African Journal of Pharmaceutical Research & Development

Vol. 14 No.3; pp. 204-209 (2022)

ANTIMALARIAL AND HEMATOLOGICAL EFFECTS OF *Dioscorea bulbifera* L. TUBERS IN MICE

PHILIP FELIX UZOR[1,*], EMEKA JAMARTY OKECHUKWU[1]

1. Department of Pharmaceutical and Medicinal Chemistry, University of Nigeria, Nsukka, 410001, Nigeria

Received 29 November, 2022; Revised 30 January, 2023; Accepted 31 January, 2023

ABSTRACT

Malaria remains a serious killer disease particularly in sub-Sahara Africa. The conventional drugs are often unaffordable and associated with resistance. Historically, medicinal plants have served as a useful source of antimalarial drugs. In the present study, the antimalarial effect of the methanol extract of *Dioscorea bulbifera* L.tuber,at three doses of 100, 200 and 400 mg/kg, was investigated in mice against *Plasmodium berghei*. Artemisinin combination therapy (ACT) was used as a standard drug. Effect of the extract on the hematological parameters including hemoglobin (Hb), packed cell volume (PCV) and white blood cell count (WBC) was also determined in mice. The extract was screened for its major phytochemical constituents. Results showed that the extract exhibited (p<0.05) a dose-dependent inhibition of parasitemia in a murine model of *Plasmodium berghei* infected mice. The extract improved (p<0.05) the hematological parameters in the infected animals. Phytochemical screening revealed the presence of alkaloids, terpenes, steroids, flavonoids, tannins and saponins in the extract. It was concluded that the crude extract of *D. bulbifera* tuber demonstrated promising antimalarial activity.

KEYWORDS: **Antimalaria activity; *Dioscorea bulbifera;* Hematological parameters; *Plasmodium berghei***

INTRODUCTION

Malaria remains one of the world's deadliest parasitic diseases. The disease is caused by protozoa of the genius *plasmodium*. The most lethal form of malaria is caused by *P. falciparum* [1]. An estimated number of 221 million new cases of malaria and 625,000 malaria-related deaths were recently reported by the WHO in 85 countries [2]. Besides the loss of lives, the disease impacts heavily on the economy of the affected regions.

Currently there is no effective vaccine against the scourge; hence the fight against the protozoan disease is mainly by vector control and chemotherapy. In addition, there is now an increasing incidence of resistance of the parasitic organisms to the commonly used antimalarial drugs including the artemisinin-based drugs [3]. Moreover, many of the currently used drugs, including artemisinin, are associated with a number of adverse effects and decreased sensitivity to the parasite [4]. As a result, there is an urgent need for new highly efficacious and affordable antimalarial agents [5].

For many years, medicinal plants have been the basis of traditional medicine systems and have served as a good source of drugs. The antimalarial drugs, quinine, obtained from Cinchona species, and artemisinin, obtained from *Artemisia annua* are clear indications of the potential of medicinal plants as a source of potent antimalarial agents [6]. The traditional reputation of a particular plant for efficacy in the treatment of malaria is a sure way for the

***Corresponding author:** philip.uzor@unn.edu.ng; +2348037008294*

selection of plants to be screened for antimalarial activity.

Dioscorea bulbifera L. (Family: Dioscoreaceae) is a vigorously twining herbaceous vine, with underground and aerial tubers in leaf axils. The tribals of Jharkhand, India, gather the tuber as one of their food supplements in the rural areas. It is used for the treatment of malaria, diarrhoea and diabetes among other ailments [7]. In addition, a related species, *D. dumetorum*, is used in south-east Nigeria in the treatment of malaria [8]. The present study was, therefore, designed to evaluate the antimalarial activity *D. bulbifera* in order to provide a scientific basis for its traditional use in malaria treatment.

MATERIALS AND METHODS

Collection of Plant Material

Tubers of *Dioscorea bulbifera* used for this study were bought from Orba market in Nsukka, south-east Nigeria. The plant was identified and authenticated by Mr. Felix Nwafor, a botanist with the Department of Pharmacognosy and Environmental Medicines, University of Nigeria, Nsukka. The voucher specimen (voucher number: PCG/UNN/096) was deposited in the herbarium of the department.

Plant Extraction and Preparation

The tubers were rinsed in clean water and shade-dried at room temperature for two weeks. The dry plant sample was ground into powder using pestle and mortar. A certain quantity (700 g) of the ground tuber was macerated in 1.7 liters of methanol (95%) at room temperature (28^{o}C) for 72 h with intermittent shaking, after which it was filtered through a cotton wool plug and the filtrate concentrated using a Rotary evaporator at 40 ^{o}C to afford the dry crude extract (coded as DB; 34% yield).

