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Effects of time of lead intoxication on some blood and oxidative stress parameters of mice experimentally infected with *Plasmodium berghei*

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Abstract

Both pollutants and parasites can affect any living organism, and their interaction is dependent on factors such as the level and duration of toxic exposure, as well as the route and time of exposure to the pollutant/parasite. This study investigated the effects of time of exposure to lead (relative to time of infection) on blood and oxidative stress parameters of mice infected with *Plasmodium berghei*. Twenty-five albino mice were used for the study. They were randomly assigned to five groups of five each, as follows: Group 1 – Normal control; Group 2 – Infected with parasite only (PO); Group 3 – Intoxicated with lead only (LO); Group 4 – Infected with the parasite before lead intoxication (PL); and Group 5 – Lead intoxication before infection with the parasite (LP). Lead intoxication was administered through drinking water, and the LP group was intoxicated six days before the *Plasmodium berghei* infection. The percentage of *Plasmodium* parasitaemia, haemoglobin (Hb) concentration, red blood cell (RBC) morphology and some oxidative stress parameters were evaluated. Results showed that the percentage of parasitaemia was significantly ($p < 0.05$) lower in the PL and LP groups compared to the PO group. Haemoglobin concentration was significantly ($p < 0.05$) lower in the LO group. The LP group had a significantly ($p < 0.05$) higher number of macrocytes, while the LO and PO groups had a significantly ($p < 0.05$) higher number of microcytes. The PO group further had significantly higher plasma level of malondialdehyde while the LP, LO and PO groups had significantly ($p < 0.05$) higher plasma catalase activity. These findings suggest that time of exposure played a crucial role in the interaction between lead intoxication and the *Plasmodium berghei* infection on the blood and oxidative stress parameters of the mice.

Keywords: Lead intoxication; *Plasmodium berghei* infection; Mice; Parasitaemia; Haemoglobin concentration; Red Blood Cell Morphology; Oxidative stress parameters.

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Introduction

Environmental pollutants refer to toxic agents or substances produced by humans or introduced into the environment through human activities. They can include a wide range of compounds, ranging from inorganic substances such as heavy metals to numerous organic compounds such as pharmaceuticals and medicines. Twenty-three heavy metals are required in small amounts to maintain health, but when their concentrations surpass the maximum acceptable limits, they become harmful (Jaishankar *et al*., 2014; Sokan-Adeaga *et al*., 2023). Most heavy metal pollution results from anthropogenic activities such as mining, smelting operations, industrial production and use, and domestic and agricultural use of metals and metal-containing compounds (Tchounwou *et al*., 2012). All organisms in the environment, including plants and all animal taxa (free-living and parasites), may be affected by heavy metal pollution (Sanchez *et al*., 2016).

Malaria, caused by the *Plasmodium* parasite, is a disease that holds a special place in the annals of public health history. It is prevalent in sub-Saharan Africa, Amazonia, and South Asia, and is a complex parasite that has proven difficult to defeat. In these regions, malaria is the leading cause of morbidity and mortality (Simwela *et al*., 2022). There is a need to continue studying the parasite's development, its host-pathogen interactions, efficacy of drugs and vaccine used against it, and the effects of the parasite's interaction with environmental pollutants (as multiple stressors) on host physiology and fitness. Since *P. falciparum*, the parasite that causes malaria in humans, does not infect rodents and *in vitro* cultures of liver and blood *Plasmodium* stages poorly translate to *in vivo* situations, rodent malaria parasites are used for studies. These rodent malaria species, which include *P. berghei, P. yoelli, P. chabaudi,* and *P. vinckei*, share a highly conserved chromosomal gene synteny with *P. falciparum*. This allows them to be used for *in vivo* studies of different aspects of the parasite's biology. (Carravieri *et al*., 2020; Simwela *et al*., 2022; Jimenez-Diaz *et al*., 2023).

