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ANTIMALARIAL AND ANTIOXIDANT EFFECTS OF *Persea Americana* (avocado) LEAF EXTRACT IN *Plasmodium berghei*-INFECTED MICE

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ABSTRACT

The emergence of drug-resistant *Plasmodium* strains necessitates the search for alternative treatment strategies, including plant-derived bioactive compounds with antimalarial and antioxidant properties. *Persea americana* (avocado) leaves have been reported to possess medicinal benefits, but their antimalarial potential remains underexplored. This study evaluates the antimalarial and antioxidant effects of *P. americana* leaf extract in *Plasmodium berghei*-infected mice. Mice infected with *P. berghei* were treated with varying doses of the extract, and body weight, rectal temperature, packed cell volume (PCV), parasitemia levels, and oxidative stress markers were assessed. Results demonstrated a significant improvement in body weight and PCV, as well as reductions in rectal temperature, parasitemia, improved parasite clearance, and enhanced antioxidant enzyme activity in treated groups compared to the untreated control. These findings suggest that *P. americana* leaf extract possesses potent antimalarial and antioxidant properties, supporting its potential as a complementary therapeutic agent. Further studies are needed to elucidate its mechanisms of action and optimize dosage for clinical applications. This study contributes to the ongoing search for plant-based alternatives in malaria treatment and oxidative stress management.

Keywords: Antimalaria, Antioxidant, *Persea Americana*, Oxidative stress

INTRODUCTION

Malaria remains a major global health concern, particularly in sub-Saharan Africa, where it causes significant morbidity and mortality (Kolawole et al., 2023). The disease is caused by Plasmodium species, with *Plasmodium falciparum* being the most lethal in humans. However, experimental models often utilize *Plasmodium berghei*, a rodent parasite, to study antimalarial interventions. The emergence of drug-resistant malaria strains has necessitated the search for alternative treatment strategies, including plant-derived bioactive compounds with potent antimalarial and antioxidant properties (Evbuomwan et al., 2025; Oluba, 2019).

Persea americana (Avocado) is widely known for its nutritional and medicinal benefits, with its polyphenolic compounds exhibiting strong antioxidant, anti-inflammatory, and antimicrobial properties (Onyedikachi et al., 2024). Recent studies have highlighted the therapeutic potential of avocado polyphenolic extract in various disease models (Eidangbe and Oluba, 2024), but its efficacy against malaria-induced oxidative stress and parasite burden remains largely unexplored. The pathogenesis of malaria is associated with increased oxidative stress, which leads to the destruction of red blood cells, organ damage, and immune system dysregulation (Mavondo et al., 2019). Plasmodium infection triggers excessive production of reactive oxygen species (ROS), causing oxidative damage to host cells and worsening disease severity. Polyphenols, as natural antioxidants, can neutralize ROS, protect cells from oxidative damage, and modulate immune responses, thereby potentially enhancing host resistance to malaria (Varela et al., 2022).

The antimalarial activity of avocado polyphenols may be attributed to multiple mechanisms, including direct inhibition of Plasmodium growth, modulation of host immunity, and protection against oxidative damage (Hamza et al., 2013). Polyphenols have been reported to interfere with parasite metabolism, disrupt heme detoxification, and modulate inflammatory cytokine responses, which are critical for parasite clearance (Pradniwat, 2024). Moreover, the antioxidant effects of avocado polyphenol may help mitigate malaria-induced anemia, liver dysfunction, and systemic inflammation, improving disease outcomes.

This study aims to investigate the antimalaria and antioxidant activities of avocado polyphenolic extract in *Plasmodium berghei*-infected mice. Specifically, it seeks to evaluate the curative effects of avocado extract against *P. berghei* infection as well as assess its effects on oxidative stress markers in infected mice. By elucidating the dual antimalaria and antioxidant effects of avocado polyphenolic extract, this research could contribute to the development of plant-based adjuvant therapies for malaria management. If proven effective, avocado extract may serve as a natural, cost-effective alternative or complementary agent to existing antimalarial drugs, especially in malaria-endemic regions where access to conventional treatments is limited. Furthermore, its antioxidant properties could help mitigate malaria-associated complications, making it a promising candidate for further pharmacological investigations.

