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Antiplasmodial Activity of Aqueous Extract from the Root Bark of Boswellia dalzielii (Burseraceae) in Mice Infected with Plasmodium berghei Nk-65

Mary Matawal Mankilik[1*], Pofi Faith Nandi[1], Joel Paul[1], Peace Ori Abaya[1], Daniel Hassan Mhya[2], Otitoju Akin Peace[3], Ishaya Yohanna Longdet[1]

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ABSTRACT

Medicinal plants continue to play a vital role in malaria management across Africa, particularly in response to the growing resistance of malaria parasites to conventional antimalarial drugs. Boswellia dalzielii has been reported to possess antimalarial activity. However, scientific evidence regarding the efficacy of its root bark remains limited. This study investigated the antimalarial potential of Boswellia dalzielii root bark extract in Swiss albino mice infected with Plasmodium berghei NK-65. The collected root bark was thoroughly washed, air-dried, pulverized, and macerated in distilled water. The extract was subsequently subjected to phytochemical screening, acute toxicity evaluation, and in vivo antimalarial assessment. Eighteen mice were randomly assigned into six groups comprising infected mice treated with a standard antimalarial drug, infected untreated mice, infected mice administered 100, 200, and 400 mg/kg body weight of the extract, and uninfected untreated controls. Parasitaemia levels, packed cell volume (PCV), body weight changes, and mean survival time were evaluated. Phytochemical analysis revealed the presence of flavonoids, tannins, saponins, balsams, carbohydrates, and phenolic compounds. Acute toxicity testing indicated that the extract was safe at the administered doses. In vivo results demonstrated a dose-dependent reduction in parasitaemia, improvement in PCV, and increase in body weight among treated mice. Administration of 400 mg/kg of the extract extended the mean survival time to 20 days, exceeding the 17-day survival period observed with the standard drug, with antimalarial activity comparable to chloroquine phosphate (25 mg/kg). These findings suggest that Boswellia dalzielii root bark is safe and contains bioactive constituents with promising antimalarial properties.

INTRODUCTION

Malaria remains one of the most significant public health challenges worldwide, with the greatest burden occurring in tropical and subtropical regions of Africa (Li *et al.,* 2024). Of the five Plasmodium species known to infect humans, namely P. falciparum, P. malariae, P. vivax, P. ovale, and P. knowlesi, P. falciparum is responsible for the most severe and life-threatening forms of the disease (Robert *et al.,* 2025). Although substantial progress has been made in malaria control, the disease continues to cause considerable morbidity and mortality, largely driven by the emergence and spread of resistance to existing antimalarial drugs (White, 2004; Zheng *et al.,* 2024). Nigeria bears a disproportionate share of the global malaria burden, accounting for approximately 27 percent of reported cases and 31 percent of malaria-related deaths, with transmission intensity particularly high in the northern parts of the country (Burrows *et al.,* 2017; Friedman-Klabanoff *et al.,* 2024; Li *et al.,* 2024; Okunlola & Oyeyemi, 2019). Despite the implementation of preventive and therapeutic strategies, including vaccines, insecticide-treated nets, seasonal malaria chemoprophylaxis, and artemisinin-based combination therapies, malaria remains endemic in many communities (Burrows *et al.,* 2017; Friedman-Klabanoff *et al.,* 2024). Medicinal plants continue to attract scientific interest as alternative or complementary sources of antimalarial agents. Boswellia dalzielii Hutch., a member of the family Burseraceae and commonly referred to as African frankincense, is widely distributed across tropical Africa, including Nigeria, Cameroon, Sudan, and Burkina Faso (Dogara *et al.,* 2022; Mbiantcha *et al.,* 2017; Sabo *et al.,* 2022; Vedekoi *et al.,* 2024). Previous studies have reported several pharmacological properties of the plant, particularly from its leaves and stem bark, such as anti-inflammatory and antimicrobial activities (Ammon *et al.,* 2010; Awada *et al.,* 2020). However, information on the antimalarial potential of the root bark is scarce. In view of this knowledge gap, the present study investigated the in vivo antimalarial activity of Boswellia dalzielii root bark extract using Plasmodium berghei NK-65-infected mice.

