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Original Article

Antimalarial Effects of the Aqueous Extract of *Entandrophragma angolense* Bark on *Plasmodium berghei* Infection in Mice

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ABSTRACT

Background: Research for new antimalarial drugs remains a permanent quest for the control of malaria. **Objective**: The present study investigates the effects of the aqueous extract of *Entandrophragma angolense* bark on *P. berghei*-induced malaria in mice. **Methods:** Eight weeks old mice, were intraperitoneally infested with 200 µl of blood, containing 1x10^6 *P. berghei*-infected-erythrocytes. Parasitaemia was determined using a 10% giemsa stained blood smear read under optical microscope (x100). The infected animals were randomized into 5 groups of 10 animals each and daily treated for 5 days with the plant extract at 125, 250 and 500 mg/kg. The normal control and malaria control received water while the chloroquine control was treated with 10 mg/kg of chloroquine. Body weight, parasitaemia and survival time were monitored daily during treatment and follow up periods. Five animals from each group were sacrificed under anaesthesia at the end of treatment (d8) and after the follow up period (d28). Venous blood was used for haematological and biochemical tests. Organs (liver, kidneys and spleen) were also collected for biochemical and histological analyses. **Results:** Administration of the aqueous extract of *E. angolense* bark to infected mice significantly inhibited parasite development ($p < 0.001$) with ED_{50} estimated at 25.32 mg/kg. The extract prevented animal from death, body weight loss, anaemia, leucocytosis, high transaminases (ALT and AST), high bilirubin, creatinine and MDA levels, oxidative stress and anatomical alteration in organs as compared to the malaria control. **Conclusion:** The *E. angolense* bark possesses antimalarial properties, supporting its use in traditional medicine to treat malaria.
Key words: Antiplasmodial activity, *E. angolense*, Malaria infection, Mice, *P. berghei*.

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INTRODUCTION

Malaria remains undoubtedly the most serious disease that afflicts mankind throughout the world with the greatest impact in the Sub Saharan Africa. In 2017, the disease burden was estimated at 219 million cases leading to approximately 435000 deaths a year over the world, in which 86% were children under 5 years of age.[1] The clinical signs of malarial illness result from the asexual stage of the malaria parasite which induces a serious red blood cells disorders making malaria a potential multisystem disease, as every organ is reached by the blood.[2] Clinical manifestations of malaria include fever, anaemia, thrombocytopenia, chills, headache, vomiting, muscle ache, anorexia, rigor, diarrhoea, abdominal discomfort, hypoglycaemia, coma associated with increased intracranial pressure (cerebral malaria), resulting from the rupture of the infected erythrocytes and the release of putative malaria toxins, which activate peripheral blood mononuclear cells and stimulate the release of different mediators.[3] Different control strategies to the disease has been developed, but the emergence and spread of *Plasmodium* multidrug-resistant strains has led to therapeutic failure, rising the urgent need to search for new, safe and effective antimalarial drugs. The two highly effective antimalarial drugs Quinine and Artemisinin derived from plants traditionally used to fight against malaria.[4,5] Indeed, various plant species are currently used for the malaria treatment in Cameroon[6] such as *Entandrophragma angolense*,(Welwitsch) C.DC (Meliaceae)[7] and could represent a promising source of new antimalarial drugs. Although its *in vitro* antiplasmodial effect against *Plasmodium falciparum* W2 strain has been demonstrated,[8] the *in vivo* antimalarial activity of the plant is yet to be confirmed. The present study reports the *in vivo* antimalarial activity of the aqueous extract of *Entandrophragma angolense* stem bark in *Plasmodium berghei*-induced malaria in mice.

MATERIALS AND METHODS

Plant material

Entandrophragma angolense plant was harvested at Dschang, Menoua Division (West Region, Cameroon) in June 2016. Plant parts such as stem bark were collected and authenticated by Mr Nana Victor, a botanist at the National Herbarium of Cameroon where a voucher specimen of the plant has been deposited under the number 29933/HNC.

Experimental animal

Eight weeks old female mice Balb/c weighing between 22 to 25 g were used for experimentation.

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The animals were housed in sanitary cages, at room temperature (23 ± 2 °C) on a 12 h light-dark natural cycle, in the animal house of the Faculty of Science of University of Yaoundé I (Cameroon). Water and food were given *ad libitum* during the experiment. The study was conducted with the approval of 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 N° FWA-IRD 0001954).

Parasites

Plasmodium berghei MRA 406 parasites from Bei Resources (USA) were used for the study. Cryopreserved parasites stored at -80°C were thawed and maintained by serial passage of blood from infected mice to naive mice.

Extraction procedure

The fresh stem bark of *Entandrophragma angolense* was cut into small pieces, air-dried in a shaded area and powdered material (500 g) were macerated in 5 L of distilled water for 24 hours, following the traditional healer instructions. The mixture was filtered and lyophilised to yield 78.81 g of crude extract (yield = 15.36% w/w). The extract was stored at -20ºC until use.

Phytochemical screening

Phytochemical screening of extract was performed according to standardized protocols to investigate the presence of some secondary metabolites such as flavonoids, alkaloids, saponins, tannins, phenols, steroids, triterpenes, glucosides and anthocyanins.[9-12]

In vivo antimalarial assay

The study was carried out on 60 female mice. They were randomly selected then 50 of them were infected through intraperitoneal inoculation with 0.2 mL of mice blood containing 1 x10^6 parasitized erythrocytes. Three days post-inoculation, thin blood smears were made from a tail cut of each mouse, fixed for 5 minutes using methanol, stained with 10% Giemsa stain in Phosphate buffer, pH 7.2 and examined microscopically under oil immersion (x100) for assessment of parasitemia. Infected animals were then divided into 5 groups of ten animals each and treated as followed: A normal control group consisted to healthy animals, treated with distilled water (10 mL/ kg). Three groups of infected animals treated with the plant extract at the doses of 125, 250 and 500 mg /kg, respectively. The malaria control received distilled water while the chloroquine control was treated with chloroquine sulfate (Sigma, Germany) at the dose of 10 mg/kg. Different solutions were orally administered once a day for 5 consecutive days during which, body weight was recorded and parasitemia was determined as previously described. At five days post-treatment, five animals from each group were sacrificed, while five others were followed up for further 20 days. During the treatment period, survival time, body weight and parasitemia were daily recorded. The survival time was recorded till the end of the follow-up period (day 29) and surviving animals were sacrificed under anesthesia of ketamine (30 mg/kg) and diazepam (10 mg/kg).

The average parasitemia was determined using following formula:

Parasitemia (%) = (Number of parasitized RBC count / Total number of RBC)× 100.[13]

The percentage inhibition of parasitemia for each dose of the plant extract was calculated as

Inhibition %= [(Parasitemia in malaria control – Parasitemia in the given group)/Parasitemia in malaria control] × 100

Artery-venous blood was collected in EDTA and dry tubes. Blood in EDTA tube served for haematological analysis while blood in dry tube was centrifuged at 360g at 4°C for 15 minutes and the serum was collected and stored at -80°C for biochemical analysis. The liver, kidneys and spleen were removed and weighed. Homogenates (20%) of liver and kidney samples were prepared in Tris-HCl buffer (pH 7.4). Organs were crushed and then the mixture was centrifuged at 360 g at 4°C for 20 min. The supernatant was collected and stored at -80 °C until biochemical tissue analysis. The remaining organs (liver, kidney and spleen) were fixed in 10% buffer-formalin for histological analysis.

