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ORIGINAL ARTICLE

Methanol extracts of *Fagara zanthoxyloides* leaves possess antimalarial effects and normalizes haematological and biochemical status of *Plasmodium berghei*-passaged mice

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Department of Biochemistry, University of Nigeria, Nsukka, Nigeria

ABSTRACT

Context: The resistance of *Plasmodium* species to many available antimalarials calls for a continuous search for newer antimalarial agents. One possible source of new antimalarials is from natural sources such as *Fagara zanthoxyloides* Lam (Rutaceae), a medicinal plant used traditionally for treating malaria in South-Eastern Nigeria, Uganda and Asia.

Objectives: To investigate the application of methanol extracts of *F. zanthoxyloides* in combating malaria infection and its associated disorders.

Materials and methods: Methanol extracts of *F. zanthoxyloides* leaves (MEFZ) were evaluated for *in vivo* antimalarial activity. MEFZ at doses of 200, 400, and 600 mg/kg/d were administered orally for 4 consecutive days (days 0–4) to *P. berghei*-infected mice. The possible ameliorative effects of MEFZ on malaria-associated organ malfunctions were also assessed.

Results: At 200, 400 and 600 mg/kg b.w., respectively, MEFZ produced 82.37% and 68.39%, 84.84%, and 90.75%, 95.95% and 92.67% chemosuppression and inhibition of *P. berghei*, respectively, comparable to 98.67% and 97.29% by combisunate, a standard antimalarial. The IC_{50} of MEFZ was estimated to be 235.23 mg/kg b.w. Similarly, treatment of parasitized mice with MEFZ significantly restored the malaria-modified haematological and biochemical status of the parasitized-MEFZ-treated mice compared with parasitized-untreated mice. MEFZ was tolerable up to 5000 mg/kg b.w dose; hence, the LD_{50} is above 5000 mg/kg b.w.

Discussion and conclusions: The results of this curative assay demonstrated that MEFZ has antimalarial effects and normalized haematological and biochemical aberrations generated by malaria. The isolation of the antimalarial principles in MEFZ is warranted; they could be lead molecules for the development of new antimalarials.

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Introduction

Malaria still remains one of the deadliest infectious diseases in the world today, causing high morbidity and mortality annually. It is endemic in tropical and sub-tropical regions including parts of Africa, Asia, and the Americas. In 2015, there were 212 million cases of malaria, leading to 429,000 deaths, most of which were children less than 5 years old (World Health Organization [WHO] 2016a). These figures rose to 216 million cases in 2016, resulting in 445,000 deaths, most of which occurred in Sub-Saharan Africa and India (World Health Organization [WHO] 2017). World Health Organization [WHO] (2018) estimated that about 3.2 billion people across 91 countries are still at risk of malaria. Global Technical Strategy for Malaria sets a target to reduce the case of malaria incidence and mortality rates at least 40% by the year 2020.

Five species of *Plasmodium* (Plasmodiidae), the causative agent of malaria, are infectious to humans. These species include *P. falciparum, P. vivax, P. malariae,* and *P. ovale.* The other specie called *P. knowlesi* is zoonotic and can be transmitted from animals to humans. *Plasmodium falciparum* is responsible for the vast majority of deaths from malaria especially in unprotected and non-immune populations (Balogun et al. 2012; Esan et al. 2014). However, in malarial studies using rodents, the *P. berghei* is the model frequently adopted because it shares common characteristics with *P. falciparum* (Ogbuehi et al. 2014).

The high mortality rate associated with malaria infection, especially in tropical and sub-tropical regions of the world, is of great concern. In addition, several reports document increase in the development and spread of resistance to antimalarials by these parasites (Nuwaha 2001; Dondorp et al. 2009; Rogers et al. 2009; Lun et al. 2014; Suresh and Haldar 2018) and toxicities associated with conventional antimalarial drugs (Peto 1989; Luzzi and Peto 1993; McGready et al. 2002; Alkadi 2007; Clark 2009; El-Dakdoky, 2009; Boareto et al. 2012; Bitta et al. 2017; Luo et al. 2018) necessitate the search for alternatives especially from natural product such as plants (Lawal et al. 2015). Research on plants has increased tremendously due to rising evidence of their medicinal potential (Odeghe et al. 2012). These health benefits

CONTACT Innocent Uzochukwu Okagu innocent.okagu@unn.edu.ng Medical Parasitology, Toxicology and Drug Discovery Unit, Department of Biochemistry, University of Nigeria, Nsukka, Nigeria

are attributed to their bioactive components that can function individually and synergistically, exhibiting a wide range of biological activities (Kumar et al. 2011). These bioactive components can be found in the leaves, seeds, bark, fruits, roots, and flowers of these plants (Osho et al. 2011).

