Toxicity Profile, Antiplasmodial and Gametocytocidal Properties of *Erigeron bonariensis* L. (anc. *Conyza bonariensis* (L.) Cronquist).

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Abstract

Ethnopharmacological relevance. *Erigeron bonariensis* L. (anc. *Conyza bonariensis* (L.) Cronquist) is a medicinal plant with traditional use in Cameroonian folk medicine and other parts of Africa for the management of ailments, including malaria.

Aim: This study focused on investigating the antiplasmodial, gametocytocidal properties and toxicity profile of *E. bonariensis*.

Materials and methods: Extracts of *E. bonariensis* were prepared by sequential maceration using n-hexane, ethyl acetate, and methanol, and their antiplasmodial properties were assessed on both sexual and asexual *Plasmodium* parasite stages. Additionally, cytotoxicity was assessed on the MDA-MB-231 breast cancer cell line, while acute and sub-chronic toxicity were evaluated using albino mice.

Results: Generally, all extracts were active against both stages of the parasites at different levels. Hexane extract exhibited good antiplasmodial activity (IC_{50} 6.02 ± 1.13 μg/mL) against asexual parasites while also demonstrating the highest gametocytocidal activity (IC_{50} 1.863 ± 0.717μg/mL). All extracts were relatively non-toxic on the MDA-MB-231 breast cancer cell line. No significant adverse effects at the tested dose were observed following an acute exposure with an LD_{50} > 5000 mg/kg BW. In the sub-chronic toxicity evaluation, no significant changes in body weight, or hematological and biochemical parameters were observed across the dosed groups compared to the control, with a p-value < 0.05. Additionally, histopathological examination of the liver indicated no signs of toxicity or abnormalities.

Conclusion: The findings show that *E. bonariensis* has effective antiplasmodial and gametocytocidal properties *in vitro* and has relatively low toxicity. This study supports the use of *E. bonariensis* as a culturally appropriate alternative for malaria management. However, further studies are needed on the safety profile and in harnessing the therapeutic potential of *E. bonariensis* for the development of novel antimalarials.

Keywords: Malaria, Medicinal plants, *Erigeron bonariensis*, Antiplasmodial, Toxicity

Introduction

Malaria continues to pose a significant global health challenge, particularly in resource-limited regions. It is responsible for over 251 million cases and has caused over 610,000 deaths across 85 malaria-endemic countries in 2023 globally (WHO, 2024). Cameroon is among the 11 countries with the highest malaria burden, contributing to 70% of global malaria cases and reporting over 2.7 million cases, according to the World Health Organization (WHO, 2024). The primary chemotherapeutic medication used to treat malaria is Artemisinin-based Combination Therapies (ACTs). However, the development and spread of drug-resistant strains of *Plasmodium falciparum* to ACTs (WHO, 2024) and to other antimalarials have impeded disease control efforts, emphasizing the critical need for alternative therapeutic agents. In response, there has been a growing interest in natural products research, particularly plant-derived compounds, which have become increasingly important in modern medicine for their potential to combat malaria and other diseases (Kamaraj et al., 2023; Nchang et al., 2023; Shinyuy et al., 2025).

Among these natural sources, *Erigeron bonariensis* L. (anc. *Conyza bonariensis* (L.) Cronquist), belongs to the Asteraceae family, and commonly known as fleabane has attracted attention due to its traditional use in folk medicine (Hanwen, 2007). Indigenous to South America, *Erigeron bonariensis* (EB) is a species of flowering plant with alternate lance-shaped leaves covered with fine hairs. EB has upright stems covered in thick hairs. At the tips of the branches are clusters of tiny, daisy-like blooms (**Figure 1**). EB flourishes throughout roadsides, meadows, pastures, and waste places thanks to its fibrous root system (Hanwen, 2007). EB is an invasive species in some parts of North America, Europe, Africa, and Asia (Hanwen, 2007). It is widely distributed across the temperate and tropical regions and has a long history of use as a traditional remedy for treating fevers related to malaria (Araujo et al., 2013). EB has a rich phytochemical composition, including flavonoids, glucosides, terpenoids, essential oils, phenolic compounds, and sesquiterpene lactones, among others (Ferreira et al., 2023; Opiyo, 2023). Due to its rich phytochemical profile, EB has demonstrated

activity against several ailments. It is known for its antifungal, antibacterial, antioxidant, analgesic, anti-inflammatory, and antimicrobial properties (Boniface and Pal, 2013). EB is used traditionally for a variety of illnesses, including asthma, bronchitis, hypertension, anti-inflammatory, antimicrobial, malaria, and diarrhea (Espinoza et al., 2020; Kamaraj et al., 2023; Opiyo, 2023). Hence, a product of high demand, especially in malaria-endemic regions and therefore, a plant of high ethnomedicinal and ethnopharmacological relevance. However, the daily massive consumption of natural plant products to manage diseases such as malaria and the integration of traditional medicine into conventional modern medicine make it imperative to determine the efficacy and document the safety profile of this natural plant product (Bandaranayake et al., 2006).

