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Microbes & Immunity

ORIGINAL RESEARCH ARTICLE

The impact of an *Arachis hypogaea*-based diet on malaria resistance and hematological indices in mice infected with *Plasmodium berghei*

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Abstract

This study evaluated the effects of different *Arachis hypogaea* dietary preparations on parasite load, hematological indices, and physiological responses in *Plasmodium berghei*-infected mice. Albino mice ($n = 45$) were randomly assigned to five groups: normal control, infected untreated control, roasted groundnut, boiled groundnut, and a combination of roasted and boiled groundnut diets. Data were analyzed using one-way analysis of variance; $p < 0.05$ indicated statistical significance. Infection resulted in a high parasite load in the untreated group, with no significant difference compared to the boiled and combined diet groups. However, the roasted groundnut group showed a reduction in parasite load and relatively higher chemosuppressive activity, although differences were not statistically significant. White blood cell counts increased significantly following infection, and dietary treatments did not restore normal levels. Similarly, red blood cell counts and packed cell volume were significantly reduced in infected mice. The roasted groundnut diet moderately improved packed cell volume compared to other treatments, but did not restore it to normal levels. Weight loss was most pronounced in untreated mice, while roasted groundnut intake showed slight mitigation. No significant effects on temperature regulation were observed. Overall, *A. hypogaea* diets did not significantly reduce parasitemia or improve hematological parameters, indicating limited therapeutic value for malaria management.

Keywords: Malaria; Parasite load; Hematological indices; *Arachis hypogaea, Plasmodium berghei*

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Citation: Okeke OA, Aniekwe GD, Ndinyelum OM, *et al*. The impact of an *Arachis hypogaea*-based diet on malaria resistance and hematological indices in mice infected with *Plasmodium berghei*. *Microbes & Immunity.* 2026;3(4):026160036. doi: 10.36922/MI026160036

Received: April 16, 2026

Revised: June 2, 2026

Accepted: June 5, 2026

Published online: July 14, 2026

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1. Introduction

Malaria is a vector-borne parasitic disease transmitted by the female Anopheles mosquito, which carries the *Plasmodium parasites*. Of all the species that can cause malaria in humans, *Plasmodium falciparum* and *Plasmodium vivax* are the most important.[1] *P. falciparum* causes the most severe form of malaria and is commonly found in Africa,

while *P. vivax* is the parasite of interest in numerous areas beyond sub-Saharan Africa.[2] It remains a major health challenge in the world due to the emergence and spread of parasite resistance to established antimalarial drugs.[3,4] Malaria disproportionately affects vulnerable populations, including children under five years of age and pregnant women, further exacerbating socio-economic disparities and hindering development efforts in endemic regions. The fight against malaria has been characterized by multifaceted approaches, ranging from vector control and case management to preventive measures and research into vaccines and novel interventions.[5]

The manifestations of malaria result from the infection of erythrocytes and can reach host tissues via the circulatory system. The interactions between infected erythrocytes and host tissues are limited to specific sites, such as the brain, lungs, liver, kidneys, and intestines.[6] A higher incidence of malaria occurs in the rainy season because more favorable conditions for mosquito breeding occur.[7] Severe malaria can lead to complications, such as anemia, thrombocytopenia, leucocytosis, antioxidant reductions, increased lipid peroxidation, lactic acidosis, coma, and death.[8]

Groundnut or peanut (*Arachis hypogaea L.*) is one of the important edible oilseed crops cultivated in the world. Groundnuts play an important role in the economy of several countries.[9,10] Groundnuts are high in oil content and, compared to other major oil seeds, are relatively low in ash and carbohydrate.[11] Fatty acids, particularly oleic and linoleic, have a large bearing on the stability and nutritional quality of groundnut oil. Groundnut plant is known for its nutritional and health benefits, with five main nutrients required by the body to maintain and repair the tissues, namely energy, protein, phosphorus, thiamin, and niacin, found in good quantity in it.[12] Groundnuts are rich in vitamins and contain at least 13 different types of vitamins that include vitamins A, B, C, and E. It is rich in 26 essential minerals, including calcium, iron, zinc, and boron.