[1] Department of Medical Biochemistry, Faculty of Basic Medical Sciences, University of Jos, Plateau State, Nigeria. Postal Code: 930001
[2] Department of Medical Biochemistry, Faculty of Basic Medical Sciences, Abubakar Tafawa Balewa University, Bauchi. Bauchi State, Nigeria. Postal Code: 440002
[3] National Biotechnology Research and Development Agency (NBRDA), FCT, Abuja, Nigeria. Postal Code: 900001
[*] Corresponding author's e-mail: mankilikma@unijos.edu.ng

LITERATURE REVIEW

Plant-based compounds have long served as important foundations for antimalarial drug development. Notable examples include quinine, derived from Cinchona species, and artemisinin, isolated from Artemisia annua, both of which exhibit strong antiplasmodial efficacy (White, 2004; Zhou & Yue, 2022). The increasing prevalence of resistance to existing antimalarial drugs has renewed interest in identifying alternative plant sources that may yield effective therapeutic agents (White, 2004; Zheng *et al.,* 2024).

Recent investigations have demonstrated the pharmacological relevance of species within the genus Boswellia. In particular, extracts obtained from the leaves and stem bark of Boswellia dalzielii have shown notable in vitro antimalarial activity against Plasmodium falciparum (Jansen *et al.,* 2020; Salihu *et al.,* 2018). These biological effects have been linked to the presence of diverse phytochemical constituents, including flavonoids, phenolic compounds, triterpenoids, and saponins (Atawodi *et al.,* 2011; Awada *et al.,* 2020; Dogara *et al.,* 2022; Mankilik *et al.,* 2021). Flavonoids and phenolics are known to exhibit antioxidant activity and membrane-stabilizing properties, which may interfere with parasite proliferation while protecting host erythrocytes during infection (Atawodi *et al.,* 2011; Dogara *et al.,* 2022). In addition, saponins have been reported to exert antimalarial effects through membrane interactions and by influencing host immune responses (Mankilik *et al.,* 2021). Monoterpenes such as alpha pinene have also been detected in substantial amounts in B. dalzielii leaves and are believed to contribute to the plant's antiparasitic activity (Dogara *et al.,* 2022).

Traditional medical practices further support the use of B. dalzielii in malaria management. Different plant parts, including the leaves, stem bark, and roots, have been utilized in ethnomedicine across several African countries for the treatment of malaria-related illnesses (Sachdeva *et al.,* 2022; Vedekoi *et al.,* 2024). While extensive research has focused on the leaves and stem bark, the root bark has received comparatively little scientific attention. This gap highlights the root bark as a potentially valuable source of unexplored antimalarial compounds (Dandashire *et al.,* 2019; Gadzama *et al.,* 2025; Salihu *et al.,* 2018; Sherifi *et al.,* 2020; Vedekoi *et al.,* 2024).

Evidence from studies on other medicinal plants further supports the investigation of B. dalzielii root bark. Experimental malaria models in rodents have shown that extracts from plants such as Myrica salicifolia, Dorstenia barnimiana, Leonotis ocymifolia, Cucumis metuliferus, and Lippia kituiensis can significantly reduce parasitaemia, enhance survival rates, prevent weight loss, maintain body temperature, and alleviate malaria associated anemia (Derebe *et al.,* 2021; Kifle *et al.,* 2020; Mankilik *et al.,* 2025; Teklu *et al.,* 2020). These observations emphasize the importance of evaluating medicinal plant extracts not only for their direct antiparasitic effects but also for their ability to provide systemic protection during malaria infection. Accordingly, assessing the antimalarial activity of B. dalzielii root bark extract using Plasmodium berghei-infected mice may reveal bioactive constituents with therapeutic relevance and contribute to future antimalarial drug development.

