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

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**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.; 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.

**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 species 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...
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 _F. zanthoxyloides_ leaves extract has antibacterial and membrane stabilizing activities. Similarly, the antibacterial and antifungal activities of _F. zanthoxyloides_ leaves extract has an antiprotease (Maximin et al. 2007); while leaves extract has anti-inflammatory (Oriowo 1982; Aloke et al. 2012) properties.

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.

**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.

**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
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 × 10⁶ 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 parasitemia level. The percentage parasitemia was determined by counting the parasitized red blood cells (RBCs) out of total RBCs in random fields of the microscope using the formula below:

\[ \text{Percentage malaria parasitaemia} \quad \% \quad = \frac{\text{Number of parasitized RBC}}{\text{Total number of RBC counted}} \times 100 \]

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

\[ \text{Day 3 Percentage Inhibition} \quad \% \quad = \frac{\text{MP of untreated} - \text{MP of treated}}{\text{MP of untreated}} \times 100 \]

\[ \text{Day 3 Malaria Chemosuppression} \quad \% \quad = \frac{\text{Day 0 MP} - \text{Day 3 MP}}{\text{Day 0 MP}} \times 100 \]

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).

| 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 IC₅₀ 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.).

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 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

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### Table 2. Acute toxicity profile of the MEFZ.

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

Results are expressed in means ± SD (n = 4); 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.

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

| Groups | Day 0 MP (%) | Day 3 MP (%) | Day 3 chemosuppression (%) | Day 3 percentage inhibition |
|--------|--------------|--------------|---------------------------|----------------------------|
| Group 1| 0.00 ± 0.00ᵃ | 0.00 ± 0.00ᵃ | –                         | –                          |
| Group 2| 45.75 ± 8.18ᵃ | 86.50 ± 5.0³ᵃ | –                         | –                          |
| Group 3| 42.50 ± 6.45ᵇᵇ | 1.15 ± 0.15ᵃᵈᵃ | 98.67 ± 9.72ᵃ | 97.29 ± 9.77ᵃ |
| Group 4| 48.25 ± 6.34ᵇᵇ | 15.25 ± 2.50ᵃᶜᵃ | 82.37 ± 5.0³ᶜ | 68.39 ± 6.07ᵈ |
| Group 5| 49.50 ± 4.20ᵇᵇ | 8.00 ± 2.16ᵃᵇᵃ | 90.75 ± 5.68ᵇ | 84.84 ± 4.86ᶜ |
| Group 6| 47.75 ± 3.86ᵇᵇ | 3.50 ± 1.00ᵇᵃ | 95.95 ± 8.05ᵃ | 92.67 ± 7.41ᵇ |

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.

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

| Groups | PCV (%) | Hb (g/dl) | RBC (×10⁶) | WBC (×10³/mm³) | Platelet (×10³/mm³) |
|--------|---------|-----------|------------|---------------|--------------------|
| Group 1| 41.50 ± 1.29ᵇ | 12.85 ± 0.25ᵇ | 10.73 ± 0.23ᶜ | 10,825.00 ± 655.11ᵇ | 16,225.00 ± 250.00ᵇ |
| Group 2| 27.00 ± 2.58ᵃ | 7.10 ± 0.26ᵃ | 5.54 ± 0.90ᶜ | 9252.00 ± 377.49ᶜ | 13,625.00 ± 1833.71ᵇ |
| Group 3| 33.00 ± 2.58ᵇᵇ | 11.55 ± 0.30ᵇᵇ | 9.56 ± 0.39ᵇᵇ | 11,400.00 ± 565.69ᵇ | 16,475.00 ± 309.5ᶜ |
| Group 4| 38.00 ± 1.63ᵇᵇ | 12.18 ± 0.49ᵇᵇ | 10.56 ± 0.63ᶜ | 11,200.00 ± 408.25ᶜ | 16,325.00 ± 590.9₀ᵇ |
| Group 5| 39.25 ± 2.22ᵈᵈ | 12.10 ± 0.29ᵈᵈ | 10.77 ± 0.16ᵇ | 10,475.00 ± 298.61ᵇ | 16,125.00 ± 727.44ᵇ |
| Group 6| 40.50 ± 1.00ᵈᵈ | 12.25 ± 0.65ᵈᵈ | 10.45 ± 0.26ᵈ | 10,775.00 ± 512.35ᵈ | 15,600.00 ± 1116.54ᵇ |

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.

