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Effects of ginger (*Zingiber officinale*) rhizome ethanolic extract on the serum biochemistry and haematology profile of mice in a four-day suppressive test against experimental *Plasmodium berghei* infection

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Abstract

The rising occurrence of resistance of malarial parasites to antimalarial drugs makes it pressingly needful to search for more and newer possible antimalarial agents. The present study investigated the effects of ginger (*Zingiber officinale)* rhizome ethanolic extract on the serum biochemistry and haematology profile of mice in a four-day suppressive test against experimental *Plasmodium berghei* infection in mice. It explored its potential as treatment for early malaria infections. Ginger rhizomes were collected, shade-dried, pulverized and then extracted using ethanol. The extract was qualitatively screened for phytoconstituents and assessed for acute toxicity. Albino mice were procured and randomly assigned to six groups, including control groups and those treated with 200, 400, 800, and 1200 mg/kg of the extract. *Plasmodium berghei* parasitized erythrocytes (1×10^7) in volume of 0.2 ml were used to infect each of the experimental mice in the infected groups by intraperitoneal injection. Mice were treated once daily with the extracts for four days post infection. Blood samples were collected on day 5 to evaluate the percentage of parasitaemia, chemosuppression, various biochemical and hematological parameters. Results showed that ginger rhizome extract contained moderate levels (++) of alkaloids, flavonoids and steroids and mild levels (+) of terpenoids, glycosides, saponins, tannins and reducing sugars. No mortality was recorded during the acute toxicity test. Treatment doses of 800 mg/kg and 1200 mg/kg of the extract significantly reduced parasitaemia and achieved high levels of chemosuppression, comparable to the positive control treated with artesunate. The malaria infection led to elevated activity of liver enzymes in serum, decreased serum proteins and altered blood cell counts. Despite the reduction in parasitaemia, ginger rhizome extract did not exhibit any significant effect on the haematological and serum biochemical parameters assayed. It was concluded that ginger rhizome ethanolic extract at the doses of 800 and 1200 mg/kg demonstrated antimalarial properties in early infection.

Keywords: *Plasmodium berghei;* Ginger (*Zingiber officinale*) rhizome extract; Four-day Antimalarial suppressive test; Serum biochemistry, Haematology.

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Introduction

Progress has been made in reducing malaria cases and deaths in sub-Saharan Africa; however, the region remains a major hotspot for the disease. According to the African Union, the goal of eliminating malaria by 2030 is not on track for most member countries (ALMA, 2025). *Plasmodium falciparum* is the most prevalent and deadly malaria parasite in sub-Saharan Africa. While *P. falciparum* is the primary cause of malaria in the region, *P. vivax* is also present and can lead to severe cases. Other malaria species, such as *P. malariae*, *P. ovale*, and *P. knowlesi* are found but are less common (WHO, 2024).

According to the WHO's recent World Malaria Report, there were an estimated 263 million cases and 597,000 malaria deaths worldwide in 2023. This number reflects an increase of about 11 million cases compared to 2022, while the number of deaths remained nearly the same. In 2023, the WHO African Region accounted for about 94% of all malaria cases and 95% of deaths. Children under 5 years old represented approximately 76% of all malaria deaths in the region. It was reported that over half of these deaths occurred in four countries: Nigeria (30.9%), the Democratic Republic of the Congo (11.3%), Niger (5.9%), and the United Republic of Tanzania (4.3%) [WHO, 2024; WHO, 2025].

The focus of malaria control in sub-Saharan Africa is on reducing morbidity and mortality, with a strong commitment to achieving malaria elimination by 2030. Key strategies include scaling up proven interventions such as insecticide-treated bed nets (ITNs) and indoor residual spraying (IRS), as well as deploying malaria vaccines and antimalarial drug therapies (Oladipo *et al*., 2022). While modern anti-malarial therapies are preferred, traditional remedies continue to play an important role in sub-Saharan Africa. Many people rely on these remedies, either on their own or in combination with modern treatments, due to limited access to contemporary anti-malarial drugs and the growing issue of drug resistance (Onukansi *et al*., 2025). Traditional medicinal plants are a vital part of malaria treatment and management in sub-Saharan Africa, because of their beneficial phytochemical constituents, which are believed to have the ability to suppress malaria parasitaemia.

Many studies have reported the suppressive activity of medicinal plants using Peter's four-day suppressive test (Peter, 1975). In an experimental study involving *P. berghei* infection in mice, doses of *Markhamia tomentosa*, *Polyalthia longifolia*, and *Trichilia heudelotii* were administered and monitored for 14 days (Bankole *et al*., 2016). *Markhamia tomentosa* and *Polyalthia longifolia* demonstrated chemosuppression of 73% and 53% of parasites, respectively, while *Trichilia heudelotii* showed no chemosuppressive activity (Bankole *et al*., 2016). In another study, the chemosuppressive activities of hydroethanolic extracts of *Anthocleista djalonensis* and *Ziziphus mauritiana* were evaluated in mice with experimental *P. berghei* infection (Attemene *et al*., 2018). The chemosuppressive activities of these extracts were observed to be dose-dependent (Attemene *et al*., 2018). Additionally, the medicinal plant *Indigofera spicata*, when administered to mice infected with *P. berghei*, elicited suppressive activity, with the highest dose showing the best results (Birru *et al*., 2017). Additionally, methanol extracts of ginger have been reported to suppress *P. berghei* during a four-day suppressive test (Biruksew *et al*., 2018).

