Antiplasmodial activity of total alkaloids and flavonoids of stem bark extracts of Enantia chlorantha *in mice*

Abdulkadir Abubakar, Nasir Shako Ahmad, Helmina Olufunmilayo Akanya, Abdullahi Abdulkadir & Asmau Niwoye Abubakar

Comparative Clinical Pathology

ISSN 1618-5641

Comp Clin Pathol
DOI 10.1007/s00580-020-03138-4

Springer

Comparative Clinical Pathology
https://doi.org/10.1007/s00580-020-03138-4

ORIGINAL ARTICLE

Antiplasmodial activity of total alkaloids and flavonoids of stem bark extracts of *Enantia chlorantha* in mice

Abdulkadir Abubakar[1] · Nasir Shako Ahmad[1] · Helmina Olufunmilayo Akanya[1] · Abdullahi Abdulkadir[1] · Asmau Niwoye Abubakar[1]

Received: 12 October 2019 / Accepted: 8 June 2020

Abstract

Plant-derived compounds have played vital roles in the management of malaria infections. Thus, *Enantia chlorantha (*Anonaceae) is one of such plants used in folklore medicine in Nigeria for the treatment of malaria. There is, therefore, a need to validate the potential in this plant by investigating its in vivo antiplasmodial activity against *Plasmodium berghei*-infected mice. The alkaloid and flavonoid extracts were extracted from the crude extract following standard procedure. The crude extract was orally administered to *Plasmodium berghei*-infected mice at 200, 400 and 800 mg/kg bodyweight (kgbw), while flavonoid fraction and alkaloid extract were each administered at a dose of 100, 200 and 400 mg/kgbw respectively for 4 days after establishment of infection. The bodyweights and packed cell volume were determined at pre- and post-treatment of infected animals, while parasitaemia was monitored daily throughout the period of treatment. Results showed that parasitaemia count was lowered in mice administered with crude and alkaloid extracts in a dose-dependent manner while the effect of the flavonoid fraction was not significant compared with infected, untreated mice ($p > 0.05$). The percentage parasite inhibition of the crude and alkaloid extracts ranged between 59–73% and 56–70% respectively, while that of the flavonoid fraction was in the range of 28–39%. The animals treated with crude and alkaloid extracts did not suffer much bodyweight loss. However, only the group treated with alkaloid extract at 400 mg/kgbw recorded an increase in PCV which was comparable with the chloroquine-treated group. The crude and alkaloid extracts prolonged the mean survival time of the treated mice compared with the untreated group. Overall, the findings from this study indicate that alkaloid extract of the stem bark of *Enantia chlorantha* may be a good source of antimalarial. In addition, the study also suggested that alkaloids and flavonoids may have synegistically ameliorated the reduction in PCV associated with *Plasmodium* infection.

Keywords Antimalarial · *Enantia chlorantha* · *Plasmodium berghei* · Crude extract · Alkaloid · Flavonoid

Introduction

Malaria is a parasitic disease caused by five *Plasmodium* parasites: *vivax*, *falciparum*, *malariae*, *knowlesi* and *ovale* (Odeghe et al. 2012). It is transmitted when a female *Anopheles* mosquito takes a blood meal from a human host. About 86% of global malaria-associated mortality occurs in pregnant women and children under the age of 5 (WHO 2013). Poor rural dwellers in tropical and subtropical areas are highly vulnerable to this attack owing to the favourable and ideal climatic condition for the reproduction and development of vectors and parasites (Greenwood et al. 2008).

Most of the conventional antimalarial drugs currently in use are faced with toxicity and inefficacy owing to the rapid resistance developed by the parasite over time (Olajide et al. 2013). This therefore calls for investigation into effective and affordable antimalarial with minimal side effects. Natural products remain one of the most widely used for the controls of malaria, particularly in rural areas where access to conventional health facilities is limited or inadequate. In certain rural areas, medicinal plants are even preferred to conventional drugs in the treatment of malaria. Medicinal plants therefore represent a bountiful source of potential candidates for the development of new alternative antimalarial drugs (WHO 2016).

Flavonoids are nearly ubiquitous in plants and are recognized as the pigments responsible for the colors of leaves,

✉ Abdulkadir Abubakar
abukadir2@gmail.com

[1] Department of Biochemistry, Federal University of Technology, P.M.B. 65 Minna Nigeria

Published online: 27 June 2020

especially in autumn. They are generally hydroxylated phenolic substances and therefore are referred to as plant polyphenols. Several bioflavonoids from dietary sources as well as from medicinal plants have been found to possess in vitro and in vivo antiplasmodial effectiveness in both sensitive and resistant strains of *P. falciparum* (Petrus 2014). It is believed that flavonoids act by inhibiting fatty acid biosynthesis in the parasite biochemistry. They also act probably by inhibiting the influx of L-glutamine and myoinositol into infected erythrocytes during the intra-erytrhocytic phase of *Plasmodium* life cycle (Rasoanaivo et al. 2011).