Evaluation of the effects of extract on physiological changes in animal

Hematological and biochemical analyse

Hematological analysis was performed on total blood sample to determine some parameters as red blood cells (RBC) count, white blood cells (WBC) count, platelets (PLT) count, haemoglobin (HGB) level and leucocytes species (lymphocytes (LYM), granulocytes (GRA) and monocytes (MON)) using hematometer Xp 3600 (Italy). Biochemical analyses were focused on glycaemia, creatinine, bilirubin, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) concentration according to the protocols provided by Fortress Diagnostics commercial kits (UK). The supernatant collected from organ was used to investigate total proteins,[14] catalase,[15] superoxide dismutase SOD,[16] malondialdehyde (MDA),[17] and reduced glutathione (GSH).[18]

Histopathological analysis of organs

The liver, kidney and spleen of each animal fixed in 10 % buffered formalin were dehydrated through passage in gradual concentrations of alcohol and then embedded in paraffin. Serial paraffin sections at 5 µm were stained with haematoxylin and eosin (HE) for examination under light microscopy brand Olympus and photography in objective 20 auricular 100 (HEx200).

Statistical analysis

The results were expressed as mean ± standard deviation (SD). The statistical analyses were performed using the Analysis of Variance (ANOVA), followed by the Tukey post-test using GraphPad Prism version 7.00 software. Values were considered significant for $p < 0.05$.

RESULTS AND DISCUSSION

Effects of Entandrophragma angolense aqueous extract on survival time of infected animals

Figure 1 summarizes the effects of plant extract on the mean survival time of animals for 28 days of experimentation. It appears that the intraperitoneal inoculation of 200 µL of 1×10^6 parasitized-erythrocytes to healthy mice significantly induced death in untreated animals from day 10th to day 26th with the loss of mice in malaria control ($p < 0.001$) group compared to normal control. Contrarily, the administration the plant extract at any dose as well as chloroquine prevented death in infected animals ($p < 0.001$) (Figure 1).

Effects of *E. angolense* on the body weight evolution of infected-animals

Untreated malaria infected mice significant put off body weight by 26.22 % ($p < 0.001$) with regards to normal control group (Figure 2) at day 8 and over the 20 days of follow-up till the death of all animals at the day 26. The daily administration of the aqueous extract of *E. angolense*

for five days, dose dependent prevented the decrease of body weight ($p < 0.001$) by 25.98%, 36.90% and 46.08% at the respective doses of 125, 250 and 500 mg/ kg related to malaria control. The administration of the aqueous extract of *E. angolense* and chloroquine for 5 days then followed up for 20 days significantly curbed body weight ($p < 0.001$) loss as compared to the malaria control and normal control.

Effects of aqueous extract of *Entandrophragma angolense* on the parasitaemia

The effects of aqueous extract of *E. angolense* stem bark on parasitemia count of infected mice are shown in Figure 3. The intraperitoneal inoculation of *P. berghei*-parasitized-erythrocytes to healthy animals showed after 3 days post induction, an average parasitemia of 8.86 % that, without treatment rose up to 18.08% at the day 8 then 60.66% at the day 26, accompanying with the death of all animals in the malaria control group. The daily administration of the extract for 5 days resulted

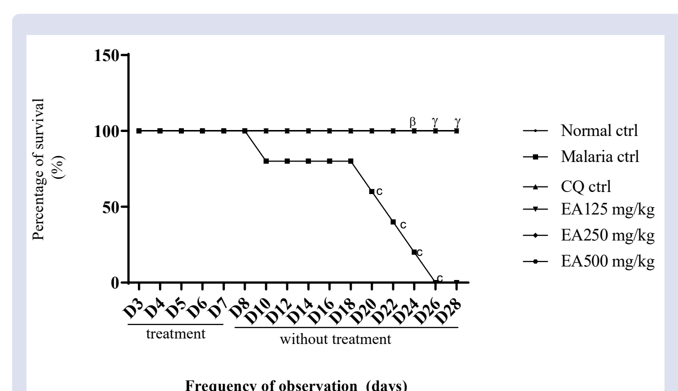

Figure 1: Effects of *E. angolense* extract on the survival time of infected animals for 28 days of experimentation.

Points express mean ± SD, n = [5-10], $^{c}p < 0.001$ significant difference as compared to normal control (Normal ctrl), Normal ctrl = uninfected animal receiving distilled water. $^{\beta}p < 0.01$, $^{\gamma}p < 0.001$ compared to malaria control (malaria ctrl) = infected mice treated with distilled water. CQ ctrl = infected mice treated with chloroquine (10 mg/kg). EA = infected animals treated with aqueous extract of *E. angolense* at 125 mg/kg (EA125 mg/kg), 250 mg/kg (EA250 mg/kg) and 500 mg/kg (EA500 mg/kg).

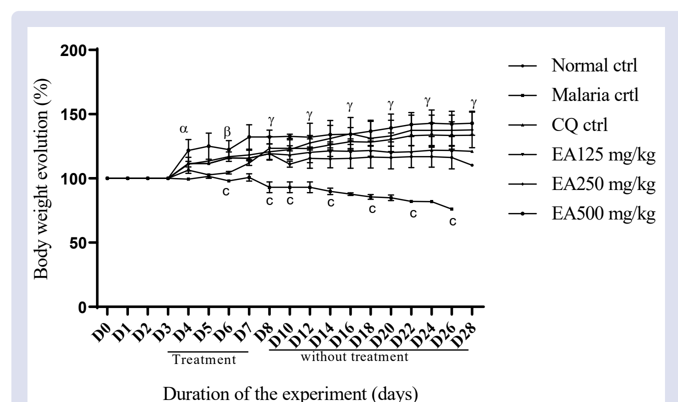

Figure 2: Effects of *E. angolense* extract on the body weight change in *P. berghei*-infected animals.

Points represent mean ± SD, n = [5-10], $^{c}p < 0.001$ compared to normal control (Normal ctrl); $^{\gamma}p < 0.001$ compared to Malaria control; Normal ctrl = Healthy mice receiving distilled water (10 mL/kg), Malaria ctrl = infected mice treated with distilled water. CQ ctrl = infected mice treated with chloroquine (10 mg/kg). EA = infected animals treated with aqueous extract of *E. angolense* at 125 mg/kg (EA125 mg/kg), 250 mg/kg (EA250 mg/kg) and 500 mg/kg (EA500 mg/kg).

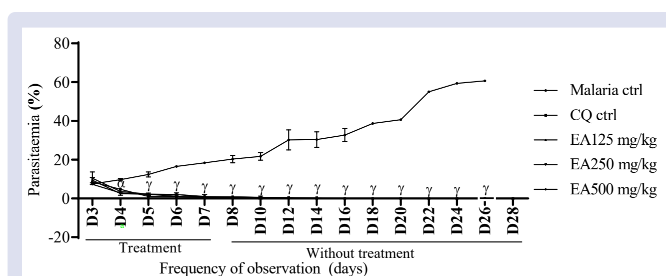

Figure 3: Effects of the aqueous extract of *E. angolense* on parasitemia in *Plasmodium berghei*-infected mice.

Each point represents mean ± SD, n = [5-10], $^{\alpha}p < 0.05$, $^{\gamma}p < 0.001$ significant difference as compared to malaria control (malaria ctrl) = infected mice treated with distilled water (10 mL/kg); CQ ctrl= Infected mice treated with chloroquine (10 mg/kg); EA 125, 250 and 500 mg/kg = infected mice and treated with *E. angolense* extract at the respective doses of 125, 250 and 500 mg/kg.

in the significant decrease in parasiteamia count by 65.44% ($p < 0.05$), 70.70% ($p < 0.01$) and 70.68% ($p < 0.01$) at the respective doses of 125, 250 and 500 mg/kg, compared to the malaria control. The percentage inhibition of parasite at the day 8 was lightly dose-dependent with 96.32%, 98.16% and 98.89% respectively at the dose 125, 250 and 500 mg/kg. The effective dose-50 (ED_{50}) of aqueous extract of *E. angolense* was estimated at 25.32 mg/kg. A complete parasite clearance was achieved at the day 10, day 14 and day 16 at the respective dose of 500, 250 and 125 mg/kg.

Effects of the aqueous extract of *E. angolense* on some hematological parameters

The effects of the aqueous extract of *E. angolense* on some hematological parameters in *P. berghei*-infected animals after 8 and 28 days of experimentation are summarized in Table 1.