When screening medicinal plants for antimalarial potential, the four-day suppressive test is crucial for determining whether the compounds in the medicinal plants can suppress early malaria infection. Therefore, in contrast to the previously reported antimalarial effects of ginger rhizome, this study evaluated the four-day

suppressive antimalarial potential of ginger ethanolic extract, and also investigated the effects of these treatments on various biochemical and haematological parameters.

Materials and Methods

Procurement and authentication of plant material: The rhizomes of *Zingiber officinale* were collected from a local market in Nsukka town, Nsukka Local Government Area, Enugu State, Nigeria. The plant materials were identified and authenticated by a taxonomist in the Department of Plant Science and Biotechnology, Faculty of Biological Sciences, University of Nigeria, Nsukka, and voucher specimens were kept in the herbarium.

Preparation of ethanol extracts: The ginger rhizomes that were procured were washed, sliced, and shade-dried separately at room temperature to a constant weight. The dried rhizome was pulverized, and 400 g of the fine powder obtained was percolated separately in 300 ml of absolute ethanol for 72 hours, followed by occasional shaking to increase the extraction capacity. It was filtered through a Whatman No. 1 filter paper and evaporated to dryness using a temperature-regulated water bath preset at 40°C. The concentrated filtrate was exposed to open air to evaporate to dryness. The extract was placed in an airtight bottle, labeled accordingly, and stored in a refrigerator at 4°C before use. A portion of the extracts were weighed and used for qualitative phytochemical screening.

Phytochemical screening of plants ethanol extracts: Qualitative phytochemical analysis was carried out on the extract using standard procedure to identify their phyto-constituents as described by Harbone (1973; 1984), Trease and Evans (1989; 1996), and Sofowora (2008). A colour chart was used to ascertain the intensity or qualitative levels of the phytochemicals present.

Procurement and preparation of standard drug: Artesunate tablets (Artesunat® GMP) from Guangzhou Madison Biotechnology Company Limited, Guangzhou, Guangdong, China was purchased from University Model Pharmacy, University of Nigeria Nsukka, Enugu State, Nigeria. The artesunate tablets were milled into fine powder and stored in airtight bottles in a refrigerator at 4°C before use. Fresh daily preparations were administered by oral intubations to the mice in the standard control group.

Procurement of *P. berghei* strain: The artesunate-sensitive strain of the rodent malaria parasite *Plasmodium berghei* NK-65 was used for the study. It was obtained from the Department of Pharmacology and Toxicology, National Institute of Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria. The strain was maintained in the laboratory for the period of the study by *in vivo* serial passage from mouse to mouse (Ebiloma *et al*., 2012). This was done at weekly intervals.

Procurement and maintenance of albino mice: Adult albino mice were procured from the Genetics and Animal Breeding Unit of the Department of Zoology and Environmental Biology, Faculty of Biological Sciences, University of Nigeria, Nsukka, Nigeria. The procured mice were acclimatized for one week before the start of the experiment. The mice were kept in stainless cages in a clean experimental animal house. They were fed a pelleted diet (Chikun Feeds®), and potable drinking water was provided *ad libitum*. The mice were maintained according to the National Research Council Guidelines on laboratory animal use (NRC, 2011). Ethical clearance for the use of the mice for the study was obtained from the Ethical Review Board, Faculty of Biological Sciences, University of Nigeria, Nsukka, Nigeria

Determination of weights of the mice: The body weights of the mice were meadured by use of a weighing balance. Changes in the body weights of the mice were taken into account while determining the dosages of the extracts and during the course of therapy.

Acute toxicity study: The acute safety assessment of the extract was performed based on the procedure described by Lorke (1983), but with modifications (Somsak *et al*., 2016). A total of 22 mice were used for the acute toxicity tests. Eighteen of the mice were randomly assigned to six groups of 3 each, and were given extract doses of 10, 100, 1,000, 1,600, 2,900, and 5,000 mg/kg of body weight respectively by oral intubations, while the remaining four were used as control. After each dosing, the mice were observed for signs of toxicity such as paw-licking, salivation, stretching of the entire body, weakness, sleep, respiratory distress, coma and death for first four hours. Subsequent observations were carried out daily at 12-hour intervals for 7 days. Food and water were available to the mice approximately an hour after treatment. There were no abnormal behavior and death in the mice given the extract. This informed the choice of the following doses for the experiment proper: 200 mg/kg, 400 mg/kg, 800 mg/kg, and 1200 mg/kg of extract.

Parasite Inoculation: A set of mice parasitized with *P*. *berghei* NK-65 strain were monitored daily by microscopy of their thin blood smear stained with Giemsa. The mice were humanely sacrificed after 6 days having been observed to show clinical symptoms of malaria, and were confirmed by microscopy through the examination of tail blood samples for 20% parasitaemia (Somsak *et al*., 2016), and with > 2×10^7 *P*. *berghei* parasitized erythrocytes (Onyishi *et al*., 2020). Blood were collected via retrobulbar plexus of the medial canthus of the mice eyes. The blood collected was diluted in normal saline in the ratio of 1:10 (1 ml of blood in 10 ml of normal saline). The diluted blood sample containing 1×10^7 *P*. *berghei* parasitized erythrocytes in volume of 0.2 ml were used to infect each of the experimental mice by intraperitoneal injection (Onwusonye and Uwakwe, 2014).