Alkaloids are a diverse group of heterogeneous compounds, containing a ring structure with a nitrogen atom located in the heterocyclic ring having a marked physiological action on humans and animals when used in small quantities. Alkaloids have been implicated in a number of pharmacological properties, many of which are used in traditional or modern medicine, or as a template for drug discovery. Examples of alkaloids with pharmacological properties reported in literatures include quinine, ephedrine, homoharringtonine, morphine, galantamine and piperine with significant antimalarial, antiasthma, anticancer, analgesic, cholinomimetic and hypoglycemic activities respectively (Pérez-Amador et al. 2007).

Alkaloids are among the most therapeutically efficient plant secondary metabolites and have been used as the major backbone in some drugs like morphine, quinine, colchicines and vincristine among others (Jigam et al. 2010). An alkaloid (9-methoxycanthin-6-one) has also been identified to be a more potent antimalarial agent than the chloroquines when tested against *Plasmodium falciparum* Gombak A isolate (Chan et al. 2004).

Enantia chlorantha (Family—Anonaceae), locally known as *Awopa*, *osupupa* or *Dokitaigbo* in (Yoruba), is commonly found along Central Africa and the west coasts of West Africa (Adesokan and Akanji 2003). The plant has been reportedly used in the management of infective hepatitis, rickettsia, fever, typhoid fever or jaundice (Gbadamosi et al. 2011). It has also been reported for antipyretic activities and antimalarial and antimicrobial effects against a wide spectrum of organisms (Adesokan et al. 2008). Although there was reported wide usage of *E. chlorantha*, no compounds have been isolated to support the use of this plant against malaria. The lack of scientific information on the use of alkaloids and flavonoids from this plant against malaria parasites necessitates this study. This study was therefore aimed at investigating the in vivo antimalarial activity of total alkaloid and flavonoid extracts from *Enantia chlorantha* stem bark.

Materials and methods

Plant material, experimental animals and the parasite

Enantia chlorantha stem bark was obtained from local herb sellers at the Kure Market Minna, Niger State, Nigeria. All the plant organs were obtained fresh and were presented to the botanist at the Department of Plant Biology, Federal University of Technology, Minna, Nigeria, for scientific identification. Chloroquine-sensitive *Plasmodium berghei* was obtained from the National Institute of Pharmaceutical Research and Development (NIPRD) Abuja, Nigeria. A total of 68 Swiss albino mice comprising of 13 mice for acute toxicity studies while 55 were used for antiplasmodial studies with a mean weight of 25.87 ± 2.34 g, obtained from NIPRD Abuja.

Table 1 Phytochemical constituents of ethanol extract of stem bark of *E. chlorantha*

Phytochemicals	Presence	Concentrations (mg/100 g)
Phenols	+	1.09 ± 0.10^{b}
Flavonoids	+	0.39 ± 0.02^{a}
Alkaloids	+	2.68 ± 0.21^{c}
Saponins	+	1.56 ± 0.23^{b}
Tannins	_	N.D.

Values are mean ± SEM of triplicate determinations. Values with different alphabets along a column are significantly ($p < 0.05$) different

Present (+), *N.D.* not detected

Preparation of crude extract

The stem bark of *E. chlorantha* was dried for 2 weeks and grounded into a coarse powder with the aid of a mortar and pestle. Two hundred and fifty grams of the grounded plant material was extracted using cold maceration with 2.5 L of 70% ethanol for 72 h. The resulting extract was filtered and concentrated using a water bath at 40 °C.

Extraction of alkaloid

Alkaloid extraction was carried out according to the method of González et al. (2014). Three hundred grams of grounded *Enantia chlorantha* bark was moistened with 100 ml of 70% ethanol and alkalified with 200 ml of ammonia solution for 24 h. The basic plant material was macerated and further

Table 2 Acute toxicity properties of crude extract of *E. chlorantha*

Groups	Dose (mg/kg)	Mortality
I	10	0/3
II	100	0/3
III	1000	0/3
IV	1600	0/1
V	2900	0/1
VI	5000	0/1
VII (normal saline)	2 ml/100 kg	0/1

