Antiplasmodial Properties of Ethanol Stem-Bark Extract of *Newbouldia Laevis* (P. Beauv) Seem in *P. Berghei* Infected Mice

Aderinola A. Adeyinka[1,2*], Ejiofor I. Jane[2], and Chindo A. Ben[2,3]

[1]Department of Pharmacology and Therapeutics, Olabisi Onabanjo University, Ago-iwoye, Nigeria; [2]Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, Nigeria, and [3]Department of Pharmacology, Kaduna State University, Kaduna, Nigeria.

***Corresponding author:** Aderinola A. Email address : aderinola.aderonke@oouagoiwoye.edu.ng/ aderonkeaderinola@gmail.com

DOI: https://dx.doi.org/10.4314/ajhs.v36i4.15

Abstract

BACKGROUND

Despite substantial improvement in the management of malaria infection over the last centuries, it remains a significant public threat especially to those in the tropical and subtropical African regions due to the emergence of drug-resistant *P. falciparum* strains, delayed diagnosis, high cost as well as contraindications of some available antimalarial medications. Hence, the need for necessary measures in terms of alternative therapeutic approaches to counter this threat is of utmost importance. The present study was aimed at determining the antimalarial potential of ethanol stem-bark extract of *Newbouldia laevis* in *P. berghei* (*NK65*) infected mice.

METHODOLOGY

The antimalarial activity of *Newbouldia laevis* stem-bark at 250, 500 and 1,000 mg/kg was investigated in mice infected with 1 x 10^7 parasitized erythrocytes using three experimental animal models: Peter's 4-day suppressive, curative and prophylactic models. Phytochemical screening of the stem bark was also conducted.

RESULTS

The extract at all tested doses of 250, 500 and 1000 mg/kg showed significant (*p<0.01*) and dose-dependent reductions in the parasitemia levels of the treated rats with percentage chemosuppresion of 90.7, 91.8 and 94% respectively in the Peters 4-day suppressive test. There were also significant (*p<0.01*) dose-dependent reductions in parasitemia density of the treated rats at all doses of 250, 500 and 1000 mg/kg in the curative and prophylactic studies compared to the distilled water group.

Phytochemical screening revealed the presence of carbohydrates, tannins, alkaloids, flavonoids, saponins, terpenoids, cardiac glycosides, steroids and anthraquinones in the crude extract.

CONCLUSION

The results suggest that ethanol stem-bark extract of *Newbouldia laevis* possesses antimalarial activity and this justifies the traditional use of the stem-bark of the plant in antimalarial herbal remedy.

Keywords: *Antimalarial, Phytochemical Screening, Chloroquine, Plasmodium Berghei, Newbouldia Laevis.*

[*Afr. J. Health Sci*. 2023 36 (4): 472-481]

Introduction

Malaria is a serious and fatal parasitic infectious disease that has become a serious public health problem, especially for people in the tropical and subtropical regions of sub-Saharan Africa (1). The disease is caused by *plasmodium* parasites; *P. falciparum*, *P. ovale*, *P. vivax*, *P. malariae* and *P. knowlesi* and is

transmitted to people through the bite of infected female *Anopheles* mosquitoes, blood transfusion or from mother to child (1-2). Malaria is characterized by chills, headache, myalgia and malaise, fatigue, abdominal discomfort, muscle and joint aches, fever, nausea and vomiting (3), respiratory distress, unconsciousness, convulsion, pulmonary oedema and coma in severe cases (1). In 2020, an estimated 241 million cases with 627,500 deaths from malaria infection were reported and 95% of these deaths were from Africa (4-5). Malaria constitutes a high economic burden to both individuals and the government due to high health care costs including payment for consultation and laboratory tests, purchase of drugs for malaria treatment, lost days of work, absence from school, cost of preventive measures, supply and staffing of health facilities, public health interventions against malaria (5-6). Many strategies for malaria control interventions including the use of long-lasting insecticidal nets (LLINs), indoor residual spray (IRS), intermittent preventive treatment in pregnancy and use of available antimalarial drugs have been put in place to ensure total eradication of the disease but these have not provided the desired outcome in many countries especially in high transmission areas (7-8). Resistance of malaria parasites to the available antimalarial drugs, mosquitoes' resistance to insecticides, adverse effects as well and high costs of some antimalarial drugs coupled with the unavailability of effective vaccines has greatly increased malaria morbidity and mortality (9). Hence, the need for newer and safer antimalarial drugs becomes imperative.

