

Original Research Article

Antimalarial potential of aqueous extract of *Cissus quadrangularis* stem in three mice models

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Abstract

Purpose: To investigate the antimalarial potential of *Cissus quadrangularis* stems using three mice models.

Methods: Ninety mice were divided into 3 models (30 each), i.e., suppressive, curative, and prophylactic activities. Six groups (n = 5) were used in each model: normal control, negative control, standard drug control, chloroquine (5 mg/kg) for suppressive and curative tests, pyrimethamine (1.2 mg/kg) for prophylactic, and three extract-treated groups (100, 200, 400 mg/kg). Extracts and controls were given orally for 4 days. Parasitaemia and haematological parameters were assessed. Acute toxicity was evaluated using Lorke's method.

Results: Phytochemical screening revealed alkaloids, flavonoids, and carbohydrates. Acute toxicity showed an LD₅₀ > 5000 mg/kg, with no observable behavioural changes or mortality. Extract exhibited a dose-dependent suppression of parasitaemia (p < 0.05). Treated groups had higher Red Blood Cell (RBC) count, Packed Cell Volume (PCV), Platelet Count (PLT count), Haemoglobin (HBG), and lower White Blood Cell count (WBC) compared with negative control (p < 0.05), indicating protection against malaria-induced anaemia and leukocytosis.

Conclusion: Aqueous extract of *Cissus quadrangularis* stem possesses antimalarial properties that could be harnessed to develop a safe, effective and cost-effective antimalarial agent.

Keywords: Antimalaria, *Plasmodium berghei*, *Cissus quadrangularis*, Aqueous, Haematological parameters

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INTRODUCTION

Malaria is a disease caused by a protozoan parasite of the genus *Plasmodium* that is spread through the bite of previously infected female *Anopheles* mosquito. There are five *Plasmodium* species that are known to cause infection in

humans: *Plasmodium falciparum*, *P. malariae*, *P. ovale*, *P. vivax*, and *P. knowlesi*, with *P. falciparum* being the most virulent and responsible for most of the mortalities and morbidities [1]. Malaria continues to pose a significant threat to human health around the tropics, increasing morbidity and mortality rates,

particularly in Africa [2]. Despite efforts to combat the disease, malaria cases have continued to rise, especially after the pandemic. The World Health Organization (WHO) states that the global malaria burden has increased with an estimated 282 million cases worldwide, representing an incidence rate of 64.0 cases per 1,000 people at risk in 2024 [2]. This represents a 2 % rise from the 2023 rate of 62.7 per 1000 population at risk.

Nigeria bore the brunt of the global malaria burden in 2024, responsible for approximately 31 % of global malaria-related fatalities [2]. This staggering proportion made Nigeria the country carrying the greatest estimated malaria case load globally (24.3 %). Furthermore, the WHO African Region, where Nigeria is located, accounted for 95 % of global malaria-related deaths, bearing the largest share of malaria mortality worldwide [2]. The emergence of *Plasmodium* strains resistant to various antimalarials (including chloroquine, sulfadoxine-pyrimethamine, mefloquine, and amodiaquine) is a big challenge that has been a threat to most malaria control programs, resulting in increased malaria morbidity and mortality [3].

Traditional herbal medicines have long been the cornerstone of disease treatment in Africa, with approximately 65 - 80 % of the global population, particularly in Africa and other developing nations, relying solely on these remedies for healthcare [2]. Africa's rich biodiversity has led to an estimated 40,000 - 45,000 plant species, of which about 5,000 are recognized for having therapeutic properties [4]. The quest for novel plant-based antimalarial drugs is urgent, driven by the rise of chloroquine-resistant and multidrug-resistant malaria parasites. Plants have long been a source of antimalarial treatments. The Cinchona tree, for example, yielded quinine, the first antimalarial drug, used to treat human malaria since 1632 [5]. Another key malaria drug, artemisinin, is derived from *Artemisia annua*. Nigeria's lush plant diversity has led to the traditional use of various plant species for medicinal purposes, including malaria treatment. Notable examples include *Alstonia boonei* (Apocynaceae) and *Azadirachta indica* (Meliaceae) [5]. *C. quadrangularis* (Gadali in Hausa) belongs to the Vitaceae family and is widely consumed and serves as a common food source in the lives of an average Indian. The edible plant is found in India, Sri Lanka, Malaya, Java, Thailand, and West Africa [6]. It is also a vital medicinal plant used in Ayurveda to treat various health issues, including malaria [6]. The plant is characterized by its simple or reniform, serrate leaves, approximately 5 cm in width, and a quadrangular fleshy stem with nodes and

internodes measuring up to 10 cm in length, exhibiting dichotomous branching [6]. Its pharmacological properties, such as antimicrobial, antioxidant, analgesic, and anti-inflammatory properties of roots, leaves and most especially the stem have been well-documented [7].

In Nigeria, Borno State, an ethnobotanical survey of anticancer plants shows that *Cissus quadrangularis* stem and roots are utilized to treat cancer (Daji in Hausa) and other ailments like fever, joint pain and gastrointestinal disturbances [8]. In Mali, traditional therapists have identified *C. quadrangularis* stem and roots as a treatment for malaria [9], highlighting its potential as a source of novel antimalarial drugs. Therefore, the present study is aimed at evaluating the antimalarial potential of aqueous extract of *C. quadrangularis* stem in a mouse model.

