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Effects of *Plasmodium berghei* NK65 Infection on Haematological and Antioxidant Parameters, CD4+ T-Lymphocyte Count, and Spleen Morphology in Mice

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ABSTRACT

Malaria is associated with haematological abnormalities, oxidative stress, and alterations in immune and splenic functions that may contribute to disease progression. This study investigated changes in parasitaemia, haematological indices, CD4+ T-lymphocyte count, antioxidant parameters, and spleen morphology in mice infected with *Plasmodium berghei* NK65. Fifteen albino mice with an average body weight of 24.20 ± 1.40 g were divided into an uninfected control group ($n = 5$) and an infected group ($n = 10$). Mice in the infected group were inoculated with chloroquine-sensitive *P. berghei* NK65 and monitored for 14 days. Parasitaemia increased progressively from 11.1% on day 4 post-infection to 27.9% on day 14. Compared with the control group, infected mice showed lower red blood cell count, white blood cell count, platelet count, haemoglobin concentration, haematocrit, mean corpuscular haemoglobin concentration, lymphocyte percentage, and neutrophil percentage, whereas mean corpuscular volume and mean corpuscular haemoglobin were higher. Infection was also associated with alterations in oxidative stress-related parameters, including superoxide dismutase, catalase, malondialdehyde, nitric oxide, and glutathione. CD4+ T-lymphocyte counts did not differ significantly between the groups. Gross examination revealed an enlarged and darker appearance of the spleen in infected mice compared with controls. These findings demonstrate that *P. berghei* NK65 infection is

accompanied by progressive parasitaemia and alterations in haematological and oxidative stress-related parameters, together with gross morphological changes in the spleen.

Keywords: *Plasmodium berghei*, malaria, parasitaemia, haematological parameters, oxidative stress, spleen, CD4⁺ T lymphocytes

1. INTRODUCTION

Malaria remains a major global health challenge despite substantial advances in prevention, diagnosis, and treatment. According to the World Health Organization, an estimated 282 million malaria cases and 610,000 malaria-related deaths occurred worldwide in 2024, with the WHO African Region accounting for approximately 94% of cases and 95% of deaths (WHO, 2025). The disease is caused by protozoan parasites of the genus *Plasmodium*, with *Plasmodium falciparum* responsible for the greatest burden of severe malaria and malaria-related mortality in Africa.

The pathogenic consequences of malaria extend beyond the presence and replication of parasites in circulating erythrocytes (Woldegerima et al., 2025). Infection is frequently accompanied by substantial haematological disturbances, including anaemia, thrombocytopenia, and alterations in circulating leukocyte populations (Duneeh et al., 2026).

These abnormalities arise through multiple mechanisms, including destruction of parasitized erythrocytes, enhanced clearance of infected and uninfected erythrocytes, altered erythropoiesis, inflammatory responses, and changes in splenic sequestration and clearance. Experimental *Plasmodium berghei* infection similarly produces abnormalities in erythropoiesis and other haematopoietic compartments, making rodent malaria models useful for investigating the systemic consequences of blood-stage malaria infection.

Oxidative stress is another important component of malaria pathogenesis. During infection, reactive oxygen and nitrogen species are generated through host immune responses and parasite-associated processes, including haemoglobin degradation and the release of free haem (Buthelezi et al., 2025; Vasquez et al., 2021). Although reactive oxygen species participate in host defence against malaria parasites, excessive oxidative activity may overwhelm endogenous antioxidant systems and contribute to lipid peroxidation and cellular injury. Consequently, changes in antioxidant defence systems, including superoxide dismutase, catalase, and glutathione, together with markers such as malondialdehyde and nitric oxide, can provide information on the biochemical disturbances accompanying malaria infection (Ojongnkpot et al., 2023).