Suppressive test to evaluate the antimalarial potency of the ginger ethanolic extract in mice infected with *Plasmodium berghei*: The standard 4-day suppressive test (Peter, 1975), which tests the ability to prevent establishment of parasite infection were carried out. Seventy mice were used for this study; they were randomly assigned to seven groups (Groups A – G) of ten each. Group A was normal control group; this group was not infected and not treated. Groups B to G were inoculated with *P*. *berghei* intra-peritoneally at the commencement of the experiment. Group B mice were treated with the standard antimalarial drug – artesunate (positive control). Group C mice were treated with distilled water (negative control). Groups D, E, F and G mice were treated with 200 mg/kg, 400 mg/kg, 800 mg/kg and 1,200 mg/kg of the ginger extracts. The mice were treated once daily for four days. On the fifth day, blood samples were collected from the tail of the mice, and percentage parasitaemia and suppression determined by comparing with the untreated group: % suppression = Parasitaemia levels of untreated group – Parasitaemia level of treated group / Parasitaemia level on untreated group × 100.

Total *Plasmodium* parasitaemia estimation: The total parasitaemia counts of each treatment group were determined on day 4, 8 and 15. The determination was done on microscopic examination of thick blood smears, by counting parasites against white blood cells (WBCs). Slides of thick blood smears were collected in duplicate for each mouse and examined microscopically for *Plasmodium berghei* parasite density. Three parts of the thick films that the white blood cells were well distributed and parasites well stained were read using 100× oil immersion objectives, while counting the WBCs and

number of parasites in each field read. The number of parasites per microlitre (µl) of blood was calculated as described by Wampfler *et al*. (2013) and Stone *et al*. (2017).

Percentage (%) suppression of *P. berghei* parasitaemia by the extract: The inhibitory efficacy of the extract was evaluated by the percentage suppression of *P. berghei* parasitaemia of the experimental mice determined at day 4. These were calculated at the end of the suppressive tests as described by Rathore *et al*. (2005).

Monitoring Relapse of *P. berghei* in the infected mice: To monitor for relapse, parasitaemia of infected mice was determined at day 15, and the percentage relapse was determined thus: % Relapse (at day 15) = Parasitaemia level of untreated group – Parasitaemia level of treated group / Parasitaemia level on untreated group × 100.

Evaluation of the haematological indices: The haematological parameters [packed cell volume (PCV), haemoglobin (Hb), white blood cells (WBCs), red blood cells (RBCs), and platelet count] of the mice blood samples dispensed in ethylene diamine tetra acetic acid (EDTA) bottles were determined on day 8 using standard procedures (Adewuyi and Olatunji, 1995; Cheesbrough, 2009). These parameters were measured for each group, recorded accordingly and compared, for the untreated and treated groups.

The PCV was determined by the microhaematocrit method (Thrall and Weiser, 2002), using microcapillary tubes (Marienfeld, Germany), microhaematocrit centrifuge (Hawksley, England) and microhaematocrit reader (Hawksley, England). The haemoglobin concentration was determined by the cyanomethaemoglobin method (Higgins *et al*., 2008), using Drabkin's haemoglobin reagent. The total white blood cell count and red blood cell counts were done by the haemocytometer method (Schalm *et al*., 1975; Thrall and Weiser, 2002), using a haemocytometer set containing an improved Neubauer counting chamber. The Rees and Ecker method of direct counting of platelets was used for platelet counting (Brown, 1976).

Evaluation of the serum biochemical indices: The serum biochemical parameters [alanine transaminase (ALT), aspartate transaminase (AST), alanine phosphatase (ALP), creatinine and total protein] of the mice blood samples were determined at day 15 using standard procedures (Sood, 2006). The serum ALT and AST activities were determined by the Reitman-Frankel colorimetric method (Reitman and Frankel, 1957; Colville, 2002), using the Quimica Clinica Aplicada (QCA) ALT/GPT test kit (QCA, Spain). The serum ALP activity was determined by the thymolpthalein monophosphate method (Roy, 1970; Colville, 2002), using TECO ALP Test kit (TECO Diagnostics, Anaheim, California, USA). The modified Jaffe method (Blass *et al*., 1974) was used to determine the serum creatinine levels. The serum total protein levels was determined by the direct Biuret method (Lubran, 1978) using a RANDOX Total Protein (TP) test kit (Randox Laboratories Ltd., Crumlin, County Antrim, UK).

Data Analysis: Data analyses were performed using IBM SPSS Statistics software version 23.0 (IBM SPSS Incorporation, Chicago, Illinois, USA). Preliminary analysis was conducted to test for linearity of the data. Normally distributed continuous data on the body weights, relative organ weights, percentage parasitaemia, biochemical and haematological parameters of treatment and control mice groups were compared using one-way analysis of variance (ANOVA) followed by Duncan multiple range test (DMRT). An independent sample t-test was used to compare the initial and final weights of mice. Results were expressed as mean ± SE (standard error of mean).

Results

Extraction yields of the dried ginger rhizome: Extraction of the 400 grams of ginger rhizome in 300 ml of ethanol yielded 63.22 grams of ginger extract (15.8% yield).

Qualitative Phytochemical Analysis Results: Phytochemical analysis of the ginger extract showed that it contained moderate amounts of alkaloids, flavonoids, and steroids, as well as low amounts of terpenoids, saponins, glycosides, tannins and reducing sugar (Table 1). Resins were absent (Table 1).

Acute oral toxicity test results: No deaths were recorded in all the mouse groups treated with the extracts during the acute toxicity study. There were no significant differences ($p > 0.05$) between the body weights before and after treatment with ginger extracts (Table 2).