The administration of the plant extract restored hematological parameters by significantly increasing RBCs count by 97.28% ($p < 0.01$), 83.68% ($p < 0.01$) and 103.92% ($p < 0.001$); HGB level by 117.47% ($p < 0.01$), 107.76% ($p < 0.01$) and 143.68% ($p < 0.001$); HCT rate by 83.11% ($p < 0.01$), 86.16% ($p < 0.05$), and 117.17% ($p < 0.001$); MCHC by 20.00% ($p < 0.01$), 22.40% ($p < 0.01$) and 16.76% ($p < 0.05$) and LYM by 9.80% ($p < 0.05$), 7.84% ($p < 0.01$) and 10.68% ($p < 0.05$) at the respective doses of 125 , 250 and 500 mg/kg compared to malaria control. The extract has also induced a significant decrease in the total WBC count by 23.94%, 44.36% and 90.84% ($p < 0.01$); GRA count by 56.56% ($p < 0.01$), 50.50% ($p < 0.05$) and 54.54% ($p < 0.01$) and monocytes count (MON) by 58.02% , 59.25% and 70.37% ($p < 0.001$) compared to malaria control (Table 1). At the end of the follow up post treatment (day 28), no significant change in hematological parameters (RBC, HGB, HCT, MCHC, WBC, LYM, GRA, and MON) was observed in animals treated with plant extract and normal control. However, a significant decrease in hemoglobin was recorded in chloroquine control ($p < 0.05$) compared to the plant extract. No significant change was observed in hematological parameters in test groups between the days 8 and 28 of experiment.

Effects of the aqueous extract of *E. angolense* on the blood glucose, liver and kidney functions

Table 2 summarizes the effects of *E. angolense* on blood glucose, some liver and kidney parameters functions in *P. berghei*-infected mice. *P. berghei*-infected mice showed after 8 days a significant decrease of glycaemia by 11.16% ($p < 0.01$) and proteins level by 46.33% ($p < 0.001$) and significant increase in ALT and AST activities by 10.58% ($p < 0.05$) and 51.47% ($p < 0.001$), respectively, in bilirubin level by 63.50 % (p

Table 1: Effects of the aqueous extract of *Entandrophragma angolense* on heamatological parameters after 8 and 28 days of follow-up.

Parameters	Nor ctrl	Mal ctrl	CQ ctrl	EA125 mg/kg	EA250 mg/kg	EA500 mg/kg
			Day 8			
RBC (10^3 /µL)	5.90 ± 0.28	3.31 ± 0.41^{a}	5.40 ± 0.45^{γ}	6.53 ± $0.23^{ß}$	6.08 ± 0.13^{α}	6.75 ± 0.64^{γ}
HGB (g/dL)	11.00 ± 0.50	5.15 ± 0.52^{c}	10.23 ± $0.58^{ß}$	11.20 ± $0.52^{ß}$	10.70 ± $0.50^{ß}$	12.55 ± 0.74^{γ}
HCT (%)	31.58 ± 1.69	17.06 ± 1.83^{b}	30.32 ± 2.60^{μ}	31,24 ± $1.70^{ß}$	31.76 ± 0.53^{α}	37.05 ± 1.93^{γ}
PLT (10^3 /µL)	257.20 ± 3.85	222.40 ± 8.76	244.75 ± 5.50	266.80 ± 5.49	202.80 ± 1.81	234.20 ± 7.15
WBC (10^3/µL)	3.26 ± 0.23	5.00 ± 0.34^{b}	2.20 ± 0.22^{μ}	3.00 ± 0.38^{α}	3.47 ± 0.72^{α}	3.15 ± 0.30^{α}
LYM (10^3/µL)	2.67 ± 0.05	1.00 ± 0.17^{b}	3.48 ± 0.33^{γ}	2.46 ± 0.33^{α}	2.50 ± 0.10^{α}	2.26 ± 0.11^{α}
GRA (10^3/µL)	0.41 ± 0.04	0.99 ± 0.08^{b}	0.43 ± $0.09^{ß}$	0.43 ± $0.05^{ß}$	0.49 ± 0.14^{α}	0.45 ± $0.11^{ß}$
MON (10^3/µL)	0.31 ± 0.00	0.81 ± 0.05^{c}	0.31 ± 0.07^{γ}	0.34 ± 0.01^{γ}	0.33 ± 0.02^{γ}	0.24 ± 0.00^{γ}
			Day 28			
RBC (10^3 /µL)	5.56 ± 0.97	Nd	4.13 ± 0.00	6.60 ± 0.00	5.09 ± 0.05	5.96 ± 0.20
HGB (g/dL)	12.68 ± 1.39	Nd	9.41 ± 0.00	12.57 ± 0.06	11.13 ± 0.54	11.5 ± 0.31
HCT (%)	33.10 ± 4.10	Nd	30.49 ± 0.00	35.55 ± 0.58	32.90 ± 1.79	31.20 ± 0.20
PLT (10^3 /µL)	342.40 ± 6.62	Nd	253.00 ± 0.00	212.00 ± 0.00	229.50 ± 2.05	209.00 ± 0.00
WBC (10^3/µL)	3.44 ± 0.28	Nd	2.80 ± 0.00	3.22 ± 0.26	3.15 ± 0.30	3.20 ± 0.66
LYM (10^3/µL)	3.07 ± 0.03	Nd	2.70 ± 0.21	2.49 ± 0.00	2.74 ± 0.18	2.52 ± 0.01
GRA (10^3/µL)	0.38 ± 0.04	Nd	0.34 ± 0.00	0.42 ± 0.00	0.40 ± 0.00	0.41 ± 0.00
MON (10^3/µL)	0.32 ± 0.00	Nd	0.31 ± 0.00	0.30 ± 0.03	0.31 ± 0.03	0.33 ± 0.00

Value represents mean ± SD, n = [5-10], Nd = not determined, $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$significant difference from the normal control (healthy mice receiving distilled water at 10 mL/kg, $^{\alpha}p < 0.05$, $^{ß}p < 0.01$, $^{\gamma}p < 0.001$ difference as compared to the malaria control (Mal ctrl) =infected mice treated with distilled water for 5 days; CQ ctrl = infected mice treated with chloroquine (10 mg/kg).EA = infected mice treated with *E. angolense* extract at the doses of 125mg/kg (EA 125 mg/kg), 250 mg/kg (EA 250 mg/kg) and 500 mg/kg (EA 500 mg/kg). Day 8 and day 28 = data recorded after the respective days 8 and 28 of the experiment. Nd = not determined. RBC = red blood cells, HGB = hemoglobin, HCT = hematocrit, PLT = platelets, WBC = white blood cells, LYM = lymphocytes, GRA = granulocytes, MON = monocytes.

<0.001), in creatinine level by 73.95% (p <0.001) compared to the normal control.

However, the daily plant extract administration for five days resulted in significant increase in blood glucose level by 16.99%, 21.16% and 20.87% ($p < 0.001$) compared to the malaria control. In comparison to the normal control, it was observed a significant increase in glycaemia in infected animals treated with extract at the dose 250 mg/kg ($p < 0.01$) and 500 mg/kg ($p < 0.05$). After the 20 days of follow up (day 28), a significant increase in the blood glucose level was noted in the animals treated with the extract at 250 mg/kg ($p < 0.01$) while that of doses 125 and 500 mg/kg decrease compared to the normal control. Interestingly, the blood glucose level significantly decreased at the dose of 500 mg/kg at the day 28 ($p < 0.001$) compared to the day 8. No significant change was observed between test groups and chloroquine control group.