The spleen also has a central role in the host response to blood-stage malaria. It contributes to the recognition and clearance of parasitized erythrocytes and coordinates cellular immune responses to infection (Wang et al., 2021). Experimental *P. berghei* infection has been associated with splenic enlargement (Imai et al., 2020), changes in splenic cellular populations (Lakkavaram et al., 2020), congestion (Onobrudu et al., 2026), and structural remodelling (Kumar & Bagai, 2014). These responses are closely related to parasite burden and the host's capacity to control infection (Wang et al., 2021). CD4⁺ T lymphocytes are also important components of antimalarial immunity through their regulation of cellular and humoral immune responses (Kurup et al., 2019). *Plasmodium berghei* is a rodent malaria parasite extensively used as an experimental model for investigating malaria pathogenesis and host responses

(Daclam et al., 2024; Matz & Kooij, 2015; Otun et al., 2024). Although rodent malaria does not reproduce every feature of human *P. falciparum* infection, *P. berghei* models provide a useful experimental system for examining parasite-associated haematological, biochemical, immunological, and organ-level changes. Previous studies have demonstrated anaemia (Azizah et al., 2023), altered erythropoiesis (Lakkavaram et al., 2020), oxidative stress (Scaccabarozzi et al., 2018), and splenic changes during experimental *P. berghei* infection (Wang et al., 2021).

Therefore, this study investigated the progression of parasitaemia and associated changes in haematological parameters, CD4+ T-lymphocyte count, antioxidant and oxidative stress-related parameters, and gross spleen morphology in mice infected with chloroquine-sensitive *P. berghei* NK65 over a 14-day period.

2. MATERIALS AND METHODS

2. 1. Experimental animals

Fifteen albino mice of both sexes, with a mean body weight of 24.20 ± 1.40 g, were obtained from the Animal Laboratory, Department of Biochemistry, University of Ilorin, Ilorin, Kwara State, Nigeria. The animals were housed in standard plastic cages and allowed to acclimatize for one week before the commencement of the experiment. They were maintained under standard laboratory conditions with access to standard mouse feed and clean tap water.

2. 2. Experimental design

Following acclimatization, the animals were divided into two groups. Group A comprised five uninfected mice and served as the control group, whereas Group B comprised ten mice infected with *Plasmodium berghei* NK65 and monitored for 14 days.

2. 3. Plasmodium berghei infection

The chloroquine-sensitive *P. berghei* NK65 strain was used to establish experimental malaria infection. Parasitaemic blood obtained from an infected donor mouse was diluted with normal saline to prepare the inoculum. Following the one-week acclimatization period, mice in Group B were inoculated with the prepared suspension of *P. berghei*-infected erythrocytes. The animals were subsequently monitored for 14 days.

2. 4. Preparation of blood smears and determination of parasitaemia

Beginning after establishment of infection, blood samples were collected from the tail of infected mice and thin blood smears were prepared on clean glass slides. The smears were air-dried, fixed with absolute methanol, stained with Giemsa stain, rinsed with clean water, and allowed to dry before microscopic examination.

Parasitaemia was determined by examining the stained blood smears under a microscope using a $\times 100$ oil-immersion objective. The numbers of infected and total erythrocytes were counted, and percentage parasitaemia was calculated as:

$$\text{Parasitaemia (\%)} = \frac{\text{Total number of erythrocytes counted}}{\text{Number of infected erythrocytes}} \times 100$$

Parasitaemia was assessed on days 4, 7, 10, and 14 post-infection.

2. 5. Blood collection

At the end of the 14-day experimental period, the animals were anaesthetized with diethyl ether, and blood samples were collected by jugular puncture into appropriately labelled sample containers for subsequent haematological and biochemical analyses.

2. 6. Haematological analysis

Haematological parameters were determined using a Sysmex KX-21N automated haematology analyser. The parameters assessed included red blood cell (RBC) count, white blood cell (WBC) count, platelet (PLT) count, haemoglobin concentration (HGB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), lymphocyte percentage (LYM), and neutrophil percentage (NEU).

