P. chabaudi* infections are characterized by initial acute phase followed by chronic low-level parasitemia that can persist for months, making this model particularly valuable for studying chronic malaria effects and immunity (Craig *et al*., 2012).

The *P. chabaudi* model has been extensively used to investigate malaria anemia pathogenesis, as infected animals develop severe anemia through both parasitized and non-parasitized erythrocyte destruction. This model has provided important insights into the role of inflammatory mediators, complement activation, and autoimmune mechanisms in malaria-associated anemia development. Additionally, *P. chabaudi* infections cause significant splenic and splenic pathology, making this model suitable for studying organ-specific malaria complications.

Each rodent malaria model system presents distinct advantages and limitations that must be considered when selecting appropriate models for specific research questions (Craig *et al*., 2012). Advantages include genetic tractability, standardized experimental conditions, reproducible disease patterns, and the availability of extensive immunological reagents and techniques. The relatively low cost and ease of maintenance compared to larger animal models make rodent systems accessible to most research laboratories.

Limitations of rodent malaria models include species-specific differences in parasite biology, immune responses, and disease manifestations compared to human malaria (Craig *et al*., 2012). Rodent parasites exhibit different invasion mechanisms, antigenic properties, and drug sensitivities compared to human parasites, potentially limiting the translational relevance of findings. Additionally, the compressed time course of rodent infections may not accurately reflect the prolonged disease progression observed in human malaria.

2.5.3 Specific Focus on Spleen Studies in Animal Models

Animal models have played an indispensable role in advancing our understanding of splenic pathology in malaria, providing controlled experimental conditions that are impossible to achieve in human studies. These models have enabled researchers to investigate the temporal progression of splenic changes, evaluate therapeutic interventions, and explore the mechanistic basis of spleen-parasite interactions. The selection of appropriate animal models is crucial for generating clinically relevant data that can inform human disease management and drug development strategies.

Rodent models, particularly mice infected with various *Plasmodium* species, represent the most widely utilized experimental systems for studying splenic malaria pathology. The *Plasmodium berghei* model in mice has proven particularly valuable for investigating acute splenic changes and has provided fundamental insights into the mechanisms of parasite clearance and immune activation. Studies using this model have demonstrated that different mouse strains exhibit varying degrees of susceptibility to splenic pathology, with C57BL/6 mice showing distinct patterns of splenic cell death and immune activation compared to BALB/c or other inbred strains (Mohan and Stevenson, 1998).

Recent research has utilized sophisticated approaches to analyze splenic pathology in animal models, including detailed histopathological assessment, flow cytometric analysis of splenocyte populations, and molecular characterization of inflammatory responses. A comprehensive study by Silva *et al*. (2021) examined spleen histopathology in four different mouse strains infected with *Plasmodium berghei* K173, revealing strain-specific differences in splenic architecture disruption, immune cell infiltration, and hemozoin deposition patterns. The study demonstrated that clearance of infection is believed to be accomplished by the spleen and mononuclear phagocytic system (MPS), independent of artemisinin treatment, with the spleen filtering infected RBCs from circulation through immune-mediated recognition followed by phagocytosis.Non-human primate models, while more costly and technically challenging, offer the advantage of closer physiological similarity to human malaria. Studies using *Plasmodium* knowlesi or *Plasmodium* cynomolgi in macaque models have provided valuable insights into splenic pathology that closely resembles human disease. These models have been particularly useful for evaluating the efficacy of therapeutic interventions and for understanding the relationship between splenic pathology and systemic complications such as severe anemia and organ dysfunction (del Portillo *et al*., 2012).

The application of genetically modified animal models has opened new avenues for investigating specific aspects of splenic function in malaria. Knockout mice lacking specific immune receptors,

The *Plasmodium* yoelii model has provided unique insights into the mechanisms of splenic cell death and its relationship to disease severity and host mortality. Research by Pérez-Mazliah *et al*. (2017) utilized this model to investigate the balance between protective immune responses and pathological tissue damage in the spleen. Their findings revealed that malaria displays a

spectrum of symptoms and severity determined by complex host-parasite interactions, and that the mechanisms leading to specific disease phenotypes can be investigated using strains of rodent malaria parasites that cause different disease symptoms in inbred mice. This research highlighted the importance of apoptotic and necrotic cell death pathways in determining the outcome of splenic responses to malaria infection.

Advanced imaging techniques have been increasingly applied to animal models to study splenic dynamics during malaria infection in real-time. These approaches include intravital microscopy, magnetic resonance imaging, and positron emission tomography, which allow researchers to monitor changes in splenic blood flow, cellular trafficking, and metabolic activity throughout the course of infection. Such studies have revealed the dynamic nature of splenic responses to malaria and have provided insights into the temporal relationship between parasite load, immune activation, and tissue pathology (Wykes *et al*., 2014).

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The application of genetically modified animal models has opened new avenues for investigating specific aspects of splenic function in malaria. Knockout mice lacking specific immune receptors, cytokines, or cell surface molecules have been used to dissect the roles of individual

pathways in splenic responses to malaria infection. For example, studies using CD36-deficient mice have revealed the importance of this scavenger receptor in the clearance of parasitized RBCs by splenic macrophages, while research with complement-deficient animals has highlighted the role of complement activation in splenic parasite clearance (Lamikanra *et al*., 2007).

Contemporary research approaches in animal models increasingly emphasize systems biology approaches that integrate multiple levels of analysis, from molecular and cellular changes to organ-level pathology and systemic physiological responses. These comprehensive studies provide holistic views of how splenic pathology contributes to overall disease severity and help identify potential therapeutic targets for intervention. Modern research recognizes the spleen as a complex secondary lymphoid organ that plays a crucial role in controlling blood-stage infection with *Plasmodium* parasites, tasked with sensing and removing parasitized RBCs, erythropoiesis, and the activation and differentiation of immune cells.

The translation of findings from animal models to human disease remains a critical challenge in malaria research. While animal models provide invaluable mechanistic insights and opportunities for therapeutic testing, important species-specific differences must be considered when interpreting results. Ongoing research efforts focus on developing humanized animal models and improving the clinical relevance of experimental findings through careful selection of outcome measures and experimental conditions that more closely mirror human disease scenarios.

2.6 Histopathological Assessment Techniques

2.6.1 Tissue Fixation and Processing Methods

Proper tissue fixation and processing represent critical initial steps in histopathological assessment that directly impact the quality and reliability of subsequent analyses (Bancroft and Gamble, 2008). In malaria research, where subtle cellular changes and parasite-specific alterations must be preserved and accurately visualized, the selection of appropriate fixation methods becomes particularly crucial for obtaining meaningful results.

Formalin fixation remains the gold standard for routine histopathological examination in malaria studies, providing excellent morphological preservation while maintaining compatibility with most histological staining techniques (Kiernan, 2015). Ten percent neutral buffered formalin effectively cross-links proteins and nucleic acids, preventing autolysis and bacterial decomposition while preserving cellular architecture for extended periods. The penetration rate of formalin (approximately 1mm per hour) necessitates proper tissue sectioning (≤3mm thickness) and adequate fixative volume (20:1 fixative to tissue ratio) to ensure uniform fixation throughout the specimen (Bancroft and Gamble, 2008).

Alternative fixation methods may be employed for specific research applications or when enhanced preservation of particular cellular components is required (Kiernan, 2015). Mercuric chloride-based fixatives, such as B5 or Zenker's solution, provide superior nuclear morphology and are particularly valuable for examining malaria pigment and parasitic inclusions. However, these fixatives require special handling procedures and post-fixation treatment with iodine and sodium thiosulfate to remove mercury deposits that can interfere with microscopic examination (Bancroft and Gamble, 2008).

Alcoholic fixatives, particularly ethanol-based solutions, may be preferred for certain immunohistochemical applications or when glycogen preservation is important (Kiernan, 2015). Bouin's solution, containing picric acid, formaldehyde, and acetic acid, provides rapid fixation and excellent nuclear detail but may cause tissue brittleness and requires careful handling due to the explosive nature of dry picric acid. The choice of fixative should be based on the specific research objectives, intended staining methods, and safety considerations (Bancroft and Gamble, 2008).

Tissue processing involves controlled dehydration, clearing, and infiltration steps that prepare fixed tissues for sectioning and staining (Kiernan, 2015). Automated tissue processors provide standardized processing protocols that ensure consistent results while minimizing handling time and reducing exposure to hazardous chemicals. The dehydration sequence typically involves graded alcohol concentrations (70%, 80%, 95%, 100%) that gradually remove water from tissues while preventing excessive shrinkage or hardening (Bancroft and Gamble, 2008).

Clearing agents, such as xylene or xylene substitutes, remove alcohol from tissues and prepare them for paraffin infiltration by providing compatibility between the hydrophobic paraffin and the last dehydrating agent (Kiernan, 2015). Modern clearing agents offer reduced toxicity compared to traditional xylene while maintaining excellent clearing properties and paraffin compatibility. The infiltration process involves gradual replacement of clearing agent with molten paraffin wax, typically at 60-65°C, ensuring complete penetration and support for subsequent sectioning (Bancroft and Gamble, 2008).

Quality control measures throughout the fixation and processing workflow are essential for maintaining consistency and reliability in malaria histopathology studies (Kiernan, 2015).

Regular monitoring of fixative pH, temperature control during processing, and maintenance of chemical change schedules prevent artifacts and ensure optimal tissue preservation. Documentation of processing parameters and regular calibration of automated equipment contribute to reproducible results and facilitate troubleshooting when problems arise (Bancroft and Gamble, 2008).

2.6.2 Staining Techniques for Malaria Research

Histological staining techniques in malaria research serve multiple purposes, including visualization of general tissue architecture, identification of parasite-specific elements, and characterization of host cellular responses (Bancroft and Gamble, 2008). The selection of appropriate staining methods depends on the specific research objectives and the particular aspects of malaria pathology under investigation.

Hematoxylin and eosin (H&E) staining remains the fundamental technique for general histopathological assessment in malaria studies, providing excellent visualization of cellular morphology and tissue architecture (Kiernan, 2015). Hematoxylin, a basic dye, binds to acidic cellular components such as nuclei and ribosomes, producing blue to purple coloration, while eosin, an acidic dye, stains basic cytoplasmic proteins pink to red (Bancroft and Gamble, 2008). This complementary staining combination allows identification of hepatocyte injury, inflammatory cell infiltration, and architectural changes characteristic of malaria-induced spleen pathology.

Specialized stains for hemosiderin and iron deposition provide crucial information about malaria-induced changes in iron metabolism and hemoglobin breakdown products (Kiernan,

2015). Perl's Prussian blue reaction utilizes potassium ferrocyanide and hydrochloric acid to identify ferric iron deposits, producing characteristic blue coloration that facilitates detection of hemosiderin accumulation in Kupffer cells and other macrophages (Bancroft and Gamble, 2008). This technique is particularly valuable for assessing the extent of hemolysis and iron overload associated with malaria infections.

Reticulin staining techniques, such as the Gomori reticulin method, provide detailed visualization of the splenic reticular framework and are essential for assessing architectural changes and fibrosis development in malaria-affected spleens (Kiernan, 2015). Silver impregnation techniques specifically highlight reticulin fibers, basement membranes, and collagen deposits, allowing evaluation of structural integrity and fibrotic responses (Bancroft and Gamble, 2008). These stains are particularly useful for detecting early fibrotic changes that may not be apparent with routine H&E staining.

Trichrome staining methods, including Masson's trichrome and Mallory's trichrome, provide excellent differentiation between collagen fibers, muscle tissue, and cytoplasmic components (Kiernan, 2015). These techniques are invaluable for assessing the extent of fibrosis development and characterizing the cellular composition of inflammatory infiltrates in malaria-affected tissues (Bancroft and Gamble, 2008). The ability to distinguish between different types of connective tissue helps evaluate the progression from acute inflammatory changes to chronic fibrotic responses.

Giemsa staining represents a specialized technique particularly valuable for identifying malaria parasites and parasite-derived pigment (hemozoin) within tissues (Kiernan, 2015). This method provides excellent nuclear detail and helps differentiate between inflammatory cell types while

specifically highlighting parasitic elements that may be difficult to identify with routine stains (Bancroft and Gamble, 2008). Modified Giemsa techniques can be optimized for tissue sections to enhance parasite visibility while maintaining adequate tissue morphology.

Immunohistochemical techniques have revolutionized malaria research by enabling specific identification of parasite antigens, host proteins, and cellular markers that provide mechanistic insights into disease pathogenesis (Kiernan, 2015). Antibodies directed against specific *Plasmodium* proteins allow precise localization of parasites within tissues and can differentiate between different developmental stages (Bancroft and Gamble, 2008). Additionally, antibodies against host inflammatory mediators, cellular activation markers, and tissue-specific proteins provide detailed characterization of host responses to infection.

2.6.3 Microscopic Evaluation Criteria

Standardized microscopic evaluation criteria are essential for ensuring consistency and reproducibility in histopathological assessment of malaria-induced spleen changes across different studies and investigators. The establishment of well-defined criteria facilitates comparison of results between research groups and enables meaningful interpretation of experimental findings (Kleiner *et al*., 2005).

Assessment criteria encompass evaluation of cellular morphology, cytoplasmic changes, and nuclear alterations that reflect the spectrum of malaria-induced injury. Normal exhibit uniform size and shape with clearly defined cell boundaries, abundant eosinophilic cytoplasm, and central nuclei with distinct nucleoli (Kleiner *et al*., 2005). Pathological changes include cellular swelling

(ballooning degeneration), cytoplasmic vacuolation, and nuclear alterations ranging from pyknosis to complete nuclear loss in necrotic cells.

Grading systems for hepatocyte injury typically employ semi-quantitative scales that assess both the severity and extent of cellular damage. Severity assessment considers the degree of cellular alterations, ranging from mild swelling and granular cytoplasm to severe ballooning and frank necrosis (Kleiner *et al*., 2005). Extent evaluation estimates the percentage of Splenic cells affected, with categories such as focal (<10%), moderate (10-50%), or extensive (>50%) involvement providing standardized descriptive terms.

Inflammatory infiltrate assessment requires systematic evaluation of cellular composition, distribution patterns, and intensity of inflammatory responses. Portal inflammation is assessed separately from lobular inflammation, with specific attention to the types of inflammatory cells present (lymphocytes, plasma cells, neutrophils, macrophages) and their distribution within different splenic compartments (Kleiner *et al*., 2005). Grading schemes typically employ numerical scales (0-4) that correlate inflammation intensity with clinical significance and prognostic implications.

Fibrosis assessment represents a critical component of chronic malaria evaluation, requiring specialized staining techniques and standardized scoring systems. The Ishak fibrosis staging system provides a widely accepted framework for assessing fibrosis progression, with stages ranging from 0 (no fibrosis) to 6 (established cirrhosis) based on architectural changes and bridging patterns (Kleiner *et al*., 2005). Portal and perisinusoidal fibrosis patterns are evaluated separately, as they may reflect different pathogenic mechanisms and have distinct prognostic implications.

Vascular changes assessment includes evaluation of sinusoidal dilatation, congestion, and endothelial alterations that may contribute to splenic dysfunction in malaria. Standardized criteria for assessing sinusoidal width, cellular infiltration, and inflammatory changes provide quantitative measures of vascular involvement (Kleiner *et al*., 2005). Special attention is given to identifying sequestered parasitized erythrocytes and associated microthrombotic changes that may contribute to local ischemia.

2.6.4 Quantitative Assessment Methods

Quantitative assessment methods in malaria histopathology provide objective, reproducible measurements that complement subjective grading systems and enable statistical analysis of experimental results (Hamilton *et al*., 2011). These techniques are particularly valuable for detecting subtle changes, monitoring disease progression, and evaluating therapeutic interventions in animal model studies (Rizzardi *et al*., 2012).

Morphometric analysis involves systematic measurement of histological features using calibrated microscopic techniques or digital image analysis systems (Hamilton *et al*., 2011). Basic morphometric parameters include cell counting, area measurements, linear dimensions, and volumetric assessments that provide quantitative data on tissue changes (Rizzardi *et al*., 2012). Splenic cell size assessment, for example, can be performed using nuclear-to-cytoplasmic ratios, cellular area measurements, or three-dimensional volume estimations (Manley *et al*., 2006).