Relative organ weight of the mice used for acute oral toxicity study: The relative kidney weight of the mice groups given ginger extract did not show any significant difference ($p > 0.05$) compared to the control, except for the 1600mg/kg group, which had a significantly higher ($p < 0.05$) relative kidney weight than the control and 100mg/kg group (Figure 1). Additionally, the relative liver weights of all ginger treatment groups did not differ significantly ($p > 0.05$) from the control (Figure 2).

Table 1. Results of the phytochemical analysis of the ginger rhizome ethanolic extract.

Phytochemicals	Levels in the Extract
Alkaloid	++
Flavonoid	++
Terpenoid	+
Steroids	++
Cardiac glycoside	+
Saponins	+
Resins	-
Tannins	+
Reducing sugar	+

+++ highly present; ++ moderately present; + mildly present

Table 2. Body weights of mice used for acute oral toxicity testing of ginger rhizome ethanolic extract.

Doses of ginger extract (mg/kg)	Mean ± standard deviation	
	Initial weight (g)	Final weight (g)
0 mg/kg	18.17 ± 4.20	18.30 ± 3.88
10 mg/kg	16.39 ± 2.24	17.09 ± 2.19
100 mg/kg	14.86 ± 0.71	13.81 ± 2.67
1000 mg/kg	19.25 ± 1.42	19.03 ± 1.20
1600 mg/kg	14.00 ± 2.67	13.59 ± 1.75
2900 mg/kg	19.89 ± 1.14	17.98 ± 3.85
5000 mg/kg	13.51 ± 3.18	14.02 ± 4.34

No significant difference ($p > 0.05$) between the initial and final body weights.

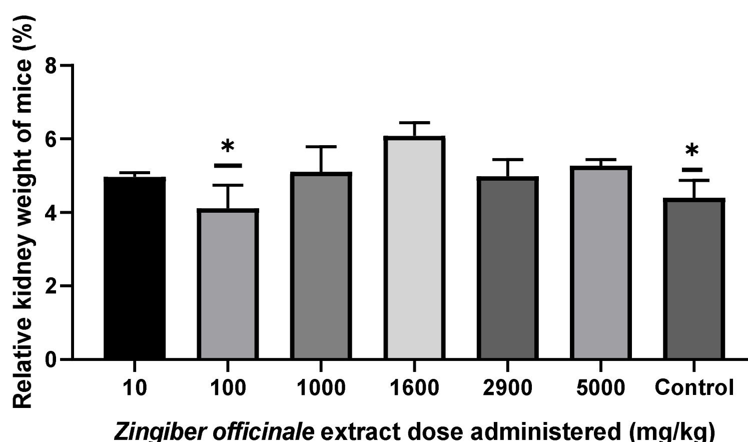

Figure 1. Relative kidney weights of mice groups given different doses of ginger extract during the acute oral toxicity study, compared to a control group that was not given extract. *Significantly lower kidney weight (p<0.05) compared to the 1600 mg/kg group.

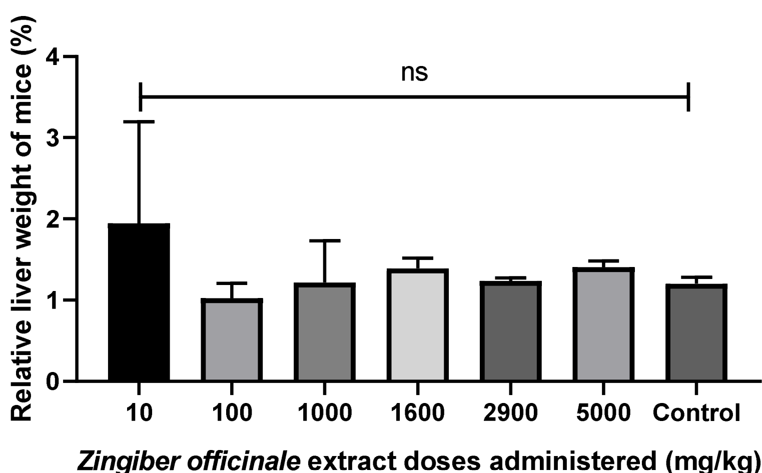

Figure 2. Relative liver weights of mice groups given different doses of ginger extract during the acute oral toxicity study, compared to a control group that was not given extract. ns – no significant difference between treatment groups and control.

Antimalarial activity of ginger extract in the four-day suppressive test: The antimalarial activity of the extract at varying doses is shown in Table 3. A significantly lower ($p > 0.05$) percentage parasitaemia was observed in all treatment groups compared to the negative control (NC). However, the percentage of parasitaemia in mice given 800 and 1200 mg/kg of ginger extract was statistically not different from that of the positive control (PC), with a high percentage of chemosuppression of > 80%, which was statistically not different from that of the PC. The percentage of parasitaemia and chemosuppression of the 200 and 400 mg/kg groups was statistically not different from each other (Table 3).

During the seven-day follow-up period, treatment with ginger extract resulted in a significantly lower ($p < 0.05$) percentage of parasitaemia in all treatment

groups compared to the negative control. However, when comparing the treatment groups, the 200 mg/kg and 800 mg/kg groups showed near similar levels of parasitaemia, which were significantly lower ($p < 0.05$) than the 400 mg/kg group. The 1200 mg/kg group had a percentage of parasitaemia not different from that of the positive control (PC) group, but it was significantly lower than the other treatment groups. Additionally, during the seven-day follow-up, the percentage of chemosuppression in the 400 mg/kg group was significantly lower at 11% compared to the other treatment groups. The 200 mg/kg and 800 mg/kg groups had a chemosuppression rate of > 30%, while the 1200 mg/kg (51%) and positive control (60%) groups showed no significant difference ($p > 0.05$).