EXPERIMENTAL

Collection, identification and preparation of plant material

The stem of *C. quadrangularis* was collected on the 15th October, 2024, from Mazah village in Jos North Local Government Area (LGA) situated in Plateau State, Nigeria, on Latitude 8° 38' 82¹¹ N and Longitude 8° 58' 65¹¹ E. The identification of the stem was confirmed and authenticated by a plant taxonomist at the Federal College of Forestry, Jos, Nigeria, and the sample was deposited in the herbarium with a voucher number: FHJ 313. The fresh stem was rinsed with water, chopped into pieces, and air-dried at room temperature for three weeks. The dried stem was then pulverized into a coarse powder using a mortar and pestle. The plant extract was prepared by mixing the powder (100 g) with one litre of hot distilled water (70 °C) and intermittently shaking at intervals for the next 24 hours. Filtration was done using Whatmann No 1 filter paper, and the resulting filtrate was evaporated at 60 °C using a hot water bath. The yield was then stored in a sealed container in the refrigerator as described by [10].

Phytochemical screening

The extract underwent phytochemical screening to identify the active phytoconstituents present in the stem of *C. quadrangularis*. The screening tested for the presence of various secondary metabolites, including alkaloids, flavonoids, phenolic compounds, saponins, terpenoids, cardiac glycosides, steroids, and tannins [11].

Animals

Male and female Swiss Albino mice weighing between 15 and 25 g were sourced and housed in the Animal Experimental Unit, Pharmacology Department, University of Jos, Nigeria. The mice were kept in clear plastic rectangular cages with ventilated racks; wood shavings were used as bedding. The animals were given four days to acclimatize at room temperature for 12 hours dark/night periodicity. The mice were given a standard rodent diet with a constant supply of water.

Acute toxicity test (LD₅₀)

The water-based extract of *C. quadrangularis* was assessed for acute toxicity in mice using a modified version of Lorke's method [12]. The initial phase involved administering the extract at doses of 10, 100, and 1000 mg/kg. The second phase involved administering doses of 1600, 2900, and 5000 mg/kg. The extracts to be administered were prepared by dilution and given according to each animal's weight. The extract was mixed with distilled water. The extract (0.2 g) was mixed in 10 mL of distilled water to yield a 20 mg/mL as the concentration.

Design

A total of ninety mice were used as the study subjects, and thirty mice were used for each test, i.e., curative, suppressive, and prophylactic. The animals were split into six groups at random, each comprising five animals – an uninfected untreated group (UU) serving as the normal control, an infected untreated group (UI) as the negative control, a standard drug (chloroquine 5 mg/kg for suppressive and curative assessments, and pyrimethamine 1.2 mg/kg for the prophylactic evaluation) treatment group as the positive control, and three groups were treated with extract doses of 100, 200 and 400 mg/kg, respectively.

Animal use and ethical consideration

This study received ethical approval from the Institutional Animal Care and Use Committee (IACUC), Ethical Committee and Animal Experimental Unit, Pharmacology Department at the University of Jos in Nigeria (approval no. REF/UJ/FPS/F17-00379).

Preparation and inoculation of the parasite

The chloroquine-sensitive *P. berghei* NK 65 was provided by the Institute for Advanced Medical Research and Training (IAMRT) at the University

of Ibadan, by inoculation of mice with infected blood. The parasite was kept in the laboratory through serial passage of uninfected mice with infected blood. A donor mouse infected with *P. berghei* NK65 (Parasitaemia level: 25 - 30 %) was euthanized with chloroform anesthesia. The collected blood was subsequently diluted using normal saline solutions to achieve a concentration of approximately 1×10^7 parasitized RBC per 0.2 mL. Each recipient mouse was then inoculated with 0.2 mL of the infected blood [13].

Suppressive activity of *C. quadrangularis*

This involved the treatment with aqueous extract of *C. quadrangularis* immediately after inoculation (early infection). The method, as described by [13], involved selecting thirty mice of either sex and divided into six groups of five. All the groups except group 1 were given 0.2 mL of the inoculum intraperitoneally. Group 3 administered orally standard antimalarial drug chloroquine phosphate (CQ) 5 mg/kg, groups 4, 5, and 6 were given oral extract doses of 100, 200, and 400 mg/kg, respectively, once daily on days 1, 2, 3, and 4. The course of parasitaemia was monitored for four days (D4 - D7) by making thin smears with the blood taken from each mouse's tail. The percentage suppression (S) of the parasite was calculated based on Eq 1.

$$S (\%) = \{(C_p - T_p)/C_p\}100 \dots\dots\dots (1)$$

Where C_p is Parasitaemia in Control and T_p is Parasitaemia in test group.

Curative activity of *C. quadrangularis*

This test was similar to the suppressive test, but with a delayed treatment regimen. The extract and standard drug were administered from Day 5 to Day 8 post-inoculation [13].

The mice were monitored for parasite clearing from Day 5 to Day 8, and the increase or decrease in parasitaemia (P) was calculated based on Eq 2.

$$P (\%) = (N_p/N_t)100 \dots\dots\dots (2)$$

Where N_p is the number of parasitized red blood cells and N_t is the total number of red blood cells

Prophylactic activity of *C. quadrangularis*

The extracts' prophylactic activity was evaluated using a method outlined by [13]. Thirty mice were divided into five separate groups, each containing 6 animals. The extract was

administered to three groups at doses of 100, 200, and 400 mg/kg, respectively. Group 1 and 2 (control 1 and 2) were given normal saline and 1.2 mg/kg of pyrimethamine (SKG Pharma Limited, Nigeria) for four consecutive days. On the fourth day, all the mice received an injection of 0.2 mL of *P. berghei*-infected blood. Then, after 72 hours (D4 - D7), slides were made and the level of parasitaemia calculated using equation 2.