Animals

Adult Swiss albino mice (20 – 28g) of both sexes were obtained from the animal unit of the Department of Zoology and Environment Biology, University of Nigeria, Nsukka. The animals were kept in standard cages at room temperature of about 25^{o}C. Dark and light cycles were maintained at 12 h each. The mice were fed with standard pellets (Grand Cereals Ltd, Enugu Nigeria) and had unrestricted access to clean drinking water. The guide for the care and use of laboratory animals was followed (according to the institutional animal ethical committee with approval number: FPSRE/UNN/21/0001).

Parasite Inoculation

Donor mouse blood infected with the *P. berghei* was obtained from the Faculty of Veterinary Medicine, University of Nigeria, Nsukka and was used for inoculum preparation. Blood sample was collected from the donor mouse by heart puncture and diluted serially in Alsever's solution to make a suspension containing about 1x10^{6} infected RBCs in every 0.2 ml suspension. This 0.2 ml suspension was injected (*i.p.)* into the experimental animals (on Day 0) to initiate infection. Infection for malaria was confirmed 72 h post infection (on Day 3).

In vivo Antimalarial Testing

The inoculated mice were randomized into six (6) groups of five (5) mice and treated according to the following groupings. The doses were chosen based on our preliminary studies (not described here) which showed the LD_{50} to be above 5000 mg/kg. Groups 1 to 3 were infected and treated with 100, 200 and 400 mg/kg of extract (DE) respectively, Group 4 animals were infected and treated with the standard drug, artemeter-lumefantrin (ACT) at 7 mg/kg body weight (calculated based on the tablet weight), Group 5 animals were the normal animals (uninfected and untreated) while Group 6 (Control group) animals were infected but untreated. The *in vivo* antimalarial testing was done in mice in a 5-day curative test model [9]. The administration of the extract/drug was done daily (p.o) from Day 3 to Day 7. Blood was obtained from each mouse on Day 3 and Day 8 via a tail cut from which thin blood smears were prepared. Smears were fixed with methanol for 5 min and stained with 10% Geimsa. The slides were observed under microscope×100 objective lens (oil immersion). The percentage parasitemia was determined by counting the parasitized red blood cells out of RBCs in random fields of the microscope and according to Eqn. 1.

$$\%\text{Parasitemia} = \frac{\text{number of parasitized RBC}}{\text{total number of RBC counted}} \times 100 \quad \text{.....Eqn. 1}$$

Determination of the Haemoglobin (Hb) Concentration

Heamoglobin (Hb) concentration was determined using cyanomethaglobin technique [10] on Days 0, 3 and 8. Whole blood (20 µl) was added to 4 ml of Drabkin's solution in a test tube in a 1:250 dilution. This was well mixed, allowed to stand for 10 min at room temperature (28^{o}C) and the absorbance was

read colorimetrically at 540 nm with Drabkin's solution as a blank.

Determination of the Packed Cell Volume (PCV)
PCV was measured as previously described [11]. Determination was done on Days 0, 3 and 8. Heparinized capillary tubes were used for collection of blood from tail of each mouse. The tubes were centrifuged in a micro-hematocrit centrifuge and PCV was determined using a standard Micro-Hematocrit Reader.

Determination of White Blood Cells (WBC) Count
The white blood cell count was determined on Days 0, 3 and 8 as described previously [11]. Whole blood (20 µl) was added to 380 µl of diluting fluid (acetic acid, with gentian violet) and mixed. The counting chamber was charged with the well mixed diluted blood and the cells were allowed to settle in a moist chamber for 3 min. The four corners of the chamber were visualized under a low power (10X) objective microscope.

Phytochemical Analysis
The crude extract (DB) was screened for the presence of different secondary metabolites following standard procedures [12].

Statistical Analysis
Data obtained were analyzed using IBM Statistical Product and Service Solutions (version 21.0 SPSS Inc., Chicago, IL, USA). The results were expressed as Mean ± Standard Error of Mean (SEM). One-way analysis of variance (ANOVA) with Dunnett's test for multiple comparisons was used to compare means across the groups. Mean values with $p<0.05$ were considered statistically significant compared to the control group.