All these plant nutrients are of great benefit to the body, which prompted the study of groundnut diets on malaria resistance and hematology in mice. Therefore, understanding the host protective mechanisms against malaria and parasite-host interactions is essential for using dietary management strategies to control malaria.

2. Materials and methods

2.1. Preparation of food materials

Groundnut and commercial pellet feed were purchased at the local Market in Awka, Anambra State, Nigeria. For roasted groundnut flour, groundnuts were cleaned and sorted, then steamed and dried. It was then roasted above 100 °C for 25 min in a gas oven, then dehulled, milled, and sieved to obtain roasted groundnut flour. For boiled groundnut flour, groundnut was cleaned and sorted, after which it was steamed at a temperature of 100 °C for 45 min and dried for two weeks before being milled and sieved to obtain boiled groundnut flour.

2.2. Animal study

A total of 45 Swiss albino mice, 10 weeks old and weighing an average of 21.91 g, were obtained from the Institute for Advanced Medical Research and Training College of Medicine, University of Ibadan. Four mice (donor mice) were obtained from the Nigerian Institute of Medical Research, Lagos. The mice were transported to the research area—the Zoology Laboratory in the Faculty of Biosciences, Nnamdi Azikiwe University, Awka—in a transportation plastic cage measuring 40 × 20 × 20 cm to ensure adequate ventilation. The mice were acclimatized for a week before the experiment began. They were housed in 15 cages in the Zoology Laboratory of the Faculty of Biosciences, Nnamdi Azikiwe University, Awka, and fed formulated food and water daily, morning and evening.[13] The vital chick mash was fed to the animals; this feed is designed to provide young animals with healthy growth and development. This is noted for its high concentration of protein, energy, vitamins, and minerals, as well as essential amino acids for protein synthesis and growth.[13] All animal experiments were conducted in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and approval was obtained from the University's institutional animal care and use committee.

2.3. Experimental design

A cross-sectional descriptive survey was conducted amongst adults in both urban and rural areas. A total of 100 participants participated, and responses were collected using a questionnaire. Statistical analysis of the questionnaire results was used to determine the average amount of food material consumed and the frequency of consumption. From the results, the animal equivalent dose was calculated using **Equation 1**:

$$HED(mg/kg) = Animal\ dose \times \frac{Animal\ K_m}{Human\ K_m} \quad (1)$$

where HED is the human equivalent dose; K_m is the constant factor for each species; the K_m ratio (animal/human) is used to simplify calculations. According to a previous study, the K_m ratio to convert the dose in a mouse (mg/kg) to the dose in a human (mg/m²) is 3.14. The experimental animals were randomly housed in 11 stainless steel

metabolic cages laid out in a complete randomized design of three treatments, replicated thrice with each replicate having three mice, and fed with commercial food (Vital Feed Broiler Starter, 18.00 ± 0.50g/100g crude protein, and 2,106.00 kcal/kg metabolizable energy; Vital Feed, Grand Cereals Limited, Nigeria) and water ad libitum daily.[13]

The experimental animals, which were of homogeneous sizes, were randomly kept in three cages (24 × 24 cm) containing three mice each and labeled as follows: Group 1: Normal control (uninfected, untreated); Group 2: Negative control (infected, untreated); Group 3: Roasted groundnut (fed with 14 g of roasted groundnut and 22 g of commercial feed prior to and after infection); Group 4: Boiled groundnut (fed with 14 g of boiled groundnut and 22 g of commercial feed prior to and after infection); Group 5: Roasted and boiled groundnut (fed with 7 g of roasted groundnut 7 g of boiled groundnut, and 22 g of commercial feed prior to and after infection).

2.4. Parasite inoculation

The infected erythrocytes for each test were collected from donor mice with 20–30% parasitemia. The mice were sacrificed through cardiac puncture, and blood was collected in a heparinized tub with an anticoagulant (0.5% trisodium citrate). The blood was then diluted with normal saline (0.9%) in a proportion of 1:4. Each mouse in every group was then inoculated with 0.2 mL of blood containing about 1.25×10^6 *Plasmodium berghei*-infected erythrocytes two weeks after feeding them with experimental feeds through the intraperitoneal route.