Parasites and hosts can react differently to heavy metal pollution in the environment. However, in most cases, host responses to parasites and pollutants are similar (Sures, 2008). For instance, a study conducted on brine shrimp *Artemia* showed that tapeworm infection made them resistant to arsenic pollution, even under increased temperature. The tapeworm parasite was found to increase antioxidant enzyme defences in the brine shrimp in the presence of the pollutant (Sanchez *et al*., 2016). Another study conducted on mice showed that lead acetate exposure three weeks before *Trypanosoma cruzi* infection enhanced the susceptibility of mice to the infection. This resulted in higher parasitemia because lead can decrease antibody production, T-cell recognition, and macrophage activity in mice (Ellis, 1996). Studies on the interaction between cadmium exposure and infection with intestinal parasites *Moniliformis moniliformis* (Acanthocephala) on stress hormone levels in rats showed that cadmium and the parasite presence in the rats significantly reduced cortisol uptake compared to the control (Sures *et al*., 2002). This affected hormone homeostasis in the rats, leading to negative health effects (Sures *et al*., 2002). In another study, lead intoxication moderately increased the burden of *Ascaris suum* infection in mice because, in the presence of lead acetate, the parasite suppressed the peritoneal macrophages of the mice (Jalčová and Dvorožňáková, 2014). However, cadmium and mercury intoxication triggered a marked reduction of parasite burden in mice. This was reported to be a result of the fact that cadmium increased the superoxide production and also stimulated the activity of oxygen radicals after *A. suum* infection (Jalčová and Dvorožňáková, 2014). Mercury intoxication

had also been reported to have a stimulative effect on macrophage metabolic activity, and subsequent *A. suum* infection moderately reduced this activity (Jalčová and Dvorožňáková, 2014). In an experimental *Schistosoma mansoni* infection in mice, El-Gohary *et al*. (2003) reported that chronic lead exposure elicited significant reductions in worm burden, tissue egg load, and ova excretion in the stool, liver, and intestine of infected mice, compared to the control group.

The effects of heavy metal pollution on responsiveness in host-parasite interactions may depend on factors such as the level and duration of the toxic exposure or the route and time of administration. A study on the effects of lead acetate, nickel chloride, and sodium selenite on the resistance towards *Klebsiella pneumonia* infection revealed that nickel administered 24 hours or 3-day pre-treatment enhanced the resistance of mice against *K. pneumonia* (Laschi-Loquerie *et al*., 1987). There are no reports in available literature on the interactions between the time of lead intoxication and *Plasmodium* parasite dynamics in both humans and animals. This present study investigated the effects of the time of lead acetate intoxication on some blood and oxidative stress parameters of mice experimentally infected with *Plasmodium berghei*.

Materials and Methods

Experimental animals: Twenty-five Swiss albino mice (7 – 9 weeks old), weighing 21 – 22 g, were used in the experiment. The mice were purchased from the Laboratory Animal House Facility, Department of Biology, Alex Ekwueme Federal University Ndufu-Alike, Ikwo, Ebonyi State, Nigeria. The mice were kept in cages with perforated plastic lids and had free access to commercially available feed and drinking water. They were acclimatized to laboratory conditions for one week before the study proper commenced.

Plasmodium Parasite used for the study: *Plasmodium berghei* (ANKA strain) in donor mice, procured from the University of Nigeria Nsukka, was used for the study. The donor mouse was euthanized using halothane and blood was immediately collected through a cardiac puncture onto a petri dish containing 2% trisodium citrate as an anticoagulant. The blood was then diluted with physiological saline (0.9%) in such a way that 1 mL of blood contained 5 $\times 10^7$ infected red blood cells (RBCs).

Lead toxicant used for the study: The lead acetate trihydrate, (Pb $(CH_3COO)_2 \cdot 3H_2O$), Product Code – 15300 (purity: 99%) used for the study was procured from Molychem, Mumbai, India.

