EFFECT OF MALARIA PARASITE ON THE KIDNEY USING ALBINO WISTAR RATS

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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

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CERTIFICATION

We the undersigned certify that this research work was carried out by OSEHEBO ELISHA 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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DEDICATION

This project is dedicated to the Lord God Almighty.

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ABSTRACT

This study investigated the effects of malaria parasite infection on kidney function using albino Wistar rats. The aim of the study was to determine kidney impairment induced by malaria through controlled infection with Plasmodium *berghei*, a rodent malaria parasite closely similar to Plasmodium *falciparum*. Sixteen male Wistar rats (130–174 g) were divided into four groups: control (uninfected), and three experimental groups infected with high (10^6 iRBCs), medium (10^4 iRBCs), and low (10^2 iRBCs) parasite doses, respectively. At the end of a 42-day experimental period, kidneys were harvested, processed, and examined histologically using hematoxylin and eosin staining. Results revealed dose-dependent renal pathology, with the high infection group showing a tendency of marked glomerular hypertrophy, tubular necrosis, vascular congestion, interstitial inflammatory infiltration, and hemosiderin casts, while moderate and mild changes were observed in the medium and low infection groups. Kidney weights however showed no significant increase in infected rats compared to controls, indicating parasitemia-related organomegaly. These findings demonstrate that malaria infection causes progressive, dose-dependent kidney damage characterized by glomerular and tubular injury, interstitial inflammation, and vascular alterations. In conclusion, malaria-associated nephropathy is a major complication of infection, and Plasmodium *berghei*-infected Wistar rats provide a reliable model for studying malaria-induced renal dysfunction and for evaluating potential therapeutic interventions.

CHAPTER ONE: INTRODUCTION

1.1 Background of Study

Malaria, an alarming and globally significant parasitic disease, continues to persist and impose a significant public health burden, particularly in regions of sub-Saharan Africa, where it remains a leading agent of morbidity and mortality (World Health Organization, 2023). Malaria is caused primarily by the parasitic protozoan Plasmodium falciparum and transmitted to humans via the bite of infected female Anopheles mosquitoes. Malaria presents with a range of clinical manifestations. While commonly characterized by fever, chills, headache, and flu-like symptoms, progression to severe malaria is a serious concern, as progression may lead ultimately to multiple organ dysfunction syndrome (MODS). Among the organs affected by severe malarial complications, the kidneys are often and significantly affected, advancing to conditions such as Acute Kidney Injury (AKI), which contributes largely to adverse clinical outcomes and mortality (Knox *et al.,* 2018; Sitprija & Eiam-Ong, 2008).

The pathogenesis of kidney damage induced by malaria is complex and multifactorial, involving host-parasite interactions and immunological responses that are not yet fully understood. However, several key mechanisms have been identified. Among these is the phenomenon of sequestration, where Plasmodium falciparum-infected red blood cells (iRBCs) adhere to the endothelial lining of capillaries, arterioles, and venules of the kidney (Naik *et al.,* 2017; Clark & Cowden, 2003). Sequestration results in microvascular obstruction, impaired tissue perfusion,

and localized hypoxia within the parenchyma of the kidneys. Also, the deposition of immunological complexes – antigens of parasite bound to host antibodies – within the glomeruli and tubular interstitium can bring about immense inflammatory responses, aiding direct renal tissue injury (Barsoum, 2012). Furthermore, the systemic inflammatory response typical of severe malaria often include an unregulated release of pro-inflammatory cytokines, frequently termed a "cytokine storm" (Kwiatkowski *et al.,* 1990). This uncontrolled release of cytokines can promote generalized endothelial dysfunction, increase vascular permeability, and directly lead to cellular damage of renal tissues (Medapalli *et al*., 2015). The metabolic stress associated with malaria infection also generates an increase of reactive oxygen, resulting in oxidative stress, which can overpower local antioxidant defenses and cause damage on renal cells and structures (Das *et al.*, 2010). Given the complexity of these multifactorial pathogenic pathways, a critical understanding and knowledge of the specific impact of varying parasite concentrations on renal pathology is vital for elucidating disease mechanisms and the development of targeted and effective therapeutic interventions aimed at preserving renal function in affected individuals.

1.2 Statement Of The Problem

Despite advancements and increased knowledge of malaria control and treatment, renal complications associated with malaria infection continue to contribute to mortality, especially in severe cases (Eiam-Ong & Sitprija, 2005). Previous research has shown the involvement of malaria in renal pathogenesis, but there is still a gap in understanding the precise effects of malaria parasite concentrations on the extent of kidney damage. Specifically, it is unclear how different parasite loads affect the severity of pathological conditions in renal tissue. This

knowledge gap hinders the prediction, prevention, and effective management of malaria-induced renal injury.

1.3 Significance Of The Study

This study is significant for several reasons. Firstly, by investigating the dose-dependent effects of malaria parasite concentration on renal pathology, it will contribute to a more thorough comprehension of the mechanisms involved in malaria-induced organ damage. This more thorough understanding could potentially bring about the identification of critical parasite thresholds that facilitates severe renal complications. Secondly, the findings will lay a foundation for future research into specific cellular and molecular pathways associated with malaria-induced kidney injury, which could inform the development of targeted therapeutic strategies aimed at mitigating renal damage. Ultimately, this research aims to improve patient outcomes and reduced morbidity associated with malaria infection.

1.4 Aim Of The Study

The primary aim of this study is to investigate the dose-dependent effects of malaria parasite infection on the kidneys of laboratory rats.

1.4 Specific Objectives Of The Study

The specific objectives are to:

1. To assess the histological changes induced by varying concentrations of malaria parasites in the kidneys of infected rats.
2. To determine the dose-dependent effects of malaria parasite concentration on the extent of kidney tissue damage.
3. To document the level of damage to the kidney in each experimental group compared to the control group.

1.5 Research Questions

This study will address the following research questions:

1. What are the histological changes observed in the kidneys of rats infected with low, medium, and high concentrations of malaria parasites compared to uninfected control rats?
2. Is there a dose-dependent relationship between malaria parasite concentration and the severity of kidney tissue damage in infected rats?
3. How does the level of kidney damage differ across the various malaria-infected groups and the uninfected control group?

CHAPTER 2: LITERATURE REVIEW

2.0 INTRODUCTION

Malaria is named after the Italian term “mal’aria”, which means “bad air” to signify association of the disease with marshy areas. It is an endemic vector-borne parasitic disease caused by protozoan parasites of the genus Plasmodium in tropical and subtropical regions worldwide (Escalante *et al*., 2019). Plasmodium has more than 200 species that infect mammals, birds, and reptiles, with malaria parasites typically exhibiting host specificity. Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae, Plasmodium ovale, and Plasmodium knowlesi are the five known species of the genus Plasmodium that causes malaria in humans. Among the five Plasmodium species responsible for malaria in humans, P. falciparum is the causative agent of severe malaria. (Fikadu and Ashenafi 2023).

2.1.1. Malaria Parasite: Overview

Malaria can be acute, fulminant or chronic. Patients present asthenia, anorexia, headache, myalgia, nausea and vomiting. P. vivax, P. ovale, and, less frequently, P. malariae are the most common causes of the acute type. Patients present with fever, which may persist from one to four days. Other symptoms include chills, sweating, anaemia, hepatomegaly, and splenomegaly. By detecting the parasite within erythrocytes on thin or thick blood films, a diagnosis can be determined using microscopy. Anaemia, adynamia, diarrhoea, jaundice, acute renal injury, hydroelectrolytic abnormalities, respiratory failure, disseminated intravascular coagulation, shock, and coma are all indicators of fulminant malaria, which is caused by P. falciparum. Given that P. ovale and P. vivax survive in the liver of quiescent forms, known as hypnozoites, disease reactivation can occur in infections caused by these pathogens. This can present as fever recurrence, anaemia, dehydration, hepatomegaly, and splenomegaly. The chronic form is linked

to P. malariae, the parasite most frequently associated with malaria-related glomerulonephritis; however, P. vivax, although regarded as a "benign" parasite with low mortality rates, has also been associated with severe disease. Age, haemodynamic abnormalities, and respiratory failure are linked to renal implications in P. vivax infections. (Silva *et al*., 2017).

2.1.2 BIphase Lifecycle Of Malaria Parasite

Anopheles mosquitoes and vertebrates are both involved in the intricate life cycle of the malaria parasite. The sporozoites in mosquito saliva reach the human host's epidermis and bloodstream during the initial stage of infection, after which they infiltrate hepatocytes to replicate asexually. Thousands of merozoites are released during this phase, also known as the hepatic or pre-erythrocytic phase, when infected hepatocytes burst.(Siciliano and Alano, 2015)

The merozoites and red blood cells (RBCs) interact during an erythrocytic infection. By altering the host cell's surface, the merozoite head aligns and abuts the erythrocyte membrane. The parasite then penetrates the erythrocyte to conduct the following asexual reproduction by forcing the cytoskeleton of the erythrocyte to reconfigure. P. falciparum and P. knowlesi attack erythrocytes of any age, however P. vivax and P. ovale primarily target younger erythrocytes. On the other hand, senescent erythrocytes are preferred by P. malariae (Ryan *et al.,* 2019). Following their invasion of red blood cells, merozoites develop into trophozoites and schizonts, which emerge from the erythrocytes to release more merozoites, infiltrate fresh RBCs, and carry on the asexual replication cycle. (Zuccala *et al.,* 2011)

Once a part of the trophozoites develop into male and female gametocytes, the sexual reproductive cycle of malaria begins. Through these gametocytes, the malaria parasite is transferred from the mammalian host to the mosquito. Mature Gametocytes are transported to

the midgut of the mosquito during an anopheles bite. In the midgut, gametocytes are transformed into active gametes, and zygotes then develop into invasive, motile ookinetes. The ookinetes eventually develop into oocysts in the basal lamina of the midgut. Following the oocyst development, the sporozoites are released and travel to the salivary gland of the mosquito. Via an infected mosquito bite, the parasite migrates to another mammalian host. (Smith *et al*., 2016).