2.5. Determination of parasite load

Parasitemia was measured by collecting a blood sample via tail bleeding for the preparation of thick and thin film smears, which were stained with Giemsa stain. Parasitemia was calculated based on the number of infected erythrocytes observed among the counted erythrocytes. The formula for parasitemia is as follows (**Equation 2**):

$$Parasitemia(\%) = \frac{Number\ of\ infected\ erythrocytes}{Total\ number\ of\ counted\ erythrocytes} \times 100 \quad (2)$$

The percentage of inhibition was calculated using the following **Equation 3**:

$$Inhibition(\%) = \frac{NC - TG}{NC} \times 100 \quad (3)$$

where NC is the mean of parasitemia in the negative control, and TG is the mean of parasitemia in the treated group (G1, G2, G3).

2.6. Body weight gain

The mice's weights were measured and recorded using a sensitive weighing scale, after which they were randomly placed into different sections of the cage within the range of body weight gain per mouse for each treatment group.[14] Weight gain was calculated as the difference between two successive weekly body weights (**Equation 4**):

$$Weight\ gain = final\ weight - initial\ weight \quad (4)$$

2.7. Temperature

The mice's temperature was measured and recorded with a thermometer, and they were then randomly placed into different sections of the cage. This was performed before and after acclimatization.

2.8. Determination of hematological parameters

The packed cell volume (PCV) was determined using the microhematocrit centrifuge method as described in a previous study.[15] White blood cell (WBC) count was obtained using the principle of calibrated capillary tube for blood sampling with a hemocytometer (**Equation 5**).[13,16,17]

$$Number\ of\ WBC\ in\ 1\ \mu L = \frac{N \times 10 \times 20}{4} \quad (5)$$

where N is the number of cells counted; 10 is the depth correction factor (mm^{-1}) of the counting chamber; 20 is the dilution ratio (1:20); and 4 is the area of the squares counted (mm^2). This can be summarized into N × 50, which gives the WBC count.

The red blood cell (RBC) count was determined using the calibrated tube method with the hemocytometer (**Equation 6**).[16,17]

$$Number\ of\ RBC\ in\ 1\ \mu L = \frac{N \times 10 \times 200}{0.2} \quad (6)$$

where N is the number of cells counted; 10 is the depth correction factor (mm^{-1}) of the counting chamber; 200 is the dilution ratio (1:200); and 0.2 is the area of the squares counted (mm^2). This is summarized into N × 10,000, which gives the RBC count.

2.9. Statistical analysis

The data on hematological indices, weight, temperature, and parasitemia of mice were analyzed to obtain the mean, standard deviation (SD), and one-way analysis of variance

using Statistical Package for Social Sciences (SPSS 25.0, IBM, United States).[18] The Tukey's Honestly Significant Difference test was used to separate mean significant differences between treatments at a 5% significant level.[19,20] The results are expressed as mean ± SD; Mean values with different alphabets as superscripts are significantly different ($p < 0.05$).

3. Results

3.1. Impact of *A. hypogaea* on malaria parasite resistance

The mean parasite load in infected, untreated mice was 6.77 ± 0.06% (range: 6.70% to 6.80%). Treatment with a combination of roasted and boiled *A. hypogaea* resulted in a mean parasite load of 6.40 ± 2.60% (range: 4.80% to 9.40%), which did not differ significantly from the untreated control. Similarly, treatment with single diets of roasted *A. hypogaea* (mean: 1.47 ± 0.31%, range: 1.20% to 1.80%) and boiled *A. hypogaea* (mean: 6.00 ± 3.70%, range: 3.80% to 10.80%) also did not significantly impact the parasite load in the infected mice (Figure 1).