Body weights and Relative organ weights during the 4-day suppressive test: When the initial and final body weights of mice infected with *P. berghei* and treated with different doses of the extract were compared, there were no significant variations ($p > 0.05$) in body weights all through the duration of the study (Table 4).

Relative organ weights during the 4-day suppressive test: The relative kidney weights of all the groups treated with ginger extract and the negative control were not significantly different ($p > 0.05$) from that of the normal control, except for the positive control (PC) group, where the relative kidney weight was significantly higher ($p < 0.05$) than that of the normal control and the groups treated with 200 mg/kg and 1200 mg/kg (Table 4). The relative liver weight of the groups treated with 1200 mg/kg, 800 mg/kg, and 200 mg/kg of ginger extract, as well as the negative control and positive control groups, were significantly higher ($p < 0.05$) than that of the normal control (Table 4). However, there was no significant difference in the relative spleen weight between the normal control, negative control and the groups treated with 200 mg/kg and 400 mg/kg of ginger extract (Table 4).

Table 3. Percentage of parasitaemia and chemo-suppressive activities of the mice groups given varied doses of ginger extract, after infection with *Plasmodium berghei*. [NC – Negative Control; PC – Positive control]

Dose (mg/kg)	Parasitaemia (%)	Chemosuppression (%)	Relapse (%)	Relapse Chemosuppression (%)
NC	49.80 ± 7.93^{a}	-	58.00 ± 6.20^{a}	-
200	17.10 ± 4.40^{b}	65.30 ± 8.71^{b}	38.00 ± 8.38^{c}	34.45 ± 12.64^{b}
400	20.00 ± 6.36^{b}	59.20 ± 15.84^{b}	50.50 ± 5.27^{b}	11.84 ± 15.48^{c}
800	6.60 ± 1.51^{c}	86.45 ± 3.82^{a}	39.10 ± 6.28^{c}	31.85 ± 13.75^{b}
1200	6.60 ± 1.51^{c}	86.45 ± 3.82^{a}	27.60 ± 4.33^{d}	51.62 ± 11.07^{a}
PC	7.30 ± 4.08^{c}	86.02 ± 6.06^{a}	23.30 ± 5.18^{d}	60.01 ± 8.22^{a}

Results expressed as mean $\pm$ SD; Mean values in a column for each extract with different alphabets as superscripts are significantly different at $p < 0.05$.

Table 4. Body weight and relative organ weights of mice groups infected with *P. berghei* and treated with varied doses of ginger extract. [NC – Negative Control; PC – Positive control]

Groups and their treatments	Body weight		Rel. kidney weight	Rel. liver weight	Rel. Spleen weight
	Initial weight	Final weight			
Normal control	18.55 ± 1.29	26.17 ± 3.68 *	1.16 ± 0.14 ^{b}	4.40 ± 0.56 ^{c}	0.52 ± 0.08 ^{c}
NC	14.00 ± 1.47	13.21 ± 1.06	1.23 ± 0.25 ^{ab}	5.13 ± 0.63 ^{b}	0.63 ± 0.16 ^{c}
200 mg/kg	21.99 ± 1.89	22.46 ± 2.28	1.13 ± 0.21 ^{b}	5.90 ± 1.26 ^{ab}	0.65 ± 0.14 ^{c}
400 mg/kg	20.07 ± 1.67	21.30 ± 2.42	1.18 ± 0.19 ^{ab}	5.00 ± 0.36 ^{bc}	0.78 ± 0.26 ^{bc}
800 mg/kg	28.13 ± 2.73	29.16 ± 3.42	1.20 ± 0.27 ^{ab}	5.52 ± 1.26 ^{b}	1.23 ± 0.82 ^{ab}
1200 mg/kg	24.63 ± 2.73	24.55 ± 2.28	1.14 ± 0.11 ^{b}	6.54 ± 1.46 ^{a}	1.48 ± 0.99 ^{a}
PC	43.20 ± 3.73	43.95 ± 3.73	1.38 ± 0.28 ^{a}	5.89 ± 1.07 ^{ab}	1.30 ± 0.44 ^{a}

Results expressed as Mean ± SD; T-test was used to compare initial and final body weights of the different treatment groups (depicted using *); Comparing relative organ weights, mean values with different alphabets as superscripts are significantly different at $p < 0.05$

Effect of *P. berghei* infection and extract treatment on serum activity of enzyme markers of liver function in the 4-day suppressive test: The effects of infection and treatment on serum activity of enzyme markers of liver dysfunction are presented in Table 5. Infection with the parasite resulted in serum ALT activity higher than that of the normal control group, but this difference was not statistically significant ($p > 0.05$). Treatment with the standard drug caused a slightly lower ALT activity, but this difference was also not significant ($p > 0.05$) compared to the normal levels. However, treatment with all doses of ginger extract, except for the 800mg/kg dose, led to lower serum ALT activity that was statistically comparable ($p > 0.05$) to the normal levels (Table 5).

Similarly, infection with *P. berghei* led to higher serum AST activity in infected mice, but this higher activity was not significantly different ($p > 0.05$) from the activity recorded for the normal control (Table 5). Treatment with the standard drug and ginger extract also led to relatively higher serum AST activity, but this difference change was not statistically significant ($p > 0.05$) [Table 5].

