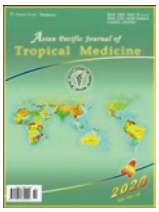

Original Article

Asian Pacific Journal of Tropical Medicine

journal homepage: www.apjtm.org

doi: 10.4103/1995-7645.280239 Impact Factor: 1.77

Antimalarial activity of the aqueous extract of *Euphorbia cordifolia* Elliot in *Plasmodium berghei*-infected mice

Raceline Gounoue Kamkumo[1]✉, Jaures Marius Tsakem Nangap[1], Lauve Rachel Tchokouaha Yamthe[2], Florence Ngueguim Tsofack[1], Patrick Valère Tsouh Fokou[3], Mariscal Brice Tchatat Tali[3], Théophile Dimo[1], Fabrice Fekam Boyom[3]

[1]*Department of Animal Biology and Physiology, University of Yaounde 1, Cameroon*

[2]*Institute of Medical Research and Medicinal Plant Studies, Yaounde, Cameroon*

[3]*Department of Biochemistry, University of Yaounde 1, Cameroon*

ABSTRACT

Objective: To evaluate the antimalarial activity of the aqueous extract of *Euphorbia (E.) cordifolia* Elliot against *Plasmodium (P.) berghei*-infected mice.

Methods: Thirty healthy Swiss mice were intraperitoneally inoculated with 200 μL of *P. berghei* parasitized-erythrocytes and divided into five groups, and then daily treated for 5 d with single dose of 10 mL/kg of distilled water for malaria control, 10 mg/kg of chloroquine for the chloroquine control and 100, 200 and 400 mg/kg of the aqueous extract of *E. cordifolia* for the three test groups. Parasitaemia was monitored by Giemsa-staining. At the end of the treatment, animals were sacrificed, and blood was collected for haematological and biochemical analyses. Organs were collected for biochemical and histopathological analyses. Statistical significance (P<0.05) was evaluated by analysis of variance followed by the Tukey post-test using Graphpad prism 7.0.

Results: *E. cordifolia* extract decreased the parasite load to 2.46%, with an effective dose (ED_{50}) of 113.07 mg/kg compared to the malaria group where the parasite load increased to (46.46±10.28)%. *E. cordifolia* extract prevented hypoglycaemia, anaemia, leucocytosis and thrombocytopenia, attenuated the increase of transaminases activities, bilirubin and creatinine rate, and improved catalase and superoxide dismutase activities, while reducing malondialdehyde contents in the liver and kidney. *E. cordifolia* extract significantly prevented histological damages observed in the malaria control group. No acute toxicity sign was observed in mice with plant extract at the dose up to 5 000 mg/kg.

Conclusions: *E. cordifolia* extract at 200 and 400 mg/kg showed significant antimalarial effects. This results support its traditional use in the treatment of malaria.

KEYWORDS: Antimalarial activity; Curative effects; *Euphorbia cordifolia*; *Plasmodium berghei*

1. Introduction

Malaria is the world deadliest parasitic infection with 216 million cases and 445 000 deaths recorded in 2016, with the Sub-Saharan Africa countries accounting for more than two thirds of the global deaths[1]. Despite substantial efforts applied to reduce the burden of this disease, the progress seems to be slowed down by the rapid emergence of drugs resistant parasites[2]. The development of an effective and safe antimalarial vaccine is the most appropriate approach to malaria prevention[3]. However, studies in that area still face polymorphism problems with parasitic strains[4,5].

Fortunately, traditional medicine potions have been used with success in the treatment of malaria since decades and are well known as important sources for antimalarial drug discovery since two of the most important first line drugs (artemisinin and quinine derivatives) for malaria control originated from medicinal plants[6]. Therefore, investigating plants used in traditional medicines against malaria can lead to alternative therapy against malaria. In this regards, *Euphorbia (E.) cordifolia* (Euphorbiaceae), with the common heartleaf sandmat, is an endemic herbal plant growing over the world. Its stem contains latex and has many adventitious roots[7]. The herb is traditionally used in population from West Region (Cameroon) in the treatment of malaria and related symptoms as

✉To whom correspondence may be addressed. E-mail: fotsingrace@yahoo.fr

How to cite this article: Gounoue Kamkumo R, Tsakem Nangap JM, Tchokouaha Yamthe LR, Ngueguim Tsofack F, Tsouh Fokou PV, Tchatat Tali MB, et al. Antimalarial activity of the aqueous extract of *Euphorbia cordifolia* Elliot in *Plasmodium berghei*-infected mice. Asian Pac J Trop Med 2020; 13(4): 176-184.

Article history: Received 3 May 2018 Revision 11 October 2019
Accepted 12 December 2019 Available online 25 March 2020

fever. This study aimed to evaluate the antimalarial activity of the aqueous extract of *E. cordifolia* Elliot against the *Plasmodium (P.) berghei*-infected mice model.

2. Materials and methods

2.1. Plant collection and extract preparation

The whole plant of *E. cordifolia* Elliot (Euphorbiaceae) was harvested in April 2016 in Yaounde-Cameroon, and authenticated by Mr Victor Nana, a botanist at the National Herbarium where a voucher specimen No. 20631/SRF Cam was deposited.