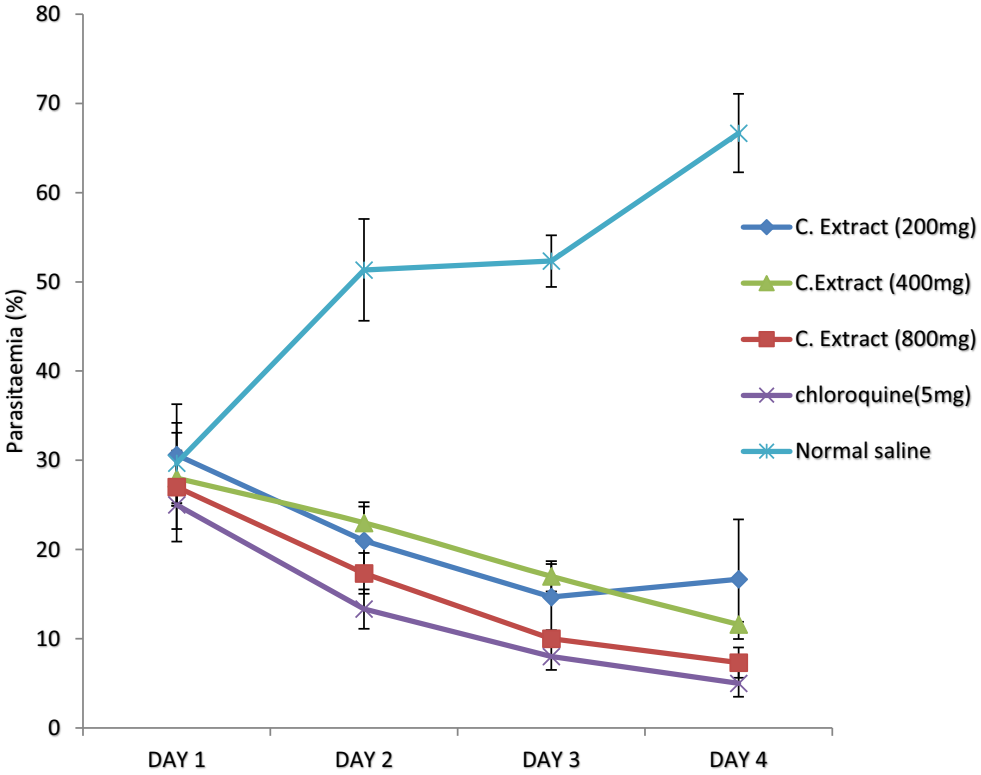

Fig. 1 Antiplasmodial activity of *E. chlorantha* crude stem bark extract in *P. berghei*-infected mice

extracted with 350 ml of 70% ethanol using a soxehelet apparatus. The crude alkaloid extract obtained after filtration was further treated with 1.0 N hydrochloric acid and then filter. Ammonia solution was added to the filtrate and the alkaloid fraction was obtained by chloroform partitioning.

Extraction of flavonoid

Flavonoid fraction was obtained from the crude extract according to the method of Jouad et al. (2001). Ten grams of methanolic extract of the plant materials was dissolved in distilled water and extracted with 250 ml saturated n-butanol. The butanolic extract was concentrated and then subjected to column chromatography, eluted with methanol to obtain the flavonoid fraction (Jouad et al. 2001).

Acute toxicity testing

Acute toxicity of the crude stem bark extract of *E. chlorantha* was carried out in two phases by oral administration of the extract to 3 Swiss albino mice per single doses of 10, 100 and 1000 mg/kgbw (phase 1) and 1 mouse per single doses of 1600, 2900 and 5000 mg/kgbw (phase II) as described by the method of Lorke (1983).

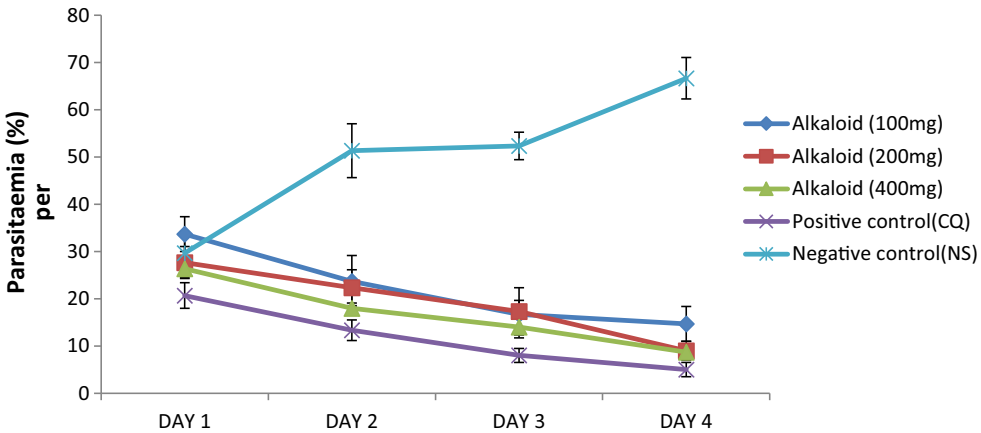

Fig. 2 Antiplasmodial activity of alkaloid fraction of *E. chlorantha* stem bark extract in *P. berghei*-infected mice