The daily intake of extract significantly prevented the rise of ALT activities by 28.35%, 27.17% ($p < 0.01$) and 38.19% ($p < 0.001$) and AST activities by 23.24% (p <0.05), 41.19% and 66.52% ($p < 0.001$) at the respective doses of 125, 250 and 500 mg/kg as compared to the malaria control. The AST concentration was significantly low in animals treated with the extract at the dose of 250 mg/kg and 500 mg/kg ($p < 0.01$) compared to those receiving the dose 125 mg/kg. At the end of follow up, no change was observed in ALT activities amongst animals. However, a significant low AST activity of 34.50%; ($p < 0.001$), 16.82% and 19.84% ($p < 0.05$) was recorded with the extract at the respective doses of 125, 250 and 500 mg/kg at the day 8 compared to the day 28**.**

A significant low creatinine concentration (p <0.001) by 74.31%, 60.03% and 72.37% was recorded at the days 8, respectively, at 125, 250, and 500 mg/kg compared to the malaria control. After the follow up period of 28 days, significant an increase in the creatinine concentration ($p < 0.01$) was observed at 125 mg/kg compared to normal control. It was also noted an increase in the creatinine concentration ($p < 0.01$) at the same dose between day 8 and day 28.

The bilirubin concentration significantly decreased at the day 8, in the treated mice with plant extract by ($p < 0.001$) whatever the extract dose compared to the normal control. No change was recorded compared to chloroquine control.

The test groups showed significant increase in protein levels ($p < 0.001$) by 53.33% and 54.54% at the respective dose of 250 mg/kg and 500 mg/kg compared to the malaria control. Proteins level was significantly enhanced in the extract at 250 and 500 mg/kg ($p < 0.001$) compared to the dose 125 mg/kg.

In comparison to the chloroquine control, it was observed a significant decrease in protein ($p < 0.05$) at 125 mg/kg. At day 28, protein concentration was restored in all treated groups and no change was observed among them.

Effects of aqueous extract of *Entandrophragma angolense* on some oxidative stress parameters

Figure 4 shows the effects of the aqueous extract of *E. angolense* on some anti-oxidative factors as superoxide dismutase (SOD) and catalase activities, reduced glutathione (GSH), nitrites (NO) and malondialdehyde (MDA) concentration in the liver and kidney of *P. berghei*-infected mice, treated for five days (day 8) and monitored for 20 days (day 28). *P. berghei*-infected mice displayed significant breakdown in SOD by 91.46% (p <0.001) in liver and 87.43% (p <0.01) in kidney, in reduced glutathione ($p < 0.001$) by 90.03% and 60.31%, in catalase activity by 65.78% and 60.64 % (p <0.001) in liver and kidney, respectively, in nitrites level by 50% ($p < 0.001$) in liver while the MDA concentration increased ($p < 0.001$) compared to the normal control after eight days. The single daily dose administration of the *E. angolense* bark extract resulted in a significant dose-dependent increase in SOD activity at the day 8, by 91.42% and 92.43% (p <0.001) in liver, by 50.41% and 55.66% (p <0.01) in kidney at the respective doses of 250 and 500 mg/kg compared to malaria control (Figure 4A). A significant enhancement in SOD activity ($p < 0.001$) was observed in liver of animals treated with plant extract at 250 and 500 mg/kg compared to the chloroquine control. The SOD activity significantly decreased in liver ($p < 0.001$) and in kidney ($p < 0.01$) of animals treated with extract at 125 mg/kg compared to those receiving extract at 250 and 500 mg/

Table 2: Effects of the aqueous extract of *Entandrophragma angolense* on blood glucose and some liver and kidney parameters function in *Plasmodium berghei*-infected mice.

	Normal ctrl	Malaria ctrl	CQ ctrl	EA125 mg/kg	EA250 mg/kg	EA500 mg/kg
			Day 8			
Blood glucose (mg/dL	114.50 ± 1.80	103.00 ± 2.21^{b}	123.40 ± 2.29$^{a,\gamma}$	120.50 ± 1.42$^{\gamma}$	124.80 ± 1.52$^{b,\gamma}$	124.50 ± 2.05$^{b,\gamma}$
ALT (UI/L)	61.21 ± 2.38	76.09 ± 0.53^{a}	59.31 ± 2.39^{ß}	55.92 ± 3.11^{ß}	56.84 ± 6.53^{ß}	48.24 ± 4.23$^{\gamma}$
AST (UI/L)	108.10 ± 1.68	222.78 ± 8.77^{b}	129.61 ± 8.85$^{\gamma}$	171.00 ± 2.10$^{\gamma}$	140.00 ± 7.11$^{\gamma}$	131.00 ± 7.10$^{\gamma}$
Bilirubin (mg/dL)	1.18 ± 0.01	3.23 ± 0,14^{c}	1.47 ± 0,05$^{\gamma}$	1.58 ± 0.01$^{\gamma}$	1.52 ± 0.03$^{\gamma}$	1.53 ± 0.02$^{\gamma}$
Creatinine (mg/dL)	0.51 ± 0.04	1.96 ± 0.04^{c}	0.56 ± 0.03$^{\gamma}$	0.50 ± 0.06$^{\gamma}$	0.90 ± 0.15$^{\gamma}$	0.54 ± 0.11$^{\gamma}$
Proteins (mg/mL)	0.65 ± 0.01	0.35 ± 0.04^{c}	0.68 ± 0.02$^{\gamma}$	0.49 ± 0.02$^{a,\mu,\pi}$	0.75 ± 0.03$^{\gamma}$	0.77 ± 0.03$^{\gamma}$
			Day 28			
Blood glucose (mg/dL	112.83 ± 1.35	nd	118.00 ± 0.00	113.50 ± 0.15$^{\varepsilon *}$	116.80 ± 1.64^{b}	111.55 ± 0.60$^{\phi\pi,\theta}$
ALT (UI/L)	61.33 ± 0.56	nd	55.81 ± 3.50	58.41 ± 2.12	57.02 ± 3.36	62.61 ± 2.33
AST (UI/L)	107 ± 1.39	nd	114.71 ± 5.94	111.99 ± 4.10$^{\theta,\#}$	116.45 ± 1.39$^{\times}$	105.28 ± 1.16$^{\times}$
Bilirubin (mg/dL)	1.36 ± 0.11	nd	1.86 ± 0.15^{b}	1.45 ± 0.02	1.57 ± 0.09	1.22 ± 0.02$^{\sharp}$
Creatinine (mg/dL)	0.51 ± 0.01	nd	0.56 ± 0.09	0.61 ± 0.06$^{b,\sharp}$	0.72 ± 0.07^{b}	0.55 ± 0.09
Proteins (mg/mL)	0.66 ± 0.02	nd	0.75 ± 0.05	0.59 ± 0.01$^{\sharp}$	0.66 ± 0.02	0.72 ± 0.04

Values represent mean ± SD, n = [5-10], $^{a}p <0.05$, $^{b}p <0.01$, $^{c}p <0.001$ significant difference from the normal control (normal ctrl), $^{ß}p <0.01$, $^{\gamma}p <0.001$ difference as compared to the malaria control (Malaria ctrl); $^{\mu}p < 0.05$, $^{\varepsilon}p < 0.01$, $^{\phi}p < 0.001$ difference compared to chloroquine control (CQ ctrl), $^{*}p < 0.05$, $^{\theta}p < 0.001$ difference in given parameter between d8 and d28. Normal ctrl = healthy mice receiving distilled water (10 mL/kg; Malaria ctrl = infected mice treated with distilled water; CQ ctrl = infected mice treated with chloroquine (10 mg/kg). EA = infected mice treated with *E. angolense* extract at the doses of 125mg/kg (EA 125 mg/kg), 250 mg/kg (EA 250 mg/kg) and 500 mg/kg (EA 500 mg/kg). Day 8 and day 28 = data recorded after the respective days 8 and 28 of the experiment. nd = not determined. AST = aspartate aminotransferase, ALT = alanine aminotransferase