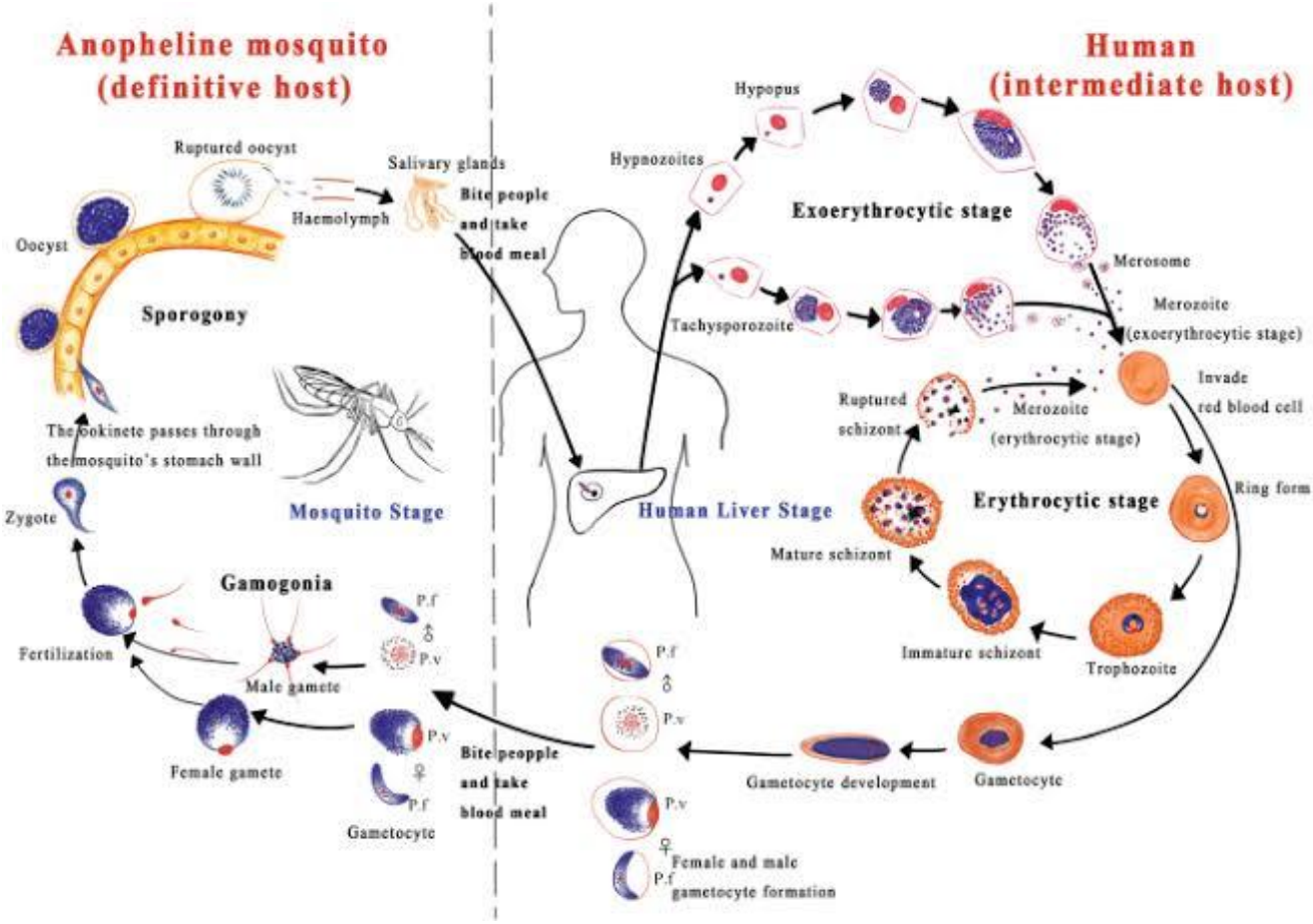


Figure 2.1: Biphase lifecycle of Malaria Parasite (Wu, 2023).

2.1.3 Renal Involvement In Malaria

According to Elsheikha *et al*., (2007), the parasitic infection first clearly associated with diseases of the kidney is Malaria, as severe malaria can induce disease in the glomeruli, renal tubules as well as the interstitial region. Malaria-induced Kidney disease occurs largely as a result of erythrocyte abnormalities. Parasitized red cells have the tendency to adhere to blood platelets, healthy erythrocytes, and the endothelial lining of capillaries, resulting in rosette formation and clumping, which obstruct microcirculation. These activities are the possible factors responsible for kidney injury, in association with hemodynamic imbalance, such as shock and hypovolemia. Parasitic activities of malaria lead to endothelial activation, this in turn results to the release of several cytokines, such as thromboxane, catecholamines, endothelin and other inflammatory mediators that are also suspected to be involved in the pathogenesis of malaria-associated kidney injury. Immune system activation in malaria undergo Th1 and Th2 response. Complement activation occurs when Th2 response is prevails in the infection by P. malariae, resulting in the deposition of immune complexes therefore leading to glomerulonephritis.

Multiple factors contribute to the emergence of these complications, including hypovolemia, vasoconstriction, haemolysis (resulting in haemoglobinuria), erythrocyte parasitemia, immune complex deposition in glomeruli, microcirculatory dysfunction (due to cytoadherence of parasitic erythrocytes), and rhabdomyolysis (which is infrequent in malaria). Additionally, hepatic dysfunction, characterised by jaundice and hepatomegaly, can lead to hyperbilirubinemia, resulting in cast nephropathy and acute kidney injury (AKI), while liver disease and its sequelae may also precipitate AKI (hepato-renal syndrome) (Kute *et al.,* 2012).

AKI is a known complication of malaria prevalent in about 40% of patients with severe infection by P. falciparum in endemic regions, leading to a high mortality rate of about 75% of cases.

Plasmodium falciparum induces the most severe variant of malaria and accounts for the majority of AKI patients. The pathophysiology of AKI in malaria is still unclear. Probable causes include immune-mediated glomerular impairment, volume depletion, and renal microcirculation obstruction induced by parasite erythrocyte sequestration. Acute tubular necrosis and, less commonly, interstitial nephritis and glomerulonephritis are the most frequent kidney histological findings in malaria, indicating a critical role of haemodynamic factors in malaria-associated AKI12 (Silvia *et al*., 2017)

2.2 Renal Structure, Physiology And Function

The kidneys are bean-shaped organs that weigh between 150 and 200 g in males and approximately 120 to 135 g in females, with medial concavity and lateral convexity. Typically, the dimensions are 10 to 12 cm in length, 5 to 7 cm in breadth, and 3 to 5 cm in thickness. Each kidney is approximately the size of a closed fist. They are located between the transverse process of T12 and L3 on the posterior abdominal wall, retroperitoneally. The two upper poles are often orientated somewhat medially and posteriorly in relation to the lower poles.(El-Reshaid and Abdul-Fattah, 2014).

Two regions make up the kidney: the cortex and medulla. The cortex consists of renal corpuscles, convoluted tubules, straight tubules, collecting ducts, and its vasculature. Straight tubules and collecting ducts continue into the cortex from the medulla to form medullary rays. Also found in the medulla is the vasa recta, which is a network of capillaries, elemental to the countercurrent exchange system. Pyramids are cone-shaped structures composed by the assemblage of tubules in the medulla. The base of the pyramids tilt towards the cortex and the apices tilt towards the hilum. The minor calyces are extensions of the papillae, which are located at the apices of the

pyramids and drain via the collecting ducts at their tips at the cribrosa area. A collecting duct and the group of nephrons that it drains is collectively known as a lobule (Soriano *et al.,* 2023)

The functional units of the kidney are the nephrons, with about 2 million nephrons per adult kidney. A nephron has a network of capillary loops termed the glomerulus, which is supplied by an afferent arteriole. It is surrounded by a double-layer of epithelium referred to as the Bowman's capsule, the glomerulus and the bowman's capsule collectively form a renal corpuscle. An efferent arteriole which drains the glomerulus becomes the vasa recta that supplies the renal tubules. The nephron has an arrangement beginning with the renal corpuscle, distally followed by: the proximal convoluted tubule, thick descending limb of the loop of Henle, thin descending limb of the loop of Henle, thin ascending limb of the loop of Henle, thick ascending limb of the loop of Henle, distal convoluted tubule, collecting tubule, cortical collecting duct, medullary collecting duct, papillary duct, minor calyx and major calyx, renal pelvis, and ureter. The tubules originate in the cortex, descend into the medulla, making a hairpin twist in the thin descending limb of the loop of Henle, and ascend adjoining the cortex at its original renal corpuscle (Scott and Quaggin, 2015).

The glomerular filtration barrier of the renal corpuscle consists of the glomerular basement membrane (GBM), the fenestrated endothelium of glomerular capillaries, and the visceral layer of Bowman's capsule, with podocytes that extend around the capillaries. The GBM is composed of the lamina rara externa, lamina rara interna, and lamina densa. The parietal layer of Bowman's capsule is composed of simple squamous epithelium, and it is separated from the visceral layer by Bowman's space. Mesangial cells are found throughout the renal corpuscle outside of the capillaries, and the juxtaglomerular apparatus is made up of specialized mesangial cells outside of the renal corpuscle, juxtaglomerular cells, and the macula densa. The thick

ascending limb continues to its original glomerulus, and forms specialized cells lining the afferent arteriole called the macula densa (Scott and Quaggin, 2015).

The kidney, ureters, and urethra make up the renal system. Ultimately, the system filters about 200 litres of fluid each day from renal blood flow, allowing excess ions, metabolic waste products, and toxins to be excreted while maintaining essential components in the blood. By adjusting the blood's concentration of water, solutes, and electrolytes, the kidney maintains plasma osmolarity. Through the renin-angiotensin-aldosterone pathway, it is essential for maintaining intravascular volume and regulating blood pressure. In addition to synthesizing erythropoietin, which promotes the production of red blood cells, it maintains the long-term acid-base balance. It also converts vitamin D to its active form and produces renin, which regulates blood pressure.