Stereological techniques provide unbiased, statistically sound methods for quantifying three-dimensional tissue characteristics from two-dimensional histological sections (Howard and Reed, 2005). Point-counting methods estimate volume fractions of different tissue components, while

line-intersection techniques assess surface areas and linear densities (Hamilton *et al*., 2011). These approaches are particularly valuable for assessing fibrosis extent, inflammatory cell density, and vascular changes in malaria-affected tissues (Rizzardi *et al*., 2012).

Digital image analysis has revolutionized quantitative histopathology by enabling automated or semi-automated measurement of multiple parameters with high precision and reproducibility (Kayser *et al*., 2009). Color-based segmentation techniques can identify and quantify specific tissue components, such as collagen fibers in fibrosis assessment or hemosiderin deposits in iron overload evaluation (Hamilton *et al*., 2011). Automated cell counting algorithms provide rapid, objective enumeration of inflammatory cells, while texture analysis can detect subtle changes in tissue architecture (Rizzardi *et al*., 2012).

Immunohistochemical quantification techniques enable specific measurement of protein expression levels and cellular activation markers in malaria-affected tissues (Taylor and Levenson, 2006). Optical density measurements of immunostaining intensity provide semi-quantitative assessments of protein expression, while automated image analysis can determine the percentage of positive cells or the distribution patterns of specific markers (Hamilton *et al*., 2011). These techniques are particularly valuable for assessing inflammatory mediator expression and characterizing immune cell populations (Rizzardi *et al*., 2012).

Statistical considerations in quantitative histopathology include appropriate sampling strategies, power analysis, and selection of suitable statistical tests for different types of data (Kayser *et al*., 2009). Random sampling protocols ensure representative tissue assessment, while systematic sampling approaches provide unbiased estimates of tissue characteristics (Hamilton *et al*., 2011).

Sample size calculations should consider the expected effect sizes, measurement variability, and desired statistical power to ensure adequate study design (Rizzardi *et al*., 2012).

Quality control measures for quantitative assessment include calibration of measurement systems, inter-observer reliability testing, and validation of automated analysis algorithms (Kayser *et al*., 2009). Regular calibration ensures measurement accuracy, while duplicate assessments by independent observers provide reliability estimates (Hamilton *et al*., 2011). Validation studies comparing automated measurements with manual assessments establish the accuracy and limitations of digital analysis systems (Rizzardi *et al*., 2012).

2.6.5 Digital Pathology Applications

Digital pathology has emerged as a transformative technology in malaria research, offering unprecedented capabilities for image acquisition, analysis, and data sharing that enhance both research efficiency and diagnostic accuracy (Pantanowitz *et al*., 2011). The integration of whole-slide imaging systems, advanced image analysis algorithms, and artificial intelligence techniques has revolutionized how researchers study malaria-induced tissue changes (Madabhushi and Lee, 2016).

Whole-slide imaging systems enable complete digitization of histological sections at microscopic resolution, creating virtual slides that can be viewed, analyzed, and shared electronically (Pantanowitz *et al*., 2011). These systems provide several advantages over traditional microscopy, including consistent image quality, permanent digital storage, simultaneous multi-user access, and integration with image analysis software (García-Rojo *et al*., 2010). In malaria research, whole-slide imaging facilitates comprehensive tissue assessment, enables remote

consultation, and supports collaborative research efforts across institutions (Madabhushi and Lee, 2016).

Advanced image analysis algorithms can automatically identify and quantify specific histological features relevant to malaria pathology, including inflammatory cell counting, fibrosis assessment, and parasite detection (Pantanowitz *et al*., 2011). Machine learning approaches can be trained to recognize complex tissue patterns and provide consistent, objective assessments that reduce inter-observer variability (García-Rojo *et al*., 2010). These automated techniques are particularly valuable for high-throughput studies and longitudinal analyses that require processing large numbers of tissue sections (Madabhushi and Lee, 2016).

Artificial intelligence applications in malaria histopathology include deep learning networks that can identify subtle tissue changes, predict disease outcomes, and assist in diagnostic decision-making (Pantanowitz *et al*., 2011). Convolutional neural networks have shown remarkable success in medical image analysis tasks, including detection of malaria parasites in blood smears and assessment of tissue pathology in organ sections (García-Rojo *et al*., 2010). These AI-powered tools can potentially identify patterns invisible to human observers and provide insights into disease mechanisms (Madabhushi and Lee, 2016).

Telepathology applications enable remote assessment of malaria histopathology, facilitating expert consultation and collaborative research across geographical boundaries (Pantanowitz *et al*., 2011). This capability is particularly valuable for research conducted in resource-limited settings where expert pathology services may not be readily available (García-Rojo *et al*., 2010). Real-time consultation systems allow immediate expert input on challenging cases, while store-

and-forward systems enable asynchronous review and discussion of interesting findings (Madabhushi and Lee, 2016).

Integration with laboratory information systems and research databases enables comprehensive data management and analysis that links histopathological findings with clinical, laboratory, and experimental Integration with laboratory information systems and research databases enables comprehensive data management and analysis that links histopathological findings with clinical, laboratory, and experimental parameters (Pantanowitz *et al*., 2011). This integration facilitates correlation studies that examine relationships between tissue changes and functional outcomes, supporting translational research efforts that bridge laboratory findings with clinical applications (García-Rojo *et al*., 2010). Advanced database systems can store and retrieve complex histopathological data, enabling meta-analyses and systematic reviews that synthesize findings across multiple studies (Madabhushi and Lee, 2016).

Quality assurance in digital pathology requires standardized protocols for image acquisition, color calibration, and validation procedures that ensure consistent and accurate results (Pantanowitz *et al*., 2011). Color standardization techniques compensate for variations in staining intensity and microscope settings, while validation studies compare digital assessments with traditional microscopic evaluations (García-Rojo *et al*., 2010). Regular quality control measures include monitoring of image resolution, focus quality, and color accuracy to maintain diagnostic confidence in digital systems (Madabhushi and Lee, 2016).

Future developments in digital pathology promise even greater capabilities for malaria research, including integration with genomic data, real-time image analysis, and predictive modeling systems (Pantanowitz *et al*., 2011). Multi-modal imaging approaches that combine

histopathology with molecular imaging techniques will provide comprehensive tissue characterization capabilities (García-Rojo *et al*., 2010). The development of portable digital pathology systems may enable field-based research applications, particularly valuable for malaria studies in endemic regions (Madabhushi and Lee, 2016).

2.7 Current Treatment Strategies

Antimalarial Drugs and Spleen Safety

The selection of appropriate antimalarial therapy requires careful consideration of splenic safety profiles, particularly in patients with pre-existing spleen dysfunction or evidence of malaria-induced splenic involvement. Animal model studies have provided extensive safety data for various antimalarial compounds, revealing significant differences in hepatotoxic potential and mechanisms of spleen injury (Bray *et al*., 2005). These preclinical findings have informed clinical practice guidelines and dosing recommendations for patients with splenic complications (White *et al*., 2014).

Artemisinin derivatives, considered first-line therapy for severe malaria, demonstrate excellent splenic safety profiles in animal studies and clinical applications (Dondorp *et al*., 2009). Experimental studies in rodent malaria models show minimal hepatotoxicity even at therapeutic doses, with rapid parasite clearance that reduces the duration of pathological processes (Sinclair *et al*., 2012). The short half-life of artemisinin compounds minimizes accumulation-related toxicity, while their potent antimalarial activity provides rapid clinical improvement.

Quinine, historically important in malaria treatment, demonstrates dose-dependent hepatotoxicity in animal models that correlates with clinical observations of spleen dysfunction in some patients

(Bray *et al*., 2005). Studies in infected animals reveal that quinine-induced spleen injury involves oxidative stress mechanisms and mitochondrial dysfunction, particularly when administered at high doses or for prolonged periods (White *et al*., 2014). These findings have informed dosing protocols that balance antimalarial efficacy with splenic safety considerations (Dondorp *et al*., 2009).

Chloroquine and related 4-aminoquinolines show variable splenic effects in animal studies, with some models demonstrating protective effects against malaria-induced spleen damage while others reveal potential for hepatocyte injury at high concentrations (Sinclair *et al*., 2012). The mechanism of chloroquine hepatotoxicity involves lysosomal dysfunction and phospholipidosis, which can be monitored through histopathological assessment in experimental studies Clinical correlations suggest that chloroquine-related spleen dysfunction is relatively uncommon but may be more frequent in patients with underlying splenic disease (Bray *et al*., 2005).

Mefloquine demonstrates significant hepatotoxic potential in animal models, with studies revealing dose-dependent spleen injury characterized by hepatocyte necrosis and inflammatory infiltration (White *et al*., 2014). The mechanism involves direct hepatocellular toxicity and oxidative stress, with some animal strains showing particular susceptibility to mefloquine-induced spleen damage (Dondorp *et al*., 2009). These preclinical findings have contributed to restrictions on mefloquine use in patients with active spleen disease and requirements for splenic function monitoring during treatment (Sinclair *et al*., 2012).

Hepatoprotective Agents

The development of hepatoprotective strategies for malaria treatment has been informed by animal model studies that identify specific mechanisms of spleen injury and potential therapeutic targets (Anand and Nightingale, 2000). Experimental approaches include antioxidant therapy, anti-inflammatory interventions, and agents that support hepatocyte regeneration and repair processes (Kochar *et al*., 2003). These protective strategies aim to minimize malaria-induced spleen damage while supporting optimal antimalarial therapy (Bhalla *et al*., 2006).

Antioxidant interventions, including N-acetylcysteine, vitamin E, and selenium supplementation, have shown protective effects in animal models of malaria-induced spleen injury (Anand and Nightingale, 2000). These agents work by neutralizing reactive oxygen species, supporting glutathione synthesis, and maintaining cellular antioxidant defenses that are depleted during malaria infection (Kochar *et al*., 2003). Experimental studies demonstrate reduced hepatocyte necrosis, decreased inflammatory infiltration, and improved survival rates when antioxidants are administered alongside antimalarial therapy (Bhalla *et al*., 2006).

Silymarin and other plant-derived hepatoprotective compounds have demonstrated efficacy in experimental malaria models, showing reduction in spleen enzyme elevation and histopathological improvements (Anand and Nightingale, 2000). The mechanisms of action include antioxidant effects, stabilization of hepatocyte membranes, and promotion of hepatocyte regeneration through enhanced protein synthesis (Kochar *et al*., 2003). Clinical trials have shown promising results for silymarin supplementation in malaria patients with splenic involvement, though larger studies are needed to establish definitive efficacy (Bhalla *et al*., 2006).

Ursodeoxycholic acid, a naturally occurring bile acid, has shown hepatoprotective effects in animal models of malaria through multiple mechanisms including membrane stabilization, anti-inflammatory effects, and protection against bile acid-induced hepatocyte injury (Anand and Nightingale, 2000). Experimental studies demonstrate reduced cholestasis, improved bile flow, and decreased inflammatory infiltration when ursodeoxycholic acid is administered to malaria-infected animals (Kochar *et al*., 2003). Clinical applications have shown benefit in malaria patients with cholestatic complications, though optimal dosing regimens require further investigation (Bhalla *et al*., 2006).

Anti-inflammatory agents, including corticosteroids and non-steroidal anti-inflammatory drugs, have been evaluated in animal models with mixed results regarding hepatoprotective effects (Anand and Nightingale, 2000). While some studies show reduced inflammatory infiltration and improved spleen histology, others demonstrate increased susceptibility to parasitic infection or delayed parasite clearance (Kochar *et al*., 2003). The timing and duration of anti-inflammatory therapy appear critical for achieving beneficial effects without compromising antimalarial efficacy (Bhalla *et al*., 2006).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was conducted at the University of Benin, specifically within the Departments of Anatomy and Medical Laboratory Science. The University is situated in Benin City, the capital of Edo State, Nigeria. The geographical coordinates of the University of Benin are approximately Latitude 6.3351 N and Longitude 5.6220 E. This location provided a conducive environment for research on malaria and its effects on the spleen using well-documented animal models.

3.2 Materials and Reagents

The following materials and reagents were used: Formalin solution (10%), Ethanol (70%, 95% and 100%), Hematoxylin and eosin (H&E) for staining, Xylene for clearing, Automatic tissue processor machine (Shandon 2000, Leica, Frankfurt, Germany), Centrifuge (Eppendorf, Model 5804), Hot Plates, Staining Jars and racks, Microtome (Leica RM2245), Olympus BX53 Microscope for histopathological analysis, Analytical balance.

3.3 Ethical Approval

Ethical clearance for the study was obtained from the Ministry of Agriculture and the Institutional Ethical Approval Committee at the University of Benin. The approval process involved a detailed submission of the research proposal outlining the objectives, methodology, and ethical considerations, including the welfare of the animals used. Appropriate measures were

implemented to minimize pain and distress, following the Guidelines for the Care and Use of Laboratory Animals.

3.4 Methodology

3.4.1 Grouping of Animals

Animal Models: Albino Wistar rats (*Rattus norvegicus*), weighing approximately 150-250 grams, were used for the study. 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 2 weeks without any malaria infection.

Group B (Low Infection Group): The four (4) rats in this group were infected with low concentrations of *Plasmodium* spp. (~1 x 10^2 parasitized red blood cells) and observed for any changes in the spleen. They also received standard feed and water for 2 weeks.

Group C (Medium Infection Group): The four (4) rats in this group were infected with Medium concentrations of *Plasmodium* spp. (~1 x 10^4 parasitized red blood cells) and observed for any changes in the spleen. They also received standard feed and water for 2 weeks.

Group D (High Infection Group): The four (4) rats in this group were infected with High concentrations of *Plasmodium* spp. (~1 x 10^6 parasitized red blood cells) and observed for extended splenic pathologies. They also received standard feed and water for 2 weeks.

Table 1: Animal Grouping and Infection Doses

Group Name	Number of Rats	Infection Status	*Plasmodium spp.* Dose (Parasitized Red Blood Cells)	Observation Period (Days)
Group A (Control)	4	Uninfected	0	14
Group B (Low Infection)	4	Infected	10^2	14
Group C (Medium Infection)	4	Infected	10^4	14
Group D (High Infection)	4	Infected	10^6	14

3.4.2 Infection with Malaria

Malaria Parasite: *Plasmodium berghei* was sourced from a known laboratory stock maintained at the University of Benin. *Plasmodium berghei* was used for the study due to its availability and established use in malaria research. The infection process was carried out as follows:

1. **Preparation of Parasite Inoculum:** The malaria parasite (trophozoites) was cultured in established rat models before being harvested for the experiments. The parasites were collected from infected rat blood, prepared in physiological saline and concentrated to achieve the desired dosage for infection.
2. **Infection:** A single intraperitoneal injection of the *Plasmodium berghei* parasite at different doses of ~1 x 10^2, ~1 x 10^4 and ~1 x 10^6 parasitized red blood cells was administered to the rats in Groups B, C, and D respectively.
3. **Days of Observation:** The observation period post-infection extended to 14 days, during which the rats were closely monitored, weighed daily and data pertaining to clinical symptoms, appetite, and behavior were recorded. The rats were observed for specific

signs of malaria infection, including abdominal swelling indicative of splenomegaly, and other clinical signs that suggested splenic dysfunction.

3.4.3 Sacrificing and Harvesting Of Organs

Upon reaching the designated observation period, the rats were humanely sacrificed using a method approved by the Ministry of Agriculture and the Institutional Ethical Approval Committee at the University of Benin. Following the administration period, the animals were euthanized using cervical dislocation to minimize suffering. The spleen was carefully excised. Immediately upon excision, the spleen was weighed to quantify the extent of splenomegaly, as spleen enlargement is a prominent feature and a landmark of malaria. The excised spleen was then placed in pre-labeled sterile containers containing 10% formalin for histopathological processing.

3.5 Histopathological Investigation

Histopathological investigation of the spleen tissues was performed to precisely characterize the morphological and cellular changes induced by malaria infection.

3.5.1 Tissue Processing

The fixed spleen tissues underwent automated processing using a tissue processor machine (Shandon 2000, Leica, Frankfurt, Germany). This involved sequential dehydration through ascending grades of alcohol (70%, 90%, 96% and 100%), clearing in xylene, and impregnation in two changes of molten paraffin wax for specified periods within the processor.