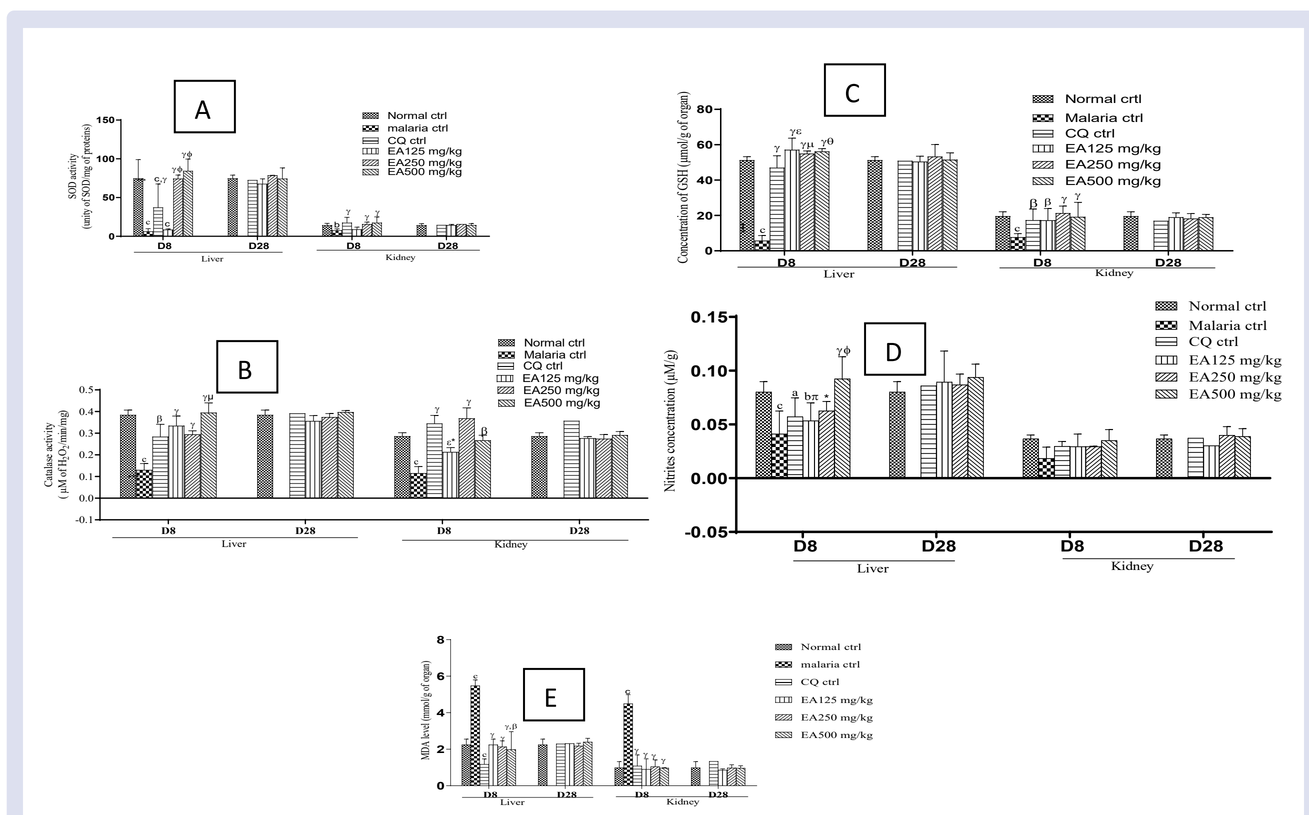

Figure 4: Effects of *Entandrophragma angolense* aqueous extract in some oxidative parameters in liver and kidney A (SOD), B (Catalase), C (Glutathione), D (nitrites) and E (MDA) of *Plasmodium berghei*-infected animals.

Each bar represents mean ± SD, n = [5-10], $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p <0.001$ significant difference as compared to the normal control (Normal ctrl); $^{ß}p < 0.01$, $^{\gamma}p < 0.001$ difference from the malaria control (malaria ctrl). $^{\mu}p < 0.05$, $^{\varepsilon}p < 0.01$, $^{\phi}p < 0.001$ difference as compared to the CQ control; $^{*}p < 0.01$, $^{\pi}p < 0.001$difference between test groups. Normal control (Normal ctrl) = healthy mice receiving distilled water. Malaria control (Malaria ctrl) = infected mice treated with distilled water for 5 days; Chloroquine control (CQ ctrl) = infected mice treated with chloroquine at 10 mg/kg; EA = infected mice treated with *E. angolense* extract at 125 mg/kg (EA125 mg/kg), 250 mg/kg (EA250 mg/kg) and 500 mg/kg (EA500 mg/kg). Day 8th and day 28th = data recorded at the respective days 8 and 28 of the experiment.

kg. After the follow-up period of 20 days, no significant change in SOD activity was observed in the liver as well as in kidneys of animals treated with the extract.

The extract induced significant increase in catalase activity ($p < 0.001$) by 60.60%, 55.17% and 66.66% in the liver, by 47.61% ($p < 0.05$), 69.44% ($p < 0.001$) and 57.69% ($p < 0.001$) in the kidney, at doses of 125, 250 and 500mg/kg respectively, compared to the malaria control (Figure 4B). In comparison to the chloroquine control, a significant decrease in catalase activity was observed in kidney ($p < 0.01$) of animal treated with extract at 125 mg/kg. Likewise, it was noticed a significant enhancement in catalase activity in the liver of animal treated with extract at 500 mg/kg ($p < 0.05$). A significant decrease in renal catalase activity was recorded in animals treated at 125 mg/kg ($p < 0.001$) and 500 mg/kg ($p < 0.05$) compared to those treated at 250 mg/kg. At the day 28, a significant decrease in catalase activity ($p < 0.05$) was observed in animals treated with plant extract compared to the chloroquine control.

The plant extract administration resulted in a significant restoration in glutathione level by an increase of 91.05%, 90.71% and 90.90% ($p < 0.001$) in the liver and by 41.75% ($p < 0.01$), 63.57% and 59.50% ($p < 0.001$) in the kidney at the respective doses of 125, 250 and 500 mg/kg compared to the malaria control (Figure 4C). It was observed a significant increase in glutathione concentration in the liver of animals treated with plant at the dose of 125 mg/kg ($p < 0.01$), 250 mg/kg ($p < 0.05$) and 500 mg/kg ($p < 0.001$) compared to the chloroquine control. After 20 further days of the follow-up, no change in glutathione level was observed among the experimental groups.

The extract absorption led to significant increase in hepatic nitrites level by 33.33% ($p < 0.05$) and 55.50% ($p < 0.001$) at the respective doses of 250 and 500 mg/kg compared to malaria control (Figure 4D). Compared to the normal control, treated animals with chloroquine (10 mg/kg) or plant (125 mg/kg) resulted in significant decrease in nitrites level by 37.50% ($p < 0.05$) and 40.01% ($p < 0.01$), respectively, in the liver. Among the animals treated with plant extract, significant increase in nitrites concentration was observed at the hepatic level in group receiving 500 mg/kg compared to those treated with the 125 and 250 mg/kg ($p < 0.001$) and chloroquine. No significant change in nitrites rate was observed in kidney of experimental animals. After additional 28-days follow-up, the nitrite levels were restored in all experimental groups.

The daily administration of a single dose of *E. angolense* extract for five days induced a significant decrease in MDA concentration in both liver and kidney by 59.02%, 61.02% and 63.83% ($p < 0.001$) in the liver and by 79.91%, 76.75% and 74.42% ($p < 0.001$) in the kidneys, at the respective doses of 125, 250 and 500 mg/kg compared to the malaria control (Figure 4E). A significant decrease in MDA concentration in the liver and kidney was recorded in chloroquine control ($p < 0.001$) compared to the malaria control. A significant increase in MDA liver ($p < 0.01$) was observed at the extract dose of 500 mg/kg compared to chloroquine control. No change in MDA level was observed within animals treated with plant extract. The hepatic and renal MDA levels did not changed among the experimental animals after 28 days of follow-up.