2. 7. Determination of oxidative stress-related parameters

Oxidative stress-related parameters assessed in this study included superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), nitric oxide (NO), and malondialdehyde (MDA).

2. 7. 1. Catalase activity

Catalase activity was determined using a colorimetric assay based on the decomposition of hydrogen peroxide by catalase. Following termination of the enzymatic reaction, residual hydrogen peroxide was quantified through a peroxidase-coupled chromogenic reaction, with absorbance measured at 520 nm.

2. 7. 2. Reduced glutathione

Reduced glutathione was determined using the method based on the reaction of sulfhydryl groups with 5,5'-dithiobis-(2-nitrobenzoic acid), as described by Ellman. Formation of the yellow-colored product was measured spectrophotometrically at 412 nm.

2. 7. 3. Superoxide dismutase activity

Superoxide dismutase activity was determined based on the ability of SOD to inhibit the autooxidation of pyrogallol under alkaline conditions.

2. 7. 4. Nitric oxide

Nitric oxide production was estimated by measuring nitrate and nitrite. Nitrate was reduced to nitrite, followed by spectrophotometric quantification using the Griess reaction at 540 nm.

2. 7. 5. Malondialdehyde

Malondialdehyde was determined using the thiobarbituric acid-reactive substances method. The reaction between MDA and thiobarbituric acid produces a chromophore that was measured spectrophotometrically at 532 nm.

2. 8. CD4+ T-lymphocyte count

CD4+ T-lymphocyte counts were determined in blood samples obtained from control and *P. berghei*-infected mice at the end of the experimental period.

2. 9. Gross examination of the spleen

Following termination of the experiment, spleens were collected from control and infected mice and examined grossly for differences in size and colour. Representative spleens from the two groups were photographed for comparison.

2. 10. Statistical analysis

Data were analysed using IBM SPSS Statistics version 20.0 (SPSS Inc., Chicago, IL, USA). Results are expressed as mean $\pm$ standard error of the mean (SEM). Differences between groups were evaluated using Student's *t*-test, with $P < 0.05$ considered statistically significant.

3. RESULTS

3. 1. Progression of Parasitaemia

Parasitaemia increased progressively throughout the 14-day observation period in mice infected with *Plasmodium berghei* NK65. The percentage parasitaemia was 11.1% on day 4 post-infection and increased to 16.3%, 24.5%, and 27.9% on days 7, 10, and 14 post-infection, respectively (Table 1).

Table 1. Percentage parasitaemia in *Plasmodium berghei* NK65-infected mice during the 14-day observation period.

Group	Day 4 post-infection	Day 7 post-infection	Day 10 post-infection	Day 14 post-infection
Infected mice (Group B)	11.1	16.3	24.5	27.9

3. 2. Haematological Parameters

The haematological parameters of the control and *P. berghei*-infected mice are presented in Tables 2 and 3. Compared with the control group, the infected group had lower mean values for red blood cell count (RBC), white blood cell count (WBC), platelet count (PLT), haemoglobin concentration (HGB), haematocrit (HCT), mean corpuscular haemoglobin concentration (MCHC), lymphocyte percentage (LYM), and neutrophil percentage (NEU).

In contrast, mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) were higher in the infected group than in the control group.

Table 2. Haematological parameters of control and *Plasmodium berghei* NK65-infected mice after 14 days.

Group	RBC ($10^3/\mu\text{L}$)	WBC ($10^6/\mu\text{L}$)	PLT ($10^3/\mu\text{L}$)	HGB (g/dL)	HCT (%)
Control (Group A)	7.63 ± 0.70	16.87 ± 8.69	840.00 ± 45.57	9.03 ± 0.98	39.87 ± 1.38
Infected (Group B)	5.40 ± 3.96	4.70 ± 4.70	617.00 ± 403.00	6.90 ± 4.90	32.80 ± 21.80

Table 3. Erythrocyte indices and differential leukocyte parameters of control and *Plasmodium berghei* NK65-infected mice after 14 days.