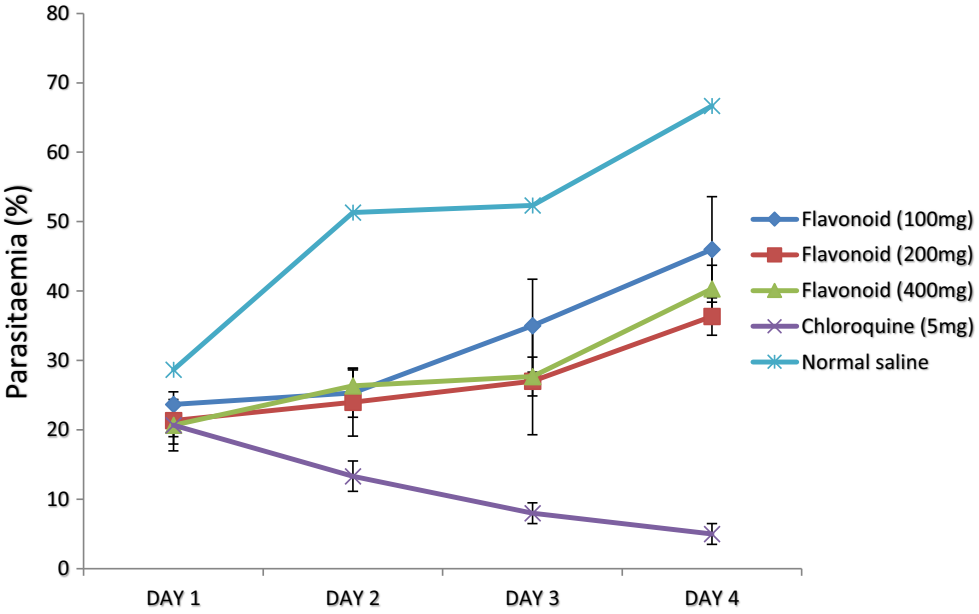

Fig. 3 Antiplasmodial activity of flavonoid fraction of *E. chlorantha* stem bark extract in *P. berghei*-infected mice

Qualitative and quantitative phytochemical analysis

The method described by Trease and Evans (1999) was employed for qualitative phytochemical analysis. In quantitative analysis, total flavonoid was determined as described by Chang et al. (2002), using quercetin to establish the calibration curve. The alkaloid was quantified by extrapolation from the standard curve using the method of Pérez-Amador et al. (2007). The method described by Oloyed (2005), was used for saponin estimation, while the total phenolic content was determined using the Folin-Ciocalteu method as described by AOAC (1984) and the result was expressed as gallic acid equivalent (GAE) in milligrams per gram material.

Parasite inoculation

Highly parasitized (20–30% parasitaemia) blood was obtained by cardiac puncture from *Plasmodium berghei*-infected mice. The blood was diluted with phosphate-buffered saline and 0.2 ml of the diluted blood was intraperitoneally inoculated into each mouse (Hilou et al. 2006).

Antiplasmodial screening of crude, flavonoid and alkaloid extracts of *E. chlorantha*

Four days of prophylactic test were used to evaluate the antimalarial properties of the crude, flavonoid and alkaloid extracts of *E. chlorantha* as described by Jigam et al. (2011). Fifty-five *P. berghei*-infected mice were randomly grouped into eleven (I–XI) of 5 mice each. Groups I–III animals were treated with 200, 400 and 800 mg/kgbw of the crude extract, while mice in groups IV–VI and VII–IX were treated with 100, 200 and 400 mg/kgbw of alkaloid and flavonoid extracts respectively. Groups X and XI received normal saline (2 ml/100 g) and chloroquine (5 mg/kgbw) to serve as negative and positive controls respectively. All the treatments were carried out orally for 4 consecutive days. Changes in bodyweight and packed cell volume (PCV) were monitored throughout the study period. The PCV was determined using the microhaematocrit method as described by Dacie and Lewis (1995). Daily parasitaemia count was carried out by preparing a Giemsa-stained thin film and viewed under a microscope as described by Jigam et al. (2011), while the

Table 3 Percent inhibition and mean survival period of *P. berghei*-infected mice treated with *E. chlorantha* stem bark crude extract

Treatment	Dose (mg/kgbw)	Parasite count	% inhibition	Mean survival period (days)
Normal saline	2 ml/100 g	53.50 ± 4.80	–	10.00 ± 0.6^{a}
Crude extract	200	22.25 ± 4.98	59.60	15.76 ± 1.5^{b}
	400	19.69 ± 4.86	62.49	24.33 ± 0.9^{c}
	800	14.67 ± 3.76	73.52	27.00 ± 1.2^{cd}
Chloroquine	5	11.49 ± 2.4	79.25	29.00 ± 0.6^{d}

Values with different alphabets along a column are significantly ($p < 0.05$) different

Table 4 Percent inhibition and mean survival period of *P. berghei*-infected mice treated with alkaloid fraction of *E. chlorantha* stem bark

Treatment	Dose (mg/kgbw)	Parasite count	% inhibition	Mean survival period (days)
Normal saline	2 ml/100 g	53.50 ± 4.80	–	10.00 ± 0.6[a]
Alkaloid extract	100	23.17 ± 5.54	56.58	16.33 ± 1.5[b]
	200	20.83 ± 5.37	62.23	23.00 ± 1.3 [c]
	400	16.75 ± 3.72	70.40	27.00 ± 1.2 [d]
Chloroquine	5	11.49 ± 2.4	79.25	29.00 ± 0.6[e]

Values with different alphabets along a column are significantly ($p < 0.05$) different

mean survival time of the mice in each treatment group was determined over a period of 29 days (D0–D28) as described by Salawu et al. (2010). The percentage inhibition of parasitaemia was calculated for each dose level by comparing the parasitaemia in infected controls with those of treated mice and the results multiplied by 100 (Salawu et al. 2010).