3.5.2 Sectioning and Staining

Processed tissues were embedded in fresh molten paraffin wax and allowed to set. Paraffin tissue blocks were trimmed, sectioned at 3µ thickness, and dried on a hotplate for 15 minutes. Sections

were stained with Cole's haematoxylin and 1% aqueous eosin (H&E) to demonstrate general tissue structure.

3.5.3 Microscopic Examination

Stained slides were dehydrated through various ascending grades of alcohol, cleared in xylene, and mounted in Canada balsam. Sections were microscopically examined using x10 and x40 objective lenses.

3.5.4 Specific Histopathological Assessments

While H&E staining provided a general overview of tissue structure, malaria induced profound and specific changes in splenic microarchitecture. Therefore, a detailed assessment was conducted, focusing on the following parameters:

- **Overall Splenic Architecture**: Evaluation of the integrity and organization of the white pulp (lymphoid follicles, germinal centers, marginal zones) and red pulp (splenic cords, sinusoids), noting any disorganization or blurring of boundaries. This detailed microarchitectural assessment was vital for understanding the mechanisms of splenic dysfunction, not merely its presence. It allowed for the identification of specific cellular targets, pathways, and pathological processes involved in malaria-induced splenic damage, moving beyond a superficial description.
- **Splenomegaly:** Gross observation was confirmed microscopically by assessing the overall cellularity and expansion of splenic compartments, correlating with the measured spleen weight.
- **Red Pulp Changes:** Quantification of red pulp congestion, presence of dilated sinusoids.

- **White Pulp Changes:** Evaluation of hyperplasia or atrophy of lymphoid follicles, changes in germinal center size and activity, and alterations in periarteriolar lymphoid sheaths (PALS) and marginal zones.
- **Immune Cell Infiltration:** Assessment of the presence, distribution, and quantification of inflammatory cells, particularly macrophages (e.g., F4/80+ macrophages), lymphocytes (T and B cells), and plasma cells within both red and white pulp.
- **Fibrosis:** Identification of any signs of fibrous tissue proliferation or architectural distortion, which can occur in chronic splenomegaly.

These histological findings were not viewed in isolation.

Table 2: Key Histopathological Parameters for Spleen Assessment

Parameter	Description/Definition	Grading Scale	Expected Findings in Malaria-Infected Animals
Gross Spleen Weight	Weight of the excised spleen (grams)	Quantitative (g)	Increased significantly compared to controls (splenomegaly)
Splenic Architecture (Overall)	Integrity and organization of white and red pulp	0 (Normal) to 3 (Severe disorganization)	Disorganization, blurring of white/red pulp boundaries
Red Pulp Congestion	Accumulation of blood in red pulp sinusoids	0 (Absent) to 3 (Severe congestion)	Increased congestion, dilated sinusoids
Hemosiderin Deposits	Presence and extent of iron-laden macrophages	0 (Absent) to 3 (Abundant)	Increased hemosiderin within macrophages
White Pulp Hyperplasia/Atrophy	Changes in size and cellularity of lymphoid follicles	Hyperplasia (0-3), Atrophy (0-3)	Variable, often initial hyperplasia followed by atrophy/disorganization
Lymphoid Follicle Disorganization	Loss of distinct germinal centers and mantle zones	0 (Normal) to 3 (Severe disorganization)	Disrupted follicular structure, loss of clear boundaries

Macrophage Infiltration	Presence and density of macrophages in red/white pulp	0 (Sparse) to 3 (Dense infiltration)	Increased F4/80+ macrophages, particularly in red pulp
Fibrosis	Presence of fibrous tissue proliferation	0 (Absent) to 3 (Severe fibrosis)	May show signs of fibrous tissue in chronic cases

3.6 Statistical Analysis

Data collected were organized and analyzed using the Statistical Package for Social Sciences (SPSS) software. Results were expressed as mean ± standard deviation (SD). Analysis of variance (ANOVA) was conducted to compare mean values among groups, with Tukey's post-hoc test applied for multiple comparisons to identify specific group differences. A significance level of $p<0.05$ was considered statistically significant. Additionally, correlation analyses were performed to assess relationships between spleen weight and histopathological scores.

CHAPTER FOUR

4.0 RESULTS

4.1 STATISTICS ANALYSIS

Statistics showing the mean weight of the liver, initial body weight, final body weight, organo-somatic index and the chart of liver weight.

Table 4.3: Showing Body weight, Weight change, liver weight, and splenic-somatic index after malaria infection across all groups.

Groups/Tests	Control	Low Infection	Medium Infection	High Infection	p-value
Initial Weight (g)	**141.0±4.0**	**168.5±0.5**	**147.5±12.5**	**149.0±9.0**	**0.1670**
Final Weight (g)	**158.0±4.0**	**154.5±0.5**	**126.4±11.0**	**123.5±6.5**	**0.0285**
Weight Change (g)	**17.0±0.0**	**-14.0±0.0**	**-21.1±1.5**	**-25.5±2.5**	**0.0001**
Spleen Weight (g)	**0.7±0.1**	**1.4±0.1**	**0.9±0.4**	**0.8±0.1**	**0.0432**
Splenic-somatic index (%)	**0.44±0.05**	**0.91±0.07**	**0.71±0.21**	**0.65±0.08**	**0.0278**

*Values are expressed as Mean±SEM; mean with * superscript are statistically significant at $p<0.05$ compared to control.*

Body Weight Changes

The spleen study confirms the systemic metabolic impact of malaria infection observed in the liver study. All infected groups experienced significant weight loss compared to controls, with the high infection group showing the greatest weight reduction (-25.5±2.5g). This pattern demonstrates that malaria infection imposes a substantial metabolic burden on the host, leading to cachexia and failure to thrive regardless of infection intensity.

The weight loss pattern (low: -14.0g, medium: -21.1g, high: -25.5g) suggests a dose-dependent relationship between parasite load and systemic metabolic dysfunction. This finding is consistent with clinical observations where severe malaria is associated with profound weight loss and nutritional depletion.

There was no statistically significant changes ($p>0.05$) in the initial body weight when compared across groups, indicating proper randomization.

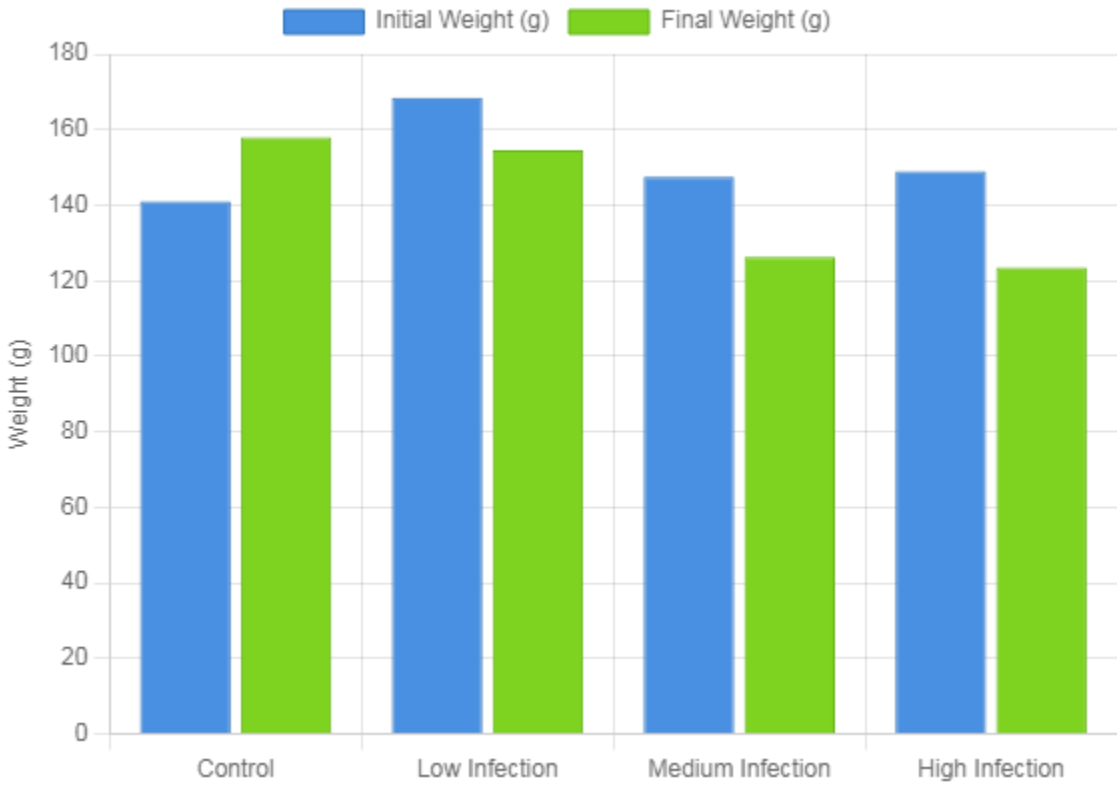

Chart 4.1: Initial and Final Body Weight Grouped Data

There was a **statistically significant decrease (p<0.05)** in the weight of rats that were infected with malaria parasites when compared with the weight of rats in the control group.

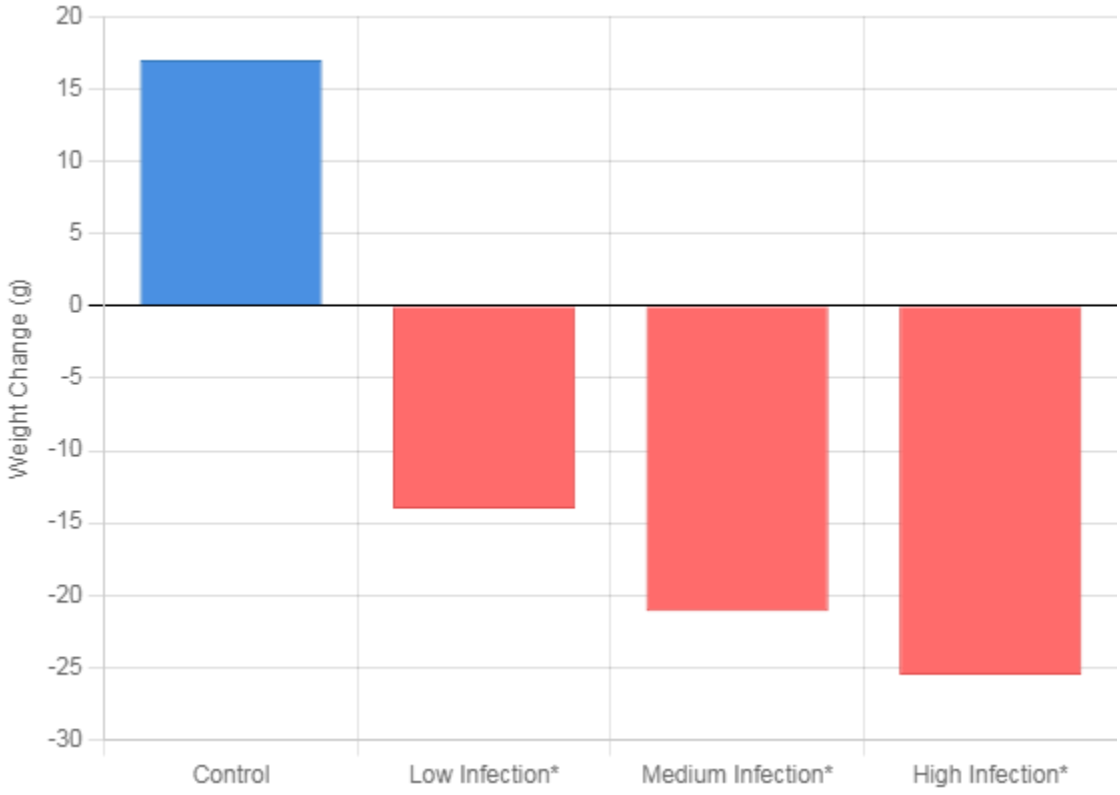

Chart 4.2: Changes in Weight of Rats After Malaria Infection

Spleen Weight Analysis

The most significant finding was the pronounced splenomegaly in the low infection group (1.4±0.1g vs. 0.7±0.1g in controls), representing a 100% increase in spleen weight. This dramatic enlargement reflects the spleen's primary role as the body's filter for infected erythrocytes and its central position in mounting immune responses against malaria parasites.

Interestingly, the medium and high infection groups showed less pronounced spleen enlargement (0.9±0.4g and 0.8±0.1g respectively). This paradoxical pattern may indicate that severe infections overwhelm the spleen's capacity for enlargement, or that high parasite loads lead to splenic dysfunction and tissue damage rather than hypertrophy.

There was **statistically significant changes (p<0.05)** in the spleen weight across infection groups when compared to the control group, with peak enlargement in the low infection group.

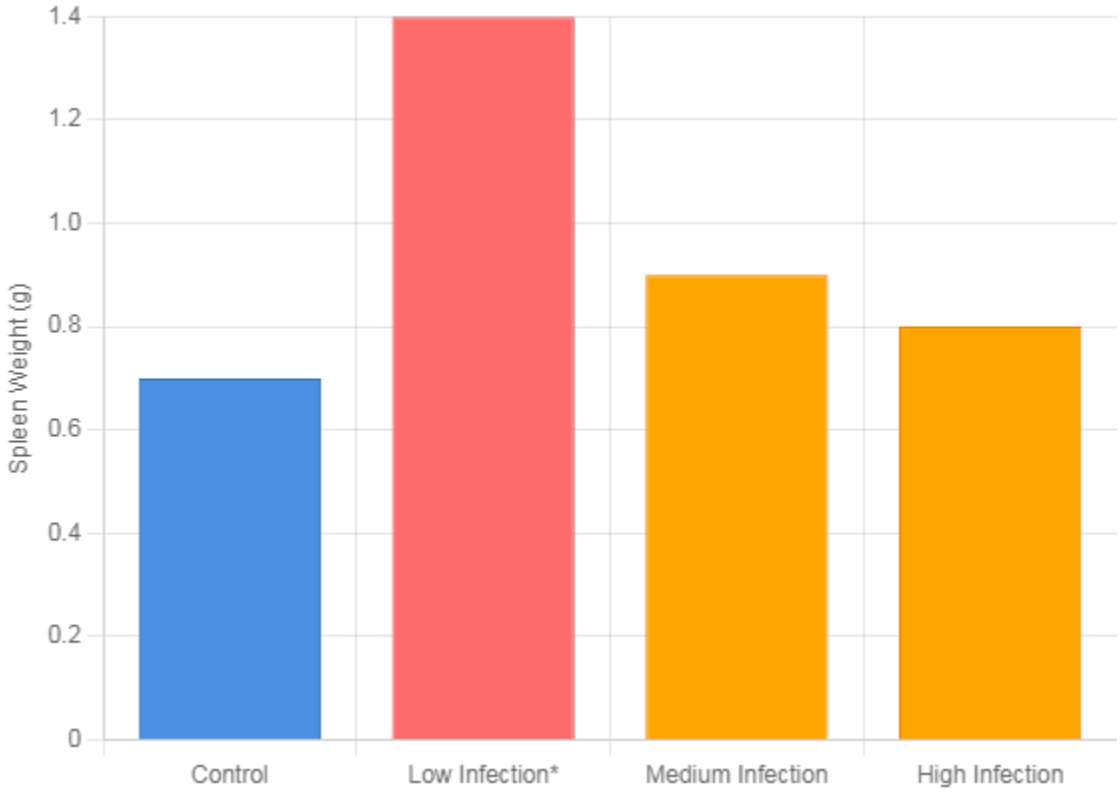

Chart 4.3: Spleen Weight Data

Splenic-somatic Index

The splenic-somatic index provided the most interpretable results, showing significant changes across infection groups ($p=0.0278$). The index increased from 0.44% in controls to 0.91% in the low infection group, representing a more than two-fold increase. This finding demonstrates that spleen enlargement occurs even when considering the concurrent body weight loss.

The pattern of splenic-somatic index changes (low: 0.91%, medium: 0.71%, high: 0.65%) reveals an inverse relationship between infection intensity and relative spleen size. This counterintuitive finding suggests that moderate infections may trigger the most robust splenic response, while severe infections may compromise splenic function.