MATERIALS AND METHOD

Plant Sample Collection

Fresh root bark of Boswellia dalzielii, characterized by a brownish coloration, was collected from Pilkong in Abwor Dyis village, Lankang District, Pankshin Local Government Area of Plateau State, Nigeria, in January 2023. The plant material, including the root bark, leaves, and flowers, was authenticated at the Herbarium of the Department of Botany, Federal College of Forestry, Jos, Plateau State. A voucher specimen was deposited for future reference under the accession number FHJ2023. The collected root bark was thoroughly cleaned to remove adhering dirt and debris, air dried at room temperature between 22 and 25 degrees Celsius, and subsequently pulverized into a coarse powder using a wooden mortar and pestle.

Preparation of Plant Extract

Four hundred grams of the powdered root bark of Boswellia dalzielii was accurately weighed using a digital weighing balance (JJ200 G and G Deutschland) and soaked in 2.25 liters of distilled water. The mixture was stirred thoroughly and allowed to stand at room temperature for one hour. After maceration, the mixture was filtered using a clean white muslin cloth. The resulting dark brown filtrate was transferred into a one-liter Pyrex beaker and concentrated on a water bath at 120 degrees Celsius. Further drying was carried out in a dry oven (Uniscope Gallenkamp, England) until a solid extract was obtained. The dried extract was stored in a clean, airtight sterile container for subsequent analyses.

Experimental Animals and Handling

Eighteen Swiss albino mice of both sexes, aged seven to eight weeks and weighing between 18.4 and 31.5 grams, were obtained from the Animal House of the Department of Pharmacology, Faculty of Pharmaceutical Sciences, University of Jos. The animals were housed in well-ventilated cages under standard laboratory conditions at room temperature. They were provided with unrestricted access to standard laboratory feed (Vital Feed ECWA Feed, Jos) and clean drinking water throughout the experimental period. All experimental procedures involving animals were conducted in accordance with the National Institutes of Health guidelines for the care and use of laboratory animals (NIH Publication No. 8023, revised 1978). Ethical approval for the study was granted by the University Research and Ethics Committee under reference number F17-00379.

Phytochemical Screening

Qualitative phytochemical screening of the aqueous

root bark extract was carried out to identify the presence of secondary metabolites using standard analytical procedures as described by Trease and Evans (2002). The extract was tested for the presence of alkaloids, flavonoids, phenolic compounds, saponins, tannins, and other relevant phytochemicals.

Acute Oral Toxicity Study

The acute oral toxicity of the aqueous root bark extract of Boswellia dalzielii was evaluated using Lorke's method (1983). Nine mice were randomly assigned into three groups, each containing three animals. The mice were fasted for twelve hours prior to extract administration. Each group received a single oral dose of the extract at 1000, 2000, and 5000 milligrams per kilogram body weight, respectively. Following administration, the animals were closely monitored for 24 hours for signs of toxicity, behavioral changes, morbidity, and mortality.

Parasite Inoculation

Donor mice infected with Plasmodium berghei NK 65 were used as the source of the parasite. Approximately ten milliliters of infected blood was collected into a beaker containing thirty milliliters of normal saline to prepare a parasite suspension. Each experimental mouse was inoculated intraperitoneally with 0.5 milliliters of the prepared Plasmodium berghei suspension to establish infection.

Experimental Groupings

Eighteen Swiss albino mice of both sexes were used for the experiment and grouped as follows;

a. Group A (Positive control)– Mice infected with 1x10-7 Plasmodium (Plasmodium berghei NK-65) strain and treated with chloroquine

b. Group B (Negative control) - Mice infected with 1x10-7 Plasmodium (Plasmodium berghei NK-65) strain and is untreated

c. Group C (Extract treated at lower dose) - Mice infected with 1x10-7 Plasmodium (Plasmodium berghei NK-65) strain and treated with 100mg/kg b.wt of Boswellia dalzieliii root-bark aqueous extract.

d. Group D (Extract treated at median dose) - Mice infected with 1x10-7 Plasmodium (Plasmodium berghei NK-65) strain and treated with 200mg/kg b.wt of Boswellia dalzieliii root-bark aqueous extract.

e. Group E (Extract treated at higher dose) - Mice infected with 1x10-7 Plasmodium (Plasmodium berghei NK-65) strain and treated with 400mg/kg b.wt of Boswellia dalzieliii root-bark aqueous extract.

f. Group F (Normal control) - Mice were uninfected and untreated (Norma control group) – they were given only feed and water ad libitum.