Results

Qualitative screening of the phytochemicals showed the presence of flavonoids, phenols, saponins and alkaloids in *E. chlorantha* ethanol stem bark extract. The quantitative phytochemical constituents of the extract showed that flavonoids had the least concentration (0.39 ± 0.32 mg/100 g) while alkaloids had the highest concentration (2.68 ± 0.21 mg/kg). The concentration of saponin and phenol were statistically ($p > 0.05$) comparable while tannin was not detected (Table 1).

There was no mortality at all the dose levels used within 24 h and after 2 weeks of acute toxicity study. The animals also showed normal physiological and behavioral features and no visible sign of toxicity was recorded (Table 2).

Figures 1, 2 and 3 show the antiplasmodial properties of the crude extract, alkaloid and flavonoid fractions of *E. chlorantha* stem bark respectively. All the treated groups recorded reduced parasitaemia in a dose-dependent manner throughout the treatment period. The significant ($P < 0.05$) decrease in parasitaemia of the treated groups was more with crude and alkaloid than flavonoid as compared with the untreated (negative control) group. Treatment with reference standard drug resulted in a significant ($p < 0.05$) reduction in parasitaemia when compared with all other treatment and negative control groups.

The percentage inhibition and mean survival period of *P. berghei*-infected mice and treated with crude, alkaloid and flavonoid are presented in Tables 3, 4 and 5 respectively. The result showed that the crude extract inhibited parasite by 59.60%, 62.49% and 73.42% at 200, 400 and 800 mg/kgbw, respectively, when compared with the untreated group (Table 3). The percentage parasite inhibition for alkaloid- and flavonoid-treated groups were 56.58%, 62.23%, 70.40% (Table 4) and 28.03%, 39.85%, 36.33% (Table 5) at doses of 100, 200 and 400 mg/kgbw, respectively, while the chloroquine-treated group (Standard control) had the highest percent inhibition (79.25%). Consequently, the parasite inhibition was highest in alkaloid-treated animals than with the flavonoid-treated animals. There was a significant increase ($p < 0.05$) in the mean survival time in all treatment groups compared with the normal saline–treated group (Tables 3, 4 and 5). However, the chloroquine-treated group had the highest mean survival time (29.00 ± 0.6 days).

Tables 6, 7 and 8 show the result of packed cell volume of *P. berghei*-infected mice treated with crude, alkaloid and flavonoid extracts of the stem bark of *Enantia chlorantha.* The result indicated that all the plant-treated mice suffered a decrease in PCV at all the dose levels with the exception of the alkaloid-treated group at 400 mg/kgbw that recorded an increase in PCV. However, the anaemia was not severe as

Table 5 Percent inhibition and mean survival period of *P. berghei*-infected mice treated with flavonoid fraction of stem bark of *E. chlorantha*

Treatment	Dose (mg/kgbw)	Parasite count	% inhibition	Mean survival period (days)
Normal saline	2 2 ml/100	53.50 ± 4.80	–	10.00 ± 0.6[a]
Flavonoid fraction	100	39.50 ± 5.14	28.03	11.00 ± 0.5[a]
	200	33.25 ± 3.34	39.85	13.67 ± 0.9[a]
	400	35.74 ± 4.14	36.33	13.20 ± 1.7 [a]
Chloroquine	5	11.49 ± 2.4	79.25	29.00 ± 0.6[b]

Values with different alphabets along a column are significantly ($p < 0.05$) different

Table 6 Packed cell volume of *P. berghei*-infected mice treated with crude extract of stem bark of *E. chlorantha*

Treatment	Dose (mg/kgbw)	Changes in PCV		
		Pre-treatment	Post-treatment	% change
Normal saline	–	54.67 ± 4.20	41.30 ± 3.9	− 29.70
Crude extract	200	61.67 ± 3.50	57.33 ± 4.33	− 6.90
	400	55.33 ± 5.80	49.67 ± 6.90	− 5.90
	800	56.67 ± 4.30	49.67 ± 5.20	− 1.79
Chloroquine	5	52.56 ± 4.20	54.67 ± 3.30	+ 1.12

compared with infected untreated control. Thus, the PCV of alkaloid fraction treated was increased by 1.09% as compared with untreated control that had 29.7% reduction in PCV.

The bodyweight of the parasitized mice treated with the crude, alkaloid and flavonoid extracts is shown in Tables 9, 10 and 11 respectively. Mice treated with crude and alkaloid extracts gained bodyweight in a dose-dependent manner whereas the flavonoid-treated group did not result to weight gain, while that of the untreated control was reduced by 37.40%. However, the percentage increase in bodyweight by mice treated with alkaloid extracts at 200 mg/kg, 400mg/kgbw and standard drug was 4.36, 5.32 and 6.06% respectively.

Discussion

The qualitative and quantitative analyses of the plant studied, revealed the presence of pharmacologically active phytochemicals such as saponins, phenols, flavonoids and alkaloids. The high antimalaria activity of the crude extract may be due to the presence of these phytochemicals particularly alkaloid and flavonoid. The high content of alkaloids in crude extracts as shown in Table 1 is in agreement with the previous study carried out by Ayoade and Akanji (2010) on phytochemical analysis of the same plant. The presence of phytochemicals in medicinal plants has been shown to be responsible for their activities in the treatment of diseases (Dubey 2014).