3.2. Chemosuppressive activity of *A. Hypogaea* on the parasite load of *P. berghei*-infected mice

The diet containing roasted *A. hypogaea* demonstrated the strongest chemosuppressive activity against *P. berghei* infection, with a mean suppression rate of 78.43% (range: 72.53%–82.35%). However, this activity did not differ significantly from that of the other treatment groups fed with boiled or mixed *A. hypogaea*. In the group that received a combination of roasted and boiled *A. hypogaea*, the mean chemosuppressive activity was only 5.88% (with a range from −38.24% to 27.41%), while the group fed only boiled *A. hypogaea* showed an even lower mean activity of 2.9% (ranging from −58.82% to 44.12%). Overall, there was no significant difference in the chemosuppressive activity among all the treatment groups (Figure 2).

3.3. Impact of *A. hypogaea* diets on white blood cell count of *P. berghei*-infected mice

Infection with the parasite resulted in a significant increase ($p < 0.05$) in WBC counts in the negative control group compared to the normal control group. However, treatment with the various groundnut diets is not significantly different ($p > 0.05$) in WBC levels, to a degree comparable among them (Figure 3).

3.4. Impact of *A. hypogaea* diets on red blood cell count of *P. berghei*-infected mice

The RBC count in normal mice was significantly higher than in the other treatment groups. Infection with the parasite resulted in a significant decrease ($p < 0.05$) in RBC levels compared with the normal group. While treatment