Antimalarial Activity (Curative Test)

The curative antimalarial activity of the aqueous extract was evaluated using Plasmodium berghei NK 65 infected mice. Animals in groups A to E were inoculated intraperitoneally on day one with 1×10^7 parasitized red blood cells suspended in 200 microliters of inoculum. Treatment commenced on day four and continued on days five, six, and seven at twenty-four-hour intervals. Mice in groups C, D, and E received the aqueous extract at doses of 100, 200, and 400 milligrams per kilogram body weight, respectively. Group A served as the positive control and was treated with chloroquine at a dose of 25 milligrams per kilogram body weight, while group B functioned as the negative control and received no treatment. Parasitaemia was assessed using May Grunwald Giemsa staining at a concentration of ten percent. The mean survival rate of infected mice was determined by calculating the average number of days each mouse survived from the day of infection until the thirtieth day, as described by Sidiki *et al.* (2023). Antimalarial activity was expressed as the percentage reduction in parasitaemia in treated groups relative to the negative control group, in accordance with the method reported by Singh *et al.* (2021). Both parasitaemia levels and percentage inhibition were calculated for each treatment group.

Determination of Packed Cell Volume

Packed cell volume was determined using a modified Wintrobe method. Blood samples were collected from the tails of the mice using heparinized microhematocrit tubes. Each tube was filled to approximately three quarters of its total capacity and sealed with crystoseal. The tubes were centrifuged at twelve thousand revolutions per minute for five minutes in a microhematocrit centrifuge, ensuring that the sealed ends were positioned outward. After centrifugation, packed cell volume values were read using a standard hematocrit reader. Baseline packed cell volume measurements were recorded prior to parasite inoculation. Packed cell volume was calculated as the ratio of the volume of erythrocytes to the total blood volume.

Measurement of Body Temperature

Rectal temperature was measured using a digital thermometer to evaluate the effect of the extract on thermoregulation during infection. Measurements were taken one hour before parasite inoculation and monitored throughout the five-day curative test period.

Measurement of Body Weight

Body weight of each mouse was recorded in grams using a sensitive digital weighing balance. Measurements were obtained three hours prior to parasite inoculation and during the course of curative treatment. Mean body weight for each experimental group was calculated by dividing the total body weight of the group by the number of mice.

Statistical Analysis

All data were expressed as mean plus or minus the standard error of the mean. Results were summarized using bar charts, with standard error of the mean indicated for each analysis.

RESULTS AND DISCUSSION

Phytochemical Profile and Implications for Antimalarial Activity

Phytochemical screening of the aqueous extract shown in Table 1 indicated the presence of flavonoids, tannins, saponins, phenols, balsam and carbohydrates, while alkaloids, terpenes, steroids, cardiac glycosides, salkowski-reactive compounds and resins were not detected. The extract yield was 250 g of chocolate-brown powder, corresponding to a 62.5% yield. The presence of flavonoids and phenolic compounds is relevant because these classes possess antioxidant and membrane-stabilizing properties that can limit oxidative damage and erythrocyte susceptibility during malaria infection (Atawodi *et al.,* 2011; Dogara *et al.,* 2022). Saponins detected have been implicated in antiparasitic effects through interactions with cell membranes and immunomodulation. These phytochemical attributes align with prior reports which documented antiplasmodial activity in other parts of Boswellia dalzielii, supporting ethnomedicinal accounts of the species (Sachdeva *et al.,* 2020; Salihu *et al.,* 2020). The organ-specific composition observed here, with terpenoids reported in leaves but absent in root bark (Dogara *et al.,* 2022), emphasizes the importance of screening individual plant parts for distinct bioactive profiles and justifies further fractionation and compound isolation from the root bark.