Medicinal plants are now becoming popular for the treatment of malaria infection owing to their accessibility and affordability. Also, the two drugs (quinine and artemisin) that have been mainly used for the treatment of malaria over the years are derived from medicinal plants; cinchona tree and *Artemisia annua* (10). Thus, medicinal plants can provide useful compounds that can be used for the development of newer, safer and more effective antimalarial drugs.

Plasmodium berghei malaria parasite has a high sensitivity to chloroquine and elicits similar signs and symptoms exhibited by human *Plasmodium* infection and has also been in use since time immemorial to study the activity of many potential antimalarial agents including chloroquine, mefloquine, halofantrine and artemisinin derivatives (11-12) was used for this study.

Newbouldia laevis also called akoko in Yoruba, Ogirisi in igbo and aduruku in Hausa is a tropical evergreen drought-resistant plant with large, glossy deep green leaves and purple and white flowers (13-14). It grows up to 7 - 15m in height in Nigeria. Different parts of the plant (leaves, stem-bark, root, flower) are widely used in folkloric medicine to treat various diseases including migraine, skin infections, fever, stomach ache, epilepsy and conjunctivitis (15); malaria and fever (16-17); dysmenorrhea (18); toothache, diarrhoea, dysentery, breast cancer, sexually transmitted diseases (STDs), anaemia, ulcer, arthritis, rheumatism, haemorrhoids, constipation, cardiovascular diseases, diabetes, cough, elephantiasis, urinary tract infection (19-24). Extracts from different parts of the *N. leavis* plant have been reported to possess anti-diabetic activity (25); anticancer activity (26); hypoglycaemic and antihypertensive effects (27-28); wound healing and antiulcer effect (29); entomocide activity (30); antioxidant activity (31-32); antimalarial activity (33-34); sedative, anticonvulsant and antidepressant activities (35) as well as antimicrobial activity (36).

Phytochemical constituents including glycosides, steroids, triterpenes, saponins, tannins, flavonoids and alkaloids have been found present in the methanol leaf extract of *Newbouldia laevis* (37). This study was aimed at investigating the antiplasmodial activity of

ethanol stem-bark extract of *Newbouldia laevis* in *P.berghei*-infected mice.

Materials and Methods

Plant material

The stem-bark of *N. laevis* was collected in March 2014 from Abeokuta, Abeokuta South Local Government Area, Ogun State, Nigeria. The plant was identified and authenticated by Malam Namadi Sunusi of the Department of Botany, Ahmadu Bello University, Zaria and a voucher specimen was deposited in the herbarium section of the Department and voucher number ABU02881 was obtained. The fresh stem bark of the plant was air dried under shade until a constant weight was obtained and then reduced to a coarse powder using a grinding machine. Five hundred grams (500 g) of powdered plant material was cold macerated in 4 litres of 90% ethanol for 72 hours with intermittent shaking and then filtered with Whatman (No. 3) filter paper. The resultant filtrate was concentrated using a rotatory evaporator and then dried over a water bath at 55˚C, this was preserved in a levelled air-tight container for future use.

Phytochemical screening

Preliminary phytochemical screening of the crude extract was carried out to assess for the presence of anthraquinones, flavonoids, alkaloids, saponins, tannins, steroids, carbohydrates, triterpenes and cardiac glycosides using the method of (38).

Animals

Adult Swiss Albino mice (20-25g) obtained from the Animal House of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria were used for the study. The animals were kept in clean cages for two weeks of acclimatization and all through the study, they were maintained under standard laboratory care and fed with a standard rodent pellet diet (Vital Feeds, Jos, Nigeria) and water *ad libitum* for the duration of the study. All experimental protocols were done with strict adherence to the Ethics Guidelines and Research Policy of Ahmadu Bello University, Zaria. Ethical Approval (ABUCAUC/2021/084) was obtained from the ABU Committee on Animal Use and Care.

Drugs and reagents

All the drugs and chemical reagents used for this study were of analytical grade and included: chloroquine (Sigma-Aldrich, USA), ethanol (BDH Chemicals Limited, Poole, England), methanol (BDH Chemicals Limited, Poole, England), Geimsa powder (Philip Harris Ltd, England).