Although EB has a long history of use and is of ethnopharmacological importance in alleviating fevers related to malaria (Opiyo et al., 2023), there is little or no data on both the safety profile and efficacy on *Plasmodium* gametocytes (the form of the parasite responsible for the disease transmission) and the asexual parasites (causes symptoms and pathology of malaria). It is therefore important to elucidate and document the toxicity profile as well as the efficacy of EB on malaria parasites. This study is focused on investigating the antiplasmodial potentials of leaf extracts of EB against the gametocytes and asexual forms of *P. falciparum* and evaluating its toxicity profile by assessing cytotoxicity, acute and sub-chronic oral toxicity. Moreover, targeting the *Plasmodium* gametocyte is crucial as it will serve as a vital tool to block transmission of malaria while also alleviating the disease pathologies. This would provide valuable insights into the potential therapeutic application of EB and substantiate its traditional use, particularly in resource-limited settings where malaria burden remains high. Leveraging EB's pharmacological properties might offer new insights into the search and development of biologically active compounds with broader application in the management of infectious diseases.

Figure 1: Field picture of EB in the Adamawa region of Cameroon

2 Materials and methods.

2.1 Collection and processing of plant material

Fresh leaves of EB were collected from the Adamawa region, Cameroon, and voucher specimens were deposited at the Limbe Botanical Gardens, authenticated, and a voucher number assigned (SCA 1678). The leaves were sorted, oven-dried at 30°C, powdered, and kept in appropriate condition for the preparation of crude extracts.

2.2 Preparation of crude extracts

Crude extracts were prepared by sequential maceration as described by Mbah et al. (2012). In brief, the plant powder was successively macerated at room temperature for three days, starting with n-hexane, followed by ethyl acetate, and finally methanol. Whatman No. 1 filter paper was used to filter the mixture, and Büchi brand Rota vapor was used for rotary evaporation under pressure to concentrate the filtrate at 30°C. After being recovered using the appropriate solvents, each crude extract was dried, weighed, and kept for further use at 4°C. Additionally, ethanol extract was prepared as described above and used to evaluate the safety profile of EB.

2.3 *Plasmodium falciparum 3D7 in vitro* culture and viability test

As described by Trager and Jensen (1976), a chloroquine-sensitive 3D7 *P. falciparum* strain was maintained *in vitro* in 3 % hematocrit (human type A or O-positive red blood cells). Complete Culture Medium (CCM) used in the assay was prepared by supplementing RPMI 1640 (Gibco, Fisher Scientific, Merelbeke, Belgium) medium containing NaHCO3 (32 mM), HEPES (25 mM), and L-glutamine with 10 % heat-inactivated human plasma, 1.76 g/L of glucose (Sigma-Aldrich, Overijse, Belgium), 44 mg/mL of hypoxanthine (Sigma-Aldrich, Overijse, Belgium), and 100 mg/L of gentamycin (Gibco, Fisher Scientific, Merelbeke, Belgium). Parasitemia was verified by microscopy using Giemsa-stained thin films. All cultures were placed in a humidified incubator at 37 °C with a standard gas mixture of 5 % O_2, 5 % CO_2, and 90 % N_2. A stock solution of crude extracts was prepared by dissolving 10 mg of extract in 1 mL DMSO, and an antiplasmodial assay was conducted using an initial extract concentration of 100 μg/mL, which was subsequently serially diluted to eight concentrations. Each extract was tested in duplicate wells with the parasites at a final volume of 250 μL, making a final DMSO concentration of < 1%. The positive control consisted of wells with parasites only, while the negative control consisted of wells with only culture medium. As a standard, a stock solution of Artemisinin (1μg/mL) (Gibco, Fisher Scientific, Merelbeke, Belgium) was made and serially diluted as described above. During a 48-hour period of incubation, plasmodial lactate dehydrogenase activity was estimated to assess parasite growth, as previously reported (Kenmogne et al., 2006), and linear regression was used to determine the IC_{50} (concentration that kills 50% of parasites). All tests were repeated at least twice.

2.3 Induction of gametocytogenesis, culture, and viability assay

Gametocyte induction and culturing were done as described elsewhere (Gebru et al., 2017). Before the start of initiation of gametocyte culture, synchronization of asexual chloroquine-sensitive NF-54 *P. falciparum* strain was performed by D-sorbitol (5% w/v) and magnetically aided magnetic cell separation with LD-MACS. Upon

synchronisation, gametocyte culture was started with a 6% haematocrit, 0.3% ring-stage parasitaemia, and a daily change of medium for 14 days. Haematocrit was dropped to 3% when a parasitaemia of 3% was reached. N-acetyl-D-glucosamine was added to a final concentration of 50 mM between days 10 and 14 to eliminate all parasite forms other than late-stage gametocytes (stage IV and V), which were then subjected to *in vitro* viability assays. Working concentrations were prepared by serially diluting the stock solution of extracts (prepared as previously described) in at least 1:500 (DMSO < 0.4 %). Methylene blue (50µM) prepared in sterile distilled H_2O was used as a gametocytocidal drug control. Medium (80µL) was introduced in each well, and 40µL of the extract/drug was added in well A and serially diluted (1:3 dilution) to well G. The last wells were used as growth control (well H). A column for the gametocyte dilution control was also prepared. Gametocyte cultures were purified with NycoPrep and MACS separation (Held et al., 2014), and an improved Neubauer counting chamber was used to quantify the parasites. 20µL of parasite suspension containing about 50000 gametocytes was added to each well. Plates were incubated for 48 h at 37°C, 5% CO_2, and 5% O_2. A luminescent assay based on the quantitation of ATP produced by the parasites (BacTiter-Glo) was used following the protocol described by Lelièvre et al. (2012) to assess the viability of gametocytes, and data were analyzed using R and the dcr-package to obtain IC_{50} values. The assay was repeated at least twice in technical duplicate.