Fagara species (Rutaceae) are deciduous and evergreen shrubs and trees native to warm temperate and subtropical regions of the world (Medhi et al. 2013), and are widely grown in the rainforest regions of Southern Nigeria. Traditional healers in Nigeria use species of the *Fagara* for the treatment of a wide range of disorders, including toothache, urinary and venereal diseases, rheumatism, and lumbago (Adesina 2005). In Uganda, the plant is widely used for the treatment of malaria and related conditions (Ogwal-Okeng et al. 2003). Some of the metabolites isolated from *Fagara* species include alkaloids, aliphatic and aromatic amides, lignins, coumarins, and sterols (Adefisoye et al. 2012). Some of these metabolites have shown cytotoxic, molluscidal, anticonvulsant, and anti-sickling effects (Sofowora and Isaacs 1971; Honig et al. 1975; Sofowora et al., 1975; Abu et al. 1981; Ouattara et al. 2009). Others include anesthetic, antibacterial (Taiwo et al. 1999; Ynalvez et al. 2012), antihypertensive, and anti-inflammatory (Oriowo 1982; Aloke et al. 2012) properties. Oyedapo and Famurewa (1995) demonstrated that extracts of *F. zanthoxyloides* Lam have antiprotease and membrane stabilizing activities. Similarly, the antibacterial and antifungal activities of roots bark were demonstrated (Anne et al. 2013, Misra et al. 2013). Antiparasitic activity of root extracts was found to be significantly active against the intracellular form of *Leishmania* major parasite (Maximin et al. 2007); while leaves extract has presented lowest anthelminthic activities on *Ascaris lumbricoides* (Barnabas et al. 2011). The nonpolar fractions from crude alkaloid displayed a good antiplasmodial effect with an IC_{50} ranging from 1.91 to 4.32 µg/mL (Gansane et al. 2010). The reverse-phase high-pressure liquid chromatography (RP-HPLC)-semipurified, and RP-HPLC-purified root extract inhibit the growth of *P. falciparum* (3D7) *in vitro*, with an IC_{50} value of 4.90, 1.00 and 0.13 µg/mL, respectively (Kassim et al. 2005).

It is, therefore, necessary to evaluate the antimalarial potentials of *F. zanthoxyloides* in order to scientifically validate the folkloric use of the plant in the management of malaria. It is also believed that a good antimalarial candidate should not only clear malaria parasite but also restores aberrations induced by malaria infection; hence, we monitored the effect of the extract on haematological and biochemical indices of parasitized mice.

Materials and methods

Plant materials

Fresh leaves of *F. zanthoxyloides* were collected in April 2018 from a habitat in Uzo-Uwani Local Government Area of Enugu State, Nigeria. The plant materials were identified and authenticated by Mr Alfred Ozioko, a taxonomist of the Bioresources Development and Conservation Program (BDCP) Research Centre, Nsukka, Enugu State, Nigeria. Voucher specimen with voucher number, #Intercedd/901 was deposited at the herbarium for reference purposes. The authenticity of the identification was also confirmed with that in 'http://www.theplantlist.org/' and 'http://www.ipni.org/' databases. The plant material was washed of dirt and air-dried for 2 weeks with turning at intervals to avoid decaying.

Study animals

Fifty-four adult healthy albino Wistar mice of average body weight of 30 ± 5 g were used for this study; 24 for the acute toxicity study and 30 for the antimalarial study. They were purchased from the Animal Breeding Unit, Faculty of Veterinary Medicine, University of Nigeria, Nsukka. The mice were acclimatized under standard laboratory condition with 12 h light/dark cycle in the Animal Farm of the Department of Biochemistry for 1 week prior to the commencement of the experiment. They were maintained on a regular feed (commercial Rodent Chow, Vital Feeds Nig. Ltd.) and portable water *ad libitum*. They received humane care throughout the experimental period in accordance with the institutional (EC approval number, UNN/FBS/2018_029A), national and international ethical recommendations for care and use of laboratory animals (National Academy of Sciences [NAS] 2011).

Chemicals and reagents

All chemicals used in this study were of analytical grade. They were products of Sigma Aldrich, St. Louis, MO (standard phytochemicals, ammonia, sodium hydroxide, ethyl acetate, acetic anhydride, ferric chloride, and formaldehyde), British Drug House (BDH), England (methanol, ethanol, hydrochloric acid, and sulphuric acid), Qualikems, India (citric acid, aluminium chloride, and phosphomolybdic acid), Fluka, Germany (lead acetate and potassium ferrocyanide) and May and Baker, England (sodium chloride, dextrose, and sodium citrate). Reagents used for haematological analysis were products of Sysmex Corporation (Kobe, Japan), kits used for biochemical assays were products of Randox and Roche Diagnostics.

Standard drug

The standard antimalarial drug used in this study was combisunate (Ajanta Pharma Ltd., Mumbai, India), an artemisinin-combination therapy (ACT) composed of arthemeter and lumefantrine in a ratio of 80:480. The drug was procured from a reputable retailer, Vegil Pharmaceutical and Drug Stores, Nsukka, Nigeria.

Extraction procedure

The dried leaves of the plant were pulverized into a coarse form using a mechanical grinder. Powdered sample (1500 g) was macerated in 4.5 L of methanol (British Drug House (BDH), England, UK) and the suspension was left standing for 72 h with occasional stirring. The suspension was thereafter filtered using a mesh. Further filtration was achieved using Whatman No. 1 filter paper to remove fine residues. The extraction process was repeated twice and all the filtrate was concentrated using a rotary evaporator at 45 °C to obtain the crude methanol extract (MEFZ) that was stored in an air-tight container at 4 °C until needed.

Phytochemical analysis of methanol extract of F. zanthoxyloides leaf

The phytochemical analysis of MEFZ was carried out in order to ascertain the presence of plant secondary metabolites in the

extract. This was done using the protocols illustrated by Trease and Evans (1989) and Harborne (1998).