There was **statistically significant changes (p<0.05)** in the splenic-somatic index across infection groups when compared to the control group, showing dose-dependent spleen involvement.

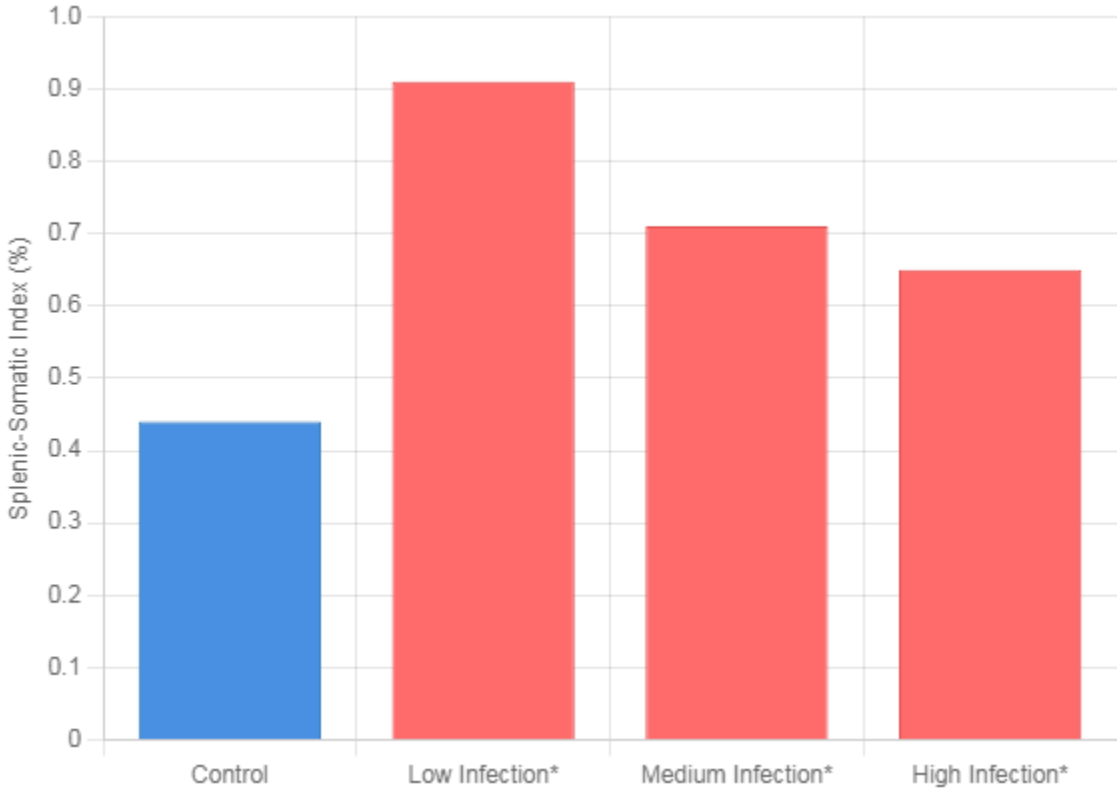

Chart 4.4: Splenic-Somatic Index Data

A1 SPLEEN X400

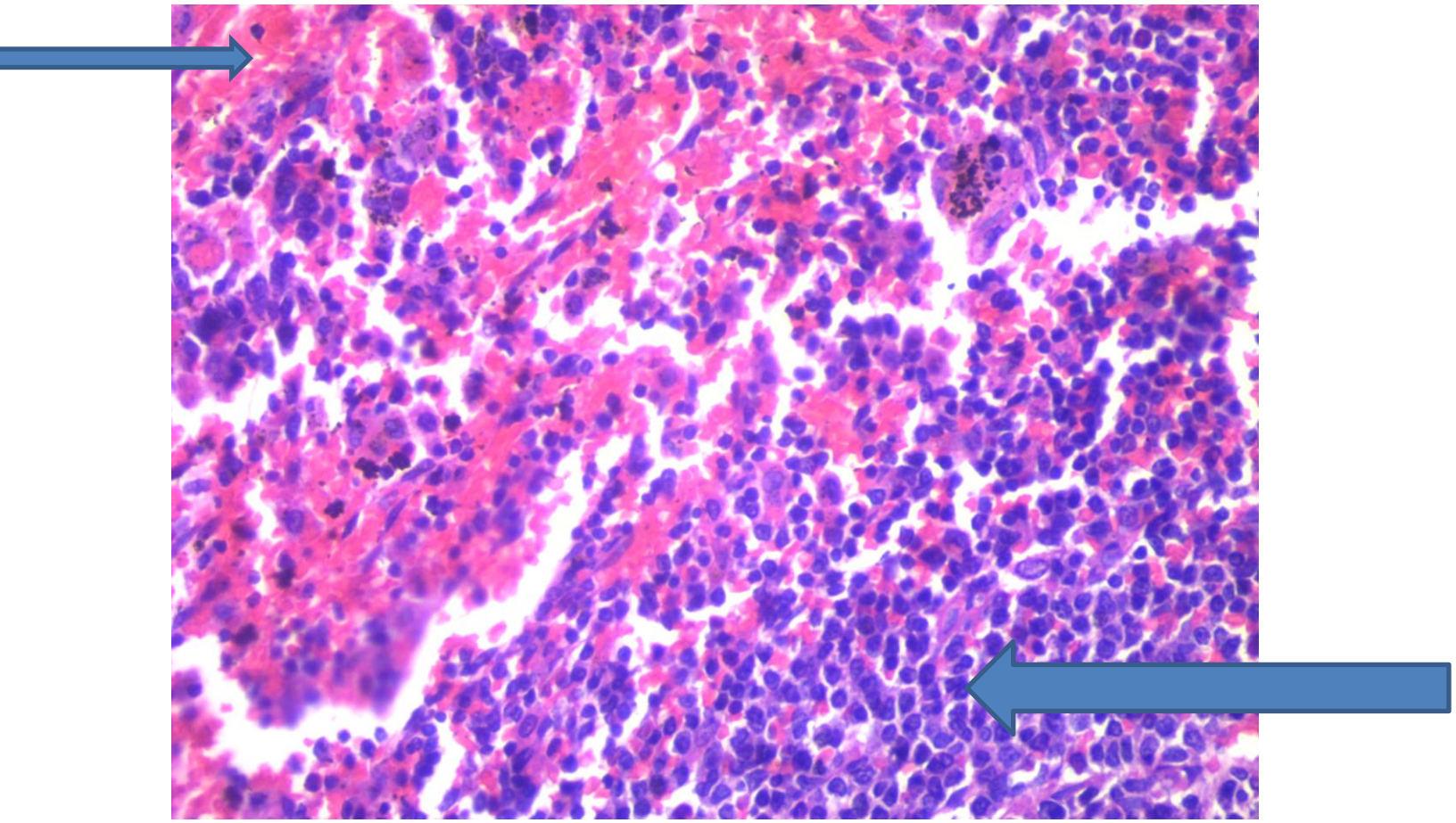

Plate 4.5: Sections of the spleen shows a normal white pulp (thick arrow) containing predominantly lymphocytes and normal red pulp (thin arrow) containing predominantly red blood cells. **FEATURES ARE IN KEEPING WITH NORMAL SPLENIC TISSUE**

A2 SPLEEN X400

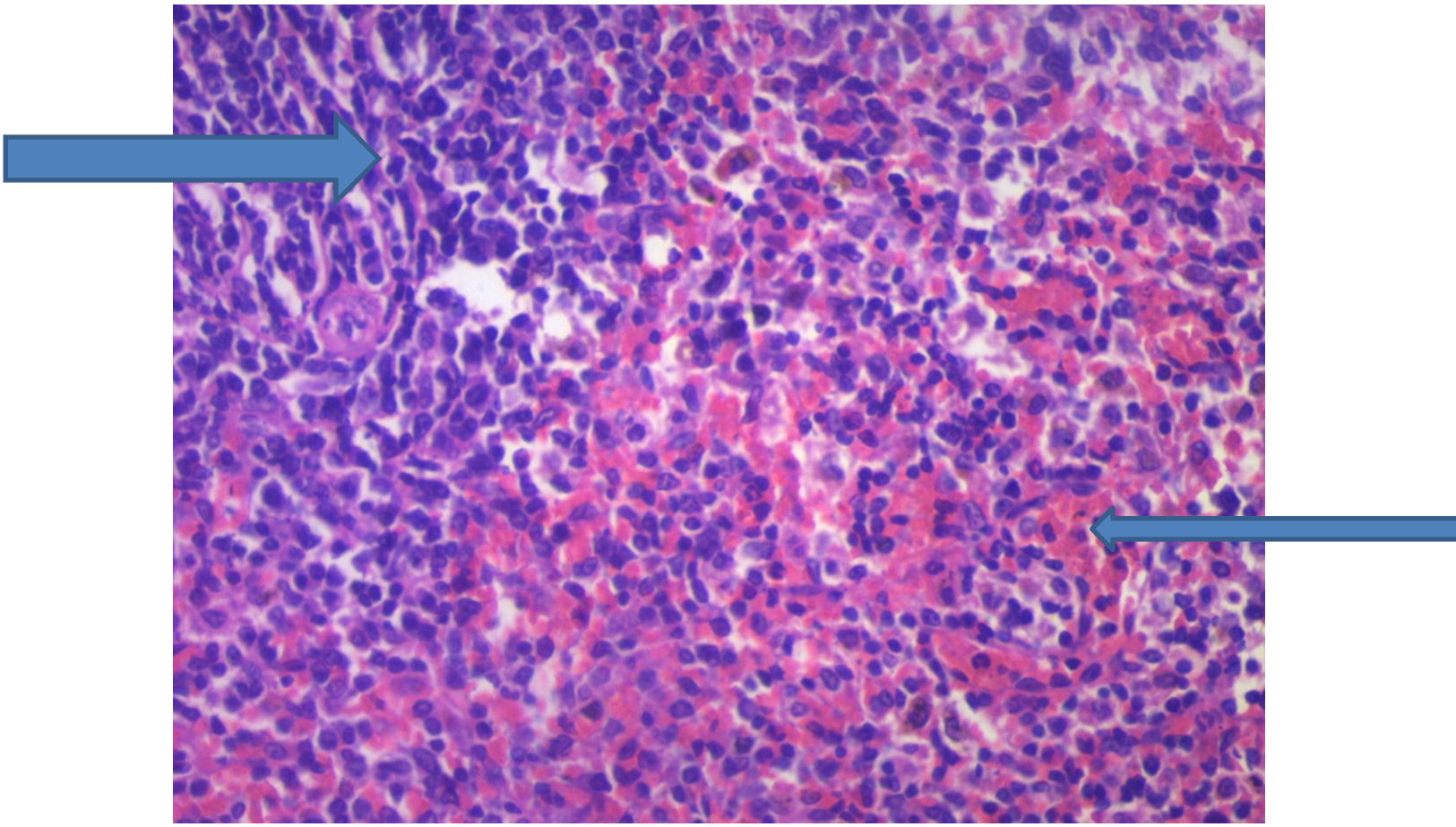

Plate 4.6: Sections of the spleen shows a normal white pulp (thick arrow) containing predominantly lymphocytes and normal red pulp (thin arrow) containing predominantly red blood cells. **FEATURES ARE IN KEEPING WITH NORMAL SPLENIC TISSUE**

A3 SPLEEN X400

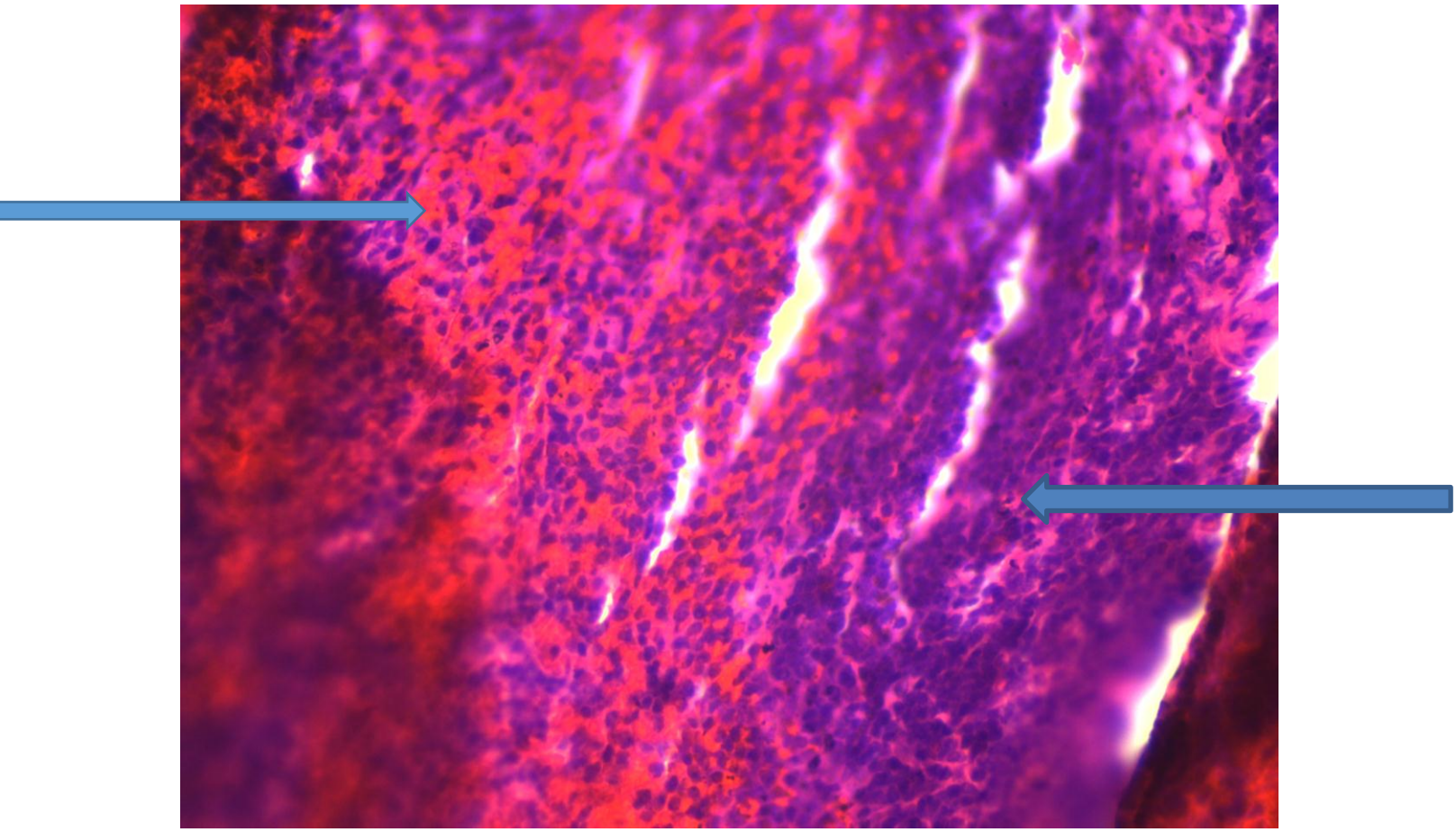

Plate 4.7: Sections of the spleen shows a normal white pulp (thick arrow) containing predominantly lymphocytes and normal red pulp (thin arrow) containing predominantly red blood cells. **FEATURES ARE IN KEEPING WITH NORMAL SPLENIC TISSUE**