2.4 Cytotoxicity assays

Using a resazurin-based assay, cytotoxicity of EB extracts was assessed on MDA-MB-231 cells (triple-negative breast cancer) that were seeded in 96-well cell culture plates at a density of 2,500 cells per well (2.5×10^4 cells/mL). Cells were allowed to grow and adhere for 24 hours (Bowling et al., 2012) and subsequently exposed to EB extracts for 72 hours in complete medium (DMEM + 10% FBS), at a final DMSO concentration of < 0.5%. Positive control wells received DMEM containing 0.5% DMSO, whereas negative control wells were treated with doxorubicin (5 µM). The plates were kept in

an incubator set at 37°C with 5% CO_2 in a humidified environment for the whole 72-hour incubation period.

Cell viability assay was performed after 72 h incubation (old media removed) by adding 75µL of resazurin reagent in each well (PrestoBlue, Thermo Fisher Scientific, ref. A13261) and fluorescence was measured using FlexStation microplate reader at an excitation/emission wavelength of 560/590 nm after 2 hours of incubation. The fluorescence values obtained were used to compute the IC_{50} values (concentration needed to inhibit 50% of the cell population relative to the controls).

2.5 Ethical clearance

Ethical clearance for work with animals was granted by the University of Buea Institutional Animal and Use Committee (UB-IACUC), Cameroon, with a permission number: UB-IACUC N°23/2024.

2.6 Animals

Three-month-old female albino mice weighing between 17-25 g obtained from the Faculty of Medicine and Pharmaceutical Sciences, University of Douala, Cameroon, were used for acute toxicity study, while 8-week-old female albino mice weighing between 18-24g were used to evaluate sub-chronic toxicity. Animals were divided into several test and control groups, maintained in standard environmental conditions (30 ± 3°C, 12 h light/ 12 h dark cycle, free access to water and food. All methods used in this study complied with the Organization for Economic Co-operation and Development (OECD) guidelines and regulations for the testing of chemicals (OECD, 2001).

2.8 Acute Toxicity

Female adult mice were starved overnight, weighed, and divided into three distinct groups of 5 animals each. These groups were as follows: a control group and two test groups. Through an oral route using a gavage, the test groups received a single dose of 2000 and 5000 mg/kg BW of ethanol extract of EB, whereas the control group

received 1 mL of distilled water per 100 g of body weight. Animals in all groups were observed for 2 hours and then allowed access to food and water. Animals were then observed once daily for the next 14 days for any mortality and other signs of toxicity, including changes in the skin and fur, eye color, convulsions, salivation, diarrhea, or coma. Body weight was measured daily throughout the experiment.

2.9 Sub-chronic toxicity

For the sub-chronic toxicity evaluation, 24 non-pregnant and nulliparous female mice were employed and randomly divided into 4 groups of 6 mice each. The test groups (group I, II, and III) received daily doses of 400, 800, and 1600mg/kg BW of ethanol extract of EB, respectively (El Kabbaoui et al., 2017), whereas the fourth group (control group) received distilled water. All treatments were administered via gavage at the same time each day over 28 days. Animals were concurrently observed for signs of abnormalities, and body weights were recorded. After this treatment period, all animals were allowed free access to water but not food overnight, then put under ether anesthesia. Blood was collected with and without anticoagulant, Ethylenediaminetetraacetic acid (EDTA) by retro-orbital puncture for hematological and biochemical studies, respectively. After blood collection, mice were sacrificed by decapitation and dissected. The liver was collected and fixed in 10% formaldehyde for histological analysis.

2.10 Hematological and biochemical parameters assessment

An automatic hematological analyzer (SysmexKX-21N) was used for hematological analysis including red blood cell (RBC) count, leukocyte (WBC) count, hemoglobin (Hb), hematocrit (HCt), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count, lymphocyte, monocyte, neutrophil, basophil and eosinophil counts (Costa et al., 2020).

Blood was centrifuged at 3000 rpm for 10min using a centrifuge (ROTOFIX 32A®, Mettich Zentrifugen, Germany) for the analysis of biochemical parameters in sera.

This included the determination of glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total cholesterol (TC), low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglyceride (TG), alkaline phosphatase (ALP), total bilirubin (TB), conjugated bilirubin (EB), total protein (TP) and albumin (ALB). Additionally, concentrations of urea and creatinine were measured to assess renal function as well as other metabolites and minerals; uric acid, Na^{+}, Cl, K^{+}, Ca^{2+}, Mg^{2+}, and inorganic phosphorus. The assays were performed using an automatic biochemical analyzer (Hitachi902, Roche) with a spin react biochemical kit.

