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Understanding the interplay of malarial pathogenesis, host immune response and oxidative stress: Implications for disease progression and therapeutic strategies

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ABSTRACT

Despite sustained efforts, malaria elimination in developing countries, particularly in Africa, remains a to be a public burden due to the evolution and emergence of resistance to most of the currently available antimalarials and insecticides. Over time, it has been argued that a thorough understanding of the parasite's biology and pathogenesis is important because it arises from a dynamic interplay between the host and the parasite. The lifecycle of the malarial parasite is complex, involving distinct developmental stages that each express specific antigens, which in turn trigger the immune system to either protect or promote pathophysiology. Malaria pathogenesis is thus a complex interplay of *Plasmodum*-induced red blood cell alterations and microvascular irregularities that lead to clinical symptoms and disease severity. Immune activation during malarial infection triggers a robust production of reactive oxygen and nitrogen species (ROS/RNS), contributing to oxidative stress, a characteristic seen during malarial infection and believed to exacerbate malarial pathophysiology. Therefore, this manuscript will examine the cellular mechanism underlying malarial pathophysiology, zoom in on oxidative stress, how it is linked to malarial severity and pathophysiology, and how it could be targeted to ameliorate ROS-mediated associated complications in malaria.

1. Introduction

Malaria is a severe and fatal disease that dates to early historical times and is defined as a hematological disease that causes the hemolysis of red blood cells (RBCs), thereby leading to the manifestation of complicated or uncomplicated clinical symptoms (Nureye and Assefa, 2020). This vector-borne disease is caused by a unicellular protozoan parasite belonging to the genus *Plasmodium* and is transmitted by the bite of an infected *Anopheles* female mosquito. About 200 *Plasmodium* species have been reported and documented, each affecting a certain range of hosts. Only five have been reported to infect humans: *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale*, and *P. knowlesi* (Sato, 2021; Milner Jr et al., 2014), with the latter also known as zoonotic malaria due to its ability to infect macaque monkeys widely distributed in Southeast Asia (Sato, 2021; CDC, 2022). Malaria caused by *P. falciparum* is the most severe form of the disease, resulting in complex and complicated symptoms that are often associated with high parasitemia and intense hemolysis due to the frequent replication and division of the parasite after red blood cell invasion, which, when not treated results in death promptly (CDC, 2022; Mojab, 2012; Ravindar et al., 2023). *P. vivax,* though overshadowed by *P. falciparum*, is still a noteworthy parasite that continues to cause mortality and morbidity (Geleta and Ketema, 2016; Opoku et al., 2019)

Globally, more than half of the world's population is at risk of being affected and infected by this disease, with over 100 countries being affected and about 200 million clinical cases and over 500 000 deaths confirmed yearly (Mawson, 2013; Percário et al., 2012). In 2023 alone, 263 million clinical cases and 579 000 deaths globally were attributed to

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malaria, with most of the cases and deaths occurring in the African WHO regions, representing 11 million more cases when compared to the cases reported in 2022 (WHO, 2024). Malaria continues to be a devastating public health burden to humans, posing a threat to clinical health and the economy due to its widespread prevalence in developing countries and regions, thereby restricting socio-economic development (Qin et al., 2020; Sato, 2021). The disease mostly affects those residing in developing countries, particularly those in rural areas, as they have limited access to healthcare. Pregnant women, children, and people with compromised immune systems are the most susceptible to the disease and are, therefore, frequently infected (Del Prado et al., 2014). Zekar and Sharman (2022) state that children under the age of five are the most vulnerable and susceptible of the group as they have an underdeveloped immune system. As such, the disease claims a child's life every 30 s. In 2023, 76 % of all the reported deaths associated with malaria were accounted for by children under the age of five (WHO, 2024).

Despite the vigorous strides and efforts aimed at malarial elimination and eradication by government and non-governmental organizations, the disease continues to strive (Opoku et al., 2019; Badmos et al., 2021; Ribeiro et al., 2023a). This is largely attributed to the development and emergence of resistance against available antimalarial drugs (chloroquine and artemisinin) and insecticides, leading to an alarming increase in reported malarial clinical cases and deaths (Opoku et al., 2019; Percário et al., 2012; Mubaraki et al., 2017). This alarming increase in the number of reported cases of resistance has, therefore, necessitated the need to find alternative strategies to circumvent the disease, including the use of sulfur, triazole, chlorine, and 4-aminoquinoline--based scaffolds and hybrids, which has been shown to improve antimalarial drug potency and the propensity to overcome resistance, development of safe and effective antimalarial drugs and effective vaccines (Ravindar et al., 2023a; Ravindar et al., 2023b; Zhao et al., 2019; Fang et al., 2019; Nyaba et al., 2018; Ribeiro et al., 2023). Moreover, Mawson (2013) and Zininga et al. (2015) state that an improved and thorough understanding of the parasites' biology and pathogenesis is imperative in developing new or alternative therapeutic options considering the emergence of antimalarial drug resistance.

1.1. *Malaria pathogenesis*

The disease begins when an infected *Anopheles* mosquito takes a blood meal from the human host and inoculates the *Plasmodium* parasite in the form of sporozoites, which reside on the skin for 4–6 h (Fig. 1). These are then activated into a state of readiness through which they enter the blood circulatory system of the host and migrate to the liver (Venugopal et al., 2020). They then invade and infect the hepatocytes using the cholesterol uptake pathway and begin to differentiate and multiply asexually for 7–10 days, during which the symptoms are not pronounced or evident (Cowman and Crabb, 2006; Yamauchi et al., 2007; Mawson, 2013). Following this period, the sporozoites emerge from the hepatocytes as merozoites encapsulated in vesicles and travel through the heart to the lungs' capillaries (Mawson, 2013). Once the vesicles burst, the released merozoites enter the blood circulatory system to infect red blood cells and multiply, marking the beginning of the parasite's blood stage (Mawson, 2013; Siciliano and Alano, 2015; Venugopal et al., 2020). As the red blood cells hemolyze and burst, more red blood cells are infected and invaded by the parasite (Siciliano and Alano, 2015).

The clinical symptoms presented by patients infected with malaria are induced by the asexual stage of the parasite that develops inside red blood cells (Buffet et al., 2011; CDC, 2025). Mawson (2013) stipulates that the manifestation of clinical symptoms results from the hemolysis of red blood cells and the release of malarial toxins. This is because as red blood cells burst, malarial toxins and debris, including hemozoin, glycophosphatidylinositol, and putative malarial toxins, are released. As shown in Fig. 1, these trigger the immune system and other pathological processes leading to the manifestation of clinical symptoms such as anaemia, headache, respiratory distress, metabolic acidosis, and, in severe cases, coma, which occurs as a result of increased intracranial

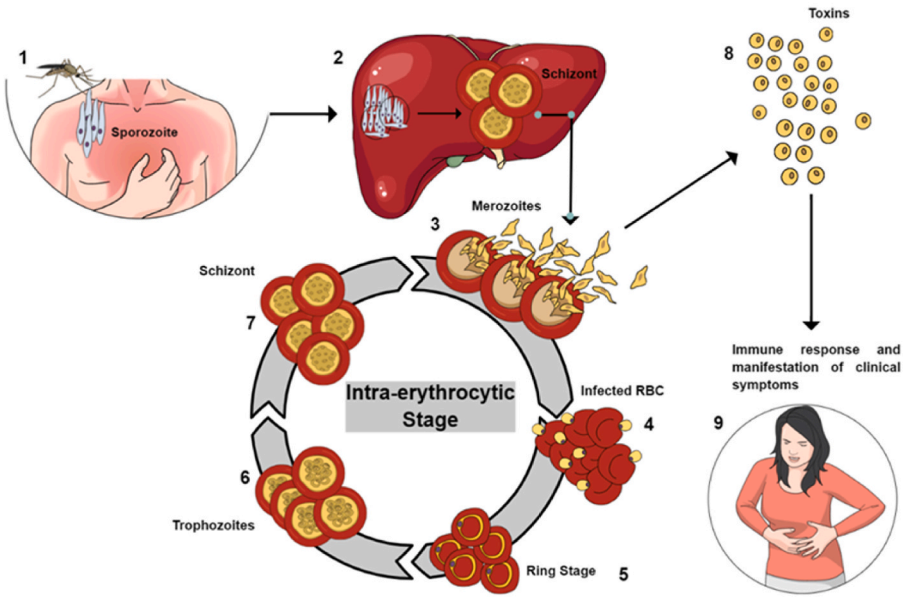

Fig. 1. Malaria life cycle and disease pathogenesis. After an infected *Anopheles* mosquito takes a blood meal from a human host, it inoculates the infective form of the parasite (sporozoites) (1) that travels into the liver, where it infects the hepatocytes. These then mature into schizonts, housing multiple merozoites (2). After this, the schizont bursts open, releasing thousands of merozoites (3) that attack and infect red blood cells (4), marking the beginning of the intra-erythrocytic stage, which is responsible for the manifestation of the clinical symptoms (9) associated with malaria. Once the merozoites infect the red blood cells, they enter a stage of reproduction and develop into a ring stage (5), trophozoites (6), and schizonts (7). The schizonts then burst open, releasing thousands of new merozoites that infect more red blood cells (3). Toxins such as the insoluble hemozoin, glycophosphatidylinositol, and putative malarial toxins (8) are released and stimulate the immune response, activating inflammatory signalling pathways and manifesting clinical symptoms (9). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

pressure (Clark and Cowden, 2003; Mawson, 2013; CDC, 2025). The pathogenesis of malaria is, therefore, a complex interplay of parasite-induced red blood cell modifications and microcirculatory abnormalities followed by local and systematic immune responses that manifest clinical symptoms and disease severity.