Experimental design: The experimental protocol was approved by the Institutional Animal Care and Use Committee of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka (FVM-UNN-IACUC-2022-0399). The 25 mice were randomly assigned to five groups of five each, as follows: Group 1 – Normal Control, mice without intoxication and infection; Group 2 (LO) – Mice intoxicated with lead acetate from day 6 to the end of the experiment (day 15); Group 3 (PO) – Mice infected with *P. berghei* only at day 0 with no lead intoxication; Group 4 (PL) – Mice infected with *P. berghei* on day 0 and intoxicated with lead acetate from confirmation of infection till the end of experiment; Group 5 (LP) – Mice given lead acetate from day 0 till the end of the experiment and infected with *P. berghei* on day 6.

Infection of the mice with *Plasmodium berghei*: The diluted blood from the donor with 30% parasitaemia was used for the infection. Each mouse in the infected groups (PO, PL and LP) were injected intra-peritoneally with 0.2 ml of the diluted blood containing 5 $\times 10^7$ infected RBCs. Seventy-two hours after infection, parasitaemia was confirmed in the infected mice.

Administration of the lead acetate: The lead acetate was administered in drinking water to the mice in Groups 2, 4 and 5 (LO, PL and LP) at 10 mg/l *ad libitum*.

Determination of Parasitaemia: A thin blood film was prepared from each infected mouse blood and stained with 10% Giemsa. The percentage of parasitemia was determined by counting five fields of approximately 100 – 200 cells in each slide, which included both infected and uninfected red blood cells (RBCs). The percentage of parasitemia was calculated on day 4 to confirm the establishment of infection and on day 16 for infected groups. To calculate the percentage of parasitemia, the number of plasmodium-parasitized RBCs was divided by the total number of RBCs counted and then multiplied by 100.

Blood sample collection: The mice were anaesthetized and sacrificed 24 hours after receiving treatment on day 15. Blood samples were taken from the heart and collected in ethylenediamine tetra-acetic acid (EDTA) bottles for the determination of haemoglobin concentration and evaluation of red blood cell morphology, while plasma derived from centrifuging the anti-coagulated blood was used to determine oxidative stress parameters.

Determination of Haemoglobin (HB) levels and Red Blood Cell Morphology: Haemoglobin concentration was quantitatively determined using Drabkin's colorimetric method (Whitehead *et al.*, 2019). Red blood cell morphology was determined using a thin blood smear stained with Giemsa and viewed under the microscope at × 100 magnification.

Determination of oxidative stress markers – Malondialdehyde (MDA) levels and Catalase activity: The oxidative stress markers were measured using plasma. Plasma MDA level was determined using the method of thiobarbituric acid, which measures MDA-reactive products, as described by Todorova *et al*. (2005). Briefly, 0.5 ml of each plasma sample was mixed with 0.5 ml of physiological solution and 0.5 ml of 25% trichloroacetic acid and centrifuged at 2,000 rpm for 20 minutes. One millilitre of the protein-free supernatant was aspirated after the centrifugation and mixed with 0.25 ml 0.5% thiobarbituric acid and heated at 95°C for one hour. After the heating, the mixture was cooled, and the intensity of the pink colour of the end fraction product was determined by a spectrophotometer at 532 nm. The MDA concentration was then calculated by comparing the absorbance of the samples with that of a blank and a standard. Catalase was estimated according to the method of Aebi (1984). Catalase degrades hydrogen peroxide which is measured directly by decrease in absorbance at 240nm.

Statistical analysis: GraphPad Prism was used to analyze the data generated. One-way analysis of variance followed by Turkey multiple comparison test was used to compare the means of groups for parameters considered. Significance was accepted at $p < 0.05$. Results were expressed as mean ± standard error.

