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Original Research Article

Antimalarial Efficacy of *Azadirachta indica* (Neem) and *Nigella sativa* (Black Seed) Seed Extracts in Mice Infected with *Plasmodium berghei*

Maria M. Adamu[1], Usman A. Saeed,[2*] Adamu B. Samaila[1], Sam M. Panda[1] , Garba A. Madugu[3], Aisha A. Jalingo[4], Umar S. Inuwa[5], Auwal M. Musa[2] Safwan A. Idris[6], Fatima B[7], Shittu A. Muhammad[8]

[1]*Department of Biological Sciences, Abubakar Tafawa Balewa University, Bauchi, Nigeria.*
[2]*Applied Entomology and Parasitology Unit,Department of Zoology, Faculty of Natural Sciences, University of Jos, P.M.B. 2084, Jos - Nigeria.*
[3]*Department of Animal and Environmental Biology. Faculty of Life Sciences Federal University Oye-Ekiti, Ekiti state Nigeria.*
[4]*Department of Plant Science and Biotechnology, Faculty of Life Sciences Bayero University, Kano, P.M.B 3011Kano Nigeria*
[5]*Department of Biological Sciences, Sule Lamido University Kafin Hausa Jigawa state Nigeria*
[6]*Department of Microbiology, Faculty of Ntural Sciences, University of Jos, P.M.B. 2084, Jos-Nigeria*
[7]*Defence Research and Development Bereau, National Defence, College, Abuja. Nigeria.*
[8]*Department of Chemistry, Faculty of Natural Sciences University of Jos, P.M.B, 2084, Jos Nigeria.*

ABSTRACT

Malaria continues to persist as a deadly parasitic infection transmitted by insects with a significant proportion of the deaths and burden accounted for in sub-Saharan Africa. Although several measures such as vector control and chemotherapy have been used in control, the disease still persist. One of the major setbacks to chemotherapy is that the *Plasmodium* Spp. are developing resistance to the currently used antimalarials. This study assessed the antiplasmodial potential of *Azadiracta indica* and *Nigella sativa* ethanol seed extracts against *Plasmodium berghei*-infected mice models. The treatments were administered separately in concentrations of 200,400 and 600mg/kg body weight and compared against a standard antimalaria drug (artemether). The findings revealed that, both extracts possessed Phytoactive compounds with antimalarial potentials and showed incremental effect on parasitaemia level with increasing concentrations of administration. Although, *A. indica* extract reduced parasitaemia to levels significantly ($p<0.05$) lower (0.78±0.08) than the positive control (14.53±0.58), the observed difference was however comparable ($p>0.05$) to that observed using *N. sativa* (2.57±0.86), and the standard drug (0.53±0.0.07). Additionally, the extracts exhibited moderate restorative effects on haematological parameters compromised by malaria infection, including PCV, Hb, and RBC counts. Furthermore, it was observed that the extracts were safe at doses up to 5000 mg/kg, with no observed toxicity. Therefore, it is suggested that both extracts possess anti-plasmodial effects that would benefit pharmacological development. Thus, necessitating further studies to identify the active compounds in the plants that were effective against the parasites and study their mechanism of actions.

Keywords: Azadirachta indica,Nigella sativa, Phytochemical, Parasitaemia, Antiplasmodial, Toxicity, Mice

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Introduction

Despite several efforts aimed at combating malaria infection, the disease still pose a significant public health threat globally, with the 2024 estimate indicating a global malaria burden of about 282 million cases with 610,000 deths. This data points to a 3% increase in malaria cases (i.e about 9 million cases) with a corresponding 2% (i.e about 12,000) increase in recorded deaths due to malaria infection compared to the 2023 records. [1] In sub-Saharan Africa, malaria burden is high, accounting for about 94% of the global malaria cases (265 million) and 95% (579,000) of all deaths resulting from malaria in 2024.