Malarial parasites have the unique ability to instigate enormous changes in host cells, and this includes loss of the normal biconcave shape of red blood cells, increased rigidity of the plasma membrane and permeability, allowing a wide spectrum of ions and other substances in and out of the cells (Artavanis-Tsakonas et al., 2003; Percário et al., 2012; Mohandas and An, 2012). Many interactions between the red blood cells and the parasite occur from when the parasite invades red blood cells to the intra-erythrocytic stage, where approximately 400 proteins encoded by the parasite are exported into the cytoplasm (Mohandas and An, 2012). According to Mohandas and An (2012), once the parasite has invaded the red blood cells, structural, biochemical, and functional modifications occur in these cells. These induced cellular and membrane changes result in the manifestation of clinical symptoms. Anaemia, a serious pathological condition, is one of the implications of malaria caused by the parasite changing adhesive and natural properties of the red blood cells, thereby leading to membrane changes and modifications, destruction of infected and uninfected erythrocytes, as well as ineffective erythropoiesis (Artavanis-Tsakonas et al., 2003; Mohandas and An, 2012). Other examples include offsetting cerebral malaria, which occurs due to the sequestration of infected erythrocytes in the microvasculature (Percário et al., 2012; Gomes et al., 2022; Venugopal et al., 2020; CDC, 2025).

1.2. Immune response during malarial infection

As is true for different infections, including malaria, the immune system is elicited to provide protection and resistance against infection-causing pathogens and toxins. Evidence suggests that the immune system plays an antiparasitic role against the *Plasmodium* parasite in all developmental stages and can also prevent and protect against malarial infection. However, the biochemical processes remain unclear (Long and Zavala, 2017). As such, the complexity of the physiology of the parasite has made it difficult to develop effective vaccines and immunotherapies against malaria due to the different developmental stages that express unique antigens (Nureye and Assefa, 2020; Belachew, 2018; Malaguarnera and Musumeci, 2002; Venugopal et al., 2020). Long and Zavala (2017) state that the antigens and regions of these proteins can trigger a robust immune response that can hinder the sporozoites seen in the liver stage of the parasite as well as the merozoites seen in the blood stage to facilitate protection against the parasite (Fig. 2). There have been contrasting ideas surrounding the immune system's response during a malarial infection to either play a protective role against the parasite or contribute to pathophysiology and disease progression (Long and Zavala, 2017; Malaguarnera and Musumeci, 2002). Evaluation of these conflicting ideas has been complex due to the complexity of the immune response, which includes an array of signals that target specific developmental stages of the parasite.

After infection, the innate immune response is elicited as the first line of defense, followed by the adaptive immune response that uses B-cells, T-cells, and antibody production within the liver and red blood stages (Long and Zavala, 2017). Infection with the parasite in the pre-blood (liver stage) or blood stage is sensed by different receptors presented by the host immune cells, resulting in an array of signalling pathways and the production of cytokines and chemokines to clear the parasite and regulate adaptive immunity (Fig. 2) (Gowda and Wu, 2018). The host system responds to different pathological diseases by recognizing conserved molecular structures that are pathogen-derived, such as the pathogen-associated molecular patterns (PAMPs) and other endogenous structures or factors produced during the infection period called danger-associated molecular patterns (DAMPs) (Gowda and Wu, 2018). The innate immune system is activated when these PAMPs and DAMPs are recognized and detected by pattern recognition receptors (PRRs) such as Toll-like receptors (TLRs), c-type lectin receptors (CLRs), and scavenger receptors (SRs). As shown in Fig. 2, this leads to the activation of signalling pathways that produce pro-inflammatory cytokines and chemokines, including tumour necrosis factor-α (TNF-α) and interleukin-1B (IL-1B), to help fight invading pathogens and infection. However, this can also lead to disease pathogenesis (Milner Jr et al., 2014; Gowda and Wu, 2018; Amorim et al., 2016; Parroche et al., 2007). Malaria is an inflammatory-driven disease; therefore, protecting the host or progression of the parasite solely depends on regulating the immune system. On the one hand, the innate immune system responds by producing a pro-inflammatory response beneficial to the host by clearing the parasite and infection. Still, a prolonged pro-inflammatory response is just as lethal as it promotes parasite pathogenesis and disease progression (Fig. 2) (Gowda and Wu, 2018; Deroost et al., 2015).

In the liver stage, the immune system targets free sporozoites and infected hepatocytes by producing antibodies that target these sporozoites and other proteins like the circumsporozoite (CPS), a protein found on the surface of the protozoan sporozoite that is important for the invasion of red blood cells. This is important as, by so doing, the immune system prevents the invasion of the hepatocytes (Long and Zavala, 2017). A study conducted by Persson and colleagues (2002) reported the production of anti-CSP antibodies within the body that protect against malaria by shedding CSP and inhibiting the infection of hepatocytes. However, these are frequently produced in individuals older than 50, while they are produced in minute amounts in children. In the liver stage of the parasite, the parasitized hepatocytes produce type-1 interferon (IFN) by sensing parasite cytosolic RNA, one of the parasite's associated molecular patterns recognized by host cells (Fig. 2) (Gowda and Wu, 2018). This type of cytokine production results in the death of parasitized hepatocytes that expose their constituents, further eliciting the immune response. Dendritic cells (DCs) and inflammatory macrophage (M φ s) cells also recognize and detect these factors, including sporozoites, which in turn stimulate toll-like receptors and inflammasome-mediated immune response (Belachew, 2018; Gowda and Wu, 2018). The immune system also activates complement fixation and phagocytosis while promoting the lysis of the infected hepatocytes using cytotoxic natural killer (NK) cells and natural killer T (NKT) cells (Belachew, 2018). $CD8^+$ T cells are also implicated in killing intrahepatic/liver parasites using type-1 interferon (IFN-γ) and other immune cells such as NK, NKT, and gamma delta T (γδT) cells (Fig. 2) (Belachew, 2018; Dinko and Pradel, 2016). Type-1 interferon is particularly important as it stimulates the production of chemokines by the hepatocytes, IFN-γ, and chemokines by NK and NKT cells, which are abundant in the liver, as well as the production of chemotaxis-mediated recruitment of M φ s, neutrophils, and lymphocytes to the site of infection (Gowda and Wu, 2018).