Results

Percentage parasitaemia: The percentage of parasitized RBCs (parasitaemia) of the PO group was significantly higher ($p < 0.05$) than that of the PL and LP groups (Figure 1). There was no significant difference ($p > 0.05$) between the percentage parasitaemia of the PL and LP groups, but the percentage parasitaemia of the LP group was relatively lower than that of the PL group (Figure 1).

Haemoglobin (Hb) concentrations: The Hb concentration of the PL group did not differ significantly ($p > 0.05$) from that of the normal control, but it was significantly ($p < 0.05$) higher than those of all other groups (Figure 2). The LO group had the lowest Hb concentration which was significantly lower than those of all other groups (Figure 2).

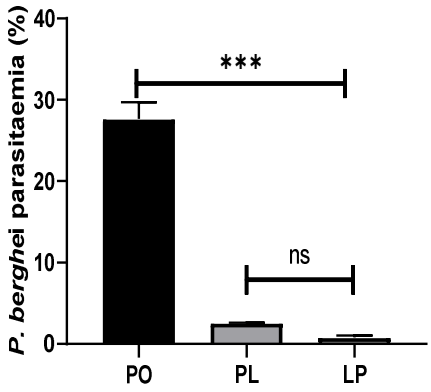

Figure 1. Percentage parasitaemia of mice infected with *Plasmodium berghei* and intoxicated with lead acetate. [PO – Infected with the *Plasmodium* parasite only; PL – Infected with the *Plasmodium* parasite before lead intoxication; LP – Intoxicated with Lead acetate before infection with the *Plasmodium* parasite].

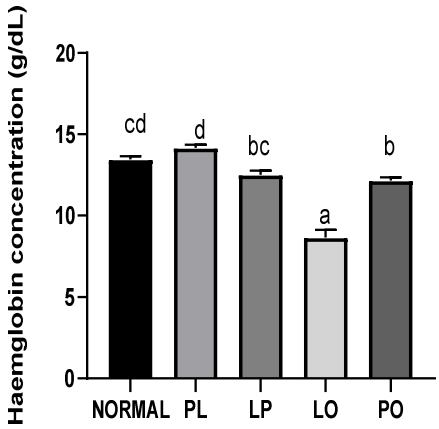

Figure 2. Haemoglobin concentration of mice groups infected with *Plasmodium berghei* and intoxicated with lead acetate, compared with normal control group. [Normal – Normal control group not given lead and not infected; PL – Infected with the *Plasmodium* parasite before lead intoxication; LP – Intoxicated with Lead acetate before infection with the *Plasmodium* parasite; LO – Intoxicated with lead only; and PO – Infected with the *Plasmodium* parasite only].

Red Blood Cell Morphology: The normocyte RBC count of the PL group was not significantly different ($p > 0.05$) from that of the normal control group, but those of LP, LO and PO groups were significantly ($p < 0.05$) lower than those of the normal control and PL groups (Figure 3). Macrocyte RBCs were not observed in the PL and PO groups, but the LP group showed a significantly ($p < 0.05$) much higher macrocyte count compared to the normal control and LO groups (Figure 4). Microcytes were not observed in the blood of the normal control mice (Figure 5). The microcyte RBC counts of the PL group did not significantly ($p > 0.05$) differ from that of the LP group, but both were significantly ($p < 0.05$) lower than those of the LO and PO groups (Figure 5). The PO group had the highest microcyte RBC counts that were significantly higher than those of all other groups (Figure 5).

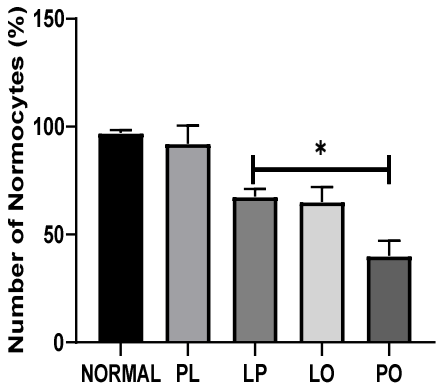

Figure 3. Normocyte red blood cell counts of mice groups infected with *Plasmodium berghei* and intoxicated with lead acetate, compared with a normal control group. [Normal – Normal control group not given lead and not infected; PL – Infected with the *Plasmodium* parasite before lead intoxication; LP – Intoxicated with Lead acetate before infection with the *Plasmodium* parasite; LO – Intoxicated with lead only; and PO – Infected with the *Plasmodium* parasite only].