Effects of *Entandrophragma angolense* aqueous extract on some organ architecture

Effects on the liver

Figure 5 illustrates micrography of the liver section in infected mouse treated with the aqueous extract of *E. angolense* for 5 days (day 8) then followed up for 20 days (day 28). On the days 8 and 28, the liver section of healthy mice presents a normal parenchyma with a well differentiated portal vein, bileduct, hepatic artery, hepatocytes separated by sinusoidal capillaries where Kupffer cells are well observed (Figures 5A and A'). The liver section of malaria control mice at the day 8 shows major damage in the tissue with aclarified parenchyma, an inflammatory focus spread along the peripheral portal space surrounded by leukocyte infiltrations and vascular congestion in the hepatic artery, Kupffer cells were stained by malarial pigment (Figure. 5B). The micrograph of the liver of chloroquine control presented a portal vein, a bile duct, a hepatic artery, hepatocytes, sinusoidal capillaries, Kupffer cells containing malarial pigment and an inflammatory zone along centrilobular vein (Figure 5C), which were no longer observed at the 28th (Figure 5C'). It was observed at day 8 in the liver of infected mice treated with the aqueous extract of *E. angolense* (125 mg/kg), an undisclosed inflammatory focus with localized leukocyte infiltration and Kupffer cells containing malarial pigment (Figure 5D). These changes disappeared at the day 28 (Figure 5D'). The micrography of the liver of infected animal receiving the plant extract at the dose 250 and 500 mg/kg presented no change in the tissue with the architecture quite similar to that of a normal control, as well as on day 8 (Figure 5E) and on day 28 (Figure 5E').

Effects on the kidney tissue

Figure 6 shows the effects of E. angolense extract on the photomicrography of the kidney section of *P. berghei*-infected mouse at day 8 and day 28. It was observed in the kidney section of healthy mouse presents a normal appearance of the renal parenchyma where glomerulus, Bowman's space, proximal and distal tubules and the collector tubes are well differentiated (Figure 6A). In the infected mouse, it was noted some alteration in kidney tissue marked by the absence of urine space, clarification in the tubules and some inflammatory sites (Figure 6B). The treatment of infected mouse with chloroquine (10 mg/kg) or with plant extract (125, 250 and 500 mg/kg) protected against anatomic damage as observed in the kidney of malaria control. The kidney section shows normal appearance of the renal parenchyma with glomerulus, Bowman's space, proximal and distal tubules are distinctly observed both on day 8 (Figures 6 C, D, E, F) and on day 28 (Figures 6 C',D', E', F').

Effects on the spleen tissue

The effects of the aqueous extract of *E. angolense* on the spleen of *P. berghei*-infected mouse and followed for 8 and 28 days are presented in Figure 7. The micrography of the spleen from healthy mouse shows normal parenchyma with distinctly white and red pulp, trabecula, central artery and splenic artery (Figure 7A). In the malaria-infected mouse, it was noted major disorganization in the parenchyma marked by the absence of differentiation of the white and red pulp; dilatation of splenic arteries and the presence of malarial pigment in parasitized red blood cells (Figure 7B). Spleen micrography of infected mice treated with chloroquine (10 mg/kg) (Figure 7C) or with aqueous extract of *E. angolense* at 250 and 500 mg/kg (Figures 7 E and F) present normal architecture with white and red pulp quite distinct as similar to the normal control, however the presence of malarial pigment both on day 8 (Figures 7 C, E, F) and on day 28 (Figures 7 C', E', F') while spleen section of animals treated with the extract at 125 mg/kg shows less pronounced disruption in the structure of the organ with the presence of malarial pigment (Figure 7 D).

The present study aimed to investigate the *in vivo* activity of the aqueous extract of *E. angolense* stem bark on *P. berghei*-infected mice. Malarial infection progressively developed in absence of treatment resulting in the death of all animals in the malaria control group at day 26 with a parasitemia up to 60.66%. Indeed, malaria is a multisystem disease that can induce serious physiological disturbances and lead to death.[2,19] The administration of the aqueous extract of *E. angolense* stem bark induced significant decrease in parasite growth with dose-dependent effect, resulting to the survival of all treated animals and the

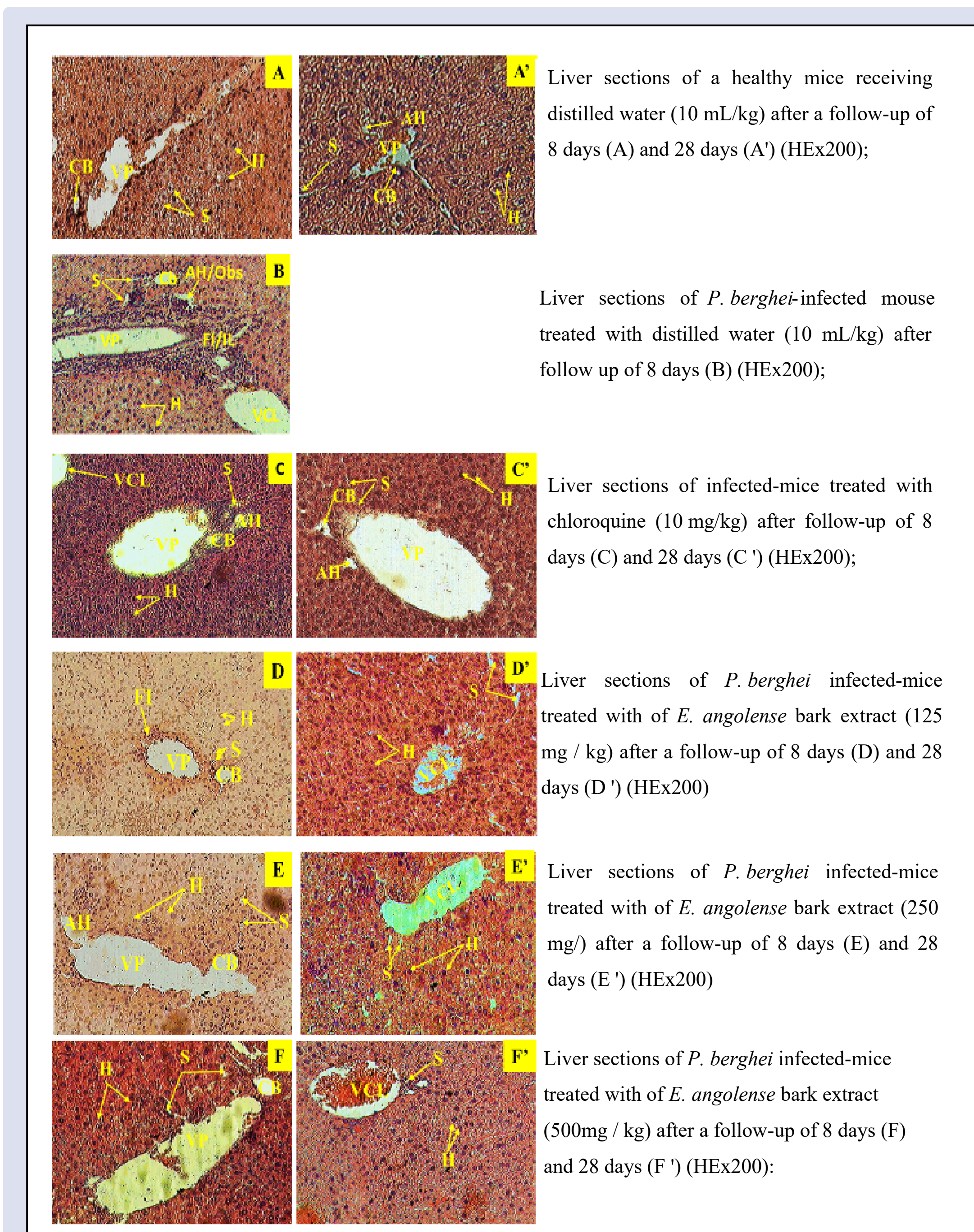

Figure 5: Effects of *E. angolense* bark on the photomicrography of the liver sections of *Plasmodium berghei*-infected mice. VP = portal vein; Cb = bile canaliculus, H = hepatocyte, AH = hepatic artery, VCL = centrilobular vein, S = sinusoidal capillary, FI / IL = inflammatory zone with leukocyte infiltration.