The second stage is the blood or intra-erythrocytic stage, which follows the merozoites' invasion of the red blood cells. In this stage, the immune system targets merozoites by producing antibodies and T cells (Fig. 2) (Belachew, 2018). The immune system is mainly mediated by antibodies in the blood stage, while the cell-mediated immune response predominates in the liver/pre-erythrocytic stage of the parasite (da Costa Lima Junior and Pratt-Riccio, 2016). Antibodies produced in this phase opsonize the merozoites, allowing them to be phagocytized, thereby inhibiting their invasion into the red blood cells (Belachew, 2018). However, they also aid in killing and blocking infected red blood cell adhesion to the endothelium and neutralizing the toxins produced by the *Plasmodium* parasite to prevent a heightened inflammatory response (da Costa Lima Junior and Pratt-Riccio, 2016). This stage of malaria is known as the pro-inflammatory cytokine response that activates macrophages, which mediate the phagocytosis of parasites and immunomodulation, as well as the production of various cytokines and growth factors. These macrophages also play a critical role in initiating, maintaining, and resolving inflammation (Fujiwara and Kobayashi, 2005; Dinko and Pradel, 2016; da Costa Lima Junior and Pratt-Riccio,

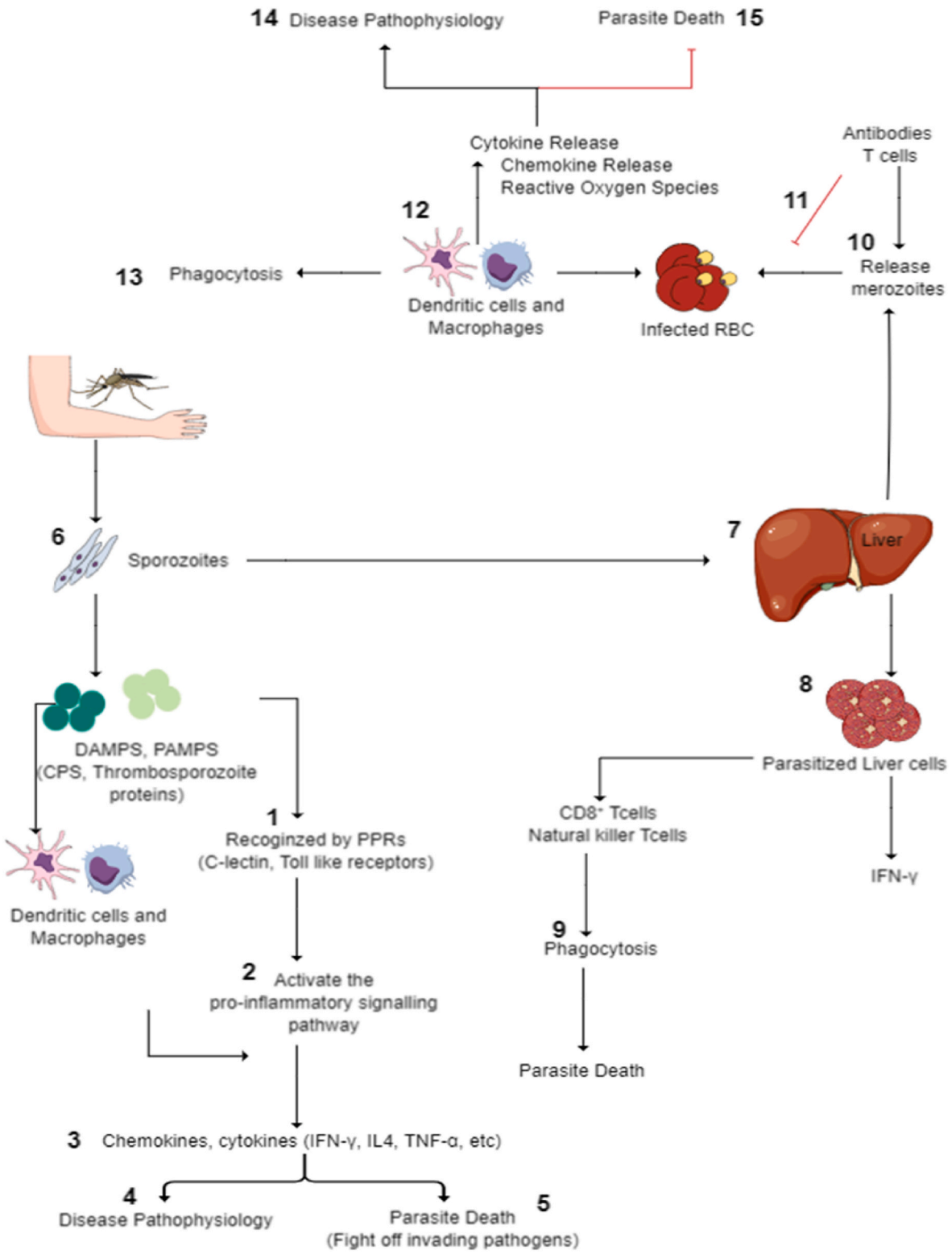

Fig. 2. Immune response to malarial infection. Pathogen-associated molecular patterns are recognized by pattern-recognizing receptors (PPRs) (1) after infection, and these respond by activating pro-inflammatory signalling pathways (2), which in turn respond by producing inflammatory cytokines and chemokines (3) that help kill the parasite (4) while promoting disease pathogenesis (5). Immune cells also recognize sporozoites (6) and respond by activating the inflammatory signalling pathway (2). However, some sporozoites (6) evade the immune system and infect the liver (7). The parasitized liver cells (8) are also recognized by immune cells and respond to this by mediating phagocytosis (9). Within the liver, the sporozoites mature and develop into schizonts that burst open, releasing merozoites (10) recognized by T-cells to prevent the invasion of the red blood cells (11). However, immune cells such as macrophages, dendritic cells, and neutrophils (12) also recognize the infected red blood cells, which respond by phagocytizing the cells (13). The activation and generation of reactive oxygen species (ROS) and pro-inflammatory cytokines lead to malaria disease pathophysiology (14) while simultaneously killing the parasite (15). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

2016). CD4^{+} T helper cells also play a significant role in this stage by producing pro-inflammatory cytokines that activate macrophages. During the blood stage of the infection, the parasite grows rapidly by reproducing in the red blood cells, leading to the induction and stimulation of the innate immune system response. Inflammatory M φ s and Dendritic cells (DCs) are then produced as the first line of defence and control of the parasite through phagocytosis (Stevenson and Riley, 2004).

As shown in Fig. 2, DCs produce cytokines (IFN-γ, TNF-α, IL-12, and IL-6) and chemokines (CXCL1, CXCL2, CCL2, CCL5, CXCL9, and CXCL10) in response to the *Plasmodium* parasite and interact with cells of the innate and adaptive immune system to clear the parasite, while simultaneously promoting disease pathogenesis (Gowda and Wu, 2018; Gotz et al., 2017; Trinchieri, 2010). Type 1 IFN primes the DCs to produce cytokines and chemokines and to activate NK, NKT, γδT, and T cells to induce IFN-γ, which promotes the pro-inflammatory Th1 response and effector T cell response (Trinchieri, 2010). The increased production of IFN-γ plays a role in controlling parasitemia and also primes M φ s and neutrophils to increase their phagocytic activity, thus decreasing the parasite burden (Gowda and Wu, 2018; Trinchieri, 2010). Although this provides a form of disease control, IFN-γ contributes to cerebral malaria and other pathologies associated with the disease, suggesting that the immune system protects against the disease but can also facilitate malarial pathogenesis (Gowda and Wu, 2018). Therefore, as illustrated in Fig. 2, the immune system's response to foreign pathogens includes producing highly reactive oxygen species (ROS), which are meant to kill invading pathogens, including malaria. However, excessive production of these ROS can lead to oxidative stress and damage to host tissues, causing bodily harm and contributing to disease pathophysiology (Marri and Richner, 2015; Narsaria et al., 2012).

1.3. Oxidative stress in malaria

As earlier stated, the immune system responds to malarial infection by activating phagocytic cells, amongst other immune cells; these respond by engaging in a respiratory burst, promoting free radical production and the subsequent generation of reactive oxygen and nitrogen species (ROS/RNS) that elicit oxidative stress (Fig. 3) (Percário et al., 2012; Gomes et al., 2022; Babalola et al., 2022). Oxidative stress is a phenomenon that results from a homeostatic imbalance in the production and accumulation of reactive oxygen species (ROS) in cells and tissues and is characterized by an imbalance between pro-oxidants and antioxidants in which the former is greater than the latter (Mylonas and Kouretas, 1999; Lavender, 1993; Yoshikawa and Naito, 2002; Pabon et al., 2003; Szyller and Bil-Lula, 2021). The key role of oxidative stress during malaria is still not well elucidated as it is not known whether it plays a protective role against the parasite or infection or functions in the pathophysiology of malaria (Pabon et al., 2003; Percário et al., 2012). As a defence mechanism, it is important in infection clearance as it has been shown that the *Plasmodium* parasite is susceptible and vulnerable to ROS (Dockrell and Playfair, 1984; Sobolewski et al., 2005). However, an accumulation of ROS and RNS at high levels can cause immense damage to a living system, especially in the vascular lining and blood-brain barrier. Still, at low to moderate levels, these have significant roles in the cells, including acting as secondary messengers in signal transduction, supporting immune system defence, combating bacterial infections, and regulating vascular tone (Ikwegbue et al., 2017; Postma et al., 1996; Vasquez et al., 2021). Therefore,