*Corresponding author. mail: usaeed93@gmail.com
Tel: 0806 459 0855

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Nearly half of the global malaria cases is accounted for by only five countries in Africa: Nigeria, Ethiopia, Mozambique, the Democratic Republic of the Congo, and Uganda, underscoring the extreme geographic concentration of the disease.[1]

Historically, efforts to effectively combat malaria have relied heavily on the strategic combination of antimalarial medications with comprehensive mosquito control measures. [2] This approach has proven essential in efforts to mitigate the geographic spread of the disease. Various vector management tools, which include the application of insecticides, necessary environmental modifications, sleeping under treated bed nets, have being key to the malaria control initiatives. [2] Despite these efforts, there have been recent setbacks that have raised concerns within the malaria control community, such as the growing problem of insecticide resistance observed in mosquito populations. [3] Of importance also is the continual threat observed on the efficacy of antimalarial drugs, posed by the recurrent development

of resistance by the *Plasmodium* parasites. Antimalarial resistance not only undermines disease control efforts but also increases the risk of transmission, treatment failures, and mortality.[4]

Herbal medicine has been a long standing practice with about 75-80% of the global population still practicing it for their primary healthcare needs; particularly in the developing counties.[5] The wide acceptance of herbal medicine is because of the perceived believe that the products without any side effects and can be easily sourced from the

environment.[6] The use of herbs as treatment remedies is long documented and is the foundation of most modern medicine.[7] *Azadirachta indica* has long been used as a component of traditional herbal medicine. The extracts are believed to posses diverse antimalarial properties, as such the parasites may not be able to develop full resistance to the components in the plant. In the past, Indian ayurvedic practitioners use the leaf extract of *A. indica*, as an oral remedy for malaria.[8] While the leaf extract has been shown to posses sterols, coumarins, glycosides, limonoids, and flavonoids.[9] with documented schizontocidal and gametocidal effects against the malaria parasite. [10] Similarly, *Nigella sativa* (Black seed), a well distributed plant in the Middle Eastern Mediterranean region, India, Turkey, Pakistan, Syria, South Europe, and Saudi Arabia [11] has become a source of interest since the discovery of its wide therapeutic potential against many ailments.[12, 13] Several among the properties of *N. sativa* include its antidiabetic, anticancer and anti-inflammatory properties.[14, 15] In the Middle East folk medicin, the plant has been reported to possess antiparasitic properties.[16] Previously, other studies have described the plant as a potential antiplasmodial agent.[17, 18]
Considering the concerns about antimalarial resistance by the *Plasmodium* parasites and the need to find alternative drug products with antimalarial property, this study assessed the antiplasmodial effect of *N. sativa* and *A. indica*, against *Plasmodium berghei*, comparing the effect of the two plants as potential candidate for antimalarial therapy.

Materials and Methods

Collection and processing of plants materials
Mature seeds of *A. indica* and *N. sativa* were obtained from local stores in Terminus Market (Lat: 9.9333 º N, Long: 8.8833 º E), Jos North Local Government Area, Plateau State (Location) on the 10th of November 2025. A botanist (Mr. Benjamin Itodo) in the Department of Plant Science and Biotechnology, University of Jos, identified the procured seeds while samples were kept in the herbarium for reference purpose with voucher numbers: UJ/PCG/HSP/16N-06 (*Nigella sativa*) and UJ/PCG/HSP/17A-03 (*Azadirachta indica*). The seeds were washed to remove debris, and air-dried under room conditions. A mechanical grinder was used to grind the dried seeds into fine powder and stored in labelled containers (Restch Company Inc. Paris France; Model SN100). [19]

Extraction of seed component
Extraction of the phytochemical components in he seed extracts of *A. incica and N. sativa* was done using the Soxhlet extraction method [20] using the Soxhlet extraction device (Model R106 S-FB USA, Glyson Company, Inc, Lewis centre USA). Each plant was extracted separately, with fifty grams (50 g) of the powdered material weighed into 500ml of the solvent (ethanol; 95% absolute, manufactured by Raizen and Valero energy, USA, conc, 100% ethanol, grade-Neat ethanol). A rotary evaporator (Sigma Aldrich USA, Model-NRE 201 USA) was then used to dry the extract, forming the stock solution used to prepare the treatments.