The whole plant was cut into smaller pieces, washed and air-dried at laboratory temperature (25±2) ℃ for 2 weeks till constant weight was recorded. Then, dried plant material was ground using a blender and plant powder was macerated in distilled water for 72 h following traditional healer's recommendations. The filtrate was freeze-dried using a lyophilizer to yield 6.34% of dark-brown *E. cordifolia* extract.

2.2. Phytochemical screening of plant extract

The phytochemical constituent of aqueous extract of *E. cordifolia* including alkaloids, anthraquinones, flavonoids, phenols, saponins, tannins, lipids, reducing sugars, phenols, terpenoids and polyterpenes were qualitatively determined as described by Harborne[8], Odebeyi and Sofowara[9], Trease and Evans[10], Sofowora[11].

2.3. Experimental animals

Two months old female Swiss albino mice weighing 25 g in average were used for the experiments. The experiments were performed in the Animal House of the Faculty of Science, University of Yaounde 1. Animals were reared in standard cages, at room temperature of (22±2) ℃ on a 12 h light-dark natural cycle. Food and water were given *ad libitum* during the duration of the experiment. The study protocol was approved by the Institutional Ethical Committee, which adopted all procedures recommended by the European Union on the protection of animals used for scientific proposes (CEE Council 86/609; Ref No. FWA-IRD 0001954).

2.4. In vivo antimalarial test

The *P. berghei* strain (MRA 406 ATCC, Manassas Virginia, USA) used in the present study was maintained by subsequent passage of infected red blood cells (RBC) from mouse to mouse. The antimalarial test was performed using a standard protocol described by Fidock *et al*.[12] with slight modification. Briefly, an infected mouse with parasitaemia up to 50% was anesthetized by intraperitoneal injection of urethane (1.5 g/kg) and blood was collected by cardiac puncture in a heparinized syringe. The collected blood was diluted to 10^6 infected red blood cells/0.2 mL with a solution of sodium chloride 0.9% and was used to infect each mouse by intraperitoneal route. The Giemsa-stained blood smears were examined microscopically under immersion oil to monitor the parasitaemia daily afre 3 d post inoculation. Infected mice were randomly divided into five groups of six animals each including three test groups receiving respectively 100, 200 and 400 mg/kg of aqueous extract of *E. cordifolia*. Malaria control group received distilled water (10 mL/kg) while chloroquine control group was treated with 10 mg/kg of chloroquine (Sigma Chemicals). A batch of six healthy mice (without infection), receiving distilled water (10 mL/kg) was used as the normal control group. To obtain the experimental extract dose, two (02) measure cups of the filtrated extract were collected according to the daily dose of healer were freeze-dried corresponding to an average dose of 200 mg/kg. This dose was subsequently used as starting point for the experiment and surrounded with a lower dose of 100 mg/kg and higher of 400 mg/kg, which is below the highest dose used by Munöz *et al*[13]. The treatment was administrated orally as a single daily dose for 5 d while the body weight and parasitaemia of each animal were subsequently determined before each treatment. Parasitaemia was determined using stained fresh 10% Giemsa solution (Sigma) in phosphate buffer (pH 7.1), and counting parasite per 100 erythrocytes under microscope using immersion oil and 100× objective.

At the end of the experimental period, the percentage of inhibition (%I) of the parasite growth was determined by the following formula: %I=[(parasitaemia of malaria control-parasitaemia of extract dose)/parasitaemia of malaria control]×100. The percentage of inhibition was used to determine the effective dose, the dose of extract that reduced parasite development by 50% (ED_{50}) by a nonlinear regression using GraphPad Prism 7.0 software (San Diego, USA).

2.4.1. Evaluation of effects of E. cordifolia extract on physiological changes induced by P. berghei in mice

Twenty-four hours after the last day of treatment, the blood glucose of each mouse was measured and they were anesthetized. Blood was collected by cardiac puncture and dispensed in dry tube for biochemical analysis and in EDTA tube for haematological parameters analyses. Haematological parameters analysis included RBC count, haemoglobin (Hb) level, haematocrit (Hct), mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, total white blood cells count (WBC), proportion of lymphocytes, monocytes and granulocytes and platelets count. Blood from dry tube were centrifuged at 1 500 × *g*, at 4 ℃ for 15 min and serum was collected, stored at -20 ℃ for

biochemical analysis. Biochemical analyses were carried out focused on creatinine, bilirubin, total proteins, alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) level according to the protocols provided with Fortress Diagnostics commercial kits (UK). Liver and kidneys were removed for histological analysis. Liver and kidney sections were ground, centrifuged and the homogenate was used to assess oxidative stress response parameters such as superoxide dismutase (SOD), malonedialdehyde (MDA), catalase and tissue protein.

2.4.2. Histopathological analysis of some detoxification organs

The liver and kidneys of each animal fixed in 10% buffered formalin were dehydrated by subsequent passage through gradual concentrations of alcohol and then embedded in paraffin. Serial paraffin sections of 5 µm were stained with haematoxylin and eosin (HE) for examination under light microscopy brand Olympus and photography in objective 20 auricular 100 (HE×200).