Furthermore, infection with the parasite was also associated with higher serum ALP activity, but this higher activity was not significantly different ($p > 0.05$) from the activity level recorded for the normal control (Table 5). Treatment with the standard drug led to a significantly lower ALP activity relative to the normal and negative control groups. The groups treated with ginger extract generally had a lower serum ALP activity, but only the groups treated with 400 mg/kg and 800 mg/kg doses of extract showed a significant difference compared to the negative control group (Table 5). Only the 800mg/kg dose had a significantly ($p < 0.05$) lower serum ALP activity when compared to the normal control group (Table 5).

Effect of *P. berghei* infection and extract treatment on serum creatinine, total protein and glucose levels in the 4-day suppressive test: Results of determinations on serum creatinine total protein and glucose levels is presented in Table 6. There was no significant difference in the serum creatinine

levels between the control and ginger extract treated groups (Table 6). Infection with the parasite led to lower serum total protein (TP) levels, and treatment with the standard drug led to a significantly higher ($p < 0.05$) TP levels, but this higher level was not significantly different ($p > 0.05$) from the levels in normal and negative control groups. Among the groups treated with varied doses of ginger extract in this study, the 200mg/kg dose led to a far lower TP levels compared to the negative control group (Table 6). However, the TP levels of all groups treated with ginger extract, except for the 200mg/kg dose, were comparable to the levels recorded for the normal control (Table 6).

Table 5. Serum enzyme activity of mice groups infected with *P. berghei* and treated with varied doses of ginger extract, compared to controls.

Groups and their treatments	Mean ± standard deviation		
	Alanine aminotransferase (IU/L)	Aspartate aminotransferase (IU/L)	Alkaline phosphatase (IU/L)
Normal Control	39.33 ± 19.43 [b]	95.00 ± 35.03 [a]	44.67 ± 1.53 [ab]
Negative Control	46.00 ± 6.08 [ab]	150.00 ± 43.51 [a]	56.00 ± 3.61 [a]
200 mg/kg	30.67 ± 6.43 [b]	123.33 ± 10.02 [a]	44.67 ± 2.52 [ab]
400 mg/kg	26.67 ± 8.96 [b]	167.00 ± 107.55 [a]	43.33 ± 2.52 [b]
800 mg/kg	65.00 ± 8.96 [a]	180.33 ± 55.43 [a]	39.69 ± 0.58 [c]
1200 mg/kg	39.33 ± 8.96 [b]	146.33 ± 49.66 [a]	41.00 ± 0.00 [bc]
Positive Control	44.33 ± 0.57 [ab]	138.67 ± 56.77 [a]	34.67 ± 3.79 [d]

Mean values with different alphabets as superscripts are significantly different at $p < 0.05$

Table 6. Serum creatinine and total protein levels of mice groups infected with *P. berghei* and treated with varied doses of ginger extract, compared to controls.

Groups and their treatments	Means ± standard deviation	
	Creatinine (mg/dl)	Total Protein (g/L)
Normal Control	1.00 ± 0.00 [a]	61.00 ± 7.94 [ab]
Negative Control	0.67 ± 0.58 [a]	58.00 ± 5.29 [abc]
200 mg/kg	0.67 ± 0.58 [a]	48.67 ± 2.31 [c]
400 mg/kg	0.67 ± 0.58 [a]	56.33 ± 3.79 [bc]
800 mg/kg	1.00 ± 0.00 [a]	66.67 ± 9.50 [ab]
1200 mg/kg	1.00 ± 0.00 [a]	58.00 ± 6.00 [abc]
Positive Control	1.33 ± 0.58 [a]	68.00 ± 1.73 [a]

Mean values with different alphabets as superscripts are significantly different at $p<0.05$

Effect of *P. berghei* infection and extract treatment on haematological parameters in the 4-day suppressive test: Results of the haematological determinations are presented on Tables 7 and 8. The mean PCV of the Negative Control (NC) group was lower when compared to the Normal Control group, but the difference was not statistically significant ($p > 0.05$). Treatment with the standard drug further decreased the PCV value in the Positive Control (PC) group, and it was significantly different from the Normal Control group ($p < 0.05$), but not the Negative Control group (Table 7). Treatment with doses of ginger extract led to higher PCV levels comparable to the Normal Control group (Table 7).

Infection with the parasite slightly decreased the HB concentration in the NC group compared to the normal control group, but this change was not statistically significant ($p > 0.05$) (Table 7). Treatment with the standard drug further decreased the HB concentration, but this decrease was comparable to the normal control and NC groups (Table 7). All doses of ginger extract had varying effects on HB but levels were statistically not different ($p > 0.05$) from the Normal Control group (Table 7).

Red blood cell (RBC) counts were relatively lower in the NC group, but this decrease was not significantly different ($p > 0.05$) from the normal group (Table 7). Treatment with the standard drug and doses of ginger extract had different effects on RBC but statistically, RBC counts were not different from that recorded for the normal control (Table 7).

The TWBC of the NC group was the lowest, but its value was not significantly ($p > 0.05$) different from that of the normal control group (Table 8). Treatment with the standard drug led to higher TWBC counts, comparable to the levels recorded for the normal control group (Table 8). Treatment with varying doses of ginger extract led to higher TWBC counts statistically comparable to that of the normal control group (Table 8).