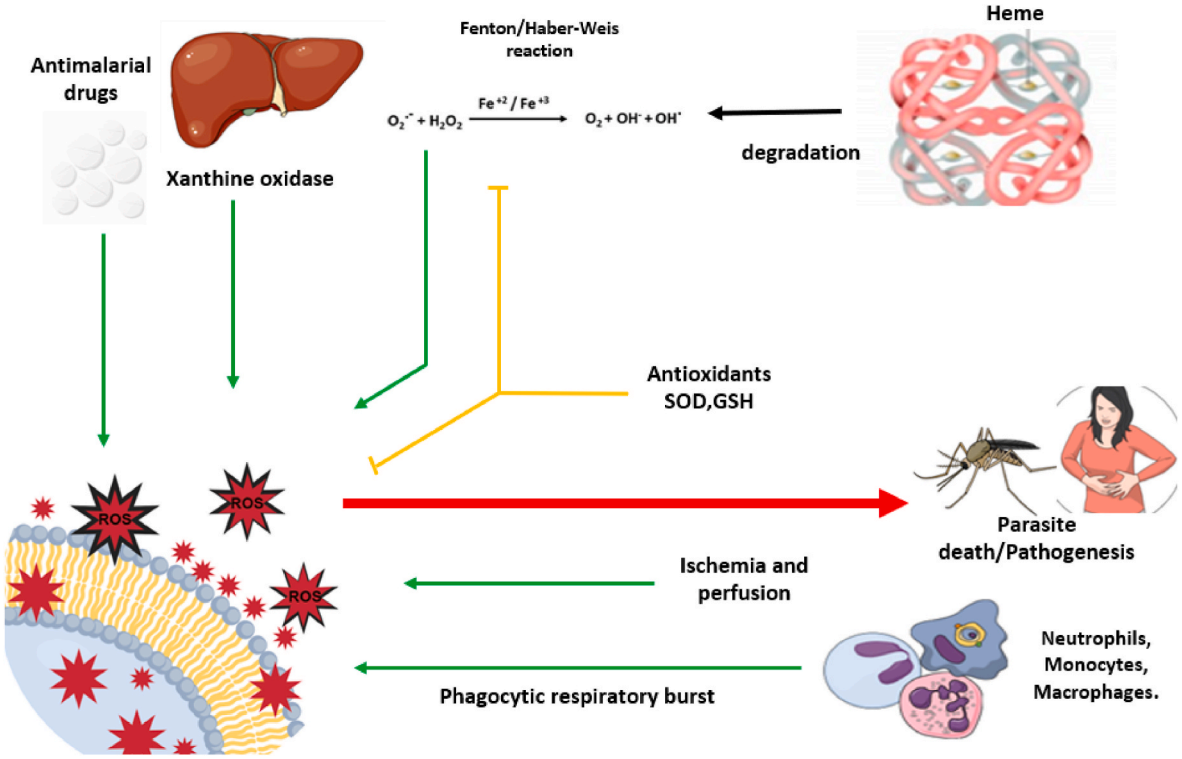

Fig. 3. Oxidative Stress during malaria infection. Various sources contribute to generating reactive oxygen and nitrogen species after malarial infection. This includes the respiratory burst by immune system cells in the form of macrophages and neutrophils that occur after the phagocytosis of infected/parasitized red blood cells; heme released during the catabolism of haemoglobin and free iron (Fe^{2+}) via the Fenton-Haber-Weiss reaction; use of antimalarial drugs, upregulation of xanthine oxidase and ischemia-reperfusion. However, the human system makes use of antioxidant enzymes such as glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD) to counteract the production of ROS and create a reduced environment, but this often falls short as the levels of produced ROS are often higher than the activity of these enzymes leading to oxidative stress which kills the parasite and promote malaria pathogenesis. Inflammation is also promoted by releasing pro-inflammatory cytokines and chemokines, leading to malaria pathogenesis. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

oxidative stress's beneficial or pathological role in the disease is highly dependent on the amount of ROS produced and where they are produced, making the role of oxidative stress during infections multicomplex (Gomes et al., 2022; Postma et al., 1996; Vasquez et al., 2021; Becker et al., 2004).

According to Percário et al. (2012), the sources of oxidative stress during malarial come from at least five sources, which include the inflammatory processes that are initiated by the host immune system in response to infections and pathogens, transition metal catalysis, the occurrence of ischemia-reperfusion syndrome that occurs from cytoadherence processes and anaemia, synthesis of the reactive oxygen species by the parasite to aid its internalization into red blood cells, and the action of widely used antimalarial drugs such as quinolines (chloroquine and mefloquine) which aim to inhibit polymerization and conversion of heme to an insoluble green pigment (hemozoin) (Wongtrakul et al., 2010). Therefore, given the above, oxidative stress can originate from the parasite, the host, or from antimalarial drugs affecting both the host and parasite.

1.4. Parasite-induced oxidative stress

During the blood stage, the *Plasmodium* parasite proteolytically degrades haemoglobin using aspartic and cysteine proteolytic enzymes inside the food vacuole; this produces active redox by-products, which causes oxidative stress to the host cells and the parasite (Becker et al., 2004; Guha et al., 2006; Goldberg and Slater, 1992). Thus, central to the generation of oxidative stress is the degradation of haemoglobin in the erythrocytic stage by the *Plasmodium* parasite as it sources amino acids for its nutrition (Henriques and de Domínguez, 2012). This is necessary as it creates space for the parasite to grow and maintain the osmotic integrity of the infected red blood cells (Lew et al., 2003). However, the degradation and catabolism of haemoglobin come with adverse effects that induce oxidative stress (Fig. 3). The degradation processes in the acidic vacuole of the parasite result in the production of free circulating heme (ferroporphyrin IX), which is a powerful free radical generator that causes cellular and molecular damage in both the parasite and the host (Percário et al., 2012).

1.5. Heme-derived oxidative stress

As indicated in Fig. 3, heme has Fe^{2+}-associated groups that induce oxidative stress by generating ROS via the Fenton and Haber-Weiss reaction (Percário et al., 2012; Becker et al., 2004). The Fe^{2+} of the heme group reacts in the presence of H_2O_2, oxidizing the iron and producing free radicals such as hydroxyl radical (OH^-) and superoxide (O_2^-) (Becker et al., 2004; Guha et al., 2006; Burda et al., 2020). When this occurs, the host increases the expression of superoxide dismutase (an antioxidant enzyme) to try and detoxify the superoxide radical, generating even more H_2O_2 and OH^- radicals (Sohail et al., 2007). The OH^--radicals mediate the killing of the parasite but can also cause damage to the host cells by rapidly reacting with DNA, lipids, and proteins, inducing cellular and molecular damage (Percário et al., 2012; Postma et al., 1996). H_2O_2 has displayed antiplasmodial activity *in vivo* in *P. yoelli* and *P. chabaudi-infected* mice subjected to intravenous injection with H_2O_2, proving that oxidative stress is detrimental to the parasite, making heme dangerous to both the parasite and the host (Dockrell and Playfair, 1984).

Therefore, both species need to find a way of neutralizing or detoxifying heme. In the mammalian system, heme is primarily produced when red blood cells burst and are detoxified via the hemopexin-mediated heme oxygenase pathway, producing biliverdin, carbon monoxide, and free iron (Guha et al., 2006). Simultaneously, the *Plasmodium* parasite converts heme into an insoluble pigment called hemozoin in biomineralization (Becker et al., 2004). However, not all the heme is converted into the insoluble pigment, and this is because some of it escapes and diffuses into the cytosol of the parasite, where it inhibits parasite enzymes, aids in the lysis of red blood cells oxidative damage, as well as stimulates inflammation thus exacerbating malarial severity (Percário et al., 2012; Becker et al., 2004). The parasite detoxifies heme that escapes crystallization to hemozoin using Glutathione (GSH), which provides free iron, likely to participate in the Fenton and Haber-Weiss reactions. Another protective mechanism against heme is the use of heme-binding proteins that protect the parasite against the toxic effects of heme (Becker et al., 2004). Even though hemozoin is not toxic to the parasite, when produced during the haemolysis of red blood cells, it can stimulate the release of cytokines such as TNF-α and IL-1 via the monocyte and macrophage system and generate ROS such as Nitric oxide (Percário et al., 2012; Moore et al., 2004). Released hemozoin may also be deposited in the liver, acting as a pro-oxidant that may cause and lead to liver damage (Guha et al., 2006). Interestingly, the *Plasmodium* parasite does not only contribute to oxidative stress via heme and hemozoin production, but the parasite itself can also produce ROS. However, there needs to be more studies in this regard. It has, nonetheless, been hypothesized that this may occur through aerobic membrane transport mechanisms, which leads to alterations in red blood cells, facilitating the entry of the parasite into them and hepatocytes (Percário et al., 2012).