The LD_{50} (> 5000 mg/kgbw) exhibited by the extract as shown in Table 2 is an indication that *Enantia chlorantha* is not toxic. According to Lorke's (1983) substances with LD_{50} values greater than 5000 mg/kgbw are considered safe. Consequently, *Enantia chlorantha*, which was administered to experimental animals up to 5000 mg/kgbw orally, without any noticeable effect is considered safe. The non-toxic nature of *Enantia chlorantha* might be the reason for its wide usage in folklore medicine.

Alkaloid with inhibitory effects on protein synthesis in *Plasmodium falciparum* has been identified from extracts of *Nigella sativa* (black seed) (Abdulelah and Zainal-Abidin 2007). In this study, a far greater antiplasmodial activity was demonstrated by the total alkaloid extract of the plant which was found to be not significantly different from chloroquine (standard antimalarial drug) (Fig. 1 and Table 3). The antiplasmodial activity could be linked to the synergism between different forms of alkaloids in the extract. The antioxidant effect of plant alkaloids may also provide different mechanistic approaches to the observed antimalarial activity.

In addition, the low parasitaemia level and prolonged survival days observed particularly in groups that received high dosage further strengthened the antimalarial efficacy of the alkaloid extract (Table 3). The fact that there was no significant difference ($p < 0.05$) in the antiplasmodial effects in groups treated with 200 mg and 400 mg/kgbw may imply that the effective dose of the alkaloid extract required to produce antimalarial activity may be below 200 mg/kgbw. In a study conducted by Nkunya et al. (2004) on the isolation of prenylated indole alkaloids from Monodora and Isolona

Table 7 Packed cell volume of *P. berghei*-infected mice treated with alkaloid extract of stem bark of *E. chlorantha*

Treatment	Dose (mg/kgbw)	Changes in PCV		
		Pre-treatment	Post-treatment	% change
Normal saline	–	54.67 ± 4.20	41.30 ± 3.9	− 29.70
Alkaloid extract	100	48.33 ± 1.20	46.33 ± 0.90	− 9.20
	200	50.67 ± 2.70	49.67 ± 2.70	− 3.26
	400	51.33 ± 2.50	52.34 ± 2.80	+ 1.09
Chloroquine	5	52.56 ± 4.20	54.67 ± 3.30	+ 1.12

Table 8 Packed cell volume of *P. berghei*-infected mice treated with flavonoid fraction of stem bark of *E. chlorantha*

Treatment	Dose (mg/kgbw)	Changes in PCV		
		Pre-treatment	Post-treatment	% change
Normal saline	–	54.67 ± 4.20	41.30 ± 3.9	−29.70
Flavonoid fraction	100	53.67 ± 4.30	42.67 ± 3.74	−20.75
	200	54.00 ± 1.50	50.33 ± 5.23	−8.57
	400	53.67 ± 3.00	49.03 ± 6.01	−3.07
Chloroquine	5	52.56 ± 4.20	54.67 ± 3.30	+ 1.12

species showed high antimalarial activities against the *P. falciparum* multidrug-resistant K1 strain with an IC_{50} value of 21 μg mL^{-1}.

The relatively high antiplasmodial activity of the alkaloid fraction from the stem bark of *E. chlorantha* as observed in this study may be due to its ability to modulate the conversion of toxic heme to hemozoin by inhibiting the biocrystallization of hemozoin, thus leading to the destruction of the parasite. Interference with the formation of complexes and biosynthesis of parasitic nucleic acids could also be implicated (Foley and Tilley 2016).

Flavonoids exert their antiplasmodial action by targeting certain functional biomolecules (proteins, enzymes, DNA etc.) that are essential for parasite survival (Almedida et al. 2011). Thus, poor antiplasmodial action of the flavonoid fraction could be due to its inability to target protein, enzymes or DNA. In addition, the concentration of flavonoids might not be sufficient enough to effect rapid and complete clearance of target parasites as was observed in this study. A high parasitaemia level observed in the animals treated with flavonoid fraction which translated into their shorter survival time is additional proof of its poor antiplasmodial activity of the fraction. A loss of activity might also have resulted from the fractionation process. Similar cases of loss of activity due to fractionation have been reported. In a study conducted by Noedl et al. (2003), the fractionation of the methanol extract of *Eucalyptus camaldulensis* (leaf) resulted in decreased antiplasmodial activity.

Saponin and phenols are primary antioxidants that scavenge free radicals and protect the cell against malarial-induced oxidative damage. These phytochemicals may be acting synergistically with the alkaloids to produce the observed antimalarial activity in this study.