Phytochemical analyses of crude seed extract of Azadirachta incica and Nigella sativa
The aqueous solution of the plant seed extracts was used for phytochemical screening of alkanoids, flavonoid, tannin, saponin, trepenes and steroids, cardiac glycoside, balsam, carbohydrates, phenol and resins using standard qualitative procedures. [21, 22]

Ethical approval
The Research and Ethics committee of the Department of Pharmacology, Faculty of Pharmaceutical Sciences, University of Jos Nigeria, reviewed the study protocol and gave the ethical approval for the study (Ethical Approval number: UJ/FPS/F17-00379)

Procurement of standard antimalarial drug
The standard antimalaria drug used in this study was Artemether (artemether and lumenfantrin, 80:480 w/w, Ajanta Pharma Ltd., India). It was obtained from a registered drug store in Terminus, Plateau State.

Procurement of study animal
Seventy-two adult Swiss albino mice of both sex, with body weights of about 30± 5g were procured from the animal house at the National Veterinary Research institute (NVRI), Vom, Plateau State and maintained on commercial feed (Chukun Feeds Nig. Ltd.) and water ad libitum in the laboratory for a week, prior to the study. The study protocol was approved by the Institutional Animal Care and use Committee (IAACUUC) Animal Experimental Unit Department of Pharmacology, Faculty of Pharmaceutical Sciences University of Jos Nigeria. Furthermore, the mice were ethically handled in following the institutional ethical recommendations for care and use of laboratory animals.

Procurement and inoculation of Plasmodium berghei
The method outlined by Enechi et al.[19] was followed in inoculating the mice model. *Plasmodium berghei*-infected blood sample was obtained from a donor mouse from the National Veterinary Research Institute (NVRI) Vom and used to prepare the inoculum. Serial dilution of the blood sample was done in Alsever's solution to obtain a suspension containing about 1 × 107 infected RBCs in every 0.2 mL suspension. Parasite inoculation of the mice in the test groups (groups 2–9) was done intraperitoneally with 0.2 mL of the prepared suspension to initiate infection. After 72 hours of parasite inoculation, parasitaemia was confirmed in the infected animals.

Acute toxicity test
The acute toxicity of the plant extracts was evaluated using a two-phase procedure as previously described.[23] A total of 36 mice were used for the assessment. In the first phase, nine mice were randomly allocated into three groups of three animals each. The respective groups received *A. indica* seed extract (AISE) orally at doses of 10, 100, and 1000 mg/kg body weight. The animals were monitored continuously for 24 h for any observable signs of toxicity and mortality. For the second phase, the remaining nine mice were randomly divided into three groups, with three animals in each group. The animals were administered higher doses of AISE at 1600, 2900, and 5000 mg/kg body weight, respectively, and subsequently observed for 24 h for clinical signs of toxicity and mortality. The acute toxicity assessment of *N. sativa* seed extract (NSSE) was conducted using the same two-phase procedure and dosing regimen described above.

On observation after the test, none of the mice exhibited any sign of toxicity and no mortality were recorded. It was therefore concluded that the lethal dose of *A. indica* and *N. sativa* seed extracts was more than 5000 mg/ kg, and therefore it is safe to consume.

Bioefficacy assay
The antimalarial experiment was conducted following the procedure described by Enechi et al.[19] Thirty-six adult Swiss mice were assigned to six groups of six animals each. However, the treatment allocation comprised nine groups, as follows:
Group 1: Normal control, administered normal saline.
Group 2: Negative control, inoculated with *P. berghei* and left untreated.
Group 3: Standard drug control, infected with *P. berghei* and treated with 140 mg/kg body weight of artemether.
Group 4: *P. berghei*-infected mice treated with 200 mg/kg body weight of AISE.
Group 5: *P. berghei*-infected mice treated with 400 mg/kg body weight of AISE.
Group 6: *P. berghei*-infected mice treated with 600 mg/kg body weight of AISE.
Group 7: *P. berghei*-infected mice treated with 200 mg/kg body weight of NSSE.

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Group 8: *P. berghei*-infected mice treated with 400 mg/kg body weight of NSSE.
Group 9: *P. berghei*-infected mice treated with 600 mg/kg body weight of NSSE.
Where, AISE =*A. indica* seed extract; NSSE = *N. sativa* seed extract
Treatment administration lasted for 4 days (from day 0-day 3). On the last day of treatment, the mice were left without feeding, and on day 4, day 4, blood sample was collected for parasitaemia count and haematological analyses. The three doses were selected based on activity-guided dose selection from the LD50 test.