Table 1: Phytochemical composition of Boswellia dalzielii root bark aqueous extract

S/No	Phytochemicals	Boswellia dalzielii's root bark aqueous extract
1.	Flavonoids	+
2.	Tannins	+
3.	Saponins	+
4.	Balsam	+
5.	Carbohydrates	+
6.	Phenols	+
7.	Alkaloids	-
8.	Terpenes	-
9.	Steroids	-
10.	Cardiac glycosides	-
11.	Salkowski	-
12.	Resins	-

NB: + signifies present and – indicate absent

Dose-Dependent Effect of Root Bark Extract on Parasitaemia Levels in Plasmodium berghei-Infected Mice

Administration of the root bark extract produced a clear, dose-dependent suppression of parasitaemia as shown in Figure 1 below. The untreated group exhibited a progressive increase in parasite density from 10% to 28% by day 5, while extract-treated groups showed an initial increase on day one followed by consistent reductions from day two to day five. Chloroquine-treated mice showed parasitaemia reduction from 1.2% to 0.2%. In the extract-treated groups, the 400 mg/kg dose produced the greatest suppression by decreasing parasitaemia from 5.3% to 0.3% by day 5 and achieving >90% inhibition. The 200 mg/kg and 100 mg/kg doses also showed reductions of ≥70%, confirming a dose-dependent antiplasmodial effect.

The biological significance of these data is that the root bark extract approaches standard-drug performance in suppressing blood-stage parasites in vivo; this performance supports prior in vitro and in vivo observations on Boswellia dalzieliii parts and reaffirms that the species contains compounds with antiplasmodial potential (Salihu *et al.,* 2020; Jansen *et al.,* 2010). Given the phytochemical profile shown in Table 1, these effects are plausibly mediated by phenolics, flavonoids and saponins acting singly or synergistically to impair parasite replication and modulate host responses (Sachdeva *et al.,* 2020; Atawodi *et al.,* 2011).

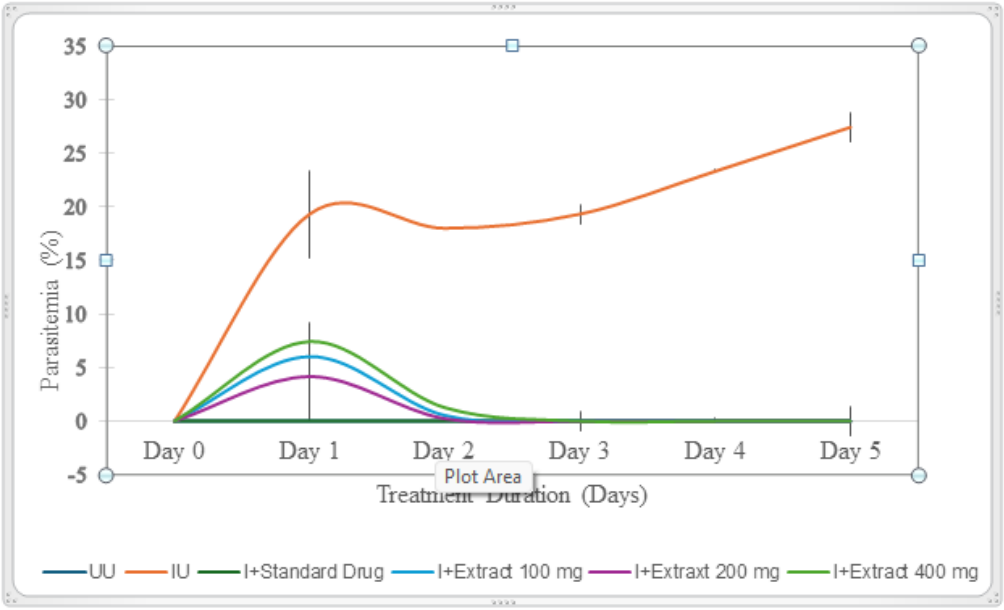

Figure 1: Effect of Boswellia dalzielii Aqueous Root-Bark Extract on Parasitaemia Levels of Mice Infected with Plasmodium berghei Nk-6

Dose Dependent Effect of Root Bark Extract on Survival of Plasmodium berghei Infected Mice