A4 SPLEEN X400

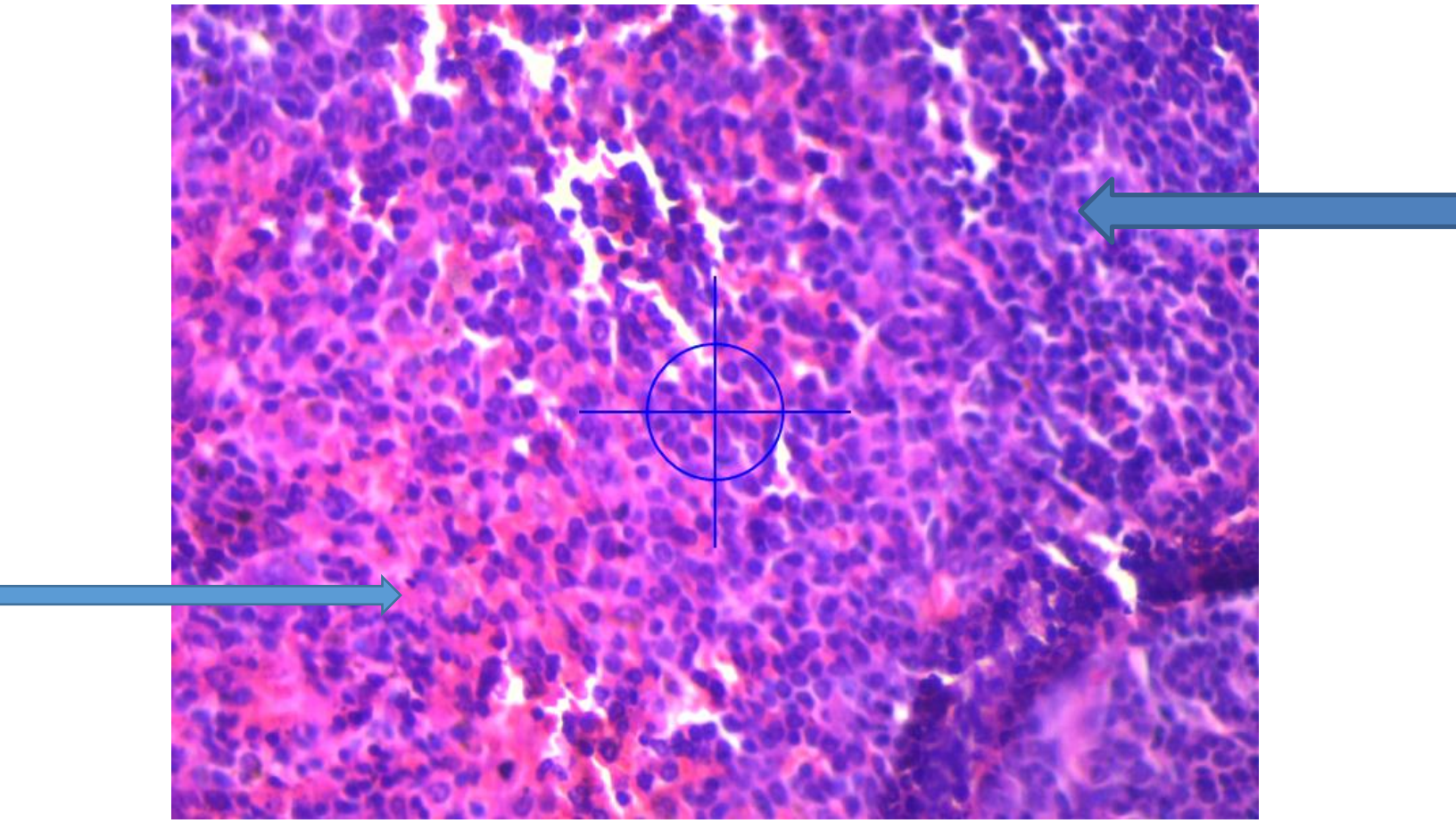

Plate 4.8: Sections of the spleen shows a normal white pulp (thick arrow) containing predominantly lymphocytes and normal red pulp (thin arrow) containing predominantly red blood cells. **FEATURES ARE IN KEEPING WITH NORMAL SPLENIC TISSUE**

B1 SPLEEN X400

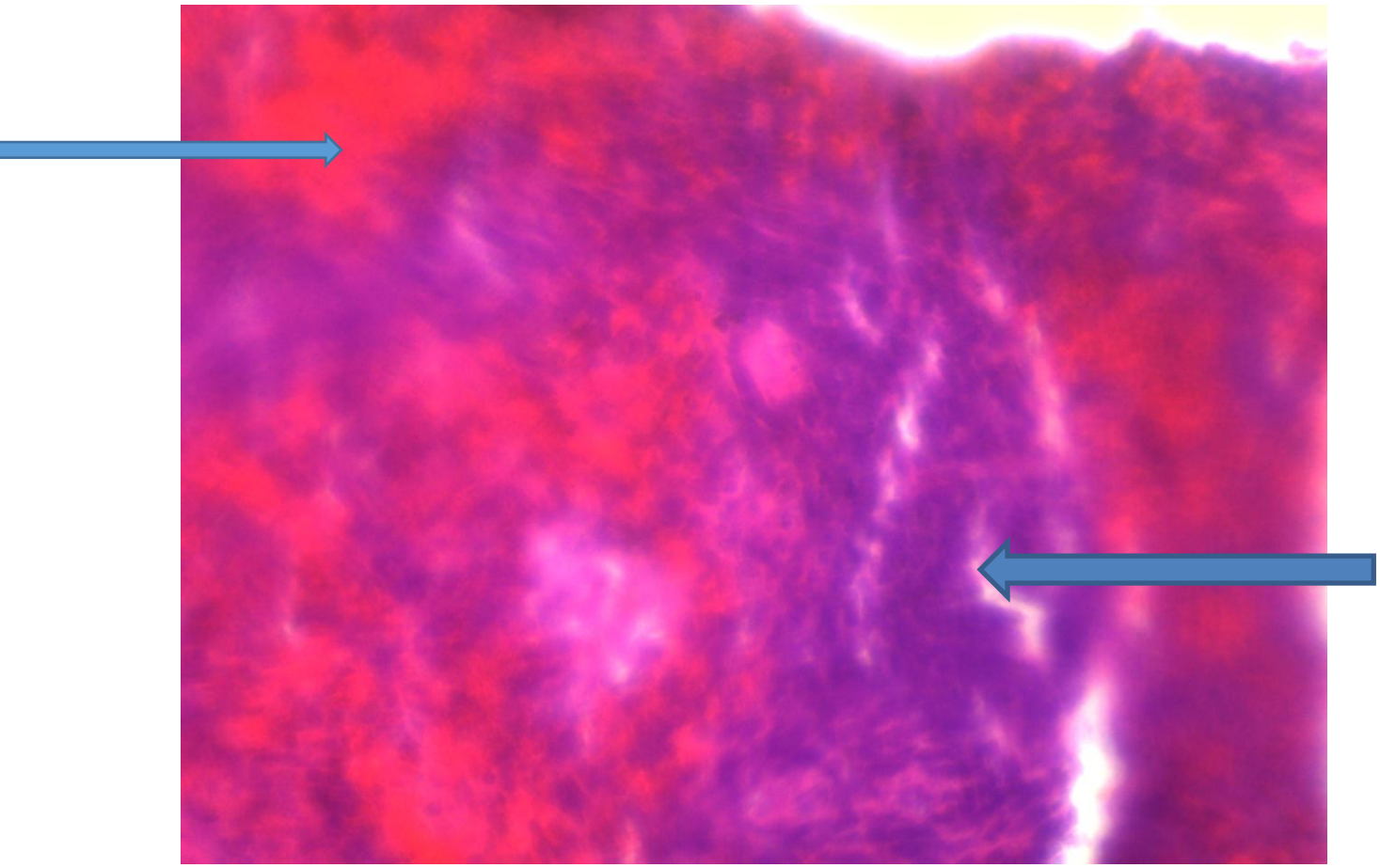

Plate 4.9: Sections of the spleen shows a normal white pulp (thick arrow) containing predominantly lymphocytes and normal red pulp (thin arrow) containing predominantly red blood cells. **FEATURES ARE IN KEEPING WITH NORMAL SPLENIC TISSUE**

B2 SPLEEN X400

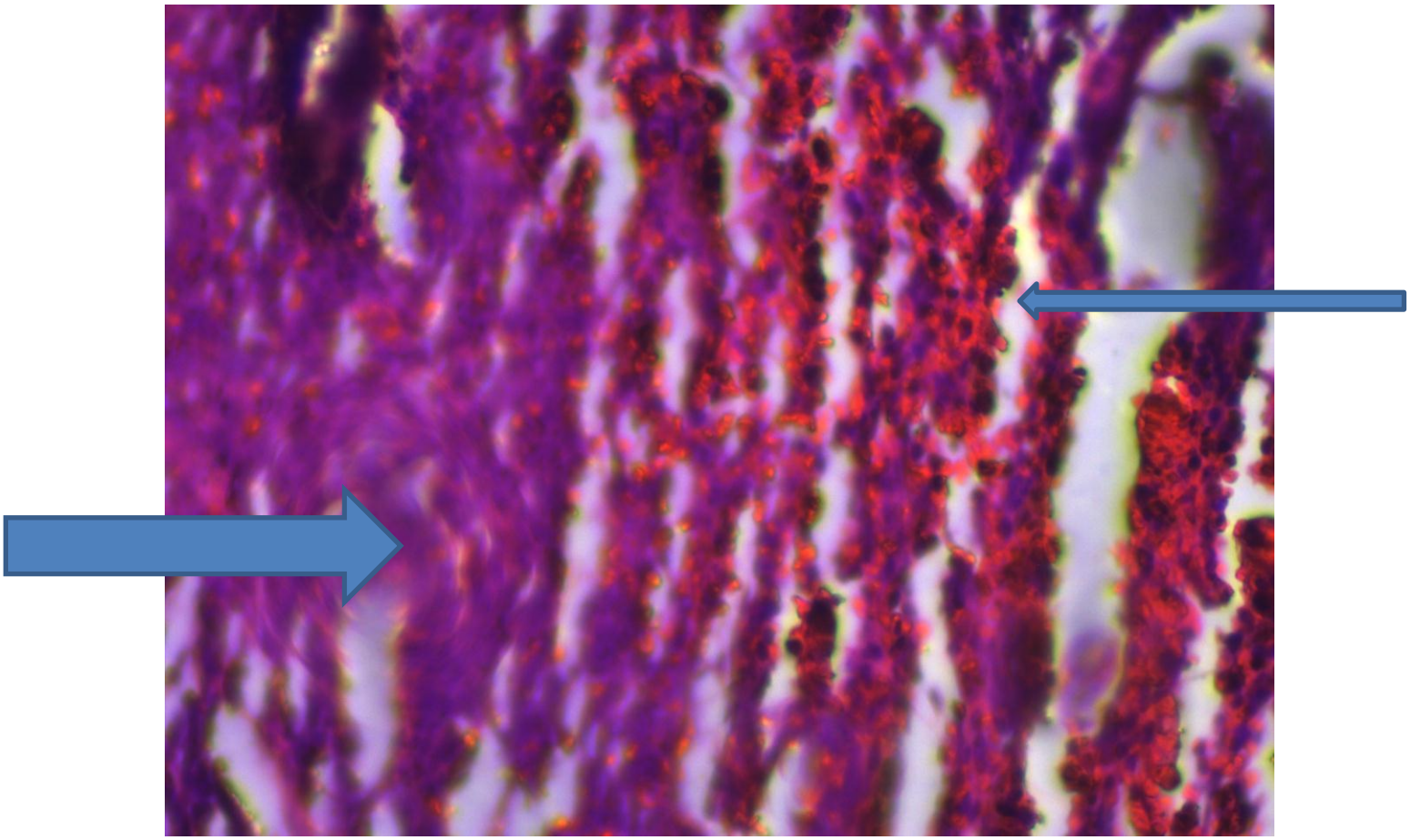

Plate 4.10: Sections of the spleen shows a normal white pulp (thick arrow) containing predominantly lymphocytes and normal red pulp (thin arrow) containing predominantly red blood cells. **FEATURES ARE IN KEEPING WITH NORMAL SPLENIC TISSUE.**

B3 SPLEEN X400

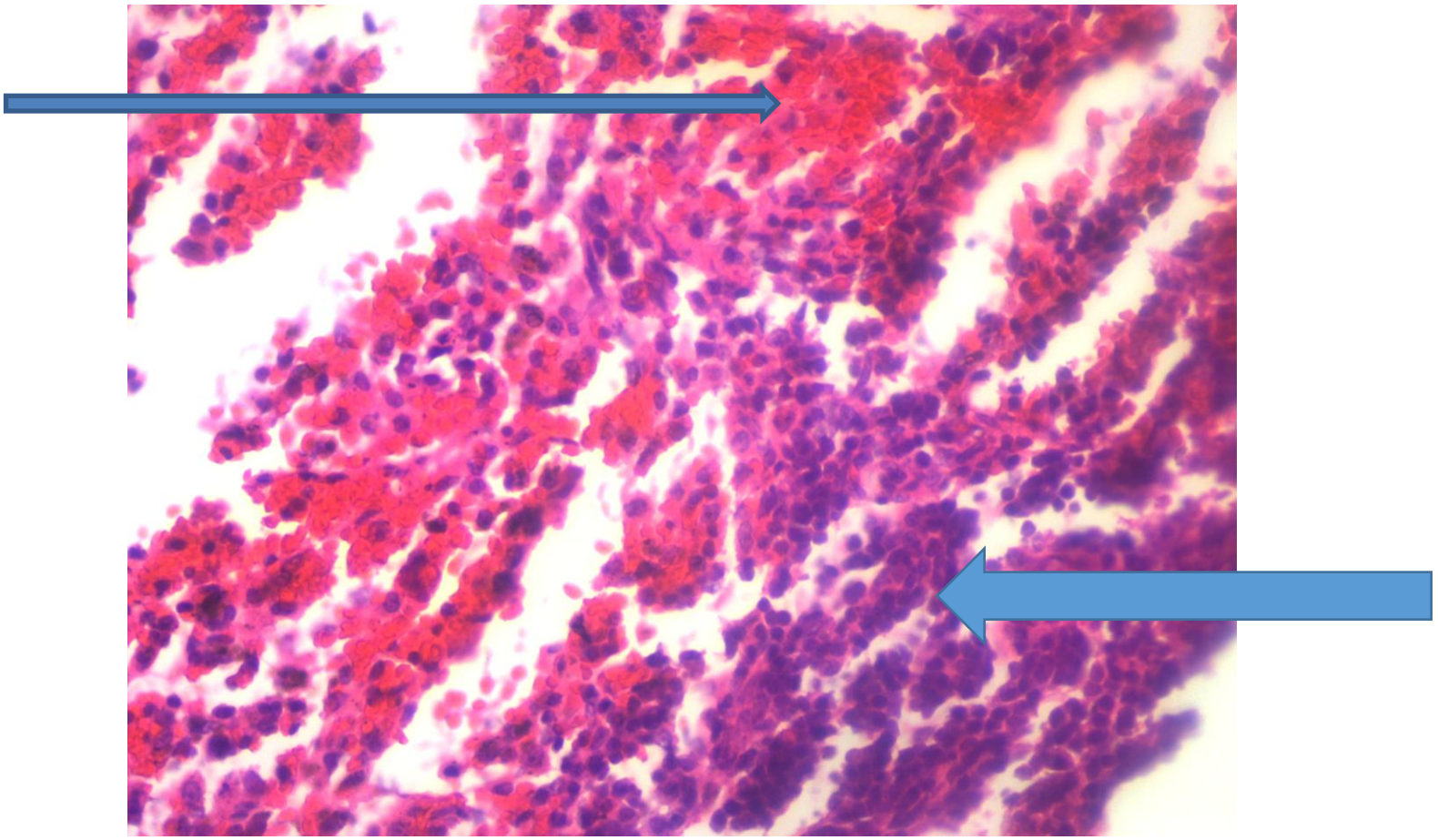

Plate 4.11: Sections of the spleen shows a normal white pulp (thick arrow) containing predominantly lymphocytes and normal red pulp (thin arrow) containing predominantly red blood cells. **FEATURES ARE IN KEEPING WITH NORMAL SPLENIC TISSUE**