2.11 Liver's histopathological evaluation

This was achieved by microscopy, wherein tissues of the liver were processed with a microtome and an automated tissue processor. Each sample was afterward embedded, placed on a glass slide, and stained with Hematoxylin and Eosin (HE). Tissues were visualized by microscopy to note changes in their structure, and pictures were taken.

2.12 Data analysis

A combination of statistical packages, including R programming, SPSS (version 25.0), and GraphPad Prism (version 10.2.0), was used for statistical analysis. RM-ANOVA was used to analyze the body weight of the mice, and one-way ANOVA, followed by Tukey's multiple comparisons, was used to analyze liver function indices and lipid parameters, hematological, and kidney parameters. A p-value of < 0.05 was considered statistically significant by comparing the test results with the control group.

3 Results

3.1. Inhibition of *P. falciparum* asexual and sexual blood stages by EB extracts

The antiplasmodial property of EB was evaluated by exposing asexual chloroquine-sensitive (*Pf*3D7) and sexual NF-54 strains of *P. falciparum* to n-hexane, ethyl acetate, and methanol extracts of EB. All three extracts demonstrated strong gametocytocidal

activity with IC_{50} values < 5 μg/mL (**Table 1**). The n-hexane extract was the most active against both the gametocytes and asexual parasites with IC_{50} values < 7 µg/mL (**Table 1**). The three extracts recorded CC_{50} values > 100 µg/mL against MDA-MB-231 cells (**Table 1).**

Table 1: Dual-stage drug sensitivity assays (n=3).

	IC_{50} (µg/mL) ± SD		**CC_{50} (µg/mL)**
Extract/standard drug	**Gametocytes**	**Asexual parasites**	**MDA- cells**
n-Hexane	1.86 ± 0.72	6.02 ± 1.13	>100
Ethyl acetate	3.53 ± 1.35	30.61 ± 4.80	>100
Methanol	2.47 ± 1.06	10.53 ± 2.82	>100
Methylene blue	0.12 ± 0.46	_	_
Artemisinin	_	0.004 ± 001	_

3.2. Acute toxicity

Following an acute study, no mortality was recorded in either the control group (vehicle) or the group that was treated with a single dose of 2000 mg/kg BW of EB during the 14-day follow-up period. However, at a higher dose of 5000 mg/kg BW of the extract, a 1/5 mortality rate was recorded on day 3 of the follow-up period. Loss of appetite, hypoactivity, and dizziness were recorded in the treatment groups (2000 and 5000 mg/kg BW) during the follow-up period. Lack of sensitivity to noise was observed in the 5000 mg/kg BW treatment group, but not in a lower dose group (**Table 2)**. Following OECD guidelines, the median lethal dose (LD_{50}) can therefore be estimated to be greater than 5000 mg/kg BW.

Table 2: An overview of toxicity signs after an acute oral administration of ethanol extract of EB.

Group	**Dose (mg/kg BW)**	**Number of mice**	**D/T**	**Symptoms of toxicity**
1	Vehicle	5	0/5	- None

2	2000	5	0/5	- Loss of appetite - Hypo-activity - Dizziness
3	5000	5	1/5	- Loss of appetite - Hypo-activity - Dizziness - Lack of sensitivity to noise

3.3. Sub-chronic toxicity of ethanol extract of EB

3.3.1. Visible indicators of toxicity and body weight

All the animals were observed for two hours post administration of ethanol extracts of EB at the different doses daily to identify signs and symptoms of toxicity and behavioral changes. This study reveals no alteration in behavior or indication of toxicity with no mortality during the study, which attests to the safety of EB following a sub-chronic oral dosing. Generally, there was an increase in the relative body weights of the animals both in the control and test groups during the 28-day study period. The highest growth rate was observed in the control group, with a relative body weight increase of up to 17%. At increasing doses, the rate of increase in the percentage body weight in the test groups was significantly lower than the control (400 mg/kg BW ($p < 0.05$) < 800 mg/kg BW ($p < 0.001$) < and 1600 mg/kg BW ($p < 0.001$)).

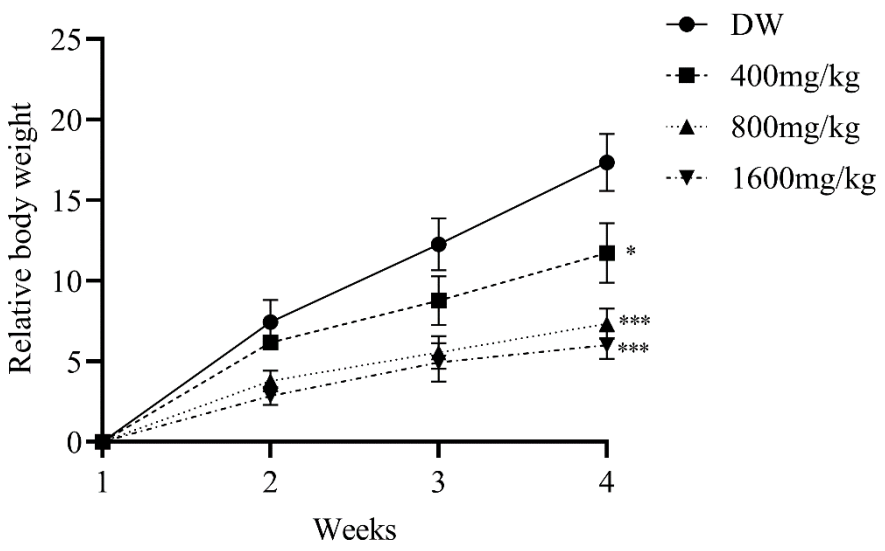

Figure 2: Effect of EB extracts on body weight of mice following sub-chronic treatment.