Determination of parasitaemia level

Malaria parasitaemia was determined by the methods described earlier by Bain [24] and Osei-Bimpong and Burthem [25] and the percentage malaria parasitemia was calculated using the relations below:

$$\% \text{ Malaria parasitaemia (MP)} = \frac{\text{Number of parasitized RBCs}}{\text{Total Number of RBCs counted}} \times 100$$

Determination of haematological status of experimental mice

The haematological indices of the test mice were determined using a haemo-autoanalyzer at the National Veterinary Research Institute, Vom.

Statistical analysis

Parasitemia levels were compared across all groups to note percentage reduction in parasitemia for each treatment group relative to the infected and untreated control. One-way analysis of variance and Tukey post hoc test was used to compare means across the groups. Similarly, variation in haematological indices were also compared across all treatment groups using One-way analysis of variance test with Tukey post hoc test. The results were presented as mean ± Standard Error (SE) of the means. Values with $p < 0.05$ were considered significant.

Results and Discussion

Phytochemical constituents of A. indica and N. sativa seed extracts

This study was conducted to assess the anti-plasmodial effect of *A. indica* (neem) and *N. sativa* (black seed) seed in rat models infected with *P. berghei*. The findings revealed that both plant extracts demonstrated dose-dependent reductions in parasitaemia and modest modulation of hematological indices, thus highlighting their potential therapeutic roles in malaria management.

Qualitative *p*hytochemical screening of the extracts (Table 1) revealed that *A. indica* seed extract contains flavonoid, tannins, saponins, steroids, cardiac glycosides, balsams, carbohydrates, phenols and renins but lacks alkaloids. On the other hand, *N. sativa* contains all the mentioned phytochemicals with the exception of balsam which was not detected in the extract.

Table 1: Result of phytochemical screening of aqueous crude seed extract of *A. indica* and *N. sativa*

Bioactive substances	**Plant**	
	A. indica	***N. sativa***
Alkanoids	-	+
Flavanoids	+	+
Tannins	+	+
Saponins	+	+
Steroids	+	+
Cardiac glycosides	+	+
Balsams	+	-
Carbohydrates	+	+
Phenols	+	+
Renins	+	+

Key: + = Present; - = Absent

The broad spectrum of secondary metabolites observed in both extracts could underlie their observed therapeutic effects in the mice models infected with *P. berghei*. Both extracts contained flavonoids, tannins, saponins, phenols, carbohydrates, cardiac glycosides, steroids, and resins, all of which are compounds known to exert biological activities including antimalarial, antioxidant, and immunomodulatory effects.[26]

A. indica extract lacked alkaloids but contained other bioactive compounds such as flavonoids, tannins, saponins, steroids, and cardiac glycosides. Similar to this finding, Biswas et al.[27] observed that the therapeutic effect of neem could be due to compounds like nimbin, nimbolide, and quercetin. Flavonoids, for instance, have been shown to inhibit parasite growth by disrupting mitochondrial function and redox balance, while saponins and tannins contribute to membrane disruption of the parasite and immune stimulation.[28]

N. sativa, on the other hand, contained all the screened phytochemicals, including alkaloids, but lacked balsams. Its richness in thymoquinone, flavonoids, and phenolic compounds is well-documented in literature as contributing to its antiplasmodial, anti-inflammatory, and antioxidant properties.[29] Alkaloids are known to possess strong antiparasitic activity, potentially giving *N. sativa* a unique edge in parasite clearance despite a slightly higher parasitaemia than *A. indica* in this study.

The variation in phytochemical profiles between the two extracts could partly explain the differences in therapeutic outcomes observed. While both plants share several active compounds, the presence of alkaloids in *N. sativa* and balsam in *A. indica* may reflect distinct mechanisms of action in the plants. Therefore, it is necessary not just to rely on the presence of such compounds but also on their abundance and interaction in determining pharmacological efficacy.

Anti-plasmodial effect of A. indica and N. sativa seed extracts

The anti-plasmodial effect of *A. indica* seed extract on parasitaemia level in the infected rats (Table 2) revealed an incremental effect on the parasites with increasing treatment concentration. The highest dosage (600mg/kg) lowered parasitaemia level to 0.53±0.07% as against what was obtained in the positive control (14.53±0.58%). However, when compared to the standard drug group (Artemeter), the difference observed was not significant ($p>0.05$). Similarly, there was no significant variation ($p>0.05$) in the anti-plasmodial effect recorded across the different treatment concentrations used.

