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Original Research Article

Baphia nitida ethanol leaf extract restores haematological and biochemical status in Plasmodium berghei-passaged rats

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Abstract

Purpose: *To investigate the effect of Baphia nitida ethanol leaf extract (BNELE) on heamatological and biochemical indices in Plasmodium berghei-infected mice.*
Methods: *A total of thirty (30) male mice were divided into 6 groups comprising of 5 mice each. Group 1 was normal control, group 2 positive control, group 3 standard group while groups 4 - 6 received 200, 400 and 600 mg/kg of BNELE, respectively. Phytochemical analysis and antimalaria effect of BNELE were investigated using standard methods. Biochemical and haematological parameters of Plasmodium berghei - infected mice given various BNELE dosages were measured using standard techniques.*
Results: *At increasing doses (200, 400, and 600 mg/kg), BNELE produced 63.16, 39.50 and 52.86 % inhibition of parasitemia in P. berghei-passaged mice, respectively, comparable to 43.75 % by 80 mg/kg artemether, a first-line anti-malarial medication. There was significant increase in red blood cell (RBC), packed cell volume (PCV), white blood cell (WBC) and hemoglobulin (HB) in mice administered varying doses of BNELE compared with those in group 2 ($p < 0.05$).*
Conclusion: *Baphia nitida ethanol leaf extract possesses antiplasmodial properties and aids the restoration of normal haematological and biochemical states in P. berghei-infected mice. However, further and more extensive in vivo studies to ascertain it full antimalarial potentials.*

Keywords: *Antimalarial, Baphia nitida, Haematological indices, Biochemical parameters, Lipid profile*

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INTRODUCTION

Parasites of the genus Plasmodium are responsible for the deadly disease called malaria, and transmissible to people through bite of an infected female anopheles mosquito. Malaria continues to be a leading cause of mortality worldwide, but poor outcomes can be averted with timely detection and immediate treatment. Global malaria fatality rate is 0.3 - 2.2 %, although cases of severe malaria can have fatality rates of 11 to 30 % in tropical climates [1]. World Health Organization oversees a worldwide malaria control program with focus on improving primary healthcare domestically, early disease screening, appropriate intervention, and disease

prevention. The prevalence of malaria has lessened over the past five years. Even though growth has dropped, the WHO's targets are still being met.

Herbal remedies serve as a precursor to most synthetic medications. With the help of technological advancements, therapeutic plants are being isolated and investigated for elemental contents. These serve as a model for development of additional analogues of herbal remedies and introduction of plant medicines [2]. *Baphia nitida plant is a* camwood*.* It was referred to by the Nigerian Igbo tribe as Aboshi, Yoruba call it Irosun, and Efik refer to it as Ubara. According to research, this plant has a variety of therapeutic activity such as hepatoprotective and anti-anaemia [3]. However, antimalarial effect of the plant has not been studied and it is being used traditionally to treat malaria in some rural areas. Consequently, this current research seeks to investigate antimalarial activity of *Baphia nitida* ethanolic leaf extract (BNELE) as well as its treatment outcome on biochemical and haematological parameters in *Plasmodium berghei*-induced rats.

EXPERIMENTAL

Materials

Plant

Fresh leaves of *Baphia nitida* leaves were procured Onuiyi community in Nsukka Local Government Area of Enugu State, Nigeria. The plant was authenticated by Mr. Felix Nwafor, a plant taxonomist at Department of Plant Science and Biotechnology, University of Nigeria Nsukka, Enugu State, and given a voucher number UNH/04/0107E. The leaves of *Baphia nitida* Load. (Fabaceae) were then kept in the herbarium for future reference.

Animals

A total of thirty (30) animals were acquired from animal house of the College of Veterinary Medicine, University of Nigeria, Nsukka with average body weights of 15 – 30 g. Animals were fed with commercial chicken grower's mash and water was administered to the animals as their normal diet ad labium for a week ahead of the experiment in the animal farm under standard laboratory settings with a 12-hour light and dark cycle. Treatment was carried out humanely in compliance with institutional, national, and international ethical criteria for the care and use of laboratory animals (approval no. UNN/FBS/2018_029A).