Acute toxicity test

The acute toxicity profile of the extract was performed in two phases following the method of Lorke (1983) with slight modification. Briefly, out of the 24 mice to be used in acute toxicity study, 12 mice divided into 3 groups of 4 mice each were used in phase I. Mice in groups 1, 2, and 3 received 10, 100 and 1000 mg/kg b.w. of extract, respectively. The mice were monitored for 24 h. From the result of phase I, higher doses were chosen for phase II. In this phase, the remaining 12 mice were divided into 3 groups of 4 mice each: mice in groups 4–6 received 1600, 2900, and 5000 mg/kg b.w. of extract, respectively. The mice were then observed for 24 h for lethality or any morphological and behavioural signs of toxicity (dullness, changes in eyes and fur appearance/colour, hyperactivity, changes in feeding patterns, sedation, etc.). Body weight of the mice 24 h post-extract administration was measured and compared with the initial body weight.

Parasite inoculation

Donor mouse blood infected with the *P. berghei* ANKA-65 obtained from Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Enugu State, Nigeria, was used for inoculum preparation. Blood was drawn from the donor mouse using heart puncture and diluted serially in Alsever's solution to make a suspension containing about 1×10^6 infected RBCs in every 0.2 mL suspension. This suspension (0.2 mL) was injected into the experimental animals intraperitoneally to initiate infection.

Experimental design for antimalarial study: a curative assay

Thirty healthy adult Wistar mice used for this curative assay were divided into six groups of five mice each: group 1 (non-parasitized rats) which served as normal control received vehicle (normal saline). Group 2 (parasite control) was infected with the malaria parasite and was untreated. Group 3 (standard control) was parasitized and treated with 140 mg/kg b.w of combisunate. Parasitized mice in groups 4–6 were treated with 200, 400, and 600 mg/kg b.w. of the extract for 4 consecutive days (day 0–day 3), a slight modification of Peters (1965) method. On the fifth day, after an overnight fast, the mice were euthanized and their blood samples were collected via cardiac puncture. Whole blood from each mouse was used for haematological analysis using Sysmex KX-21N and serum derived from the remaining blood sample was used for analyses of lipid profile, liver function test and serum electrolyte levels in Roche Cobas 6000 chemistry analyzer. In addition, lipid peroxidation status was determined spectrophotometrically by measuring the level of the lipid peroxidation product, malondialdehyde (MDA) based on the method of Wallin et al. (1993), and superoxide dismutase (SOD) and catalase (CAT) activities were assayed based on the methods of Xin et al. (1991) and Aebi (1983), respectively. The reduced glutathione concentration was determined based on the method of Kings and Wootton (1959), all as contained in Randox commercial kits.

Determination of parasitemia level in experimental animals

The level of parasitemia in the experimental mice was determined using microscopic techniques (World Health Organization [WHO] 2016b; Bain 2017; Osei-Bimpong and Burthem 2017). Thick blood smears were prepared on days 0 and 3 from tail blood, Giemsa stained and examined under a high-power microscope (×100 oil immersion resolution) to determine the parasitaemia level. The percentage parasitaemia was determined by counting the parasitized red blood cells (RBCs) out of total RBCs in random fields of the microscope using the formula below:

$$\text{Percentage malaria parasitaemia (\%)} = \frac{\text{Number of parasitized RBC}}{\text{Total number of RBC counted}} \times 100$$

The day 3 percentage inhibition and percentage chemosuppression were calculated using the formulas below.

$$\text{Day 3 Percentage Inhibition (\%)} = \frac{\text{MP of untreated} - \text{MP of treated}}{\text{MP of untreated}} \times 100$$

$$\text{Day 3 Malaria Chemosuppression (\%)} = \frac{\text{Day 0 MP} - \text{Day 3 MP}}{\text{Day 0 MP}} \times 100$$

where MP is the percentage malaria parasitaemia

Statistical analysis

Raw data obtained from the laboratory were analyzed using one-way analysis of variance (ANOVA) to compare means across groups and Student's *t*-test to compare mean body weight during acute toxicity test and parasitemia levels after 4 d of MEFZ treatment in Statistical Product and Service Solution (SPSS), version 20.0 (Chicago, IL). The results were presented as mean ± SD in tables. Mean values with $p < 0.05$ were considered significant.

Results

Phytochemical constituents of the extract

The phytochemical analysis of the extract showed the presence of flavonoid (1592.593 ± 8.903 mg/100 g), terpenoids (905.297 ± 10.140 mg/100 g), phenols (699.139 ± 2.607 mg/100 g), and alkaloids (156.098 ± 4.527 mg/100 g) in high concentration. However, the presence of glycosides (128.223 ± 0.933 mg/100 g) and tannins (13.513 ± 1.084 mg/100 g) was detected in moderate concentrations, while steroids (0.710 ± 0.146 mg/100 g) and saponins (0.649 ± 0.110 mg/100 g) were detected in low concentrations (Table 1).

Table 1. Phytochemical composition of MEFZ.

Phytochemicals constituents	Concentration (mg/100 g)
Flavonoid	1592.593 ± 8.903
Terpenoids	905.297 ± 10.140
Phenols	699.139 ± 2.607
Alkaloids	156.098 ± 4.527
Glycosides	128.223 ± 0.933
Tannins	13.513 ± 1.084
Steroids	0.710 ± 0.146
Saponins	0.649 ± 0.110

Results are expressed in mean ± SD of triplicate determinations.

Acute toxicity profile of the MEFZ

The result of an acute toxicity test showed that MEFZ was not lethal even at the highest dosage (5000 mg/kg body weight) administered. There was no significant behavioural change within 24 h of acute toxicity study. Similarly, there was no significant change in body weight of the mice 24 h post-extract administration in both phases of the study (Table 2). These suggest that the lethality dose is above 5000 mg/kg body weight.