Group	MCV (fL)	MCH (pg)	MCHC (g/dL)	LYM (%)	NEU (%)
Control (Group A)	52.83 ± 3.57	11.87 ± 0.61	22.57 ± 1.68	55.03 ± 27.57	11.67 ± 6.00
Infected (Group B)	67.35 ± 9.05	13.25 ± 0.65	19.90 ± 1.70	42.20 ± 42.20	7.80 ± 7.80

Values are expressed as mean $\pm$ SEM.

3. 3. CD4+ T-Lymphocyte Count

The mean CD4+ T-lymphocyte count was 9.50 ± 0.50 in the control group and 10.50 ± 1.50 in the *P. berghei*-infected group (Table 4). The difference between the two groups was not statistically significant ($P > 0.05$).

Table 4. CD4+ T-lymphocyte counts of control and *Plasmodium berghei* NK65-infected mice after 14 days.

Group	CD4+ T-lymphocyte count
Control (Group A)	9.50 ± 0.50
Infected (Group B)	10.50 ± 1.50

Values are expressed as mean $\pm$ SEM.

3. 4. Antioxidant and Oxidative Stress-Related Parameters

The antioxidant and oxidative stress-related parameters are presented in Table 5. Superoxide dismutase (SOD), catalase (CAT), nitric oxide (NO), and glutathione (GSH) values were lower in the infected group than in the control group. In contrast, malondialdehyde (MDA) concentration was higher in the infected group. SOD decreased from 1.3250 ± 0.095 in the control group to 0.4745 ± 0.406 in infected mice, while CAT decreased from 0.8450 ± 0.095 to 0.6950 ± 0.095 . Similarly, NO decreased from 11.7250 ± 0.095 to 4.5950 ± 0.095 , and GSH decreased from 4.0250 ± 0.095 to 3.7350 ± 0.095 . By contrast, MDA increased from 0.1850 ± 0.095 in the control group to 0.4050 ± 0.095 in the infected group.

Table 5. Antioxidant and oxidative stress-related parameters of control and *Plasmodium berghei* NK65-infected mice after 14 days.

Group	SOD	CAT	MDA	NO	GSH
Control (Group A)	1.3250 ± 0.095	0.8450 ± 0.095	0.1850 ± 0.095	11.7250 ± 0.095	4.0250 ± 0.095
Infected (Group B)	0.4745 ± 0.406	0.6950 ± 0.095	0.4050 ± 0.095	4.5950 ± 0.095	3.7350 ± 0.095

Values are expressed as mean $\pm$ SEM.

3. 5. Gross Morphology of the Spleen

Gross examination revealed visible differences between spleens obtained from control and *P. berghei*-infected mice after 14 days. Spleens from infected mice appeared enlarged and darker than those from the control group (Figure 1).



Figure 1. Gross appearance of spleens from control and *Plasmodium berghei* NK65-infected mice after 14 days.

The spleen from the infected group showed increased apparent size and darker coloration compared with that from the uninfected control group.

4. DISCUSSION

The present study examined the progression of parasitaemia and associated haematological, oxidative stress-related, immunological, and gross splenic changes in mice infected with *Plasmodium berghei* NK65. Parasitaemia increased progressively during the 14-day observation period, reaching 27.9% by day 14. This progressive increase confirms successful establishment of blood-stage infection and provides the biological context for the changes observed in the infected animals.

4. 1. Haematological Changes

Malaria is characteristically associated with disturbances in haematological homeostasis, particularly anaemia, thrombocytopenia, and alterations in circulating leukocyte profiles (Okagu et al., 2022). Here, *P. berghei*-infected mice showed lower RBC count, haemoglobin concentration, and haematocrit than uninfected controls. These findings are consistent with malaria-associated anaemia, which has also been demonstrated in experimental *P. berghei* infection (Azizah et al., 2023; Lakkavaram et al., 2020).