Parasite

Blood samples were drawn from donor mice that had *Plasmodium berghei* ANKA 65 strain infections which were obtained from College of Veterinary Medicine, University of Nigeria, Nssukka. Standard inoculums of 1×10^7 parasitized erythrocytes from a donor mouse were administered intraperitoneally to experimental mice in doses of 0.2 mL each.

Equipment

The equipment used in this experiment consists of the following: UV-Vis Spectrophotometer (Jenway 6305, China), Centrifuge (4000 rpm, Abman), Centrifuge (Model AR 3130, China), , Pasteur Pipette, Refrigerator (Kelvinator, Germany), Colorimeter (E1 312 Model, Japan), Microlux micropipette, Rotary Evaporator (Model Modulyo 4k, Edward, England), Water bath (Gallenkamp, England).

Standard drug

The standard antimalarial medication utilized in this research was artemether (Ajanta Pharma Ltd. India) which is a combination of artemether and lumefantrin, 80:480 w/w respectively.

Procedures

Extraction techniques

Fresh leaves of *Baphia nitida* weighing 2000 g were gathered and cleaned. The leaves were dried under a shade while being agitated occasionally to prevent decay. Leaves were pulverized into fines and 1000 g of the powder was macerated in 3.5 L of 80 % ethanol using a maceration flask for 72 h while being agitated. Thereafter, the preparation was filtered through a muslin cloth and placed in a flat bottom flask. Whatman No. 1 filter paper was used for additional filtration to get rid of tiny residues. The *Baphia nitida* ethanolic leaf extract (BNELE) of the plant was obtained by centrifugation (4000 rpm, Abman model) at 80 °C. Resulting crude extract was kept in an appropriately labelled, sterile, screw-capped container at 4 °C until required for use.

Quantitative phytochemical analysis of BNELE

Secondary metabolites of methanolic extract of *Baphia nitida* leaves were quantified using standard phytochemical examinations [4].

Parasite inoculation

For this work, a mouse model of *Plasmodium berghei* ANKA-65 was used. Minor modifications were made to the approach of Esan [5] to estimate percentage of parasitemia (Mp). *Plasmodium berghei*-infected donor mouse blood was acquired from College of Veterinary medicine at the University of Nigeria, Nsukka. For infection, 10 drops of parasitized blood from tails of infected mice were combined with 2 mL phosphate buffer (pH 7.2). Thereafter, 0.2 mL of parasitized red blood cells (pRBCs) was injected intraperitoneal into the animals.

Design

Group 1: Normal control (Not infected, not treated).

Group 2: Positive control (Mice infected with *Plasmodium berghei*, not treated).

Group 3: Standard drug control (*Plasmodium berghei* infected mice + 80 mg/kg of artemeter).

Group 4: *Plasmodium berghei* infected mice + 200 mg/kg body weight of *Baphia nitida* extract.

Group 5: *Plasmodium berghei* infected mice + 400 mg/kg body weight of *Baphia nitida* extract.

Group 6: *Plasmodium berghei* infected mice + 600 mg/kg body weight of *Baphia nitida* extract.

Parasitized mice in groups 4-6 were administered varying doses of BNELE for 4 days. On 5th day, following an overnight fast, a cardiac puncture was used to get blood samples used for parasitemia, haematological and biochemical tests.

Determination of parasitemia

The method of Kalra *et al* [6] was used to calculate percentage parasitemia. On slides, thin smears made from infected mice were developed, stained with Leishman stain and allowed to dry before being fixed with methanol and counter-stained. Thereafter, slides were submerged in water for 3 mins, rinsed for approximately 5 mins, and then left to drain. Using a microscope (x100) and immersion oil in 10 distinct fields on every slide, the slides were carefully examined to determine the amount of RBC harbouring the malaria parasite (nRBC). Percentage parasitaemia (P) was calculated using Eq 1.

$$P = (nRBC/tRBC)100 \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

where tRBC is the total number of RBC counted.