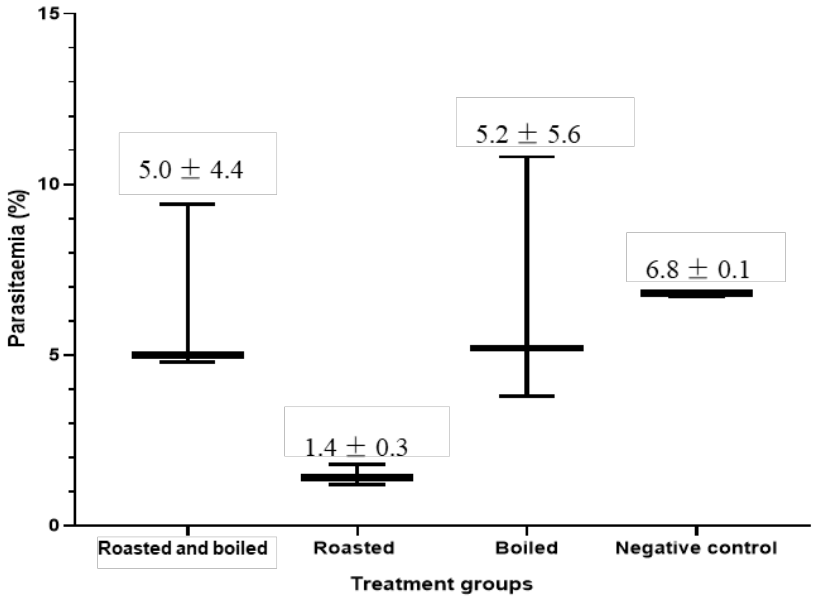

Figure 1. Impact of *A. hypogaea* on parasite load of *P. berghei*-infected mice

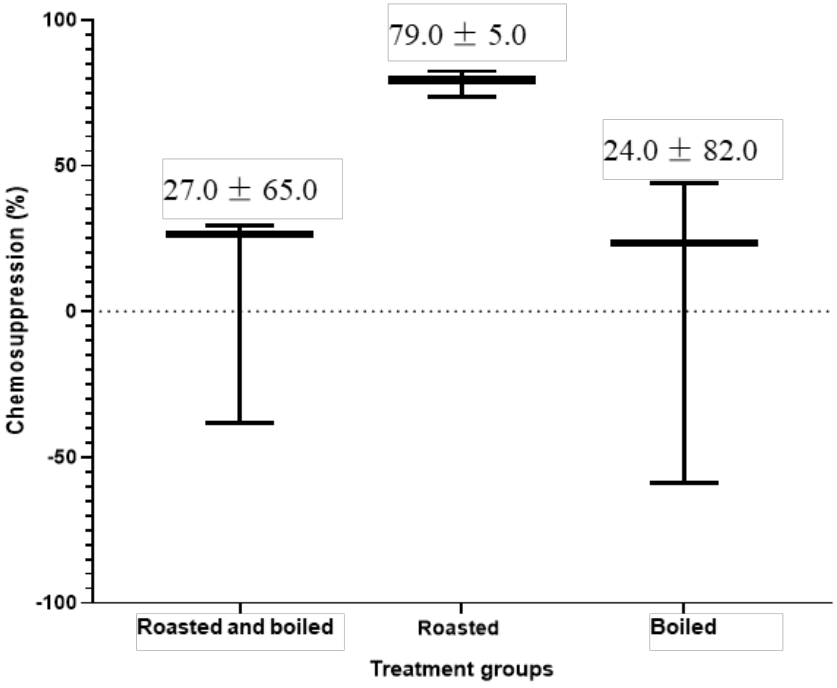

Figure 2. Chemosuppressive activity of *A. hypogaea* diets on *P. berghei*-infected mice

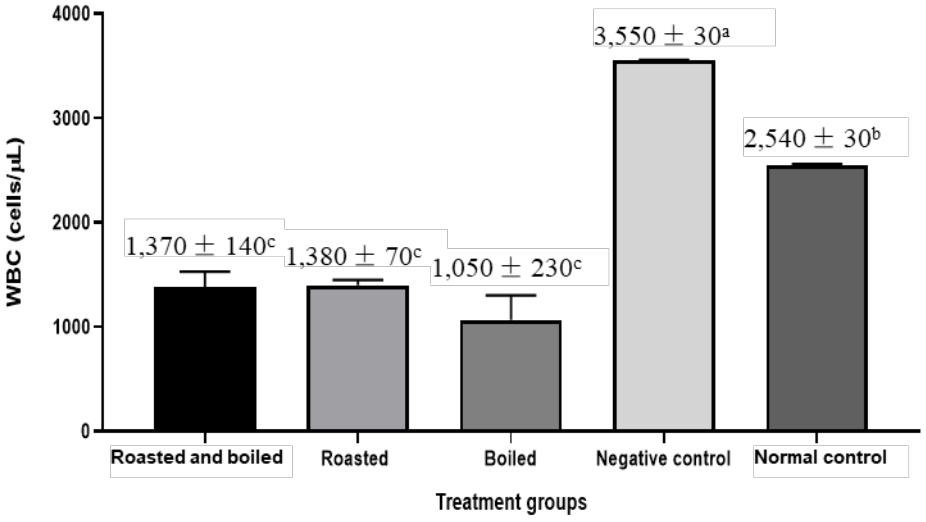

Figure 3. Impact of *A. hypogaea* diets on white blood cell (WBC) count of *P. berghei*-infected mice
Note: [a,b,c]Groups with statistically significant differences ($p < 0.05$).

with roasted groundnuts aimed to increase RBC in the mice, the results were not comparable ($p < 0.05$) to those of the normal group, and the RBC remained below ($p < 0.05$) that of the negative control. Additionally, other groups fed different groundnut diets did not show any improvement ($p < 0.05$) in RBC levels (Figure 4).

3.5. Impact of *A. hypogaea* diets on packed cell volume of *P. berghei*-infected mice

The PCV of the normal group was significantly higher ($p < 0.05$) compared to other treatment groups. Infection with the parasite caused a significant decrease ($p < 0.05$) in parasite load. The PCV of the group fed roasted groundnuts was significantly higher ($p < 0.05$) than that of the groups fed a combination of roasted and boiled groundnuts and boiled groundnuts only. However, feeding infected mice different groundnut diets did not improve ($p > 0.05$) PCV to levels above the negative control or bring it in line with the normal group (Figure 5).

3.6. Impact of *A. hypogaea* diets on body weight and temperature of *P. berghei*-infected mice

The negative control group experienced significantly greater weight loss ($p < 0.05$). Mice treated with roasted *A. hypogaea* showed an increase in weight that was significantly higher than that of the negative control group; however, this increase was not substantial enough to differ significantly from the other *A. hypogaea*-fed groups. In the groups of mice that were fed a combination of roasted and boiled *A. hypogaea*, as well as those that received boiled *A. hypogaea* only, weight loss was observed (Table 1). Among the groups fed *A. hypogaea*, only the group that consumed the roasted form of the diet demonstrated a temperature change that was comparable to normal levels at the end of the study ($p > 0.05$) (Table 1).