Several mechanisms may contribute to anaemia during malaria infection. These include rupture of parasitized erythrocytes during parasite replication, increased clearance of infected and uninfected erythrocytes, inflammatory alterations in erythropoiesis, and ineffective or suppressed erythropoietic responses (Okagu et al., 2022). Experimental *P. berghei* infection has similarly been shown to disrupt erythropoiesis, with alterations that overlap with features of anaemia of chronic disease (Lakkavaram et al., 2020).

The lower platelet count observed in infected mice is also consistent with the thrombocytopenic changes frequently associated with malaria. Malaria-associated thrombocytopenia is multifactorial, and proposed mechanisms include increased peripheral platelet destruction, splenic sequestration, immune-mediated destruction, coagulation-related consumption, and alterations in platelet production (Lacerda et al., 2011; Okagu et al., 2022).

The infected animals also showed lower WBC, lymphocyte, and neutrophil values than controls. Changes in circulating leukocyte profiles have been described in malaria and may reflect altered production, redistribution, sequestration, activation, and migration of immune cells during infection (Okagu et al., 2022). The present observations therefore suggest that the haematological effects of *P. berghei* infection extended beyond the erythrocyte compartment.

In contrast, MCV and MCH were higher in infected animals, whereas MCHC was lower. Alterations in erythrocyte indices may accompany malaria-associated anaemia and disturbed erythropoiesis (Lakkavaram et al., 2020; Okagu et al., 2022). However, because our study assessed these haematological parameters at a single terminal time point and did not directly evaluate erythropoiesis or erythrocyte morphology, the mechanisms responsible for these differences cannot be established from the current data.

4. 2. CD4+ T-Lymphocyte Count

CD4+ T lymphocytes play an important role in immunity to malaria by coordinating cellular and humoral immune responses. During blood-stage infection, CD4+ T cells contribute

to cytokine-mediated regulation, activation of other immune-cell populations, and the development of effective antibody responses against malaria parasites (Kurup et al., 2019).

In the present study, however, the CD4⁺ T-lymphocyte count did not differ significantly between infected and control mice. This finding indicates that, under the experimental conditions used, 14 days of *P. berghei* NK65 infection did not produce a detectable change in the measured CD4⁺ T-lymphocyte count.

This result should not be interpreted as evidence that CD4⁺ T-cell responses were functionally unaffected. Malaria infection can influence CD4⁺ T-cell activation, differentiation, effector function, cytokine production, and tissue distribution, and such changes are not necessarily reflected by the total number of circulating CD4⁺ cells (Kurup et al., 2019). These processes were not assessed in this work; therefore, the current findings should be interpreted specifically in relation to the measured CD4⁺ T-lymphocyte count.

4. 3. Oxidative Stress and Antioxidant Parameters

Oxidative stress is an important component of malaria pathogenesis. Reactive oxygen species are generated through both host- and parasite-associated processes, including host inflammatory and phagocytic responses, parasite metabolism, haemoglobin degradation, and the release of haem. Although reactive species may contribute to antiparasitic defence, excessive production can disturb cellular redox homeostasis and promote oxidative damage to host cells and tissues (Vasquez et al., 2021).

In this study, infected mice had lower SOD, catalase, GSH, and NO values than control mice, whereas MDA was higher. The combination of lower antioxidant indices and increased MDA is consistent with disturbance of redox homeostasis during malaria infection. Alterations in antioxidant defence and increased lipid peroxidation have also been reported in experimental *P. berghei* infection (Chua, 2012).

SOD and catalase constitute important components of the enzymatic antioxidant defence system. SOD catalyses the conversion of superoxide radicals to hydrogen peroxide, whereas catalase contributes to the removal of hydrogen peroxide. GSH is an important component of intracellular antioxidant defence and contributes to the maintenance of cellular redox homeostasis.

Alterations in these antioxidant systems may therefore affect the capacity of host cells to counter increased oxidative activity during malaria infection (Vasquez et al., 2021).

MDA is a product of lipid peroxidation and is widely used as an indicator of oxidative damage to membrane lipids.