MATERIALS AND METHODS

Plant material

Fresh avocado leaves were collected in Ekpoma, Nigeria. It was identified and authenticated by Prof. E.I. Aigbokhan, a botanic expert in the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria.

Ethical consideration and approval

All animal experiments were conducted in full accordance with the Guidelines for the Care and Use of Laboratory Animals, as well as the National Research Council's *Guide for the Care and Use of Laboratory Animals* (National Research Council, 2010). Ethical approval for the study was obtained from the Ambrose Alli University Research Ethical Review Committee. Furthermore, the study was reported in strict adherence to the Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) 2.0 guidelines (Percie du Sert et al., 2020).

Extraction procedure

The leaves were thoroughly rinsed under running tap water to eliminate surface debris and air-dried for two weeks in a well-ventilated, shaded area at ambient temperature (approximately 25°C). Once dried, they were pulverized using a kitchen blender and sieved to obtain a fine powder. The crude extract was prepared using the cold maceration method. A total of 500 g of the powdered plant material was macerated in distilled water within a 3 L glass jar for 48 h, with continuous shaking on a horizontal laboratory shaker (Thermoforma, USA) and occasional stirring at room temperature. The mixture was initially filtered through a muslin cloth and subsequently passed twice through a 125 mm Whatman No. 1 filter paper (Wagtech International Ltd., England) to obtain the crude aqueous extract. To enhance yield, the residual plant material underwent three additional maceration cycles. The filtrate was then concentrated using a rotary evaporator (Bibby Scientific Ltd., Staffordshire, UK) at 45 rpm and 60°C, followed by lyophilization in a freeze-dryer (LTE Scientific Ltd., Oldham, UK). The resulting crude extract was stored in a screw-capped glass vial at 4°C until further analysis.

Experimental animals and treatment

Thirty (30) male Swiss albino mice, aged 8 to 12 weeks and weighing between 19.5 and 23.8 g, were housed in standard plastic cages under controlled laboratory conditions (25±5°C, 12-hour light/dark cycle) in the Animal Holding Unit of the Department of Medical Biochemistry, Ambrose Alli University, Ekpoma, Nigeria. The mice were handled in accordance with ethical guidelines and provided with standard pelletized rodent feed and water *ad libitum*. They were randomly assigned to five experimental groups (n=7) (Table 1). All treatments were administered via oral gavage using a stainless steel feeding cannula.

Table 1: Experimental grouping and dosage

Groups (n=6)	Treatments 135-137
NC	Normal control, non-infected mice administered with distilled water
INF	Infected and untreated, administered with distilled water
INF + CQ	Infected and treated with standard drug – 10 mg/kg chloroquine (CQ) phosphate
INF + AE_{100}	Infected and treated with 100 mg/kg bw avocado leaf extract
INF + AE_{200}	Infected and treated with 200 mg/kg bw avocado leaf extract

Preparation of standard inoculums and parasite inoculation

A chloroquine-sensitive *Plasmodium berghei* NK65 strain, obtained from the Drug Research Laboratory, IAMRAT, University of Ibadan, Nigeria, was used to induce experimental malaria in mice. Parasitized blood was collected from four donor *P. berghei*-infected mice with parasitemia levels of 20–30%. Following parasitemia assessment, the donor mice were euthanized using 2% isoflurane, and blood was collected via retro-orbital venipuncture using a heparinized hematocrit capillary tube. The collected blood was transferred into a Falcon tube containing 4 mL of anticoagulant citrate dextrose solution, Solution A, USP (2.13% free citrate ion). The blood was then diluted with physiological isotonic saline (0.9%) according to the donor mice's parasitemia levels (Hilou et al., 2006), ensuring that 1 mL of blood contained approximately 5×10^7 *P. berghei*-infected red blood cells (iRBCs). Each experimental mouse was subsequently injected intraperitoneally with 0.2 mL of the prepared blood suspension, delivering approximately 10^7 *P. berghei*-iRBCs per mouse (Evbuomwan et al., 2025).