Preparation of slides

Mice were bled by snipping the tip of the tail using a pair of scissors. The tail was gently squeezed to express a drop of blood, which was then collected on a labelled, clean, grease-free microscopic slide [13]. A thin smear was then made by placing the blood-containing slide on a flat surface and using a second slide as a spreader. The spreader was touched to the blood drop, and the blood was allowed to spread along its edge, maintaining a 45° angle between the spreader and the slide; it was firmly pushed to spread the blood on the slide. The smears were allowed to dry on a rack, and the slides were fixed by dipping in absolute alcohol and allowed to dry. The fixed slides were then stained with a 10 % Giemsa solution for 30 minutes. Following staining, they were rinsed with clean water, air-dried, and examined microscopically using oil immersion.

Collection of blood samples

The collection of blood samples was done according to the method described by [12]. Following the final dose, at the conclusion of the study, mice were allowed to go for 24 hours with only water. The animals were weighed and anaesthetized with chloroform soaked in cotton wool. They were sacrificed and dissected to expose the cardiac cavity of the heart. Between 3-5 mL of blood was drawn using a 5 mL sterile syringe by cardiac puncture and carefully discharged into an ethylenediaminetetraacetic acid (EDTA) bottle. The bottle was covered, and the blood was mixed with the EDTA to avoid clotting. The sample bottles were labelled accordingly and were taken for haematological analysis.

Haematological analysis

All the blood parameters were analyzed using a standard protocol [13]. The Parameters were Red Blood Cell (RBC) count, Packed Cell Volume (PCV), Platelet Count (PLT count), Haemoglobin (HBG), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH)

level, Mean Corpuscular Haemoglobin Concentration (MCHC), White Blood Cell count (WBC), neutrophil, monocyte, lymphocyte, and eosinophil.

Statistical analysis

The data collected was input into Microsoft Excel for cleaning and coding, and thereafter transferred to IBM SPSS version 17.0 for statistical analysis. One-way ANOVA statistics were used to identify if there was a significant difference across parasitaemia levels as well as haematological parameters across the different treatment groups. Where a significant difference was recorded, the Bonferroni post hoc test was used to separate mean pairs that differed significantly from each other. The results were deemed statistically significant when $p < 0.05$.

RESULTS

Phytochemical screening of the *C. quadrangularis* stem aqueous extract (Table 1) shows flavonoids, alkaloids, and carbohydrates. A yield of 26 g was obtained from 200 g of crude extract. The oral administration of aqueous extract of *C. quadrangularis* showed no sign of toxicity, as shown in Table 2. Doses of 10, 100, and 1000 mg/kg were given in the first phase, and it was observed that there was no behavioural change and there was no mortality recorded. The second phase involved administering 1600, 2900, and 5000 mg/kg doses, with observations like the first phase. The animals were very active, with no respiratory distress, no mortality, and food and water intake were normal.

Figures 1 to 3 show the impact of the aqueous extract of *C. quadrangularis* on parasitaemia levels in mice. The result of the suppressive test performed using aqueous *C. quadrangularis* stem extract (Figure 1) revealed an overall significant difference in the mean parasitaemia level across the different concentrations of treatments administered ($F(4,55) = 18.597$, $p < 0.001$). Compared to the untreated control (UI), parasitaemia levels declined consistently, with the lowest record found in mice treated with chloroquine (CQ). Throughout the study, no significant change was noted in the level of parasitaemia across the different treatments administered ($p > 0.05$), even though on days three and four, parasitaemia levels were significantly higher ($p < 0.05$) in the untreated control group (UI; 11.87 ± 0.03 and 13.70 ± 0.06 , respectively) based on the Bonferroni post hoc test. Although on day 4, parasitaemia level was least in mice treated with Chloroquine ($1.73 \pm$

0.29), compared to those administered with different concentrations of the extract, the difference observed was not significant ($p > 0.05$).

The result of the curative test performed (i.e., Figure 2) revealed an overall significant difference in the mean parasitaemia level across the different concentrations of treatments administered ($F(4,55) = 32.311$, $p < 0.001$). Compared to the untreated control (UI), parasitaemia levels declined consistently, with the lowest record found in mice treated with Chloroquine (CQ). Although at day 7 of treatment, parasitaemia level was slightly higher in groups administered 200 mg/kg of *C. quadrangularis* (8.30 ± 1.13) based on Bonferroni post hoc test compared to the administration at 400 mg/kg (4.47 ± 0.60) and Chloroquine (4.20 ± 0.38 ; $p < 0.05$), and on day 8, parasitaemia levels remained statistically similar ($p > 0.05$) across all treatment concentrations as well as the Chloroquine, even though the treatment with Chloroquine (1.60 ± 0.31) and 100 mg/kg (4.13 ± 0.64) of the extract yielded lower parasitaemia levels.

The prophylactic test showed a significant difference in mean parasitaemia levels across treatment concentrations ($F(4, 55) = 22.139$, $p < 0.001$). Compared to the untreated control group, which had high parasitaemia levels on days 5 (9.20 ± 0.06) % and 6 (11.87 ± 0.03) %, the treatment groups (including Chloroquine) showed no significant difference across different doses. However, on day 7 of treatment, parasitaemia level was significantly lower in mice administered Chloroquine (1.80 ± 0.26) % compared with those treated with 100 mg/kg (5.47 ± 1.22) % of the extract. Although the untreated control group exhibited a significantly elevated parasitaemia level ($p < 0.05$; 13.80 ± 0.06) % compared to what was recorded in each treatment group.