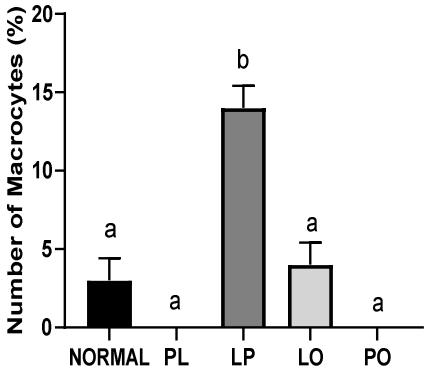

Figure 4. Macrocyte red blood cell counts of mice groups infected with *Plasmodium berghei* and intoxicated with lead acetate, compared with a normal control group. [Normal – Normal control group not given lead and not infected; PL – Infected with the *Plasmodium* parasite before lead intoxication; LP – Intoxicated with Lead acetate before infection with the *Plasmodium* parasite; LO – Intoxicated with lead only; and PO – Infected with the *Plasmodium* parasite only].

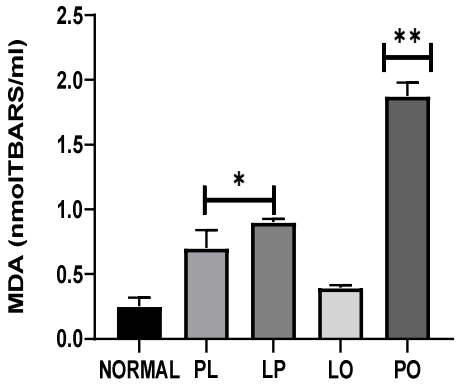

Figure 6. Plasma malondialdehyde levels of mice groups infected with *Plasmodium berghei* and intoxicated with lead acetate, compared with a normal control group. [Normal – Normal control group not given lead and not infected; PL – Infected with the *Plasmodium* parasite before lead intoxication; LP – Intoxicated with Lead acetate before infection with the *Plasmodium* parasite; LO – Intoxicated with lead only; and PO – Infected with the *Plasmodium* parasite only].

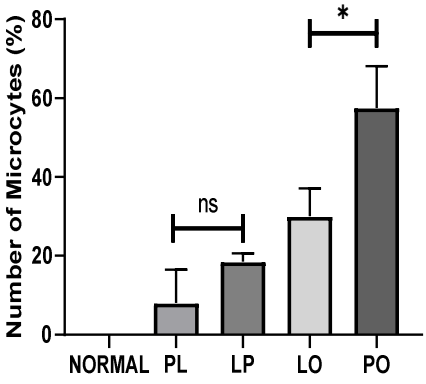

Figure 5. Microcyte red blood cell counts of mice groups infected with *Plasmodium berghei* and intoxicated with lead acetate, compared with a normal control group. [Normal – Normal control group not given lead and not infected; PL – Infected with the *Plasmodium* parasite before lead intoxication; LP – Intoxicated with Lead acetate before infection with the *Plasmodium* parasite; LO – Intoxicated with lead only; and PO – Infected with the *Plasmodium* parasite only].