Curative (Rane's) test

The Rane's curative efficacy of *P. americana* (avocado) leaf extract against established malaria infection in mice was assessed using a slightly modified version of the method previously described by Oluba et al. (2020). On day 0 (D0), mice were inoculated with *P. berghei*. After 72 hours post-infection, baseline parasitemia was determined, and treatment commenced. The extract was administered orally once daily for five consecutive days (D3 to D7) at 24-hour intervals between doses.

Parasitaemia measurement

Parasitemia was monitored using Giemsa-stained thin blood smears prepared from tail snips of each mouse. For the 4-day suppressive test, smears were examined on day 4 (D_4); for the prophylactic test, parasitemia was assessed on day 7 (D_7), 72 h post-infection; and for the curative test, monitoring was conducted from D3 to D_7 following infection establishment. Slides were examined under a light microscope (×100, oil immersion objective) by an experienced, blinded microscopist. Animal survival was assessed using Kaplan–Meier survival analysis, with comparisons made using the log-rank (Mantel-Cox) test. Percentage parasitemia and percentage inhibition were calculated using Equations 1 and 2.

$$\% \text{ Parasitaemia} = \frac{\text{Number of parasitised erythrocytes}}{\text{Total number of erythrocytes}} \times 100\% \quad (1)$$

$$\% \text{ Inhibition} = \frac{(\text{Mean parasitaemia of untreated group} - \text{Mean parasitaemia of treated group})}{\text{Mean parasitaemia of untreated group}} \times 100\% \quad (2)$$

Body weight and rectal temperature changes

Monitoring changes in rectal temperature and body weight is essential for assessing the effectiveness of medicinal plant extracts against malarial infection. Body weight was measured using a sensitive digital weighing balance (Want Balance Instrument Co., Ltd., Jiangsu, China) before infection, during the infection, and after the treatment period. Rectal temperature was recorded using a digital rectal thermometer (Omron Healthcare Co., Ltd., Japan). Both weight and temperature measurements were taken on day 0 (D_0), day 4 (D_4), and day 7 (D_7).

Packed cell volume (PCV) measurement

Packed cell volume (PCV) was measured to evaluate the efficacy of the test extract in preventing hemolysis caused by parasite multiplication within red blood cells. Blood samples were collected from the tail of each mouse using heparinized capillary tubes and centrifuged in a Microhematocrit centrifuge (Hawksley and Sons Ltd., Lancing, Sussex, England) at 11,000 rpm for 7 min. PCV was then determined using a standard Microhematocrit Reader (Hawksley and Sons Ltd., Lancing, Sussex, England). Equation 3 (Mekonnen, 2015) was applied to estimate PCV levels on different days across the experimental models: D_0, D_4, and D_7

in the curative test; D_0, D_4, D_7, and D_9 in the prophylactic test; and D_0, D_4, and D_6 in the 4-day suppressive test.

$$PCV = \frac{\text{Volume of erythrocytes in a given volume of blood}}{\text{Total blood volume}} \quad (3)$$

Blood collection and serum preparation

Blood samples were collected from representative animals into 5 mL ethylenediaminetetraacetic acid (EDTA) tubes for hematological analysis and plain bottles for biochemical assays. Collection was performed via retro-orbital venipuncture following euthanasia with 2% isoflurane. Blood in plain bottles was allowed to clot for 30 minutes before centrifugation at 5000 rpm for 15 minutes to separate the serum. The obtained serum was then stored at -20°C for subsequent biochemical analyses and assessment of proinflammatory markers.