2.5. Acute toxicity assay

The acute oral toxicity of the plant extract was investigated using the Organization for Economic Co-operation and Development [OCDE (2001)] protocol, guideline 423 with slight modifications. Briefly, 8 healthy female mice were used for acute oral toxicity studies. The aqueous extract of *E. cordifolia* was orally administered at single dose of 5 000 mg/kg body weight to 4 animals, the 4 others receiving distilled water at 10 mL/kg. The animals were then, observed continuously for behavioural and autonomic profiles for 2 h and for any signs of toxicity or mortality up to 14 d.

2.6. Statistical analysis

All data were expressed as mean±standard deviation (SD). Statistical significance was evaluated by analysis of variance (ANOVA) followed by the Tukey post-test using Graphpad prism software version 7.0. Difference was considered significant at $P<0.05$.

3. Results

The phytochemical screening of the aqueous extract of *E. cordifolia* revealed the presence of alkaloids, anthraquinones, glycosides, flavonoids, saponins, tannins and polyphenols.

3.1. Effect of E. cordifolia on parasitaemia level

The dose dependent reduction of *P. berghei* parasitaemia in each mouse by the aqueous extract of *E. cordifolia* is summarized in Figure 1. The parasitaemia in the malaria control group was found to rise from (7.98±0.59)% at day 3 (D3) to (46.46±10.28)% at day 8 (D8), indicating a successful establishment of the infection. The daily administration of a single dose of *E. cordifolia* plant extract to infected animals during the 5 experiment days led to a significant and gradual reduction of parasitaemia from day 4 at doses of 200 and 400 mg/kg ($P<0.01$) to day 8. The percentage of inhibition of parasite growth by at the end of the treatment was 34.71%, 94.70% and 92.27% ($P<0.01$) at the respective doses of 100, 200 and 400 mg/kg, with the effective dose 50 (ED_{50}) evaluated at 113.07 mg/kg.

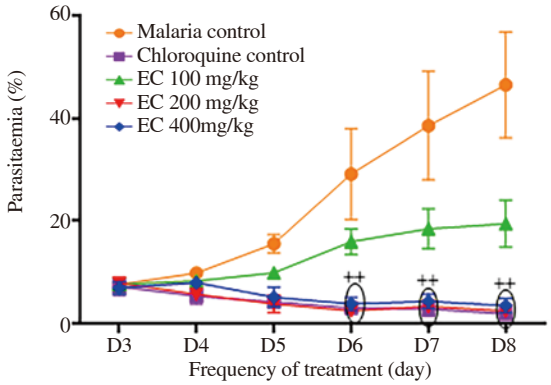

Figure 1. Effect of *Euphorbia cordifolia* on parasitaemia level in infected mice. Each point represents an average±SD; (n=6); $^{**}P<0.01$ compared to the malaria control. EC=*Plasmodium berghei*-infected animal, treated with aqueous extract of *Euphorbia cordifolia* at the dose 100 mg/kg (EC100 mg/kg), 200 mg/kg (EC 200 mg/kg) and 400 mg/kg (EC 400 mg/kg).

3.2. Acute toxicity of the extract

No toxicity signs or death were observed with the extract administration, indicating that the oral lethal dose-50 (LD_{50}) of the aqueous extract of was greater than 5 000 mg/kg.

3.3. Effect of E. cordifolia on some haematological parameters

Inoculation of mouse with *P. berghei* infected RBC resulted in a significant decrease of the RBC count ($P<0.05$), Hb rate ($P<0.01$), Hct level ($P<0.01$), platelets rate ($P<0.01$) in the malaria control group as compared to the normal control group after 8 d (Table 1). Moreover, malaria infection also induced a significant increase in the WBC count ($P<0.05$) in malaria control. Conversely, the daily administration of the aqueous extract of *E. cordifolia* at the dose of 200 mg/kg for 5 d to infected animals significantly increased the RBC count by 47.47% ($P<0.01$), Hb level (48.60%, $P<0.05$), Hct rate by 46.49% ($P<0.01$) and platelet count (10.34%, $P<0.05$). Extract at the same dose significantly decreased ($P<0.05$) the WBC count. In comparison to the malaria control, it was observed that except for Hb, haematological parameters such as RBC, Hct and Plt in infected mice treated with chloroquine (10 mg/kg) significantly increased ($P<0.05$) whereas leucocytes count decreased (Table 1).

Table 1. The effects of *Euphorbia cordifolia* on some haematological parameters in infected mice.