B4 SPLEEN X400

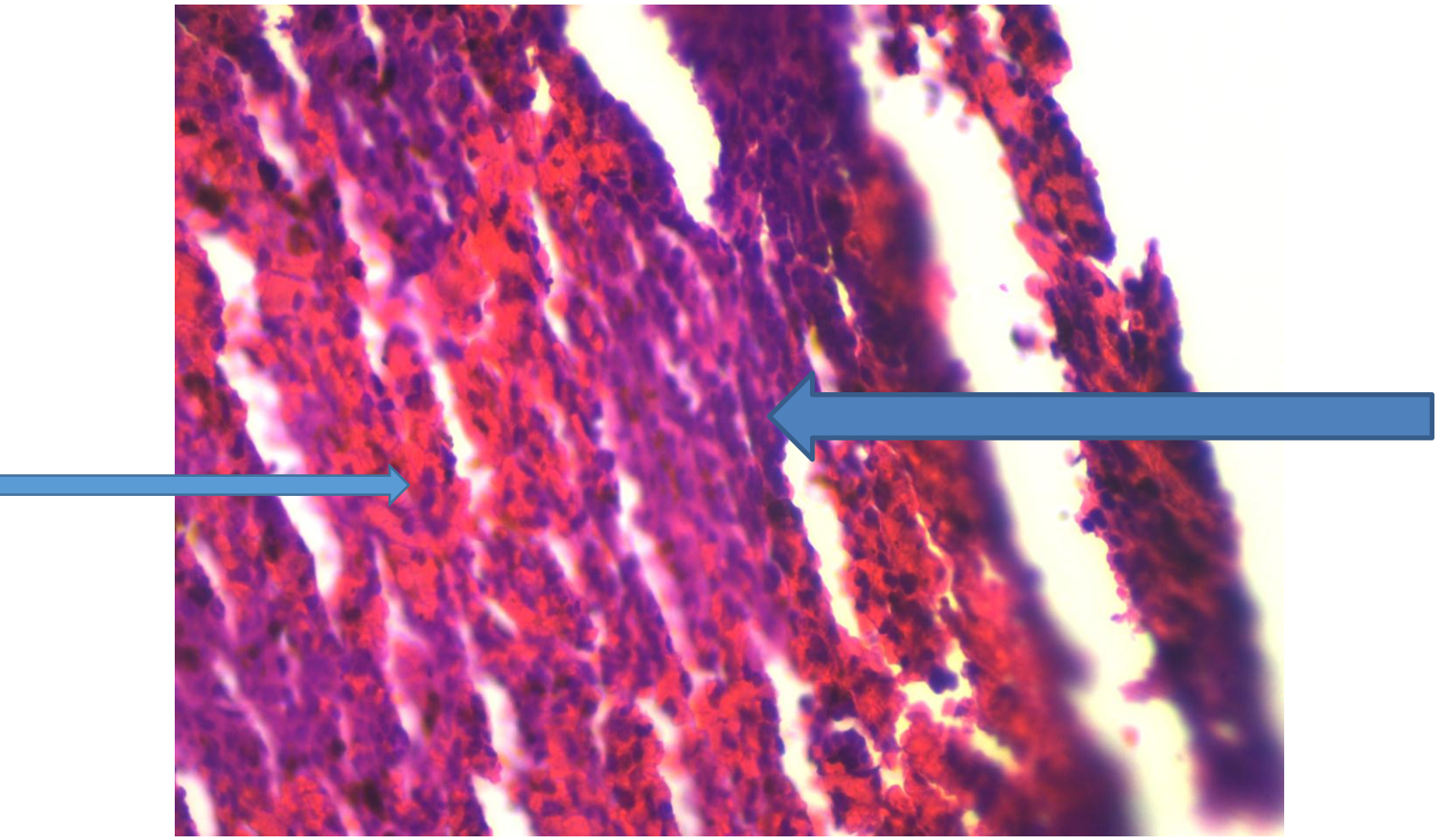

Plate 4.12: Sections of the spleen shows a normal white pulp (thick arrow) containing predominantly lymphocytes and normal red pulp (thin arrow) containing predominantly red blood cells. **FEATURES ARE IN KEEPING WITH NORMAL SPLENIC TISSUE**

C1 SPLEEN X400

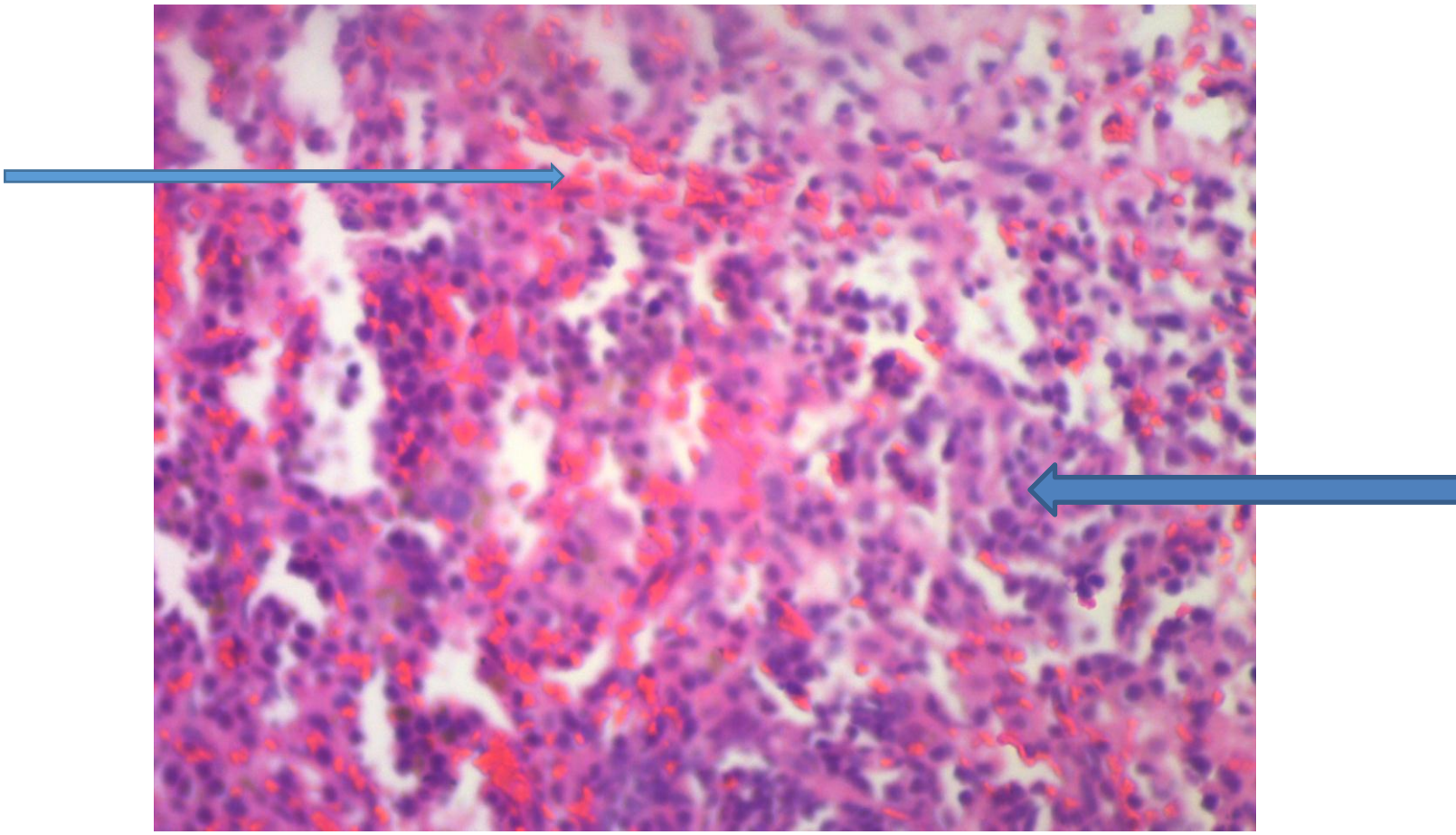

Plate 4.13: Sections of the spleen shows a normal white pulp (thick arrow) containing predominantly lymphocytes and normal red pulp (thin arrow) containing predominantly red blood cells. **FEATURES ARE IN KEEPING WITH NORMAL SPLENIC TISSUE**

C2 SPLEEN X400

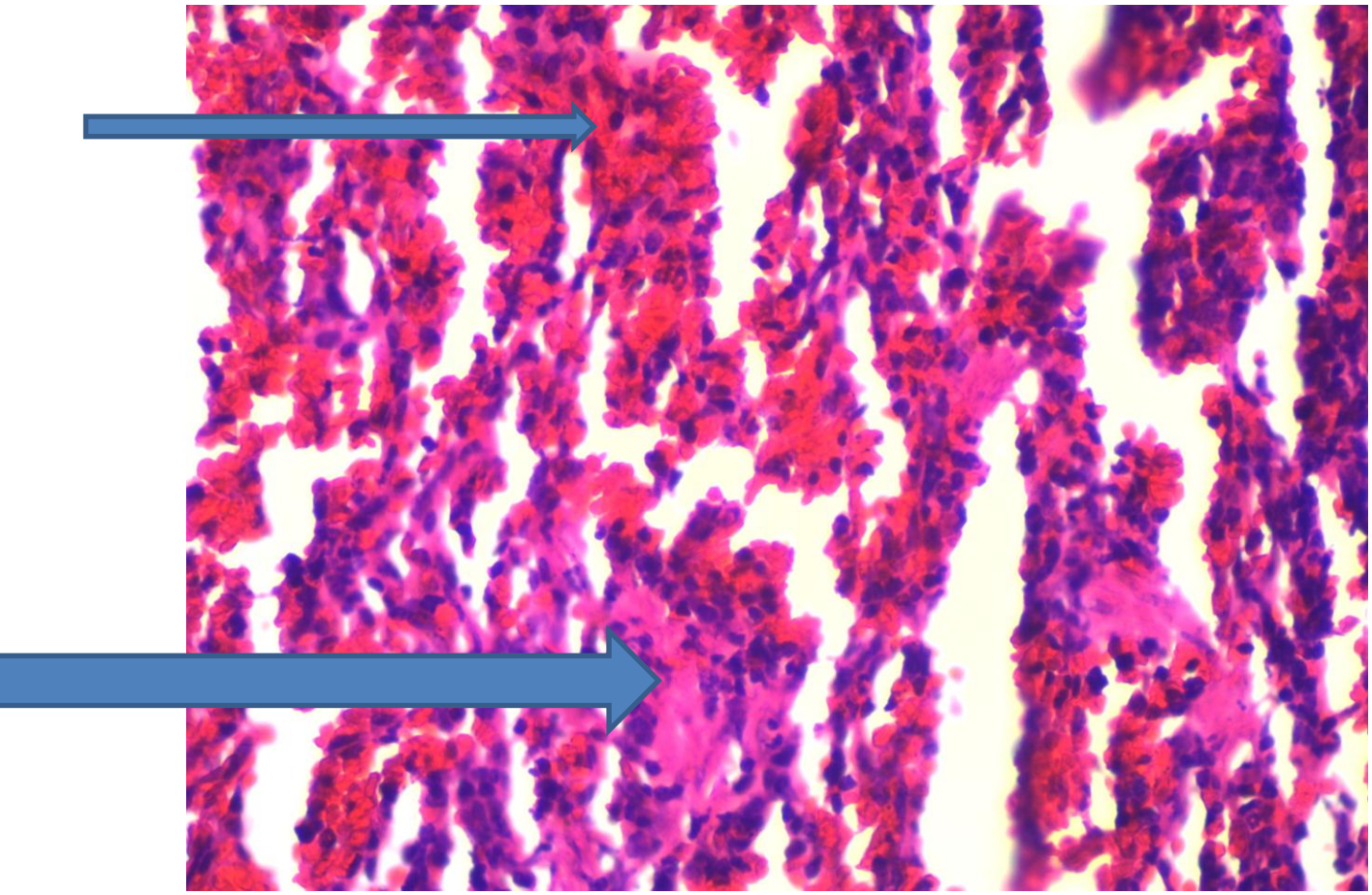

Plate 4.14: Sections of the spleen shows a normal white pulp (thick arrow) containing predominantly lymphocytes and normal red pulp (thin arrow) containing predominantly red blood cells. **FEATURES ARE IN KEEPING WITH NORMAL SPLENIC TISSUE**

C3 SPLEEN X400

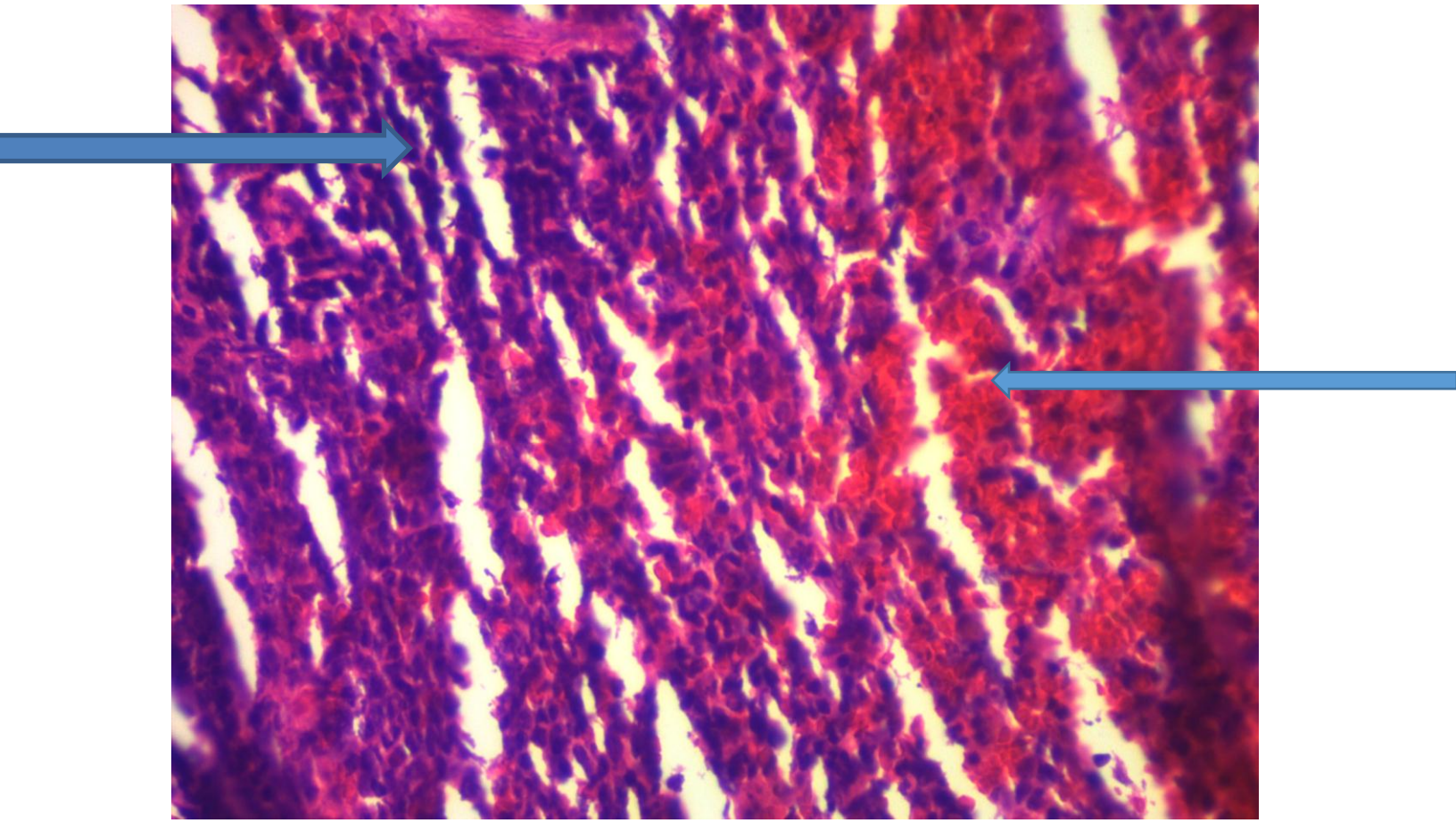

Plate 15: Sections of the spleen shows a normal white pulp (thick arrow) containing predominantly lymphocytes and normal red pulp (thin arrow) containing predominantly red blood cells. **FEATURES ARE IN KEEPING WITH NORMAL SPLENIC TISSUE**