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

| Groups | ALT (U/L) | AST (U/L) | AST/ALT (%) | ALP (IU/L) | GGT (IU/L) | Glutathione S-transferase (GST) (U/L) | Total Bilirubin (mg/dL) |
|--------|-----------|-----------|-------------|------------|-----------|---------------------------------------|------------------------|
| Group 1| 59.2 ± 2.0³ᵃ | 31.2 ± 0.9³ᵃ | 53.8 ± 1.8⁰ᵃ | 120.0 ± 1.3³ᵃ | 20.0 ± 0.5³ᵃ | 0.0 ± 0.0³ᵃ | 0.0 ± 0.0³ᵃ |
| Group 2| 64.0 ± 2.5³ᵇᵇ | 35.0 ± 1.2³ᵇᵇ | 55.8 ± 1.9⁰ᵇᵇ | 130.0 ± 1.5³ᵇᵇ | 25.0 ± 0.6³ᵇᵇ | 0.0 ± 0.0³ᵇᵇ | 0.0 ± 0.0³ᵇᵇ |
| Group 3| 70.0 ± 2.0³ᶜᶜ | 40.0 ± 1.2³ᶜᶜ | 57.8 ± 1.8⁰ᶜᶜ | 140.0 ± 1.6³ᶜᶜ | 30.0 ± 0.7³ᶜᶜ | 0.0 ± 0.0³ᶜᶜ | 0.0 ± 0.0³ᶜᶜ |

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.

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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 ME of Z. officinale 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 ± 4.32a | 62.25 ± 2.63a | 91.50 ± 2.65b | 5.53 ± 0.42b |
| Group 2 | 107.75 ± 4.195a | 96.25 ± 6.34a | 112.50 ± 5.05c | 7.08 ± 0.79c |
| Group 3 | 90.25 ± 2.63a | 88.25 ± 3.30b | 97.50 ± 3.87b | 5.14 ± 0.59b |
| Group 4 | 83.00 ± 3.83b | 67.75 ± 3.10a | 91.00 ± 5.77c | 5.64 ± 0.28c |
| Group 5 | 76.00 ± 3.37 | 68.50 ± 6.25a | 92.25 ± 4.35b | 4.88 ± 0.57b |
| Group 6 | 84.50 ± 12.48 | 64.00 ± 2.16 | 84.50 ± 3.42b | 5.29 ± 0.72b |

Results are expressed in means ± SD (n = 5); Mean values with different letters as superscripts in a column are considered significant at $p < 0.05$.

**Table 6. Effect of ME of Z. officinale on antioxidant status of malaria passaged albino mice.**

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

Results are expressed in means ± SD (n = 5); mean values with different letters as superscripts in a column are considered significant at $p < 0.05$.

**Table 7. Effect of ME of Z. officinale 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 ± 1.71a | 85.75 ± 4.03a | 17.25 ± 2.22a | 82.25 ± 6.65a |
| Group 2 | 16.25 ± 4.65b | 100.75 ± 2.59ab | 86.25 ± 5.80c | 96.00 ± 2.83c |
| Group 3 | 51.75 ± 3.10c | 90.50 ± 3.42ab | 24.50 ± 3.42b | 83.75 ± 3.86ab |
| Group 4 | 64.00 ± 3.65c | 93.25 ± 7.63ab | 16.75 ± 0.96c | 90.25 ± 3.86bc |
| Group 5 | 61.75 ± 3.10c | 92.25 ± 5.32ab | 16.25 ± 4.65b | 84.00 ± 5.66bc |
| Group 6 | 67.50 ± 3.00 | 92.75 ± 2.50 | 20.50 ± 3.42ab | 81.50 ± 1.91a |

Results are expressed in means ± SD (n = 5); Mean values with different letters as superscripts in a column are considered significant at $p < 0.05$.

**Table 8. Effect of ME of Z. officinale on some electrolyte concentrations in malaria-passaged mice.**

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

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 the 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 as 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 IC₅₀ 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 (Srivilajjaroen et al. 2002; Parida et al. 2014) while fagaronine is demonstrated to inhibit plasmodial DNA topoisomerasases 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 bio-assays used for evaluating the antimalarial activity. Complete blood count (CBC) testing is one of the primary tests in physical examination of patients. It is a cost-effective test that determines the number of cells in the blood. Hematology is a key part of the physical examination of patients. CBC is a common test that determines the number of cells in the blood. Haematological indices such as platelet counts, total white blood cell count, red blood cell count, packed cell volume and haemoglobin level are common bio-assays used for evaluating the antimalarial activity. Complete blood count (CBC) testing is one of the primary tests in physical examination of patients. It is a cost-effective test that determines the number of cells in the blood. Hematology is a key part of the physical examination of patients. CBC is a common test that determines the number of cells in the blood. Haematological indices such as platelet counts, total white blood cell count, red blood cell count, packed cell volume and haemoglobin level are common...
concentrations in MEFZ. Chaib 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, Ikpekézu 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, Ogbadoyo 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 osmolality, 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 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.

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