1.6. Host-induced oxidative stress

1.6.1. Xanthine oxidase

Xanthine oxidase (XO), an unimolecular homodimeric enzyme with a molecular weight of 270 kDa, is a potent source that exacerbates the oxidative stress seen during malarial infection (Fig. 3). This purine catabolizing enzyme facilitates the oxidation of hypoxanthine to xanthine and xanthine to uric acid with subsequent generation of H_2O_2 and O_2^- (Schmidt et al., 2019). Physiologically, XO is a resident enzyme found mainly in the liver, intestinal epithelium, and vascular endothelium, where it attaches via the sulfated glycosaminoglycan, making its presence in the serum very rare (Iwalokun et al., 2006; Battelli et al., 2001). However, it has been reported that the enzyme is highly elevated and found in high concentrations in hemolytic diseases such as sickle cell disease, sepsis, and malaria (Schmidt et al., 2019). Elevated levels of this enzyme have been observed in several *Plasmodium* studies, especially in patients suffering from cerebral and severe malaria, suggesting that this enzyme contributes significantly to the pathophysiology of this disease (Vasquez et al., 2021; Ty et al., 2019).

The elevation of XO has been attributed to lysis of the hepatocytes with high concentrations reported in the serum of patients with liver disease and hepatic injuries such as chronic hepatitis, jaundice, and cholestatic disorders than in healthy controls (Iwalokun et al., 2006; Pacher et al., 2006). However, no correlation between the high concentration of XO and tissue damage has been seen, suggesting that the enzyme is released during hepatic damage and lysis and does not induce the liver diseases mentioned above. Hypoxia, a condition characterized by low oxygen levels and seen during malarial infection and induced by red blood cell destruction, is another possible cause of the elevation of XO. This is because hypoxia upregulates and modulates Xanthine oxidoreductase, a term used to encompass two interconvertible enzymes, Xanthine dehydrogenase (XDH) and Xanthine oxidase (XO) (Pacher et al., 2006). Hypoxia elevates XO by increasing hypoxanthine production via the purine degradation pathway, where ATP is broken down to hypoxanthine. Oxidation of XO substrates increases the oxidative burden and subsequent production of ROS in the host (Fig. 3), which is detrimental to cells and the body (Schmidt et al., 2019; Parks et al., 1983; Berman et al., 1991). Shuttling electrons are produced during the oxidation of purines to reduce oxygen to H_2O_2 and O_2^-, resulting in the formation of reactive nitrogen species (RNS) such as peroxynitrite anion ($ONOO^-$) via diffusion-limited reactions between the O_2^- derived from XO and NO (Schmidt et al., 2019; Radi et al., 1991; Beckman et al., 1990). The formation of $ONOO^-$, a stable and potent oxidant, leads to the oxidation of nonproteins and non-sulfhydryl

proteins (Schmidt et al., 2019; Radi et al., 1991; Beckman et al., 1990). This has been shown to induce alterations in the cell signalling pathways, loss of integrity in the endothelial barrier, disrupting vascular homeostasis and subsequent vascular dysfunction (Schmidt et al., 2019; Radi et al., 1991; Beckman et al., 1990; Osarogiagbon et al., 2000). Hydroxyl (OH^-) is another powerful free radical that is produced during the oxidation of purines by XO via the Fenton-Haber reaction, where O_2^- oxidizes Fe^{2+} to Fe^{3+}, which ultimately reduces H_2O_2 to OH^-, a free radical that induces cellular damage, mutations, and cell death (Beckman et al., 1990).

XO can be seen as a factor that aids in the severity of malaria by producing ROS and other reactive oxygen intermediates (ROIs), which are implicated in the pathogenesis of malaria by having pathogenic roles and eliciting inflammatory signalling pathways that aid in the pathogenesis of malaria (Schmidt et al., 2019; Iwalokun et al., 2006; Ty et al., 2019). This is because the ROS produced during the oxidation of purines by XO has been shown to induce a strong inflammatory cytokine response characterized by an increase in the cytokine IL-1B, which increases the number of circulating neutrophils and heightens inflammatory events (Schmidt et al., 2019; Ty et al., 2019). Moreover, XO has also been reported to play a protective role either through the direct or indirect use of free radicals and does so by modulating parasitemia, arresting parasite growth, or killing the parasite (Fig. 3) (Iwalokun et al., 2006). When incubated with XO, Berman and colleagues (1991) showed that XO inhibited the parasite's growth and attributed this to the ability of the enzyme to oxidize purines and hindering nucleic acid synthesis. This is because the *Plasmodium* parasite is a purine auxotroph, i.e., it cannot synthesize its own purines *de novo* and relies solely on the purine salvage pathway, which, when blocked, leads to death or suppressed growth (Berman et al., 1991; de Koning et al., 2005; Tewari et al., 2019).

On the other hand, XO-induced ROS has also been implicated in killing the parasite since it is susceptible to ROS (Fig. 3) (Vasquez et al., 2021; Greve et al., 1999). A study by Dockrell and Playfair (1983) showed that OH^- decreases parasitemia *in vivo* in mice infected with *P. yoelii* and *P. chabaudi*. The death of *P. yoelii* and *P. berghei* parasites *in vitro* was also observed. Later, pre-incubation of the *P. yoelii* parasite in the presence of XO and subsequent infection of mice with the parasite showed a stark decrease in replication and low-parasitemia in comparison to mice infected with untreated parasites, concluding that XO-derived ROS tempers with the survival of malaria parasites (Dockrell and Playfair, 1983).

1.6.2. Phagocytic respiratory burst

As previously mentioned, upon the invasion of human host cells by the *Plasmodium* parasite or any other pathogen, the body responds by mounting a complex network of immune responses to curb and eliminate the invading pathogen and subsequent infections. This includes using immune cells that help fight the invading pathogens through phagocytosis, a process that involves the cellular uptake of foreign particles and their subsequent destruction (Fig. 3) (Chua et al., 2021). This process is executed by neutrophils, monocytes, and macrophages, which are recognized as the principal phagocytes after they have activated and phagocytized the invading pathogens (Vasquez et al., 2021; Potter et al., 2005). Thereafter, nicotinamide adenine dinucleotide phosphate reduced (NADPH) oxidase is activated and responds by producing ROS (O_2^-, H_2O_2), leading to the elimination and death of the invading pathogen (Dockrell and Playfair, 1983; Ofek et al., 1995). The process of phagocytosis is categorized into opsonic and non-opsonic phagocytosis, with opsonic phagocytosis characterized by the coating of the parasites or antigens with host proteins or opsonin (antibodies, collectins, etc.) followed by the binding of these complexes to immune cells to facilitate internalization. On the other hand, non-opsonic phagocytosis is characterized by the direct binding of phagocyte receptors to pathogenic antigens (Chua et al., 2021; Ofek et al., 1995; Mosser and Zhang, 2011). This process is important in clearing up parasites and foreign particles as upon internalization, a phagosome is created to which toxic substances such as ROS and degradative enzymes are released, leading to the death of the invading pathogen and prevention of further infection and disease pathogenesis. However, phagocytosis may also lead to the production of ROS, which has detrimental effects on the host as much as they do on the parasite (Figs. 2 and 3).

Following the infection of the human host by the *Plasmodium* parasite, the host uses a population of phagocytic cells that aim to eliminate the parasite. After the sporozoites have invaded the human host, entered the circulatory system, and migrated to the liver, they become opsonized by antibodies, and the first phagocytes they encounter are Kupffer cells, which phagocytize the antibody-opsonized sporozoites, preventing the development of the blood-stage infection (Chua et al., 2021). Although this is the case, it has been reported that *in vitro,* the malarial sporozoites actively evade and transverse the Kupffer cells, preventing phagosomal acidification (Pradel and Frevert, 2001; Cha et al., 2015). Through this safe passage, the sporozoites can infect the host's hepatocytes and initiate malarial infection, suggesting that they escape the process of phagocytosis and phagocyte-derived ROS. Moreover, there have been contrasting ideas in this regard, as a study by Cha and colleagues (2015) showed that the Kupffer cells play a pivotal role in mediating malarial infection through the expression of the CD68 surface protein on Kupffer cells, which are inhibited by the p38 protein. This is said to block the binding of the sporozoites to the Kupffer cells, thus creating a free passage of sporozoites to initiate the liver stage of the disease. Usynin et al. (2007) also state that sporozoites suppress the respiratory burst from the Kupffer cells by increasing cyclic adenosyl monophosphate (cAMP), inhibiting phagocytosis and ROS generation. Contrary to this, another study argues that Kupffer cells reduce malarial infection by expressing a triggering receptor expressed on myeloid cells 2 (TREM2), a receptor important for the uptake of apoptotic and foreign particles as well as stimulation of inflammatory pathways, thereby limiting the expansion of the parasite (Gonçalves et al., 2013).