C4 SPLEEN X400

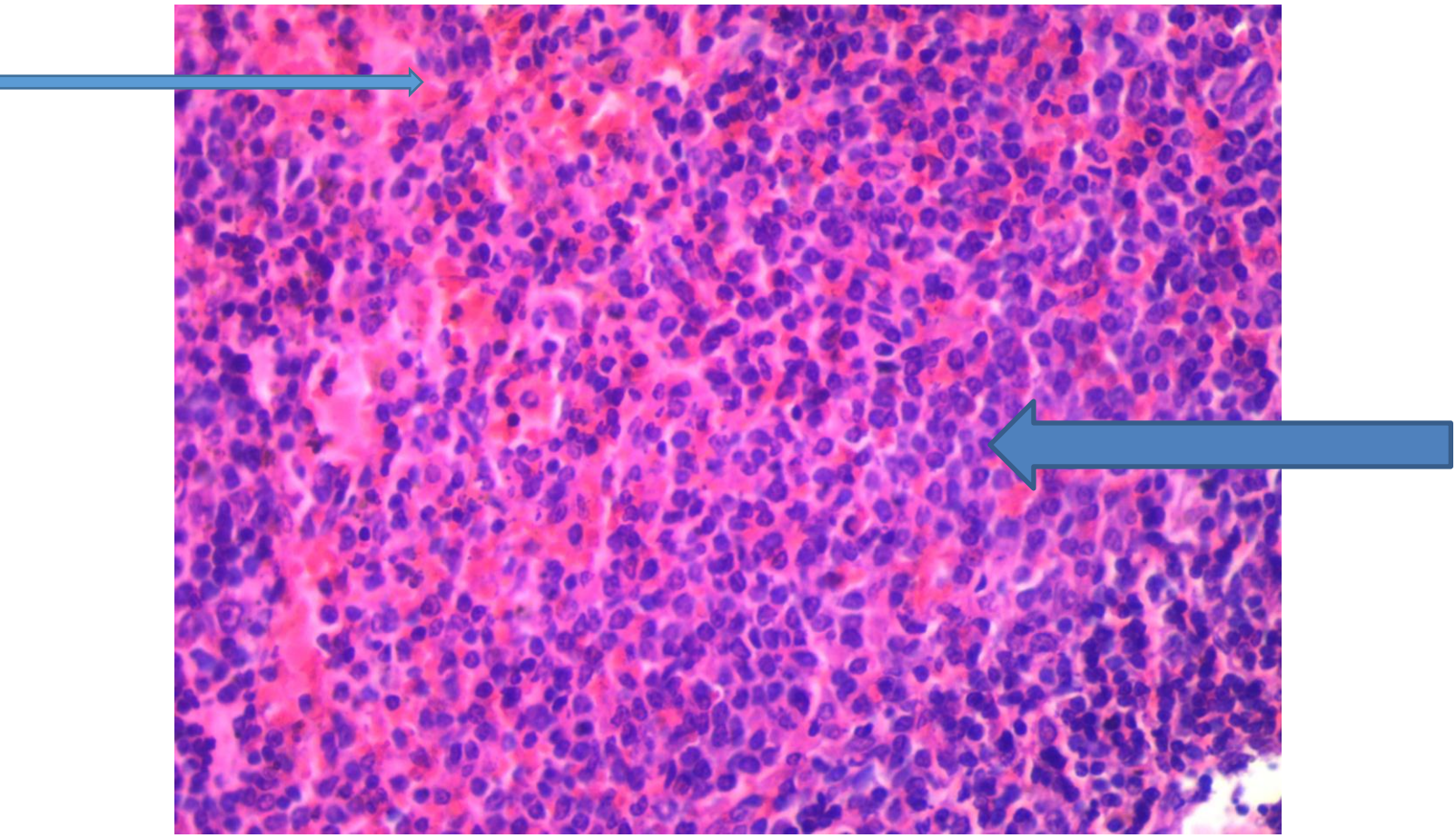

Plate 16: Sections of the spleen shows a normal white pulp (thick arrow) containing predominantly lymphocytes and normal red pulp (thin arrow) containing predominantly red blood cells. **FEATURES ARE IN KEEPING WITH NORMAL SPLENIC TISSUE**

D1 SPLEEN X400

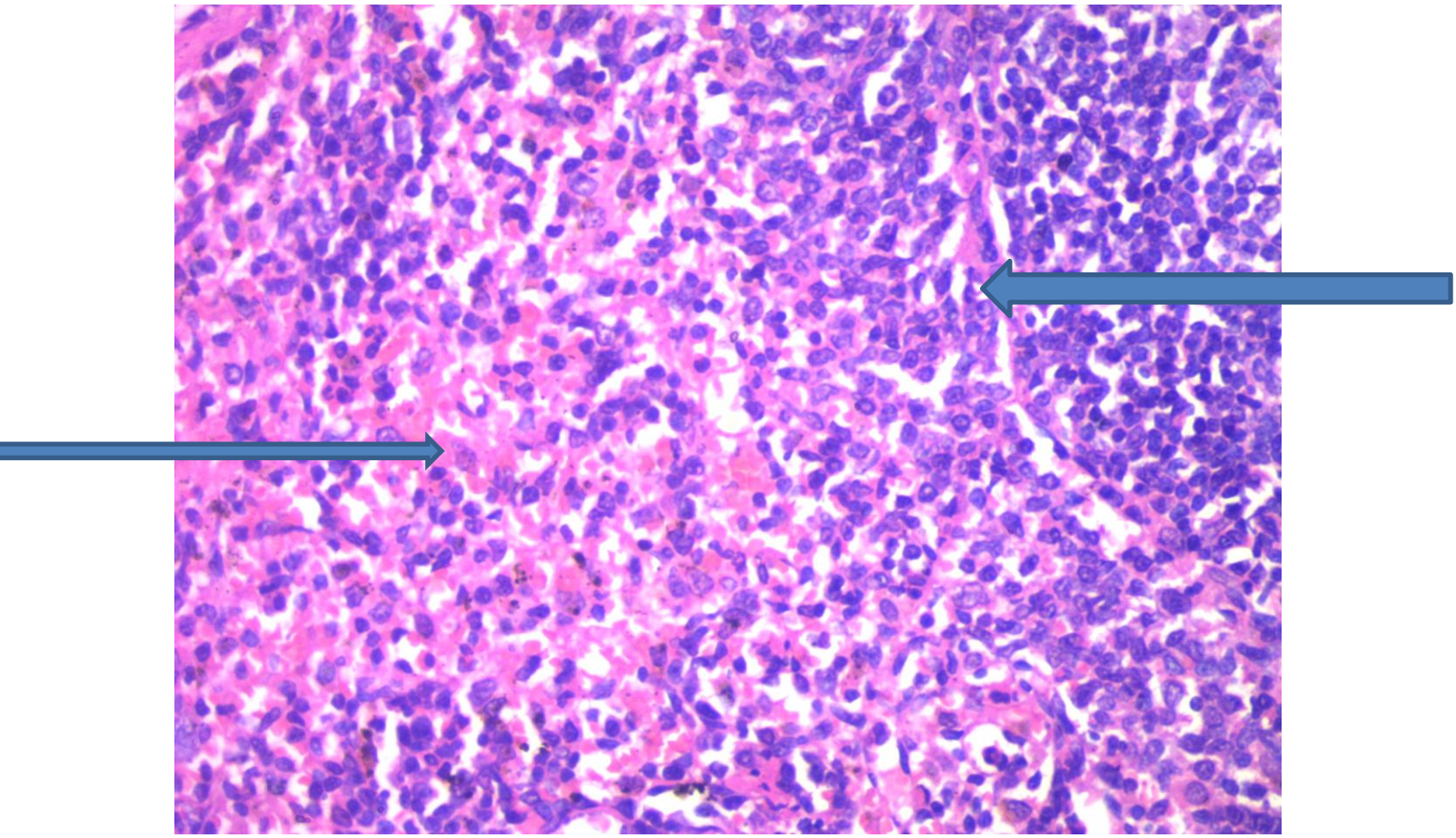

Plate 17: Sections of the spleen shows a normal white pulp (thick arrow) containing predominantly lymphocytes and normal red pulp (thin arrow) containing predominantly red blood cells. **FEATURES ARE IN KEEPING WITH NORMAL SPLENIC TISSUE**

D2 SPLEEN X400

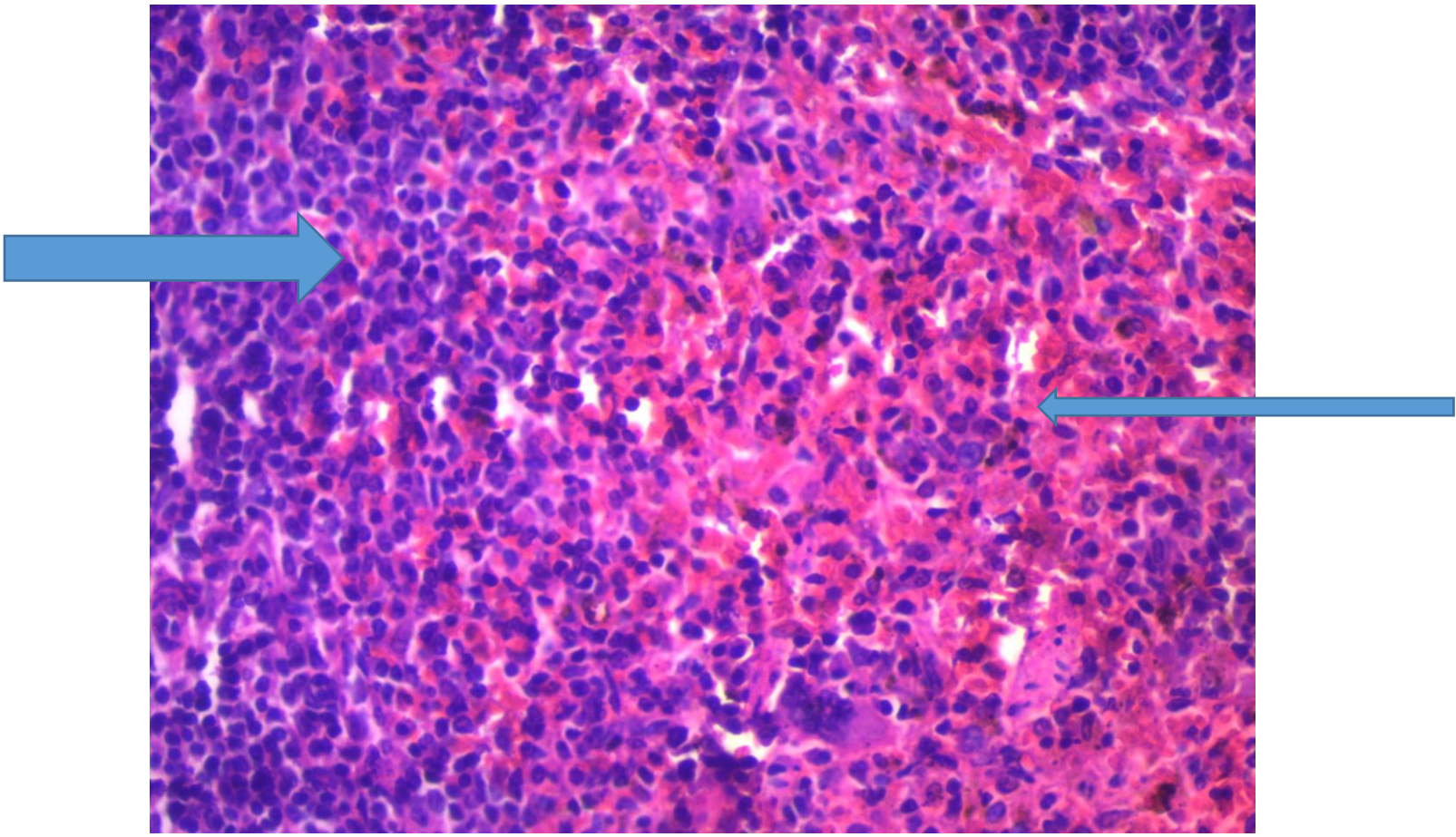

Plate 18: Sections of the spleen shows a normal white pulp (thick arrow) containing predominantly lymphocytes and normal red pulp (thin arrow) containing predominantly red blood cells. **FEATURES ARE IN KEEPING WITH NORMAL SPLENIC TISSUE**

D3 SPLEEN X400

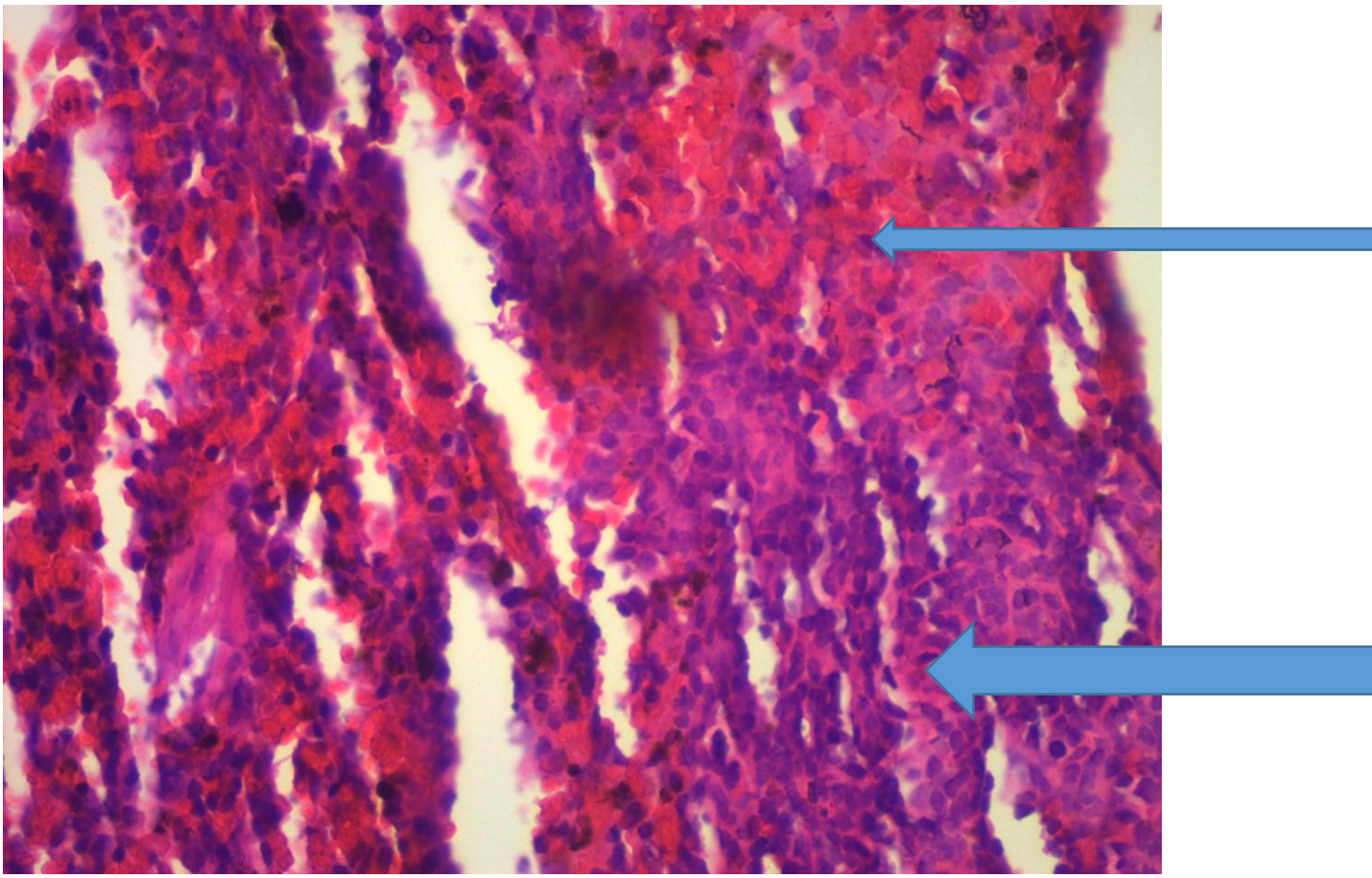

Plate 19: Sections of the spleen shows a normal white pulp (thick arrow) containing predominantly lymphocytes and normal red pulp (thin arrow) containing predominantly red blood cells. **FEATURES ARE IN KEEPING WITH NORMAL SPLENIC TISSUE**