The analysis of the haematological parameters of the mice in the three test groups indicated the following: the suppressive test (Table 3) showed that the RBC, PCV, PLT, HGB and MCHC of the

C. quadrangularis groups were significantly elevated ($P < 0.05$) relative to the negative control group. Moreover, that of the CQ group exceeded the negative control significantly ($p < 0.05$). Worth of note is that the value of *C. quadrangularis* (200 mg/kg) RBC $6.09 \pm 6.29 \times 10^{12}$ L was close to the value of CQ RBC $6.55 \pm 0.28 \times 10^{12}$ L. In Table 4, the white blood cells, neutrophil, monocyte, lymphocyte, and eosinophil values of the CQ and *C. quadrangularis* groups exhibited a significant decrease ($p < 0.05$) in relation to the negative control. The curative test in Table 5 showed that the RBC, PCV, MCH, PLT, HGB and MCHC of the negative control were significantly decreased ($P < 0.05$) when compared with the values of the CQ and *C. quadrangularis* groups. The values of white blood cell, neutrophil, monocytes, lymphocytes and eosinophil exceeded both the CQ (positive control) group and the *C. quadrangularis* groups significantly ($p < 0.05$), as shown in Table 6.

The values of RBC, PCV, MCH, PLT, HGB, and MCHC (Table 7, prophylactic test) of the *C. quadrangularis* groups were significantly higher ($p < 0.05$) than the negative control, whereas the CQ group exhibited lower values ($p > 0.05$) compared to the negative control. MCV was significantly higher ($p < 0.05$) in the negative control group than in the CQ and *C. quadrangularis* groups. The WBC, neutrophil, monocyte, lymphocyte and eosinophil of the CQ and *Cissus quadrangularis* groups were significantly lower ($P < 0.05$) when compared with the negative control (Table 8).

DISCUSSION

The safety of drugs and medicinal plants in animals and humans is crucial. To assess the safety of the experimental plant, an acute toxicity test was carried out on experimental animals. An LD₅₀ above 5000 mg/kg was recorded with no behavioural changes, clinical abnormality or mortality of any animal in the groups.

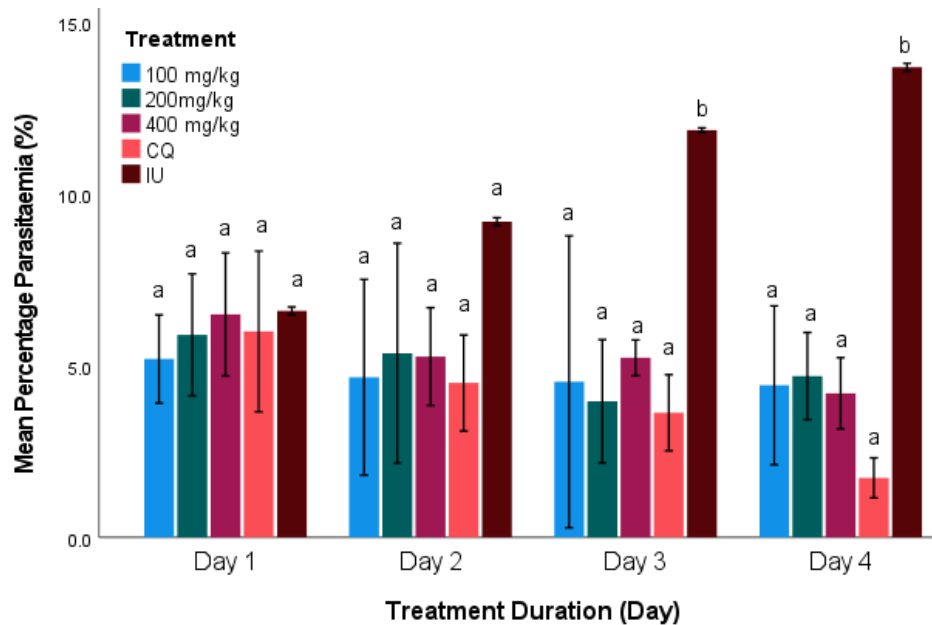
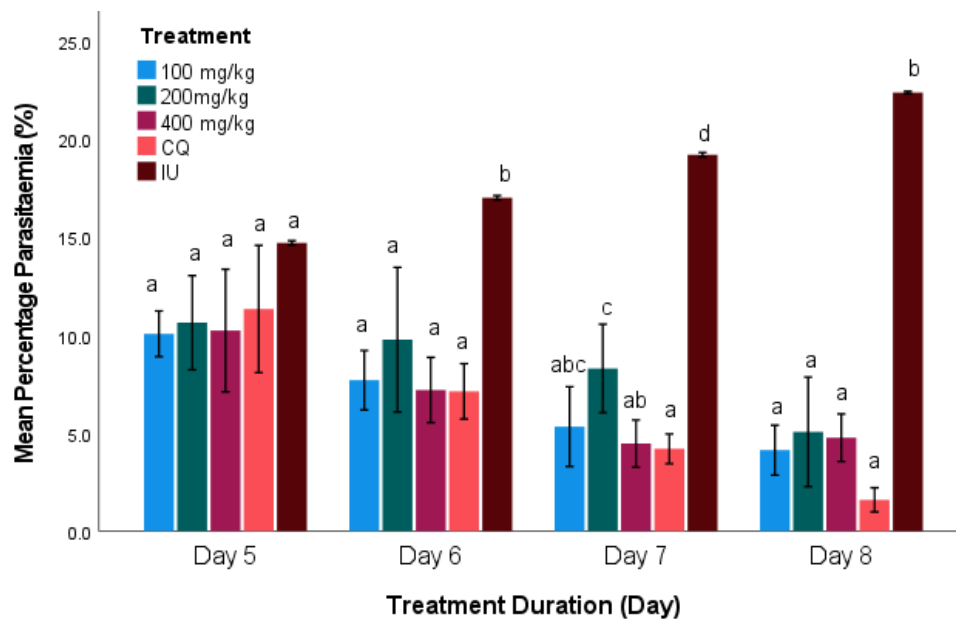
Table 1: Phytochemistry of aqueous extract of *Cissus quadrangularis* stem