Similar finding was also observed for *N. sativa* seed extract (Table 3), with the highest dosage (600mg/kg) lowering parasitaemia level to 0.40±0.00% as against what was observed in the positive control (14.53±0.58%). When compared to the standard drug group (Artemeter), the difference observed at treatment concentration of

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400mg/kg and 600mg/kg did not differ significantly (p>0.05) with the standard drug. However, the dosage, 200mg/kg recorded a significantly lower (p>0.05) anti-plasmodial effect compared to the other treatment concentrations used.
Overall, the result indicated that *A. indica* was more effective (0.78±0.08%) compared to *N. sativa* (2.57±0.86%) extract in surpressing parasitaemia level in the infected rat modesl (Table 4). However, the result of the statistical analysis performed showed that the anti-plasmodial effect of the extracts did not differ significantly (p>0.05).

Table 2: Effect of *Azadirachta indica* seed extracts on parasitaemia levels in mice

Treatment Concentration (mg/kg)	Mean parasitaemia±SE
200	1.00±0.00[a]
400	0.80±0.12[a]
600	0.53±0.07[a]
Artemeter (140mg/kg)	0.53±0.07[a]
Positive Control	14.53±0.58[b]
Normal Control	0.00±0.00

$F_{(4,10)}$ =530.815, p<0.001
Note: Values in the same column followed by different superscripts are significantly different at p<0.05

Table 3: Effect of *Nigella sativa* extracts on parasitaemia levels in mice

Treatment Concentration (mg/kg)	Mean parasitaemia±SE
200	5.67±0.35[b]
400	0.73±0.07[a]
600	0.40±0.00[a]
Artemeter (140mg/kg)	0.53±0.07[a]
Positive Control	14.53±0.58[c]
Normal Control	0.00±0.00

$F_{(4,10)}$ =394.495, p<0.001
Note: Values in the same column followed by different superscripts are significantly different at p<0.05

Table 4: Comparative effect of *Azadirachta indica* and *Nigella sativa* seed extracts on parasitaemia level in infected mice

Treatment	Mean parasitaemia ± SE
Azadirachta indica Extract	0.78±0.08
Nigella sativa Extract	2.57±0.86

t=-1.729, df=16, p=0.103

The significant reduction in parasitaemia observed in mice treated with both extracts in this study has been reported previously, and affirms the antiplasmodial potential of these botanicals. The highest dosage (600 mg/kg) of *A. indica* extract reduced parasitaemia to 0.53 ± 0.07%, which is comparable to the standard drug artemether (0.53 ± 0.07%) and significantly lower than the untreated control (14.53 ± 0.58%). This observation corroborates the findings of Biswas et al.[27] who reported potent antiplasmodial activity of neem extracts due to its bioactive limonoids such as azadirachtin and nimbolide, which interfere with parasite development and erythrocyte invasion. Similarly, *N. sativa* at 600 mg/kg reduced parasitaemia to 0.40 ± 0.00%, a level statistically indistinct from artemether, reinforcing earlier reports by Ojueromi et al.[29] which demonstrated that thymoquinone, the principal active component of *N. sativa*, disrupts parasitic replication and enhances host immunity. However, a significant difference was noted at the 200 mg/kg dosage, which recorded a less pronounced parasitaemia reduction (5.67 ± 0.35%), indicating a threshold dose may be required for therapeutic efficacy.
Comparatively, the two extracts did not outperform each other significantly even though *A. indica* showed higher parasitaemia suppression compared to *N sativa.* this could be due to the similar phytochemical constituents observed in both extracts. Both extracts contained flavonoids, tannins, saponins, phenols, and cardiac glycosides, but *N. sativa* lacked balsam, a compound with reported antimicrobial properties.[30] The presence of these phytochemicals, especially flavonoids and phenols, may contribute to antimalarial action by inducing oxidative stress in the parasite or interfering with its metabolism.[26]
In terms of safety, the acute toxicity results revealed that neither extract caused observable toxicity or mortality up to 5000 mg/kg, confirming their wide therapeutic safety margin. This supports previous studies.[31, 32] indicating that both neem and black seed extracts are generally safe at high oral doses in rodent models.