Malaria parasite

Chloroquine-sensitive *Plasmodium berghei* (NK65) was used for this study and was obtained from the Department of Biochemistry and Nutrition, Nigerian Institute for Medical Research (NIMR), Yaba, Lagos. The parasite was maintained by continuous passage of mice every 4 days with 0.2 mL intraperitoneal injection of 1×10^7 infected erythrocytes of donor mice.

Parasite inoculation

The infected blood collected with a heparinized capillary tube from the tail vein of *a P. berghei* donor mouse with about 20-25% parasitaemia level (both the percentage parasitemia and the erythrocyte count was determined) was used to infect a clean mouse. Each mouse used in the experiment was inoculated intraperitoneally with 0.2 mL of the infected blood containing about 1×10^7 *P. berghei* parasitized erythrocytes.

Evaluation of antiplasmodial activity of ethanol stem-bark extract of *Newbouldia laevis* in mice

Suppressive Test (4-day early infection): The method described by (39) was used for this test. Thirty (30) mice in five treatment groups of six mice each were used. Each mouse was inoculated intraperitoneally with 0.2 mL standard inoculum containing approximately 1×10^7 *P. berghei*-infected

erythrocytes at the commencement of the experiment (day 1). Three hours after inoculation of the parasite, the mice in the three treatment groups (B, C and D) received the extracts orally in doses of 250, 500 and 1000 mg/kg for four consecutive days, while the two control groups, the negative control (group A) received distilled water (10 ml/kg) and the positive control (group E) received 5mg/kg chloroquine phosphate respectively. On the fifth day, drops of blood samples were taken from the tail of each mouse. Thin blood films were made from the collected blood samples on a microscopic slide, air-dried and fixed with absolute methanol. The blood films were stained with 3% Giemsa solution at Ph 7.2 and examined under the microscope. The parasitemia was determined by counting the number of parasitized erythrocytes in 4 random fields using an x100 objective lens.

Percentage parasite suppression relative to the negative control group was calculated for each dose according to (40) as reported by (41) using the formula below:

% Suppression = Average parasitaemia in control - Average parasitaemia in treated group ×100 Average parasitemia in control

Curative test. The method described by (40) was used for this test. Forty (40) mice were inoculated intraperitoneally with 0.2 mL of diluted blood sample containing approximately 1×10^7 *P. berghei* infected blood and kept for 72 hours for parasitaemia establishment. Parasitaemia level was assessed on the 3rd day and thirty (30) mice with 20 – 25% parasitaemia level were selected and divided into five treatment groups of six mice per group. They were then orally treated daily with distilled water (10 ml/kg) group A, ethanol crude extract of *Newbouldia laevis* stem-bark at doses of 250, 500 and 1000 mg/kg (group B, C and D) and chloroquine (5 mg/kg) group E for 4 consecutive days. Thin blood films were prepared each day from the tail of each mouse for 7 days from day 0 of the experiment and stained with 3% Giemsa solution. The percentage chemosuppression relative to the negative control was determined for each dose as previously described.

The mice were kept for 28 days and checked daily until day 28 for Mean survival time in days as shown in the formula below:

$$MST = \frac{\text{Sum of survival time of all mice in a group (days)}}{\text{The total number of mice in that group}}$$

Prophylactic test. The repository activity of the crude extract was investigated using the method described by (40). Thirty mice were randomly divided into five groups (A, B, C, D and E) of six mice per group. The mice were first pre-treated daily with distilled water (10 mL/kg) group A, ethanol crude extract of *Newbouldia laevis* stem-bark at doses of 250, 500 and 1000 mg/kg (group B, C and D) and chloroquine (5 mg/kg) group E for 4 consecutive days and were then inoculated intraperitoneally with 0.2 mL of diluted blood sample containing approximately 1×10^7 *P. berghei* infected blood on the 5th day. Parasitaemia level was assessed after 72 hours of inoculation by blood smear. The percentage chemosuppression relative to the negative control was determined as previously described (40).

Statistical analysis

Data were analysed using one-way analysis of variance (ANOVA) followed by Dunnett's Post hoc test. The results were expressed as mean ± standard error of the mean (SEM) and presented as tables. The results were considered statistically significant at $p < 0.05$.

Results

Phytochemical constituents of ethanol stem-bark extract of *Newbouldia laevis*

The phytochemical constituents present in the ethanol crude extract of *Newbouldia laevis* are carbohydrates, tannins, alkaloids, flavonoids, saponins, terpenoids, cardiac glycosides, steroids and anthraquinones (Table 1).