1.7. Neutrophils

Amongst other immune cells, neutrophils have been shown to play a role in clearing up the malarial parasite (Ty et al., 2019; Aitken et al., 2018. Neutrophils are abundant in human circulation and the first line of defence against infections and pathogens (Segal, 2005). These cells play a pivotal role in infection clearance by making use of phagocytosis, generation of ROS (Fig. 3), formation of neutrophil extracellular traps (NETs), as well as activation and regulation of the immune system through the secretion of chemokines and cytokines (Aitken et al., 2018; de Souza et al., 2012; Dupré-Crochet et al., 2013; Babatunde and Adenuga, 2022). Neutrophils clear pathogens using a respiratory burst of ROS, and this is because these cells have NADPH oxidase enzyme located on their cells' plasma and phagosomal membrane. The ROS produced can rapidly diffuse across the neutrophil membrane, making it available in both the intracellular and extracellular compartments, enabling ROS to kill phagocytized and extracellular pathogens (Fig. 3) (Chua et al., 2021; Aitken et al., 2018; Babatunde and Adenuga, 2022). According to Kharazmi et al. (1987), neutrophils exposed to *P. falciparum* merozoites and antigens of the parasite increase in levels of oxidative burst through the overproduction of ROS, which has been shown to inhibit the asexual growth and multiplication of the parasite *in vitro* through O_2^- and hypochlorous acid rather than H_2O_2 and superoxide (Kharazmi et al., 1987). Studies have also shown that neutrophils from children infected with malaria are more potent at inhibiting the *Plasmodium* parasite than those in healthy controls (Brown and Smalley, 1981; Robinson, 2008). These consume more oxygen than normal, confirming the activation of the production of ROS via oxidative burst (Brown and Smalley, 1981; Robinson, 2008). Neutrophils phagocytize opsonized merozoites, hemozoin, and infected erythrocyte cells, with the phagocytosis of merozoites leading to the release of ROS and that of hemozoin believed to impair neutrophil function to engage in a respiratory burst through

hemolysis-derived heme and induction of heme oxidase-1 (HO-1) among other factors (Chua et al., 2021; Babatunde and Adenuga, 2022; Cunnington et al., 2012). Thus, although oxidative stress and phagocytic bursts can be a limiting and compromising factor during malaria, they can also benefit the parasite by promoting its growth and multiplication at low levels or when hindered (Vasquez et al., 2021).

1.8. Monocytes

Monocytes play a pivotal role in the red blood stage of malaria by clearing the parasite and protecting the host from malarial development through phagocytic burst, cytokine production, and antigen presentation (Figs. 2 and 3). However, these cells have also been implicated in malarial pathogenesis by promoting inflammation and sequestration of infected red blood cells in organs such as the lungs and brain (Ortega-Pajares and Rogerson, 2018; Dobbs et al., 2017). These cells remove and phagocytize opsonized and non-opsonized merozoites and infected erythrocytes, thus controlling parasitemia (Chua et al., 2021; Ortega-Pajares and Rogerson, 2018; Dobbs et al., 2017). Monocytes are differentiated into clinical subsets (classical, inflammatory/intermediate, and non-classical). The intermediate and non-classical monocytes exhibit more phagocytic activity (Dobbs et al., 2017). Antonelli et al. (2014) revealed that intermediate monocytes are more efficient in phagocytosis of *P. vivax*-infected red blood cells. Another study by Sponaas and colleagues (2009) showed the migration of monocytes from the bone marrow to the spleen and decreased parasitic burden through phagocytosis and subsequent ROS production after mice infection by *P. chabaudi*. These cells inhibit the rapid division and multiplication of the *Plasmodium* parasite by secreting inflammatory cytokines and chemokines (Fig. 2). However, excessive production of these factors leads to pathogenesis (Ortega-Pajares and Rogerson, 2018). High levels of pro-inflammatory cytokines such as (TNF-α, IL-1β, IL-6, IL-8, IL-12, IFN-γ) and chemokines (CCL2, CCL5, CXCL9, CXCL10), with lower regulatory cytokines (IL-10, TGFβ, PGE2) have also been noted and believed to exacerbate malarial pathogenesis (Ortega-Pajares and Rogerson, 2018; Spaulding et al., 2016; Vasquez et al., 2021). Monocytes in children with acute infection from *P. falciparum* exhibit an increase in the production of pro-inflammatory TNF-α, IP-10 (CXCL10), IFN-ɣ, and IL-6 with a decrease in the phagocytosis of infected erythrocytes (Spaulding et al., 2016). Implications in the pathogenesis of malaria include being key players in severe anaemia, acute lung injury, and placental malaria (Ortega-Pajares and Rogerson, 2018).

Furthermore, other cells, like macrophages, are also implicated in malaria. They are activated by the ROS produced during infection, allowing the macrophages to produce more inflammatory cytokines, such as TNF-α and IL-6, amongst other cytokines (Fig. 2) (Vasquez et al., 2021). These cells also produce oxidative substances, leading to the death of parasitized erythrocytes and the parasite itself (Fig. 3) (Vasquez et al., 2021; Forman and Torres, 2002).

1.9. Antimalarial drugs as a source of oxidative stress

The use of antimalarial drugs has a long and evolving history, starting with the discovery of quinine (QN) from the bark of a Cinchona tree in 1820 (Percário et al., 2012; Pillat et al., 2020). Although its mechanism of action is still debated, it is believed to act as a schizonticide that targets the asexual blood stage of the parasite by interfering with DNA replication, a process essential for parasite growth and reproduction (Percário et al., 2012). However, due to its widespread overuse, the drug's antimalarial efficacy was challenged by the emergence of resistance by the parasite, rendering it less potent (MVV, 2025; Pillat et al., 2020). Following this, synthetic drugs using the acridine and quinoline structure were developed, leading to the subsequent formation of chloroquine (CQ), hydroxychloroquine (HCQ), and 3-methyl--chloroquine, among other drugs (Percário et al., 2012; Pillat et al., 2020; MVV, 2025). CQ quickly became the mainstay and cornerstone therapeutic and prophylactic regimen for eradicating malaria owing to its low cost, availability, and effectiveness. However, it was also hampered by the emergence of resistance by the parasite (Blouland et al., 2002; MVV, 2025). This prompted the development of others, such as sulfoxide-pyrimethamine and derivatives, mefloquine, and artemisinin (MVV, 2025).

Antimalarial drugs are developed and designed to kill the *Plasmodium* parasite and prevent its propagation by exploiting its susceptibility to ROS and/or targeting essential metabolic pathways critical for the survival of the parasite (Percário et al., 2012; Gomes et al., 2022; Sussmann et al., 2017; Egwu et al., 2021; Akanbi et al., 2010). However, for some, their mechanism of action remains to be debated (Herraiz et al., 2019; Egwu et al., 2021). This is shown by the several mechanisms of actions that have been postulated for chloroquine: (1) formation of CQ-DNA complex, which in turn interferes with DNA and RNA replication of the parasite, (2) modulating immune response, (3) inhibiting peroxidative degradation of heme, as well as the widely accepted hypothesis suggesting the (4) inhibition of the heme detoxification pathways which subsequently leads to ROS generation and oxidative stress to both the host and the parasite (Herraiz et al., 2019; Egwu et al., 2021; Zhou et al., 2020; Percário et al., 2012). CQ has also been demonstrated to induce oxidative stress, shown by the induction of DNA damage in the kidney and liver of rats, elevated levels of thiobarbituric acid (TBARS - an indicator of lipid oxidative), and increased carbonyl proteins (an indicator of protein oxidation) in the brain and kidneys after prolonged use of CQ (Giovanella et al., 2015). It has also been shown to elevate oxidative stress by increased levels of lipid peroxidation and reduced antioxidant levels (Glutathione, vitamin C, and B-carotene) (Farombi et al., 2003; Akanbi et al., 2010). This, therefore, suggests that CQ exacerbates oxidative stress through the production of ROS that arrest *Plasmodium* growth but can be detrimental to the human host.