RESULTS

Effect of Extract on Parasitemia
The effects of the extract on the parasitemia are shown in Table 1. The parasitemia level of the infected but untreated group (Control group) on Day 8 was the highest (79.60±5.4%) suggesting that there was increment in the parasitemia (on Day 8) with respect to the level on Day 3. There was inhibition in the parasitemia level in the extract treated groups. Lowest parasitemia was observed in the group of infected mice which received 400 mg/kg dose of the extract. The extract produced a dose-dependent significant ($p<0.001$) inhibition of parasitemia ranging from 8.68 to 84.36% while that of the standard drug, ACT was 69.04%.

Effect of Extract on Hematological Parameters
Results show that there was reduction of the heamoglobin concentration across the groups as a result of the malaria (beside the normal group animals). The extract dose-dependably produced a significant ($p < 0.05$) improvement in the heamoglobin level similar to the effect of ACT (Table 2). Similarly, the extract dose-dependently improved ($p<0.5$) the PCV level of the animals after the lowering by the malarial infection (Table 3). In addition, the malaria infection caused a rise in the WBC count but this effect was reversed ($p<0.001$) by the extract in a dose-depend manner (Table 4). For the three biochemical parameters, the effect of the extract was similar to those of the standard ACT drug. The standard ACT as well as the extract, particularly at 400 mg/kg dose, brought the HB, PVC and WBC count to normal level on Day 8 (i.e. one day after the last administration of treatments).

Results of Phytochemical Analysis
All the tested phytochemicals including alkaloids, terpenes, steroids, flavonoids, tannins and saponins were found to be present in the extract.

DISCUSSION

The plant extract, particularly at higher doses, produced a good parasitemia inhibition as determined on the Day 8. This effect was comparable to that of ACT. *In vivo* antiplasmodial activity can be classified as moderate, good, and very good if an extract displayed percentage parasitemia suppression equal to or greater than 50% at a dose of 500, 250 and 100 mg/kg bodyweight per day, respectively [13]. Thus, the extract could be considered to possess a very good antimalarial activity since inhibition by the doses of 200 mg/kg and 400 mg/kg was over 50%. The antimalarial activity of the plant has also be recently reviewed [14]. A related species *D. dumetorum* is used in south-east Nigeria in the treatment of malaria [8].

By decreasing parasitemia, drugs lead to subsequent recovery of symptomatic malaria. They reduce parasitemia through various ways like reducing parasite nutrient intake, interfering with parasite metabolic pathways like heme metabolic pathway. Drugs also negatively affect parasite reproduction and growth [15]. However, the mode of antiplasmodial action of the extract was not studied in the present study.

Table 1: Effect of extracts on parasitemia of *Plasmodium berghei*-infected mice in the curative antimalarial model

Treatment	% Parasitemia		% Inhibition of parasitemia
	Day 3	Day 8	
100 mg/kg DB	62.20±1.46*	56.80±3.29***	8.68
200 mg/kg DB	57.80±2.15**	12.40±0.74***	78.55
400 mg/kg DB	66.60±1.28	10.40±0.81***	84.38
Standard drug (ACT)	78.80±3.58	24.40±5.02***	69.04
Normal	0	0	0
Control	71.20±2.47	79.60±5.4	-11.80

Data are mean ± standard error of mean (SEM) (n = 5); *P<0.05,**p<0.01, *** p<0.001 p< 0.05 as compared with control group (one way ANOVA followed by Dunnett's test, 2-sided), n = 5, DB = extract of *Dioscorea bulbifera* tuber; Normal group (uninfected and untreated); Control group (infected but untreated)

Table 2: Effect of the extracts on the hemoglobin concentration of *Plasmodium berghei*-infected mice

Treatment	Hb (mg/dL)		
	Day 0	Day 3	Day 8
100 mg/kg DB	11.14±0.13	7.78±0.56	10.3±0.48***
200mg/kg DB	11.22±1.13	8.32±0.43	11.04±0.15***
400mg/kg DB	11.20±1.7	7.7±0.37	11.64±0.09***
Standard drug (ACT)	11.28±0.71	7.84±0.29	11.22±0.13***
Normal	11.08±0.07	11.84±0.38***	11.68±0.13***
Control	10.98±0.13	7.06±0.28	6.90±0.31

Data are mean ± standard error of mean (SEM) (n = 5). p< 0.05 as compared with control group (one way ANOVA followed by Dunnett's test, 2-sided). DB = extract of *Dioscorea bulbifera* tuber; Normal group (uninfected and untreated); Control group (infected but untreated