The higher MDA concentration observed in infected mice is therefore consistent with enhanced lipid peroxidation. Similar increases in lipid peroxidation together with alterations in antioxidant defence have been demonstrated in *P. berghei*-infected mice (Chua, 2012). Taken together, the lower antioxidant indices and higher MDA concentration observed in this study support the presence of altered redox homeostasis during infection.

The lower NO value observed in infected mice should be interpreted cautiously. Nitric oxide has complex and context-dependent roles in malaria, with potential immunoregulatory, antiparasitic, vascular, and pathological effects.

Its production and biological consequences may vary according to parasite species or strain, host factors, stage and severity of infection, tissue compartment, and the method used to assess NO or its metabolites (Vasquez et al., 2021). Here, we measured NO-related biochemical levels, but it was not designed to establish the mechanism underlying the observed difference.

4. 4. Gross Splenic Changes

The spleen has a central role in blood-stage malaria because it contributes to the recognition, retention, and clearance of parasitized or otherwise altered erythrocytes and serves as an important site for the development of immune responses against malaria parasites. Consequently, substantial splenic changes, including splenomegaly, alterations in splenocyte composition, and histopathological remodelling, have been demonstrated in experimental *P. berghei* infection (Wang et al., 2021).

We found that spleens obtained from infected mice appeared enlarged and darker than those from uninfected controls. These gross changes may reflect increased splenic activity associated with the retention and clearance of altered erythrocytes, changes in immune-cell populations, vascular alterations, and tissue remodelling during infection (Wang et al., 2021). Structural changes in the spleen have also been reported following *P. berghei* NK65 infection (Kumar & Bagai, 2014).

This splenic observation should, however, be interpreted as a gross morphological finding. Spleen weight, splenic index, splenocyte composition, and histopathological changes were not assessed in the present study; therefore, the nature and severity of the underlying splenic alterations cannot be determined from gross appearance alone.

4. 5. Overall Interpretation and Study Limitations

Taken together, the findings indicate that progressive *P. berghei* NK65 parasitaemia was accompanied by changes in several haematological indices, alterations in antioxidant and oxidative stress-related parameters, and visible changes in spleen morphology. The overall pattern is consistent with previous experimental evidence showing that *P. berghei* infection can produce disturbances in erythropoiesis and haematological homeostasis, oxidative balance, and splenic structure and cellular composition (Lakkavaram et al., 2020; Wang et al., 2021).

Several limitations should nevertheless be considered when interpreting the results. Our study involved a relatively small number of animals and unequal numbers of mice in the control and infected groups. Measurements were largely obtained at the end of the 14-day experimental period, limiting assessment of the temporal relationship between increasing parasitaemia and the other measured parameters. In addition, the splenic findings were based on gross examination without quantitative spleen weight or histopathological assessment. These limitations do not negate the observed findings but restrict conclusions regarding the mechanisms and severity of the changes associated with infection.

5. CONCLUSIONS

Plasmodium berghei NK65 infection in mice was associated with a progressive increase in parasitaemia over the 14-day observation period and alterations in several haematological and oxidative stress-related parameters. Infected mice showed lower RBC, WBC, platelet, haemoglobin, haematocrit, MCHC, lymphocyte, and neutrophil values, together with changes in erythrocyte indices. Lower SOD, catalase, GSH, and NO values and a higher MDA concentration were also observed, indicating disruption of oxidative balance during infection. CD4⁺ T-lymphocyte counts did not differ significantly between infected and control animals.

Gross examination further revealed enlargement and darker coloration of the spleen in infected mice. Overall, the findings demonstrate that experimental *P. berghei* NK65 infection is accompanied by haematological and oxidative stress-related disturbances as well as gross splenic changes. Further studies incorporating larger sample sizes, serial assessment of these parameters, quantitative evaluation of spleen enlargement, and histopathological examination would provide a more detailed understanding of the systemic changes associated with infection.

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