Parameters	Normal control	Malaria control	CQ control	EC 100 mg/kg	EC 200 mg/kg	EC 400 mg/kg
RBC (10^6/mm^3)	7.56±0.54	4.36±0.40^{+}	6.22±0.69*	5.74±0.84^{+}	6.43±3.12**	5.33±1.30
Hb (g/dL)	15.70±3.13	8.95±1.92^{++}	11.90±2.46	10.22±2.41	13.30±2.44*	10.90±4.25
Hct (%)	73.10±8.49	41.40±13.20^{++}	67.30±4.23**	61.66±8.31^{+*}	60.65±4.99**	58.32±10.70*
MCV (fl)	109.20±30.00	135.33±18.70	126.00±36.9	109.00±18.40	125.00±31.50	123.60±27.6
MCH (pg)	107.90±2.11	75.20±4.75^{+}	120.70±2.93	42.90±2.56	111.00±3.02	105.90±3.75
MCHC (g/dL)	19.96±0.69	17.93±1.22	19.28±0.39	19.65±0.15	17.62±0.77	16.80±1.62
WBC (10^3/mm^3)	10.44±1.04	21.70±10.60^{+}	8.50±3.80**	14.80±6.48*	10.90±0.58*	8.36±2.99**
Lym (%)	5.73±0.66	9.90±0.77^{+}	4.62±1.03	7.90±2.97	5.17±1.17	3.83±1.65
Mon (%)	0.74±0.11	3.06±0.52^{++}	1.37±0.32	1.15±0.28	1.90±0.39	1.82±0.47
Granul (%)	0.64±0.14	5.70±0.54^{++}	1.54±0.26	1.80±0.75	2.45±0.85	1.96±0.29
Plt (10^3/mm^3)	742.40±10.62	442.60±51.23^{++}	551.60±15.09*	572.00±32.00*	488.40±26.70*	437.20±15.19

Values were expressed by mean±SD (n=6); ^{+}P<0.05, ^{++}P<0.01 compared to the normal control; $^{*}P$<0.05, $^{**}P$<0.01 compared to the malaria control. CQ control=infected mice and treated with chloroquine (10 mg/kg). EC=*Plasmodium berghei*-infected animal, treated with aqueous extract of *Euphorbia cordifolia* at the dose 100 mg/kg (EC100 mg/kg), 200 mg/kg (EC 200 mg/kg) and 400 mg/kg (EC 400 mg/kg). MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; Lym: lymphocytes; Mon: monocytes; Granul: granulocytes; Plt: platelets count.

3.4. Effect of extract on blood glucose level

The parasite infection with *P. berghei* caused a significant decrease in blood glucose level in the malaria control (P<0.01), and test groups (P<0.05) as compared to the normal control (Figure 2). However, as compared to the malaria control, the administration of *E. cordifolia* extract exhibited a dose dependent increase in blood glucose level by 88.09% (P<0.05), 92.85% (P<0.01) and 111.90% (P<0.01) at the respective doses of 100, 200 and 400 mg/kg. Furthermore, administration of chloroquine to infected animals (chloroquine control) induced a significant increase in blood glucose level (P<0.01) compared to the malaria control.

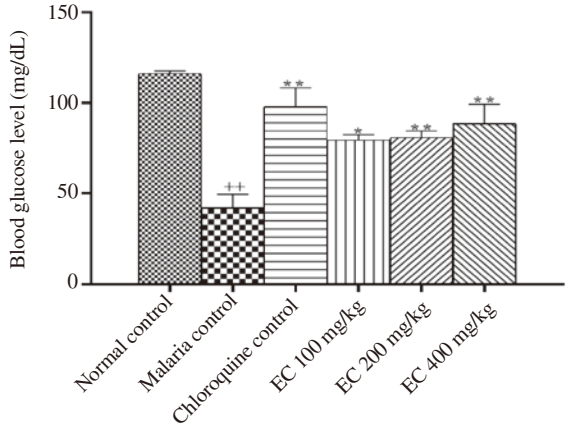

Figure 2. Effect of extract administration on blood glucose level in *Plasmodium berghei* infected mice. Bars are expressed as mean±SD; (n=6); ^{+}P<0.05, ^{++}P<0.01 compared to the normal control; $^{*}P$<0.05, $^{**}P$<0.01 compared to the malaria control. EC=*Plasmodium berghei*-infected animal, treated with aqueous extract of *Euphorbia cordifolia* at the dose 100 mg/kg (EC100 mg/kg), 200 mg/kg (EC 200 mg/kg) and 400 mg/kg (EC 400 mg/kg).

3.5. Effect of E. cordifolia on liver and renal function

The intraperitoneal inoculation of 1×10^6 RBC parasitized by *P. berghei*, resulted in a significant increase in serum transaminase (ALAT and ASAT) activities (P<0.01), bilirubin (P<0.01) and creatinine levels (P<0.01) compared to the normal control after 8 d of experiment (Figure 3A, 3B and 3C). The daily administration of the *E. cordifolia* extract for 5 d significantly protected the infected animals from the increase of ALAT activities by 92.33%, 93.32% and 93.32% (P<0.01) at the respective doses of 100, 200 and 400 mg/kg, and from the ASAT activities by 87.00%, and 64.41% at the doses of 200 and 400 mg/kg, respectively. A significant decrease in the ASAT level (P<0.05) was observed with the extract at 200 mg/kg compared to 100 mg/kg. Treatment of infected animals with chloroquine (10 mg/kg) resulted in a significant decrease (P<0.01) in ALAT (84.79%) and ASAT (83.61%) compared to the malaria control (Figure 3A). The plant extract as well as chloroquine also significantly decreased (P<0.01) the level of serum bilirubin in infected mice (Figure 3B). Similar observation was done with serum creatinine level (P<0.01) where the treatment with extract at the doses of 200 mg/kg and 400 mg/kg and chloroquine (10 mg/kg) significantly decreased its level (Figure 3C).