Malondialdehyde (MDA) concentration determination

Serum malondialdehyde (MDA) levels were measured as an indicator of lipid peroxidation. A thiobarbituric acid/trichloroacetic acid/hydrochloric acid (TBA/TCA/HCl) solution was prepared by diluting the stock solution four-fold, with butylhydroxytoluene added to the final concentration. The aqueous lipid suspension and blank were mixed with the TBA/TCA/HCl reagent in a 2:1 (v/v) ratio, thoroughly mixed, and heated in a boiling water bath for 5 minutes before cooling. The mixture was then centrifuged at 3000 rpm for 10 min at 37°C. Absorbance was measured at 535 nm, and MDA levels were calculated using Equation 4.

$$\text{MDA concentration } (\mu M) = \frac{As - Ab * Df}{1.56 \times 1000000 \times \text{protein concentration}} \quad (4)$$

A_s = Absorbance of the sample; A_b = Absorbance of the blank; DF = Dilution factor

1.56×10^6 = extinction coefficient of lipid hydroperoxides.

Nitric oxide (NO) determination

The sample was prepared by diluting 40 µL of the original sample with deionized water to a final volume of 190 µL. Subsequently, 10 µL of $ZnSO_4$ solution was added to the diluted sample, vigorously mixed, and incubated at room temperature for 15 minutes. The mixture was then centrifuged at 3000 rpm for 5 minutes, and the supernatant was collected. For further processing, 0.5 g of washed and dried cadmium (Cd) beads was placed in a centrifuge tube, and 150 µL of the deproteinized sample was added. The tube was incubated overnight at room temperature with continuous agitation. After incubation, the sample was transferred to a clean Eppendorf tube and centrifuged again at 3000 rpm for 5 minutes. Next, 100 µL of the prepared sample was seeded into a microplate in duplicate. To each well, 50 µL of sulphanilamide (p-amino-benzene-sulphonamide) in 3N HCl was added, followed by 50 µL of N-(1-Naphthyl) ethylenediamine dihydrochloride in H_2O. The plate was gently shaken for 5 minutes at room temperature, and absorbance was measured at 540 nm.

Catalase (CAT) activity determination

Catalase catalyzes the decomposition of hydrogen peroxide (H_2O_2), a by-product of cellular metabolism, into water (H_2O) and oxygen (O_2) (Equation 5) (Mavelli et al., 1982). For the control reaction, 1 mL of phosphate-buffered saline (0.01 M, pH 7.4) and 0.1 mL of H_2O_2 were combined in a tube, incubated for 5 minutes, and then mixed with 1 mL of dichromate/acetic acid reagent. For the sample reaction, 0.05 mL of serum sample was added to 1 mL of phosphate buffer (pH 7.4). The mixture was incubated at 37°C for 5 minutes, after which 0.1 mL of H_2O_2 was added, mixed thoroughly, and further incubated at 37°C for 1 minute. Subsequently, 1 mL of dichromate/acetic acid reagent was added. Both tubes were left to stand at room temperature for 10 minutes before measuring absorbance at 405 nm. Catalase activity was calculated using Equation 6.

$$2H_2O_2 \xrightarrow{CAT} 2H_2O + O_2 \quad (5)$$

The standard curve was generated by serially diluting a 100 µmol/L H_2O_2 standard solution with double-distilled water in a descending gradient to obtain final concentrations of 50, 40, 30, 20, 10, 5, 1, and 0 µmol/L.

$$\text{CAT activity (U/mg protein)} = \frac{\Delta A \times \text{Reciprocal of slope}}{\text{Reaction time} \times \text{Volume of sample}} \times f \div Cp \quad (6)$$

ΔA: Absolute OD ($OD_{Control} - OD_{Sample}$)

f: Dilution factor of sample before test

C_p: Concentration of protein in sample

1 unit is defined as the amount of catalase (CAT) in 1 g of tissue protein that decomposes 1 µmol H_2O_2/min at 37°C.