Effect of Ethanol Stem-bark extract of *Newbouldia laevis* on Early *P. berghei* Infection in Mice

A significant ($p \leq 0.05$) dose-dependent reduction in parasitaemia level was observed with the ethanol crude extract of *Newbouldia laevis* at all doses used when compared with the distilled water control group. The standard drug showed radical suppressive effect of 100% compared to the control (Table 2).

Effect of Ethanol Stem-bark Extract of *Newbouldia laevis* on Established *P. berghei* Infection in Mice

A dose-dependent reduction in parasitaemia level was observed with the ethanol stem-bark extract of *Newbouldia laevis*, this was significant ($p \leq 0.05$) only at 1,000 mg/kg. However, the standard drug (chloroquine) showed a far better effect compared to the control (Table 3).

Mice Survival Time After 4-Day Treatment with Newbouldia laevis Extract for P. berghei Infection

The infected mice of the distilled water control group died within 12 days of infection, while the crude extract-treated infected mice group showed a longer mean survival time (Table 4).

Prophylactic Effect of Ethanol Stem-bark Extract of *Newbouldia laevis* in *P. berghei* Infected Mice

A dose-dependent prophylactic effect was observed with the ethanol stem-bark extract of *Newbouldia laevis*, significant ($p \leq 0.05$) only at 500 and 1,000 mg/kg. The standard drug, (chloroquine) completely prevented parasitaemia occurrence (Table 5).

Table 1:
Phytochemical Constituents in Ethanol Stem-bark Extract of *Newbouldia laevis*

Constituents	Test Reagent	Inference
Cardiac glycosides	Keller-Killiani	+
Saponins	Frothing	+
Triterpenes (Terpenoids)	Lieberman-Burchard's	+
Steroids	Lieberman-Burchard's	+
Flavonoids	Shinoda	+
Tannins	Ferric chloride	+
Alkaloids	Dragenddorf Mayer	+
Anthraquinones	Bontrager's	+
Carbohydrates	Molisch	+

Key: + (present), - (absent)

Table 2:
Suppressive Activity of Ethanol Stem-bark Extract of *Newbouldia laevis* on Early *P. berghei* Infection in Mice

Treatment group	Doses (mg/kg)	Mean Parasitemia ± SEM	Percentage Chemo-suppression (%)
Distilled H_2O	10 (ml/kg)	104.75 ± 41.96	0
ECE	250	9.67 ± 2.39 *	90.77
ECE	500	8.55 ± 2.06 *	91.84
ECE	1000	6.25 ± 1.97 **	94.03
Chloroquine	5	0.0**	100

Key: ECE = ethanol crude extract; n = 6; Statistics: One Way ANOVA followed by Dunnett's Post hoc test at *$p \leq 0.05$, **$p \leq 0.01$, compared to control

Discussion

The plant *Newbouldia laevis* is one of the medicinal plants that is been used in folkloric medicine for the management of malaria infection due to its acclaimed antimalarial activity. This study focussed on the phytochemical screening and antiplasmodial activity of ethanol stem-bark extract of *Newbouldia laevis* against *P. berghei* infection in mice.

Secondary metabolites such as carbohydrates, tannins, alkaloids, flavonoids, saponins, terpenoids, cardiac glycosides, steroids and anthraquinones were found in the crude extract. The result obtained from the phytochemical screening of *Newbouldia laevis* stem-bark is in line with the findings of (37) who reported the presence of similar phytochemicals in *Newbouldia laevis* leaf.

Table 3:
Curative Activity of Ethanol Stem-bark Extract of *Newbouldia laevis* on *P. berghei*-induced Parasitaemia in Mice

Treatment group	Doses (mg/kg)	Mean Parasitemia ± SEM	Percentage Chemo-suppression (%)
Distilled H_2O	10 (ml/kg)	92.93 ± 16.91	0.00
ECE	250	60.95 ± 2.62	34.41
ECE	500	54.05 ± 19.72	41.84
ECE	1000	50.68 ± 17.12*	45.48
Chloroquine	5	13.87 ± 75.25*	85.07

Key: ECE = ethanol crude extract; n = 6; Statistics: One Way ANOVA followed by Dunnett's Post hoc test at *$p \leq 0.05$, compared to control