complete parasite clearance at day 28, accompanied with the ED_{50} of the extract estimated at 25.32 mg/kg. These results indicate antiplasmodial properties of the plant, which could be classified as a very good antimalarial activity (< 100 mg/kg).[20,21] This activity could be assigned to either one or a combination of compounds such as alkaloids, phenols, saponins and flavonoids contained in the plant extract. Alkaloids act by inhibiting the biosynthesis of fatty acids, useful for the parasite growth while flavonoids exert a cytoprotective effects.[22] Saponins and phenols have also been reported to act through the detergent effect on cell membrane or by inhibiting protein synthesis in parasite.[23] The body weight decline, hypoglycemia and anemia observed in untreated animal have been described as major impairment in malarial infection resulting from high parasitemia. It is known that, *Plasmodium* asexual stage, modifies some membrane function of the host cell that becomes more permeable to molecules, facilitating the entry of glucose and other nutrients required for parasite growth and multiplication.[24] Likewise, tissue anoxia induces by higher parasitemia could also lead to high glucose utilization into lactate production, causing the decrease in blood glucose levels as observed in the present study.[25] The parasite infects large number of cells which are then destroyed in the spleen resulting in hemolytic anemia. The extract administration not only protected the animals from physiological disturbance during treatment, but also for

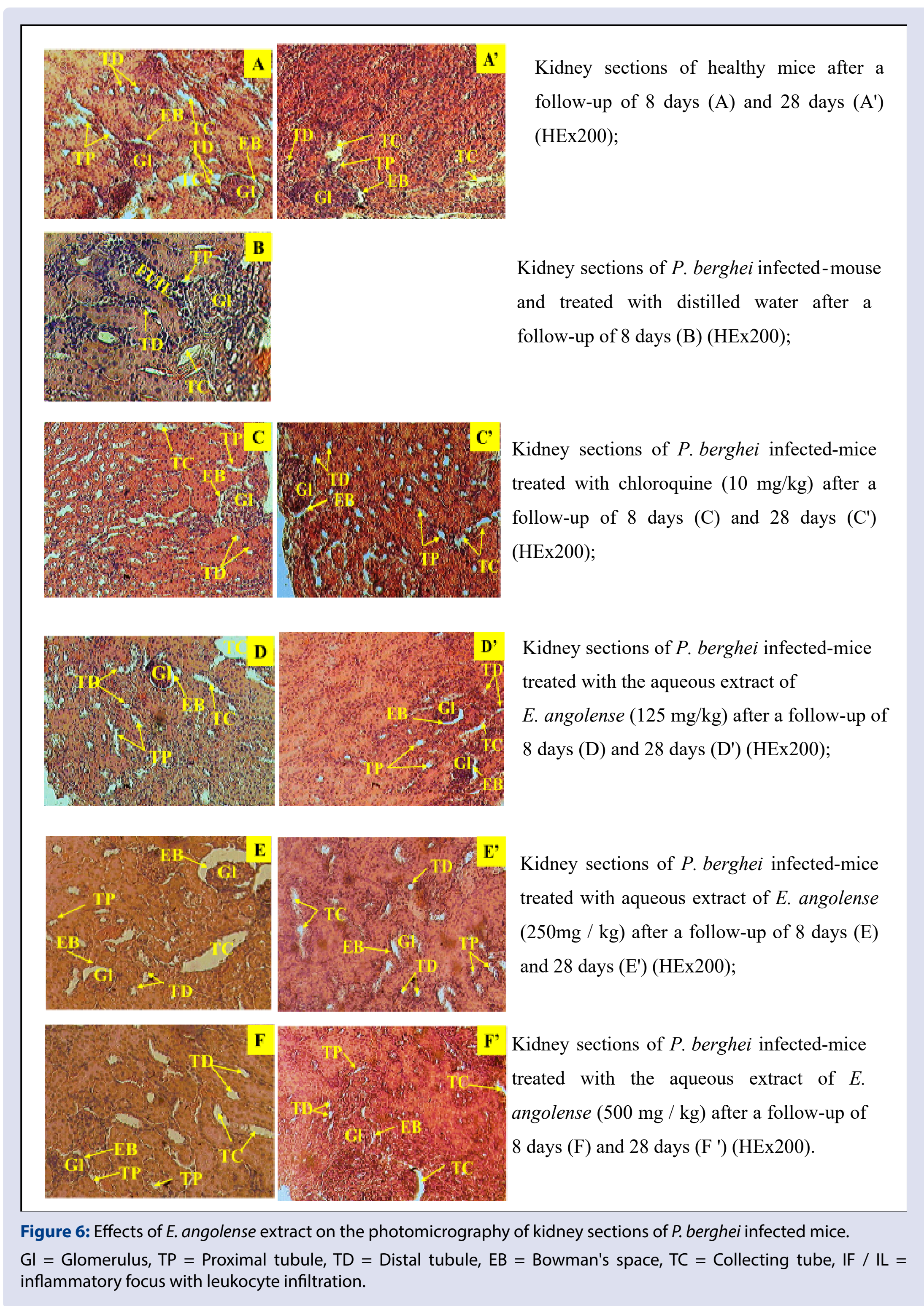

Figure 6: Effects of *E. angolense* extract on the photomicrography of kidney sections of *P. berghei* infected mice.

Gl = Glomerulus, TP = Proximal tubule, TD = Distal tubule, EB = Bowman's space, TC = Collecting tube, IF / IL = inflammatory focus with leukocyte infiltration.

the follow-up indicating the ability of the extract to prevent anemia and to inhibit plasma uptake of glucose by the parasite.

Plasmodium infection has resulted in a significant increase in transaminase activities (ALT and AST), total bilirubin and creatinine levels at the end of the 8th day of experiment, expressing renal and hepatic function failure. Liver dysfunction was accompanied by hepatomegaly, inflammatory zones in the liver parenchyma, leukocyte infiltration around centrolobular vein. This dysfunction has been reported in malarial infection and could result from sequestration of plasmodium-parasitized red blood cells in the capillaries, causing clogging of capillaries in most organs and the resultant ischemia can lead to organ dysfunction.[26] In addition, hemolysis contributes to the rising of bilirubin in malarial infection.[27] Renal damage could also proceed from precipitation of hemoglobin crystals in the renal tubules due to the intravascular hemolysis which impair glomerular filtration and induce high secretion of creatinine.[28,29] The plant extract treatment of infected animals has prevented these organ impairments, showing the ability of plant to kill malaria parasite and thwart its deleterious effects. Indeed, its constituents such as flavonoids and tannins