Constituents	Test	Aqueous extracts
Alkaloids	Mayers	++
Saponins	Fehlings	-
Tannin	Lead sub-acetate	-
Flavonoids	Lead sub-acetate	++
Carbohydrates	Iodine	++
Steroids	Libermann	-
Anthraquinones	Bontrogens	-
Cardiac glycosides	Libermann	-

Note: + slightly present, ++ moderately present, +++ present in high concentration, - not detected

Table 2: Mortality rate of mice 48 hours after the administration of aqueous extract of *Cissus quadrangularis* stem

Dose mg/kg	Number of animals tested	Mortality (%)
10	3	0
100	3	0
1000	3	0
1600	3	0
2900	3	0
5000	3	0

**Figure 1:** Levels of Parasitaemia in *Plasmodium berghei* NK65 infected mice on day 0 and treated on the same day (0-3) with the aqueous fraction of *C. quadrangularis* stem and chloroquine phosphate (suppressive test). Bars followed by different letters above in each treatment duration differ significantly ($p < 0.05$)**Figure 2:** Parasitaemia levels in *Plasmodium berghei* NK 65 infected mice on day 0 and treated on day 5-8 with the aqueous extract of *C. quadrangularis* stem and chloroquine phosphate (curative test). Bars followed by different letters above in each treatment duration differ significantly ($p < 0.05$)

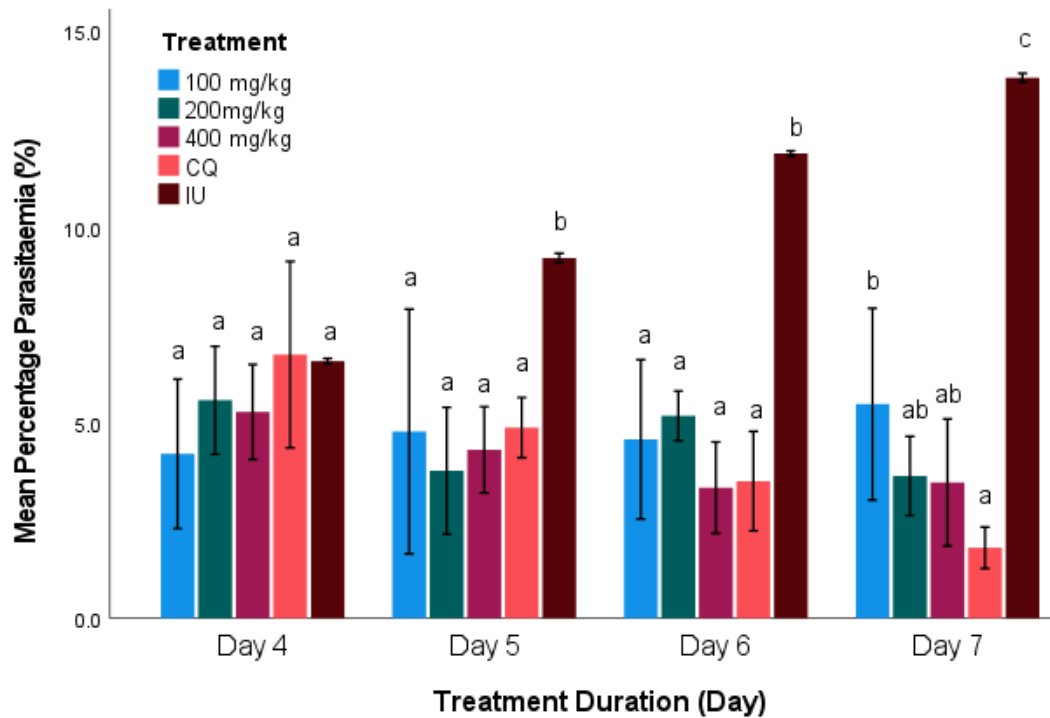


Figure 3: Parasitaemia levels in *Plasmodium berghei* NK 65 infected mice on day 3 and treated on day 0-3 with aqueous extract of *C. quadrangularis* stem and pyrimethamine (prophylactic test). Bars followed by different letters above in each treatment duration are significantly different at $p < 0.05$

Table 3: Red blood cells and related parameters of *Plasmodium berghei* NK65 infected mice on day 0, treated on day 1-4 with aqueous extract of *C. quadrangularis* stem (suppressive test)