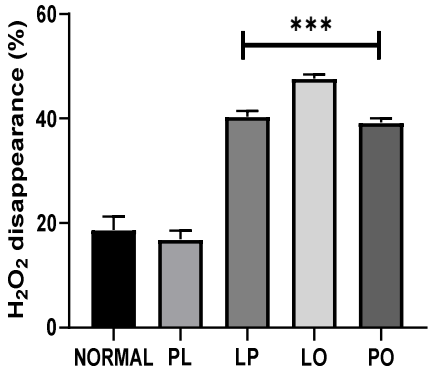

Figure 7. Plasma catalase activity of mice groups infected with *Plasmodium berghei* and intoxicated with lead acetate, compared with a normal control group. [Normal – Normal control group not given lead and not infected; PL – Infected with the *Plasmodium* parasite before lead intoxication; LP – Intoxicated with Lead acetate before infection with the *Plasmodium* parasite; LO – Intoxicated with lead only; and PO – Infected with the *Plasmodium* parasite only].

Oxidative Stress Parameters: The plasma MDA levels of PL, LP and PO groups were significantly ($p < 0.05$) higher than those of the normal control and LO groups, with the plasma MDA level of the PO group being the highest and being far significantly ($p < 0.05$) higher than those of all other groups (Figure 6). However, the plasma catalase activity of LP, LO and PO groups was significantly ($p < 0.05$) higher than those of the normal control and PL groups, with the plasma catalase activity of the LO group being the highest (Figure 7).

Discussion

The significantly lower levels of percentage parasitaemia recorded for the two lead-intoxicated groups in this study relative to the group not intoxicated with lead is believed to be due to the effects of high blood lead levels on hepcidin expression (Mukisa *et al.,* 2020). High levels of lead in blood is reported to upregulate hepcidin expression, which decreases available serum iron important for parasite survival leading to a low parasite burden (Mukisa *et al.,* 2020). Similar lower parasite burdens have been reported in mice infected with *Schistosoma* and exposed to lead acetate (El-Gohary *et al*., 2003). On the contrary however, cadmium and mercury exposure in mice infected with *A. suum*, and lead exposure in mice infected with *T. cruzi*, have been shown to cause enhanced *A. suum* burden and *T. cruzi* parasitaemia (Ellis, 1996; Jalčová and Dvorožňáková, 2014). Although there was a reduction in parasitaemia in the two lead-intoxicated-*Plasmodium* infected groups (PL and LP), the percentage of parasitaemia in the LP group was lower, implying that the time of intoxication with lead had a significant effect. It has similarly been reported earlier by Laschi-Loquerie *et al*. (1987) that exposure of mice to 8 or 12 mg/kg lead acetate for 3 days before infectious challenge with *K. pneumonia* enhanced their resistance to the infection.

Both LO and PO groups had a significantly lower haemoglobin concentration when compared to the other groups. Lower haemoglobin levels following lead exposure and *Plasmodium* infection in animals has earlier been reported (Asangha *et al*., 2017; Helegbe *et al*., 2009; Ibrahim *et al*., 2012; Ilesanmi *et al*., 2022; Kanu *et al*., 2022). Lead had been reported to inhibit the body's ability to synthesize haemoglobin by interfering with various enzymatic steps in the heme synthesis pathway, thus reducing the haemoglobin concentration (CDC, 2023). On the other hand, during its blood stage, *Plasmodium* degrades haemoglobin from the host, causing a reduction in haemoglobin concentration (Elliott *et al*., 2008). Although the haemoglobin level in LP was not significantly different from the normal level, it was lower than that in PL. This could be a result of time of lead intoxication before the parasite infection. In the PL group, the effect of lead intoxication after infection was not seen, as the haemoglobin concentration in this group was comparatively higher; this suggests that the presence of the *Plasmodium* parasite may have interfered with the action of lead on haemoglobin concentration.

Normocyte RBC counts were found to be significantly lower in the LO, PO and LP groups in this study. This suggests that mice in these groups have a lower number of normal-sized RBCs compared to mice in the normal control and PL groups (Cleveland Clinic, 2022). Studies on lead intoxication and *Plasmodium* infection in mice have also reported low normocyte RBC counts in treatment groups compared to the normal (Helegbe *et al*., 2009; Ibrahim *et al*., 2012). However, in the case of the LP group, the significantly lower normocyte RBC count could further be attributed to the longer exposure to lead-contaminated drinking water.