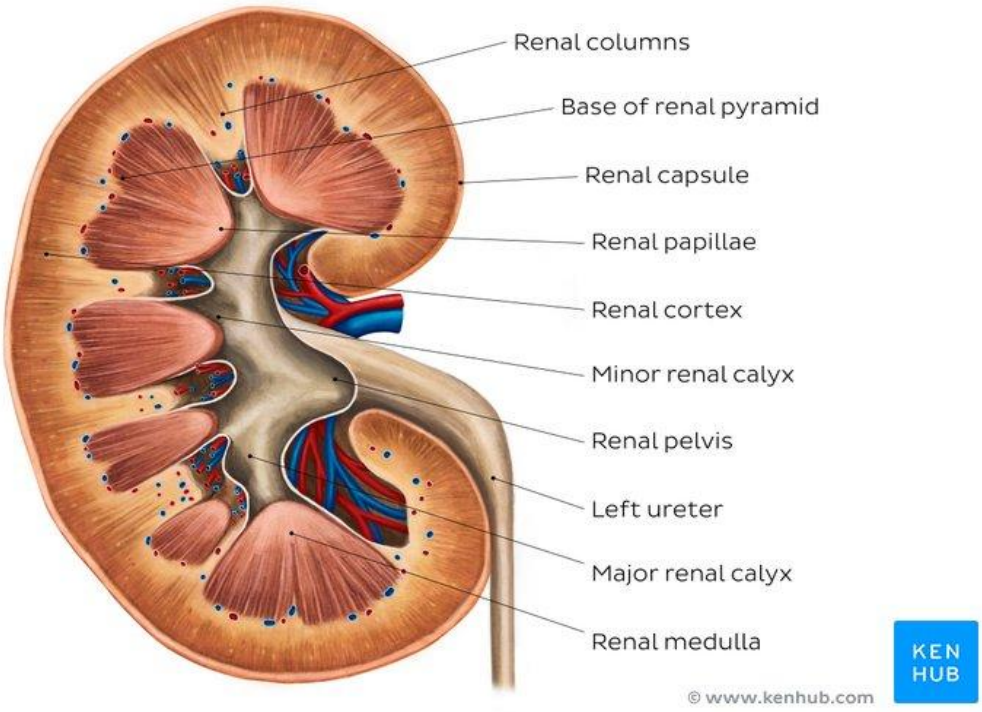


Figure 2.2: Internal overview of the human kidney. (https://www.kenhub.com. Kidneys: Anatomy, function and internal structure | Kenhub).

2.3.1 Rationale For Using Albino Wistar Rats As A Model

In order to understand host-parasite interactions in malaria, evaluate therapeutic treatments, and clarify disease causes, animal models are essential. While non-human primates exhibit the most physiological similarities to human malaria, availability, cost, and ethical concerns significantly limit their use. Due to the genetic tractability, simplicity of handling, and other characteristics, rodent models—especially those that use Plasmodium berghei and Plasmodium yoelii in mice and rats—have become useful substitutes (Gozalo *et al*., 2024).

Malaria is a disease with a complex life cycle that requires a comprehensive approach in developing effective treatments, vaccines, and control strategies. The use of animal models has proved invaluable in malaria research, providing insights into the disease's pathogenesis, immune

responses, and potential interventions. A thorough understanding of malaria requires an understanding of the interactions between the parasite, its vector, and the human host. Direct experimentation on humans is, however, constrained by ethical and practical considerations. Hence the need for rodent models that allow detailed genetic and immunological studies to non-human primate models that closely mimic human malaria, animal models bridge this gap, providing controlled environments to investigate the disease's mechanisms and potential solutions and facilitating comprehensive research that advances our understanding and ability to combat malaria. Several aspects illustrate the irreplaceable nature of animal models in malaria research:

Rodents, particulaly albino whistar rats, serve as the primary hosts in malaria research. Rodent models are therefore a cornerstone of malaria research, offering a versatile and practical platform for studying the disease's biology, host-pathogen interactions, and potential interventions. This detailed account explores the various Plasmodium species infecting rodents, the host strains used, and their applications in different research areas. The choice of Plasmodium species and rodent strain is crucial as it can influence the course of infection, immune response, and the outcome of experimental interventions.

A common model organism in malaria research is the rodent-specific malaria parasite Plasmodium berghei (Sato, 2021). It is a useful model given that its life cycle is well-characterized and it shares many biological characteristics with human malaria parasites. While Plasmodium falciparum and Plasmodium vivax are human malaria parasites, P. berghei naturally infects rodents, particularly some African rodent species like the thicket rat (Thamnomys rutilans). It is essential in the study of malaria because it allows for investigation of the various aspects of the disease in a controlled laboratory setting.

Models of P. berghei have significance in a number of important aspects of malaria research. By utilising these models to investigate the parasite's life cycle, particularly stages in the liver and blood, researchers can analyse the efficacy of potential drugs and vaccines. Additionally, the genetic tractability of P. berghei facilitates the exploration of gene function and the discovery of novel therapeutic targets. Understanding the multifaceted biology of malaria and developing efficient defence strategies have been made practicable by the information obtained from P. berghei models. Its importance comes from a combination of host specificity, genetic tractability, and experimental modification amenability (Torre *et al*., 2018).

Different strains of P. berghei exhibit distinct characteristics, such as virulence, drug sensitivity, and transmission efficiency. Early studies by Yoeli identified diverse strains of P. berghei (Otun *et al*., 2024), establishing the foundation for further research into the genetic makeup and phenotypic variations of these strains. Additionally, P. berghei can now be precisely modified genetically via gene deletion, knock-in, and tagging strategies with to developments in transgenic techniques (Otun *et al*., 2024). Finding critical molecular pathways that underpin the pathophysiology of malaria and studying the biology of parasites have been made simpler by this genetic flexibility.(Daclam *et al.*, 2024)

2.3.2 Advantages And Limitations Of Albino Whistar Rats In Malarial Studies

Albino Wistar rats which are used in many research experiments have the following peculiarities of body structure. The Wistar rat is an outbred albino rat. This breed was produced at the Wistar Institute in 1906 for use in biological and medical research, and it is famously the first rat developed to serve as a model organism during a time when scientists largely used the house mouse.

Advantages

Albino wistar mice provide the following advantages: the animals are easy to procure and quite homogeneous. The ease with which Albino Wistar rats may be obtained is a further benefit for employing them as a model. Researchers from various institutions have no trouble obtaining these rats as they are readily available in large numbers and are sold by the majority of organisations that engage in the selling of laboratory animals. Accessibility reduces the possibility of experiment variability brought on by genetic variation between many animals. Rather, one can be certain that they will be working with animals that share a comparable history, which is crucial for doing effective and reliable research.

Additionally, Albino Wistar rats are easy to handle and show little reactivity, which makes them appropriate for research where animal interaction is frequently required. Albino Wistar rats are also known for being reproducible in lab settings. For research results to be legitimate and reliable, this uniformity is essential.

Limitations

Due to their genetic homogeneity, albino wistar rats offer many advantages for use as research animals. However, they also have some serious drawbacks, such as their vulnerability to light sensitivity, spontaneous tumour growth, and behavioural and physiological changes triggered on by stress. Compared to other rat species, these rats have a tendency to become more agitated and nervous. The results of the experiment may be affected by this. Stress can alter a rat's hormone levels, immunological system, and behaviour. For instance, it may have an impact on the function of specific genes related to inflammation or metabolism, which may complicate research on these topics. Another problem is that albino wistar rats frequently develop

mammary gland tumours on their own. This may mislead the results of studies on certain disease conditions or therapies.

2.3.3 Albino Whistar Rats In Malaria

In vivo studies and models of human disease have made considerable use of malaria parasites that infect rodents, such as mice and rats. These investigations have focused on four species of rodent malaria parasites (RMPs), namely P. berghei, P. yoelii, P. chabaudi, and P. vinckei, which were first isolated in Central African thicket rats on several different occasions. Although these parasite species and the human-infecting P. falciparum (Carlton *et al*., 1998b) have a substantially conserved chromosome gene interaction, there are minor variations in their host cell preferences, life cycle duration, and stage-specific morphologies. Similar to human P. falciparum and P. malariae, P. chabaudi and P. vinckei can infiltrate mature red blood cells and cause severe parasitaemia. Similarly, in the manner that humans are infected with P. vivax and P. ovale, P. berghei and P. yoelii are typically limited to reticulocyte invasion. However, the underlying biology of Plasmodium, which infects humans and rodents, is essentially conserved.

This has facilitated the examination of various facets of malaria parasite development, host–pathogen interactions, therapeutic efficacy assessments, and vaccine research that would otherwise be unattainable with P. falciparum in vitro. RMPs also provide a number of advantages, such as the availability of an extensive range of genetic manipulation systems, the simplicity of handling in rodents, and the experimental tractability of all life cycle stages in laboratory settings. However, RMPs and their hosts differ from their human analogues (humans and P. falciparum). Because of the characteristics of their hosts, the finer molecular features may differ from those of humans who are their hosts, notwithstanding considering that they might offer significant insights into the conserved aspects of parasite biology. Therefore, by directly comparing these

models to human parasites, the application should be appropriately suited to the biological question being studied. (Craig *et al.*, 2012; De Niz and Heussler, 2018).

2.4 Pathophysiology Of Malaria Induced Renal Dysfunction

The incidence of severe malaria is approximately two million cases with almost 430,000 deaths per year (World Health organization, 2016). A multisystem disease with distinct clinical presentations in adults and children describes this medical emergency. Recent research, however, indicates that acidosis, kidney failure, and cerebral involvement are independent predictors of death in both adults and children. Malaria pathology frequently targets the kidney, resulting in a range of clinical manifestations, from minor proteinuria to severe acute kidney damage (AKI). Infected red blood cells (pRBCs), the parasite, host immune responses, and systemic implications of the disease all interact intricately in the pathophysiology of malaria-induced renal dysfunction (MRD). It is essential to fully understand these mechanisms in order to create targeted strategies for therapy.

2.4.1 Pathogenesis Of Malaria Induced Renal Dysfunction

1. Microvascular obstruction

Plasmodium falciparum is the primary cause of severe malaria due to its ability to cause end-organ dysfunction by inducing infected red blood cells (RBCs) to cytoadhere to the vascular endothelium. It is uncertain however if other plasmodium species may induce coma, nevertheless they can cause severe disease and AKI (Anstey *et al*., 2012).

2. Sequestration of Parasitized Red Blood Cells (pRBCs)

Heterogeneous microcirculation obstruction and tissue hypoxia are triggered by cytoadhesion, which cause sequestration of parasitised RBCs in the capillaries and postcapillary venules.