As shown in Figure 2, mean survival time increased progressively with rising doses of the root bark extract, indicating a clear dose dependent response. Mice treated with 100, 200, and 400 milligrams per kilogram body weight survived for an average of 15, 17, and 20 days, respectively. A statistically significant improvement in survival time was observed at the highest dose when compared with the untreated group ($P < 0.05$). Mice administered chloroquine survived for an average of 16 days, demonstrating that treatment with 400 milligrams per kilogram of the extract resulted in a longer survival period than the standard antimalarial drug. These findings indicate that Boswellia dalzielii root bark possesses considerable therapeutic potential in slowing the progression of malaria infection. Mean survival time represents an integrated measure that reflects both suppression of parasitaemia and protection against malaria associated systemic damage. Therefore, the observed extension of survival suggests that the extract confers benefits beyond short term parasite reduction. The prolonged survival may be attributed to reduced oxidative stress, preservation of erythrocyte integrity, and stabilization of host metabolic processes during infection. Similar survival enhancing effects have been reported for other medicinal plant extracts evaluated in Plasmodium berghei infected mouse models (Kifle *et al.,* 2020; Derebe *et al.,* 2021). The present findings expand this body of evidence by demonstrating comparable protective effects

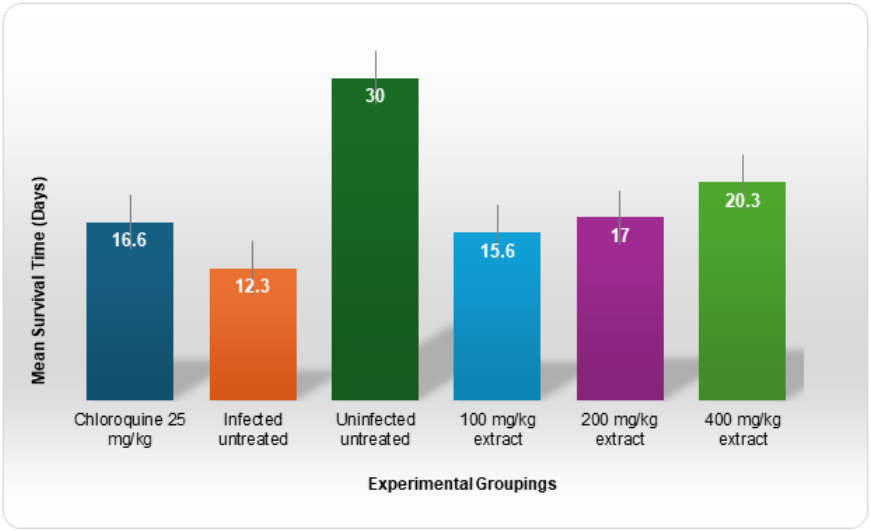

Figure 2: Effect of Boswellia dalzielii Aqueous Root-Bark Extract on Survival Rate of Mice Infected with Plasmodium berghei Nk-65

specifically associated with the root bark of Boswellia dalzielii.

Effect of Dose-Dependent Protection of Packed Cell Volume by Boswellia dalzielii Root Bark Extract in Plasmodium berghei-Infected Mice

The packed cell volume (PCV) declined in infected mice due to malaria, with extract treatment reducing this loss in a dose-dependent manner as shown in Figure 3. Boswellia dalzielii aqueous root bark extract demonstrated PCV protection in a dose-dependent manner across the 100, 200, and 400 mg/kg doses. Although chloroquine produced a stronger PCV-stabilizing effect overall, extract-treated mice exhibited less PCV decline than negative controls, indicating that the extract mitigates malaria-induced haemolysis. The preservation of PCV is clinically relevant because malaria-associated anaemia contributes substantially to morbidity. The Boswellia dalzielii extract's ability to slow PCV decline suggests protective effects on erythrocyte membranes or reduced oxidative haemolysis, consistent with the antioxidant properties of phenolics and flavonoids identified in Table 1 (Atawodi *et al.,* 2011; Dogara *et al.,* 2022).