Antimalarial effects of MEFZ

As shown in Table 3, after 72 h post-inoculation of the mice with *P. berghei* (day 0), there was no significant ($p > 0.05$) difference when the parasitaemia levels of passaged mice (groups 2–6) were compared. However, after treatment for 4 consecutive days, the results show that percentage parasitemia decreased significantly ($p < 0.05$) in the MEFZ-treated groups when compared to the parasite control group. The extract also showed a dose-dependent percentage chemosuppression and percentage inhibition of *P. berghei*. At 600 mg/kg b.w., MEFZ produced 95.95% and 92.67% chemosuppression and percentage inhibition of *P. berghei*, respectively, comparable to 98.67% and 97.29% by combisunate, a standard antimalarial. The IC_{50} of MEFZ was estimated to be 235.23 mg/kg b.w. Hence, the antimalarial effect of the extract at 600 mg/kg b.w. is comparable with that by standard antimalarial drug (combisunate at 140 mg/kg b.w.).

Table 2. Acute toxicity profile of the MEFZ.

Groups	Mortality	Behavioral changes	Body weight (g)	
			Pre-treatment	Post-treatment
Phase I				
Group 1	0/3	Nil	27.88 ± 1.42^{a}	27.92 ± 2.91^{a}
Group 2	0/3	Nil	32.90 ± 1.33^{a}	32.95 ± 1.03^{a}
Group 3	0/3	Nil	32.20 ± 1.53^{a}	32.13 ± 1.20^{a}
Phase II				
Group 4	0/3	Nil	28.65 ± 1.33^{a}	29.08 ± 1.29^{a}
Group 5	0/3	Nil	30.20 ± 1.54^{a}	30.21 ± 1.88^{a}
Group 6	0/3	Nil	28.20 ± 1.67^{a}	28.21 ± 1.30^{a}

Results are expressed in means ± SD ($n = 4$); mean values with different lowercase letters as superscripts across a row are considered significant at $p < 0.05$.
Note: The alphabets in superscript are statistical codes signifying the statistical relationships between two variables. Same alphabet as superscript means that there was no statistical difference between the body weights before and after extract administration.

Effect of MEFZ on the haematological indices of P. berghei-parasitized mice

The results of the effects of MEFZ on the haematological indices of *Plasmodium* infected mice are presented in Table 4. Infection with malaria parasite induced significant ($p < 0.05$) decreases in packed cell volume (PCV), haemoglobin (Hb) concentration, and red blood cell (RBC), white blood cell (WBC) and platelet counts of the parasitized and untreated mice when compared with normal control. However, treatment of parasitized mice with MEFZ restored the altered haematological indices comparable to that of normal control.

Effect of the MEFZ on the liver status in P. berghei-parasitized mice

As shown in Table 5, the activities of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline

Table 3. Percentage malaria parasitaemia, chemosuppression, and percentage inhibition in *P. berghei* passaged mice.

Groups	Day 0 MP (%)	Day 3 MP (%)	Day 3 chemosuppression (%)	Day 3 percentage inhibition
Group 1	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	–	–
Group 2	45.75 ± 8.18^{bA}	86.50 ± 5.0^{eB}	–	–
Group 3	42.50 ± 6.45^{bB}	1.15 ± 0.15^{dA}	98.67 ± 9.72^{a}	97.29 ± 9.77^{a}
Group 4	48.25 ± 6.34^{bB}	15.25 ± 2.50^{cA}	82.37 ± 5.05^{c}	68.39 ± 6.07^{d}
Group 5	49.50 ± 4.20^{bB}	8.00 ± 2.16^{bcA}	90.75 ± 5.68^{b}	84.84 ± 4.86^{c}
Group 6	47.75 ± 3.86^{bB}	3.50 ± 1.00^{bA}	95.95 ± 8.05^{a}	92.67 ± 7.41^{b}

Results are expressed in means ± SD ($n = 5$); Mean values with different lowercase letters within a column and mean values with uppercase letters as superscripts across a row are considered significant at $p < 0.05$.
Group 1 = Normal control (normal saline).
Group 2 = Parasite control (malaria-passaged untreated).
Group 3 = Standard control (malaria-passaged and treated with 140 mg/kg b.w of combisunate).
Group 4 = Malaria-passaged and treated with 200 mg/kg b.w. of the extract.
Group 5 = Malaria-passaged and treated with 400 mg/kg b.w. of the extract.
Group 6 = Malaria-passaged and treated with 600 mg/kg b.w. of the extract.
Note: The alphabets in superscript are statistical codes signifying the statistical relationships between two variables. Same alphabet as superscript means that there was no statistical difference between the body weights before and after extract administration.

Table 4. Effect of the MEFZ haematological indices in *P. berghei*-infected mice.

Groups	Haematological indices				
	PCV (%)	Hb (g/dl)	RBC ($\times 10^{6}$)	WBC ($\times 10\,mm^{3}$)	Platelet ($\times 10\,mm^{9}$)
Group 1	41.50 ± 1.29^{d}	12.85 ± 0.25^{c}	10.73 ± 0.23^{c}	$10{,}825.00 \pm 655.11^{b}$	$16{,}225.00 \pm 250.00^{b}$
Group 2	27.00 ± 2.58^{a}	7.10 ± 0.26^{a}	5.54 ± 0.90^{a}	9325.00 ± 377.49^{a}	$13{,}625.00 \pm 1833.71^{a}$
Group 3	33.00 ± 2.58^{b}	11.55 ± 0.30^{b}	9.56 ± 0.35^{b}	$11{,}400.00 \pm 565.69^{c}$	$16{,}475.00 \pm 309.57^{b}$
Group 4	38.00 ± 1.63^{c}	12.18 ± 0.49^{bc}	10.56 ± 0.63^{c}	$11{,}200.00 \pm 408.25^{bc}$	$16{,}325.00 \pm 590.90^{b}$
Group 5	39.25 ± 2.22^{cd}	12.10 ± 0.29^{bc}	10.77 ± 0.16^{c}	$10{,}475.00 \pm 298.61^{b}$	$16{,}125.00 \pm 727.44^{b}$
Group 6	40.50 ± 1.00^{cd}	12.25 ± 0.65^{c}	10.45 ± 0.20^{c}	$10{,}775.00 \pm 512.35^{b}$	$15{,}600.00 \pm 1116.54^{b}$