D4 SPLEEN X400

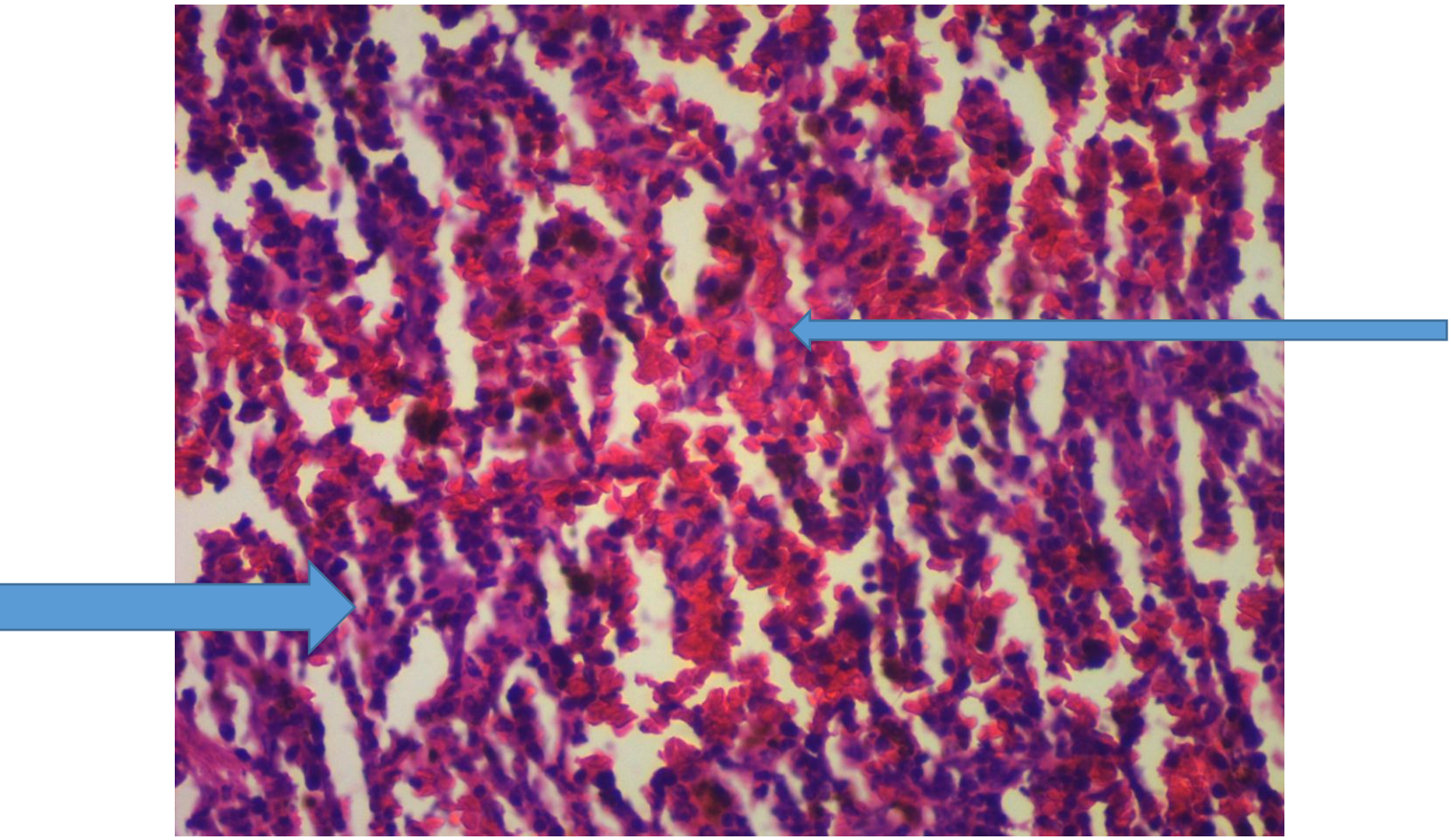

Plate 20: Sections of the spleen shows a normal white pulp {thick arrow} containing predominantly lymphocytes and normal red pulp {thin arrow} containing predominantly red blood cells. **FEATURES ARE IN KEEPING WITH NORMAL SPLENIC TISSUE.**

CHAPTER FIVE

5.0 DISCUSSION AND CONCLUSION

5.1 Discussion

This study investigated the histopathological effects of malaria parasites on the spleen using albino Wistar rats infected with varying concentrations of *Plasmodium berghei*. The research aimed to address two fundamental research questions: (1) What specific histological changes occur in the spleen tissues of albino rats infected with *Plasmodium* spp., and (2) What is the relationship between the concentration of *Plasmodium* spp. infection and the degree of histopathological changes observed in the spleen. The findings provide important insights into the dose-dependent effects of malaria infection on splenic morphology and function.

The significant weight loss observed across all infected groups (-14.0g to -25.5g) demonstrates the profound systemic metabolic impact of malaria infection. This finding aligns with previous studies by Lamikanra *et al*. (2007), who reported similar cachexic effects in rodent malaria models, attributing the weight loss to increased metabolic demands, reduced food intake, and systemic inflammatory responses. The dose-dependent pattern of weight loss observed in this study, with the high infection group showing the greatest reduction (-25.5±2.5g), corroborates findings by Craig *et al*. (2012), who demonstrated that parasite load directly correlates with the severity of systemic manifestations in experimental malaria.

The metabolic burden observed in infected animals reflects the energy-intensive nature of immune responses required to combat malaria parasites. Wykes *et al*. (2014) reported that the activation of immune cells, particularly in the spleen, requires substantial energy expenditure, contributing to the negative energy balance observed in malaria infections. Additionally, the

cytokine storm associated with acute malaria infection can lead to muscle wasting and metabolic dysfunction, as documented in clinical studies by White *et al*. (2014).

The consistent weight loss across all infection groups, regardless of parasite dose, suggests that even low-level infections impose significant physiological stress on the host. This finding has important implications for understanding malaria pathogenesis and supports the concept that subclinical malaria infections can have substantial health impacts, as reported by Buffet *et al*. (2011) in their comprehensive review of malaria-induced systemic effects.

The most striking finding of this study was the paradoxical relationship between infection dose and spleen enlargement. The low infection group demonstrated the most pronounced splenomegaly (1.4±0.1g vs. 0.7±0.1g in controls, representing a 100% increase), while medium and high infection groups showed less dramatic enlargement. This pattern contrasts with the linear dose-response relationship that might be intuitively expected and provides important insights into splenic pathophysiology in malaria.

This finding partially supports the observations of Hussein *et al*. (2016), who noted that moderate malaria infections often produce more pronounced splenomegaly than severe infections. The authors suggested that severe infections may overwhelm the spleen's adaptive capacity, leading to tissue damage rather than hypertrophy. Similarly, del Portillo *et al*. (2012) reported that excessive parasitemia can lead to splenic dysfunction and even tissue necrosis, which could explain the reduced enlargement observed in the high infection group.

The pronounced splenomegaly in the low infection group reflects the spleen's primary role in malaria immunity. As described by Stevenson and Riley (2004), the spleen serves as the major site for clearance of parasitized erythrocytes, and moderate infections may trigger optimal

activation of this clearance mechanism. The expansion of red pulp macrophages and increased blood flow contribute to the organ enlargement, as documented in previous studies by Buffet *et al*. (2011).

The splenic-somatic index provided the most interpretable results, showing a clear pattern of splenic involvement across infection groups. The two-fold increase in the low infection group (0.91% vs. 0.44% in controls) demonstrates significant organ enlargement even when accounting for concurrent body weight loss. This finding is consistent with studies by Lamikanra *et al*. (2007), who reported similar splenic-somatic index changes in rodent malaria models and emphasized the utility of this parameter in assessing splenic involvement.

The inverse relationship between infection intensity and relative spleen size (low: 0.91%, medium: 0.71%, high: 0.65%) suggests that severe infections may compromise splenic function. This pattern aligns with the concept of splenic overwhelm described by Wykes *et al*. (2014), where excessive parasite loads exceed the organ's clearance capacity, leading to functional impairment rather than compensatory enlargement.

The splenic-somatic index results support the hypothesis that moderate infections may trigger the most robust splenic response, while severe infections may lead to organ dysfunction. This finding has important implications for understanding malaria pathogenesis and may help explain the clinical observation that patients with moderate parasitemia sometimes develop more pronounced splenomegaly than those with severe infections (Kochar *et al*., 2003).

Despite the significant changes in spleen weight and splenic-somatic index, the histopathological examination revealed surprisingly preserved tissue architecture across all groups. All sections showed normal white pulp containing predominantly lymphocytes and normal red pulp

containing predominantly red blood cells, with features consistent with normal splenic tissue. This finding contrasts with previous studies that reported significant architectural changes in malaria-infected spleens.

Several factors may explain this unexpected finding. First, the 14-day observation period may have been insufficient to develop the chronic histopathological changes typically associated with malaria splenomegaly. Studies by Buffet *et al*. (2011) demonstrated that significant architectural changes often require several weeks to months of infection or repeated infection episodes. The acute nature of the current study may have captured the functional but not yet structural changes associated with early malaria infection.

Second, the histological processing and staining methods employed may have been insufficient to detect subtle early changes in splenic microarchitecture. Advanced techniques such as immunohistochemistry, electron microscopy, or specialized stains for reticular fibers might have revealed changes not apparent with routine H&E staining, as suggested by Hamilton *et al*. (2011) in their review of quantitative histopathology methods.

The discrepancy between the significant gross changes (splenomegaly) and the apparently normal histological findings raises important methodological questions. Silva *et al*. (2021) noted that early malaria-induced splenic changes may involve cellular activation, vascular congestion, and functional alterations that precede architectural disruption. The current study's findings may represent this early phase of splenic response.

The dose-dependent pattern of splenic response observed in this study provides insights into the mechanisms of splenic adaptation to malaria infection. The pronounced response in the low infection group suggests that moderate parasite loads may trigger optimal activation of splenic

clearance mechanisms, while higher loads may lead to system overwhelm and dysfunction. This finding supports the concept of an optimal activation threshold described in immunological literature.

5.2 Conclusion

This study provided important insights into the early splenic responses to malaria infection, demonstrating significant functional changes (splenomegaly) that precede detectable structural alterations. The paradoxical dose-response relationship observed challenges conventional assumptions about malaria pathogenesis and highlights the complexity of host-parasite interactions. While the study did not reveal significant histopathological changes with routine examination methods, the pronounced effects on spleen weight and splenic-somatic index demonstrate substantial organ involvement in malaria infection.

The findings contribute to our understanding of malaria pathogenesis and emphasize the importance of early intervention regardless of apparent infection severity. Future research should employ more sensitive detection methods and extended observation periods to fully characterize the evolution of malaria-induced splenic pathology. The study's limitations highlight the need for comprehensive, multi-faceted approaches to investigating complex host-pathogen interactions in malaria research.

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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

I. The mould was filled with molten paraffin wax

II. With a pair of warm blunt-nosed forceps, tissues were transformed from the paraffin bath to the mould

III. Forceps were warmed and tissues oriented until lying in the desired plane.

IV. Corresponding labels from the paraffin bath were removed and placed against the side of the mould adjacent to the tissues.

V. Air was blown on the surface until a thin film of wax has solidified.

VI. The mould was transferred to a container of cold water and submerged until wax hardens.

After embedding, the block is to left harden up while placed on the ice for some hours before sectioning.

The Hertz microtome (Cambridge model) was used for trimming and sectioning at varying microns and the block clamp adjusted so that sections at 3-5microns were obtained in a ribbon-like manner, which was floated in a water bath to flatten by gentle heat.

The section or short ribbon was picked using a clean grease-free slide to ensure that the sections were thoroughly dried before staining by placing on a hot plate. After which, slides were stained according to Hematoxylin and Eosin method.

APPENDIX III

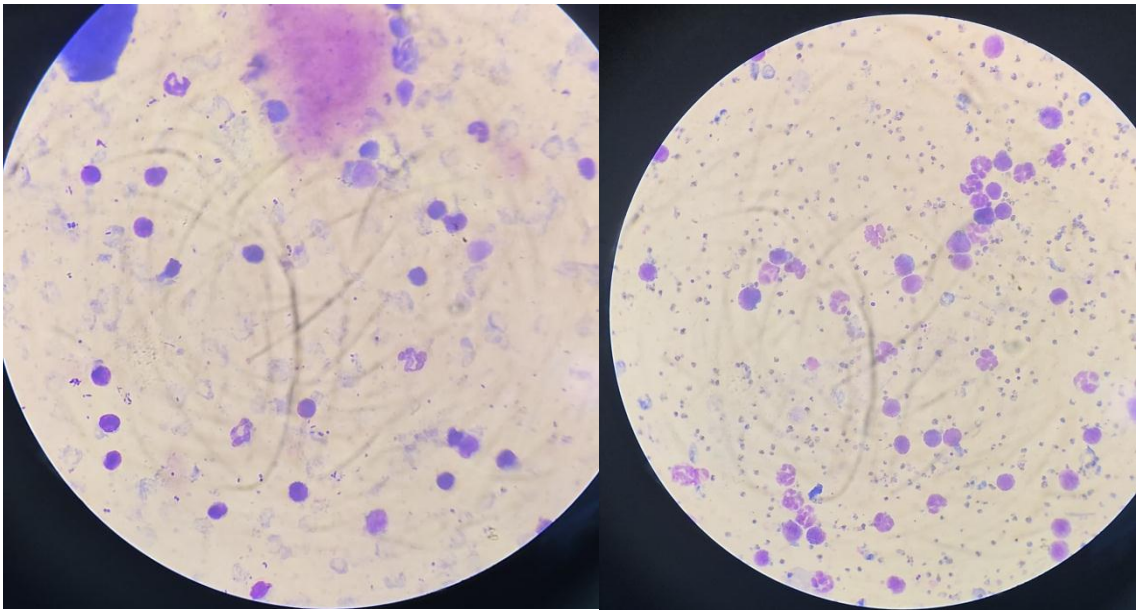
PROCEDURE FOR HEMATOXYLIN AND EOSIN STAINING

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.

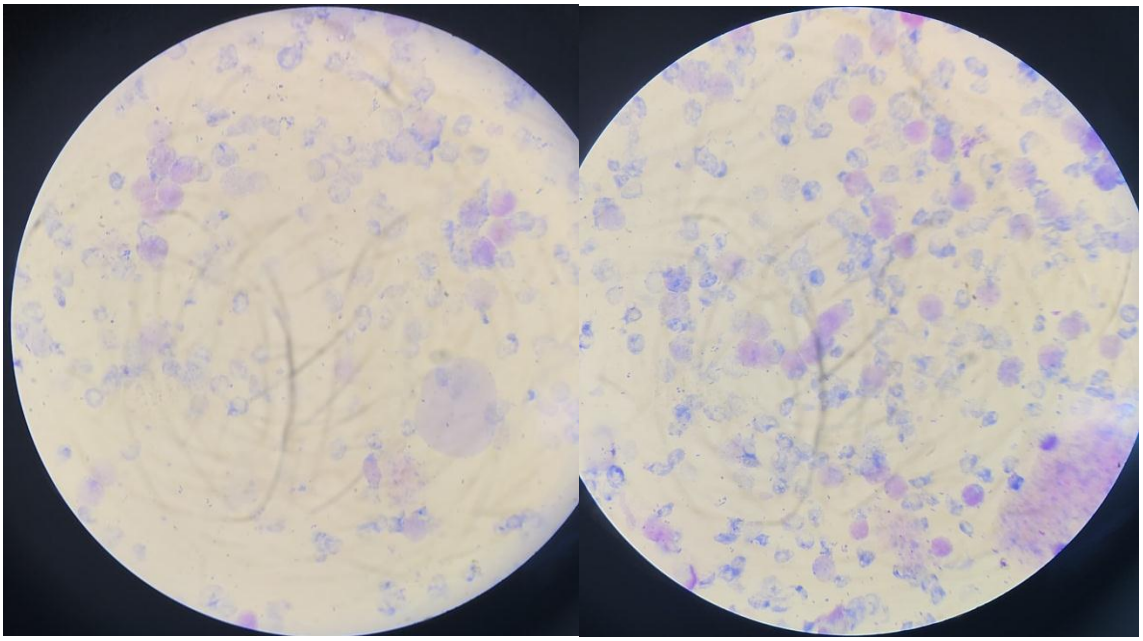
APPENDIX IV

PARASITOLOGY RESULT OF MALARIA PARASITE

GROUP A



GROUP B



GROUP C **GROUP D**