Although debated, the majority of antimalarial drugs (atovaquones, quinolines, artemisinin) kill the parasite by exerting their pro-oxidant mechanisms through the direct production of ROS and/or the inhibition of essential enzymes and molecules that aim to restore the redox homeostatic balance (Percário et al., 2012; Gomes et al., 2022; Pal, 2023; Egwu et al., 2021; Zhou et al., 2020). The mechanism of artemisinin (mainline of defense) and its derivative has been elucidated and since accepted (Zheng et al., 2024; Egwu et al., 2021). This drug's potent and fast-acting antimalarial activity is due to the presence of endoperoxide linkage, which becomes activated by hemozoin. This then leads to the production of carbon-centered free radicals that affect the parasite physiology by targeting proteins, nucleic acids, and lipids essential to the parasite's survival (Zheng et al., 2024; Egwu et al., 2021; Nsonwu-Anyanwu et al., 2019). Artemisinin has also been postulated to reduce the levels of antioxidant enzymes in the parasite, thereby inducing oxidative stress (Nsonwu-Anyanwu et al., 2019; Vasquez et al., 2021; Cui and Su, 2009). This is in line with a study by Nsonwu-Anyanwu et al. (2019), who reported elevated levels of oxidative stress in malaria patients (treated and untreated), shown by elevated levels of MDA, total plasma peroxide (TPP), and oxidative stress index (OSI) and low levels of antioxidants [GHS, total antioxidant capacity (TAC)] when compared to healthy controls. A similar trend was also observed when comparing malaria patients treated with antimalarials (Artemisinin combination therapy and derivatives) to controls, and this was attributed to the generation of ROS via parasite-antimalarial interaction. However, when comparing the treated malarial patients versus untreated, a trend characterized by low parasitemia/parasite density, TPP, OSI, and high levels of TAC was observed (Nsonwu-Anyanwu et al., 2019). This showed the parasite-clearing effect of ACTs, which led to less generation of ROS and the potential to increase serum TAC levels (Nsonwu-Anyanwu et al., 2019; Erel et al., 1997). Therefore, this suggests that while essential for combating and treating malaria, antimalarial drugs (including ACTs) can also exacerbate the malarial pathophysiology through their ability to induce oxidative stress.

Though this is the case, it is worth noting that partial resistance by

the *Plasmodium* parasite has been reported in some parts of the world against the golden standard drug, artemisinin, and partner drugs (Zhou et al., 2020; Ravandir et al., 2023a WHO, 2025). This is worrying as there is no better alternative currently (Ravandir et al., 2023a), warranting the urgent development of novel antimalarials and alternative strategies could help circumvent the catastrophic effect that could arise from this (Pal, 2023; Ravandir et al., 2023; Souza et al., 2023). Therefore, continued efforts to develop effective vaccines, find new novel antimalarial drugs from medicinal plants, structural modification of the scaffolds of the currently available antimalarials, and drug hybridization and derivation are strongly suggested. However, since the review focuses on oxidative stress and how it exacerbates malaria pathophysiology, potential mitigation strategies will be briefly discussed.

1.10. Oxidative stress and the development of malarial pathogenesis

It has been shown through several studies that the level of oxidative stress is highly elevated during malaria and that it plays a crucial role in the systematic complications of malaria and malarial severity (Gomes et al., 2022; Narsaria et al., 2012; Vasquez et al., 2021; Ty et al., 2019). Although these mechanisms have not been characterized or fully elucidated, it is possible that oxidative stress caused by the increased production of ROS causes direct severity or complications by affecting the host cells and organs or is indirectly involved through the activation and increase in inflammatory responses (Vasquez et al., 2021; Ty et al., 2019). ROS produced during infections are secreted extracellularly, exposing the host to oxidative stress. Since there is a tight link between oxidative stress and inflammation, the ROS produced can activate and induce inflammatory signals through the nuclear factor kappa B ($NF\text{-}K_B$), resulting in cytokine secretion (Ty et al., 2019; Spaulding et al., 2016; Thomas, 2017). Although this is the case, it is worth noting that the role of oxidative stress in malarial infection is still debated, as some authors suggest a protective role while others suggest that it plays a role in the pathogenesis of malaria.

The human host has developed different antioxidant defence mechanisms to help curb the effect of the increased generation of free radical species in response to infections and involves three interdependent systems: enzymatic, small molecules, and metal chelation (Percário et al., 2012). The most important antioxidant enzymes are GSH-Px (Glutathione peroxidase), catalase (CAT), and Superoxide dismutase (SOD), which act on oxidants to make them less potent. However, there is an exception of some highly reactive oxidants implicated in the pathophysiology associated with malaria, such as OH^- and $ONOO^-$ (Percário et al., 2012; Gomes et al., 2022; Ezzi et al., 2017). The amount of oxidative stress seen during either *P. falciparum* or *P. vivax* infection goes hand in hand with a decrease in antioxidant levels, showing that there is a homeostatic imbalance between the ratio of oxidants and antioxidants (Pabon et al., 2003; Vasquez et al., 2021; Ezzi et al., 2017). This could be attributed to some of the antioxidant enzymes' inability to reduce the oxidants produced to less reactive compounds or the saturation of the antioxidant enzymes or systems (Narsaria et al., 2012; Ezzi et al., 2017). Gomes and colleagues (2022) state that the antioxidant systems often fall short of eliminating these ROS, suggesting that oxidative stress does play a role in the pathophysiology of malaria, as increased oxidative stress accompanied by a decrease in antioxidant activity has been seen in children with severe malaria (Narsaria et al., 2012).

Oxidative stress and its implication in malaria severity may be through the actions of these oxidants on the host organs and tissues, especially the brain and lungs (Gomes et al., 2022; Fabbri et al., 2013). Compared to healthy controls, an increase in oxidative stress has been seen in *P. vivax* patients through the increased production of malondialdehyde (MDA), a marker of oxidative stress and a by-product of lipid peroxidation (Babalola et al., 2022; Fabbri et al., 2013). The increased production of MDA indicates lipid peroxidation by free radicals against the membrane of red blood cells and hepatocytes. It has additionally been observed *in vitro* that *P. falciparum* induces lipid peroxidation on the surface of red blood cells, reducing the deformability of infected and uninfected RBCs. Reduced deformability in the latter relates to the high levels of death and mortality of children and adults suffering from malaria, which further suggests the role of oxidative stress in malarial severity (Vasquez et al., 2021; Dondorp et al., 2002; Araujo et al., 2008). Increased rigidity of red blood cells presents a significant challenge contributing to microcirculatory obstruction and anaemia caused by increased splenic removal of uninfected RBCs (Becker et al., 2004). In addition to the anaemia seen during malarial infection, lipid peroxidation causes the cells to lose their elasticity and ability to be selectively permeable, which increases their fragility and causes their receptors to be defective. The cells, therefore, develop a shortened life span and subsequently die. Due to the loss of elasticity and shortened life span, anaemia prevails (Erel et al., 2001). Although rare in malaria, liver damage or injury is also believed to be caused by ROS generated by *P. falciparum*. This is shown by the increase in the alkaline phosphatase (ALP) enzyme in the serum of malaria patients, suggesting the offset of the hepatic stage of the parasite (Ezzi et al., 2017). Disturbance and damage in the hepatocyte membrane are accompanied by the leaking of this enzyme from the hepatocytes. Elevation of ALP is also associated with cholestasis, a causative effect of malarial infection (Ezzi et al., 2017).

Thrombocytopenia, a hematological condition characterized by low platelet count (<150 000 platelets/uL), is another pathological condition associated with malaria and linked to oxidative stress (Percário et al., 2012; Vasquez et al., 2021; Araujo et al., 2008; Shashirekha, 2006). This condition has been seen in patients infected with either *P. falciparum* or *P. vivax* and is the most common hematological condition in patients infected with malaria (Araujo et al., 2008; Erel et al., 2001; Shashirekha, 2006). Amongst some potential causes of this condition, such as immune mechanisms, splenic function alterations, and direct interaction with the *Plasmodium* parasite and platelets, is oxidative stress (Araujo et al., 2008; Shashirekha, 2006). A study by Araujo et al. (2008) showed that there was a significant increase in platelet MDA in *P. vivax* patients with thrombocytopenia compared to healthy controls. Since MDA is a marker of oxidative stress, this suggests that oxidative stress plays a significant role in the pathogenesis of thrombocytopenia during malarial infection.

Furthermore, Erel et al. (2001) also found a negative correlation between platelet count and platelet lipid peroxidation, marked by an increase in platelet MDA and a decrease in the platelet's antioxidant enzymes (SOD and GSH-Px). Thus, the role of oxidative stress in thrombocytopenia pathogenesis may be because the platelet membrane is thinner than red blood cells, making platelets susceptible to ROS, which become damaged structurally and functionally (Araujo et al., 2008, 2022). Disruption of the membrane integrity of platelets causes the receptors on their surface to malfunction, leading to their subsequent dysfunction and impairment. Therefore, an increase in oxidative stress, as seen during malarial infection, may mean more damage to the platelets, resulting in decreased platelet counts and the offset of thrombocytopenia.