3.6. Effect of E. cordifolia extract on antioxidant parameters

Antioxidant parameters such as MDA, superoxide dismutase, catalase activities and tissue protein were analysed 8 d after infection. As compared to normal control group, infected animals showed a significant increase in lipid peroxidation (P<0.01) in the liver and kidney tissues (Figure 4A and 4B). However, the daily administration of the plant extract significantly reduced the lipid peroxidation (P<0.05) by 36.24% and 34.96% at the respective doses of 200 and 400 mg/kg in the liver and by 53.33% (P<0.01) in the kidney at 200 mg/kg. Malaria also significantly decreased the SOD level (P<0.01) in liver and kidney (Figure 4C). However, the daily administration of 200 mg/kg and 400 mg/kg showed a reversal effect by increasing SOD level (P<0.01) by 180.31% and 215.00% in liver

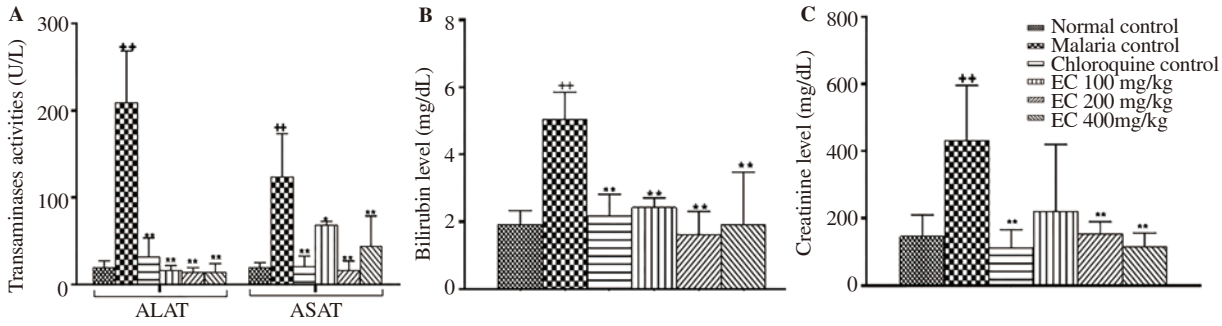

Figure 3. Effects of *Euphorbia cordifolia* extract on liver and kidney functions (A) ASAT and ALAT, (B) bilirubin level, (C) creatinine level. Bars are expressed as mean±SD; (n=6); ^{+}P<0.05, ^{++}P<0.01 compared to the normal control; $^{*}P$<0.05, $^{**}P$<0.01compared to the malaria control. EC=*Plasmodium berghei*-infected animal, treated with aqueous extract of *Euphorbia cordifolia* at doses of 100 mg/kg (EC100 mg/kg), 200 mg/kg (EC 200 mg/kg) and 400 mg/kg (EC 400 mg/kg).

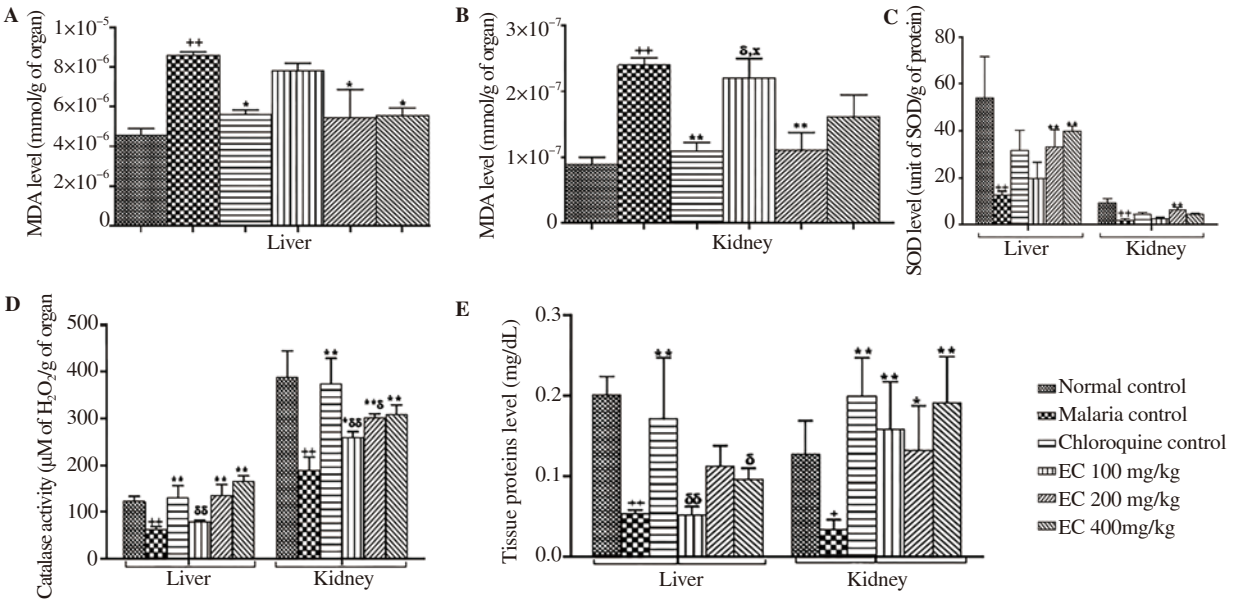

Figure 4. Effects of the aqueous extract of *Euphorbia cordifolia* on some oxidative stress parameters (A) MDA liver, (B) MDA kidney, (C) SOD, (D) catalase and (E) total protein in liver and kidney tissues of infected mice. Bars are expressed as mean±SD; (n=6); ^{+}P<0.05, ^{++}P<0.01 compared to the normal control; $^{*}P$<0.05, $^{**}P$<0.01 compared to the malaria control. P<0.05, P<0.01 compared to the chloroquine control; $^{\delta}P$<0.05, $^{\delta\delta}P$<0.01 compared between plant extract doses. EC=*Plasmodium berghei*-infected animal, treated with aqueous extract of *Euphorbia cordifolia* at doses of 100 mg/kg (EC100 mg/kg), 200 mg/kg (EC 200 mg/kg) and 400 mg/kg (EC 400 mg/kg).