Microcirculatory flow is believed to be further impeded by increased rigidity of both infected and uninfected RBCs, clumping of infected RBCs (platelet-mediated autoagglutination), and uninfected RBCs adhering to infected RBCs (rosette formation), in addition to flow obstruction by sequestered parasitised RBCs (Dondorp *et al*., 2004). Research on individuals with severe malaria who have AKI reveals endothelial alterations in both glomerular and peritubular capillaries on histopathology, decreased renal cortical blood flow, and increased kidney size (Atalabi et al., 2013). AKI and the demand for renal replacement therapy (RRT) in adults with severe malaria are closely linked to cell-free haemoglobin and lipid peroxidation indicators. An increased heme-to-hemopexin ratio was linked to haemoglobinuria, stage 3 AKI, and 6-month mortality in children with severe malaria. (Plewes *et al.*, 2018)

Glomerular Damage And Immune Complex Deposition

Various kinds of glomerulopathy, which are characterised by structural and functional damage to the glomeruli, the kidney's principal filtration units, are typically brought on by malaria infection. One important mechanism in this process is immune complex deposition (Silvia *et al*., 2017). The host immune system produces antibodies against parasite antigens during malaria. These antibodies may produce immunological complexes with circulating parasite antigens, which subsequently settle in the glomeruli, particularly in the mesangial and subendothelial regions. A localized inflammatory response develops within the glomerulus as a result of the deposition of these immune complexes, which also activates the complement system and triggers inflammatory cells. The glomerular filtration barrier may eventually be compromised by this inflammatory cascade, which may eventually lead resulting in mesangial cell growth, podocyte destruction, and thickening of the glomerular basement membrane. Proteinuria and, in extreme situations, haematuria are symptoms of the ensuing rise in glomerular permeability, which

suggests a serious impairment of renal filtration integrity. Both acute and chronic types of malaria-associated kidney disease may be aggravated by this immune-mediated injury. (Naqvi, 2015).

Systemic Inflammation and Cytokine Storm

Pro-inflammatory cytokines such as interleukin-6 (IL-6), interferon-gamma (IFN-γ), and tumour necrosis factor-alpha (TNF-α) are released when a malaria infection triggers an acute inflammatory response. This "cytokine storm" is a major factor to renal damage and other multi-organ failure. (Andersson *et al*., 2000) In the kidney, these cytokines directly cause endothelial cell activation and destruction, which increases vascular permeability and results in microvascular dysfunction. Further causing damage to renal parenchymal cells, they also promote the migration and activation of inflammatory cells, including neutrophils and macrophages, which can release reactive oxygen species and proteases. Prolonged inflammation can cause extensive cellular damage and apoptosis in the glomeruli and renal tubules, compromising their functionality and increasing the development of AKI. Thus, in malaria, the systemic inflammatory response serves as a critical activator of renal injury. (Clark *et al*., 2006).

Oxidative Stress and Reactive Oxygen Species (ROS)

Oxidative stress is a major contributor to renal damage, as the infection causes an imbalance between the body's antioxidant defence mechanisms and the production of reactive oxygen species (ROS) due to the metabolism of haemoglobin by parasites, the release of free heme during haemolysis, and the activation of phagocytic cells during the immune response (Elphinstone *et al*., 2016). These highly reactive molecules can cause extensive damage to cellular components, including lipids, proteins, and DNA, resulting in membrane peroxidation,

enzyme inactivation, and cellular dysfunction in renal cells. The kidney, with its high metabolic rate and rich blood supply, is especially susceptible to oxidative damage.. Research has explicitly associated oxidative stress to the observed renal damage by demonstrating elevated levels of oxidative stress markers and decreased antioxidant enzyme activity in the kidneys of malaria-infected individuals and animal models. Acute tubular necrosis and compromised glomerular function are primarily caused by reactive oxygen species destruction of cellular integrity. (Vasquez and Rodriguez, 2021).

Coagulation Abnormalities

Hypercoagulability triggered by malaria infection frequently disrupts the coagulation cascade. In small arteries, particularly those in the kidney, this can show up as disseminated intravascular coagulation (DIC), which is characterized by widespread coagulation activation and the consequent production of microthrombi. Localized ischaemia and renal tissue infarction result from the accumulation of fibrin and platelet aggregates in renal capillaries, which further decrease blood flow. Ironically, bleeding tendencies may result from the intake of platelets and clotting factors during this procedure, which would complicate the underlying medical situation. The severity and progression of AKI in malaria are made worse by the vicious cycle that is produced by the interaction of inflammation, endothelial activation, and coagulation abnormalities. (Pantep Angchaisuksiri, 2014)

Metabolic Acidosis and Hypovolemia

Renal failure is also primarily caused by systemic effects resulting from severe malaria, such as metabolic acidosis and hypovolemia. Lactic acidosis can result from severe malaria due to reduced hepatic lactate clearance, increased anaerobic glycolysis, and impaired tissue perfusion.

Acidosis directly lowers myocardial contractility, which decreases cardiac output and results in systemic hypotension, thereby significantly reducing renal perfusion (English *et al*., 1997). Additionally, hypovolemia and prerenal azotemia can result from fluid losses induced by fever, vomiting, diarrhoea, and decreased oral intake. Hypovolemia and metabolic acidosis both decrease renal blood flow and effective circulation volume, which puts the kidneys at risk for ischaemic injury and worsens pre-existing damage (Leopold *et al.,* 2019). Despite not having direct parasitic effects, these systemic conditions create a dangerous environment which severely impairs kidney function. (Possemiers *et al*., 2021).

2.5 Dose-dependent Relationship Between Parasitemia And Renal Dysfunction

According to data from a study by (Euclides *et al.*, 2020), acute kidney damage (AKI) is a common adverse reaction in malaria patients and may be related to the parasitemia levels experienced by these patients. In endemic regions, AKI, a known consequence of malaria, may adversely impact approximately 40% of patients with severe disease caused by P. falciparum, contributing to a high fatality rate of almost 75% of cases. The majority of AKI cases are caused by P. falciparum, which also induces the most severe type of malaria. Malaria accounts for over 10% of patients admitted with AKI in several parts of the world. (Koopmans *et al*., 2015; Silvia *et al*., 2017). There is a strong correlation between renal impairment and the level of malaria parasitemia, especially in cases of severe Plasmodium falciparum infections. An known risk factor for acute kidney injury (AKI) is higher parasitemia.

As previously stated, the pathogenesis of malaria involves a number of interrelated pathways. Significant microvascular sequestration occurs when parasitized red blood cells adhere to the endothelial lining of glomerular and peritubular capillaries due to increased parasite biomass. Localised ischaemia and tubular necrosis result from this physical blockage, which lowers renal

blood flow. Additionally, free haemoglobin, known to be nephrotoxic and can precipitate in the renal tubules, is released when red blood cells are rapidly destroyed (haemolysis), resulting to further damage. Renal pathophysiology is also influenced by the immunological response of the host. Pro-inflammatory cytokines are produced as a result of the systemic inflammatory response that the parasites and their byproducts (such as hemozoin) induce. These cytokines have the ability to directly damage kidney cells and aggravate microvascular dysfunction. The primary outcome of this sequence of events is a reduced glomerular filtration rate (GFR). (Plewes *et al*., 2018)

Its significance as a prognostic biomarker is shown by the association between parasitemia and renal failure. Elevated blood urea nitrogen (BUN) and serum creatinine are essential diagnostic markers of possible or confirmed renal failure, commonly linked to high parasite loads, which frequently surpass 100,000 parasites/μL. Hence, quantitative PCR may help guide the administration of intravenous anti-malarial medication and predict the outcome of patients with P. falciparum infections.

2.6.1 Histopathological Changes In The Kidney Due To Malaria Infection

In endemic regions, AKI, can occur in approximately 40% of patients with severe disease caused by P. falciparum, contributing to a high fatality rate of almost 75% of cases. A large percentage of AKI cases are caused by P. falciparum, which also induces the most severe variant of malaria. Malaria accounts for more than 10% of patients admitted with AKI in various regions of the globe.

Oligo-anuria (46-76% of cases), severe metabolic acidosis, and hypercatabolic condition are the most typical clinical manifestations of P. falciparum malaria-associated AKI. Recent research

has identified the following as risk factors for AKI in malaria: advanced age, hospital-acquired secondary infection, hyperbilirubinemia, referral from another hospital, the need for inotropic medications, and factors linked to mortality: leukocytosis, oligo-anuria, hyperkalaemia, jaundice, altered consciousness, and P. falciparum infection (as opposed to P. vivax infection). Electrolyte abnormalities include hyperkalaemia, which is linked to haemolysis, rhabdomyolysis, and acidosis in addition to AKI, and hyponatraemia, which appears to be common in malaria-associated AKI, occurring in 30–50% of patients.

Acute tubular necrosis is the primary kidney histological feature in malaria, with interstitial nephritis and glomerulonephritis occurring less commonly. This suggests that haemodynamic parameters play a major role in malaria-associated AKI. In 2017, (Silvia *et al*., 2017) Glomerulonephritis is rarely observed in P. falciparum infection patients, and it appears that children are more susceptible to this consequence. Although the exact prevalence of glomerulonephritis in malaria is unknown, it is thought to be approximately 18%.

Malaria often results with hypergammaglobulinemia. When the microbe interacts with the innate immune system, it activates B cells, which in turn produce immunoglobulins, resulting in immunosuppression secondary to cytokine production which depletes macrophages and T cells. Immunoglobulin synthesis results in the creation of immunological complexes, which are associated with glomerular injury. Although there is ongoing debate over the molecular mechanisms of malarial nephropathy, it is hypothesised that granulocyte-macrophage colony-stimulating factor (GM-CSF), TNF-α, IL-1α, IL-6, and IL-10 play a role.

IgM, IgG, and C3 deposits have been detected in P. falciparum malaria through immunofluorescence. IgA deposits in malaria-associated AKI have also been discovered in

more recent research; these deposits clear during the acute stage of the infection. Children infected with P. falciparum have also been reported to develop eosinophilic glomerulonephritis (Katsoulis *et al*. 2021). The presence of granular, fibrilar, and amorphous material is linked to electron-dense deposits in the subendothelial and mesangial regions. Although their function is yet unknown, autoantibodies have also been found in patients with glomerulonephritis associated with malaria, according to results from electronic microscopy..

According to a study by (Wichapoon et al. 2014), glomerular cells were more frequent in the AKI group when glomerular proliferation was assessed morphologically by identifying mesangial cells, endothelial cells (ECs), and podocytes in the control, non-AKI, and AKI groups. Additional alterations included the presence of PRBCs in the capillaries, thickening of basement membrane congestion, and protein accumulation in the Bowman's capsule. Acute tubular necrosis, interstitial nephritis, and glomerulonephritis make up the range of morphological alterations associated with acute kidney injury caused by malaria. Infections with P. falciparum exhibit these alterations. However, vivax infections might also exhibit comparable alterations. Histologically, acute tubular necrosis (ATN) is the most reliable characteristic. ATN is triggered by the associated hypovolemia and hypoperfusion of the kidney. Tubular changes such as cloudy swelling, hemosiderin granular deposits and acute kidney injury are seen in malaria.