4. Discussion

The present study evaluated the effects of differently processed *A. hypogaea* diets on parasitemia, hematological parameters, and physiological indices in *P. berghei*-infected mice. The untreated infected group exhibited a high parasite load with minimal variability, indicating consistent infection across subjects. Although the combined roasted and boiled diet showed a slight reduction in mean parasite load, the wide variability suggests no significant impact on parasite burden, consistent with reports that high-fat diets may modulate immune responses rather than directly reduce parasitemia.[21,22]

The roasted *A. hypogaea* diet showed the highest mean chemosuppressive activity against *P. berghei* infection, resulting in a substantial reduction in parasite levels. The range is quite narrow, showing consistent suppression effectiveness within this group. A combination of roasted and boiled *A. hypogaea* shows much lower suppression with a wide range. Boiled *A. hypogaea* exhibited the lowest mean chemosuppressive activity with an even wider and more variable range. The consistent and high suppression achieved by roasted groundnut compares favorably

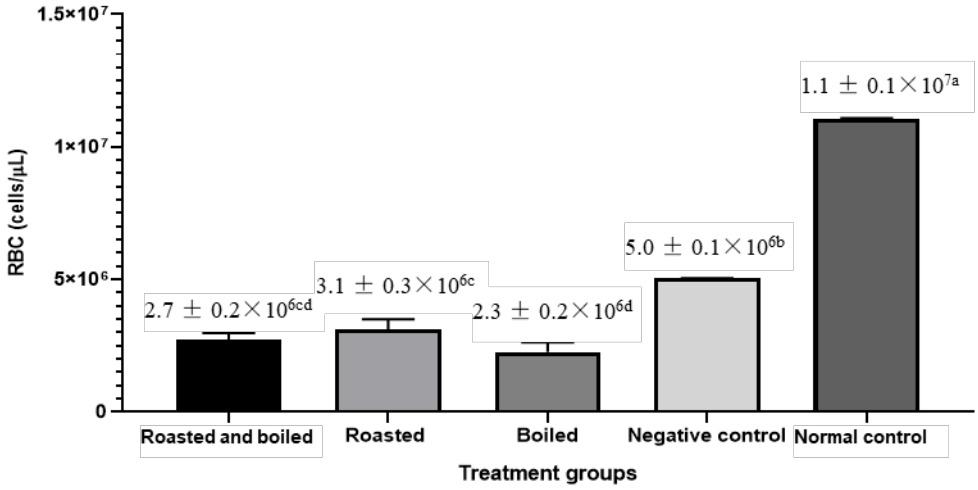

Figure 4. Impact of *A. hypogaea* diets on red blood cell (RBC) count of *P.berghei*-infected mice
Note: [a,b,c,d]Groups with statistically significant differences ($p < 0.05$).