Platelet counts were significantly higher in the group treated with the standard drug (positive control) when compared with the group treated with 1,200 mg/kg of the extract, but none was significantly ($p > 0.05$) different from the normal control (Table 8).

Table 7. Packed cell volume (PCV), haemoglobin (Hb) concentration and red blood cell (RBC) counts of mice groups infected with *P. berghei* and treated with varied doses of ginger extract, compared to controls.

Groups and their treatments	Means ± standard deviation		
	PCV (%)	Hb conc. (g/dl)	RBC counts (10^6/µl)
Normal Control	35.00 ± 5.29^{ab}	14.67 ± 1.53^{ab}	7.33 ± 1.16^{ab}
Negative Control	30.67 ± 0.58^{bc}	13.67 ± 0.58^{ab}	6.67 ± 0.58^{b}
200 mg/kg	38.00 ± 1.73^{a}	15.67 ± 1.53^{a}	8.00 ± 0.00^{a}
400 mg/kg	33.33 ± 2.08^{abc}	15.67 ± 3.22^{a}	6.67 ± 0.58^{b}
800 mg/kg	29.33 ± 3.22^{bc}	10.67 ± 2.89^{b}	6.33 ± 0.58^{b}
1200 mg/kg	34.67 ± 3.79^{ab}	13.67 ± 1.16^{ab}	7.33 ± 0.58^{ab}
Positive Control	28.00 ± 2.65^{c}	11.00 ± 3.06^{b}	6.33 ± 0.58^{ab}

Mean values with different alphabets as superscripts are significantly different at $p < 0.05$

Table 8. Total white blood cell and platelet counts of mice groups infected with *P. berghei* and treated with varied doses of ginger extract, compared to controls.

Groups and their treatments	Means ± standard deviation	
	Total WBC (10^3/µl)	Platelet count (10^3/µl)
Normal Control	7.33 ± 2.08 [ab]	334.67 ± 38.79 [ab]
Negative Control	4.33 ± 1.53 [b]	351.67 ± 9.50 [ab]
200 mg/kg	8.67 ± 3.22 [ab]	328.33 ± 9.71[ab]
400 mg/kg	11.33 ± 2.08 [a]	334.67 ± 11.93 [ab]
800 mg/kg	6.00 ± 1.00 [ab]	363.67 ± 39.80 [ab]
1200 mg/kg	9.67 ± 5.03 [ab]	321.00 ± 12.00 [b]
Positive Control	8.67 ± 4.62 [ab]	372.67 ± 23.96 [a]

Mean values with different alphabets as superscripts are significantly different at $p < 0.05$

Discussion

Earlier reports in available literature has detailed the phytochemicals that were recorded in the present study in the ethanolic extract of ginger rhizome: the extract contained alkaloids, flavonoids, tannins, terpenoids, and saponins, whose anti-bacterial, anti-inflammatory, anti-allergic, analgesic, antioxidant, and anticancer qualities are well-known (Kela *et al.*, 2021; Ibrahim, 2024; Oyetunji and Nwadike, 2024). Furthermore, earlier reports on ginger rhizome extract have demonstrated antimalarial activity. The antimalarial potential of ginger was reportedly associated with a phenolic compound called 6-gingerol (Yousfi *et al.*, 2021).

The ginger rhizome extract caused no mortality in the oral acute toxicity study, and no significant differences in body weight or relative organ weight were recorded between groups given the extract and the control group. This is thought to be as a result of the fact that ginger is generally regarded as safe spice/herbal medicine. The absence of mortality and abnormalities aligns with previous reports indicating that rats gavaged with ginger powder showed no signs of mortality or abnormalities (Rong *et al.*, 2009). In an earlier reported seven-day acute toxicity study involving a dosage of 2000 mg/kg of ginger extract, no deaths or abnormalities were recorded by Benny (2021).

In the four-day suppressive test, the ginger extract at 800 mg/kg and 1200 mg/kg exhibited a chemosuppressive activity of over 80%, similar to that of the standard drug group. Seven days' post-treatment, the ginger extract demonstrated a chemosuppressive effect of over 50% at the 1200 mg/kg dose, which was comparable to the chemosuppression achieved by the positive control. This suggests that the antimalarial activity of ginger extract is primarily effective at higher doses. The finding that ginger's chemotherapeutic activity is restricted to high doses aligns with previous reports indicating that the suppressive effect of ginger extract increases with higher dosages (Biruksew *et al.*, 2018).

Treatment with the ginger rhizome extract during the four-day suppressive test did not have any significant effect on the body weight of the animals. The kidney weight of the

animals was also not considerably affected by the infection or treatment with the extract. However, treatment with 200 mg/kg, 800 mg/kg, and 1200 mg/kg of ginger extract, as well as the standard drug, was able to reduce liver enlargement caused by the infection. In contrast, the 800 mg/kg and 1200 mg/kg doses of ginger extract, along with the standard drug, did not effectively reduce spleen enlargement due to the infection. The lack of a significant difference in kidney weights between the infected and treated groups suggests that the infection had no pronounced effect on the kidneys of mice in this study. This result is consistent with a previous study where the extract of *Distemonanthus benthamianus* Baill did not cause any noticeable changes in kidney weight compared to the negative control group (Ayisi *et al*., 2021). The lack of significant differences in the body weight of animals in this study suggests that the density of parasites associated with the infection and/or the acute nature of the study did not have a noteworthy impact on the nutritional status of the mice. Furthermore, it may not have stimulated the production of large amounts of pro-inflammatory cytokines, such as TNF-α, which are associated with weight loss in malaria infections (Su *et al*., 2018). This finding contradicts reports indicating that ginger extract failed to protect infected mice from weight loss (Biruksew *et al*., 2018).