Group	RBC ($\times 10^{12}/L$)	PCV	MCH (pg)	PLT ($\times 10^9/L$)	HGB (g/dL)	MCV (fL)	MCHC (g/dL)
UU	7.11 $\pm$ 0.289	37.96 $\pm$ 8.05	21.37 $\pm$ 1.259	788.00 $\pm$ 90.996	15.33 $\pm$ 14.621	70.63 $\pm$ 2.115	33.00 $\pm$ 23.065
IU	2.97 $\pm$ 0.124	18.70 $\pm$ 0.916	15.53 $\pm$ 0.202	179.00 $\pm$ 14.526	4.30 $\pm$ 0.472	63.56 $\pm$ 0.762	24.56 $\pm$ 0.569
CQ5mg/kg	6.55 $\pm$ 0.286 ^b	34.00 $\pm$ 2.000 ^b	16.86 $\pm$ 0.520 ^b	488.00 $\pm$ 138.750 ^b	11.00 $\pm$ 0.152 ^b	49.76 $\pm$ 1.821 ^b	33.83 $\pm$ 0.202 ^b
100mg/kg	5.06 $\pm$ 0.083 ^{bc}	23.70 $\pm$ 0.115 ^{bc}	15.96 $\pm$ 0.290 ^{bc}	382.00 $\pm$ 120.950 ^{bc}	8.06 $\pm$ 0.088 ^{bc}	46.76 $\pm$ 0.545 ^{bc}	34.10 $\pm$ 0.472 ^{bd}
200mg/kg	6.09 $\pm$ 0.295 ^{bc}	28.90 $\pm$ 1.493 ^{bc}	16.13 $\pm$ 0.338 ^{bc}	350.67 $\pm$ 141.060 ^{bc}	9.83 $\pm$ 0.405 ^{bc}	47.40 $\pm$ 1.290 ^{bc}	34.10 $\pm$ 0.400 ^{bd}
400mg/kg	5.26 $\pm$ 0.276 ^{bc}	26.06 $\pm$ 1.618 ^{bc}	16.46 $\pm$ 0.185 ^{bc}	637.33 $\pm$ 252.630 ^{bd}	8.66 $\pm$ 0.545 ^{bc}	49.50 $\pm$ 0.709 ^{bc}	33.26 $\pm$ 0.569 ^{bc}
P-values	<0.0001	0.0002	0.1263	0.3603	<0.0001	<0.0001	<0.0001

Note: RBC (Red Blood Cell count), PCV (Packed Cell Volume), MCH (Mean Corpuscular Haemoglobin), platelet count, haemoglobin levels, and MCV (Mean Corpuscular Volume). Data are presented as mean $\pm$ SEM (n = 5). ^a $P < 0.05$ vs uninfected untreated group; ^b $P < 0.05$ vs infected untreated group; ^c $P < 0.05$ vs standard drug group; ^d $P > 0.05$ vs standard drug

Table 4: White blood and related parameters of *Plasmodium berghei* NK65 infected mice on day 0, treated on day 1-4 with aqueous extract of *C. quadrangularis* stem (suppressive test)

Group	WBC ($\times 10^9/L$)	NEU (%)	MON (%)	LYM (%)	EOS (%)
UU	3.83 $\pm$ 0.817	22.15 $\pm$ 7.253	0.30 $\pm$ 0.057	71.10 $\pm$ 0.950	1.23 $\pm$ 0.088
IU	17.43 $\pm$ 1.770	67.90 $\pm$ 1.210	3.30 $\pm$ 1.480	77.30 $\pm$ 1.550	14.50 $\pm$ 1.970
CQ5mg/kg	4.67 $\pm$ 0.913 ^a	54.66 $\pm$ 5.424 ^a	0.56 $\pm$ 0.366 ^a	40.96 $\pm$ 5.608 ^a	3.30 $\pm$ 0.600 ^a
100mg/kg	13.05 $\pm$ 1.536 ^{ad}	45.00 $\pm$ 3.630 ^{ac}	0.77 $\pm$ 0.480 ^{ad}	49.30 $\pm$ 5.580 ^{bd}	4.45 $\pm$ 3.625 ^{ac}
200mg/kg	4.80 $\pm$ 0.935 ^{ad}	55.70 $\pm$ 7.919 ^{ad}	0.33 $\pm$ 0.145 ^{ac}	42.00 $\pm$ 8.580 ^{bd}	11.20 $\pm$ 0.321 ^{ac}
400mg/kg	8.95 $\pm$ 4.970 ^{ad}	58.66 $\pm$ 4.045 ^{ad}	0.36 $\pm$ 0.088 ^{ac}	37.86 $\pm$ 4.826 ^{ac}	2.46 $\pm$ 0.952 ^{ac}
P-values	0.0755	0.0596	0.0608	0.0037	0.0154

Note: WBC (White Blood Cell count), NEU (Neutrophils), MON (Monocytes), LYM (Lymphocytes), and EOS (Eosinophils). Data are presented as mean $\pm$ SEM (n = 5), ^a $P < 0.05$ vs. uninfected untreated group; ^b $P < 0.05$ vs. infected untreated group; ^c $P < 0.05$ vs. standard drug group; ^d $P > 0.05$ vs standard drug

Table 5: Red blood cell indices of *P. berghei* NK65 infected mice at baseline (day 0) and following treatment with *C. quadrangularis* stem aqueous extract from day 5-8 (curative test)