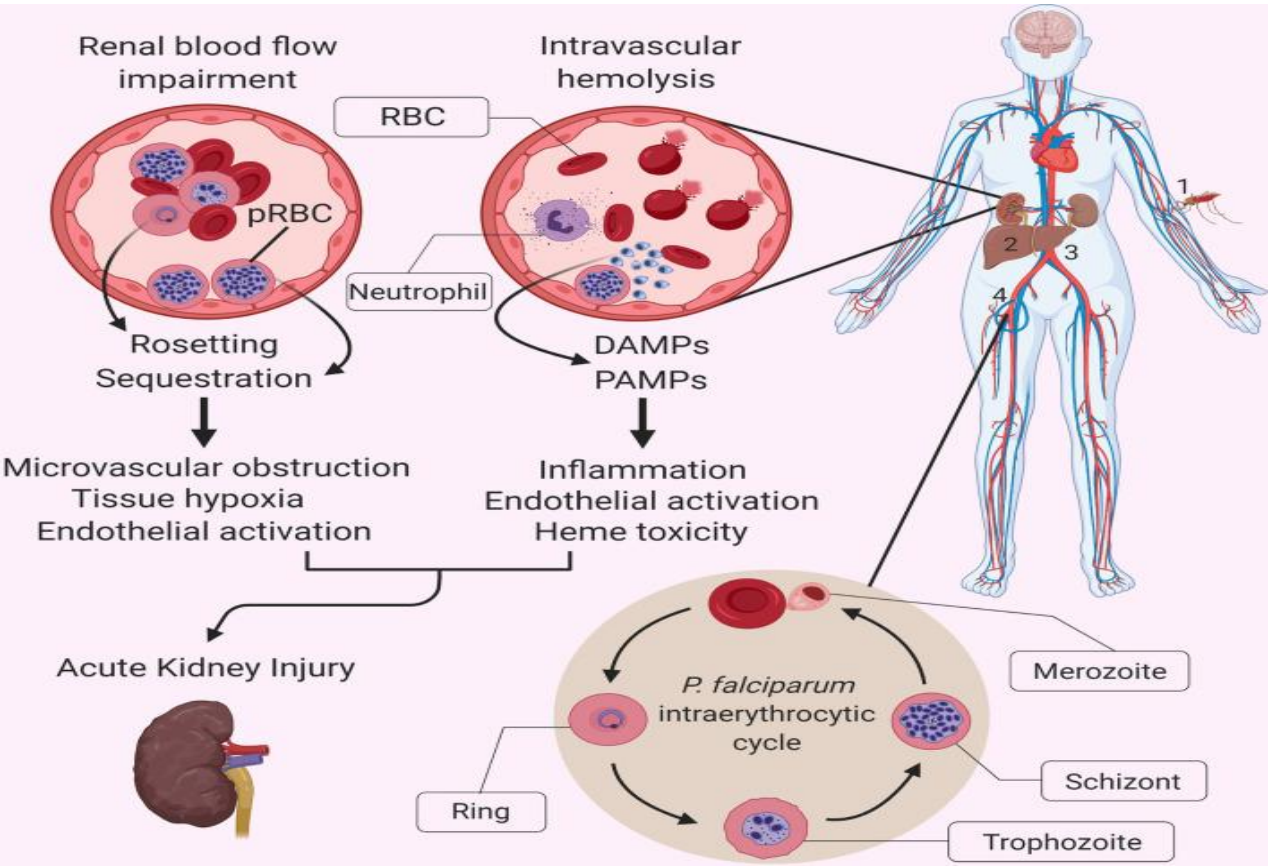


Fig. 4: *Histopathological features observed in AKI (Biorender.com. Katsoulis et al., 2021).*

2.6.2 Histopathological Assessment Techniques

Histopathological analysis is still essential for understanding the cellular and structural changes induced by malaria, particularly in organ systems like the kidneys in rodent models. To visualize and evaluate pathological changes, a number of common and advanced techniques are used.

1. Eosin and haematoxylin (H&E) Staining: The most used histology method is H&E staining given that it renders tissue architecture relatively simple to visualise. Eosin cytoplasmic and extracellular proteins a pink, while haematoxylin gives nuclei a deep blue or purple stain. H&E is essential for detecting inflammatory infiltrates, interstitial oedema, and renal necrosis in malaria investigations (Wichapoon et al., 2014).

2. Immunohistochemistry: By identifying parasite antigens such as Plasmodium lactate dehydrogenase (pLDH) or Histidine-rich protein-2 (HRP-2), IHC can directly detect the presence of malaria parasites in kidney tissue. This supports the theory that the kidney damage was caused by parasites (Genrich *et al*., 2007).

3. Analysis of Digital Images: Using convolutional neural networks and other machine learning models, digital image analysis utilize microscopic pictures of tissue biopsies and Giemsa-stained blood smears to identify parasites and host cellular damage to detect manifestations of malaria and related kidney necrosis (Maturana *et al*., 2023). By automating the processes of parasite detection and quantification, cell segmentation, and infected cell classification, these AI-powered systems—which may be incorporated into smartphone applications—enable objective diagnosis and kidney damage and parasitemia monitoring. (Yoon *et al*., 2021).

4. Special Stains and Markers

Special stains like Masson's Trichrome (for fibrosis), Periodic Acid-Schiff (PAS) (for glycogen), and TUNEL assay (for apoptosis) are used in addition to H&E to highlight certain structural changes. When combined, these techniques offer a more thorough evaluation of malaria-induced kidney impairment.

2.7 Antimalarial Drug Efficacy On Malarial Induced Renal Dysfunction

Chloroquine is a classical malaria medication. As a result the drug resistance issue, it appears to be outdated at present time. Considering the renal toxicity of chloroquine, there fails to be any conclusive proof that it may be administered for the treatment of malaria. However, the challenge of kidney toxicity is observed with long-term usage, which is not the case with malaria (Ngaha, 1982). Patients with underlying terminal kidney dysfunction who consumed

chloroquine may experience severe acute megaloblastic anaemia, exfoliative dermatitis, and symptomatic pancytopenia as an important adverse effect (Thorogood, 2007).

Quinine is an antimalarial drug that is effective. An allergy to this drug may occur, and renal issues may be observed. Acute renal failure, liver damage, anaemia, thrombocytopenia, neutropenia, and neurological problems can all be observed in cases of allergies. (Howard, 2003).

The breakthrough antimalarial drug, artesunate, has been shown to be effective and to be not subject to drug resistance. It is additionally employed as an alternative additional therapy for cancer. A number of studies have centered on its possible renal toxicity, with reversible nephrotoxicity observed in a rat model. Artesunate decreases glomerular filtration rate and increases kidney blood flow and urine excretion of Na, Cl, and K. However, it is still safe for short-term low dosage use in the treatment of malaria, and an animal experimental study has demonstrated the clinical efficacy of artesunate in the treatment of nephrotic syndrome. (Wiwanitkit, 2015).

2.8 Gaps In Literature And Future Research

In developing countries, malaria is a deadly parasite disease with a high morbidity and mortality rate. Malaria has a diverse aetiology, and its clinical presentations can vary from severe and complicated to mild and simple to asymptomatic. The clinical severity of malaria has been extensively studied, but less is known about silent infections. Because it has a substantial impact on the dynamics of transmission, asymptomatic malaria continues to provide a problem for malaria control measures. It is necessary to have a full understanding of how hosts and parasites interact to produce various therapeutic outcomes. Although AKI is known to increase the risk of long-term issues like neurocognitive impairment and death, little is understood about the

structural and functional alterations that cause these consequences and how they affect health-related quality of life. (Laishram, 2012).

Future research should focus on developing robust animal models that mimic human pathology, utilizing advanced technologies like organ-on-a-chip models, identifying specific molecular pathways contributing to renal damage, and developing new diagnostic biomarkers and more sensitive treatments for acute kidney injury in malaria patients (Claure-Del, 2023).

CHAPTER THREE: MATERIALS AND METHODS

3.1 Study Area

This study was conducted specifically within the Animal House in the Department of Anatomy, and the Histopathology Laboratory in the University of Benin Teaching Hospital, both in Benin City, Edo State. Edo State lay between longitude 06°04′E and 06°43′E and latitude 05°44′N and 07°34′N, with a land mass of about 17,450 sq. km, located in the south-south geopolitical zone of Nigeria, and with a population of 4.7 million people (World Gazetteer, 2007). The facility provided a controlled environment necessary for experimental infection studies, including standard housing conditions for laboratory animals (temperature 22–28°C, 12-hour light/dark cycle, and unrestricted access to food and water). The animal house complied with institutional ethical standards and national regulations for the care and use of laboratory animals.

3.2 Collection of Parasite Material

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

3.2.1 Parasite Inoculum Preparation

The inoculum was prepared following the modified method of Pedroni et al. (2006). Donor mice previously infected with Plasmodium berghei were used to harvest parasitized erythrocytes once parasitemia reached approximately 20–30%. Blood was collected via cardiac puncture into anticoagulated tubes, then diluted in sterile phosphate-buffered saline (PBS, pH 7.2) to obtain a

concentration of 1×10^7 infected red blood cells (iRBCs) per 0.2 mL. This suspension was used for inoculation into experimental rats via intraperitoneal injection.

3.3 Animal Care

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

3.4 Ethical Clearance

Ethical approval for this research was obtained from the Ethics Committee of the Ministry of Agriculture and Natural Resources, Edo State, Nigeria. All procedures involving animals conformed strictly to the guidelines for the care and use of laboratory animals.

3.5 Experimental Design

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

Group A (Control): This group consisted of 4 rats that received only standard feed and water for a duration of fourty-two (42) days without any malaria infection.

Group B (High Infection Group): The four (4) rats in this group were infected with high concentrations of Plasmodium spp. (~1 × 10^6 parasitized red blood cells) and observed for kidney pathologic symptoms. They also received standard feed and water for a duration of fourty-two (42) days.

Group C (Medium Infection Group): The four (4) rats in this group were infected with medium concentrations of Plasmodium spp. (~1 × 10^4 parasitized red blood cells) and observed for kidney changes. They also received standard feed and water for a duration of fourty-two (42) days.

Group D ((Low Infection Group): The four (4) rats in this group were infected with low concentrations of Plasmodium spp. (~1 × 10^2 parasitized red blood cells) and observed for changes in kidney function. They also received standard feed and water for a duration of fourty-two (42) days.

At the end of the 42-day experimental period, four (4) rats per group were humanely euthanized via unconsciousness. The kidneys were harvested and fixed in 10% neutral buffered formalin (NBF) for 24 hours.