Infection with malaria parasites produces decrease in PCV while the administration of the antimalarial extracts leads to significant improvement ($p < 0.05$) in the PCV values (Kotepui et al. 2014). The underlying causes of anaemia as observed in this study may due to increased loss of infected erythrocytes through parasite-enhanced phagocytosis, or reduced erythropoiesis and dyserythropoiesis (Lamikanra et al. 2009). The observation of the effect of the alkaloid extract (Table 4) in PCV reduction when compared with the untreated group is similar to that of the effect of crude extract. The dose-dependent improvement in PCV of the alkaloid extract–treated group could be due to mediated destruction of parasitized red blood cells, protection of the new red blood cells in bone marrow or improvement of erythropoiesis as reported by Lamikanra et al. (2009).

The reduction in the body weight of the infected mice is expected because malaria infection is normally accompanied by loss of appetite. The alkaloid extract of the stem bark of *E. chlorantha* caused a significant ($p < 0.05$) weight gain in mice (Table 5). Alkaloids have been shown to ameliorate acute fluid loss, lipolysis and proteolysis which are usually associated with weight

Table 9 Bodyweight of *P. berghei*-infected mice treated with crude extract of stem bark of *E. chlorantha*

Treatment	Dose (mg/kgbw)	Changes in body weight		
		Pre-treatment	Post-treatment	% change
Normal saline	–	23.07 ± 4.77	16.79 ± 3.41	− 37.40
Crude extract	200	24.52 ± 3.22	24.86 ± 2.66	+ 1.36
	400	30.02 ± 2.74	30.39 ± 3.12	+ 1.22
	800	36.72 ± 3.17	37.39 ± 4.92	+ 1.79
Chloroquine	5	28.32 ± 3.69	30.15 ± 3.61	+ 6.06

Table 10 Bodyweight (g) of *P. berghei*-infected mice treated with alkaloid extract of stem bark of *E. chlorantha*

Treatment	Dose (mg/kgbw)	Changes in body weight		
		Pre-treatment	Post-treatment	% change
Normal saline	–	23.07 ± 4.77	16.79 ± 3.41	− 37.40
Alkaloid extract	100	20.09 ± 0.96	20.21 ± 2.15	+ 0.59
	200	21.67 ± 2.81	22.66 ± 2.11	+ 4.36
Chloroquine	400	24.04 ± 4.37	26.89 ± 3.30	+ 5.32
	5	28.32 ± 3.69	30.15 ± 3.61	+ 6.06

loss in malaria infection as was earlier reported by Jeremiah and Uko (2007).

Conclusion

The alkaloid extract of the stem bark of *Enantia chlorantha* exhibited a direct plasmocidal activity against *P. berghei* and also ameliorated the reduction in PCV and weight loss commonly associated with parasite infection.

Compliance with ethical standards

Conflict of interest The authors declared that they have no conflict of interest.

Informed consent Not applicable.

Ethical approval The principles governing the use of laboratory animals as laid out by the Federal University of Technology, Minna Committee on Ethics for Medical and Scientific Research and also existing internationally accepted principles for laboratory animal use and care as contained in the Canadian Council on Animal Care Guidelines and Protocol Review were duly observed.

References

Abdulelah HAA, Zainal-Abidin BAH (2007) *In vivo* anti-malarial tests of *Nigella sativa* (black seed) different extracts. Am J Pharmacol Toxicol 2(2):46–52

Adesokan AA, Akanji IA (2003) Effect of Admininistration of aqueous extract of *Enantia chlorantha* on the activity of some enzymes in the small intestines of rats. Niger J Biochem Mol Biol 18(2):103–105

Adesokan AA, Yakubu MT, Owoyele BV, Akanji MA, Soladoye A, Lawal OK (2008) Effect of administration of aqueous and ethanolic extracts of *Enantia chlorantha* stem bark on brewer's yeast-induced pyresis in rats. Afr J Biochem Res 2(7):165–169

Almedida RB, Pereira AC, Cortes SF, Lemos VS (2011) Vascular effects of flavonoids. Curr Med Chem 16:107–118

AOAC (1984). Association of analytical chemists (14th edn), Arlington, pp 187–188

Ayoade AA, Akanji MA (2010) Antimalarial bioactivity of *Enantia chlorantha* stembark in medicinal plants. Phytochem Pharmacol Ther 1:441–447

Chan KL, Choo CY, Abdullah NR, Ismail Z (2004) Antiplasmodial studies of Eurycoma longifolia Jack using the lactate dehydrogenase assay of Plasmodium falciparum. J Ethnopharmacol 9:223–227

Chang CC, Yang MH, Wen H, Chern JC (2002) Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Anal 10(3):178–182

Dacie JV, Lewis SM (1995) Practical haematology. Elsevier Chuchill Livington, Eighth edition

Dubey NK (2014) Plants as a source of natural antioxidants. Seerapt, J., Alexandra, L., & Shankari, W. (Editors). Croydon, U.K, CPI group Ltd, 6-10

Foley M, Tilley L (2016) Quinoline antimalarials: mechanisms of action and resistance. Int J Parasitol 4:23–27

Gbadamosi IT, Moody JO, Lawal AM (2011) Phytochemical screening and proximate analysis of ethnobotanicals used as antimalerial remedies in Ibadan Nigeria. J Biosci 44:2967–2971