Table 4:
The Mean Survival Time of Mice Infected with *P. berghei* Parasitaemia Following 4-day Treatment with Ethanol Crude Extract of *Newbouldia laevis* Stem-bark

Treatment group	Dose (mg/kg)	MST (days)
Distilled water	10ml/kg	12.2±0.2
ECE	250	18.5±2.6
ECE	500	20.6±1.7
ECE	1000	22.1±2.1*
Chloroquine	5	27.6±1.5**

Key: ECE = ethanol crude extract; n = 6; Statistics: One Way ANOVA followed by Dunnett's Post hoc test at *$P \leq 0.05$, and **$P \leq 0.01$, compared to distilled water (DW) control group.

Table 5:
Prophylactic Effect of Ethanol Stem-bark Extract of *Newbouldia laevis* on *P. berghei* Infection in Mice

Treatment group	Doses (mg/kg)	Mean Parasitemia ± SEM	Percentage Chemo-suppression (%)
Distilled H_2O	10 (ml/kg)	142.5 ± 23.23	0
ECE	250	57.50 ± 11.09	59.65
ECE	500	24.25 ± 8.74*	82.98
ECE	1000	14.00 ± 2.65**	90.18
Chloroquine	5	0.00 ± 0.0 **	100

Key:ECE = ethanol crude extract; n = 6; Statistics: One Way ANOVA followed by Dunnett's Post hoc test at *$p \leq 0.05$ and **$p \leq 0.01$, compared to control

The therapeutic effects derived from several medicinal plants have been attributed to the presence of some phenolic compounds such as flavonoids, phenolic acid, pro-anthocyanidins and tannins (42). The pharmacological activities of ethanol stem-bark extract of *Newbouldia laevis* observed in this study may be due to the presence of one or combinations of some of these secondary metabolites such as flavonoids, alkaloids and terpenoids.

The ethanol stem-bark extract of *Newbouldia laevis* demonstrated antiplasmodial activity in mice with *P. berghei*-induced parasitaemia. The crude extract produced a significant chemo-suppressive effect slightly lower than the 100% chemo-suppressive effect of chloroquine used as a standard antimalarial drug in the early infection study. There was dose-dependent curative chemo-suppressive activity of the crude extract with a more pronounced effect at the highest dose used in the established infection study, the chloroquine group showed a far better curative effect. The result of this study suggests that the crude extract of *Newbouldia laevis* stem-bark possesses good antiplasmodial activity and was able to reduce the parasitaemia density of the treated mice. The mice in the distilled water control group died within 12 days of infection as against the mice in the crude extract which survived much longer. The longer mean survival time of crude extract of *Newbouldia leavis* may be due to the extract's ability to reduce the parasitemia density of the treated mice and this also suggests that the crude extract possesses good antiplasmodial activity.

The crude extract exhibited dose-dependent prophylactic effects, and the standard drug (chloroquine) showed 100% chemo-prevention of parasitaemia occurrence. The result from the chloroquine-treated group agrees with the work of (11) who reported that *Plasmodium berghei*, NK65 is highly sensitive to chloroquine. The antiplasmodial effects of this plant in this study corroborated with the traditional use of the plant in the management of malaria in many parts of Nigeria. The secondary metabolites such as alkaloids that are present in the crude extract of *Newbouldia leavis* stem-bark have been reported to exhibit antiplasmodal activity by blocking plasmodial protein synthesis; flavonoids are known to possess anti-inflammatory, anti-parasitic and anti-carcinogenic properties while terpenoids including sesquiterpenes have been reported to be involved in endoperoxidation reactions (43-44). The presence of these metabolites may be implicated in the antiplasmodial activity exhibited by the crude extract of this plant. The results of antiplasmodial activity obtained from this study are consistent with the result of (33) who reported dose-dependent antiplasmodial activity in the mice treated with *Newbouldia laevis* leaf extract. Similar dose-dependent antiplasmodial activity was also reported by (45) in mice treated with aqueous extract of *Newbouldia laevis* stem-bark.

Conclusion

The results obtained from this study suggest that ethanol stem-bark extract of *Newbouldia laevis* possesses antimalarial activity and this justifies the traditional use of the stem-bark in antimalarial herbal remedy.

Conflict of interest: None declared

Acknowledgements: The authors appreciate the technical staff of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, Nigeria for their contributions and for providing support and access to research facilities.

Source of Funding: Self-funded

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