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**	**21**
Group D (High Infection)	**4**	**Infected**	**10^6**	**21**
Group C (Medium Infection)	**4**	**Infected**	**10^4**	**21**
Group D (low Infection)	**4**	**Infected**	**10^2**	**21**

3.6 Processing of Histology Sample

3.6.1 Histological Technique

Procedure:

Histopathologically, to detect inflammation, the kidney tissues were processed, sectioned and stained using hematoxylin and eosin staining techniques to demonstrate general tissue structure and then viewed microscopically. The procedure involved includes:

Harvesting Tissue: The kidney tissues were harvested from the rats and immediately put in a fixative. After 24 hours of fixation, the kidney tissues were cut up and sectioned into thin slices of 3mm by size, using a rotary microtome.

Tissue processing using automatic method: Sequences for automatic tissue processing were as follows:

For histological evaluation, the kidney was selected for its profound susceptibility to pathological alterations induced by malarial infection. The kidney, a paired organ located retroperitoneally, is responsible for producing urine through the processes of filtration, reabsorption, and secretion. It is also responsible for it's central role in fluid and electrolyte homeostasis, waste excretion, and endocrine function. Structurally, it is composed of two primary regions: an outer cortex and an inner medulla. The cortex contains the glomeruli, which are responsible for blood filtration, and the convoluted tubules, which modify the filtrate. The medulla contains the loops of Henle and the collecting ducts. Histopathological evaluation of the kidney provides critical insight into the health of the renal parenchyma, as distinct pathological changes such as acute tubular necrosis and immune complex glomerulonephritis are hallmarks of severe malarial nephropathy. Glomerular changes, including deposition of immune complexes or the sequestration of parasitized red blood cells in capillary lumens, are indicative of malarial kidney injury. Tubular changes such as degeneration, regeneration, or the presence of hemoglobin casts resulting from hemolysis are critical markers of acute kidney injury associated with P. falciparum malaria. The interstitial tissue, containing fibroblasts, capillaries, and inflammatory cells, can exhibit signs of edema, fibrosis, or infiltration by lymphocytes and plasma cells, providing additional markers of chronic pathology. Similarly, the renal vasculature, vital for maintaining glomerular filtration pressure, is an important histological marker, as

vascular congestion, endothelial swelling, or arteriolar hyalinosis frequently accompany malarial renal injury. Following collection, the kidney tissues were fixed in 10% neutral buffered formalin (NBF), prepared from commercial formalin (37–40% formaldehyde), sodium dihydrogen phosphate monohydrate, disodium hydrogen phosphate anhydrous, and tap water, maintaining a neutral pH of ~7.0 to prevent formalin pigment formation and ensure morphological preservation (Bancroft & Gamble, 2008). Tissues were immersed in NBF for at least 24 hours at room temperature to achieve thorough penetration and stabilization of renal architecture, preventing autolysis and minimizing artifacts. After fixation, dehydration was carried out by sequential immersion in graded alcohols (70% and 90% ethanol), followed by two rounds of absolute alcohol, each lasting one hour and using a volume 20-50 times the tissue volume. The samples were then cleared in two changes of xylene for 60 minutes each, removing residual alcohol and preparing the renal parenchyma for paraffin infiltration. Paraffin wax impregnation was performed at wax melting temperature, using tissue-to-wax ratios of 1:25–30, with two changes of molten wax, each lasting 1 hour, to ensure complete infiltration of tubular and glomerular structures. Finally, the kidney tissues were embedded in labeled cassettes with molten paraffin wax, solidified, and cooled rapidly using a cold plate. The hardened blocks were trimmed and sectioned at 0 and 3 µm thickness respectively,using a digital rotary microtome (Histoline MR3000, Italy). Sections were mounted on clean, grease-free slides and subjected to hematoxylin and eosin (H&E) staining, enabling detailed evaluation of glomerular morphology, tubular integrity, interstitial space, and pathological alterations indicative of Plasmodium-induced renal injury.

3.6.2 Staining of Processed Tissues

Tissue sections prepared for general histological evaluation were stained using the Ehrlich's Haematoxylin and Eosin (HandE) staining technique, following the method outlined by

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

Procedure For Hematoxylin And Eosin Staining

Tissue sections were initially dewaxed by immersing them in two changes of xylene, each for 2 minutes, to remove paraffin wax. This was followed by rehydration through a descending alcohol series, starting with absolute alcohol for 2 minutes, then 90% alcohol for 1 minute, and finally 70% alcohol for 1 minute. The slides were then rinsed under running tap water for 1 minute to remove residual alcohol. After rehydration, the sections were stained with hematoxylin for 10 minutes to highlight nuclear structures. Excess stain was removed by brief rinsing in distilled water for 30 seconds, followed by differentiation in 1% acid alcohol for 15 seconds to enhance contrast. The slides were then rinsed thoroughly in distilled water for 5 minutes to stop the differentiation process. Subsequently, the tissues were counterstained with 1% eosin for 5 minutes to visualize cytoplasmic and extracellular components. After staining, the sections were rinsed in running tap water for 30 seconds, then dehydrated through ascending grades of alcohol 70%, 90%, and 100% for 1 minute each. Dehydrated slides were cleared in two changes of

xylene for 2 minutes each to remove alcohol and make the tissue transparent. Finally, the sections were mounted using DPX mounting medium and examined microscopically under an objective lens to assess histological features (Braimah *et al*., 2024).

3.6.3 Microscopy And Photomicrography

Tissue sections were examined at 40× and 100× magnifications using an Olympus CX23 binocular light microscope, equipped with an integrated LED illumination system to ensure consistent and high-contrast visualization of histological features. For image documentation, photomicrographs were captured using an Olympus BX53 trinocular microscope fitted with an Olympus DP74 high-resolution digital camera. The setup was connected to a computer via Olympus cellSens imaging software, which facilitated accurate acquisition and processing of the microscopic images.

Table 2. Parameters for Kidney Histopathology in Malaria-Infected Animals

Parameter	Description/Definition	Grading Scale	Expected Findings in Malaria-Infected Animals
Glomerular Integrity	Structural integrity of the glomeruli, including the basement membrane and capillaries	0 (Normal) to 3 (Severe damage)	Glomerular hypertrophy, proliferation of endothelial and mesangial cells, widening of the Bowman's space, and capillary loop collapse
Tubular Necrosis/Damage	Death or damage to the renal tubules, including the proximal and distal convoluted tubules	0 (Absent) to 3 (Severe damage)	Swelling or flattening of tubular epithelial cells, loss of brush borders, tubular dilation, and presence of protein casts
Interstitial Infiltration	Presence and extent of inflammatory cells in the interstitial space	0 (Absent) to 3 (Severe infiltration)	Increased numbers of mononuclear cells (lymphocytes,

	between the tubules		macrophages, plasma cells), and neutrophils; interstitial edema
Intratubular Hemoglobin/Hemosiderin Casts	Accumulation of hemoglobin or hemosiderin within the renal tubules	0 (Absent) to 3 (Abundant casts)	Presence of granular, often brown casts within tubular lumens, indicating hemolysis
Vascular Changes	Changes in the blood vessels of the kidney, such as arterioles and venules	0 (Normal) to 3 (Severe changes)	Thickening of the vessel walls, endothelial swelling, and microthrombi formation
Glomerular Hypertrophy/Atrophy	Changes in the size and cellularity of the glomeruli	Hypertrophy (0-3), Atrophy (0-3)	Variable, often initial hypertrophy followed by atrophy in severe or chronic cases

3.6.4 Statistical Analysis

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

CHAPTER FOUR: RESULTS

4.0 RESULTS

The effects of malaria parasite on body weight and kidney weight of Albino Wistar rats were evaluated statistically. Data were expressed as mean ± SEM, and group comparisons were assessed using one-way ANOVA. The initial and final body weights showed no significant differences across the groups ($p > 0.05$). Kidney weights were similar in groups A, B, and C (0.45 ± 0.03 to 0.45 ± 0.05 g), while group D showed a slightly lower weight (0.38 g), though this was not statistically significant ($p = 0.36$).

Table 4.1: Body and Organ Weights of Albino Wistar Rats Exposed to Malaria Parasite

Parameters	**A(Mean±SEM)**	**B(Mean ± SEM)**	**C(Mean± SEM)**	**D(Mean± SEM)**	**p-value**
Initial Weight (g)	**142.00 ± 7.14**	**135.50 ± 3.97**	**145.75±9.63**	**145.50 ± 5.72**	**0.706**
Final Weight (g)	**142.98 ± 5.97**	**129.48 ± 2.33**	**138.10 ± 10.02**	**139.55 ± 5.64**	**0.537**
Kidney (g)	**0.45 ± 0.03**	**0.45 ± 0.03**	**0.45 ± 0.05**	**0.38 ± 0.03**	**0.36**

Values are presented as mean ± Standard error of mean. Significant at $p < 0.05$ (ANOVA).