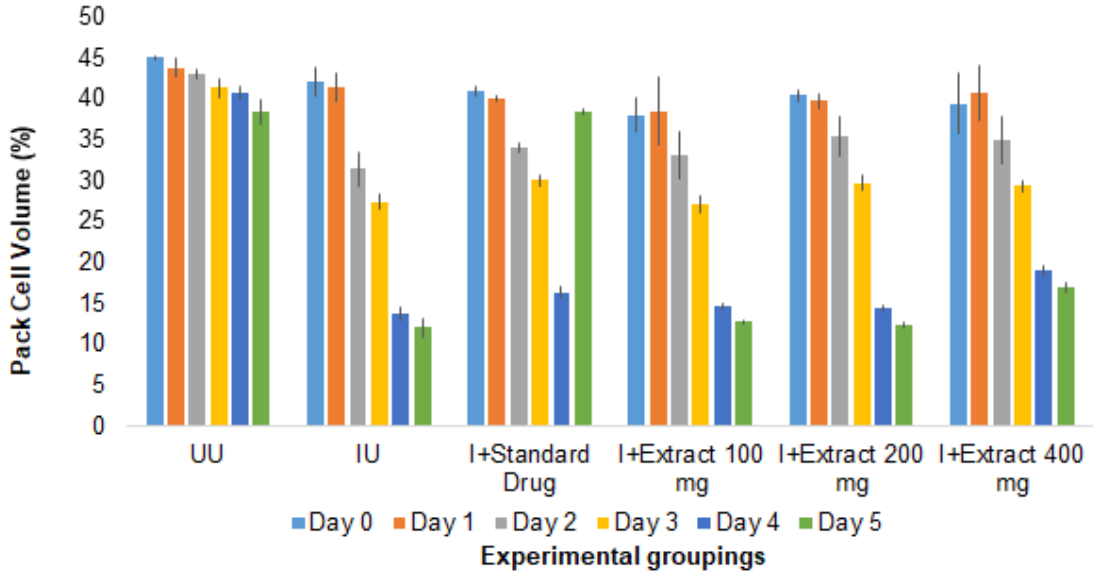

Figure 3: Effect of Boswellia dalzielii Aqueous Root-Bark Extract on Packed Cell Volume (PCV) of Mice Infected with Plasmodium berghei Nk-65

Effect of Extract on Body Temperature of Mice Infected with Plasmodium berghei Nk-65

Rectal temperature decreased progressively in untreated mice, while extract administration blunted this decline and produced only mild fluctuations across all doses (Figure 4). Extract-treated and chloroquine-treated mice both showed prevention of pronounced temperature drops, with only slight decreases observed as infection progressed. Maintenance of body temperature during infection is an indicator of preserved metabolic and thermoregulatory function. The extract's stabilizing effect is consistent with reports that effective antimalarial agents mitigate hypothermia in rodent models (Chilombe *et al.,* 2022; Protsiv *et al.,* 2020).

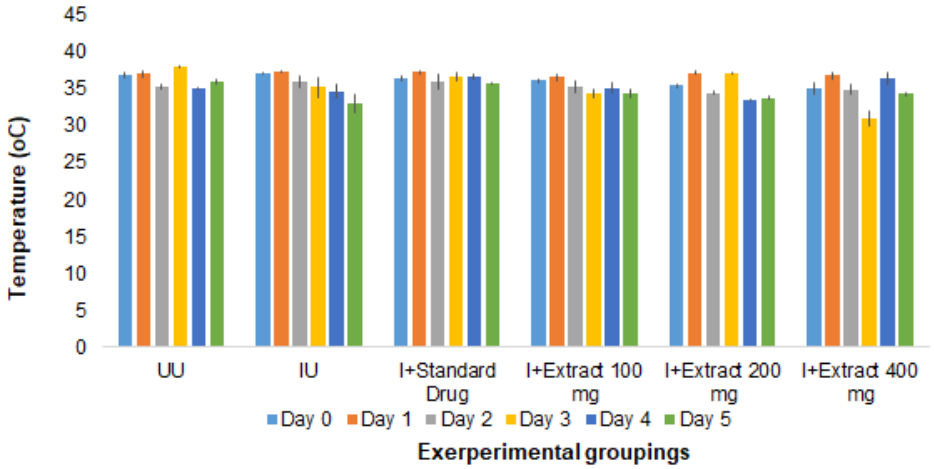

Figure 4: Effect of Boswellia dalzielii Aqueous Root-Bark Extract on Temperature of Mice Infected with Plasmodium berghei Nk-65