Determination of biochemical and haematological parameters

The method of Ochei and Kolhatkar [7] was used to determine haematological indices. Using a commercial QCA kit, total serum cholesterol concentration was calculated following the procedure of Allain *et al* [8]. Concentration of serum HDL-cholesterol and triacylglycerol were evaluated using the method of Albers *et al* [9]. Low-density lipoprotein (LDL) cholesterol was determined as the difference between total cholesterol and cholesterol content of the supernatant after precipitation with polyvinyl sulphate (PVS) in polyethylene glycol monomethyl ether. Levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) was measured following method by Reitman and Frankel [10]. Concentration of alkaline phosphatase (ALP) was measured following method of Klein *et al* [11] using a Randox kit. Catalase activity was investigated using established method [12]. Superoxide dismutase (SOD) activity [13] and glutathione concentration was also investigated [14].

Statistical analysis

Data were analyzed using one-way and two-way analysis of variance (ANOVA) with Statistical Product and Service Solution (SPSS) software version 22.0. Outcomes were recorded as mean ± standard deviation (SD). Mean values with $p < 0.05$ were considered statistically significant.

RESULTS

Table 1: Quantitative phytochemical composition of BNELE (n = 3)

Phytochemicals	Concentration (mg/100g)
Total phenolics	7766.13±258.410
Reducing sugar	3189.85±326.329
Flavonoids	920.15±14.075
Alkaloids	587.64±17.078
Terpenoids	376.25±3.719
Tannins	48.97±2.222
Steroids	27.41±3.719
Glycosides	2.40±0.600

Note: Results are reported as mean ± SD

Phytochemical composition of BNELE

Quantitative phytochemical analysis of BNELE showed high concentrations of total phenolics (7766.13 ± 258.410 mg/100 g) and reducing sugar (3189 ± 326.329 mg/100 g). Steroids

(27.41 ± 3.719 mg/100g) and glycosides (2.40 ± 0.600 mg/100 g) were present in trace amounts.

Effect of BNELE on parasitemia

Before treatment, percentage parasitemia levels of passaged rats (groups 2 to 6) did not differ significantly ($p > 0.05$). However, following treatment with artemether and BNELE, percentage parasitemia of groups 3 to 6 significantly reduced ($p < 0.05$) as compared to positive control (Table 2).

Effect of BNELE on haematological indices

Group 2 animals (*Plasmodium berghei*-infected mice not treated) had significantly lower ($p < 0.05$) levels of PCV, HB, RBC, and WBC compared to group 1 (Normal control) mice. However, there was a significant increase in PCV, HB, WBC, and RBC of the mice given different dosages of BNELE compared to group 2 ($p < 0.05$; Table 3).

Effect of BNELE on antioxidant parameters and lipid peroxidation indices

When compared to groups 3 - 6 of treated mice, group 2 (*Plasmodium berghei*-infected) mice showed a significant decrease ($p < 0.05$) in GSH, SOD, and CAT levels. Concentration of MDA however, differs significantly ($p < 0.05$) between group 2 (untreated) mice and groups 3 - 6 (treated) mice (Table 4).

Table 2: Effect of BNELE on percentage parasitemia in *Plasmodium berghei*-infected mice (N=3)

Group	Before treatment	After treatment
1	0.00±0.000^{a}	0.00±0.000^{a}
2	76.67±11.547^{b}	70.00±10.000^{c}
3	53.33±40.414^{b}	30.00±10.000^{b}
4	63.33±15.275^{b}	23.33±20.816^{b}
5	66.66±15.275^{b}	40.00±0.000^{b}
6	70.00±10.000^{b}	33.00±5.770^{b}

Note: Results were expressed as means ± SD. At 95 % confidence interval, there was a significant difference between groups with different superscripts ($p < 0.05$)

Effect of BNELE on liver function parameters

Liver function parameters revealed that AST ALT, ALP, and TB levels of mice infected with *P. berghei* without treatment (group 2) increased significantly compared to standard medication and BNELE ($p < 0.05$). Following administration of artemether and BNELE, groups 3-6 had their liver condition restored to normal compared to regular group (Table 5).

Effect of BNELE on lipid profile

Group 2 (untreated) had significantly lower ($p < 0.05$) HDL levels compared to groups 3-6 (treated). Furthermore, LDL levels of group 2 mice significantly increased ($p < 0.05$). Also, group 2 (untreated) had significantly higher cholesterol and TAG concentrations compared to groups 3-6 (treated) (Table 6).