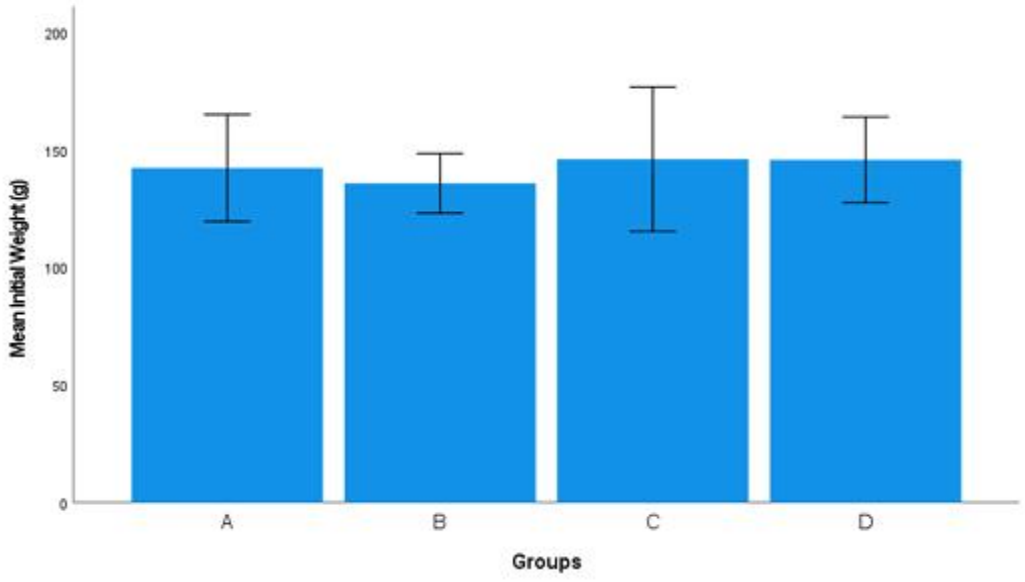


Chart 4.1 Mean initial weights of wistar albino

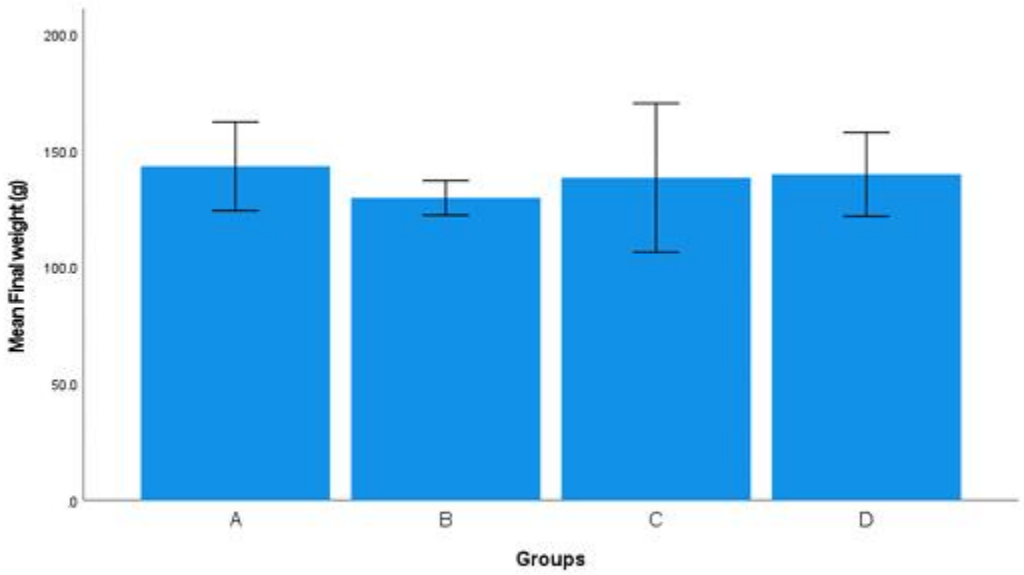


Chart 4.2: Mean final weights of albino whistar rats exposed to malaria Parasites

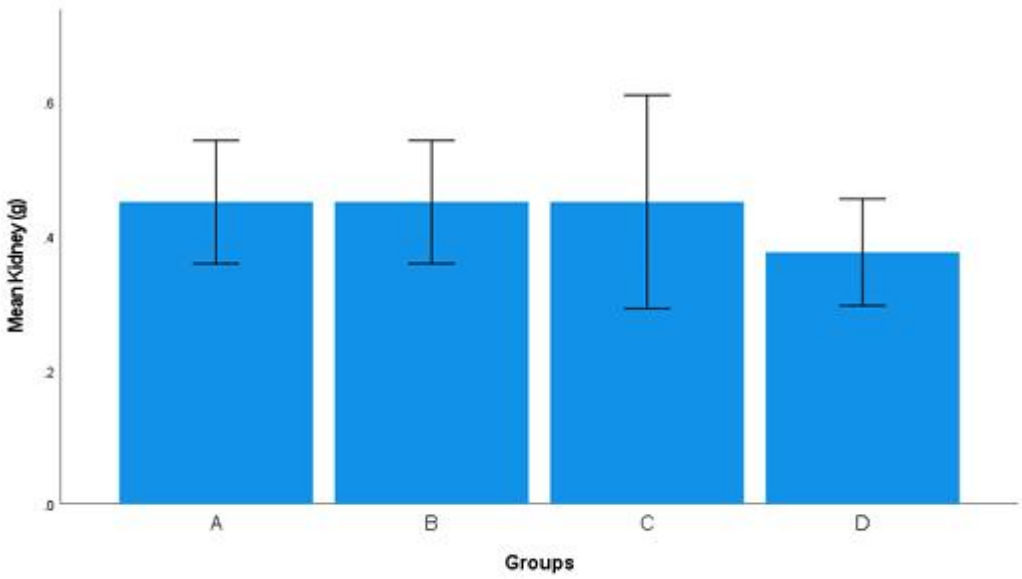
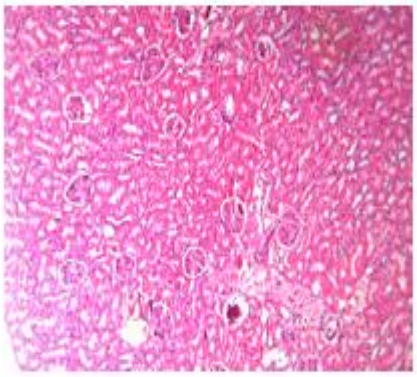


Chart 4.3: Mean kidney weights of albino whistar rats exposed to malaria Parasite

4.1 Histopathological Changes: The histopathological alterations observed in the kidneys of Albino Wistar rats following malaria parasite infection are presented in the photomicrographs below.

A1 KIDNEY X100



A1 KIDNEY X400

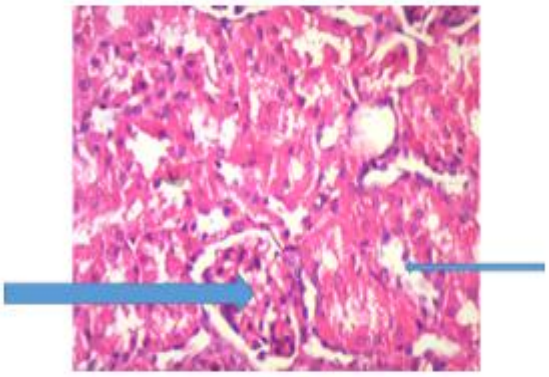
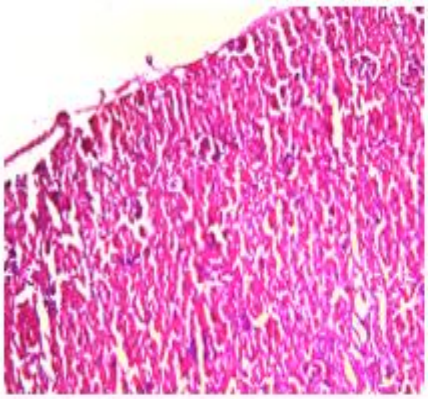


Plate 4.1 Section of the kidney from group A shows normal glomeruli (thick arrow) containing normal mesangium, blood vessels and epithelium. The tubules (thin arrow) are oval shaped and lined by cuboidal epithelium with some tubules containing pale eosinophilic material. Features are in keeping with **NORMAL KIDNEY**

B1 KIDNEY X100



B1 KIDNEY X400

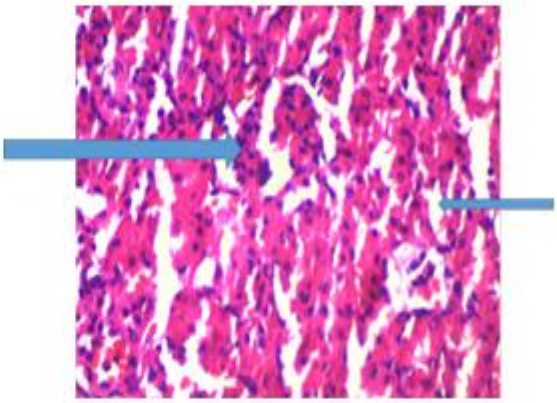
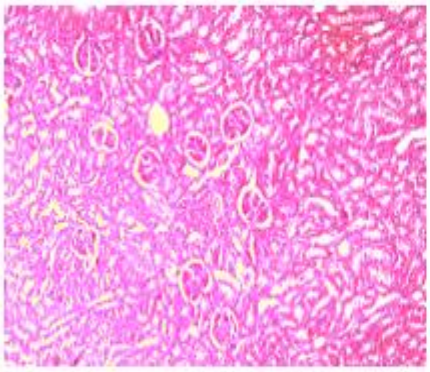


Plate 4.2 Section of the kidney from group B shows normal glomeruli (thick arrow) containing normal mesangium, blood vessels and epithelium. The tubules (thin arrow) are oval shaped and lined by cuboidal epithelium with some tubules containing pale eosinophilic material. Features are in keeping with **NORMAL KIDNEY**

C2 KIDNEY X100



C2 KIDNEY X400

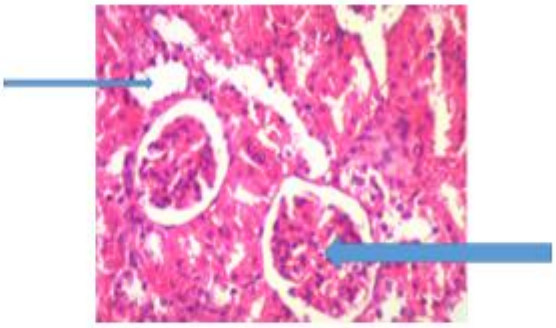


Plate 4.3 Section of the kidney from group C shows normal glomeruli (thick arrow) containing normal mesangium, blood vessels and epithelium. The tubules (thin arrow) are oval shaped and lined by cuboidal epithelium with some tubules containing pale eosinophilic material. Features are in keeping with **NORMAL KIDNEY**

D2 KIDNEY X100

D2 KIDNEY X400

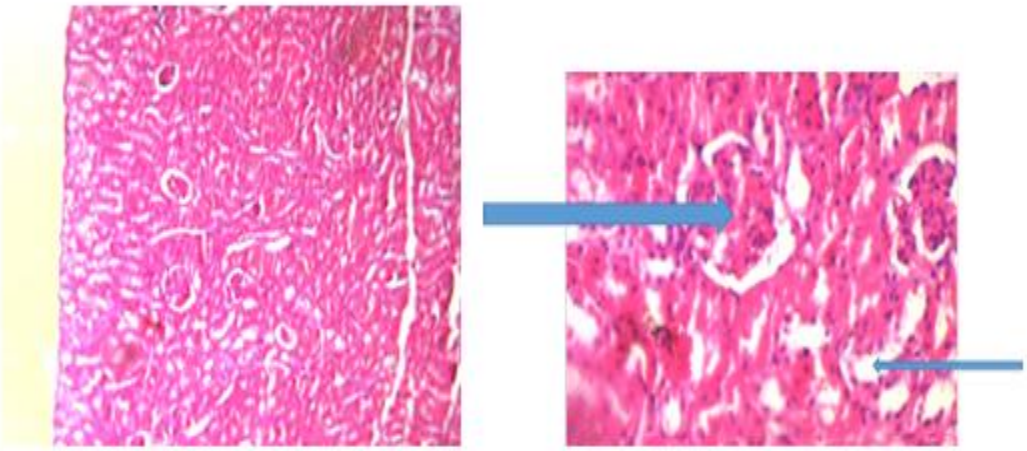


Plate 4.4 Section of the kidney from group D shows normal glomeruli (thick arrow) containing normal mesangium, blood vessels and epithelium. The tubules (thin arrow) are oval shaped and lined by cuboidal epithelium with some tubules containing pale eosinophilic material. Features are in keeping with **NORMAL KIDNEY**

CHAPTER FIVE

5.0 Summary of Findings

The effects of malaria parasite infection on the kidneys of albino Wistar rats were investigated in this study. A total of sixteen rats were divided into four groups: low infection, medium infection, high infection, and uninfected control. Plasmodium *berghei* administration was used to create parasitemia, and kidneys were harvested for histological analysis at the conclusion of the study.