and by 130.41% and 92.08% (P<0.01) in the kidney, respectively. Of note, the *Plasmodium* infection significantly decreased (P<0.01) the catalase activity in the liver and kidney as compared to the normal control (Figure 4D). The administration of the plant extract significantly reversed this effect (P<0.01) by 113.16% and 160.18% in liver and 59.78% and 62.96% in kidney at the respective dose of 200 and 400 mg/kg. The tissue proteins rate was significantly reduced (P<0.01) in liver and kidney (P<0.05) in *Plasmodium*-infected animals compared to the normal control (Figure 4E). However, it was observed a significant increase in the protein level (P<0.01) in mice treated with plant extract. Daily administration of chloroquine (10 mg/kg) significantly decreased the MDA level (P<0.05) and increased the protein level (P<0.01) and the catalase and SOD activities in comparison to the malaria control and plant extract at100 mg/kg as well as in the liver and kidney tissue.

3.7. Effect of E. cordifolia extract on the histology of the major organs of detoxification

Figures 5 and 6 show the effects of plant extract on some organs of detoxification in *P. berghei*-infected mice. Figure 5A shows the histology of the liver of a normal mouse. This section shows a distinct centro-lobular vein, bile duct and hepatocytes separated by the sinusoids. The liver of the untreated animal infected by *P. berghei* (malaria control) is enlarged and has a slatey-gray appearance (Figure 5B) showing a disorganized parenchyma with sinusoid dilation, a diffuse infiltration of leucocytes with inflammatory sites around centrolobular vein, a vascular congestion and Kupffer cells containing malarial pigment. Pigments were also found in parenchymal cells. The liver section from infected animals and treated with chloroquine (Figure 5C) shows a slight dilation of

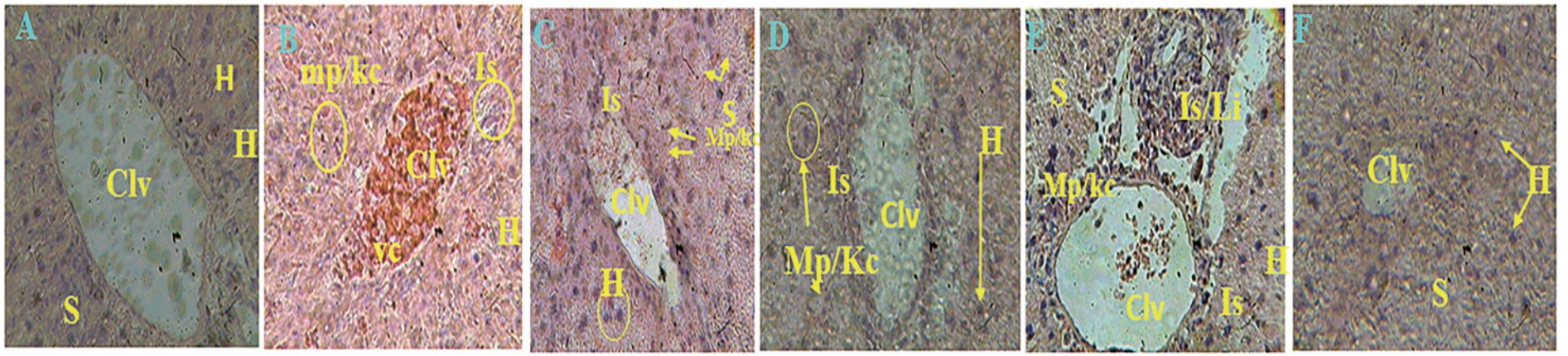

Figure 5. Effect of *Euphorbia cordifolia* on lliver morphology of malaria-infected animal (A) normal control; (B) malaria control; (C) chloroquine control; malaria infected mouse treated with plant extract at the (D) dose 100 mg/kg, (E) dose 200 mg/kg and (F) 400 mg/kg. H=hepatocytes; Clv=Centro lobular vein; S=sinusoid; Vc=vascular congestion; Li=leucocyte infiltration; IS=Inflammatory site; Mp/Kc=malarial pigment in Kupffer cells. H&E 20×.