Hepatomegaly and splenomegaly were observed in the infected mice in this study. These lesions are typical of malaria, and are attributed to the presence of infected erythrocytes that are phagocytosed by macrophages in the spleen and Kupffer cells in the liver (Wilson *et al*., 2009). The varying doses of ginger extract that was administered were unable to protect against hepatomegaly and splenomegaly in this study. This was also observed in the group treated with the standard drug. These results suggest that while the extract and standard drug may have some antimalarial activity against parasites in the circulating blood, they may not be effective against those in the tissue. A similar finding was reported in a study of Noni leaves (*Moringa citrifolia*) in *P. berghei* infected mice, where the extracts were unable to prevent splenomegaly and hepatomegaly (Rahayu *et al*., 2021).

The activity of liver enzymes (ALT, AST, and ALP) in the infected groups was significantly higher: this suggests that the malaria parasite causes liver damage during its liver stage of development within the host hepatocytes (Scaccabarozzi *et al*., 2018). Administration of the ginger extract was able to restore the elevated levels of ALT to normal. This protective effect may be attributed to the presence of bioactive compounds in the extract (Akbari *et al*., 2019), which not only suppressed the parasite levels but also mitigated liver damage caused by the parasite. In another study, it was found that treatment of *Plasmodium*-infected mice with an ethanolic extract of *Terminalia macroptera* significantly reduced elevated liver enzyme levels (Sidiki *et al*., 2023).

In this study, infection with the parasite was found to lead to lower serum levels of total protein. This lowering of serum total protein levels is believed to be a result of the infection's impact on liver function, which adversely affected protein synthesis and led to lower hepatic protein synthesis. However, treatment with a ginger rhizome extract was found to restore the serum total protein level to normal, suggesting that the extract possesses antioxidant and anti-inflammatory properties that can combat oxidative stress and tissue damage caused by the malaria parasite. This helps to preserve hepatic protein synthesis levels in infected mice (Asmilia *et al*., 2020; Atanu *et al*., 2021). This result is consistent with a previous study where ethanolic extracts of *Euphorbia hirta* and *Vernonia amgydalina* were found to lead to higher serum total protein levels in treated

groups compared to the untreated infected group in a *Plasmodium berghei* infection (Ajayi *et al*., 2017).

The absence of a significant *Plasmodium* infection effect on the negative control group's creatinine level in comparison to the treatment groups indicates that the rodent malaria parasite typically has no discernible effect on mice's creatinine levels, suggesting that the parasite did not cause significant damage to the kidneys in this model (Onyishi *et al*., 2020). This result agrees with the result of a study where *P. berghei-infected* mice were treated with goat bile and it was observed that all mice groups had creatinine levels within the normal range (Arwati *et al*., 2021).

Treatment with the ginger rhizome extract successfully restored the lowered packed cell volume (PCV), haemoglobin (HB), and red blood cell (RBC) count in some of the infected mice groups. Additionally, the extract normalized the reduced total white blood cell (TWBC) and platelet counts caused by the infection. The lower RBC, HB, and PCV levels in the infected groups is believed to be as a result of red blood cell breakdown. This results from the parasite entering and multiplying inside the RBCs, leading to their rupture and a continuous cycle of destruction. As a result, the levels of these parameters are lowered (Mohandas and An, 2013). However, the administration of certain doses of ginger extract was found to improve these reduced levels. This may be due to the extracts' potential erythropoietic stimulating properties, which can promote the production of RBCs. This counteracts the anaemia caused by the malaria parasite, which actively destroys RBCs within the host. Another further possible explanation for the improvement in these parameters in some of the infected groups that were treated could be that the plant extracts increased the production of red blood cells in the bone marrow. This could possibly be achieved by mimicking or enhancing the action of erythropoietin (EPO), a hormone responsible for RBC production (Ikese *et al*., 2020; Lakkavaram *et al*., 2020).

In this study, the total white blood cell counts in the infected and untreated mice group was lower than that of other groups. This lowered total white blood cell count contrasts with the reports of some earlier published studies on *P*. *berghei* infection in mice. It is thought that the observed drop seems to be the result of leukocytes being localized to the spleen and other marginal pools, away from peripheral circulation (McKenzie *et al*., 2005). Treatment with ginger rhizome extract improved the white blood cell count to normal levels and even above normal. This probably suggests that the ginger rhizome extract have anti-plasmodial properties, such that as the parasites decrier in the tissues, the WBCs return to blood (Kanu *et al*., 2022).

The lack of statistically significant differences in platelet counts among all mouse groups in this study suggests that the parasite does not have a notable effect on platelet levels. This finding is consistent with a study that reported on the antimalarial efficacy and safety of *Senna occidentalis* (L.) root extract in *Plasmodium berghei*-infected BALB/c mice, which also found no significant difference in platelet counts between untreated and infected mice compared to other groups (Mogaka *et al*., 2023).

In conclusion, ethanolic extract of ginger rhizome, as used in this study is rich in bioactive components and is safe for use as herbal medicine. The extract especially at a higher dose, demonstrated potential antimalarial properties in early malaria infection and restored to normal some of the alterations in serum biochemistry and haematology that were associated with *Plasmodium berghei* infection in the mice.

Conflict of Interest

The authors declare they have no conflict of interest in this study.

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