3.3.2. Effect of ethanol extract of EB on Liver function indices and lipid parameters

In the sub-chronic toxicity study of ethanol extract of EB, biochemical analysis of serum samples showed that the serum levels of marker enzymes did not change significantly in the test groups compared to the group treated with distilled water, except serum AST, ALT, ALP, and total cholesterol (TC) levels. Generally, there was a significant decrease ($p < 0.05$) in the serum levels of AST, ALT, ALP, and TC in the test groups (400, 800, and 1600 mg/kg BW) compared to the distilled water-treated group **(Table 3).**

Table 3: Liver function indices of mice treated with ethanol extract of EB for 28 consecutive days.

		Ethanol extract of EB		
	Normal group	**400mg/kg BW**	**800mg/kg BW**	**1600mg/kg BW**
Glu (g/L)	0.57 ± 0.02	0.57 ± 0.05	0.58 ± 0.03	0.55 ± 0.02
AST (IU/L)	227.31 ± 3.93	216.58 ± 2.62**	210.65 ± 4.25***	206.09 ± 4.01***
ALT (IU/L)	52.78 ± 1.05	50.08 ± 1.54	44.04 ± 1.02***	40.42 ± 0.88***
ALP (IU/L)	337.92 ± 1.25	318.66 ± 11.69*	315.38 ± 8.18***	301.86 ± 5.10***
TP (g/L)	72.14 ± 0.69	70.62 ± 0.62	71.94 ± 0.50	71.14 ± 0.50

ALB (g/L)	20.22 ± 0.11	20.77 ± 0.68	20.46 ± 0.18	20.49 ± 0.10
TB (mg/L)	6.03 ± 0.13	6.43 ± 0.70	6.85 ± 0.08	6.47 ± 0.73
EB (mg/L)	3.67 ± 0.08	3.22 ± 0.40	3.16 ± 0.53	3.10 ± 0.30
TC (g/L)	0.63 ± 0.01	0.63 ± 0.02	0.57 ± 0.03***	0.59 ± 0.03***
LDL (g/L)	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00
HDL (g/L)	0.53 ± 0.01	0.51 ± 0.03	0.51 ± 0.02	0.51 ± 0.02
TG (g/L)	1.42 ± 0.14	1.49 ± 0.20	1.44 ± 0.11	1.49 ± 0.11

Data was analyzed by one-way ANOVA, followed by Tukey's multiple comparison test, and presented as mean ± SEM with *$p < 0.05$, ***$p < 0.001$, significantly different from the control group. Glu: glucose, ALT: alanine aminotransferase, AST: aspartate aminotransferase, ALP: alkaline phosphatase, ALB: albumin, TP: total protein, TB: total bilirubin, EB: conjugated bilirubin, TG: triglyceride, TC: total cholesterol, LDL: low density lipoprotein, HDL: high density lipoprotein.

3.3.3. Effect of ethanol extract of EB on hematological parameters

There were no significant modifications in hematological parameters of test groups compared to the distilled water-treated group after a twenty-eight-day treatment of animals with different doses of EB, except for WBC count, which increased significantly ($p < 0.05$) in the group treated with the dose 600 mg/kg BW relative to the distilled water-treated group. RBC count was also seen to increase significantly ($p < 0.001$) in all the treatment doses (400, 800, and 1600 mg/kg BW). There was a general increase in all the other hematological parameters of EB-treated mice as compared to the distilled water-treated group (**Table 4**). However, these increases were not significant.

Table 4: Effect of EB ethanol extract on hematological parameters in mice

		Ethanol extract of EB		
Parameter	**Normal group**	**400 mg/kg BW**	**800 mg/kg BW**	**1600 mg/kg BW**
WBC (X103 U L^{-1})	11.18 ± 0.13	10.78 ± 0.15	11.56 ± 0.03	12.07 ± 0.89 *
RBC (X106 Ul^{-1})	6.02 ± 0.15	6.72 ± 0.13***	6.72 ± 0.11***	6.87 ± 0.23 ***
Hemoglobin (G/Dl)	11.66 ± 0.27	11.90 ± 0.31	12.05 ± 0.127	11.57 ± 0.47
Hematocrit (%)	34.14 ± 0.40	34.72 ± 0.49	34.31 ± 0.24	33.79 ± 0.73