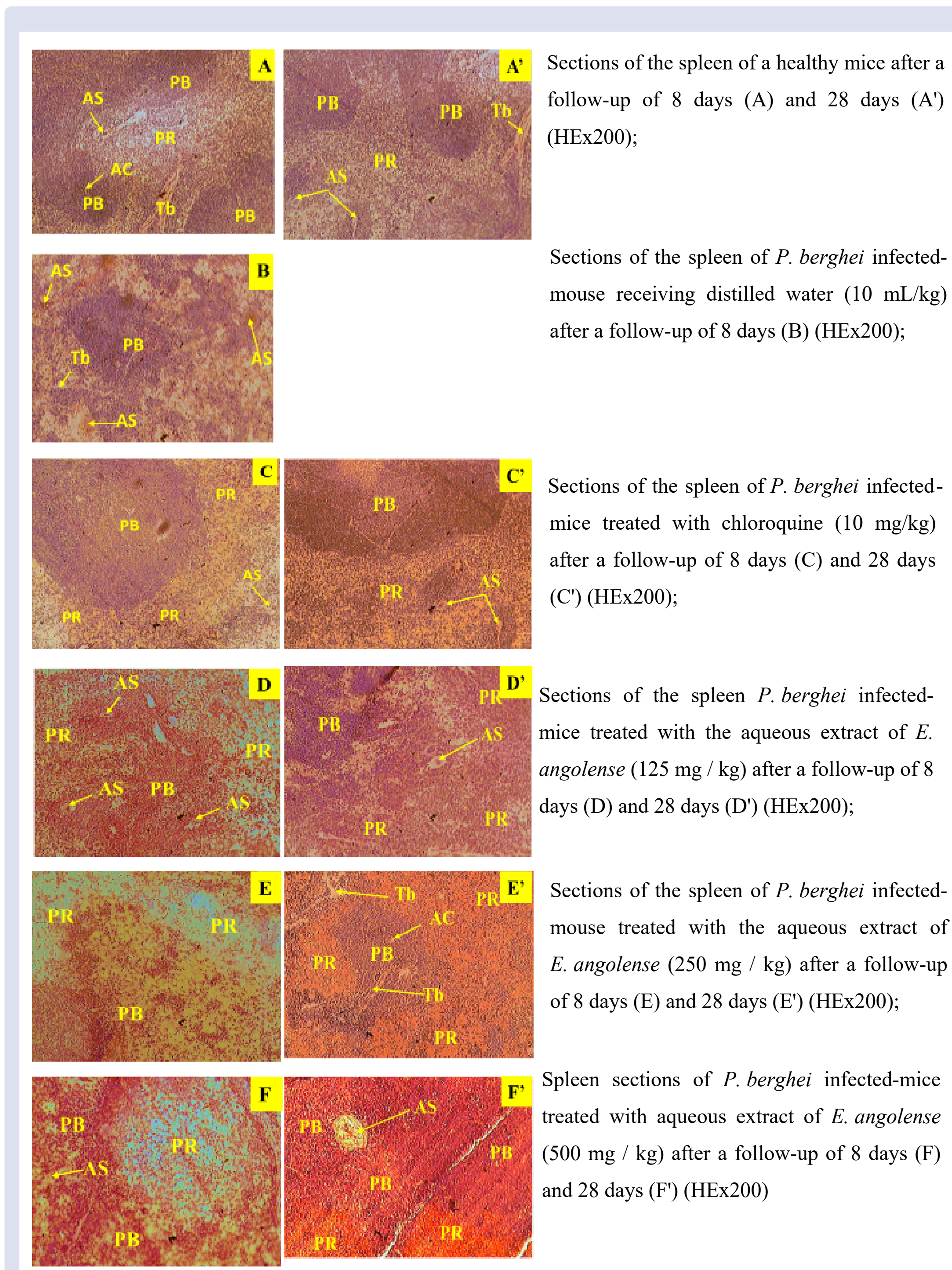

Figure 7: Effects of *E. angolense* extract bark on the photomicrography of spleen sections of *P. berghei* infected-mice. PR = red pulp, PB = white pulp, AS = splenic artery, Tb = trabecular, AC = central artery.

respectively protect cells from damages[30,31] and form complexes with macromolecules to promote the regeneration of tissues.[32,33]

The degradation of hemoglobin into amino acids by the parasite during malaria generates large amount of reactive oxygen species (ROS) through the oxidation of Fe^{2+} to Fe^{3+}.[34,35] This phenomenon was expressed in the present study by the significant decrease in antioxidant defenses such as catalase, SOD, reduced glutathione and nitrite (NO) whereas MDA concentration increased as seen in malaria control group. MDA is conventional evidence of lipid peroxidation resulting from massive hemolysis and hepatocyte cell membrane. The administration of the aqueous extract of *E. angolense* for five days significantly increased catalase, SOD and reduced glutathione activity as well as MDA, suggesting that the extract could mop free radicals produced during the degradation of hemoglobin by plasmodium. The maintenance of these parameters at values close to normal 20 days after stopping treatments suggests that our extract would have long-term protective effects on the tissues of the body. This ability of the extract would be due to the presence of compounds with antioxidant effects such as tannins and

phenols. The tannins are a group of compounds which acts as primary antioxidant or garbage from free radicals.[36] The phenolic compounds act as antioxidants through their redox properties, allowing them to act as reducing agents, donors of hydrogen.[37] These results corroborate with those obtained by Ayoola et al.[38] demonstrating antioxidant properties of the methanolic extract of *E. angolense* bark.

In addition, no toxicity signs was revealed in the acute toxicity test of the aqueous extract of *E. angolense* bark and no death was observed in animals after 14 days of experimentation at the dose of 5000 mg/kg, indicating that the lethal dose 50 (LD_{50}) of the extract is greater than 5000 mg/kg with classification as less or not toxic substance according to the Globally Harmonized Classification System (GHS).[39]

CONCLUSION

This study reports the *in vivo* efficacy and safety of the aqueous extract of *E. angolense* stem bark on *P. berghei*-infected mice with a complete parasite clearance that led to the survival of all treated animal. The plant extract also protected from anemia, leucocytosis, liver, kidney and spleen damages, the decrease in antioxidant defense and prevented architectural damage in organs. However, further experiments are required to fully explore the antimalarial potential of *E. angolense* bark prior to its classification as an alternative substance in malaria control.

ACKNOWLEDGEMENT

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ABBREVIATIONS

ALT = alanine aminotransferase, AST = aspartate aminotransferase, EDTA = Ethylene diamine tetracetic acid, RBC = red blood cells, HGB = haemoglobin, HCT = Haematocrit, WBC = white blood cells, LYM = lymphocytes, MON = monocytes, GRA = granulocytes, PLT = platelets, GSH = reduced glutathione, SOD = superoxide dismutase, MDA = Malonedialdehyde, NO = nitrite oxide, ROS = reactive oxygen species, LD_{50} = lethal dose 50, ED_{50} = Effective dose 50.

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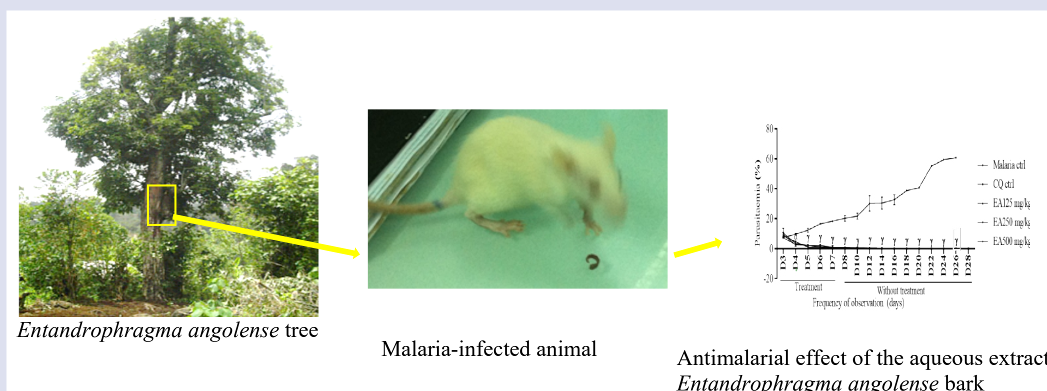
GRAPHICAL ABSTRACT



Entandrophragma angolense tree

Malaria-infected animal

Antimalarial effect of the aqueous extract *Entandrophragma angolense* bark

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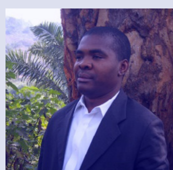

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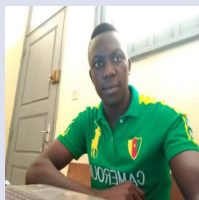

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Fabrice FEKAM BOYOM, Full Professor in Analytical Biochemistry at University of Yaounde 1 and the Head of AntiMicrobial and Biocontrol Agents Unit focused on Drug discovery against pathogenic protozoa, fungi, and bacteria. His team has developed standard approaches to investigate potential anti-infective agents in vitro and in vivo. The team has identified potent antimalarial and antifungal plant extracts. In addition, their recent work has identified highly promising anti-Toxoplasma hits that are currently under hit-to-lead development at the AntiMicrobial Agents Unit. From their work, they have published over 35 peer-reviewed papers within the last five years.

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