Table 3: Effect of the extracts on the packed cell volume (PCV) of *Plasmodium berghei*-infected mice

Treatment	PCV (%)		
	Day 0	Day 3	Day 8
100 mg/kg DB	40.6±1.07	32.2±1.28	33.40±0.92***
200mg/kg DB	41.6±0.67	29.00±0.94	38.00±0.83***
400mg/kg DB	43.2±0.58	30.8±1.85	40.40±0.67***
Standard drug (ACT)	41.40±0.74	32.60±1.60	39.80±0.66***
Normal	43.20±0.86	42.80±0.86***	43.80±0.37***
Control	41.80±0.58	28.60±1.32	24.00±1.44

Data are mean ± standard error of mean (SEM) (n = 5). p< 0.05 as compared with control group (one way ANOVA followed by Dunnett's test, 2-sided). DB = extract of *Dioscorea bulbifera* tuber; Normal group (uninfected and untreated); Control group (infected but untreated

Table 4: Effect of the extracts on the white blood cell (WBC) count of *Plasmodium berghei*-infected mice

Treatment	WBC count ×10⁴ (µL⁻¹))		
	Day 0	Day 3	Day 8
100 mg/kg DB	1.06±0.00	1.26±0.00	1.33±0.00**
200mg/kg DB	1.06±0.00	1.12±0.00	1.13±0.00***
400mg/kg DB	1.05±0.01	1.18±0.00	1.07±0.00***
Standard drug (ACT)	1.10±0.02	1.31±0.00	1.06±0.00***
Normal	1.12±0.00	1.07±0.00***	1.04±0.00***
Control	1.09±0.00	1.28±0.00	1.47±0.00

Data are mean ± standard error of mean (SEM) (n = 5). p< 0.05 as compared with control group (one way ANOVA followed by Dunnett's test, 2-sided). DB = extract of *Dioscorea bulbifera* tuber. Normal group (uninfected and untreated); Control group (infected but untreated

In the present study, administration of the extract significantly reduced WBC and triggered an increase in Hb and PCV as a result of increased production of RBC, thus, suppressing hemolytic damage to RBC. PCV was measured to evaluate the effectiveness of the crude extract in preventing hemolysis due to escalating parasitaemia level. The underlying cause of anemia includes the clearance and/or destruction of infected RBCs, the clearance of uninfected RBCs and erythropoietic suppression [11].

Screening for antimalarial activity of plant crude extracts is the first step in isolation of new molecules with potent activity [16.] Biological activity is attributed to the presence of various secondary metabolites in plants. In addition, presence of more than one class of secondary metabolites in a given plant extract determines the nature and extent of its biological activity [17]. In the present study, the observed antimalarial activity of the extract could be attributed to the presence of alkaloids, flavonoids, terpenes, steroids or saponins. In a previous phytochemical study, a gas chromatography flame ionization detector (GC-FID) analysis of *D. bulbifera* revealed seven components in which rutin had the highest concentration while phytate had the lowest. Other constituents identified in the study include lunamarine, ribalinidine, tannin, phenol and kaempferol [18]. Presence of these secondary metabolites has been shown to be responsible for biological activity. Alkaloids have been known to exhibit antiplasmodial potentials by blocking protein synthesis in *P. falciparum*. A prominent example of an alkaloidal antimalarial drug is quinine [6]. In addition, flavonoids have been reported to chelate the nuclei acid base pairing of the parasite [19]. Moreover, in a recent study, quercetin has been identified as a major active compound responsible for the antimalarial activity of *D. bulbifera* [20].

CONCLUSION

The results of the present study have presented evidence for the antimalarial potentials of *D. bulbifera*. The study provided a scientific basis for the use of the plant in ethnomedicine for malaria treatment. Any or a combination of the numerous phytoconstituents could be responsible for the observed activity. Further research is envisaged in our laboratory towards the isolation of the bioactive phytoconstituents of the plant.

ACKNOWLEDGEMENT

The authors are grateful to Ebube Uzor for typesetting the manuscript.

REFERENCES

1. Pham TT, Lamb TJ, Deroost K, Opdenakker G, Van den Steen PE. Hemozoin in Malarial Complications: More Questions Than Answers. Trends in Pharmacology, 37(3), 2021: 226-39.
2. World Health Organization. World Malaria Report 2022.https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2022 (Accessed on December 12, 2022).
3. Woodrow CJ, White, NJ. The clinical impact of artemisinin resistance in Southeast Asia and the potential for future spread. FEMS Microbiology Reviews, 41 (1), 2017: 34–48.