Dose Dependent Effect of Root Bark Extract on Survival of Plasmodium berghei Infected Mice

As shown in Figure 2, mean survival time increased progressively with rising doses of the root bark extract, indicating a clear dose dependent response. Mice treated with 100, 200, and 400 milligrams per kilogram body weight survived for an average of 15, 17, and 20 days, respectively. A statistically significant improvement in survival time was observed at the highest dose when compared with the untreated group ($P < 0.05$). Mice administered chloroquine survived for an average of 16 days, demonstrating that treatment with 400 milligrams per kilogram of the extract resulted in a longer survival period than the standard antimalarial drug. These findings indicate that Boswellia dalzielii root bark possesses considerable therapeutic potential in slowing the progression of malaria infection. Mean survival time represents an integrated measure that reflects both suppression of parasitaemia and protection against malaria associated systemic damage. Therefore, the observed extension of survival suggests that the extract confers benefits beyond short term parasite reduction. The prolonged survival may be attributed to reduced oxidative stress, preservation of erythrocyte integrity, and stabilization of host metabolic processes during infection. Similar survival enhancing effects have been reported for other medicinal plant extracts evaluated in Plasmodium berghei infected mouse models (Kifle *et al.,* 2020; Derebe *et al.,* 2021). The present findings expand this body of evidence by demonstrating comparable protective effects specifically associated with the root bark of Boswellia dalzielii.

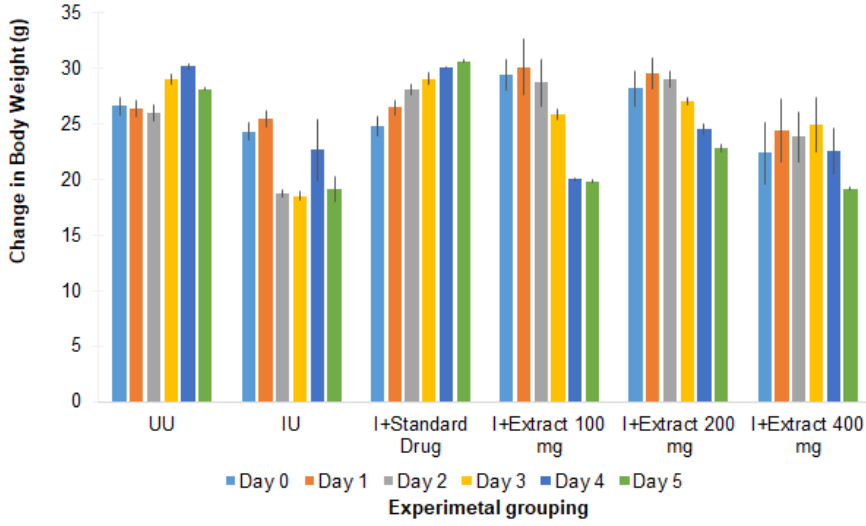

Figure: 5 Effect of Boswellia dalzielii Aqueous Root-Bark Extract on Body Weight of Mice Infected with Plasmodium berghei Nk-65

CONCLUSION

The aqueous root bark extract of Boswellia dalzielii demonstrated notable antimalarial activity in mice, producing dose-dependent suppression of parasitaemia, prolonged survival, and protected clinical markers such as the packed cell volume, body weight, and body temperature. These findings support the traditional medicinal use of the plant and highlight the potential of natural products in addressing malaria, particularly in the context of rising drug resistance. Further studies focusing on isolation of bioactive compounds, elucidation of mechanisms of action, and comprehensive pharmacological profiling are warranted to advance the development of Boswellia dalzieliii–based antimalarial therapies.

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