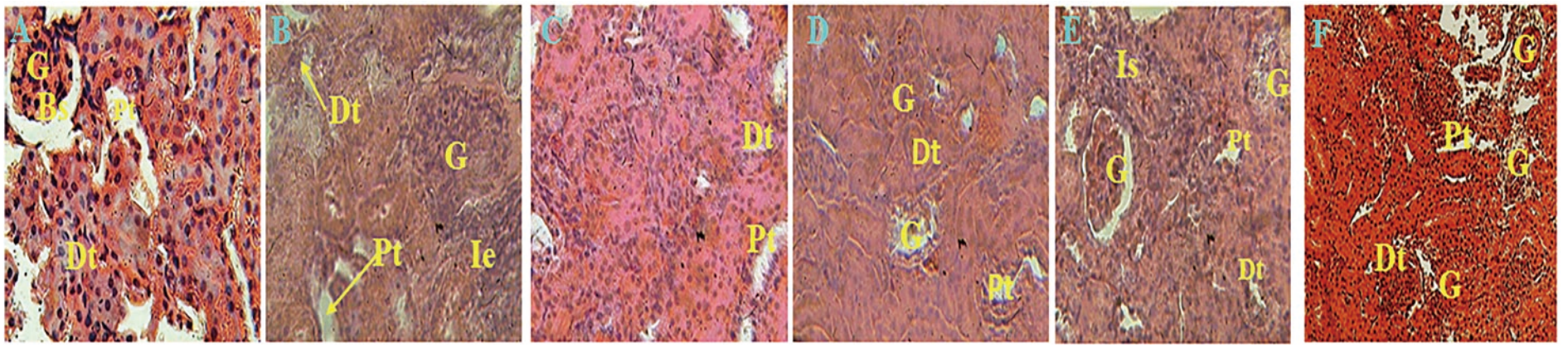

Figure 6. Effect of *Euphorbia cordifolia* on kidney morphology of malaria-infected mouse. (A) normal control; (B) malaria control; (C) chloroquine control; infected mouse treated with plant extract at the (D) dose of 100 mg/kg, (E) dose 200 mg/kg and (F) 400 mg/kg. G=Glomerulus; Bs=Space of Bowman's capsule; Pt=Proximal convoluted tubule; Dt=Distal convoluted tubule; Ie=Interstitial edema. H&E 20×.

sinusoids and abundant malarial pigment in the parenchyma. The liver of infected mice treated with *E. cordifolia* aqueous extract at the dose of 100 mg/kg for 5 d (Figure 5D) showed a large inflammatory sites around the centrolobular vein, malaria pigment and a clarification in sinusoids in the parenchyma. These architectural changes were considerable reduced at the doses 200 (Figure 5E) and 400 mg/kg (Figure 5F).

The examination of the kidney of healthy mice (Figure 6A) presented a normal architecture with the presence of Bowman's capsule, proximal tubule and distal tubule. The histological section of the kidneys of the infected and untreated mouse (Figure 6B) showed an architectural change in renal tissue with absence of Bowman's capsule and interstitial oedema. The renal tissue of the infected mice treated with chloroquine at 10 mg/kg (Figure 6C) showed an anatomical structure fairly close to that of normal mouse, although some inflammatory sites were still present. In mice treated with the plant extract at the dose of 100 mg/kg (Figure 6D), some alterations were observed such as the absence of bowman space in the glomerulus; while the dose of 200 mg/kg (Figure 6E) and 400 mg/kg (Figure 6F) restored the normal architecture.

4. Discussion

Malaria is one of most prevalent disease under the tropics with high mortality and morbidity. Given the current trend in parasite drug resistance, there is an urgent need to search for alternative therapies for management of malaria. The results achieved in this study showed that the intraperitoneal inoculation of 10^6 of *P. berghei*-parasitized RBC resulted in a 46% increase of parasitaemia 8 d after inoculation in malaria control group. Conversely, the oral administration of the aqueous extract of *E. cordifolia* to infected animals for 5 d significantly reduced the parasitaemia level indicating the antimalarial property of this plant. This antimalarial potency of *E. cordifolia* extract could be associated with the presence of alkaloids and phenols acting by inhibition of phosphodiesterase and known as inhibitors of the fatty acids biosynthesis pathway needed for successful growth of malaria parasite[14-16]. The decrease of the blood glucose level in the plasmodial infection is well described during malaria and may be due to an impairment of hepatic gluconeogenesis, leading to an important drop of blood glucose level in untreated individuals[17]. Haematological alterations resulting in the decrease of RBC count, Hb level, Hct, mean of RBC haemoglobin rate, and mean haemoglobin concentration levels observed in infected animals are some conventional signs of anaemia[18]. During malaria infection, *Plasmodium* invades the host cells and shorten the lifespan of RBC through the digestion of Hb using glucose, oxygen and hemozoin formation and finally the bursting of the erythrocytes during the development of their asexual blood stage[19-21]. The treatment of animals with the plant extract significantly improved the haematological parameters, partially restored the blood glucose level, showing that the *E. cordifolia* extract is capable to inhibit the growth of parasites as confirmed by the decrease of parasitaemia and also reduce some damages caused by malaria and therefore, protect animals from death. The anti-anaemia activity of plant extract could exert by promoting the regeneration of tissues, decreasing the permeability of blood capillaries or increasing the resistance of cells to haemolysis[22], through alkaloids, tannins, flavonoids and anthraquinones known to improve the resistance of erythrocytes to the haemolysis induced by *Plasmodium*[23]. Phenol components play an important role against oxidative damage in RBCs, through a possible interaction between flavonoids and RBC membrane, generally targeted by lipid peroxidation[24]. A significant increase in WBC was recorded in infected mice as otherwise previously reported in malaria infection[25]. This may be attributed to the parasite and their pigment (hemozoin) playing a key role in malaria immunopathology[26]. The leucocytosis significantly decreased in correlation to the reduction of the parasitaemia after treatment with plant extract. The level of platelets count significantly decreased in *P. berghei*-infected animals. The thrombocytopenia in malaria infection could be associated to platelets consumption as a part of disseminated intravascular coagulation, an excessive removal of normal or immunologically deranged platelets by the hypertrophied reticuloendothelial system or as a result of splenic pooling of platelets and decrease in platelet life span[27]. The treatment of infected mice with the *E. cordifolia* extract significantly protected infected mice from the immune cells and platelets dysregulation, through the presence of alkaloids, tannins and phenolic components that act by detoxification of enzymes and modulating effect on the immune system[28].