Results are expressed in means ± SD ($n = 5$); Mean values with different letters as superscripts in a column are considered significant at $p < 0.05$.
Note: The alphabets in superscript are statistical codes signifying the statistical relationships between two variables. Same alphabet as superscript means that there was no statistical difference between the body weights before and after extract administration.

phosphatase (ALP) as well as total bilirubin concentration were found to be significantly ($p < 0.05$) higher in parasitized-untreated mice when compared to normal control. Parasitized mice treated with 200, 400, and 600 mg/kg b.w. of MEFZ as well as the standard drug have significantly ($p < 0.05$) lower serum AST, ALT, and ALP activities, and total bilirubin concentration when compared with group 2 (parasitized and untreated mice. There was no significant ($p > 0.05$) difference when the liver status of the parasitized-MEFZ-treated mice was compared with that of the normal mice (group 1). This implies that the extract normalized the liver damage generated by malaria infection.

Effect of MEFZ on the antioxidant and lipid peroxidation status of P. berghei*-parasitized mice*

Table 6 reveals that the activities of SOD and catalase, as well as GSH concentration reduced significantly ($p < 0.05$) in the parasite control group when compared to parasitized-treated groups, while the MDA concentration exhibited significant ($p < 0.05$) increase in group 2 when compared to treated mice. However, treatment of parasitized mice with MEFZ normalized the antioxidant and lipid peroxidation status of the mice.

Effect of MEFZ on the lipid profile of P. berghei*-parasitized mice*

The result of the lipid profile in Table 7 shows that the mean total cholesterol levels of mice in groups 3–6 were non-significantly ($p > 0.05$) lower than the values for mice in group 2. There was non-significant ($p > 0.05$) elevation in the HDL concentration of mice in all the parasitized mice treated with graded doses of MEFZ and standard drug when compared to the parasite control group. However, LDL concentration of mice in groups 3–6 were non-significantly ($p > 0.05$) lower than those of parasite control. Triacylglycerol (TAG) concentrations of mice in groups 3–6 were non-significantly ($p > 0.05$) lower than parasite control.

Effect of MEFZ on some electrolyte concentrations in malaria passaged mice

As shown in Table 8, the concentrations of potassium and chloride ions of parasite control mice were significantly ($p < 0.05$) lower, while their sodium ion level was significantly ($p < 0.05$) higher when compared with parasitized-MEFZ-treated mice.

Discussion

Malaria remains an overwhelming infectious disease with significant health challenges in African and other endemic countries globally (Lawal et al. 2015). In the present study, MEFZ showed

Table 5. Effect of the MEFZ on the liver status in *P. berghei*-infected mice.

Groups	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	Total bilirubin (mg/dl)
Group 1	74.00 ± 4.32^{a}	62.25 ± 2.63^{a}	91.50 ± 2.65^{b}	5.53 ± 0.42^{a}
Group 2	107.75 ± 4.19^{d}	96.25 ± 6.34^{c}	112.50 ± 5.07^{c}	7.08 ± 0.79^{b}
Group 3	90.25 ± 2.63^{c}	88.25 ± 3.30^{b}	97.50 ± 3.87^{b}	5.14 ± 0.59^{a}
Group 4	83.00 ± 3.83^{b}	67.75 ± 3.10^{a}	91.00 ± 5.77^{b}	5.64 ± 0.28^{a}
Group 5	76.00 ± 3.37^{a}	68.50 ± 6.25^{a}	92.25 ± 4.35^{b}	4.88 ± 0.57^{a}
Group 6	84.50 ± 12.48^{a}	64.00 ± 2.16^{a}	84.50 ± 3.42^{a}	5.29 ± 0.72^{a}

Results are expressed in means ± SD ($n = 5$); Mean values with different letters as superscripts in a column are considered significant at $p < 0.05$.
Note: The alphabets in superscript are statistical codes signifying the statistical relationships between two variables. Same alphabet as superscript means that there was no statistical difference between the body weights before and after extract administration.

Table 7. Effect of MEFZ on lipid profile of malaria-passaged albino mice.