Group	RBC (L)	PCV	MCH (pg)	PLT (L)	HGB (g/dL)	MCV (fL)	MCHC (g/dL)
UU	7.11±0.289	37.96±8.05	21.37±1.259	788.00±90.996	15.33±14.621	70.63±2.115	33.00±23.065
IU	2.97±0.124	18.70±0.916	15.53±0.202	179.00±14.526	4.30±0.472	63.56±0.762	24.56±0.569
CQ5mg/kg	5.80±0.435 ^b	35.86±0.433 ^b	17.23±1.267 ^b	498.00±60.103 ^b	9.93±0.611 ^b	49.96±2.238 ^b	34.50±2.122 ^b
100mg/kg	5.91±0.236 ^{bd}	30.93±2.080 ^{bc}	16.43±0.296 ^{bc}	1030.70±59.845 ^{bd}	9.73±0.554 ^{bc}	52.20±1.501 ^{bd}	31.50±0.585 ^{bc}
200mg/kg	6.19±0.294 ^{bd}	31.63±1.533 ^{bc}	16.10±0.288 ^{bc}	710.00±86.153 ^{bd}	9.96±0.617 ^{bd}	51.10±0.624 ^{bd}	31.43±0.393 ^{bc}
400mg/kg	4.99±0.639 ^{bc}	26.00±4.095 ^{bc}	16.56±0.288 ^{bc}	756.33±26.400 ^{bd}	8.30±1.127 ^{bc}	51.63±1.590 ^{bd}	32.13±0.721 ^{bc}
p-values	0.0010	0.0026	0.4333	0.0109	0.0009	0.0004	0.0008

Note: Data are presented as mean ± SEM (n = 5), ^aP < 0.05 vs. uninfected untreated group; ^bP < 0.05 vs. infected untreated group; ^cP < 0.05 vs. standard drug group; ^dP > 0.05 vs standard drug

Table 6: White blood cell indices and related parameters of *P. berghei* NK65 infected mice on day 0 and following treatment on day 5-8 with aqueous extract of *C. quadrangularis* stem (curative test)

Group	WBC (x10 ⁹ /L)	NEU (%)	MON (%)	LYM (%)	EOS (%)
UU	3.83±0.817	22.15±7.253	0.30±0.057	71.10±0.950	1.23±0.088
IU	17.43±1.770	67.90±1.210	3.30±1.480	77.30±1.550	14.50±1.970
CQ5mg/kg	5.51±5.963 ^a	45.80±11.143 ^a	0.60±0.251 ^a	51.80±11.870 ^a	1.60±0.556 ^a
100mg/kg	6.92±1.858 ^{ac}	62.13±5.938 ^{ad}	0.60±0.057 ^{ae}	34.90±5.505 ^{ac}	2.03±0.384 ^{ad}
200mg/kg	24.99±14.300 ^{bd}	50.60±8.649 ^{ad}	0.36±0.088 ^{ac}	47.33±8.407 ^{ac}	1.23±0.584 ^{ac}
400mg/kg	9.99±3.878 ^{ac}	47.63±11.023 ^{ad}	0.23±0.088 ^{ac}	50.30±10.610 ^{ac}	1.36±0.218 ^{ac}
p-values	0.4808	0.3296	0.0447	0.0550	<0.0001

Note: Data are presented as mean ± SEM (n = 5); ^aP < 0.05 vs uninfected untreated group; ^bp < 0.05 vs. infected untreated group; ^cP < 0.05 vs. standard drug group; ^dp > 0.05 vs standard drug

Table 7: Red blood cell and related parameters of *Plasmodium berghei* NK65 infected mice on day 4 and treated on days 1-4 with aqueous extract of *C. quadrangularis* stem (Prophylactic test)

Group	RBC (L)	PCV	MCH (pg)	PLT (L)	HGB (g/dL)	MCV (fL)	MCHC (g/dL)
UU	7.11±0.289	37.96±8.05	21.37±1.259	788.00±90.996	15.33±14.621	70.63±2.115	33.00±23.065
IU	2.97±0.124	18.70±0.916	15.53±0.202	179.00±14.526	4.30±0.472	63.56±0.762	24.56±0.569
PM1.5mg/kg	5.20±0.465 ^b	35.66±0.783 ^b	16.10±0.300 ^b	478.33±23.190 ^b	8.36±0.617 ^b	47.73±1.053 ^b	33.80±0.305 ^b
100mg/kg	5.01±0.455 ^{bc}	24.06±1.572 ^{bc}	16.20±0.416 ^{bd}	374.33±14.460 ^{bc}	8.06±0.656 ^{bc}	48.06±1.568 ^{bd}	33.80±0.709 ^{be}
200mg/kg	5.15±0.292 ^{bc}	25.36±0.920 ^{bc}	17.06±0.533 ^{bd}	315.33±15.240 ^{bc}	8.76±0.284 ^{bd}	49.33±1.167 ^{bd}	34.60±0.251 ^{bd}
400mg/kg	7.33±0.531 ^{bd}	34.36±1.889 ^{bc}	15.90±0.305 ^{bc}	299.67±0.881 ^{bc}	11.73±0.636 ^{bd}	47.26±1.048 ^{bc}	34.17±0.078 ^{bd}
P-values	0.0003	<0.0001	0.1236	0.0741	<0.0001	<0.0001	<0.0001

Note: Data are presented as mean ± SEM (n = 5), ^aP < 0.05 vs. uninfected untreated group; ^bP < 0.05 vs. infected untreated group; ^cP < 0.05 vs. standard drug group; ^dP > 0.05 vs standard drug

Table 8: White blood cell indices and related parameters of *P. berghei* NK65 infected mice on day 4 following treatment on day 0-3 with aqueous extract of *C. quadrangularis* stem (Prophylactic test)