A significantly high number of macrocyte RBCs were recorded for the LP group in this study, indicating a higher presence of large-sized red blood cells in this group. This suggests that mice in the LP group may have suffered from macrocytic anaemia (Cleveland Clinic, 2023a). This condition was not observed in any other group in the study, suggesting that it could be a result of the preliminary administration of lead before *Plasmodium* infection in this group.

Microcytes are red blood cells that are smaller than normal in size, and microcytosis is a condition where an animal or individual has smaller-than-normal-sized red blood cells (Cleveland Clinic, 2023b). The presence of microcytes usually suggests a defect in red blood cell/haemoglobin formation (Bain, 2017). In this study, microcytes were not observed in the normal control group, and the percentage of microcytes found in the PL and LP groups were comparable. This suggests that the mice in the PL and LP groups may not have suffered from haemoglobin formation defects, as evidenced by their haemoglobin content which was statistically not different from that of the normal group. However, the LO and PO groups had a significantly higher population of microcytes than the other groups, suggesting a possible defect in haemoglobin formation in these mice. Other earlier studies have reported microcytic anaemia in cases of lead exposure and *Plasmodium* infection (Sama *et al*., 2021; Wani *et al*., 2015).

In the present study, plasma MDA levels in the PL, LP and PO groups were significantly higher than those of LO and normal control, indicating that these groups suffered more oxidative stress caused by lipid peroxidation (El-Gohary *et al*., 2003). Previous studies (Ercal *et al*., 1996; Fan *et al*., 2020) have shown that lead exposure can increase oxidant levels, but this was not observed in this study. The LO group had plasma MDA levels comparable to that of the normal control, suggesting that short-term lead exposure did not elevate MDA levels as reported by Dobrakowski *et al* (2017). It is believed that the higher plasma MDA levels in the PL and LP groups was triggered by the presence of *P. berghei* in the blood. Increased blood concentrations of MDA in *Plasmodium* infections have been earlier reported (Sharma *et al.*, 2012; Dogruman-Al *et al.,* 2015; Mueangson *et al.*, 2023).

Significantly high plasma catalase activity was recorded for the LP, LO, and PO groups in this study. It is well-established that lead intoxication and *Plasmodium* infection reduce blood catalase activity. Catalase works by breaking down hydrogen peroxide into water and oxygen, thereby detoxifying it (Nandi *et al*., 2019). The high plasma catalase activity observed in the LO group in the present study is contrary to earlier published reports. In an earlier study, Adegbesan and Adenuga (2007) reported an increase in catalase activity following lead intoxication. They suggested that the reason for this high plasma catalase activity could be due to the production of radicals like H_2O_2 and superoxides. This could explain the high plasma catalase enzyme activity recorded for the LO group in this particular study. The high plasma catalase activity seen in PO mice could be due to the increased production of reactive oxygen species by the immune system, as well as the synchronized release of superoxide during haemoglobin degradation by the malarial parasite (Sohail *et al*., 2007). Earlier reports by Sohail *et al*. (2007) and Ozojiofor *et al*. (2021) found that serum catalase activity was high in the *P. falciparum* and *P. vivax*-infected patients/groups, respectively. The high plasma catalase activity recorded in the LP group in comparison to the PL group could be attributed to the effect of the time of lead intoxication.

In conclusion, the results of this study suggest that the time of lead intoxication significantly affected the course of *Plasmodium* infection in the mice. Lead intoxication reduced the susceptibility to *Plasmodium* parasite

establishment and significantly affected the red blood cell morphology, especially when the lead was administered before the *Plasmodium* infection. Though the time of lead intoxication did not affect the MDA levels, higher catalase activity was recorded for the group given lead before *Plasmodium* infection.

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Conflict of Interest

The authors declare no conflict of interest.

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