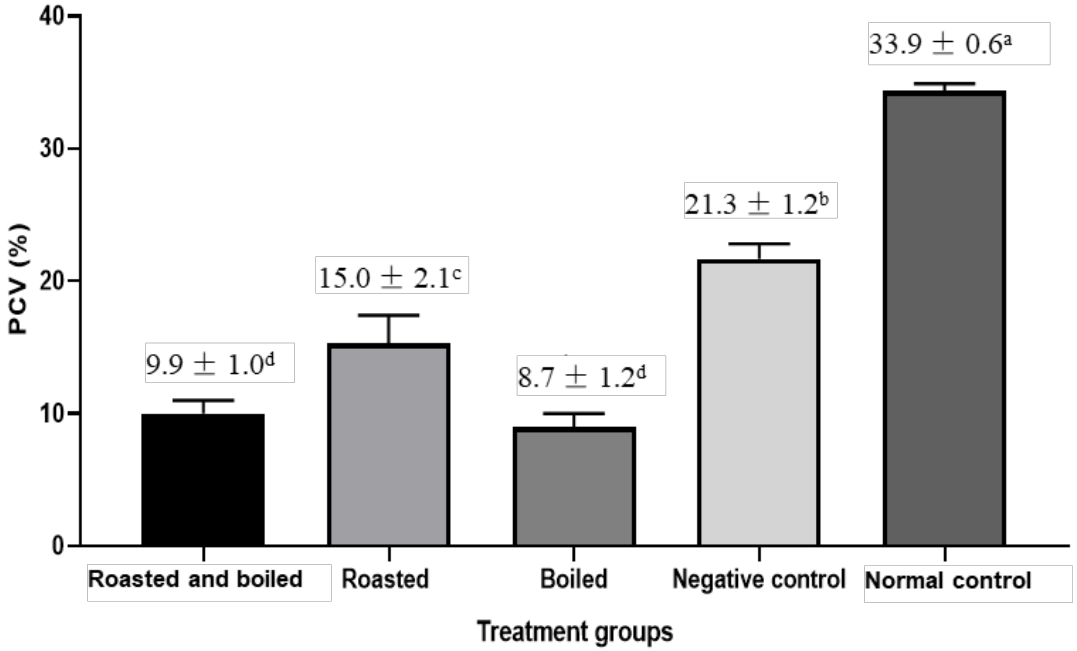

Figure 5. Impact of *A. hypogaea* diets on packed cell volume (PCV) of *P. berghei*-infected mice
Note: [a,b,c,d]Groups with statistically significant differences ($p < 0.05$).

Table 1. Impact of *A. hypogaea* diets on body weight and temperature of *P. berghei*-infected mice

Groups	Weight of animals (g)			Temperature of animals (°C)		
	Initial	Final	Change in weight	Initial	Final	Change in Temp.
Roasted and boiled	27.77 ± 1.45[cd]	26.97 ± 0.55[c]	−0.80 ± 0.90[b]	37.00 ± 0.90[a]	38.30 ± 0.50[a]	1.30 ± 0.40[b]
Roasted	30.47 ± 1.85[bc]	30.77 ± 1.65[b]	0.30 ± 0.20[b]	37.07 ± 0.85[ab]	37.80 ± 0.60[ab]	0.70 ± 1.45[ab]
Boiled	26.47 ± 0.06[d]	25.00 ± 1.30[c]	−1.47 ± 1.35[b]	37.50 ± 0.10[b]	35.87 ± 1.85[b]	−1.63 ± 1.75[b]
Negative control	37.63 ± 0.06[a]	30.33 ± 0.06[b]	−7.30 ± 0.10[c]	37.43 ± 0.06[a]	36.83 ± 0.06[a]	−0.60 ± 0.10[ab]
Normal control	31.77 ± 0.06[b]	35.37 ± 0.06[a]	3.60 ± 0.00[a]	38.93 ± 0.06[a]	39.07 ± 0.12[a]	0.13 ± 0.06[ab]

Note: [a,b,c,d]Groups with statistically significant differences ($p < 0.05$).

with that of boiling groundnut, suggesting reliable antiplasmodial properties. Comparable antiplasmodial effects have been reported for several plant-derived treatments. Previous studies demonstrated that phytogenic preparations, ethanolic leaf extracts, and *Ajuga bracteosa* showed dose-dependent chemosuppression of *P. berghei* in mice, with up to 77.7% suppression at higher doses and prolonged survival, indicating substantial parasite reduction similar to that of roasted *A. hypogaea*'s effect.[23,24]

Infection caused a significant increase in WBC counts in the negative control group compared to the normal control group. Several studies on the effects of an oil-rich diet have shown that infections lead to a significant increase in WBC counts compared with control groups.[25–27] Antibodies, produced by WBCs, can restrict the growth of blood-stage parasites, leading to their increase and the progression of clinical symptoms through several mechanisms, such as obstructing erythrocyte invasion.[28–30] Groundnut diets did not improve immune normalization as measured by WBC counts, implying a limited effect on controlling or modulating systemic inflammation or immune activation triggered by the infection. This aligns with Chauhan *et al.*[31], who reported that groundnut diets did not improve immune normalization, as measured by WBC counts, suggesting limited effects on controlling systemic inflammation or immune activation triggered by infection.