The tissue hypoxia cause by malaria infection induces an activation of the natural host defence that generates large amounts of reactive oxygen species, causing an imbalance between the formation of oxidizing species and the activity of antioxidants which can lead to the death of the parasites[29,30]. The oxidative stress induction was also described as electrons produced during the oxidation of Fe^{2+} into Fe^{3+} following the Hb degradation by the parasites[31]. Moreover, antioxidant enzymes (catalase and SOD), and molecules such as proteins significantly decreased while lipid peroxidation (MDA) increased during malaria infection[32]. Meanwhile, the plant extract induced a protective effect by increasing the activity of antioxidant enzymes and molecules. This activity can be explained by the presence of phenols, flavonoids and tannins that are able to trap free radicals[33].

In the present study, higher level of transaminases and hyperbilirubinemia were observed in untreated infected animals.

Temporary hepatic dysfunction is a current change in malaria infection characterized by the increase of relative liver weight and liver enzyme activities. The changes in liver may result from alteration in blood flow through the organ as parasitized RBC adhere to endothelial cells, blocking the sinusoids and obstructing the intrahepatic blood flow. Likewise, liver damage could be also due to the leakage of some hepatic cells which were killed or injured by the immune response progress and/or by abnormal cell activation induced by the parasites[34]. The role of radical oxygen species in the liver impairment has been linked to the leakage of some enzymes. This observation is emphasized by a significant increase in MDA and the level of transaminases. Hyperbilirubinemia caused the impairment of drainage capacity in the liver because of reticulo-endothelial blockage and disturbance of hepatocyte microvilli[35]. Likewise, the histological analysis of liver from malaria infected animal showed a significant enlargement. This reticuloendothelial hyperplasia expressed by general architectural disorganization of liver with inflammatory sites, hepatocyte necrosis, vascular congestion, malarial pigment contained into the Kupffer cells and bile stasis is suggestive of inflammatory reaction in the tissue. The bile stasis is due to impairment of bilirubin transport because of reticulo-endothelial blockage and disturbance of hepatocyte microvilli[36]. Our study showed that the daily administration of aqueous extract of *E. cordifolia* improved not only serum transaminases level but also significantly decreased the concentration of MDA. *E. cordifolia* could exert its action by inactivating lipid peroxidation reactions and by reducing free radical generation due to its antimalarial action.

Otherwise, the increase of creatinine level recorded in the infected animal is a conventional sign of renal damage expressing an acute renal failure. The renal damage observed in the malaria infection could be multifactorial in origin, including direct effect of parasite when attaching to a specific receptor on the cell membrane which results in pathophysiological alterations followed by renal ischaemia and acute tubular necrosis[37]. The formation of antigen-antibody complexes and their deposition in the basal membrane causes an overload of the kidney and a reduction in the purification capacity of this organ, which is already abnormally stressed by the increase of haemolysis. This renal failure was manifested by interstitial oedema and tubular thinning which was corrected by the treatment with the plant extract, indicating the antimalarial activity of the aqueous extract of *E. cordifolia*.

The intraperitoneal inoculation of *P. berghei* in mice induced plasmodial infection with higher blood parasitaemia which led to anaemia, thrombocytopenia, hypoglycaemia, alteration of hepatic and renal function and has generated oxidative stress with tissue damage. The administration of *E. cordifolia* aqueous extract for 5 d reduced parasitaemia, prevented anaemia, thrombocytopenia, blood glucose level decrease, oxidative stress and tissue damage and restored hepatic and renal function. The results of the present study demonstrate the antimalarial activity of the aqueous extract of *E. cordifolia* supporting its use in Cameroonian traditional medicine to cure malaria. Studies are in progress to investigate the mechanisms of the plant extract activity on the inhibition of parasite development in infected mice.

Acknowledgments

The authors are grateful to Mr Nana Victor, botanist at the Cameroon National Herbarium for the authentication of the plant species, the "Pathologie-Cytologie-Development" team through Mrs Catherine Cannet for histological analyses.

Authors' contributions

RKG, TD and FFB designed the study and collected the plant; RGK, MJTN, LRTY and carried out the study. RKG and MJTN drafted the manuscript. RKG, TNT, PVTF and MBTT performed calculations and data analysis. PVTF, DT and FFB critically revised the manuscript. All the authors contributed to the final version of the manuscript.

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