Most significant findings were as follows:

Initial and final body weights did not differ significantly across the groups, as determined by statistical analysis ($p > 0.05$). However, kidney weights were similar in control, low, and medium infectioned groups, while group B (high infection) had a slightly reduced kidney weight (0.38 g) than the other groups, although this difference was not statistically significant ($p = 0.36$).

2. Histopathological Findings: Microscopic analysis showed that infections caused tubular degeneration, glomerular congestion, thickening of basement membranes, and inflammatory cell infiltration, along with various histological changes in the kidneys. In comparison to the control group, the damage was evident in the medium and high infection groups.

3. Dose-Dependent Effect: Histopathological data indicated a dose-dependent association, with higher parasite concentrations resulting in more severe kidney changes, although the relationship was not statistically significant in organ weight analysis.

5.1 Discussion of Findings

The findings of this study align with previous conclusions that are discussed in Chapter Two. According to Silva *et al.* (2017), malaria-induced renal disease might fail to show up as gross organ weight changes immediately, but it is more evident histologically. This is supported by the lack of significant weight chanPges in both body and kidney weights. Second, the observed histological alterations validate the role of malaria in renal impairment. Malaria parasites, especially P. *falciparum* and its rodent analogue P. *berghei*, are closely linked to acute kidney injury (AKI), as reviewed by Elsheikha *et al*. (2007) and Kute *et al.* (2012). This is due to mechanisms like cytoadherence of parasitized erythrocytes, immune complex deposition, cytokine production, and oxidative stress. The findings in this study, such as tubular necrosis and glomerular congestion, are in line with those mechanisms.

Third, research like Plewes *et al*. (2018) and Euclides *et al*. (2020) have shown that high parasite loads are important predictors of acute renal failure in malaria, and the dose-dependent connection indicated by the results is consistent with those findings. The study's high-infection group's more severe histological alterations support these findings.

Finally, the use of albino Wistar rats as an experimental model proved efficient, confirming previous research (Gozalo *et al*., 2024; Sato, 2021) that rodent malaria models offer dependable insights into the pathophysiology of malaria and effective treatment methods. The model's translational effectiveness was further demonstrated by the observation that, in spite of several limitations, it replicated the distinctive histological features of malaria-induced nephropathy that were reported in human analyses.

In summary, the discussion emphasises that kidney injury caused by malaria often shows up evidently at the histological level and not readily in organ weight changes. The outcomes of this investigation thus add additional evidence to the body of knowledge regarding renal impairment linked to malaria.

5.2 Conclusion

This study finds that, even when there are no appreciable changes in organ weight, malaria parasite infection induces histological abnormalities in the kidneys of albino Wistar rats. These changes—which include glomerular congestion, tubular degeneration, and inflammatory cell infiltration— demonstrate thethe susceptibility of renal tissues are to malaria-induced damage. Additionally, a dose-dependent effect is shown by the fact that the severity of these changes rises with parasite concentration. This result aligns with the larger body of research on acute kidney injury linked to malaria, where parasite burden has been found to be a significant predictor of renal outcome. Kidney involvement should be regarded as a key complication in the clinical therapy of malaria since malaria infection offers a serious danger to renal integrity.

5.3 Recommendations

5.3.1 Research Recommendations

1. Future studies should employ larger sample sizes and both molecular and biochemical indicators (such as creatinine and blood urea nitrogen) to accurately evaluate kidney damage.
2. 2. Longitudinal studies are required for understanding how kidney damage progresses over time and how easily it may be reversed with treatment.

3. 3. Advanced methods such as electron microscopy and immunohistochemistry should be employed to better understand the cellular and molecular processes behind malaria-induced kidney damage.
4. 4. Comparative studies using several Plasmodium species and host strains may increase the knowledge of species-specific renal effects.

5.3.2 Clinical Recommendations

1. Patients with malaria, especially those with high parasitemia levels, require routine renal function screening.
2. 2. To prevent acute kidney damage (AKI) from developing, clinicians should employ early diagnostic techniques to identify kidney dysfunction linked to malaria.
3. 3. In addition to antimalarial treatment, supplementary therapies that target oxidative stress and immune-mediated kidney damage should further be investigated.

5.3.3 Public Health Recommendations

1. To reduce high parasite burdens in endemic populations, malaria control efforts should prioritise preventive measures including vector control and prophylaxis.
2. Awareness of the potential of problems with renal function following malaria should be integrated into public health education initiatives to promote prompt treatment-seeking behaviour.
3. The mortality and long-term morbidity linked to malaria-induced nephropathy may be decreased by introducing renal medical evaluations into malaria care procedures.

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APPENDIX I

The instrument used for this research is as follows:

1. Animal House: during the time of feeding.

a. Weighing balance

b. Feed (pellets)

c. Plastic cage

d. ISOL disinfectant

e. Feeding flat plate

f. Feeding water troughs

g. Indian ink

2. For Sacrificing

a. Hand gloves

b. Chloroform

c. Sterile Lancets

d. Cotton wool

e. Universal containers

f. Dissenting set

g. Sterile containers

h. Formalin

3. Histology Laboratory

a. Microtome blade

b. Rotary microtome

c. Embedding machine

d. Automatic tissue processor

e. Cold plate

f. Tissue basket

g. Tissue moulds

h. Spatula

i. Water bath

j. Hot plate

k. Block holder

l. Slides and cover slips

m. Stain (Haematoxylin and eosin)

n. Pencil

o. Dibutylphthalate polysterene xylene (DPX),

p. Xylene, alcohol and water

q. Binocular microscope

APPENDIX II

PROCEDURE FOR PARAFFIN WAX EMBEDDING

I. The mould was filled with molten paraffin wax

II. Tissues were transferred from the paraffin bath to the mould using a pair of clean forceps

III. Tissues were oriented properly in the mould using a tamper.

IV. The tissue casette bearing the label (with the cap removed) was placed on the mould and extra wax was dispensed until it was properly filled.

V. It was transferred to the cold plate to allow the wax to solidify.

VI. After sufficient hardening, it was deblocked and scraped to remove excess wax.

The Rotary microtome was used for trimming at 0 microns and sectioning at 3-5 microns, 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 (Absolute alcohol, 90% alcohol and 70% alcohol respectively), for 1 minute each.

3. The slides were washed in running tap water for one minute.

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 about 15seconds.

7. The slides were blued in distilled water for 5minutes.

8. The sections were counterstained with 1% eosin for 5minutes

9. The sections were washed in running tap water for 30seconds

10. Sections were dehydrated bypassing through ascending grades of alcohol (70%, 90%, and Absolute alcohol respectively), for 1minutes each.

11. The sections were cleared in two changes of xylene for 2minutes each

12. The sections were mounted using Dibutylphthalate Polystyrene Xylene (DPX) and coverslip.

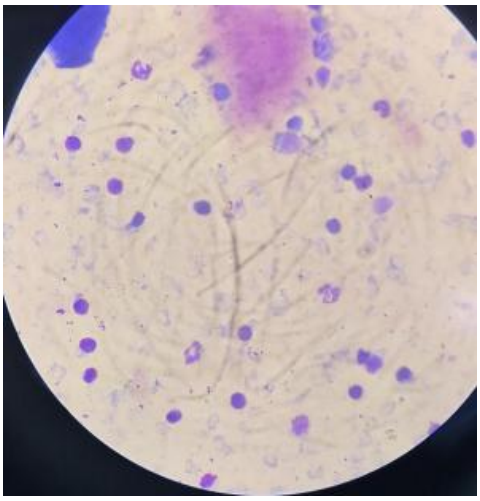
APPENDIX IV

IMAGES OF THE ALBINO WHISTAR RATS DURING THE RESEARCH

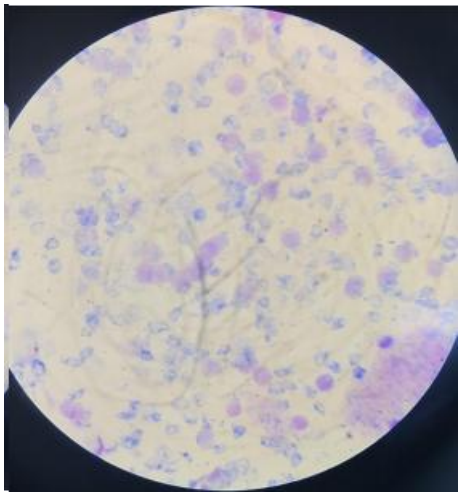
APPENDIX V

PARASITOLOGY RESULT OF MALARIA PARASITE

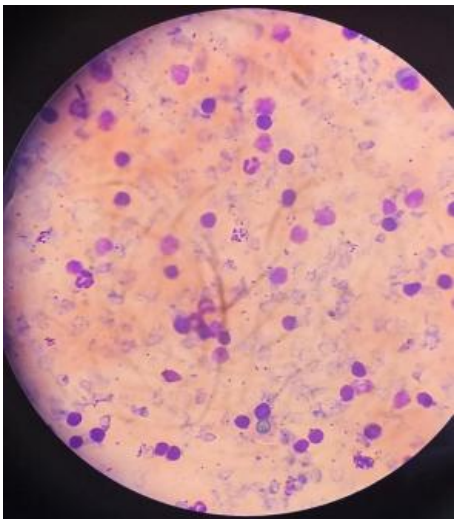
GROUP A



GROUP B



GROUP C



GROUP D