Table 3: Effect of BNELE on haematological markers in *Plasmodium berghei*-infected mice

Group	Haematological markers			
	PCV (%)	***HB (g/dL)***	***RBC ($\times10^{12}$/L)***	***WBC ($\times10^{9}$/L)***
1	40.000±1.155^{a}	15.390±1.978^{a}	6.567±0.788^{b}	6.933±0.481^{a}
2	29.333±0.882^{b}	8.657±1.105^{b}	3.400±0.100^{a}	6.800±1.405^{b}
3	38.333±2.603^{c}	15.873±1.773^{ac}	6.533±0.167^{b}	6.133±0.267^{ac}
4	40.667±3.712^{a}	16.260±1.113^{c}	6.967±0.186^{bc}	8.033±0.088^{c}
5	43.667±2.404^{ac}	16.007±0.802^{c}	6.733±0.145^{b}	7.867±0.088^{c}
6	41.667±0.667^{a}	16.950±0.512^{c}	6.633±0.088^{b}	7.967±0.088^{b}

Note: Results are expressed in means ± SD

Table 4: Effect of BNELE on antioxidant parameters and lipid peroxidation indices (n = 3)

Group	GSH (mg/dL)	SOD (IU/L)	CAT (IU/L)	MDA (mg/dL)
1	2.463±0.767^{a}	11.027±0.091^{b}	3.270±0.418^{b}	1.453±0.210^{a}
2	1.680±0.499^{b}	5.925±0.082^{c}	1.077±0.645^{a}	3.033±0.007^{b}
3	2.113±0.199^{a}	11.008±0.052^{b}	3.370±0.419^{b}	1.043±0.009^{a}
4	1.663±0.380^{ac}	11.189±0.081^{b}	5.940±0.204^{c}	1.690±0.023^{ac}
5	2.183±0.296^{a}	11.345±0.696^{ab}	6.203±1.921^{c}	1.707±0.049^{ac}
6	2.060±0.381^{ac}	11.200±1.877^{b}	4.997±0.188^{b}	1.730±0.006^{d}

Note: Results are expressed in means ± SD. There was significant difference between groups with different superscripts ($p < 0.05$)

Table 5: Effect of BNELE on liver function parameters (N=3)

Group	Liver Function Parameters			
	AST (IU/L)	***ALT (IU/L)***	***ALP (IU/L)***	***TB (mg/dL)***
1	27.667±2.848^{b}	19.333±0.667^{c}	32.333±0.333^{b}	0.970±0.004^{b}
2	36.000±1.528^{a}	39.333±1.764^{b}	55.667±10.528^{a}	1.343±0.299^{a}
3	25.000±2.517^{b}	29.000±7.024^{a}	27.667±3.930^{bc}	0.997±0.122^{b}
4	21.333±2.667^{bc}	28.067±2.126^{a}	24.167±5.271^{c}	0.859±0.298^{b}
5	21.333±1.764^{bc}	26.100±1.258^{a}	20.363±1.462^{c}	0.867±0.087^{b}
6	21.000±1.732^{a}	28.467±2.691	14.857±2.306^{a}	0.837±0.148^{b}

Note: Results are reported as means ± SD. Groups with different superscripts are significantly different ($p < 0.05$)

Table 6: Effect of BNELE on lipid profile in *Plasmodium berghei*-infected mice (n = 3)

Group	Lipid profile			
	HDL (mmol/L)	***LDL (mmol/L)***	***CHOL (mmol/L***	***TAG (mmol/L)***
1	2.233±0.233^{b}	3.600±0.306^{b}	5.433±0.809^{b}	2.000±0.208^{c}
2	1.367±1.367^{a}	7.700±0.902^{a}	9.600±1.106^{a}	5.200±0.100^{a}
3	2.533±0.120^{bc}	3.267±0.088^{b}	4.133±0.219^{b}	1.267±0.033^{b}
4	2.567±0.233^{bc}	3.167±0.338^{b}	5.033±0.240^{b}	1.700±0.551^{b}
5	2.600±0.351^{bc}	3.167±0.167^{b}	5.700±0.379^{bc}	2.000±0.208^{c}
6	2.613±0.176^{bc}	3.333±0.033^{b}	4.033±0.296^{b}	1.900±0.153^{bc}

Note: Results are reported as mean ± SD

DISCUSSION

The findings of *Baphia nitida* ethanolic leaf extract (BNELE) phytochemical investigation demonstrated the presence of flavonoids, tannins, glycoside, alkaloids, total phenols, terpenoids, reducing sugar, and steroids. These phytochemicals may be responsible for the antimalarial activity because they have been proven to exhibit antiplasmodial effect [15]. Presence of alkaloids and terpenoids is fundamental because they are used as precursors in synthesis of antimalarial drugs such as artemisinin and quinines.