Cerebral malaria is another fatal complication and manifestation of the clinical symptoms seen in patients infected with malaria, especially those infected with *P. falciparum* (Imai et al., 2014). Characterized by body aches, delirium, coma, and impaired consciousness, cerebral malaria has been linked to several possible causes, which include inflammatory response processes, sequestration of parasitized red blood cells in the microvasculature, and adherence of the cells in the endothelial lining of the brain, causing the formation of cerebral malaria which is a major cause of morbidity in children infected with malaria (Percário et al., 2012; Ofek et al., 1995). Oxidative stress has also been associated with this complication; hence, the increased literature reporting on the clinical role of oxidative stress in the manifestation of cerebral malaria *in vivo* using mouse models (Vasquez et al., 2021). Depending on the lesions in the brain cells and parenchyma, the imbalance in the redox

status in the human host can lead to cognitive and physical impairments (Gomes et al., 2022). It has also been shown that oxidative stress and its associated damage are implicated in the pathogenesis of neurodegenerative disorders and encephalopathy associated with sepsis and that these pathologies include cerebral malaria (Nyaba et al., 2018; Bermpohl et al., 2005). This was demonstrated by Nyaba et al. (2018), who showed increased levels of MDA were observed in mouse models (C57BL/6 J) when infected with *P. berghei*, with the effect reversed when treated with a plant with antioxidant and antimalarial activity, suggesting the possibility of exploiting the use of antioxidants to reverse or mitigate ROS-mediated complications in malaria.

Oxidative stress in malaria and potential mitigation strategies

Though the role of oxidative stress and malarial severity is still not clearly understood, evidence suggests that an imbalance in the redox status and homeostasis plays a role in malarial pathophysiology and severity. This suggests that restoring oxidative homeostasis during malarial infection can benefit the host. As such, there is a growing number of studies that have focused their attention on finding strategies that could help address the issue of *Plasmodium*-induced oxidative stress and its associated complications (Gomes et al., 2015; Isah and Ibrahim, 2014; Percário et al., 2012; Sussmann et al., 2017; Kotepui et al., 2023). Some of the strategies that have been proposed include the use of antioxidants as supplements or adjuvants (in combination with antimalarial drugs) (Pal, 2023; Gomes et al., 2015; Isah and Ibrahim, 2014). Antioxidants, as supplements or adjuvants, are a promising avenue in addressing oxidative stress and the damage it causes during malarial infection (Isah and Ibrahim, 2014). This is due to the knowledge and understanding that antioxidants can neutralize and scavenge free radicals, thus maintaining redox homeostasis, which can protect against oxidative damage. As such, researchers have looked into several antioxidants, including Vitamin C, E, D, *N*-acetylcysteine (NAC), melatonin, folate, deferoxamine, as well as plants with antioxidant activity (Gomes et al., 2015; Gomes et al., 2022; Isah and Ibrahim, 2014; Sussmann et al., 2017; Kotepui et al., 2023).

1.11.1. Vitamin E

Vitamin E is one of the most studied antioxidants because it is speculated to confer protection against malaria (Isah and Ibrahim, 2014; Kotepui et al., 2023). However, findings from a study by Awodele and colleagues (2007) suggested otherwise. Their results showed that vitamin E reduced parasite clearance (i.e., increased parasitemia) by retarding or interfering with the action of artesunate, thereby antagonizing its effect. This antagonizing effect may also be attributed to the parasite's depleting antioxidant levels and using the vitamin to its benefit. This is because the *Plasmodium* parasite has been shown to synthesize its own vitamin E and use it to ameliorate ROS (Sussmann et al., 2017). This is in line with Kotepui et al. (2023), who state that vitamin E is highly reduced in patients with malaria and those with severe malaria than in healthy controls and that this occurrence could be due to the parasite depleting the antioxidant or using it to counteract the effect of ROS. This then warrants a careful selection of antioxidants to use.

N-acetylcysteine

NAC, an acetylated variant of the amino acid L-cysteine, is another powerful antioxidant with a wide spectrum of biological activities, including acting as an antidote for paracetamol poisoning (Millea et al., 2009; Cotgreave, 1997). NAC has been suggested as a supplement that could be beneficial during malarial pathogenesis (Treeprasertsuk et al., 2003), potentially because of its ability to scavenge ROS and replenish GSH and cysteine levels (Charunwatthana et al., 2013). However, contrasting results have been noted. In a study by Watt et al. (2002), NAC reduced malarial severity in malarial patients treated with quinine by normalizing serum lactate levels. However, when used in combination with artesunate, it was shown to have no measurable impact (Charunwattana et al., 2013), while a study by Arreesrisom et al. (2007) noted an antagonistic effect of NAC on the drug, thus warranting careful consideration. A study by Gomes et al. (2015) demonstrated that NAC only reduced parasitemia by 7–10 days in mice infected with *P. berghei* and that it increased the trolox equivalent antioxidant capacity and slightly reduced TBARS levels, suggesting the potential use of NAC as an adjuvant and supplement that can ameliorate ROS-associated pathophysiologies of malaria. To further support this, Reis et al. (2010) showed that NAC and Desferrioxamine (another antioxidant), combined with chloroquine, reduced cognitive impairment, a ROS-mediated injury associated with cerebral malaria.

Plants with antioxidant activity

Another strategy that has been employed to help restore the host's oxidative homeostasis is the use of natural products from medicinal plants, potentially through the regulation of the host's immune response, improvement of the antioxidant defense system, or by directly inhibiting parasite growth and development using other mechanisms (Gomes et al., 2022; Laryea and Sheringham, 2021; Sadiq et al., 2017). This strategy appears promising due to its dual action of being able to scavenge ROS and reduce oxidative stress while also killing the parasite. As such, a growing number of studies have investigated medicinal plants' antioxidant and antimalarial properties to ameliorate malaria and its associated symptoms, with most studies showing promising results. For instance, Al-adhoery et al. (2010) showed that the methanol extract of *Piper betle* had antioxidant and schizonticidal impact against *P. berghei.* A similar trend was also observed with *Acacia nilota* (L) del (Sadiq et al., 2017), extract of *Celtis africana*, *Physalis micrantha* (Laryea and Sheringham, 2021), *Terminalia avicennioides* (Omonkhua et al., 2013), and *Sonchus arvensis* (Wahyuni et al., 2023). Moreover, to show that this has the ability to address ROS-mediated pathologies, a study by Mubaraki et al. (2017) showed that mouse models (C57BL/6) with cerebral malaria caused by *P. berghei* ANKA (PbA) had an increased production of MDA and decrease in the antioxidant level and that treatment with *Zizyphus spina-christi* suppressed occurrence. Even so, it should be noted that these studies have only been limited to *in vitro* and *in vivo*, with few to no clinical trials, and that the bioactive compounds that could be responsible for the observed activity have not been isolated. Therefore, more research still needs to be done.

Conclusion

Malaria is a complex and life-threatening disease that continues to be a persistent public health burden. As part of the immune system response to malarial infection, a robust inflammatory response and oxidative stress characterized by high levels of ROS/RNS are seen. Although the host tries to combat this through the use of antioxidants, the host system often falls short, causing an oxidative imbalance characterized by increased levels of ROS. While the exact role of oxidative stress in malarial infection remains debated, it functions as a double-edged sword. Oxidative stress, through generating reactive oxygen species (ROS), mounts protective immunity and reduces parasitemia, thus protecting against the disease. However, excessive ROS production and prolonged activation of inflammatory pathways exacerbate tissue damage, worsening the disease's severity. Therefore, a deep comprehension of the intricate relationship between redox mechanisms and how they could be regulated is required. This understanding could lead to developing novel therapeutic strategies, including using antioxidants, which have been shown to reduce ROS-mediated damage during malaria. However, careful consideration and more research are needed to balance antioxidants' efficacy and potential side effects during malaria.

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CRediT authorship contribution statement

Muzi Nicolas Buthelezi: Writing – original draft, Investigation, Data curation. **Kgaugelo Josephine Masia:** Writing – original draft, Investigation, Data curation. **Priscilla Masamba:** Writing – original draft, Visualization, Investigation, Data curation. **Mthokozisi Blessing Cedric Simelane:** Writing – review & editing, Supervision, Project administration, Conceptualization. **Abidemi Paul Kappo:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Ethical approval

Not Applicable.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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