Groups	HDL (mg/dl)	TAG (mg/dl)	LDL (mg/dl)	Total cholesterol (mg/dl)
Group 1	61.75 ± 1.71^{c}	85.75 ± 4.03^{a}	17.25 ± 2.22^{a}	82.25 ± 6.65^{a}
Group 2	16.25 ± 4.65^{a}	100.75 ± 2.50^{abc}	86.25 ± 5.80^{c}	96.00 ± 2.83^{c}
Group 3	51.75 ± 3.10^{b}	90.50 ± 3.42^{ab}	24.50 ± 3.42^{b}	83.75 ± 3.86^{ab}
Group 4	64.00 ± 3.65^{c}	93.25 ± 7.63^{a}	16.75 ± 0.96^{a}	90.25 ± 3.86^{bc}
Group 5	61.75 ± 3.10^{c}	92.25 ± 5.32^{bc}	16.25 ± 4.65^{a}	84.00 ± 5.66^{ab}
Group 6	67.50 ± 5.00^{c}	92.75 ± 2.50^{c}	20.50 ± 3.42^{ab}	81.50 ± 1.91^{a}

Results are expressed in means ± SD ($n = 5$); Mean values with different letters as superscripts in a column are considered significant at $p < 0.05$.
Note: The alphabets in superscript are statistical codes signifying the statistical relationships between two variables. Same alphabet as superscript means that there was no statistical difference between the body weights before and after extract administration.

Table 8. Effect of MEFZ on some electrolyte concentrations in malaria-passaged mice.

	Electrolyte concentrations (mmol/l)		
Treatment group	Potassium (K^+)	Sodium (Na^+)	Chloride (Cl^-)
Group 1	5.13 ± 0.22^{cd}	129.50 ± 3.41^{b}	7.79 ± 0.71^{a}
Group 2	3.20 ± 0.16^{a}	146.00 ± 6.73^{a}	6.50 ± 0.50^{b}
Group 3	4.23 ± 0.26^{b}	129.75 ± 8.26^{b}	7.43 ± 0.56^{a}
Group 4	4.60 ± 0.50^{bc}	132.75 ± 2.50^{ab}	7.15 ± 0.31^{a}
Group 5	4.35 ± 0.20^{cd}	132.67 ± 3.06^{b}	7.80 ± 0.87^{b}
Group 6	4.64 ± 0.29^{d}	128.40 ± 3.65^{b}	7.92 ± 0.41^{a}

Results are expressed in means ± SD ($n = 5$); mean values with different letters as superscripts in a column are considered significant at $p < 0.05$.
Note: The alphabets in superscript are statistical codes signifying the statistical relationships between two variables. Same alphabet as superscript means that there was no statistical difference between the body weights before and after extract administration.

Table 6. Effect of MEFZ on antioxidant status of malaria passaged albino mice.

	Lipid peroxidation and antioxidant status			
Groups	MDA conc. (mg/dL)	SOD activity (IU/L)	Catalase activity (IU/L)	GSH conc. (mg/dL)
Group 1	8.493 ± 0.200^{a}	3.422 ± 0.470^{b}	1.679 ± 0.195^{b}	5.220 ± 0.635^{a}
Group 2	14.859 ± 0.748^{b}	2.784 ± 0.333^{a}	1.131 ± 0.040^{a}	4.858 ± 0.525^{a}
Group 3	8.308 ± 1.885^{a}	4.801 ± 0.415^{c}	1.654 ± 0.236^{b}	5.281 ± 0.422^{a}
Group 4	7.597 ± 1.089^{a}	3.620 ± 0.378^{b}	1.578 ± 0.267^{b}	5.144 ± 0.791^{a}
Group 5	7.485 ± 0.995^{a}	4.597 ± 0.448^{c}	1.535 ± 0.413^{b}	5.482 ± 0.210^{a}
Group 6	7.836 ± 0.561^{a}	4.902 ± 0.483^{c}	1.585 ± 0.074^{b}	5.582 ± 0.272^{a}

Results are expressed in means ± SD ($n = 5$); mean values with different letters as superscripts in a column are considered significant at $p < 0.05$.
Note: The alphabets in superscript are statistical codes signifying the statistical relationships between two variables. Same alphabet as superscript means that there was no statistical difference between the body weights before and after extract administration.

a dose-dependent reduction in percentage malaria parasitaemia and, hence, an increase in parasite clearance. This result agrees with the views of Medhi et al. (2013) who classified *F. zanthoxyloides* as a medicinal plant with antimalarial properties. The presence of phytochemicals such flavonoids, terpenoids, phenols, alkaloids, glycosides, tannins, steroids, and saponins in the extract which have earlier been shown to have antiplasmodial effects (Saxena et al. 2003; Khaomek et al. 2008), could be responsible for the antimalarial effect observed in this study. Specifically, the presence of alkaloids in this plant is in accordance with the report of Elujoba et al. (2005), who described the main active ingredients in *F. zanthoxyloides* as alkaloids: berberine, fagaronine, chelerythrine, canthin-6-one, and benzoic acid derivatives. Kassim et al. (2005) showed that fagaronine also inhibited *P. falciparum* growth *in vitro* with an IC_{50} value of 0.018 µg/mL. Fagaronine has also been shown to inhibit the growth and cell differentiation of human erythroleukaemia K562 cells and L1210 murine leukaemia cells (Prado et al. 2004). The presence of tannins, saponins, and flavonoids agrees with the report of Adefisoye et al. (2012). These compounds are known to be biologically active and have been shown to possess antimicrobial (Okeke et al., 2001) and antimalarial effects. Takanishi et al. (1999) reported that fagaronine, at a dose of 75 mg/kg b.w. showed cytotoxic activity against transplantable murine tumour P-388 cells. Berberine-rich plants have been shown to exhibit antimalarial activity (Caraballo et al. 2004; Chandel et al. 2015). Similarly, berberine and its analogs have been shown to exhibit antimalarial activity and as well, protect organs of malaria infected mice (Silikas et al. 1996; Iwasa et al. 1998; Dkhil et al. 2015a, 2015b). The mechanism of antimalarial activity of the extract may be attributed to the presence of berberine and fagaronine earlier reported in the extract (Elujoba et al. 2005). Berberine has been shown to inhibit plasmodial telomerase activity (Sriwilaijareon et al. 2002; Parida et al. 2014) while fagaronine is demonstrated to inhibit plasmodial DNA topoisomerases I and II. Fagaronine also acts as a DNA intercalating agent (Larsen et al. 1993).