González R, Hellgren U, Greenwood B, Menéndez C (2014) Mefloquine safety and tolerability in pregnancy: a systematic literature review. Malar J 13:75–80

Greenwood BM, Fidock DA, Kyle DE, Kappe SHI, Alonso PL, Collins FH, Duffy PE (2008) Malaria: progress, perils, and prospects for eradication. J Clin Investig 118:1266–1276

Table 11 Bodyweight of *P. berghei*-infected mice treated with flavonoid fraction of stem bark of *E. chlorantha*

Treatment	Dose (mg/kgbw)	Changes in bodyweight		
		Pre-treatment	Post-treatment	% change
Normal saline	–	23.07 ± 4.77	16.79 ± 3.41	− 37.40
Flavonoid fraction	100	22.00 ± 1.39	16.79 ± 1.40	− 31.0
	200	21.40 ± 0.58	17.72 ± 0.64	− 20.70
Chloroquine	400	25.54 ± 3.20	22.78 ± 5.36	− 12.11
	5	28.32 ± 3.69	30.15 ± 3.61	+ 6.06

Hilou A, Nacoulma OG, Guiguemde TR (2006) In vivo antimalarial activities of extracts from *Amaranthus spinosus* L. and *Boerhaavia erecta* L. in mice. J Ethnopharmacol 103(2):236–240

Jeremiah ZA, Uko EK (2007) Depression of platelet counts in apparently healthy children with asymptomatic malaria infection in a Nigerian metropolitan city. Platelets 18(6):469–471

Jigam AA, Akanya HO, Ogbadoyi EO, Dauda BEN (2010) *In vivo* antiplasmodial, analgesic and anti-inflammatory effects of the root extracts of *Acacia nilotica* del (Leguminosae). Asian J Exp Biol Sci 1(2):315–320

Jigam AA, Abdulrazaq UT, Egbuta MN (2011) *In-vivo* antimalarial and toxicological evaluation of *Chrozophoras enegalensis* A. Juss (euphorbiaceae) extracts. J Appl Pharm Sci 1(10):90–94

Jouad H, Lacaille-Dubois MA, Lyoussi B, Eddouks M (2001) Effect of flavonoids extracted from *spergulariapurpurea* pers. On arterial blood pressure and renal function in normal and hypertensive rats. J Ethnopharmacol 7:159–163

Kotepui M, Phunphuech B, Phiwklam N, Chupeerach C, Duangmano S (2014) Effect of malarial infection on haematological parameters in population near Thailand-Myanmar border. Malar J 13(1):218–224

Lamikanra AA, Theron M, Kooij TWA, Roberts DJ (2009) Hemozoin (malarial pigment) directly promotes apoptosis of erythroid precursors. PLoS One 4(12):e8446

Lorke D (1983) A new approach to practical acute toxicity testing. Arch Toxicol 54:275–287

Nkunya MH, Makangara JJ, Jonker SA (2004) Prenylindoles from Tanzanian Monodora and Isolona species. Nat Prod Res 18:253–258

Noedl H, Faiz MA, Yunus EB, Rahman MR, Hossain MA, Samad R, Miller RS, Pang LW, Wongsrichanalai C (2003) Drug resistant malaria in Bangladesh: an *in vitro* assessment. Am J Trop Med Hyg 68(2):140–142

Odeghe OB, Uwakwe AA, Monago CC (2012) Antiplasmodial activity of methanolic stem bark extract of *Anthocleista grandiflora* in mice. Int J Appl Sci Technol 2(4):18–23

Olajide O, Idowu D, Okolo S, Orishadipe A, Sunday T (2013) Phytochemical and antioxidant properties of some Nigerian medicinal plant. Am J Sci Ind Res 2:12–17

Oloyed OI (2005) Chemical profile of unripe pulp of Carica pagaya. Pak J Nutr 4:379–381

Pérez-Amador MC, Muñoz Ocotero V, García JM, Castañeda AR, González E (2007) Alkaloids in *Solanum torvumSw* (Solanaceae). Int Exp Bot 76:39–45

Petrus AJA (2014) The abundant redox-active dietary molecules of life. Asian J Chem 26(16):4929–4939

Rasoanaivo P, Wright CW, Willcox ML, Gilbert B (2011) Whole plant extracts versus single compounds for the treatment of malaria: synergy and positive interactions. Malar J 10(1):4–10

Salawu Oluwakanyinsola A, Tijani Adeniyi Y, Babayi H, Nwaeze Angela C, Anagbogu RA, Agbakwuru VA (2010) Anti-malarial activity of ethanolic stem bark extract of *Faidherbia Albida* (Del) a. Chev (Mimosoidae) in mice. Arch Appl Sci Res 2(5):261–268

Trease GE, Evans WC (1999) Pharmacology (12thed.). Bailliere Tindal, pages 683-685

WHO (2013) World malaria report. World Health Organization: Geneva, Switzerland. http://apps.who.int/malaria/wmr2013/

WHO (2016) World malaria report. World Health Organization. Geneva, Switzerland, http://apps.who.int/malaria/wmr2016/