Platelets (X103 μl^{-1})	543.66 ± 7.44	530.66 ± 8.33	535.83 ± 8.22	542.83 ± 24.88
Lymphocytes (%)	59.26 ± 0.32	59.46 ± 4.44	61.16 ± 3.57	64.79 ± 3.81
Monocytes (%)	12.58 ± 0.65	12.68 ± 1.10	12.99 ± 0.48	12.94 ± 0.55
Granulocytes (%)	20.82 ± 0.36	20.98 ± 0.73	20.91 ± 0.55	21.00 ± 0.43
Neutrophils (%)	21.53 ± 0.31	21.29 ± 0.22	21.66 ± 0.38	21.44 ± 0.43
Eosinophils (%)	2.30 ± 0.20	2.29 ± 0.13	2.515± 0.07	2.48 ± 0.10
Basophils (%)	0.00 ± 0.00	0.00 + 0.00	0.00 ± 0.00	0.00 ± 0.01
MCV (Fl)	55.54 ± 0.38	55.55 ± 0.39	55.55 ± 0.32	56.04 ± 0.41
MCH (Pg)	16.73 ± 0.13	16.65 ± 0.19	16.66 ± 0.20	16.60 ± 0.22
MCHC (G/Dl)	28.92 ± 0.14	28.89 ± 1.03	29.40 ± 0.63	28.79 ± 0.74

Data were presented as mean ± SEM (n=6 animals/group). *p < 0.05, ***p < 0.001, significantly different from the distilled water treated group using one-way ANOVA, followed by Tukey's multiple comparison test. Where WBC is white blood cell, MCV mean corpuscular volume, RBC red blood cell, MCH mean corpuscular hemoglobin, and MCHC mean corpuscular hemoglobin concentration.

3.3.4. Effect of ethanol extract of EB on kidney parameters and other metabolites

Some kidney parameters were significantly altered. For example, blood concentration of urea, uric acid, Ca^{2+}, and Mg^{2+} decreased significantly p < 0.05–0.001) in the EB-treated groups relative to the distilled water-treated group **(Table 5).** There was a general depletion in the serum levels of the other kidney parameter (creatinine) and other metabolites (Na^{+}, Cl^{-}, $K^{+,}$ and Inorganic P), though not significant.

Table 5: Effect of oral administration of EB extract on kidney parameters and other metabolites in mice treated for 28 consecutive days.

		Ethanol extract of EB		
	Normal group	**400mg/kg BW**	**800mg/kg BW**	**1600mg/kg BW**
Creatinine (mg/L)	7.08 ± 0.12	7.63 ± 0.46	7.17 ± 0.38	7.05 ± 0.44
Urea (mg/ L)	0.39 ± 0.01	0.31 ± 0.05***	0.31 ± 0.01***	0.25 ± 0.02 ***
Uric acid (mg/ L)	43.15 ± 0.52	40.95 ± 0.72 *	40.32 ± 0.63 **	37.53 ± 0.84 ***
Na^{+} (mEquiv./L)	131.06 ± 0.50	130.13 ± 3.66	131.55 ± 2.59	131.71 ± 1.38

Cl^- (mEquiv./L)	81.90 ± 1.02	80.00 ± 1.28	81.62 ± 2.24	80.98 ± 0.95
K^+ (mEquiv./ L)	2.79 ± 0.21	2.62 ± 0.35	2.69 ± 0.23	2.70 ± 0.29
Ca^{2+} (mg/ L)	74.21 ± 0.43	71.28 ± 0.42*	69.88 ± 2.28 **	70.04 ± 0.72 ***
Mg^{2+} (mg/L)	16.23 ± 0.16	15.19 ± 0.46	15.11 ± 0.56 *	15.26 ± 0.60 *
Inorganic P (mg/L)	30.60 ± 1.21	28.52 ± 1.08	29.00 ± 0.50	30.91 ± 0.79

Data were presented as mean ± SEM (n=6 (3 females+3males)/group). *p < 0.05, **P < 0.01, ***p < 0.001, significantly different from the distilled water treated group using one-way ANOVA, followed by Tukey's multiple comparison test.

3.3.5. Histopathological assessment of the liver

No pathological abnormalities were observed in the liver profile of the test groups compared to the distilled water-treated group. For example, after 28 days of treatment, there was no significant distortion of the liver cytoarchitecture at all doses of EB relative to the distilled water-treated group. Additionally, analysis of the histological profiles of the liver showed that the control or distilled water-treated group had normal liver profiles comprising hepatocytes well arranged in cords radiating from a central vein. **(Figure 3).**