Haematological indices such as platelet counts, total white blood cell (WBC) count, red blood cell (RBC) count, packed cell volume (PCV) and haemoglobin (Hb) level are common biomarkers of malarial infection and frequently monitored as indicators of drug efficacy against plasmodial infection. Haemoglobin has the physiological function of transporting oxygen to tissues of the animal for the oxidation of ingested food so as to release energy for the other body functions as well as transport carbon dioxide out of the body (Ugwuene 2011; Isaac et al. 2013). Similarly, PCV is an indicator of the body's ability to transport oxygen and absorbed nutrients. An increased PCV shows a better transportation capacity of the red blood cells (Isaac et al. 2013). Packed cell volume is used to assess anaemia, erythrocytosis, haemodilution, and haemoconcentration (Ugwu et al. 2013). A decrease in PCV indicates anaemia (Ugwu et al. 2013; Briggs and Bain 2017). Changes in RBC count are the most typical features of malarial infections and anaemia is the most common complication associated with malaria infection (Erhart et al. 2004). The ability of the MEFZ to restore Hb level, PCV and RBC count in parasitized-MEFZ-treated mice (groups 4, 5, and 6) when compared with parasitized-untreated mice suggests that the extract possess erythropoietic activity. In the same vein, the ability of MEFZ to normalize the reduction in WBC in the parasitized-MEFZ-treated mice when compared with parasitized-untreated showed that the extract has immunostimulatory effects. This may explain why there was a reduction percentage in malaria parasitaemia in parasitized-MEFZ-treated mice. In addition, MEFZ significantly ($p < 0.05$) increased the platelet count of parasitized-MEFZ-treated mice when compared to parasitized-untreated. This result agrees with the opinion of Ifeanyichukwu and Esan (2014) that thrombocytopenia (low platelet count) usually disappears with the treatment of malaria infection. Malaria has been reported as the major cause of thrombocytopenia in malaria endemic areas (Ifeanyichukwu and Esan 2014). Platelets engulf malaria parasites and get damaged in the process and thus are removed from circulation, leading to a reduction in platelet count during malaria (Ifeanyichukwu and Esan 2014). The haematopoietic effects observed in this study could be attributed to the phytoconstituents such as alkaloids, tannins, glycosides, and terpenoids. These phytoconstituents have been shown to increase the release of erythropoietin, the hormone that boosts RBC production as well as stimulates stem cells to divide into blood cells. Koffuor et al. (2012) demonstrated that plants rich in alkaloids, tannins, glycosides, and terpenoids exhibited haematopoietic effect in rabbits.

In parasitized mice, liver enzyme activities in the serum increase due to disruption of the liver membrane by malaria parasite during exo-erythrocytic stage as well as the products of their damage to RBCs during erythrocytic stage which affect the liver. However, treatment with MEFZ significantly ($p < 0.05$) reduced the activities of liver marker enzymes (AST, ALP, and ALT) in the serum of parasitized-MEFZ-treated mice when compared with the parasitized-untreated. This is in line with the previous report that leaf extract of *F. zanthoxyloides*, at high concentrations normalizes these enzymes activities in the serum (Enechi and Ugwu 2013). The normalization of liver function enzymes observed may be linked with the membrane stabilization and maintenance of hepatocyte integrity potentials (Oyedapo and Famurewa 1995), by preventing the leakage of liver enzymes into circulation. Reduction in RBC destruction by malaria parasite due to the antiplasmodial effect of MEFZ might be responsible for the reduction in serum levels of total serum bilirubin in the parasitized-MEFZ-treated mice when compared to parasitized-untreated mice (Trampuz et al. 2003).

In malaria infection, free radicals are generated from various sources such as host immune response, parasite metabolic activity, and haemoglobin degradation. When the level of free radicals overwhelms the body's antioxidant defence, oxidative stress – a condition implicated in malaria complications results (Sandro et al. 2012). The antiplasmodial and antioxidant activities of many plants have been linked to the presence of alkaloids, terpenoids, flavonoids and some other phenolic compounds in them (Momoh and Longe 2014). The significant reduction in the MDA level of parasitized-MEFZ-treated mice when compared to parasitized-untreated mice shows that the extract reduced the level of lipid peroxidation. George et al. (2012) reported that aqueous extract of *Aframomum sceptrum* (Oliv. & Hanb.) K Schum (Zingiberaceae) reduced the MDA level of parasitized mice. MDA is a major oxidation product that indicates the status of lipid peroxidation which is implicated in many pathologic conditions (Noori 2012). Also, treatment of parasitized mice with MEFZ significantly ($p < 0.05$) increased the activity of antioxidant enzymes (SOD and CAT) and concentration of GSH when compared with parasitized-untreated mice. This is also in line with the report by George et al. (2012) that aqueous extract of *A. sceptrum* increased antioxidant status of malaria-passaged mice. This dose-dependent increase in both enzymatic and non-enzymatic antioxidant potentials could be attributed to phytoconstituents such as flavonoids and phenols detected in high

concentrations in MEFZ. Chaaib et al. (2003) have shown that the root of the plant possesses antioxidant effects. Generally, the biological system protects itself against the damaging effect of reactive species by the action of free radical scavengers and chain terminating enzymes such as SOD, CAT, and GSH (Rahman et al. 2012).