Superoxide dismutase (SOD) activity determination

The evaluation of superoxide dismutase (SOD) activity followed the protocol described by Misra and Fridovich (1972). This method quantifies the reduction in the autoxidation rate of pyrogallol (2,3-dihydroxy phenol), a reaction that is rapidly catalyzed in the presence of superoxide anions ($O_2^{\bullet-}$). The assay principle is based on the ability of SOD to convert $O_2^{\bullet-}$ into hydrogen peroxide (H_2O_2) and molecular oxygen (O_2), thereby inhibiting the autoxidation of pyrogallol, which would otherwise form yellowish-brown oxidation products. The kinetic reaction was monitored by measuring absorbance at 560 nm at 30-s intervals over 2.5 min, starting from time zero, using a microplate reader. Absorbance readings were taken five times, and SOD activity was calculated using Equation 7.

$$\text{SOD Activity (U/mL)} = \frac{\% \text{ Inhibition of pyrogallol autoxidation}}{50\%} \quad (7)$$

One unit of superoxide dismutase (SOD) activity is defined as the amount of enzyme required to achieve 50% dismutation of the superoxide radical ($O_2^{\bullet-}$), effectively inhibiting 50% of the autoxidation of pyrogallol. To determine SOD content in enzyme units, the percentage inhibition of pyrogallol autoxidation is calculated and then divided by 50. This provides the SOD activity in units per volume of the sample.

Glutathione peroxidase (GPx) activity determination

Glutathione peroxidase (GPx) catalyzes the reduction of hydrogen peroxide (H_2O_2) and organic hydroperoxides by utilizing reduced glutathione (GSH) as a substrate, producing water (H_2O) and oxidized glutathione (GSSG). The enzymatic activity of GPx is typically assessed by measuring the consumption of GSH in the reaction. To estimate GPx activity, the decrease in GSH levels is determined using Ellman's reagent (5,5'-dithio-bis-(2-nitrobenzoic acid), DTNB). DTNB reacts with free thiol groups in GSH to produce a stable yellow-colored 5-thio-dinitrobenzoic acid (TNB) anion, which can be quantified spectrophotometrically at 412 nm. The rate of this reaction is proportional to the GPx activity in the sample.

$$H_2O_2 + 2GSH \xrightarrow{GPx} 2H_2O + GSSG \quad (8)$$

Statistical analysis

Data were analysed using One-way analysis of variance (ANOVA) and represented as mean ± SEM of six determinations. Data were statistically analysed using GraphPad Prism v9 (GraphPad Software Inc., San Diego, California, USA). Tukey's post hoc test was used to evaluate statistical significance for grouped data at $p < 0.05$.

RESULTS AND DISCUSSION

Effects on bodyweight, rectal temperature, and pack cell volume

In Rane's curative test, treatment of *P. berghei*-infected mice with 100 and 200 mg/kg *bw* of AVE exerted a significant ($p < 0.05$) effect on body weights (Figure 1a) and rectal temperatures (Figure 1b) beginning from day 4 in comparison with infected untreated control. Infection with P. berghei typical of malaria infection leads to weight loss due to anorexia, metabolic disturbances, and increased energy expenditure caused by systemic inflammation. The reduction in weight loss or weight stabilization observed in avocado leaf extract-treated groups suggests that the extract may have protective effects, potentially mitigating infection-induced metabolic disruptions. Malaria-induced fever results from the release of pyrogens during schizogony, leading to fluctuations in body temperature (Hirako et al., 2025). The potential ability of avocado leaf extract to maintain normal temperature or reduces fever spikes observed in this study could be indicative of the extract antipyretic or anti-inflammatory properties, possibly due to bioactive compounds modulating immune responses. Similarly, the extract exerted a significant ($p < 0.05$) effect on the PCV of the treated mice in comparison with the untreated group on D4 and D7 (Figure 1c). These data indicate that therapeutic intervention with the plant can avert weight loss and improve body temperature and PCV in malaria-infected mouse models. Malaria-induced hemolysis usually results in a decline in PCV, leading to anemia, a hallmark of severe malaria (Tiiba et al., 2023). The augmentation in PCV due to avocado leaf extract treatment suggests that the extract may prevent excessive erythrocyte destruction or enhance erythropoiesis, possibly through antioxidant and anti-inflammatory mechanisms.