Anti-parasitemia studies showed potent anti-malaria activity of BNELE. From the result, *P. berghei* passaged mice left untreated (parasite control) showed high parasitemia load after inoculation. However, administration of graded doses of BNELE decreased parasitemia load significantly ($P < 0.05$). Also, BNELE exhibited effective inhibition of parasitemia comparable to standard antimalarial drug (artemether) at a mid-dose (400 mg/kg b.w) and was significantly greater than that of standard drug at a high dose (600 mg/kg b.w). This effect is attributed to the rich phytochemical composition in BNELE. Perhaps, the plant extract may have scavenged free radicals and attenuated oxidative stress which play critical roles in malaria infection. These findings corroborate those of Ihekwereme *et al* [16], who revealed that hydroethanolic stem extract of *Baphia pubescens* had strong antiplasmodial action in albino mice infected with *Plasmodium berghei*.

Baphia nitida ethanolic leaf extract (BNELE) exhibited a significant increase in red blood cell (RBC), packed cell volume (PCV) and haemoglobin levels compared to positive control ($p < 0.05$). Haemolysis of malaria-infected RBCs, breakdown of infected cells in spleen, erythropoietic suppression, dyserythropoietic, and oxidative stress, which promotes membrane fragility, contribute to RBC loss in untreated mice. Loss of RBCs decreases PCV and haemoglobin. A similar study on *Zehneria scabra* revealed that at higher doses, there was significant increase in PCV [17] and this suggests that some phytochemicals present in the extract might possess hematopoietic properties.

High density lipoprotein (HDL) in infected and untreated mice reduced significantly compared to healthy control mice. Also, administration of varying doses of BNELE resulted in significantly higher HDL levels and lower LDL, cholesterol and TAG concentrations compared to untreated animals. This outcome is consistent with research by Enechi *et al* [18], who investigated antimalarial activities of *Fagara zanthoxyloides* leaves and revealed that treatment with extract resulted in greater HDL concentrations than control group.

In this study, significantly higher MDA values in parasitized and untreated mice compared to healthy control (group 1) mice indicated increased lipid peroxidation. Levels of antioxidant enzymes (CAT and SOD) were, however, significantly lower in groups that were not treated compared to groups that received various dosages of BNELE. Antioxidants aid in repairing

oxidative damage caused by free radicals in the body. Because they prevent oxidation even at low levels, these antioxidants possess a range of physiological activities in the body. Radicals are scavengers, and antioxidant constituents of plant materials change them into less reactive forms.

Concentrations of AST, ALT, ALP, and total bilirubin were significantly higher ($p < 0.05$) in parasitized and untreated groups compared to healthy control group. Results of this investigation demonstrated that after treatment with various dosages of BNELE, liver damage and changes in liver function parameters were restored to normal. These effects are due to various phytochemicals present which increase natural detoxifying enzymes, protect the liver from harm, and enhance liver enzymes in blood.

CONCLUSION

This study demonstrates that *Baphia nitida* ethanol leaf extract possesses antimalarial activity by effectively inhibiting parasitemia progression in *Plasmodium berghei*-infected mice. Also, BNELE restores haematological parameters, lipid profile, and liver status following treatment. *Baphia nitida* could serve as a potential source of leads for development of new drugs in the treatment of plasmodial infection.

DECLARATIONS

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Ethical approval

None provided.

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 associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Conceptualization: Chinelo Nkwocha; Data collection: Chidimma Azi, Florence Nduka; Methodology: Chisom Ogbuonye, Helen Agbatai; Project administration: Chinelo Nkwocha, Florence Nduka; Supervision: Chinelo Nkwocha; Writing-original draft: Christian Eze, Nkemakolam Nwafor; Writing-review and editing: Chisom Ogbuonye, Chidimma Azi.

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