Effect of A. indica and N. sativa seed extracts on the haematological indices of treated mice
The effect of the seed extract of *A. indica* on the haematological parameters of the treated mice is contained on Table 5. With the exception of white blood cell count, Neutrophil, and Eosinophil count, all other parameters were higher in the normal control group, compared to the positive control group. PCV level, WBC, N, and M count increased with increasing dosages of treatment administration. However, the difference observed was not significantly different (p>0.05) from that observed from the standard drug group. In the treatment group with *N. sativa* (Table 6), similar result was also observed. However, with the exception of RBC, N and L, which increased with treatment concentration, other parameters decreased as the dosage of administration was increased. Furthermore, PCV values were unaffected across all treatment concentrations used. Compared to the standard treatment however, the differences observed across all the parameters tested did not differ significantly (p>0.05).

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Table 5: Effect of *Azadirachta indica* extract on haematological parameters of mice infected with *P. berghei*

Treatment Concentration (mg/kg)	Mean±SE								
	PCV (%)	Hb (g/dl)	RBC (x10^12/L)	WBC (x10^109/L)	N (%)	L (%)	M (%)	E (%)	B (%)
200	29.33±0.88[a]	9.30±0.32[a]	5.80±0.23[a]	4.90±0.70[a]	40.33±1.20[a]	57.00±1.53[a]	1.00±1.00[a]	1.00±0.00[a]	0.00±0.00[a]
400	31.67±0.0.33[a]	8.47±0.15[a]	5.90±0.32[a]	5.53±0.28[a]	45.00±3.79[a]	52.00±3.46[a]	0.00±0.00[a]	1.33±0.33[a]	0.00±0.00[a]
600	30.33±0.88[a]	9.23±0.24[a]	5.50±0.35[a]	5.13±0.07[a]	41.67±1.76[a]	56.00±5.51[a]	1.00±0.58[a]	0.67±0.33[a]	0.00±0.00[a]
Artemeter (140mg/kg)	31.00±0.58[a]	9.57±0.32[a]	5.77±0.47[a]	5.13±0.32[a]	40.0±3.61[a]	61.33±3.18[a]	1.33±0.88[a]	0.67±0.67[a]	0.00±0.00[a]
Positive Control	29.00±0.58[a]	9.10±0.36[a]	5.30±0.25[a]	6.43±0.41[a]	51.00±9.54[a]	45.67±8.69[a]	0.00±0.00[a]	3.67±0.33[b]	0.00±0.00[a]
Normal Control	35.00±0.58	10.77±0.38	6.57±0.23	6.06±0.47	26.00±7.94	69.33±5.36	0.00±0.00	0.00±0.00	0.00±0.00

Note: SE=Standard Error, PCV=Pack Cell Volume, Hb=Haemoglobin, RBC=Red Blood Cell, WBC=White Blood Cell, N=neutrophil, L=Lymphocyte, M=Monocyte, E=Eosinophile, B=Basophil

Values in the same column followed by different superscripts are significantly different at $p<0.05$

Table 6: Effect of *Nigella sativa* extracts on haematological parameters of mice infected with *P. berghei*