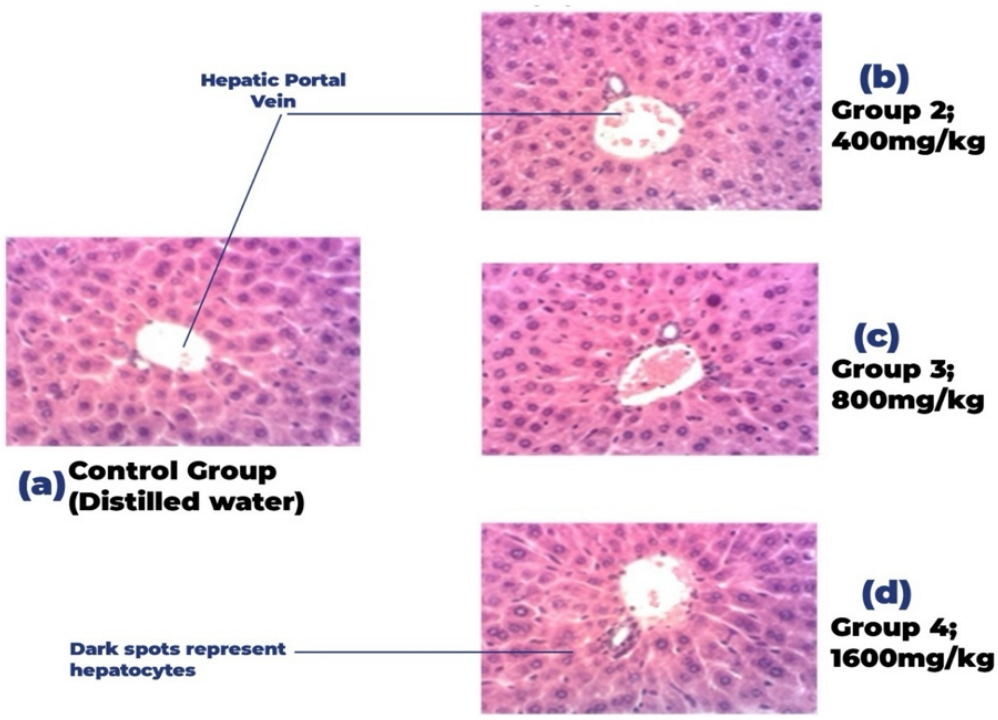

Figure 3: Histological section of the liver of mice subjected to various treatments with the ethanol extract of EB.

4 Discussion

E. bonariensis (EB) is a medicinal plant with traditional use in Cameroonian folk medicine and other malaria-endemic countries. However, there is limited information on the safety profile and efficacy of EB against *P. falciparum* gametocytes. This study focused on investigating the safety profile of EB by assessing the acute and sub-chronic exposure of ethanol extract of EB in an appropriate animal model and investigating the *in vitro* antiplasmodial activity and gametocytocidal activity. As mentioned earlier, n-hexane, ethyl acetate, and methanol extracts of EB were prepared by sequential maceration and subjected to *in vitro* assay to assess their antiplasmodial properties on both sexual and asexual chloroquine-sensitive NF-54 and 3D7 *P. falciparum* strains, respectively. Additionally, ethanol extract was prepared and used for both acute and sub-chronic oral toxicity assessment.

According to the classification describe by Jansen et al. (2017), antiplasmodial activity levels of plant crude extract are conventionally categorized as follow; highly active ($IC_{50} \leq 5$ μg/mL), good activity (IC_{50} 5 - 15 μg/ mL), moderate activity (IC_{50} 15 - 30 μg/mL), weak activity (IC_{50} 30 - 50 μg/mL) and lack of activity with $IC_{50} \geq 50$ μg/mL. In this context, the n-hexane and methanol extracts demonstrated good activity against the asexual *Pf3D7* strain, whereas the ethyl acetate extract showed weak activity. Interestingly, all extracts demonstrated high gametocytocidal activity against the *P. falciparum* NF-54 strain. The rich phytochemical composition of EB, especially flavonoids and phenolic acids, could account for the observed anti-plasmodial properties. For example, phenolic compounds such as caffeic acid and chlorogenic acid are present in EB (Barhoumi et al., 2013) and hence, could contribute to the plant's antimalarial effects. By inducing oxidative stress, phenolic compounds are known to disrupt the life cycle of the malaria parasite and diminish its virulence (Mamede et al., 2020). Additionally, quercetin and kaempferol, which are naturally occurring compounds in EB (Barhoumi et al., 2013), have been shown to interfere with the growth of *P. falciparum* (Shinyuy et al, 2023). The reported antimalarial effect may also be possibly explained by the presence of conyzine, an alkaloid isolated from EB (Opiyo, 2023) that could interfere with parasite metabolism and protein production, leading to death.

In the acute toxicity study, the aim was to classify acute toxicity of EB extract after a single exposure according to the Globally harmonized system of classification and labelling of chemicals (GHS). The acute toxicity of the ethanolic extract of EB was evaluated by administering a single dose of 2000 mg/kg BW and 5000 mg/kg BW to mice. During the 14 days post administration, there were no overt signs of toxicity in both the control and the test groups. No mortality was observed in the test group (2000 mg/kg BW of extract), while one death was recorded in the test group (5000 mg/kg BW) on day 3. Following the 14-day post administration, both the test group and the distilled water-treated groups showed no significant signs of toxicity. Behavioral and physical parameters such as grooming, locomotion, and physical appearance

remained normal, indicating that EB is well tolerated in an acute exposure, with an LD_{50} > 5000 mg/kg BW. This agrees with the findings of Zalabani et al. (2012), who reported an LD_{50} > 5000 mg/kg BW after an acute oral administration of methanol extracts of EB in mice.

A repeated daily oral administration of the ethanolic extract of EB in mice for 28 consecutive days was performed to determine the subchronic effect of EB. In this study, the ethanolic extract of EB demonstrated a safety profile as evidenced by the absence of significant changes in the general behavior or body weight of mice after a subchronic oral exposure. The fact that there were no significant differences or appreciable variations in the percentage of body weight between the test and the distilled water-treated or control groups indicates that the ethanolic extract had no direct impact on the animals' overall health. There was a significant increase in the relative body weight of mice in the control group during the experiment. Treatment with different doses of the extract resulted in a significant reduction in the growth rate of mice compared to the control group. This may likely be due to a decrease in appetite and or slowdown in metabolism, evidenced by the significant decrease in liver parameters, including total cholesterol (TC, synthesized mainly in the liver).