Normal (uninfected) mice have the highest RBC counts, serving as baseline healthy controls. *P. berghei* infection causes a reduction in RBC counts, which can bring about anemia/hemolysis due to parasitic infection.[32,33] This aligns with established knowledge that *P. berghei* infection causes anemia in mice by reducing RBC counts through a combination of hemolysis of infected RBCs and suppression of RBC production due to inflammatory responses.[34]

The effect of a roasted groundnut diet was to increase PCV, thereby preventing or reducing anemia, but PCV remained below that of normal mice, indicating that the diet was unable to fully restore RBC volume or count to healthy levels during infection. This is similar to a prior study by Uduchi *et al.*[34], which also reported that the effects of another phytogenic on PCV during infection are beneficial but insufficient to fully restore PCV volume to healthy baseline levels. The results of this study showed that the normal group had the highest PCV. Among infected groups, the group fed roasted groundnuts showed a higher PCV ($p < 0.05$) than the groups fed a combination of roasted and boiled groundnuts or boiled groundnuts only. This shows that roasted groundnuts better preserve PCV relative to the other dietary treatments. In support of Chauhan *et al.*[31], the PCV percentage for the fresh groundnut group was the highest, followed by fried, controlled, and boiled groundnut groups. This shows that roasted groundnuts may offer some moderate benefit in mitigating PCV reduction caused by infection, the improvement is insufficient to fully reverse or prevent anemia associated with the parasite infection.

The negative control group (infected and untreated) experienced greater weight loss compared to at least some other groups. This indicates that infection or lack of treatment adversely affected their weight. Mice treated with roasted *A. hypogaea* showed a significant increase in weight relative to the negative control, consistent with previous reports, suggesting a protective or restorative effect of this diet on weight maintenance or gain.[35,36] The groups fed the combination (roasted and boiled) and the boiled-only *A. hypogaea* diets experienced weight loss, indicating less benefit for weight loss compared to the roasted-only diet.

Only the group fed the roasted *A. hypogaea* diet showed a body temperature change that was comparable to normal (non-infected or healthy) levels by the end of the study. No direct or specific research was found that confirms or contradicts the observation that only the group fed a roasted *A. hypogaea* diet showed body temperature changes comparable to those of normal (non-infected or healthy) individuals. Existing studies mostly focus on the nutritional and sensory effects of roasting groundnuts, demonstrating that roasting at moderate temperatures preserves nutrients and enhances flavor without any negative impacts. While roasting groundnuts improves nutritional quality and flavor, the specific effect of roasted groundnut diets on body temperature remains unverified and requires further targeted research.[37]

5. Conclusion

This study demonstrates that the roasted *A. hypogaea* diet exhibits moderate antiplasmodial and supportive physiological effects in *P. berghei*-infected mice, surpassing those of boiled and combined preparations. However, none of the dietary interventions effectively restored parasitemia or hematological parameters to normal levels. These findings suggest that while roasted groundnuts may provide supportive benefits, they are insufficient as a standalone therapy for malaria. Further studies should explore its potential synergistic effects with standard antimalarial drugs and investigate the mechanisms underlying its bioactivity.

Acknowledgments

The researchers are thankful to the laboratory technologists and other lecturers in the faculty who contributed to this research.

Funding

None.

Conflict of interest

The authors declare they have no competing interests.

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Ethics approval and consent to participate

Ethical approval for the use of experimental animals in the research experiment was obtained from the Animal Research Ethics Committee of Nnamdi Azikiwe University, Awka (NAU/AREC/2025/0062).

Consent for publication

Not applicable.

Availability of data

Data used in the study can be obtained from the corresponding author upon reasonable request.

Further disclosure

The paper has been uploaded to or deposited in a preprint server (bioRxiv; doi: 10.64898/2026.04.03.716360).

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