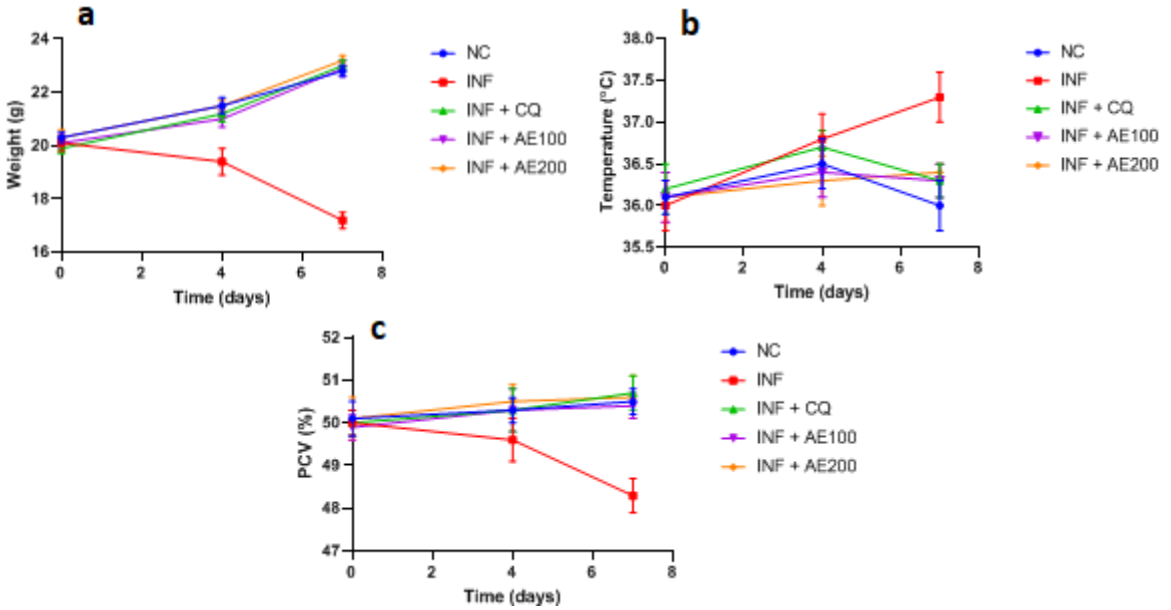

Figure 1: (a) Body weight, (b) rectal temperature, and (c) pack cell volume (PCV) of *Plasmodium berghei*-infected mice treated with avocado leaf extract. Results are means ± SEM of six determinations. Values in the same row carrying different superscripts are statistically significant. Note: NC, normal mice administered distilled water; INF, *P. berghei*-infected mice administered distilled water; INF + CQ, *P. berghei*-infected mice administered chloroquine; INF + AE_{100}, and INF + AE_{200}, *P. berghei*-infected mice administered 100 and 200 mg/kg bw of avocado leaf extract, respectively.

Effects on parasitemia and parasite inhibition

The study investigated Rane's curative effect of the extract on established malaria *in vivo*. Administration of AVE (at 100 and 200 mg/kg *bw*) significantly ($p < 0.05$) reduced parasitaemia level dose-dependently, in comparison with the untreated group (Figure 2a). Parasitaemia in the extract–treated groups measured on D_7 was lower than parasitaemia measured on previous days, showing that continued treatment with the extract caused a reduction of parasitaemia from the peak measured at D_4. The analysis of the inhibition activity of the extract shows that the extract exhibited the highest curative effect at a dose of 200 mg/kg bw on D7 with a parasitaemia inhibition effect of 80.4% (Figure 2b). Plasmodium berghei infection leads to a progressive increase in parasitemia levels, contributing to disease severity. The decrease in parasite load following the administration of avocado leaf extract suggests that the extract possesses antimalarial properties. The observed reduction in parasitaemia may be attributed to the phytoconstituents present in the plant, particularly alkaloids and flavonoids, which have been reported to inhibit the growth and proliferation of malaria parasites (Chaniad et al., 2023; Kumatia et al., 2023). A potential mechanism of action involves the extract preventing red blood cell (RBC) invasion, restricting the parasite's access to essential nutrients, or exerting a direct cytotoxic effect on the parasite (Gupta et al., 2022). Additionally, the extract may have contributed to parasite clearance by indirectly stimulating the host immune response, thereby modifying the properties of infected RBCs (Afolayan et al., 2020). Collectively, these findings suggest that the extract contains bioactive compounds with significant antimalarial potential and may serve as a basis for the development of novel antimalarial agents while also enhancing survival outcomes in the treated animals.