Group	WBC (L)	NEU (%)	MON (%)	LYM (%)	EOS (%)
UU	3.83±0.817	22.15±7.253	0.30±0.057	71.10±0.950	1.23±0.088
IU	17.43±1.770	67.90±1.210	3.30±1.480	77.30±1.550	14.50±1.970
CQ5mg/kg	7.60±2.435 ^a	68.70±3.066 ^b	1.30±0.513 ^a	27.10±1.644 ^a	2.40±1.950 ^a
100mg/kg	14.69±5.647 ^{ad}	33.80±6.173 ^{ac}	1.86±1.268 ^{ad}	62.26±6.370 ^{ad}	1.86±1.288 ^{ac}
200mg/kg	14.19±6.418 ^{ad}	38.13±16.541 ^{ac}	1.83±0.835 ^{ad}	58.46±16.388 ^{ad}	1.26±0.498 ^{ac}
400mg/kg	9.84±0.580 ^{ad}	37.56±17.207 ^{ac}	1.03±0.371 ^{ac}	41.93±2.209 ^{ad}	4.77±2.355 ^{ad}
P-values	0.4745	0.1118	0.5609	0.1011	0.0016

Note: Data are presented as mean ± SEM (n = 5), ^aP < 0.05 vs. uninfected untreated group; ^bP < 0.05 vs. infected untreated group; ^cP < 0.05 vs. standard drug group; ^dP > 0.05 vs standard drug

This, according to [14] classification table, an LD₅₀ between 5000 - 15000 mg/kg is practically non-toxic. This result showed that *C. quadrangularis* stem is relatively safe and can therefore be used for medicinal purposes. Studies in the practice of traditional medicine and use of medicinal plants have indicated that *C. quadrangularis* has been used to treat various ailments, including colds, pain, asthma, loss of appetite, and malaria [10,11]. The observed effect may be due to the alkaloids present in the plant, as indicated by the current findings.

The effect of *C. quadrangularis* stem extract on haematological parameters was assessed, revealing notable changes in treated mice. The aqueous extract induced significant haematological alterations, suggesting its potential influence on blood cell production and regulation. The increase in RBC, PCV, and HGB in groups treated with *C. quadrangularis* extract in our study aligns with previous studies [15], suggesting the extract may contain phytochemicals that stimulate erythropoiesis, boosting red blood cell production.

Conversely, as parasitaemia increased in the infected untreated group, RBC, PCV, and HGB levels significantly decreased, likely contributing to anaemia in the mice, evident from symptoms like paleness and watery blood. This highlights the extract's potential in mitigating malaria-induced anaemia. This finding aligns with [16], who similarly observed decreased RBC, PCV, and HGB levels in mice infected with *P. berghei*, accompanied by physical signs of anaemia, mirroring malaria-induced anaemia in humans.

The infected, untreated control group showed a significant rise in white blood cell (WBC) counts. This surge in WBC count is a natural response of the body's immune system, which mobilizes WBCs as a primary defense mechanism to combat invading pathogens such as bacteria, viruses, fungi, and parasites. This finding is consistent with the results of [16], who used a different plant and showed a marked rise in WBC count corresponding to higher parasitemia levels.

White blood cells of chloroquine and *C. quadrangularis*-treated group decreased compared to negative control. This decrease in white blood cells after treatment with chloroquine and *C. quadrangularis* indicates immunomodulatory effects possibly shared by chloroquine and the investigated plant extracts during *Plasmodium berghei* infection. Chloroquine acts primarily by interfering with the parasite's digestive vacuole functions, inhibiting heme polymerization critically for parasite survival. Certain plant extracts may contain bioactive compounds that exert antimalarial effects, leading to reduced parasite loads and subsequent immune modulation, as evidenced by decreased white blood cell counts as observed in the current study. This aligns with previous study results [17,18]. This suggests that these extracts may help mitigate malaria-induced immune responses, potentially alleviating disease severity.

CONCLUSION

The oral administration of aqueous extract of *C. quadrangularis* stem significantly improves the haematological parameters in mice. The significant increase in red blood cell parameters shows that the plant may possess anti-anaemic properties. The infected, untreated mice showed a significant increase in the white blood cells, indicating the presence of invading parasites. The treatment with chloroquine and plant extracts showed a notable drop in WBC count, indicating that the plant may have the same effect as chloroquine has on *Plasmodium berghei*. The findings from this study confirm the antimalarial potential of the *C. quadrangularis* stem extract. Further studies should isolate the bioactive compounds, explore their mechanisms, and assess their safety and efficacy through clinical trials.

DECLARATIONS

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Ethical approval

This study received ethical approval from the Ethical Committee and Animal Experimental Unit, Pharmacology Department, University of Jos, Nigeria (approval no. REF/UJ/FPS/F17-00379).

Use of Artificial intelligence/Large language models

The authors confirm that AI-based tools were used solely for language editing and manuscript polishing purposes (e.g., grammar, phrasing, and clarity). No AI tools were used to generate content, interpret data, or influence study design, results, or conclusions. All intellectual contributions, study analyses, and interpretations reported in the manuscript are the original work of the authors.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Obed Nanjul Goselle and Bernard Malau Matur conceived the project. Azonchi Arin Hassan and Joseph Julfa Nuhu Nden performed experiments and organized the data. Nannim Nanvyat and Barineka Mbede Wilson Nwibari performed data analysis and interpretation. Nannim Nanvyat and Obed Nanjul Goselle drafted the initial manuscript, with Benard Malau Matur and Naomi Nari James Rugu providing critical reviews. All authors contributed and consented to the submission in its current form.

Availability of data and materials

Data supporting the study's findings can be obtained on request from the corresponding author.

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