Infection of mice with malaria parasite decreased the HDL level when compared with normal control. Kiru et al. (2018) reported that malaria infected human subjects had higher LDL, TAG, HDL, VLDL, and total cholesterol levels when compared with control. Meanwhile, treatment of parasitized mice with MEFZ significantly ($p < 0.05$) increased the HDL concentration when compared with parasitized-untreated mice. On the other hand, treatment of parasitized mice with MEFZ reduced the TAG, LDL, and total cholesterol concentrations in the parasitized and treated mice when compared with parasitized-untreated mice. In children with malaria by *P. vivax*, Dias et al. (2016) reported significantly lower levels of total cholesterol, LDL, and HDL, and significantly higher levels of VLDL and TAG. Dungdung et al. (2018) reported that the total cholesterol, HDL and LDL were significantly decreased while TAG and VLDL were not significantly changed in *P. falciparum* malaria patients. Changes in serum lipid profile and lipid metabolism are due to a whole range of partially disease-specific mechanisms (Khovidhunkit et al. 2004). The extent of serum lipid profile changes during malaria infection and their underlying biological mechanisms remain unclear. A meta-analysis by Visser et al. (2013) showed that changes in serum lipids are a characteristic feature of malaria. Patients with malaria present hypocholesterolemia, decreased HDL level, and elevation in the LDL level, which are accompanied by increased levels of TAGs and very low-density lipoproteins (VLDL). Such lipid abnormalities are transient, occurring in the most prevalent species of *Plasmodium* as well as in complicated or non-complicated cases. This is because lipids play a crucial role in the metabolism of *Plasmodium,* in both phases of its life cycle (Visser et al. 2013). The actual mechanisms behind the observed dyslipidaemia in malaria may be partly host related (i.e., related to an acute phase response to parasite invasion (Rosenson et al. 2007), parasite-related (Holz 1977; Hanscheid et al. 2007) or a combination of these two. However, it was observed that malaria infection induced dyslipidaemia which was normalized in this study by treatment of parasitized mice with MEFZ.

To evaluate the effect of the extract on the kidney dysfunction generated by malaria infection, serum electrolyte concentrations of parasitized-MEFZ-extract-treated mice and parasitized-untreated mice were assessed and compared. On the one hand, the result revealed a significant ($p < 0.05$) decrease in the potassium and chloride levels, and a significant ($p < 0.05$) increase in sodium level of the parasitized-untreated mice when compared with normal control. Result of the present study agrees with the report of Baloch et al. (2011) that found decreased chloride level but increased sodium and potassium levels in malaria-infected human individuals when compared with control. Similarly, Ikekpeazu et al. (2010) and Kiru et al. (2018) reported decreased sodium and potassium levels but no significant difference in chloride level when malaria infected human subjects were compared with control. On the other hand, Ogbadoyi and Gabi (2015) and Ani (2015) found no significant difference when the sodium, chloride and potassium levels of malaria infected human subjects were compared with the controls. It appears that alteration in serum electrolyte indices in malaria infection is dependent on the species of the malaria parasite (Jasani et al. 2012), parasite density (van Wolfswinkel et al. 2010), and immunological status as well as other genetic make-up of the host. In general, malaria infection alters serum electrolyte balance, suggesting that malaria infection induces renal dysfunction. Treatment of parasitized mice with MEFZ restored the electrolyte levels to normal. Electrolytes are particles or solutes found throughout the body in fluids and are important kidney markers. They carry electrical charges and regulate acid–base balance, fluid balance, and osmolarity, and promote neuromuscular functions or fluidity. They also distribute the body's fluid and water between the compartments (Stooper 2015).

The result of an acute toxicity test of MEFZ showed that the extract was not lethal even at the highest dosage (5000 mg/kg body weight) administered. There was no morphological, behavioural or significant body weight change observed within 24 h of acute toxicity study. The result of this study suggests that the lethal dose of the extract is above 5000 mg/kg body weight. This result contradicts the report of Ogwal-Okeng et al. (2003) who showed that methanol extract of *F. zanthoxyloides* root-bark is lethal at doses above 2000 mg/kg body weight. The variation between these findings could be attributed to the differences in the part of the plant used as well as the soil in which the plant is grown. Ogwal-Okeng et al. (2003) used root-bark while the present study used leaves. Roots of plants are usually more toxic than other parts of the plants because of higher bioaccumulation of toxicants from the soil. The toxicity becomes severe when the plant is grown in contaminated soil such as heavy metal contaminated and oil polluted soil. In any case, the present study showed that MEFZ has a high safety profile.

Conclusions

This study demonstrated that the methanol extract of *F. zanthoxyloides* leaves has antimalarial potentials. It, hence, justifies the ethno-medicinal use of *F. zanthoxyloides* in the management of malaria. This effect might be attributed to the presence of pharmacologically active principles in the extract which may have acted singly or in synergy with one another to exert the antimalarial activity observed in this study. The study also showed that the extract could also offer protection against oxidative stress and lipid peroxidation associated with malaria infection. The plant extract also significantly restored altered haematological and biochemical indices induced by malaria. The isolation and the characterization of the antimalarial principles in this extract are warranted. The active ingredients also should be subjected to clinical trials as they might facilitate the development of a new generation of effective antimalarial agents.

Disclosure statement

No potential conflict of interest was reported by the authors.

ORCID

Innocent Uzochukwu Okagu http://orcid.org/0000-0002-9424-7535

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