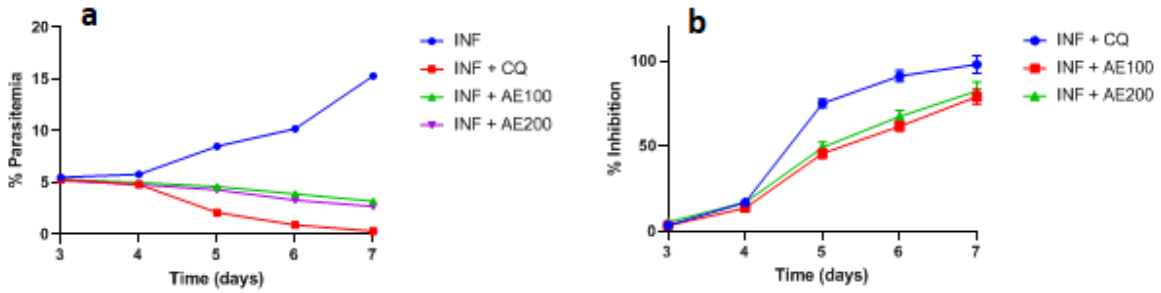

Figure 2: Effect of avocado leaf extract treatment on (a) Percentage parasitemia, and (b) parasite inhibition in *P. berghei*-infected mice. Results are means ± SEM of six determinations. Values in the same row carrying different superscripts are statistically significant. Note: NC, normal mice administered distilled water; INF, *P. berghei*-infected mice administered distilled water; INF + CQ, *P. berghei*-infected mice administered chloroquine; INF + AE_{100}, and INF + AE_{200}, *P. berghei*-infected mice administered 100 and 200 mg/kg bw of avocado leaf extract, respectively.

Effects on lipid peroxidation and antioxidant enzymes

The data in Table 1 show the effects of avocado leaf extract (AE) on oxidative stress markers and antioxidant enzyme activities in *P. berghei*-infected mice. *P. berghei*-infected mice (INF) exhibited a significant increase in malondialdehyde (MDA) levels (0.81 ± 0.01 µM/mL), indicating heightened lipid peroxidation due to oxidative stress from the infection. Treatment with chloroquine (INF + CQ) and avocado leaf extract at both 100 mg/kg (INF + AE_{100}) and 200 mg/kg (INF + AE_{200}) significantly reduced MDA levels compared to the INF group, suggesting a protective effect against lipid peroxidation. The INF group showed a marked increase in NO levels (0.09 ± 0.00 µM/mL), indicating an inflammatory response. Both chloroquine and avocado leaf extract significantly reduced NO levels, with AE_{100} and AE_{200} showing similar effects (0.04 ± 0.00 µM/mL), suggesting anti-inflammatory potential. The activities of catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) were significantly elevated in the INF group compared to the normal control (NC), likely as a compensatory response to oxidative stress. Treatment with CQ and avocado leaf extract (AE_{100}, AE_{200}) restored these enzyme activities closer to normal levels, suggesting an antioxidative effect. The AE_{100} and AE_{200} groups had slightly higher antioxidant enzyme activities than the CQ group, indicating that avocado leaf extract might provide additional benefits in mitigating oxidative damage. Malaria is characterized by heightened inflammation and oxidative stress. During *Plasmodium* spp. infection, excessive production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) compromises the host's endogenous antioxidant defense system, leading to redox imbalance and oxidative stress—common features of *Plasmodium* spp. infections (Evbuomwan et al., 2025). The increased ROS/RNS generation arises from hemoglobin degradation by the parasite (Counihan et al., 2021) and the host immune response, which triggers ROS/RNS production within phagosomes through a mechanism known as respiratory (oxidative) burst (Vasquez et al., 2021). Additionally, ROS released extracellularly during this process further exacerbates oxidative stress in the infected host (Nandi et al., 2022).