Biochemical assessments focused on markers of liver and kidney function. A significant reduction in the activities of AST, ALT, ALP, and the level of total cholesterol (TC) in the test groups, with respect to the control group, indicates the hepatoprotective and lipid-lowering properties of the extract. Reduction in AST and ALT activities indicates a decrease in liver metabolic activity, leading to decreased synthesis of enzymes; this may occur at the level of nucleic acid metabolism or protein synthesis. Additionally, a decrease in total cholesterol (TC) suggests that the plant extract may have cholesterol-lowering properties by modulating lipid metabolism or reducing the synthesis of cholesterol.

In our present study, there were no appreciable variations in the serum creatinine levels between the treatment and control groups, whereas only urea was significantly decreased. During deamination, ammonia (NH_3) is removed from the blood by

conversion into urea, and an increase may be because of high glomerular filtration (Olaniyan et al., 2016). A decrease in urea concentration with sub-chronic toxicity administration of ethanol extract of EB suggests efficient kidney function produced by the extract for the removal of NH_3. Also, we observed a progressive decrease in the level of uric acid. Excess uric acid is caused by defects in the metabolism of purine nucleotides. The observed reduced production of uric acid in the treated mice points to either a decline in nucleic acid catabolism or increased excretion; however, decreased synthesis (mainly in the liver) agrees with the decrease in hepatic enzymes and TC. Estimating the levels of electrolytes (K^+, Na^+, Cl^-, Ca^{2+}, Mg^{2+}, and inorganic phosphorus) in serum may be important in the assessment of renal function since the outcome of the regulatory mechanism of osmotic balance and ionic charges can be determined by the levels of electrolytes in the blood (Opoku-Okrah et al., 2015). Sodium is the major cation of the extracellular fluid, where it regulates water-electrolyte balance and protects the body against excessive fluid loss. Potassium is the major intracellular cation with a similar role to that of Sodium. In the extracellular and intracellular fluid, sodium and potassium, respectively, are the main cations that control the water-electrolyte balance and shield the organism from excessive fluid loss. Sodium and potassium levels were not significantly different in the test groups relative to the control group, confirming that the plant extract had little or no impact on the water-electrolyte balance in mice. However, the extract could specifically impact divalent cation homeostasis, possibly through hormonal and/or renal function, evidenced by the depletion in Ca^{2+} and Mg^{2+} ions.

From the findings of Mukinda and Syce (2007), the hematopoietic system is more susceptible to harmful substances, and therefore, assessing the effect of the ethanol extract of EB on the hematopoietic system is important to further validate its safety profile. This is possible through the evaluation of hematological parameters after a sub-chronic oral administration in mice (Adebayo et al., 2017). Results from this study indicate that, except for RBCs and neutrophils (decreased concentration), WBCs, lymphocytes, and hemoglobin (increased concentration) that differ sufficiently in the

test groups compared to the distilled water treated group, most of the investigated hematological parameters did not show significant variation. The mean increase in WBCs level in all test groups is most likely induced by inflammation which could be partly confirmed by the mean increase in Lymphocyte levels in the test groups The mean rise in WBCs levels in the test groups may partially supports the hypothesis that inflammation is the cause of the mean increase in lymphocyte levels in all test groups (Yu et al., 2020). The mean increase in WBC counts in this study indicates that EB may possess immunomodulatory properties (Berinyuy et al., 2015). In our investigation, the mice treated with the plant extract showed a progressive increase in the RBC count from group 2 (400 mg/kg BW) to group 4 (1600 mg/kg BW) and a mean increase in the hemoglobin levels ($p < 0.05$). Therefore, these increases are not attributed to any sign of toxicity; the mean increase in hemoglobin in all 3 treatment groups suggests that the ethanol extract of EB likely stimulated erythropoiesis.

It was found that mice in the test and distilled water-treated groups had normal hepatocytes and portal tracts in their livers after a macroscopic and histological assessment. No lesions or abnormal pathology in the profile of the liver susceptible to the graded doses of plant extract were seen. These are good indicators for the liver functions, further suggesting that oral treatment with EB does not alter hepatocytes. Additionally, the absence of histopathological changes is a key indicator that the biochemical and hematological alterations were probably mild and likely reversible.

5 Conclusion

These findings demonstrate that EB has strong antiplasmodial and gametocytocidal properties *in vitro*, which suppress or inhibit parasite growth and could interrupt transmission. With an oral LD_{50} value > 5000 mg/kg BW, the ethanol extract of EB can be considered practically nontoxic for a single dose or short-duration administration. Additionally, the findings from the 28-day subchronic toxicity experiment further confirm the safety of this plant extract, as the plant extract did not induce damage to hepatocytes, alter blood parameters, alter kidney profile parameters, or affect the body

weight of animals during subchronic administration. In this context, this study supports the use of EB as a culturally sound alternative for managing malaria. However, further studies are needed to fully understand the safety profile and harness the therapeutic potential of EB for the development of novel antimalarials.

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