4. Adaka IC, Uzor PF, Nwodo NJ. Bioactivity guided fractionation of *Icacina trichantha* Oliv. (Icacinaceae) tuber for antimalarial activity against *Plasmodium berghei* infected mice and GC-MS profile of bioactive fraction. Indian Journal of Traditional Knowledge, 20(4), 2021: 902-12.
5. Bhat GP, Surolia N. In vitro antimalarial activity of extracts of three plants used in the traditional medicine of India. American Journal of Tropical Medicine and Hygiene, 65(4), 2001: 304–8.
6. Uzor PF. Alkaloids from plants with antimalarial activity: a review of recent studies. Evidence-Based Complementary and Alternative Medicine. Volume 2020, Article ID 8749083, 17 pages.
7. Marandi RR, Britto SJ, George M, Minj E. Pharmacognostic, fluorescent, antibacterial and phytochemical analysis of tuber of *Dioscorea bulbifera* L. from Jharkhand. Journal of Pharmacognosy and Phytochemistry. 5(1), 2016: 08-14.
8. Dike IP, Obembe OO, Adebiyi FE. Ethnobotanical survey for potential anti-malarial plants in south-western Nigeria. Journal of Ethnopharmacology. 144(3), 2012: 618–26.
9. Okpe O, Habila N, Ikwebe JA, Upev VA, Okoduwa SIR, Isaac OT. Antimalarial Potential of *Carica papaya* and *Vernonia amygdalina* in Mice Infected with *Plasmodium berghei*. Journal of Tropical Medicine. Volume 2016, Article ID 8738972, 6 pages.
10. Ochei J, Kolhatkar A. Medical Laboratory Science, Theory and Practices, Tata McGraw-Hill. 2008: pp. 311-47.
11. Bantie L, Assefa S, Teklehaimanot T, Engidawork E. In vivo antimalarial activity of the crude leaf extract and solvent fractions of *Croton macrostachyus* Hocsht. (Euphorbiaceae) against Plasmodium berghei in mice. BMC Complementary and Alternative Medicine. 14, 2014: 79.
12. Yadav RNS, Agarwala M. Phytochemical analysis of some medicinal plants. Journal of Phytology. 3(12), 2011: 10-14.
13. Fentahun S, Makonnen E, Awas T, Giday M. In vivo antimalarial activity of crude extracts and solvent fractions of leaves of *Strychnos mitis* in *Plasmodium berghei* infected mice. BMC Complementary and Alternative Medicine. Volume 17, 2017: Article number 13.
14. Kundu BB, Vanni K, Farheen A, Jha P, Pandey DK, Kumar V. Dioscorea bulbifera L. (Dioscoreaceae): A review of its ethnobotany, pharmacology and conservation needs. South African Journal of Botany. 140, 2021:365-74.
15. Ziegler HL, Stærk D, Christensen J, Hviid L, Hagerstrand H, Jaroszewsk ZW. In vitro P. falciparum drug sensitivity assay: inhibition of parasite growth by incorporation of stomatocytogenic amphiphiles into the erythrocyte membrane. Antimicrobial Agents and Chemotherapy. 46, 2002: 1441–6.
16. Pan WH, Xu XY, Shi N, Tsang SW, Zhang HJ. Antimalarial Activity of Plant Metabolites. International Journal of Molecular Sciences. 19(5), 2018: 1382.
17. Wang YF, Ni ZY, Dong M, Cong B, Shi WQ, Gu YC, Kiyota H. Secondary Metabolites of Plants from the Genus Saussurea. Chemistry and Biodiversity. 7, 2010: 2623–59.
18. Odeghe OB, Monanu MO, Anacletus FC. Phytochemistry of *Achomanes Difformis* Stem, *Dioscorea Bulbifera* Stem Bark, *Fadogia Cienkowskii* Leaf, *Hannoa Klaineana* Stem and *Vitex Simplicifolia* Leaf. Advances in Multidisciplinary and Scientific Research. 2(1), 2016:1-8.
19. Rudrapal M, Chetia D. Plant Flavonoids as Potential Source of Future Antimalarial leads. Systematic Reviews in Pharmacy. 8(1), 2017:13-18.
20. Chaniad P, Mungthin M, Payaka A, Viriyavejakul P, Punsawad C. Antimalarial properties and molecular docking analysis of compounds from *Dioscorea bulbifera* L. as new antimalarial agent candidates. BMC Complementary Medicine and Therapies. 21, 2021:144.