The observed depletion in catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) activities, alongside elevated malondialdehyde (MDA) and nitric oxide (NO) levels, indicates oxidative tissue damage and proinflammatory responses following *P. berghei* infection in mice. This aligns with previous studies reporting dysregulation of oxidative markers in malaria (Oluba, 2019; Oluba et al., 2020). Oxidative stress induction promotes the generation of superoxide anion ($O_2\bullet^-$) via the Fenton or Haber–Weiss reaction (Burda et al., 2020). To counteract this, the host upregulates SOD expression as a primary defense mechanism against $O_2\bullet^-$ (Karagianni and Bazopoulou, 2024). However, when excess $O_2\bullet^-$ is not efficiently converted by SOD into hydrogen peroxide (H_2O_2), it reacts with NO to form peroxynitrite ($ONOO^-$), a highly reactive species that exacerbates oxidative damage and plays a crucial role in inflammation (Bartesaghi and Radi, 2018). The elevated NO levels, coupled with reduced SOD activity, underscore the severity of oxidative stress in untreated animals.

Furthermore, the increased MDA levels in the untreated group suggest significant lipid peroxidation, a hallmark of oxidative tissue damage. The amelioration of these oxidative markers following treatment with avocado leaf extract highlights its potential to mitigate lipid peroxidation and restore redox homeostasis. These findings corroborate previous reports on the antioxidant protective effects of medicinal plants (Eidangbe and Oluba, 2024).

Table 2: Effect of avocado leaf extract on lipid peroxidation level and antioxidant enzymes activities in *Plasmodium berghei*-infected mice

Treatment groups	MDA (µM/mL)	NO (µM/mL)	CAT (U/mL protein)	SOD (U/mL protein)	GPx (U/mL protein)
NC	0.25 ± 0.02^a	0.02 ± 0.00^a	0.17 ± 0.01^a	0.15 ± 0.001^a	0.21 ± 0.001^a
INF	0.81 ± 0.01^c	0.09 ± 0.00^c	0.33 ± 0.03^c	0.28 ± 0.001^c	0.39 ± 0.001^b
INF + CQ	0.26 ± 0.02^a	0.02 ± 0.00^a	0.15 ± 0.01^a	0.15 ± 0.001^a	0.23 ± 0.002^a
INF + AE_{100}	0.33 ± 0.02^b	0.04 ± 0.00^b	0.25 ± 0.02^b	0.19 ± 0.001^b	0.24 ± 0.002^a
INF + AE_{200}	0.32 ± 0.01^b	0.04 ± 0.00^b	0.23 ± 0.01^b	0.18 ± 0.001^b	0.24 ± 0.001^a

Results are means ± SEM of six determinations. Values in the same row carrying different superscripts are statistically significant. Note: MDA, malondialdehyde; NO, nitric oxide; CAT, catalase; SOD, superoxide dismutase; GPx, glutathione peroxidase; NC, normal mice administered distilled water; INF, *P. berghei*-infected mice administered distilled water; INF + CQ, *P. berghei*-infected mice administered chloroquine; INF + AE_{100}, and INF + AE_{200}, *P. berghei*-infected mice administered 100 and 200 mg/kg bw of avocado leaf extract, respectively.

CONCLUSION

Overall, these results suggest that Persea americana leaf extract may provide antimalarial benefits by reducing weight loss, stabilizing temperature, and preventing severe anemia in Plasmodium berghei-infected mice. In addition, P. americana leaf extract exhibits notable antimalarial activity, evidenced by reduced parasitemia and high parasite inhibition rates. This supports its potential use in developing alternative or complementary antimalarial therapies, possibly due to its phytochemical constituents with antiparasitic and immunomodulatory effects.

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