Treatment Concentration (mg/kg)	Mean±SE								
	PCV (%)	Hb (g/dl)	RBC (x10^12/L)	WBC (x10^109/L)	N (%)	L (%)	M (%)	E (%)	B (%)
200	30.33±1.20[a]	10.17±0.38[a]	5.97±0.12[a]	5.13±0.29[a]	37.00±1.15[a]	44.67±3.18[a]	1.00±1.00[a]	3.00±1.00[a]	0.00±0.00[a]
400	30.33±0.0.67[a]	9.23±0.18[a]	5.77±0.32[a]	5.23±0.52[a]	52.00±0.58[a]	41.00±1.53[a]	0.00±0.00[a]	1.00±1.00[a]	0.00±0.00[a]
600	30.33±0.1.20[a]	8.80±0.36[a]	6.60±0.31[a]	4.90±0.17[a]	41.33±0.67[a]	45.67±3.71[a]	1.00±1.00[a]	2.00±1.00[a]	0.00±0.00[a]
Artemeter (140mg/kg)	31.00±0.58[a]	9.57±0.32[a]	5.77±0.47[a]	5.13±0.32[a]	40.0±3.61[a]	61.33±3.18[a]	1.33±0.88[a]	0.67±0.67[a]	0.00±0.00[a]
Positive Control	29.00±0.58[a]	9.10±0.36[a]	5.30±0.25[a]	6.43±0.41[a]	51.00±9.54[a]	45.67±8.69[a]	0.00±0.00[a]	3.67±0.33[b]	0.00±0.00[a]
Normal Control	35.00±0.58	10.77±0.38	6.57±0.23	6.06±0.47	26.00±7.94	69.33±5.36	0.00±0.00	0.00±0.00	0.00±0.00

Note: SE=Standard Error, PCV=Pack Cell Volume, Hb=Haemoglobin, RBC=Red Blood Cell, WBC=White Blood Cell, N=neutrophil, L=Lymphocyte, M=Monocyte, E=Eosinophile, B=Basophil

Values in the same column followed by different superscripts are significantly different at p<0.

In this study, haematological indices were employed to assess the systemic effects of treatment using the plant extracts on infected animals. *Plasmodium* infection typically induces anaemia and leukocyte alterations due to hemolysis, splenic sequestration, and immune activation. [33] This was evident in the untreated infected mice, which showed reduced PCV (29.00%), Hb (9.10 g/dL), and RBC (5.30 x10^{12}/L) compared to the normal control group. Conversely, treatment with *A. indica* and *N. sativa* improved these indices modestly. For instance, *A. indica* at 400 mg/kg raised PCV to 31.67%, while *N. sativa* at 600 mg/kg increased RBC count to 6.60 x10^{12}/L. Though these increases were not statistically significant ($p > 0.05$), they approached normal ranges and mirrored the effect of artemether, suggesting that the extracts may help mitigate malaria-induced haematological damage. Furthermore, the observed neutrophilia and eosinophilia in the positive control group (Neutrophils: 51.00%; Eosinophils: 3.67%) diminished following treatment with both extracts. This trend suggests an anti-inflammatory or immune-modulating effect, as excessive eosinophils and neutrophils in malaria have been linked to immune dysregulation and oxidative damage. [34] Both extracts, especially *A. indica*, appeared to normalize lymphocyte counts and reduce eosinophils within or close to normal ranges (0–4%), which is indicative of a restorative influence on immune homeostasis. Furthermore, comparing the haematological indices observed in the study to the normal ranges in murine models (Normal ranges: PCV: 35–45%; Hb: 12–18 g/dL; RBC: 6.3–10 x10^{12}/L), it is observed that although treatment with the respective extracts did not fully restore these parameters to baseline, they prevented the severe derangements seen in the untreated group. This partial restoration reflect both the antiparasitic and cytoprotective effects of the extracts and agrees with the antioxidant and anti-inflammatory actions reported for neem and black seed. [35, 36] This study also adds credence to previous studies that have reported that phytochemical constituents in plants possess therapeutic activity against malaria infection in mice models.[37]

Conclusion

This study demonstrated that seed extracts of *Azadirachta indica* and *Nigella sativa* possess promising antimalarial potential, evidenced by significant reduction in parasitaemia levels in *Plasmodium berghei*-infected mice. The highest tested doses of both extracts achieved parasitaemia suppression comparable to artemether, suggesting their therapeutic relevance.

Phytochemical screening confirmed the presence of several bioactive constituents including flavonoids, tannins, saponins, phenols, and glycosides, which are likely responsible for the observed bioactivity. Additionally, the extracts exhibited moderate restorative effects on haematological parameters compromised by malaria infection, including PCV, Hb, and RBC counts. Furthermore, it was observed that the extracts were safe at doses up to 5000 mg/kg, with no observed toxicity, reinforcing their traditional use and supporting further pharmacological development. While *A. indica* showed slightly better parasitaemia suppression than *N. sativa*, both exhibited significant therapeutic potential. Therefore, further studies into their mechanisms, formulation, and possible combination therapies is recommended.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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